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TABLE OF CONTENTS

ASSOCIATION AFFAIRS

OTHER TECHNICAL PAPERS PRESENTED AT THE 1961 CONVENTION

Effects of pesticides on eggs and larvae of oysters (C. virginica) and clams (V. mercenaria). . HARRY C. DAVIS and HERBERT HIDU

Gametogenesis and spawning of the European oyster, O. edulis, in waters of Maine . . . . . VICTOR L. LOOSANOFF

Survival and growth of larvae of the European oyster, O. edulis, at lowered salinities . . . .HARRY C. DAVIS and ALAN D. ANSELL

Water transfer in oysters during processing . . . . . E. A. FIEGLER and A. F. NOVAK

Use of chemically-treated clutch for increased production of seed oysters . . . . . C. L. MACKENZIE, JR., V. L. LOOSANOFF and W. T. GNEWUCH

Longevity of the southern oyster drill, Thais haemastoma . . . . . PHILIP A. BUTLER

Field tests of a chemical method for the control of marine gastropods . . . . . H. C. DAVIS, V. L. LOOSANOFF and C. L. MACKENZIE, JR.

The effects of salinity and temperature on egg laying and larval development of the flatworm, Stylochus ellipticus . . . . . RICHARD C. TONER

Predation by the flatworm, Stylochus ellipticus, on young oysters . . . WARREN S. LANDERS

Laboratory technique of hard clam culture . . . . . V. L. LOOSANOFF

Recent developments in oyster mortalities in Delaware Bay . . . . . HAROLD H. HASKIN, DONALD KUNKLE and W. A. RICHARDS

The status of MSX in Virginia—a review of field conditions . . . . . JAY D. ANDREWS and JOHN L. WOOD

Mortality rates in Chincoteague Bay among oysters of several origins . . . . . .FRED W. SIELING

Life cycle stages of MSX . . . . . JOHN MYHRE

Studies of MSX transmission in oysters . . . . . WALTER J. CANZONIER

Curdle disease of oysters in Virginia..J. D. ANDREWS and J. L. WOOD

Suspended matter within one-half inch of the bottom as related to the food of scallops . . . . . NELSON MARSHALL

Filter-feeding by the soft-shell clam, Mya arenaria; preliminary experiments . . . . . JOHN W. BLAKE

The arterial and venous systems of the visceral mass of the American oyster . . . . . ALBERT F. EBLE

Procedure and techniques in an oyster larval and spatfall programme . . S. E. VASS and W. B. STALLWORTHY

An experiment in the selection of oyster brood stocks . . . . . JOHN J. GALLAGHER and PHILIP A. BUTLER

PANEL DISCUSSIONS

MEASUREMENT OF SHELL GROWTH IN OYSTERS BY WEIGHING IN WATER[1]

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ABSTRACT

Extensive use of a modification of the Havinga method of weighing oysters in water, using a Type K7 T GD Mettler balance with suspension attachments, has shown that weekly weighing of numbered individuals reveals individual differences in rate of shell deposition which correlate with feeding, parasite infections, etc. The method is quick, sensitive, and accurate.

Methods of measurement of growth in oysters have become stereotyped despite widespread recognition of the inaccuracies involved. Usually one or more linear measurements, air weight, volume or some combination or derivative of these ($L \times W$, L^3) is used. All of these are based on shell growth. No method has been developed for assessing meat growth except by sacrificing part of a population. This has led in growth studies to strong emphasis upon sampling of populations. But individuals in populations of oysters are notoriously variable. Being irregular in shape, oysters of the same weight can be quite variable in linear dimensions. Also, oysters with the same origin, history, and treatment can vary widely in weight at a given age (Butler 1956b).

Few studies have considered growth of individual oysters because representative specimens are difficult to choose and marking and measuring many individuals is tedious. The state of health of experimental oysters is seldom determined. (See Hopkins and Menzel 1952, Menzel and Hopkins 1955). Group samples of populations do not reveal the real parameters of environmental, hereditary, and health factors, and without individual records the techniques available for statistical analyses of variation are quite limited.

Another weakness of conventional methods of measuring growth is the long period required to obtain detectable changes in size. Even

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1 Contribution from the Virginia Institute of Marine Science No. 110.

in fast-growing species such as oysters, a month may be the minimum period for measurable changes in size.

Many changes in external environment and internal metabolism can occur in a month, and it becomes very difficult to determine and describe the conditions in which a certain growth occurred.

Another feature of most growth studies is the concern with practical applications in terms of how long it takes a certain stock to reach marketable size in given waters. Hopkins and Menzel (1952), and Hopkins, Mackin and Menzel (1953) have given excellent accounts of the factors which affect growth and yield in the Gulf of Mexico. McHugh and Andrews (1955) and Andrews and McHugh (1957) have discussed the same subjects for Chesapeake Bay. Butler (1952a), concerned with the practical problem of potential meat yields, uses the ratio of total volume of the oyster to shell volume as an index. Weights, volumes, and linear measurements are quite satisfactory for many field problems, but these same techniques have often been applied without adequate precision in basic studies of the causes of variations in growth.

The purpose of this paper is to call attention to a method which permits quick and accurate measurement of shell growth at short intervals. Daily shell growth was first demonstrated by Havinga (1928) by weighing oysters immersed in water. In this country Hewatt (1951 and 1952) first used the method in Louisiana studies in 1949 and started its use in Virginia in 1951. Havinga's paper was not discovered by us until 1955, although the method was originally suggested to Hewatt by Dr. P. Korringa. In Virginia many years of weekly and bi-weekly weighings indicate continuous calcification of shell throughout the year except in winter. The work of Wilbur and Jodrey (1952) with radioisotopes has shown that measurable shell deposition occurs in periods of only hours. Havinga's method measures total calcification of shell over short periods of time. Perhaps because the title emphasized growth rather than methods, Havinga's paper has seldom been referred to in the literature. Not only has the method been ignored but important conclusions about growth rates have been overlooked.

DESCRIPTION OF METHOD

The weight of live oysters suspended in water consists essentially of shell weight. The specific gravity of oyster meats is very close to that of salt water. Consecutive in-water weighings measure shell deposition. In-water weight of an oyster is approximately half the weight in air.

The mechanics of handling oysters for weighing in water are quite simple due to the oyster's ability to maintain watertight closure for long periods. Nevertheless, precaution should be taken to keep oysters in water prior to weighing except for brief periods of transfer and cleaning. Only weak oysters and those numbed by cold are slow to close when disturbed. At Gloucester Point oysters are held in trays suspended in the York River. Before being lifted, trays are jiggled under water to ensure closure of oysters. Oysters selected for weighing must be free of injuries and crevices which prevent tight closure. This has been no problem with young oysters which are by far the best subjects.

Selection and preparation of oysters is important. Oysters with crumbly shells and those infested with shell-boring organisms should be avoided. If infested oysters must be used, they should be treated with brine solution or other chemical solutions for removal of shell pests (MacKenzie and Shearer 1961 , Shearer and MacKenzie 1961). In our experiments, fairly large oysters of 15 g (30 g in air) or more have been used for disease studies. However, small young oysters are more sensitive indicators of environmental changes, since growth rate decreases rapidly with increase in size (Andrews, unpublished data). Also, young oysters in the Chesapeake area are usually relatively free of diseases, hence spat and yearlings provide the most satisfactory material for most experiments.

All fouling organisms and loose shell must be removed initially and oysters must be recleaned before each weighing. This has not been difficult except for short periods in spring and fall when barnacle sets are abundant. Weekly weighing minimizes the cleaning problem. It is most important to remove all calcareous growth. Breakage of new "bill" or "shoot" shculd be avoided but is not important, because calcium deposition is slight in new fragile shell.

Oysters are given individual numbers in various colors of "Mark-tex" ink after quick-drying the shells with a fan. The felt-nib quick-dry marking pens now common in drug stores have not been tried extensively but appear to be even better.

Constant conditions for weighing should be sought. Each investigator should note small errors from temperature, salinity and volume changes in the vessel used for submersion. Oysters should be kept in running water throughout preparation to maintain ambient temperatures. Sharp changes in water temperatures may cause air bubbles to form on oysters. At Gloucester Point ambient salinities of the York River are used throughout the weighing process for weekly changes are usually small. Complete submersion is essential.

At first a triple-beam balance was adapted with a grid pan suspended in a large finger bowl. Now a Mettler balance (Type K7 T GD) is used with suspension attachments which can be tared for direct reading of oyster weights (Fig. 1). Oysters can be weighed almost

Fig. 1. Arrangement of Mettler scale for suspension weighing in water. Suspension equipment is tared permitting direct reading of oyster weights.

as rapidly as one linear measurement can be made. Immersed weighing is less convenient in the field although the triple-beam balance has been used on docks.

Weights are estimated to the nearest 0.01 g on a scale which reads in 0.1 g. Weights can be replicated easily within 0.05 g even after oysters have been removed from water for some time. A change in weight of less than 0.1 g in a week has become one indicator of "sick" oysters in my work. "Good" growth for a 15 g oyster in water

is 1 g per week. The regularity of increase in weights from week to week and the persistence of healthy oysters in depositing shell throughout the warm season is illustrated in Fig. 2. This shows a

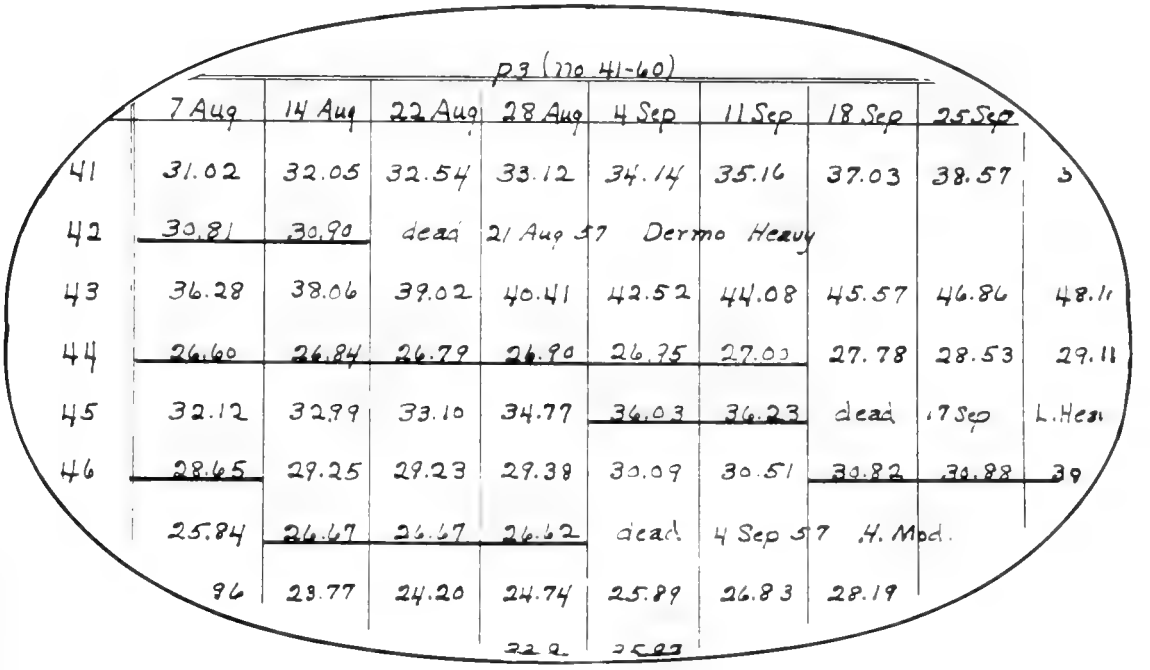

Fig. 2. Example of data sheet for weighing oysters in water. Underlining indicates periods of "sickness" as indicated by lack of growth.

portion of a data sheet in late summer when Dermocystidium (Andrews and Hewatt 1957) was active. Growth of several individual oysters is shown in Fig. 3 to indicate continuity of growth throughout the warm season and variation in patterns with the health of oysters. Further examples can be found in Hewatt (1951).

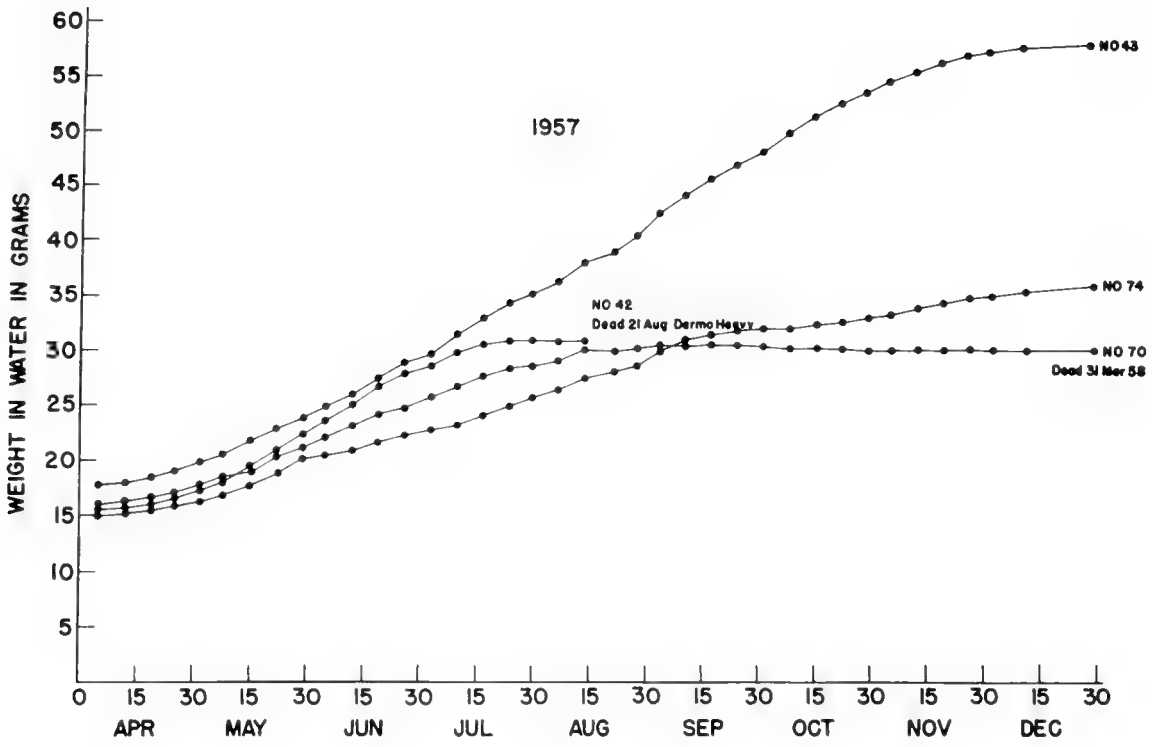

Fig. 3. Progressive weekly weights of four oysters: fast and slow growers, and two "sick" oysters. No. 74 was probably sick too but did not die.

USES OF THE IN-WATER WEIGHING TECHNIQUE

Measurement of Short-term Growth

Short-term measurement of growth is the most important advantage of the method. The environmental factors (internal and external) regulating growth of oysters are continually changing. The shorter the period of growth which can be measured, the easier it is to describe the conditions which produced this growth. One week has proven to be an adequate period for useful sorting of oysters by their growth potentials. Havinga's method does not equal radioisotope methods in sensitivity and shortness of interval to obtain measurable growth (Wilbur 1960), but does permit observations of growth under more favorable and more natural conditions. In contrast, linear measurements and air weights may be meaningful only by months or seasons, and the sensitivity decreases with age and size of oysters even more rapidly than for in-water weighing.

Assessment of Variations in Growth Between Individuals

Most investigators are aware of wide variations in growth between individual oysters (Butler 1952b and Walne 1958). Yet individual oysters are often used to measure pumping rate, feeding rate and other physiological characteristics with no more check on growth potential than that provided by the investigator's intuition. Variation in a group can not be adequately measured without individual records.

Detection of "Sick" or Weak Oysters

Hewatt (1951) and Menzel and Hopkins (1955) demonstrated that oysters sick with infections of Dermocystidium marinum stopped increasing in weight and eventually died. I have successfully used cessation of shell growth as a basis for separating sick oysters from healthy ones where other diseases and parasites are involved as well as in Dermocystidium studies.

Effects of Diseases and Parasites on Oysters

The effects of experimental and natural infections can be followed by weight changes in oysters since shell deposition seems to be closely linked to health and well-being. See Menzel and Hopkins (1955). However, physiological states may occur in healthy oysters in which shell deposition does not occur. Individuals with no apparent cause for lack of growth have been suspected of harboring undiagnosed disease.

Effects of Various Treatments

The time is fast approaching when oysters will be exposed to various chemicals and treatments designed to control predators and diseases. Salt brine, fresh water, chlorinated hydrocarbons, and heavy oils are being used or proposed already. Injuries, food combinations, pollution, and many other conditions of stress or benefit can probably be followed in terms of oyster reaction by the in-water weighing method.

Index of Suitability of Environment

Oysters are among the most efficient filter feeders in terms of quantity of water processed. As a sampling device for measuring plankton content of water, they may be as useful as nets and pumps. Although efficiency of collection is high, the kinds and quantities of

food used are not so well understood. Nevertheless, a group of oysters is a sensitive indicator of quality of the environment as expressed by in-water weights. Two or three weighings over a period of several weeks will give a useful evaluation of currently existing conditions for oyster growth in an area. Many kinds of comparisons of environment may be possible in respect to growth, fattening, pollution, and other factors. For example, in 1961 oyster growth in the York River was stopped for about one month in mid-summer during a conspicuous and extensive "red tide" bloom.

A Check on Experimental Conditions

Weighing in water is a scientist's tool rather than a practical measure of growth and yield. It is being used at the Virginia Institute of Marine Science to check laboratory conditions in experiments such as conditioning for spawning, suitability of aquarium habitats, studies of fecal and pseudofecal deposition, and food studies. "Control" groups can be held in natural waters for comparison. It appears that oysters without food do not deposit appreciable amounts of shell.

DISCUSSION

Havinga (1928) anticipated many of the uses of the weighing techniques and his paper should be consulted by anyone using the method. A very careful description of methods is included. I concur in his observations about the irregularity of fresh shoot or bill formation and its relative unimportance as a sign of oyster growth. I have not found it necessary to be as cautious as Havinga was about handling oysters. I have observed no interference with growth from necessary handling. The few hours of interruption while the oysters were being weighed or numbered merely deprived them of that time for feeding.

It is important to realize that oysters thicken their shells throughout life by additions to the entire inner surface of the valves. This is apparently much less true of many other pelecypods in which the valves remain almost uniform in thickness with most additions of new shell at the edges. Even in oysters Wilbur and Jodrey (1952) provide evidence that calcification is more rapid near the borders of the valves, but the adjustments of shell shape discussed by Korringa (1951) and Galtsoff (1954) may modify this pattern. Injuries may also alter the rate and pattern of shell deposition. In Virginia no change of weight occurs between late December and early April when temperatures are usually below 5° C. However, Galtsoff (1958) reports that injuries or obstructions will induce shell repairs in mid-winter at very low temperatures.

The technique of weighing in water has been tried with hard clams, Mercenaria mercenaria, without much success. Calcification is apparently much slower than in oysters. For example, hard clams held in trays without substratum become infested with Polydora websteri and are incapable of covering the resulting mud blisters satisfactorily. The weighing method requires rapid daily deposition of calcium salts and water-tight closure of shells. The oyster exhibits these characteristics to a high degree. Other tightly-closing mollusks should be tried.

Although the capacity of oysters to repair shells appears to be large, there have been no striking examples of exceptional rates of shell deposition. There is no indication that the usual rates of deposition of fast-growing oysters can be exceeded—even if repair is urgent. The fastest rates of deposition have been observed in apparently healthy oysters with continuous growth over many weeks.

My observations indicate that oysters deposit shell only when feeding satisfactorily. Oysters held in aquaria with limited food do not add enough shell to be measured by the weighing technique. Orton's observations (1925) of continued shell growth in the absence of food probably referred to production of new bill, which is primarily organic in composition. The Havinga technique provides a means of determining the total deposition of calcium salts for rather short periods in favorable environments. This should provide a quantitative base line for investigations of calcification in mollusks.

It is important to determine by radioisotope methods whether shell deposition is intimately related to food metabolism. Observations that shell deposition can be interrupted by sickness, failure to pump, or lack of food, suggest a rather close tie between calcification and food collection. Collection by separate techniques of concurrent data on pumping rates, amount of food collected and weights in water should provide new insights into oyster metabolism. Since diapedesis (emigration of leucocytes to outer surfaces) is frequently observed in oysters held out of water for some time, there may be an excretory function too in shell deposition.

Recent work (Bevelander 1952) indicates that considerable confusion exists as to the source of calcium salts for shell deposition. It is recognized that the amount deposited is too great to be stored in tissues or to be derived from food alone. However, when food is being collected, large volumes of water are being passed over and through the tissues providing ample opportunities for absorption

or diffusion of calcium salts in marine species. It would be quite surprising if shell deposition occurred to any extent while oysters are closed.

In summary, weighing in water is a sensitive technique for measuring individual variations in growth over short periods. The method permits selection of optimal conditions and the most satisfactory individuals for experimental studies. The disadvantages are failure to indicate meat quality, necessity for cleaning oysters carefully before each weighing, and a lack of usefulness in studying old eroded oysters.

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TECHNIQUES IN VISUALIZATION OF ORGAN SYSTEMS IN BIVALVE MOLLUSKS

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ABSTRACT

Three-dimensional representation of organ systems greatly increases the ability of the observer to visualize ramifications and relationships to other parts, and may even be indispensable. Of techniques tried in the present research, the more useful are reported in detail: embedding and sectioning of shell and entire specimens; rubber molds of the shell cavity; and vinyl acetate injections of the vascular and digestive systems, with subsequent plastic embedding and sectioning of corrosion preparations and cleared preparations.

INTRODUCTION

This paper presents a merging of techniques independently utilized in research programs at the University of Delaware and at Rutgers—The State University of New Jersey. At the former institution the emphasis has been upon techniques for studying shell structure and the organs attached to or secreting the shell; at the latter university the initial goal was to delineate the circulatory system and its relationship to other organ systems. Thus, the techniques used in each research program are logically merged because they are largely complementary, with both studies including relationships to other organ systems.

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2 The research conducted at the Trenton Junior College was supported by a National Science Foundation Science Faculty Fellowship (#68032) and by the Trenton Junior College.

Counsel in the two research programs has been most welcome and is gratefully acknowledged.

In the formative state of the research at the University of Delaware, Dr. Philip A. Butler, Biological Laboratory, U. S. Fish and Wildlife Service, Gulf Breeze, Florida, provided information on sectioning of shells. When problems arose in the embedding of whole clams in plastic, Dr. Robert A. Littleford, Ward's Natural Science Establishment, Inc., Rochester, New York, offered useful suggestions. Student assistants, Guyer W. Hartranft and Richard L. P. Custer, have done most of the embedding and sectioning and much of the credit for the refinement of the techniques described here is due to their hard work and ingenuity.

Dr. Leslie A. Stauber, Chairman, Department of Zoology, Rutgers—The State University, first suggested the possibility of clearing injected oysters; he has been, in addition, in his role as major professor to one of us (A.F.E.), a constant source of advice and guidance in every phase of the work on oyster circulation. Mr. Michael Sommerstein, presently an undergraduate at the University of Cincinnati, pioneered the early work on the vinyl acetate circulatory casts of oysters at Trenton Junior College.

PRESENTATION OF TECHNIQUES

The shell of a bivalve mollusk provides a useful topographic framework for descriptions of the positions and relationships of other organ systems. We will begin, therefore, with a description of techniques for preparing shells for study and then proceed to other organ systems.

The following sections and subsections are indexed, using the Dewey Decimal System, to aid the reader.

1.0 Shell Structure

1.1 Objective.

The study of bivalve shells reveals information on the comparative anatomy and ecology of bivalves and on the relation of shell growth and structure to environmental conditions (Shuster, 1957). While both surficial (inner and outer) and sectional views of shells are required, sections have been most useful in the study of growth patterns and finer structure of shells.

1.2 Microsections of Mollusk Shell.

Preparation of thin shell sections for megascopic analysis consists of three steps: 1) slicing the shell or cutting it to the size needed, 2) mounting the section on the glass slide, and 3) grinding section to proper thickness, and polishing it.

The shell is first encased in a supporting medium for ease in slicing the shell with a diamond cut-off saw. Several types of plaster have been tried, including dental gypsum, but good results can be obtained with a fine white gauging plaster. Ice blocks of embedded specimens are often easier to handle. We cut a block containing the shell into slices not less than 6 mm thick. Position of the shell in a plaster block is marked during embedding to allow the operator to cut the shell along predetermined lines of the shell (e. g., anterior to posterior or dorsal to ventral). When both valves are desired in the section, they should be filled with plaster and then pressed together and set before being placed in a block. After a gypsum block is cut into slices, each slice must be cut up to the edge of the shell so that the plaster can be removed without damaging the shell.

Before mounting the shell slice on a glass slide the side of the shell to be mounted should be ground flat with no. 220 grit and polished with no. 440 or no. 600 grit. Then a slide of adequate size (1 x 3 inch or 2 x 3 inch microscope slide or 3 1/4 x 4 inch lantern slide) is chosen so that the specimen can be mounted securely. The slide is placed on a hot plate preheated to 250-275 F, depending upon the mounting medium (we use a stick form of a thermoplastic cement—no. 70C LAKESIDE BRAND, manufactured by Hugh Courtright & Co., 7600 South Greenwood Ave., Chicago 19, Ill.). The melted cement is formed into a small mound on the slide in the shape of the shell slice. The shell is laid very slowly onto the mound of cement, as if placing a cover slip on a wet medium, to prevent any trapped air under the shell. Slight pressure on the shell at this time also may help remove the air. The preparation is removed from the hotplate to cool, and is then ready to be ground down.

In the case of fragile shell or a preparation involving both valves in juxtaposition, the cement should be placed up around the sides of the shell to provide extra support. This is essential with oyster shell since it tends to be more friable than other specimens.

The shell slices are ground on a lapidary's grinding wheel to the desired thickness, using great care to prevent the shell from

fracturing. When the shell slice is 10 mm or more in thickness, the first grinding is done with no. 120 grit until a thickness of about 5 mm is reached. Care must be taken to insure even thickness over the whole section. After this thickness is reached, both the shell and the grinding surface are thoroughly washed to remove all traces of the no. 120 grit and a base of no. 220 grit is put onto the grinding surface. When a thickness of 1 mm is reached, a no. 440 grit is used until the shell section becomes transparent. During this stage, only a very little pressure can be applied, lest the shell fly apart. The final polish to remove any pits or scratches is done with a cloth buffing wheel and a compound of tin oxide and the "Hanson Van Winkle" Brown (4M-30) buffing cake. After this the slide may be heated slightly to remove the haze on the cement. For extra protection spray the slide with clear plastic, such as Krylon acrylic lacquer, or use Canada balsam and a cover slip. The shell is now ready for microscopic study.

We make SnO_2 polishing cakes by melting a Hanson Van Winkle 4M-30 cake of buffing compound at a temperature not over 300 F. To about 200 cc of melted cake, we add 50-75 cc of SnO_2 (polishing grade). Stir thoroughly and pour into a one-pint cylindrical ice cream carton. After the mixture is cooled, the cake may be removed and used immediately.

2.0 Whole and Sectioned Specimens

2.1 Objective.

Plastic-embedded whole mounts and sections of specimens are being used, in addition to fresh or preserved material, to enable us to better visualize relationships of the various organ systems.

The following paragraphs describe a method for preparing live animals for embedding in plastic, and for sectioning and polishing of plastic-embedded specimens for study.

2.2 Relaxing Live Animals.

In addition to $MgSO_4$ we have used propylene phenoxetol (Goldschmidt Chemical Corporation, 153 Waverly Place, New York 14, N. Y.). To prepare, vigorously shake 5 cc of propylene phenoxetol with 15-20 cc of sea water to produce a fine emulsion. This relaxative should not be added to the sea water containing specimens, until the animals are actively siphoning (Owen, 1955).

If specimens are not siphoning, it is possible to force relaxing agent into them by drilling into the shell, which is not very satisfactory, or by injecting relaxing agent into the animal. Injection of relaxing agent produces better results. Injection is done with a veterinary syringe with a no. 20 hypodermic needle, after prying the shell open or finding a spot near the siphon where the shell cannot close tightly. It is best to inject directly into the muscles. This will produce completely relaxed animals, which are necessary for good embedding.

2.3 Fixing Specimens.

When the animals are completely relaxed they are fixed in F.A.A. (45 cc Formalin, 45 cc Acetic Acid, 900 cc 70% Alcohol in a gallon jar is enough to fix two or three large clams). The animals are kept in this solution for 24 hours. After fixation, animals are washed in running water for about five hours or until the fixing agent cannot be detected.

2.4 Preserved Animals.

Following washing, the specimens are dehydrated in a series of alcohol baths—30, 50, 75, and 95%—leaving the clam in each for 24 hours. The animals are put in a vacuum in the 75% and 95% alcohol baths. They should be left in these baths until air bubbles cease to issue from the tissues. In order to remove further any water and air left in the tissues after dehydration with alcohol, place animals in two changes of a 100% solution of commercial acetone and leave the specimen in each bath for 24 hours.

2.5 Embedding Specimens.

We have used an unsaturated polyester resin, Ward's Bio-plastic (Ward's Natural Science Establishment, Inc., P. O. Box 1712, Rochester 3, N. Y.), to embed specimens. Following acetone dehydration, specimens are placed in uncatalyzed plastic, put into a desiccator and subjected to negative pressure until all acetone has evaporated out of the specimen. This may take 24 hours or longer. Because large bubbles of acetone may come off fast enough to cause a specimen to float, it is best to evacuate the desiccator slowly at first. Water aspirators are satisfactory "vacuum pumps."

After all the acetone has evaporated, the desiccator is opened and the specimens are removed from the uncatalyzed plastic and drained with the open end down for a period of one hour. During this

time they should be replaced in the desiccator so that they will not take on water from the air.

Bivalves are embedded open end up. Specimens drained of uncatalyzed plastic are placed into a mold in which a supporting layer of catalyzed plastic has been poured. This layer should be about 3 cm thick when large specimens are embedded. A vertical probe pushed into the foot of the clam and connected by rubber bands to a horizontal stick resting on the top of the mold can be adjusted to hold the clam in the desired position.

While the supporting layer hardens the mold should be covered. The supporting layer of plastic should have approximately 0.1% catalyst and 0.05% accelerator, to speed jelling time.

After the supporting layer has hardened the specimen is anchored and the probes can be removed. Catalyzed plastic is added to fill mold 3 cm above the top edge of the clam. Amount of catalyst used in this step is very important. Catalyst added to a mold 14 x 10 x 12 cm is less than 1 cc. After the catalyzed layer is poured, the whole mold is put back into the desiccator which is evacuated for 2 to 3 hours to remove any trapped air from the specimen so that there will be no air pockets in the finished block. The small amount of catalyst increases the jelling time to between 5 and 8 hours. No accelerator is added, lest the block harden while in the desiccator. After the catalyzed plastic has jelled, it should be left at room temperature for 24 hours or longer. This is necessary to dissipate the heat of polymerization. If this heat is not dissipated cracks may appear while the block is being cured in the oven. The long curing process is necessary to minimize shrinkage, since shrinkage of the plastic can cause enough internal stress to crack the block.

2.6 Curing Plastic.

The plastic should be fairly hard before it is oven-cured. If the block is rubbery after the 24-hour period, it is best to wait longer before curing. Curing should be done at 100 F for one day, but if the block is not hard at this time, raise temperature to 120 F for an additional day. After the block is cured, slow cooling at a few degrees per house in the oven is necessary.

2.7 Sectioning Blocks.

After curing, the block is removed from the mold (see notes on molds for removing process). Before sectioning the block is made square on a belt sander using no. 50 grit belt. The block is then ready to be cut on a diamond cut-off saw. Using a guide, the block can be cut at any desired thickness—one centimeter or thicker is best. The block is fed through the saw slowly with ample water lubrication on block and blade. Blocks are sectioned in numerous ways, principally the three major axes of the specimen, to show the relationship of organ systems to shell.

2.8 Polishing Cut Surfaces.

After the block is cut into sections, it is necessary to eliminate scratches from the block surfaces and give them a high polish. The sections are first sanded on a no. 50 grit, a 6-inch belt sander (slower speeds of belt are best for sanding plastic). This first sanding can be omitted if saw marks are not too large. After sanding with no. 50 grit belt, change to no. 120 belt to remove the no. 50 scratches. From no. 120 belt it is best to go to a no. 220 belt, then no. 440 grit on a lapidary wheel, or a no. 440 belt to eliminate scratches.

The no. 440 grit leaves a haze on the blocks which can be removed with a buffing wheel and a series of buffing compounds. A red rouge (Hanson Van Winkle Co., Mattawan, N. J.) is put on the buffing wheel. The section is dampened and a paste of no. 600 grit is put on the section and buffed off. This is repeated until the haze is removed. Next a paste of no. 1000 grit is put on the section and buffed off. Now the section is ready for high gloss buffing. A special tin oxide block buffing compound (see earlier description in thin-sectioning of shells) is put on the buffing wheel. This should give a high gloss finish on the plastic. To help protect section from scratches due to handling, the section can be polished with Hi-Lite furniture wax, obtainable at any hardware store.

2.9 Notes on Molds.

The best mold material is double strength glass, cut to the desired size. For smaller specimens such as small squid, fish, or leeches, microscope glass slides can be used. To make square molds, the pieces of glass are held in a right-angle picture frame clamp while being glued together with DuPont Duco cement at the

junctures to form a water-tight mold. After the glue has hardened the mold should be covered inside and out with Bio-Plastic mold-releasing compound, painted on with a small camel's hair brush, and allowed to dry. Before plastic is poured into the mold, it is a good idea to fill it with water and check for leaks. Removal of plastic block from mold after curing can be done in several ways without breaking the glass. In most instances the plastic blocks will shrink enough so that when the mold is turned upside down the block falls out. If this does not happen, the mold must be taken apart. To soften glue that holds glass together, use a small brush and paint acetone around the edges. The glue then can be scraped off easily with a knife and the mold can be taken apart.

It is especially important when embedding clams and other bivalves that the capacity of the mold be much greater than the specimen. A good rule to follow is that a mold for plastic embedding should be large enough to form a border from two to three cm around the specimen. Molds should be made accordingly. The reason for this is that internal shrinkage between shell and plastic can cause enough force to crack the block if there is not ample plastic surrounding the specimen.

3.0 Body Spaces and Lumen of Organs

3.1 Objective.

Injection of colored materials into the lumens of organs and into body spaces is an excellent technique for studying the ramifications and connections of such passageways. Most of our effort (Eble, 1958) has been devoted to a study of the circulatory system of the Eastern American oyster, Crassostrea virginica (Gmelin).

3.2 Injecting Specimens.

The techniques involved in injecting the circulatory system of the oyster with vinyl acetate followed by either corrosion or cleared preparations are described at length elsewhere (Eble, 1962).

The injection of the digestive system of bivalve mollusks with vinyl acetate involves practically the same procedures as those described for the circulatory system. A rubber tube is used, instead of a needle, as a catheter to aid in the injection. The rubber tube, slightly larger in diameter than the opening being entered, is pushed into the esophagus as far as it will go. The same procedure, usually with a

smaller-diameter tube, is used for injecting the vinyl acetate through the rectal end of the alimentary canal.

Actively pumping bivalves that are kept in filtered sea water will be easily injected because there is less material in the lumen of the tract to block the plastic.

Specimens injected with a radio-opaque substance can be X-rayed. Radio-opaque vinyl acetate is very useful in X-ray photography of the blood vessels. It can be made by adding iodoform to the plastic, using one part iodoform to nine parts of vinylite. This does not change the setting properties of the plastic but renders it opaque to X-rays.

Red and blue vinyl acetate (Ward's) are satisfactory, but the yellow vinyl acetate becomes almost transparent when embedded in a polyester plastic.

3.3 Embedding Specimens.

Both corrosion and cleared specimens have been embedded in plastic. Actually, fixed specimens can be dehydrated and placed directly into the plastic which acts like a clearing agent. Specimens cleared in cedar oil are not satisfactory for embedding as the oil interferes with the diffusion of the plastic into the tissues.

Corrosion specimens have a tendency to become soft as well as to float in the embedding plastic. A three-step pouring of the plastic into the mold obviates most of this difficulty. First, the basement layer of catalyzed plastic is poured and permitted to harden. A second layer is poured, but only deep enough to accommodate the depth of the specimen. At this stage the corrosion preparation is carefully positioned within the non-hardened second layer. Because the vinyl acetate tends to soften quickly and because many of the vascular branches are very fine, the placement should be correct on the first try. The final plastic layer is poured after the second layer of plastic jells sufficiently to "anchor" the specimen.

4.0 Shell Cavity

4.1 Objective.

Internal molds convert the "negative" features of the inner shell surface into a positive impression of the extent of the soft parts

of the bivalve. This information is useful in visualizing or reconstructing the soft body of mollusks, especially in the study of fossil species (Shuster, 1960).

4.2 Internal Molds.

A silicone rubber (Silastic RTV 501 and 501 Catalyst A—Dow Corning Corporation, Midland, Michigan), has been used to obtain internal molds of bivalve shells with excellent results. A release agent, 3 to 5% solution of household detergent, is painted upon the shell surfaces. When valves are tightly closing, as in Mercenaria, it is sufficient to pour the freshly mixed rubber fluid and catalyst into each valve and to press them together, squeezing out all air and excess rubber. In those species which do not have tightly closing valves, a gypsum "boat" is made by pressing one valve into plaster which is setting. This forms a wall around the valve which helps to retain the rubber poured into the shell cavity.

5.0 Evaluation of Techniques

5.1 Sectioning Specimens.

Shells and specimens frozen into blocks of ice are easily sectioned using a diamond wheel, cut-off machine. This procedure usually is preferred over embedding in gypsum, when cutting shells for thin-section preparations. It is only when sectioning thin-shelled species such as the soft-shell clam, Mya arenaria L., that plaster-embedding is used, because the entire slice of shell and plaster can then be secured to a glass slide.

5.2 Plastic Embedding.

This technique is costly and time-consuming and should be contemplated only if a study warrants such expenditure. The specimens prepared in such a manner are permanent, however, and when sectioned provide excellent material for a three-dimensional study of relationships of organ systems.

Due to the difficulty in eliminating air from entire specimens, it is best to cut bivalves into two sections in a body area where all parts on either side of the line of sectioning are attached to the respective body half. When embedding the cut surfaces are positioned to face upward, thus permitting better penetration of plastic and evacuation of air.

In toto staining of specimens prior to embedding produces excellent results, particularly when a stain like toluidine blue O is used.

5.3 Injecting Specimens.

The use of corrosion preparations supplemented by cleared specimens is practically indispensable in the study of the vascular system of any animal. The resultant cast from a corrosion preparation gives an accurate, three-dimensional picture of the system involved that has the additional feature of being a permanent record. It can be stored in a glass jar or embedded in bioplastic. The plastic (vinyl acetate) easily fills even the finest branches of the circulatory system but does not penetrate to the "capillaries" (peri-intestinal, peri-gastric sinuses in the oyster), hence the arterial system can be clearly delimited from the venous system. The special value of the cleared specimen is that the injected vessels can be seen in relationship to the other body organs, hence their supply and drainage can be easily worked out. Thus the two types of preparations, corrosion and cleared, supplement one another very well.

5.4 Internal Mold.

These preparations, made with a silicone rubber, provide several kinds of information, particularly for fossil species. A mold reveals: 1) the volume of the shell cavity, hence an indirect determination of the size of the enclosed animal, 2) muscle attachments and other markings on the internal surface of the shell, 3) extent of tissue into the hinge plate and ligament area, 4) three-dimensional representation of the enclosed animal, and 5) a sectional view of the animal when the rubber mold is cut into strips, using a sharp knife or razor.

5.5 Further Study Aids.

Even though, as in the case of the oyster, body shapes are variable and specimens of different sizes have been injected, "transparency" slides (e. g., 35 mm) are useful in delineating a composite picture of the injected system.

Using a slide projector, the transparencies can be projected upon drawing paper fixed to a wall. By changing the angle of the projector to the wall from the usual perpendicular facing, axes of the

specimen can be enlarged or distorted so that a composite can be easily traced on the paper. This technique is also helpful in visualizing the magnitude and type of variation from one specimen to another. The size of the projected image is varied whenever necessary in the superimposing of images, by moving the projector toward or away from the drawing paper.

It should be obvious that each of the techniques and procedures described above are of further use when the special preparations are compared with living specimens. The appearance of the living animal in juxtaposition with the prepared specimens often reveals new information.

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THE FEEDING OF THE BAY SCALLOP, AEQUIPECTEN IRRADIANS

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ABSTRACT

The abundance of benthic and tychopelagic diatoms in the stomachs of bay scallops, Aequipecten irradians, is considered an indication that much of the food is the microflora, detritus, bacteria and organic matter that is common in the water immediately adjacent to the bottom. Shell flapping and suction from ciliary currents pull similar foods directly from the bottom and from the surfaces of the upper valves.

Table 1 is a summary of the kinds and numbers of diatoms identified from the stomachs of 99 scallops taken from six different stations in March, June and September and preserved in 5 per cent sea water formalin. Each collection contained approximately an equal number of seed and older age-group scallops. The diatoms, characteristically rather well preserved in the stomachs, may be digested too poorly to be of significance in nutrition; yet, assuming that these diatoms accompany other water-borne food materials ingested by scallops, they are useful indicators of the source of the food supply. From the habitat information in papers by Ghazzawi (1933-34), Hunt (1925), Hustedt (1955), Hustedt and Aleem (1951) and Smyth (1955) we have grouped the diatoms in Table 1 under two headings: characteristically planktonic and characteristically benthic and/or tychopelagic, the latter consisting of forms primarily associated with the benthos but mixed with plankton in shallow water. Table 1 shows that the benthic-tychopelagic group predominates in the stomach contents.

Concurrently with each collection a water sample was taken not more than two inches above the bottom inhabited by the scallops. These samples contained a predominance of planktonic forms. The contrast between these water samples and the gut content, and the greater proportion of benthic types in the stomachs, suggest four possible feeding practices as noted and evaluated below.

1 Contribution No. 62 from the Graduate School of Oceanography of the University of Rhode Island. This study was supported in part by a grant from the National Science Foundation.

Table 1. Count of diatoms from the stomachs of 99 specimens of Aequipecten irradians from three stations in the Niantic River, Connecticut, two stations in Pt. Judith Pond, R. I., and one station in Charlestown Pond, R. I. March, June and September collections are represented

Identified diatoms regarded as benthic and/or tycho-pelagic forms	Stomach contents	% of the total that were identified	% of total count
Melosira	823	22.5	10.5
Aulocodiscus	2	0.0	0.0
Fragilaria hyalina	(1 strand)		
Grammatophora angulosa	1	0.0	0.0
G. marina	44	1.2	0.6
G. oceanica	3	0.1	0.0
Licmophora	198	5.4	0.2
Plagiogramma	2	0.0	0.0
Striatella unipunctata	24	0.6	0.3
Synedra	31	0.8	0.4
Cocconeis	2147	58.8	27.4
Amphora	14	0.4	0.2
Gyrosigma	3	0.1	0.0
Navicula	22	0.6	0.3
Pleurosigma	30	0.8	0.4
Nitzschia longissima	38	1.0	0.5
Surirella	12	0.3	0.2
	3394	92.6	41.0
Identified diatoms regarded as planktonic forms			
Actinoptychus	16	0.4	0.2
Coscinodiscus	3	0.1	0.0
Skeletonema costatum	143	3.9	1.8
Rhizosolenia	56	1.5	0.7
Biddulphia	14	0.4	0.2
Chaetoceros	11	0.3	0.1
Auliscus	3	0.1	0.0
Asterionella	3	0.1	0.0
Thalassionema	7	0.2	0.1
	256	7.0	3.1
Diatoms identified only to family			
Coscinodiscaceae	582		7.4
Actinodiscaceae	2		0.0
Fragilariaceae	298		3.8
Acnanthaceae	13		0.2
Naviculaceae	2853		36.4
Nitzschiaceae	431		5.5
	4179		53.3

1. Scallops may raise food from the bottom by shell flapping and by strong currents created by the cilia. Scallops, placed in trays in which diatomaceous earth had settled to the bottom, quickly showed on their gills mucous strings tinted pink from the diatoms. Within three hours these scallops were expelling the "earth" as feces and pseudofeces.

2. Scallops may consume some of the rich microscopic flora growing on their upper shell surfaces. Three shells examined showed numerous diatoms of the kinds commonly found in the stomachs. A different species predominated on each of the shells. It was observed that feces and pseudofeces are readily deposited on the shell surface. From the shell these undigested diatoms may be recirculated through the gut.

3. Scallops probably derive much of their food directly from the water at the level of the incurrent mantle opening, roughly 1/4 inch off the bottom. Using a sampler capable of taking water as close as 1/4 inch from the bottom we have taken numerous samples at depths of 1/4 inch, 1/2 inch, 1 inch and 1 foot from the bottom. Frequently there is a sharp decline, as illustrated in Table 2, in the ratio of benthic-tychopelagic to planktonic diatoms above the 1/2 inch level.

4. Scallops may feed selectively for benthic and tychopelagic forms. Though the observations immediately above obviate the need to postulate selective feeding, it may be that the benthic types are favored because of their relatively small size and simple surface outlines. Incidentally, differences between feces and pseudofeces show, as would be expected, that larger forms carried in the incurrent water are not ingested.

Recognizing the above possibilities, it is clear that much of the food must be of benthic origin. Detritus, very conspicuous in the stomachs, and bacteria are among the benthic components readily stirred into the bottom layers of water. Such organic matter is probably very important in the nutrition of the scallop.

Table 2. Relative abundance of diatom species from water samples taken at various depths over the scallop flats in the Niantic River, July 6, 1961[1]

Identified diatoms regarded as benthic and/or tycho-pelagic	1 foot from bottom 50 ml filtered		1 inch from bottom 50 ml filtered		1/2 inch from bottom 50 ml filtered		1/4 inch from bottom 50 ml filtered		Bottom 2 ml filtered	
	Alive	Dead[2]	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Melosira	10	10	19		23	3	16	28	32	8
Grammatophora marina		3	1	1		3	1	4	5	3
Licmophora		3		11		2	3	8	17	8
Striatella		3				7		2		
Synedra		2		6		3		10	22	16
Fragilaria		1		2		11		2		3
Acnanthes		1						1	20	34
Cocconeis		13	1	11	1	9	1	27	3	9
Amphora	2			4		1	1	4		
Navicula	1	22		31	16	42	15	35	240	131
Pleurosigma		1		3		1	3	4	3	8
Nitzschia longissima			6			1				
Surirella		2		1						
Filamentous diatom							70		50	
Unknown pennate				2			2	2		
Totals	13	61	27	72	40	83	112	127	392	220
% of total count for each depth	32	91	11	81	60	86	80	82	93	97
Identified diatoms regarded as planktonic										
Actinoptychus				2			2	11	8	
Cosinodiscus		5		4	2	14	1	13	1	7
Thalassiosira								4		
Rhizosolenia	1	1								
Chaetoceros	25		229	7	25		26		11	
Gossleriella	1									
Asterionella				2						
Totals	27	6	229	15	27	14	29	28	20	7
% of total count for each depth	68	9	89	19	40	14	20	18	7	3

1 Samples were concentrated on millipore filters. Four squares of the grids were counted on each filter.
2 Empty diatom "shells."

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SURVIVAL AND GROWTH OF LABORATORY-REARED NORTHERN CLAMS (MERCENARIA MERCENARIA) AND HYBRIDS (M. MERCENARIA X M. CAMPECHIENSIS) IN FLORIDA WATERS

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ABSTRACT

The Florida State Board of Conservation delivered laboratory-reared "baby" clams, shipped by air from Milford, Connecticut, to eight locations on the Gulf and Atlantic coasts of Florida where interested persons planted them for growth studies. From mid-November 1960 to mid-June 1961, hybrid clams (Mercenaria campechiensis X M. mercenaria) grew in length from 0.125 inch or less to a maximum of 1.5 inch, and northern clams (M. mercenaria) grew from 0.25-0.5 inch to a maximum of 1.75 inch. This is faster growth than in northern states. Many clams were lost, or were killed by predators and unfavorable environmental factors such as low salinity.

INTRODUCTION

Recorded hardshell clam production began in Florida in 1880, increased significantly in 1908 with the exploitation of large clam beds in Collier and Monroe Counties near the Ten Thousand Islands, grew steadily until the peak year of 1932, remained at a high level through most of World War II and plummeted to a beginning low by 1950 with cessation of the clam industry in Collier and Monroe Counties. Since 1950 production has increased modestly. Table 1 shows production for selected years from 1880 to 1960 (taken from Fishery Statistics Digests, U. S. Fish and Wildlife Service).

The reason or reasons for the disappearance of the clam population in the Ten Thousand Islands area are obscure. Hurricanes, red tides, freshwater and mechanical harvesting have been blamed but nothing has been proved. Since the shutdown of the three canning plants at Marco in Collier County, Sarasota County has been the leading producer of clams on the west coast and in the state.

Invariably the densest concentrations of clams on the west coast of Florida are found on firm, sticky mud bottoms with an abundance of attached sea grasses, either Thalassia testudinum Konig, turtle grass, Diplanthera wrightii Ashers, Cuban shoalweed, or both.

1 Contribution No. 58.

Table 1. Clam production in Florida (pounds)*

Year	East Coast	West Coast	Total
1880	5,000		5,000
1908	57,000	182,000	239,000
1923	5,000	602,000	607,000
1930	49,840	661,736	711,576
1932	12,000	1,108,812	1,120,812
1940	6,700	701,100	707,800
1945	3,000	687,700	690,700
1950	900	4,440	5,300
1955	6,300	15,700	22,000
1960	2,134	23,893	26,027

* 5.20 pounds of meat per U. S. Standard Bushel (Florida East Coast)
8.00 pounds of meat per U. S. Standard Bushel (Florida West Coast)

Clam production on the east coast of Florida has been concentrated from Volusia County northward but yields have never approached the peak years of 1908 and 1930 since 1932.

In Florida waters clam growth is not normally interrupted by low winter temperatures. Consequently, clams reach marketable size more quickly than in temperate zone waters. With this knowledge and the prospect of decreased production in other areas and increased demand locally and nationally, interest in clams and clam culture has grown in Florida. The State has recognized the potential in planned aquaculture of clams, especially since the development of dependable techniques for culturing supplies of seed clams by Dr. Victor Loosanoff and his colleagues at the Milford, Connecticut, laboratory of the U. S. Fish and Wildlife Service.

PROCEDURE

Dr. Loosanoff brought chilled baby clams in plastic bags to Miami by commercial airline on the night of 11 November, 1960; the clams were placed under refrigeration that night and delivered by two State airplanes the next day to three locations along the Atlantic (east) coast (Fig. 1) and to Tallahassee where Dr. Winston Menzel accepted a much larger quota for growth and other studies at the Oceanographic

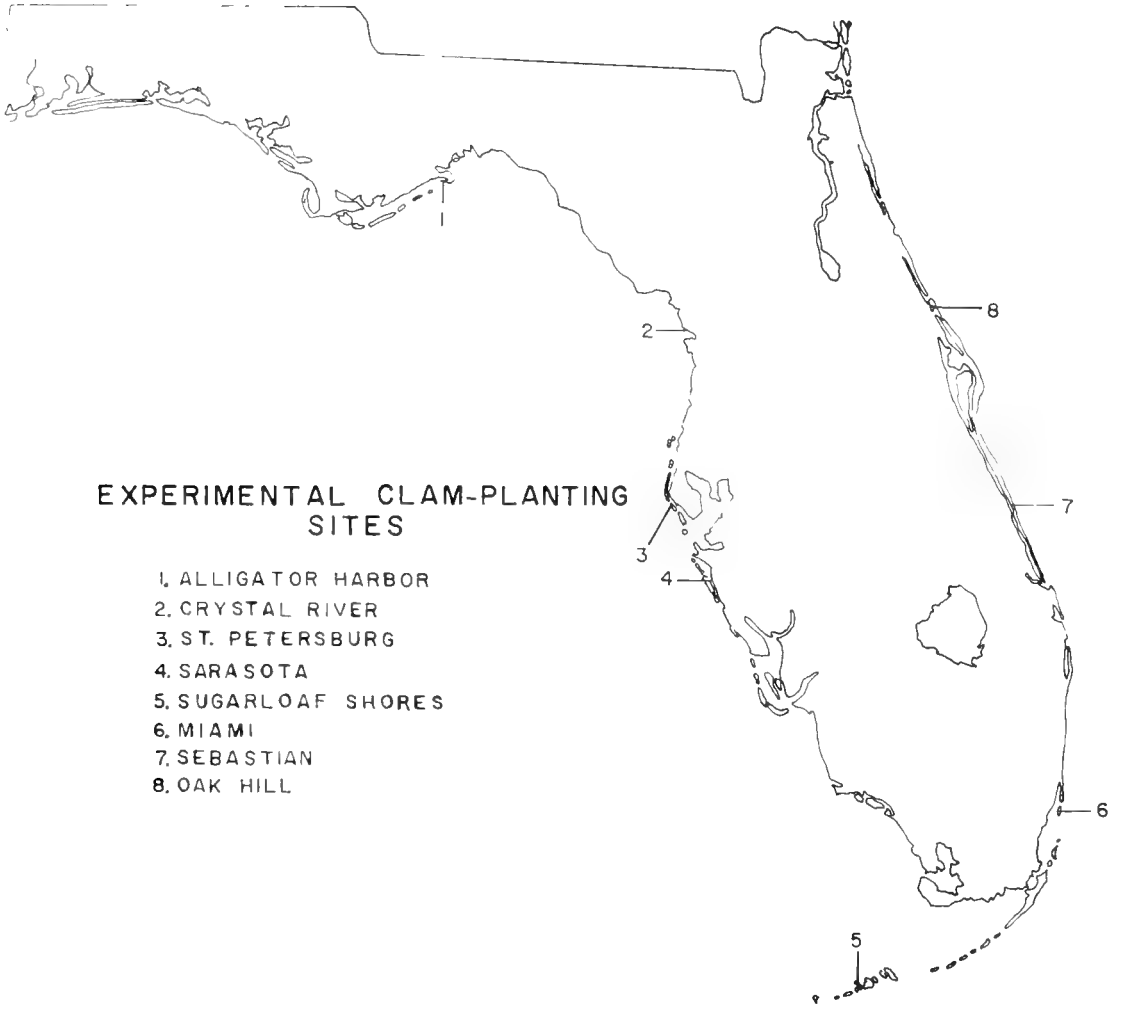

Fig. 1. Outline map of Florida showing places mentioned in text.

Institute of Florida State University. On 12 November 1960 the remaining baby clams were delivered by automobile to Sarasota and St. Petersburg. On 16 November 1960 half of the clams held in a bay at St. Petersburg were delivered by automobile to Crystal River.

Specifications prescribed by Drs. Menzel and Loosanoff for building screened-top boxes to protect the baby clams against predation had been sent to all prospective recipients. Only those who had built the boxes received clams, excepting Durbin Tabb, Biologist, University of Miami Marine Laboratory, who requested some to seed directly in a small tidal lagoon near the laboratory.

Each recipient received about 600 Mercenaria mercenaria (0.25 to 0.5 inch) and 2000 hybrids, male Mercenaria campechiensis x female Mercenaria mercenaria (0.0625 to 0.125 inch). The six locations chosen (except Crystal River) were ones where water salinities would not be expected to be less than 20 o/oo, the recommended minimum for clams.

Separate discussions of locations and results follow (see map, Fig. 1).

RESULTS

Alligator Harbor (Franklin County): Dr. Winston Menzel received a grant from a seafood company for a cost feasibility study of raising hatchery clams to marketable sizes. Results at Alligator Harbor will be reported at a later date.

Crystal River (Citrus County): D. C. Crawford, vocational agricultural teacher, and his students at the local high school placed their clams in Crystal Bay, between the more saline waters of the open Gulf and Crystal River. Recorded salinities have ranged from 13.0 o/oo to 28.0 o/00. On 16 November 1960 when the clams were placed, the salinity was 17.0 o/oo. Unfortunately, all but one box was lost and the clams in this were badly depleted by accidents in handling, so that results have been inconclusive.

St. Petersburg (Pinellas County): Bonnie Eldred, Biologist, Florida State Board of Conservation Marine Laboratory provided space for baby clams under the dock at her Madeira Beach home on the western shore of Boca Ciega Bay about one-quarter mile north of Johns Pass that connects to the Gulf of Mexico and assures a good tidal flow. Depending on tides, water depths range from one to four feet.

Salinities are very constant and rarely go below 30.0 o/oo or above 34 o/oo. The hybrid clams tripled or quadrupled in size and the northern hardshells doubled in size by 19 February 1961 when they were shifted to extra boxes to prevent overcrowding. Mortality was negligible and virtually confined to M. mercenaria at that time. Water temperatures had increased markedly during a record-breaking heat wave during February 1961. By the middle of June 1961 the hybrids had grown to maximum lengths of 1.5 inch, the northern hardshells to 1.75 inch. Approximately three-quarters of the hybrids had survived but one-half of the northern clams were lost when the screened lid of one box was dislodged and the clams were washed out or eaten by predators. A number of young clams have been found in the bottom near the boxes. They are larger than the ones in the boxes and presumably are the northern clams that were lost since there were no clams in this area previously.

Sarasota (Sarasota County): Ralph Davis, a planning and recreational specialist for Sarasota County, placed his quota of clams in a small salt-water pond behind his home on the eastern shore of Little Sarasota Bay. Mr. Davis had previously studied clam culturing techniques for two weeks at Milford, Connecticut. Baby clams that he had brought from Milford grew about one inch from 1 April 1960 until 5 August 1960 when they were killed by sudden freshwater intrusion into the small pond. Salinities generally range from 27.0 o/oo to 31.0 o/oo in this section of Little Sarasota Bay. By the middle of June 1961 hybrids planted 12 November 1960 had grown to maximum lengths of 1.5 inches and northern hardshells to maximum lengths of 1.25 inches. Since then some of the clams have been placed on the open bottom and enclosed by chicken wire. These clams appear to be growing faster than the ones remaining in protective boxes.

Sugarloaf Key (Monroe County): John Sammy, a crawfish fisherman, placed his quota of clams at the tidal cutoff between Sugarloaf and Saddlebunch Keys about 10 miles ENE of Key West. This location beside the Straits of Florida has near oceanic salinities and no fluvial drainage. On 30 May 1961 Sammy reported that he had found 10 or 12 large brown worms, six to eight inches long, but no hybrid clams or even dead shells in the protective boxes that were tight and intact with hardware cloth screening. Of his original 600 northern hardshell clams, 278 were still alive and ranged in length from 0.3 to 0.7 inch.

Miami (Dade County): The clams that were seeded in a small tidal lagoon without protective boxes apparently did not survive predation. During a check made in April, biologists from the University of Miami failed to find even single valves at Virginia Bay.

Sebastian (Indian River County): Mr. L. L. Fraunfelder, an engineer at the Cape Canaveral missile-testing center, placed his quota of clams on 12 November in the Indian River just north of the Sebastian River bridge on U. S. 1. Seven days later 50 per cent of the hybrids and 10 to 15 per cent of the northern hardshells were dead. No dead clams were found on 26 November. Salinities in this section of the Indian River range from 21 to 29 o/oo. By the middle of June hybrid clams had grown to maximum lengths of 1.25 inch and northern hard clams had grown to lengths of 1.375 inch. The sand was changed regularly to rid the protective boxes of crabs that had apparently entered when very small.

Oak Hill (Volusia County): Norman Jeffries, a veteran oysterman from New Jersey, placed his quota of baby clams near his Indian Mound Fish Camp at the northern end of Mosquito Lagoon, about 13 miles south of Ponce de Leon inlet that lies between Daytona Beach and New Smyrna Beach. Salinities generally range from 23.0 o/oo to 32.0 o/oo unless there are torrential rains and large upland runoff such as accompanied hurricane Donna and lowered salinities enough to kill oysters. Clam growth was measured on 19 January 1961 and it was found that the hybrid clams had grown as large as the northern hard clams, which had nearly doubled in size themselves. By 15 May 1961, the hybrids and northern hard clams had grown to one inch in length. Five hundred of the northern hard clams and 1800 of the hybrids were still alive.

SEASONAL GROWTH OF THE NORTHERN QUAHOG, MERCENARIA MERCENARIA AND THE SOUTHERN QUAHOG, M. CAMPECHIENSIS, IN ALLIGATOR HARBOR, FLORIDA

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ABSTRACT

Monthly shell length measurements were made of Mercenaria mercenaria for 3.5 years and of M. campechiensis for 2 years, in Alligator Harbor, Franklin County, Florida. The northern clams were laboratory-reared natives of Long Island Sound. They showed the best growth recorded for any locality, with greatest growth in spring and fall, less in winter, and least in summer. The southern clams, which originated in Alligator Harbor, grew faster than the northern species, with greatest growth in spring and fall, almost as much in summer, and least in winter. M. mercenaria grew from a length of 3 mm to a length of 67 mm in 3.5 years, and from 16.2 mm initial length to 67.0 mm in 3 years. M. campechiensis grew from 16.5 initial length to 74 mm in 2 years.

INTRODUCTION

In March, 1958, several hundred thousand laboratory-reared northern quahogs, Mercenaria mercenaria, mostly under 5 mm in shell length, were obtained from the Biological Laboratory of the U. S. Fish and Wildlife Service at Milford, Connecticut, through the courtesy of Dr. V. L. Loosanoff, Laboratory Director, whom the writer wishes to thank for aid and helpful suggestions in this study. The clams were planted in depths of about one foot at mean low water in Alligator Harbor, Franklin County, Florida, near the site of the marine laboratory of the Oceanographic Institute. The clams were first planted in boxes of sand covered with wire, and a small number of the native southern quahog, M. campechiensis, set in these boxes during the spring of 1959. Monthly shell length measurements were made of the northern species from March, 1958, and of the southern species from August, 1959. The two species of clams were planted under conditions as nearly identical as possible.

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1 Contribution No. 184, Oceanographic Institute, Florida State University.

METHODS

The northern clams were planted in various types of cages, mainly for protection against predators (Menzel, 1960). The most satisfactory protective cage for clams under about 10 mm in length was a wooden box four inches deep, filled with sand and covered with asphaltum-treated quarter-inch mesh wire. A small strip of plastic screen wire (#12) was placed around the edges of the box to prevent the small clams from washing out. As the clams became larger the quarter-inch mesh wire was replaced with wire of half-inch mesh. When the clams reached a size of about 25 mm they were planted on the bottom and covered by a frame of half-inch mesh wire pressed over them.

Clams up to about 10 mm in length were planted in concentrations of from 200 to 500 per box in boxes with 2.5 square feet of surface area. Clams from about 10 to 25 mm long were planted in concentrations of 100 to 200 in boxes of this size. Clams over 25 mm were planted in concentrations up to (but usually less than) 50 per square foot.

The southern quahogs collected from the sand-filled M. mercenaria growth boxes were first planted in an 8-inch diameter finger bowl buried in the bottom and covered with a wire frame. These clams were collected during the months of July and August, 1959, and were first measured reliably in August. They were not measured again until December, 1959. By February, 1960, additional southern quahogs had been assembled and growth data from these have been averaged with those of the first group.

Measurements of length to the nearest 0.5 mm were made with vernier calipers near the middle of each month except November, 1960. Because the periods between measurements were not all of the same number of days, the average daily growth was calculated and this figure was multiplied by the number of days in the month. The calculated growth was added to the size of the preceding month.

Surface temperature recordings were made at irregular intervals and at various times of the day. The temperature ranges given are based on from 5 to approximately 30 readings per month. Density measurements were taken occasionally with a hydrometer and converted to salinity by Knudsen's tables. Salinity ranged from 26 o/oo to 35 o/oo.

RESULTS

An initial length of 3.0 mm was selected for M. mercenaria, as this was the closest to the average (2.8 mm) when the clams were secured in March, 1958 (Table 1, Fig. 1). The average length of the 23 M. campechiensis when measured in August 1959 (Table 1, Fig. 1) was 16.5 mm. No examination was made in November, 1960, and the growth in December, 1960, is for the two months (Table 1).

The northern quahog had an average increase in shell length from 3 mm to 32.6 mm the first year, to 49.6 mm the second year and to 61.5 mm the third year (Table 1, Fig. 1). The southern species grew from 16.5 mm to 54.3 mm the first year of measurements, and to 74.2 mm the second year (Table 1, Fig. 1). Direct comparison between the two species is difficult because of the different starting dates and initial sizes. In Table 1 the two species are compared, with sizes and monthly growth, although M. campechiensis is one year later than M. mercenaria and conditions of food and temperature would not be the same. In August, 1958, M. mercenaria averaged 13.3 mm and by August, 1959, this species averaged 41.2 mm, an increase of 27.9 mm, whereas M. campechiensis grew from an average of 16.5 mm in August, 1959, to 54.3 mm in August, 1960, an increase of 37.8 mm. Fig. 1 shows that the southern species grew about as much in two years as did the northern species in three.

M. mercenaria had the greatest shell increase during the spring and fall when the temperature was roughly between 15 C and 25 C. Growth was less during the colder months and least during the warmest period (Fig. 2). M. campechiensis grew best during the spring and fall; growth continued fairly rapidly during the warmer period and slowed during the colder period (Fig. 3). Both species showed the greatest increment in shell size when they were younger and smaller (Figs. 2 and 3).

DISCUSSION

Mercenaria mercenaria had a greater annual growth in this area of Florida than in localities recorded by Gustafson (1954) in Maine, Belding (1931), Pratt (1953) and Pratt and Campbell (1956) in Massachusetts, Haskin (1949) in New Jersey, Haven and Andrews (1956) in Virginia, Chestnut (1952) and Chestnut, Fahy and Porter (1956) in North Carolina. The greater annual growth is undoubtedly due to the continued growth in winter, and absence of the hibernation found in the more northern waters.

Table 1. Average monthly size, standard error of mean, millimeter increase and number of clams measured of Mercenaria mercenaria and M. campechiensis. (M. mercenaria from March, 1958, through August, 1961; M. campechiensis from August, 1959, through August, 1961.)

	Mercenaria mercenaria				Mercenaria campechiensis				
Date	Number	Size, mm	S. E.	Increase, mm	Date	Number	Size, mm	S. E.	Increase, mm
3/58	181	3.0	0.1	-					
4/58	176	5.4	0.1	2.4					
5/58	158	9.0	0.2	3.6					
6/58	578	11.0	0.1	2.0					
7/58	244	12.3	0.2	1.3					
8/58	317	13.3	0.2	1.0	8/59	23	16.5	0.6	-
9/58	213	16.2	0.3	2.9	-	-	-	-	-
10/58	381	19.8	0.2	3.6	-	-	-	-	-
11/58	238	23.4	0.2	3.6	-	-	-	-	-
12/58	244	28.1	0.2	4.7	12/59	23	33.2	0.7	16.7
1/59	601	29.9	0.2	1.8	1/60	37	35.0	0.7	1.8
2/59	285	30.9	0.2	1.0	2/60	37	35.3	0.7	0.3
3/59	538	32.6	0.4	1.7	3/60	35	37.1	0.7	1.8
4/59	232	35.3	0.4	2.7	4/60	34	39.2	0.7	2.1
5/59	214	38.0	0.4	2.7	5/60	42	43.2	0.7	4.0
6/59	268	39.4	0.4	1.4	6/60	39	47.0	0.7	3.8
7/59	348	41.1	0.3	1.7	7/60	35	50.4	0.7	3.4
8/59	404	41.2	0.3	0.1	8/60	34	54.3	0.6	3.9
9/59	342	41.6	0.4	0.4	9/60	32	58.5	0.7	4.2
10/59	378	42.7	0.3	1.1	10/60	32	60.1	0.7	1.6
11/59	372	45.1	0.3	2.4	-	-	-	-	-
12/59	368	46.5	0.3	1.4	12/60	32	65.3	0.6	5.2
1/60	325	48.1	0.3	1.6	1/61	31	66.0	0.6	0.7
2/60	204	48.4	0.4	0.3	2/61	32	66.0	0.7	0.0
3/60	213	49.6	0.4	1.2	3/61	32	67.3	0.7	1.3
4/60	200	51.6	0.4	2.0	4/61	32	68.4	0.7	1.1
5/60	199	53.0	0.3	1.4	5/61	26	69.5	0.8	1.1
6/60	147	53.7	0.5	0.7	6/61	26	71.3	0.8	1.8
7/60	204	54.9	0.5	1.2	7/61	26	73.7	0.9	2.4
8/60	200	55.2	0.5	0.3	8/61	23	74.2	0.9	0.5
9/60	162	56.2	0.4	1.0					
10/60	136	57.5	0.7	1.3					
11/60	-	-	-	-					
12/60	191	60.1	0.6	2.6					
1/61	167	60.9	0.7	0.8					
2/61	112	61.2	0.7	0.3					
3/61	157	61.5	0.6	0.3					
4/61	156	63.2	0.7	1.7					
5/61	153	65.0	0.7	1.8					
6/61	153	65.9	0.7	0.9					
7/61	148	67.0	0.7	1.1					
8/61	139	67.0	0.7	0.0					

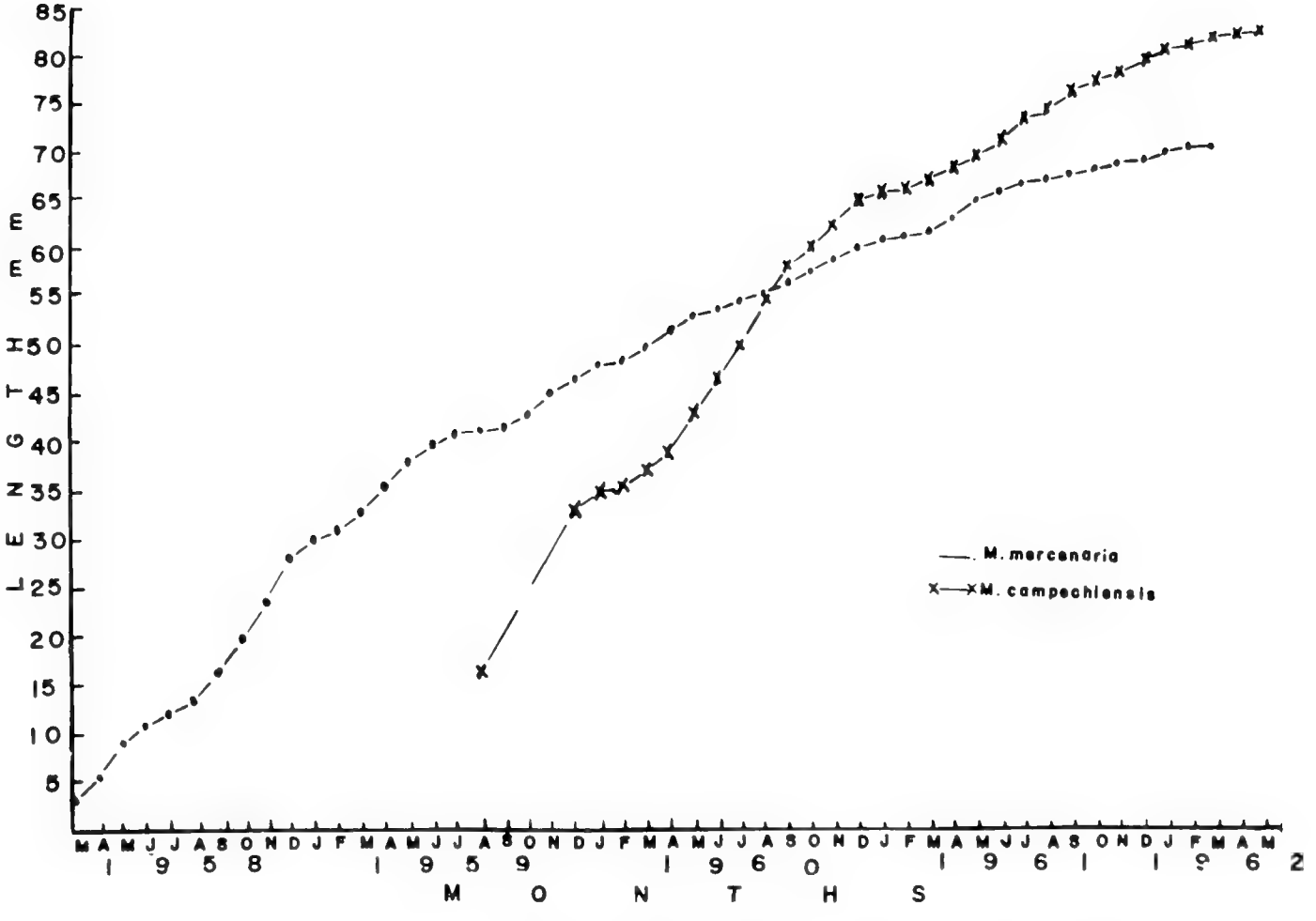

Fig. 1. Monthly size of M. mercenaria and M. campechiensis.

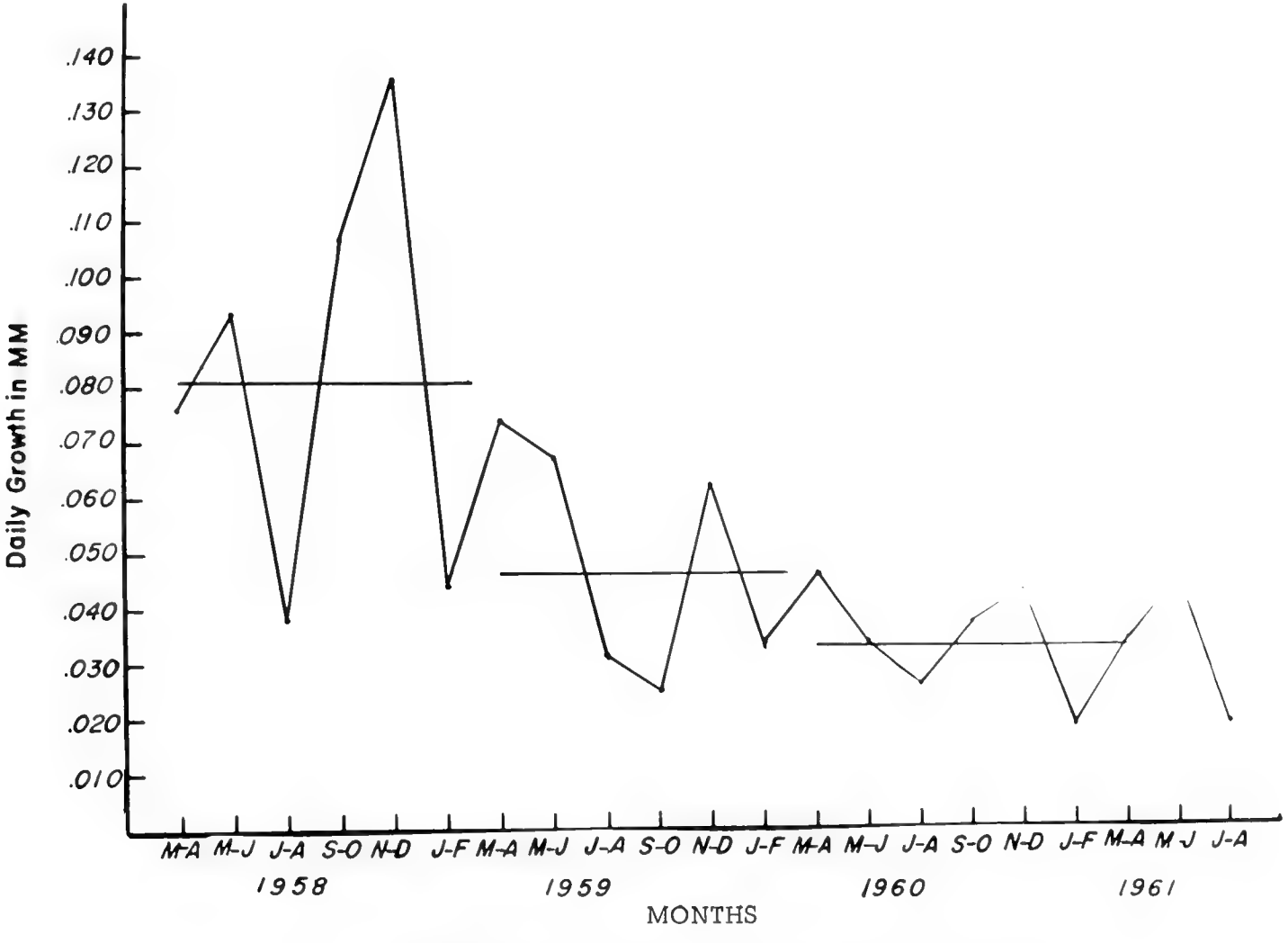

Fig. 2. Average daily growth during bi-monthly periods of Mercenaria mercenaria . (Horizontal lines = yearly average daily growth.)

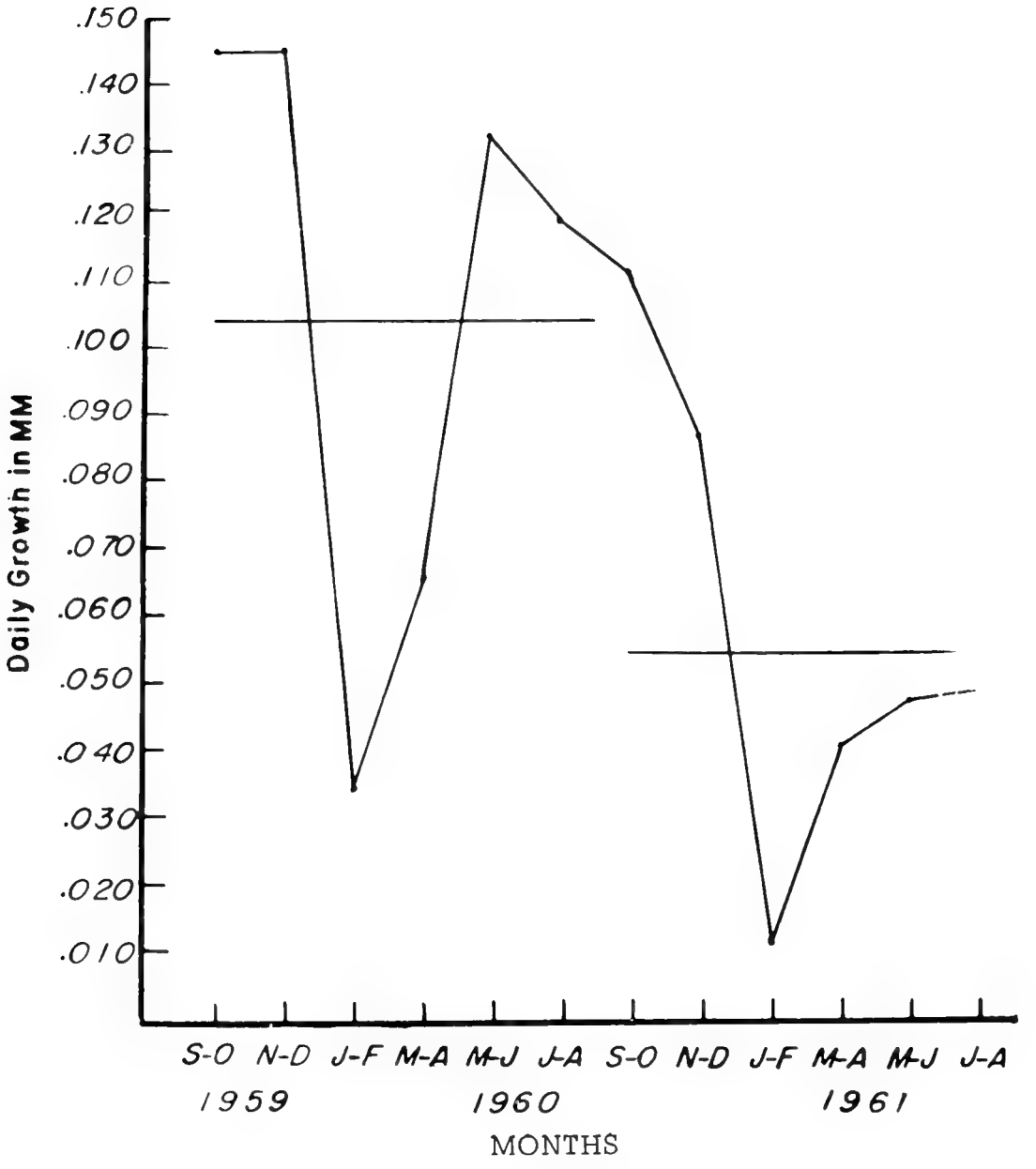

Fig. 3. Average daily growth during bi-monthly periods of Mercenaria mercenaria. (Horizontal lines = yearly average daily growth.)

Table 2. Monthly surface temperature ranges (C°) at Alligator Harbor, Franklin County, Florida

	Months	C°
1958	March	15.0 - 19.0
	April	17.0 - 26.0
	May	22.0 - 27.0
	June	25.0 - 29.0
	July	28.0 - 32.0
	August	28.0 - 33.0
	September	27.0 - 31.0
	October	17.0 - 30.0
	November	16.0 - 24.0
	December	9.0 - 18.0
1959	January	6.0 - 13.0
	February	9.0 - 14.0
	March	12.0 - 16.0
	April	15.0 - 23.0
	May	21.0 - 28.0
	June	23.0 - 29.0
	July	25.0 - 35.0
	August	29.0 - 33.0
	September	27.0 - 31.0
	October	18.0 - 28.0
	November	16.0 - 21.0
	December	11.0 - 18.0
1960	January	7.0 - 13.0
	February	9.0 - 15.0
	March	7.0 - 19.0
	April	18.0 - 26.0
	May	20.0 - 28.0
	June	25.0 - 30.0
	July	27.0 - 31.0
	August	27.0 - 32.0
	September	26.0 - 30.0
	October	18.0 - 27.0
	November	15.0 - 25.0
	December	9.0 - 17.0
1961	January	8.0 - 16.0
	February	11.0 - 18.0
	March	12.0 - 20.0
	April	14.0 - 21.0
	May	18.0 - 26.0
	June	23.0 - 30.0
	July	26.0 - 30.0
	August	27.0 - 31.0

The southern species has a faster annual growth rate than the northern species when both are grown in Alligator Harbor. Haven and Andrews (1956) found that the southern quahog grew faster than the northern species in Virginia waters. It may be that the southern quahog is a more vigorous species with a faster "natural" growth rate.

The greatest shell increase in both species, but especially in M. mercenaria, was in the spring and fall. Grice (1956) found that copepods, which are indicators of phytoplankton abundance, were most abundant in August. Marshall (1955) found the highest values for chlorophyll in spring and fall but found relatively high values in summer, greater than in winter. August was usually the period of least growth for the northern species, whereas M. campechiensis showed almost as great shell increase during this period as in the faster growing months.

It is not known whether temperature, food or some other factor was critical in the growth of the two species during the various months. M. campechiensis is native to the area and it may be assumed that it is better adapted than M. mercenaria, native to Long Island, where the temperatures never attain the high levels found in this area. The spring and fall temperatures coincide roughly with the summer temperatures of the area where the northern species is native. The northern species thrives on the food found in Alligator Harbor as evidenced by the good growth.

Another factor that may account for the relatively poor growth of the northern species during summer is turbidity which is high in Alligator Harbor during this period. Hoagland (1958) found up to 69 mg/liter of dried weight, retained by a millipore filter, and Grice (1956) found Secchi disk readings of less than 50 cm during summer months in Alligator Harbor. Loosanoff (1961) presented data on the effect of turbidity on clams and oysters. He found that high turbidity (at levels that may occur normally in some southern waters) is highly injurious to clams and oysters native to Long Island Sound. Perhaps the high summer turbidity in Alligator Harbor adversely affects the northern species, whereas the native species is adapted to it. It would be interesting to determine seasonal growth rates of M. mercenaria native to southern waters and hence possibly better adapted to high turbidity.

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INDEX OF CONDITION AND PER CENT SOLIDS OF RAFT-GROWN OYSTERS IN MASSACHUSETTS

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ABSTRACT

Oysters were suspended from a Fiberglas raft in Taylors Pond, Chatham, Massachusetts from September 1959 to October 1960. The index of condition showed a low of 8.8 in July 1960, just after spawning, and highs of 14.6 and 14.5 in October 1959 and September 1960 respectively. The average monthly index for the 13-month period was 12.2. The seasonal cycle of per cent solids was similar to that reported for oysters in Upper Chesapeake Bay. Oysters must be planted on the bottom at the end of the first year of suspension to make shells thicken and to eliminate losses due to oysters breaking away from strings during the second year of suspension.

At the 1960 meeting of the National Shellfisheries Association, I reported (Shaw, 1962) on a study dealing with the possibility of commercially rearing oysters, Crassostrea virginica, suspended from rafts in the tidal waters of Massachusetts. The results of the experiment demonstrated that seed oysters, which were suspended from a raft for their first year of life and then planted on the bottom for an additional year, grew large enough to sell on the half-shell market. In these waters it normally takes from 4 to 5 years for seed oysters to reach a market size of 3 inches when bottom culture is used exclusively.

The question of quality of the meat of raft-grown oysters as compared with oysters grown on the bottom remained unanswered. The purpose of this note is to present the results of an experiment conducted from the fall of 1959 to the fall of 1960 on the index of condition and per cent solids of oysters suspended from a raft. To evaluate the data, the index of condition and the percentage of solids of the raft-grown oysters were compared with those reported for oysters grown in other areas.

DESCRIPTION OF AREA AND METHODS

The experiment was conducted in Taylors Pond, West Chatham, Massachusetts. The pond is about 400 yards long and 200 yards wide. Its depth ranges from 1 to 9 feet at mean low water. The range of tide is about 4 feet. From 1959 through 1960, salinity varied from 28.2 o/oo to 31.5 o/oo, surface water temperature fluctuated from a summer high of 26.7 C to a winter low of -0.1 C, and average pH was 7.9.

The oysters used in the experiment came from Mill Creek, a tidal outlet of Taylors Pond. The creek runs for about one-half mile before emptying into Nantucket Sound. Its bottom is hard sand changing to soft mud near the banks. Several sand bars, which are exposed at low tide, are found along the length of the creek.

Seed oysters for the experiment were obtained by placing chicken-wire bags, each containing one-half bushel of bay scallop shells, on several sand bars. In 1958, setting occurred between July 18 and July 23. On September 2 of that year, samples of spat were strung on either polyethylene tubing, nylon rope, or parachute cord and suspended from a Fiberglas raft (Shaw, 1960). Of the three materials, the nylon rope proved the most satisfactory.

The index of condition was computed by dividing the dry weight of the meat (grams) by the volume of the shell cavity (milliliters) x 100 (Grave, 1912; Higgins, 1938). The per cent solid of the oyster meat was determined by dividing the total dry weight of meat by the total wet weight of the meat x 100 (Engle, 1950). Once a month from September 1959 to October 1960 (except for January 1960), 50 raft oysters were cleaned of all fouling plants and animals. The following measurements were taken: total volume, shell volume, wet weight of meat and dry weight of meat. The oysters were treated individually and a mean, standard deviation, and standard error of the mean for the group was determined in each category.

RESULTS

Fig. 1-B shows the seasonal changes of the index of condition for raft oysters. In September 1959, the index was 13.3. This value rose to 14.6 in October. During the winter when the oysters were hibernating the index showed little change though a slight decline was observed. On examination of Fig. 1-C it is apparent that the decline was not significant. Between March and April the index showed a sharp decline. The drop occurred when the water temperature rose from 2.5 C to 9.5 C (Fig. 1-A).

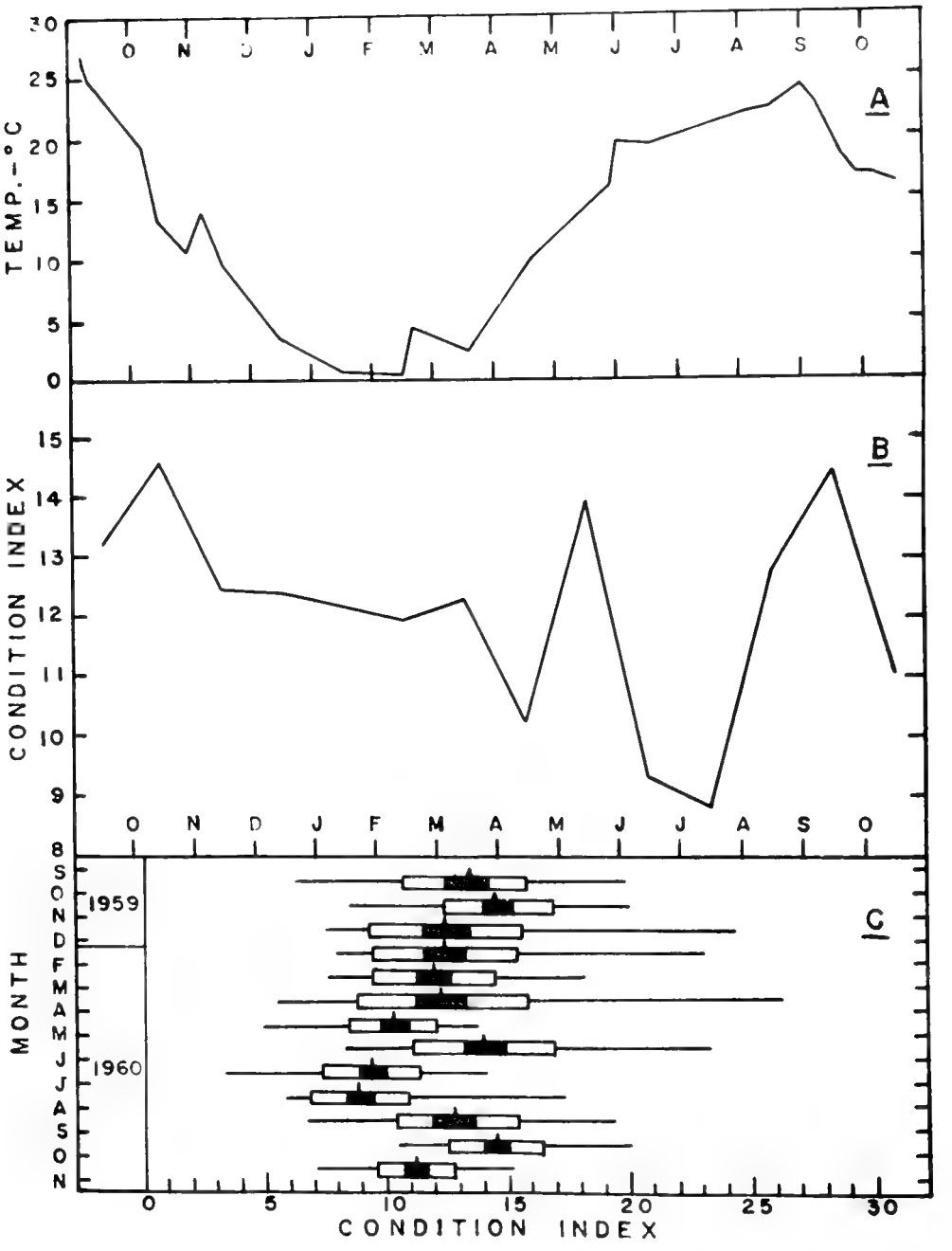

Fig. 1. A. Changes in water temperature in Taylors Pond, 1959-1960. B. Monthly index of condition of raft-grown oysters from September 1959—October 1960. C. Graphic analysis of index of condition: The monthly range, horizontal line; the mean (M), small narrow triangle; 2 standard errors of the mean, the black bar on either side of M; and one standard deviation, one-half black bar plus white bar on either side of M.

From April 18 to May 16 the index of the raft oysters climbed significantly from 10.3 to 14.0. From examination of the raft-grown oysters, this period corresponded to the time when gonadal development was taking place. As the temperature approached 20 C in the middle of June, initial spawning occurred. The oysters continued to show signs of spawning through the first part of July. A corresponding drop in index of condition took place during this period from 14.0 in mid-May to 8.8 in mid-July. Following spawning the index rose rapidly to 14.5 by September 19. The index then decreased significantly to 11.2 in October. The index of condition was never below 8.8. The average for the 13-month period was 12.2.

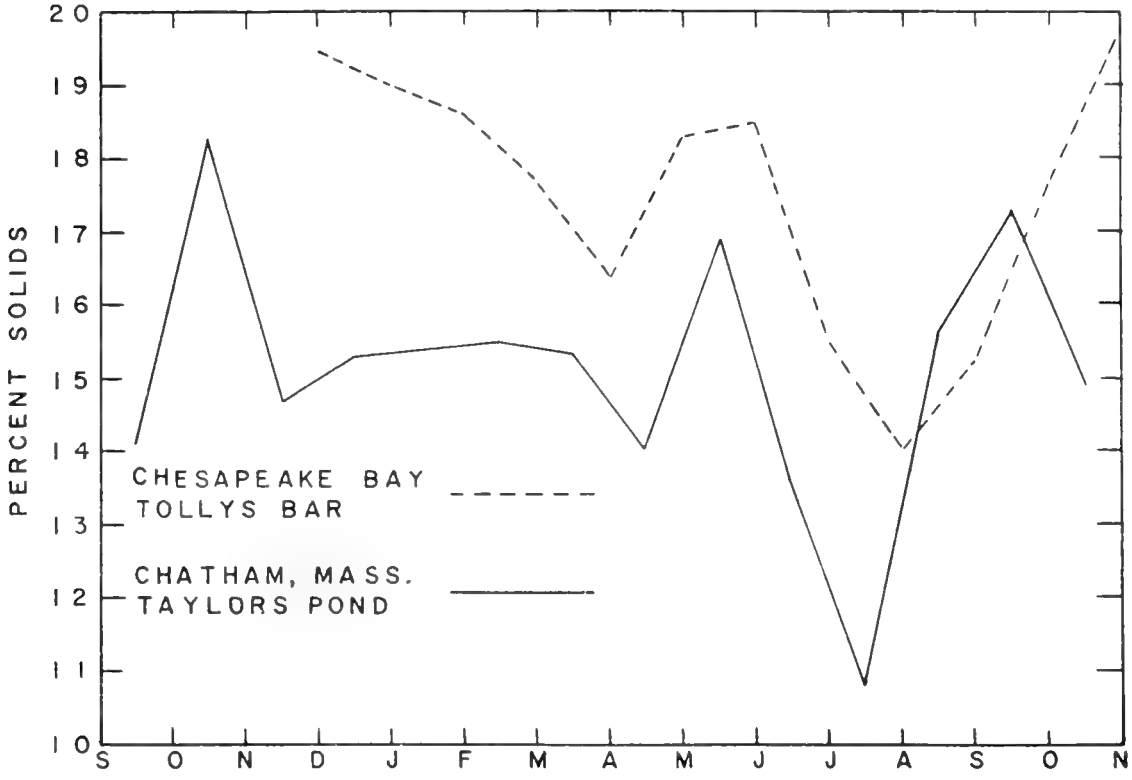

Fig. 2. Monthly averages of per cent solids for raft-grown and Upper Chesapeake Bay oysters.

In Fig. 2 the seasonal cycle of the per cent solids of raft oysters is compared with that found by Engle (1958) for oysters growing on Tollys Bar, Upper Chesapeake Bay, Maryland. The seasonal cycles of per cent solids for the two groups appear to be similar. In both, there is a winter decline which is followed by an early spring increase. During the summer a decline related to spawning occurs, and then an increase in the per cent solids takes

place during the fall. The cycles of per cent solids and index of condition for the oysters suspended from the raft were also found to follow similar seasonal patterns.

DISCUSSION AND SUMMARY

Medcof and Needler (1941) observed that the index of condition of Canadian oysters showed little change when the water temperature was below 5 C. The index was observed to fall at water temperatures between 5 C and 15 C and then rise again at water temperatures between 15 C and 20 C. These authors further found that above 20 C spawning took place among the Canadian oysters with a corresponding fall in the index. As the water temperature fell from 20 C to 15 C, fattening occurred and the index increased rapidly, but when the temperature dropped below 15 C the index also dropped.

The seasonal cycle of the condition index for raft oysters was similar to that found for Canadian oysters. The one exception was the sudden fall in the index of the suspended oysters in September and October 1960 when the water temperature was dropping from 20.5 C to 16.6 C. At corresponding temperatures the index of the Canadian oysters was found to increase. It should be mentioned that the index of condition for raft oysters was determined for just one year and if more years could have been included some modification in this pattern might have been found. It is doubtful, however, that the seasonal cycle of the per cent solids would vary to any extent since it compared closely with that found by Engle (1958) over a seven-year period.

The index of condition for raft oysters was generally much higher than that reported for oysters in Chesapeake Bay (Galtsoff, 1947; Engle, 1950; Haven, 1959). The indices for the suspended oysters also compared favorably and, in many cases, were higher than those found for Canadian oysters, C. virginica, grown in Bras d'Or Lakes, N. S., and Shediac Bay, N. B. (Medcof and Needler, 1941). On the basis of the comparison it can be stated that the index of condition of raft oysters was high.

Though the condition of raft oysters was high, the thinness of shell due to rapid growth made them unsuitable for the half-shell market. It is therefore necessary to place them on the bottom at the end of the first year in order that the shells will thicken. It was also observed in this experiment that many oysters broke away from

the strings and fell to the bottom during the second year of suspension. The danger of such losses increases the need for planting the raft oysters on suitable grounds at the end of the first year of suspension.

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MORTALITY IN PACIFIC OYSTER SEED

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With a Statistical Appendix by W. E. Ricker

ABSTRACT

Mortality in Pacific oyster seed is due mainly to silting and to competition for space on the cultching medium. This takes place within a year of planting and silting mortality probably occurs within a few months. Competition for space may vary with the rate of growth and rate of silting mortality. The possibility of reducing these losses is discussed.

Growers of oysters, whether Eastern or Pacific, have no illusions about the fact that mortality occurs in their seed. In British Columbia there has been little appreciation of the extent of mortality, the time, or the cause. This has, in part, been due to the fact that the industry thinks in terms of volume rather than numbers until the point where oysters are shucked. The Pacific oyster grower, in contemplating yields of 7 to 10 bushels for each bushel of seed planted, tends to congratulate himself when he thinks of his eastern counterpart with an approximate one-to-one return.

In terms of numbers, however, the British Columbia grower suffers average mortalities of 75 per cent or more during the period from planting to harvesting. This amount of loss warrants examination. For this purpose three sources of data are available.

1. Mortality of High Count Seed

Five cases of Japanese oyster seed containing 422,000 spat counted when packed in Japan were planted at the 3-foot tide level in Ladysmith Harbour on mud-gravel ground with a thin layer of silt. The firmness and lack of silt provided ground that would be considered somewhat better than average. Estimates of the number surviving were made at yearly intervals for three years. Assuming no mortality in transit the successive total mortalities at survival intervals were 87.5 per cent, 91.5 per cent and 91.7 per cent. Allowing for shipping

mortality the loss during the first year is considerable in contrast to that during the remaining two years. This provided an indication of the approximate amount and time of mortality.

2. Raft Culture Mortality

During work on the development of raft culture techniques applicable to British Columbia conditions information was obtained on the survival of spat on hanging strings. With the cultch off the bottom the problem of silting was eliminated along with significant predator activity. Mortality in one study amounted to about 60 per cent in a period of 12 months while in another one over a period of 8 months the mortality was only 35 per cent. The main difference was a reduced number of spat per unit of cultch and increased size of spat, in addition to a shorter time period, in the second trial.

3. Designed Experiment

The information derived from the two described studies indicated a designed experiment combining the essentials of each one. Simply, this was a comparison of the mortality of seed grown on the bottom with that grown off the bottom.

The experiment was arranged in a 2 x 2 Latin Square replicated four times. Each treatment (on bottom, off bottom) was repeated twice in each of the four blocks which were laid out at equal intervals on a predator-free intertidal beach in Ladysmith Harbour between the 6-foot and the 1-foot tide levels.(Mean sea level 8.0 ft, range 16 ft.)

Each square consisted of two 3 x 3 ft plots on the bottom and two 3 ft x 3 ft x 4 inch trays of 10 gauge, one-inch mesh galvanized wire trays held about 12 inches off the bottom by posts driven into the beach. The bottom plots were fenced by six inches of one-inch mesh chicken wire to prevent possible movement of the seed out of the plot.

The spat on 16 random groups of 100 shells of 1956 Japanese unbroken seed was counted. Each group of shells was allotted by chance (random numbers) to one of the 16 plots.

The seed was placed on the trays on 28 April 1956, and removed for counting on 17 November 1956. The tabulated results (survival) are shown in Fig. 1. The blocks and plots are arranged in the figure in the same relative position they held on the beach.

6-foot tide level

1238 (994) 80.2%	1406 (844) 55.6%
1518 (1015) 66.7%	2269 (1186) 52.3%

	Mean Survival	Mean Mortality
Tray	66.2	33.8
Bottom	61.1	38.9

2386 (1236) 51.4%	217 (753) 35.6%
1532 (548) 35.8%	1953 (1194) 61.1%

Tray	56.2	43.8
Bottom	35.7	64.3

1489 (1087) 73.8%	1827 (521) 25.8%
1670 (506) 30.3%	1956 (1325) 67.7%

Tray	70.7	29.3
Bottom	29.4	70.6

1-foot tide level

1330 (952) 71.5%	1385 (221) 15.9%
1470 (420) 28.6%	2133 (1224) 54.4%

Tray	64.4	35.6
Bottom	22.2	77.8
Total:		
Tray	69.4	35.6
Bottom	37.1	62.9

Fig. 1. Top number: original spat count April 27, 1956; bracket number: survival to November 17, 1956; T : tray.

Since the tray survival was nearly double bottom survival there is little doubt that the difference between the two treatments accorded the seed is significant. This is confirmed by a simple analysis of variance (Table 1).

Table 1.

Source of variation	D. F.	S.S.	M.S.	F.	P.
Blocks	3	995.2	331.7	-	-
Treatment	1	2,889.0	2,889.0	18.98**	< 0.01
Error	11	1,675.2	152.3	-	-
Total	15	5,559.4			

The significant factors contributing to seed mortality in the two treatments may be expressed as follows:

Bottom mortality = natural + competition for space + silting = 62.9%

Tray mortality = natural + competition for space = 35.6%

silting = 27.3%

Substituting the experimental values and cancelling out the common factors of natural mortality and competition, an estimation of 27.3 per cent is obtained for silting mortality. This assumes, though it is not strictly true, that natural mortality and competition for space are equal for the two treatments. There is no evidence to suggest appreciable natural mortality so the tray mortality of 35.6 per cent may be accepted as a measure of mortality due to competition for space. This is illustrated in Table 2 where lowest mortality on the trays is shown to occur where the spatfall per unit of area, and consequently, competition for space, is least.

Table 2.

Spat count (100 shells)	Survival	Per cent survival	Per cent mortality
2,386	1,236	51.4	48.6
2,269	1,186	52.3	47.7
2,133	1,224	57.4	42.6
1,956	1,325	67.7	32.3
1,953	1,194	61.4	38.6
1,489	1,087	73.8	26.2
1,330	952	71.5	28.5
1,238	994	80.2	19.8

Of interest is the relationship between mortality (Fig. 1), particularly on the bottom, and tidal height, for there is a marked silting gradient down the experimental beach as there is on most sloping beaches in British Columbia. Silt tends to be deposited and remain at the lower tidal levels due largely to reduced wave action. Greater wave action keeps the higher levels clear. Statistical analysis indicated there is a near-significant difference in mortality between blocks with reference to the bottom plots (Table 3), correlating well with the known degree of silting.

Table 3. Test of significance of differences between blocks in percentage mortality (data from the Appendix)

Effect	D. of F.	S. S.	M. S.
Blocks	3	995.2	331.7
Error	8	699.4	87.4
Total	11	1,694.6	

$F = \frac{331.7}{87.4} = 3.79$ d. f. 3, and 8 P = ca. 0.06

A similar correlation was obtained from raft culture studies where presumably the main source of mortality is competition for space. In Appendix A, Dr. W. E. Ricker has developed a more sophisticated analysis of the data.

This discussion of spat mortality is reduced to its simplest terms for it is much more complicated. The degree of natural mortality with the two treatments is not known though it is unlikely to be high. The relationship between silting mortality and competition for space is probably quite complex and how they interact probably depends largely on the rate of growth: the greater growth is, the less silting mortality, but the higher is the rate due to competition for space.

ECONOMICS

The economic implications are fairly obvious. The greater the seed mortality the greater the amount of seed required for a given production.

Silting mortality may be reduced by using special seed grounds, for instance, Olympia oyster dikes produce from 2 to 3 times the average industry yield of Pacific oysters. It is possible to create seed ground. In British Columbia there is considerable ground that is submarginal for a full Pacific oyster culture but could grow seed most effectively.

Competition for space on the cultching medium is inevitable but it may be reduced by using smaller units of cultch.

APPENDIX A

STATISTICAL ANALYSIS

The scheme below gives the percentage mortality in each box of the experiment, and the various groupings used. The raised trays ("treated" samples) are marked "T".

Actually, row 1 and row 2 were always side by side, as were column 1 and column 2, so that no detectable differences were expected to result from relative positions in rows or columns. However, partly to test this expectation, and partly to show how a moderately complex analysis can be done, the contributions of rows and columns are separated out below.

	Column 1	Column 2	Totals	Totals	
Row 1	80(T)	56	136		
				255	Block 1
Row 2	67	52(T)	119		
Row 1	51(T)	36	87		
				184	Block 2
Row 2	36	61(T)	97		
Row 1	73(T)	28	101		
				199	Block 3
Row 2	30	68(T)	98		
Row 1	71(T)	16	87		
				173	Block 4
Row 2	29	57(T)	86		
Totals	437	374	811		

The first step is to compute the correction term and total sum of squares:

Correction term = $811^2/16 = 657721/16 = 41107.6 = C$

Total sum of squares = $\frac{80^2 + 56^2 + 67^2 + \ldots + 57^2}{1} = 46667 - C = 5559.4.$

The sum of squares for the obvious groupings are as follows:

S.S. for columns $= \frac{437^2 + 374^2}{8} - C = 247.0$; d.f. = 1

S.S. for rows $= \frac{411^2 + 400^2}{8} - C = 7.5$; d.f. = 1

S.S. for treatments $= \frac{513^2 + 298^2}{8} - C = 2889.0$; d.f. = 1

S.S. for blocks $= \frac{255^2 + 184^2 + 199^2 + 173^2}{4} - C = 995.2$; d.f. = 3

The figures in each block of four can be combined by two's in 3 ways: horizontally, vertically and diagonally. This gives a measure of the interaction of block differences with row effects (I, below), with column effects (II), and with treatment effects (III), respectively.

I. Sum of squares for rows (r) + blocks (b) + r - b interaction

$$= \frac{136^2 + 119^2 + 87^2 + \ldots + 86^2}{2} - C = 1094.9$$

Effects	S.S.	d.f.
r + b + r - b interaction	1094.9	7
r	7.5	1
b	995.2	3
r-b interaction (by difference)	92.2	3

II. Sum of squares for columns (c) + blocks (b) + c-b interaction

$$= \frac{147^2 + 108^2 + 87^2 + \ldots + 73^2}{2} - C = 1594.9$$

Effects	S.S.	d.f.
c + b + c - b interaction	1594.9	7
c	247.0	1
b	995.2	3
c-b interaction (by difference)	352.7	3

III. Sum of squares for treatments (t) + blocks (b) + t-b interaction

$$= \frac{132^2 + 123^2 + 112^2 + \ldots + 45^2}{2} - C = 4859.9$$

Effects	S.S.	d.f.
t + b + t-b interaction	4859.9	7
t	2889.0	1
b	995.2	3
t - b interaction (by difference)	975.7	3

IV. The breakdown above is summarized as follows:

Effects	d.f.	S.S.	M.S.
1. Treatments	1	2889.0	2889.0
2. Columns	1	247.0	247.0
3. Rows	1	7.5	7.5
4. Blocks	3	995.2	331.7
5. t-b interaction	3	975.7	325.2
6. c-b interaction	3	352.7	117.6
7. r-b interaction	3	92.2	30.7
Total	15	5559.3	

As mentioned above, the design of the experiment suggests there should be no real difference between columns or rows, hence, also no interactions between these and the blocks. If so, items 2, 3, 6 and 7 above should be added to obtain the best estimate of random variability in the experiment:

Effects	d.f.	S.S.	M.S.
c + r + c-b + r-b	8	699.4	87.4

This is now used to test the significance of the block effect and the block-treatment interaction (the treatments themselves were tested with sufficient precision in Table 1, but they, too, could be tested in this way):

For blocks, $F = \frac{331.7}{87.4} = 3.79$; d.f. = 3, 8; $P \sim 0.06$

For b-t interaction, $F = \frac{325.2}{87.4} = 3.72$; d.f. = 3, 8; $P \sim 0.06$

Both of the above are only slightly below the conventional 5 per cent level of significance.

It is noteworthy that the mean squares for the columns and (to a less extent) the column-block interaction are much larger than those for rows and row-block interaction. It is perhaps imaginable that the two first-mentioned differences could be real, because of some net "set" of the current along the beach, for example. If so, they should be removed from the random error estimate, which latter then becomes the sum of items 3 and 7, giving a mean square of 24.9 for 4 degrees of freedom. Using this in the F test, the block differences and b-t interaction yield P values about 0.02—0.03. However, the more conservative F values first quoted are sufficient to demonstrate the likelihood of real differences between blocks (namely, decreased survival with increasing depth) and a real interaction between blocks and treatments (the raised trays doing more to reduce mortality in the deeper water than in the shallower). The probable reason for the direct effect is increased silting in deeper water, as explained earlier. The interaction also is quite reasonable, for the greater mortality in controls at the lower levels gives more scope for the "treatment" to be effective.

Designs similar to the above should have wide application in shellfish culture experiments. The one used here could be made to have a wider applicability with little or no extra work, simply by varying the conditions in the rows or the columns. For example, the two columns could be set out on different beaches instead of side by side; or in the two rows the spat might be set out at different densities. In fact, both these could be varied simultaneously, though there would then be some risk of inflating the error estimate because of possible column-block and row-block interactions, hence making it difficult to recognize block or even treatment effects. Another technical improvement, in an experiment where mortalities were really severe, would be to use survival rates instead of mortality rates in the analysis; or better, to use the logarithms of survival rates, which are proportional to the instantaneous mortality rates.

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DISTRIBUTION OF OYSTER MICROPARASITES IN CHESAPEAKE BAY, MARYLAND, 1959-1960

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ABSTRACT

Six-hundred and sixty-three oysters were collected for histological study from 165 oyster bars in the Maryland portion of Chesapeake Bay during surveys in the spring and fall of 1959 and 1960. The following endoparasites were found: "MSX" in 12, Dermocystidium marinum in 13, Ancistrocoma sp. in 4, Hexamita sp. in 4, Bucephalus cuculus in 12, and Nematopsis ostrearum in 82 oysters; 543 oysters contained no recognizable parasites. Nine oysters were dead or weak when collected. With the exception of Pocomoke Sound where 25.5% of the oysters from all bars sampled during the fall survey of 1960 were infected with "MSX" and where approximately 12% of the oysters had died within the two-week period before the survey, no extensive mortality of oysters was observed during the surveys. "MSX" is an undescribed microorganism believed to be the cause of economically disastrous mortalities of oysters in Delaware and Chesapeake Bays.

INTRODUCTION

Since 1959, in cooperation with the Chesapeake Biological Laboratory and the Maryland Department of Tidewater Fisheries, the Bureau of Commercial Fisheries Biological Laboratory, now located in Oxford, has been conducting studies on the identity, geographic and temporal distribution, relative abundance, and methods of dispersal of microendoparasites of oysters, Crassostrea virginica (Gmelin), and on the association of these parasites with mortality of oysters in the Maryland portion of Chesapeake Bay. These investigations were undertaken as part of a larger study of diseases associated with excessive mortality of oysters in the middle Atlantic area of the United States in recent years.

This paper reports the results of a search for parasites in the tissues of oysters collected during surveys in the upper Chesapeake Bay in 1959 and 1960.

Spring and fall surveys of 1959 were made primarily to study the composition of oyster bars in the Maryland portion of Chesapeake Bay and to determine the degree of survival of oyster set of the year and mortality of older oysters. The surveys of 1960 reported in this paper were carried out primarily to search for oyster mortalities and possible parasites associated with these deaths.

METHODS

Oysters were collected with oyster dredges by personnel from each of the laboratories using the following research vessels: the Cobia, Chesapeake Biological Laboratory; the Maryland, Department of Tidewater Fisheries; and the Alosa, Bureau of Commercial Fisheries.

One hundred and thirty-five oyster bars (Fig. 1a) were sampled during the spring of 1959 over a period of 3 1/2 weeks, and three to five oysters from each bar were preserved in Zenker-formol fixative. Histological slides were prepared of 141 of these oysters, a minimum of one from each bar.

In the fall of 1959, 123 oyster bars (Fig. 1b) were examined over a period of two weeks for evidence of oyster mortality. Only a few oysters were collected for histological examination.

Thirty oysters were taken from each of 75 oyster bars (Fig. 1c) during the five-week spring survey of 1960. These 2350 oysters were opened and examined for: number of Polydora and mud blisters; degree of penetration by the boring sponge, Cliona; number of shell and tissue ulcers; degree of obstruction of valve edges by mussels, oysters and other fouling organisms; color of fresh tissues; general condition of soft parts; and quantity of recent shell growth. Using these observations as a basis, the three or four poorest oysters from each oyster bar were fixed in Zenker-acetic fixative. Samples of 20 oysters collected at random from each of several oyster bars were also preserved.

Four hundred and twenty oysters dredged from 14 oyster bars (Fig. 2) during the fall of 1960 were opened and examined in the same manner as those collected in the spring survey of 1960. Fifteen oysters from each bar were fixed in Zenker-acetic fixative.

After fixation and washing, oysters were processed for histological examination as described by Burton (1961). During the summer and fall of 1960, 646 of these were sectioned, and 1292 histological slides were prepared. Sections, one to three per oyster, were cut transversely halfway between the promyal chamber and the anterior tip of the

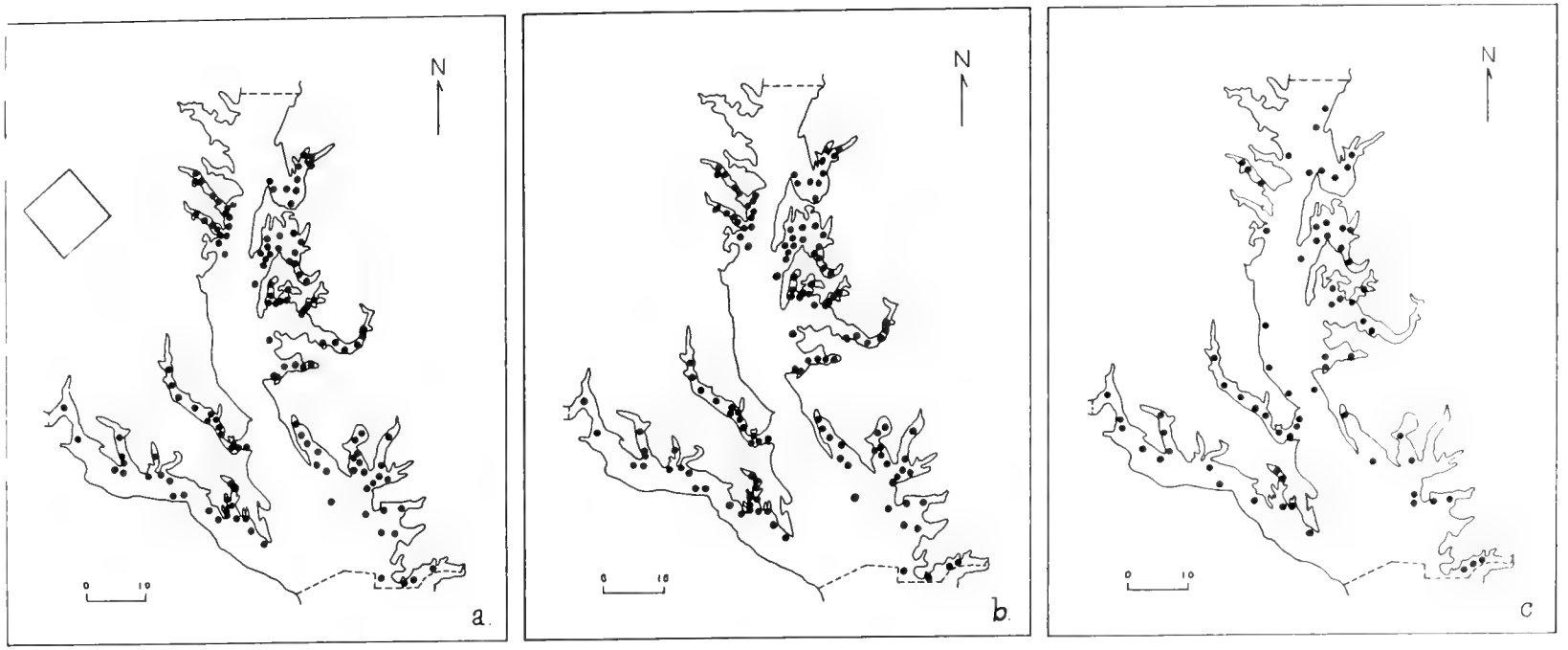

Fig. 1. Chart of Maryland portion of Chesapeake Bay showing location of oyster bars sampled. Each dot represents one oyster bar. Scale in miles. Square shows location of Washington, D. C.

a. Oyster bars sampled during spring survey of 1959.
b. Oyster bars sampled during fall survey of 1959.
c. Oyster bars sampled during spring survey of 1960.

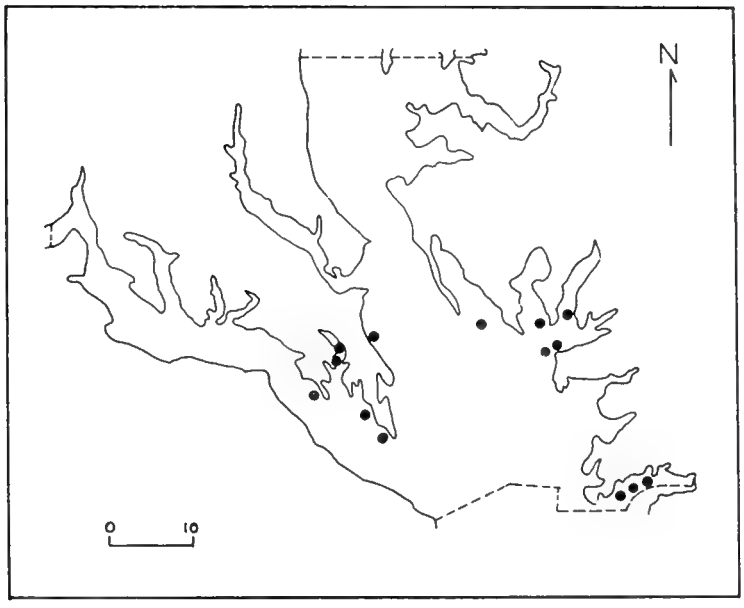

Fig. 2. Chart of portion of Maryland-Chesapeake Bay showing location of oyster bars sampled during the fall survey of 1960.

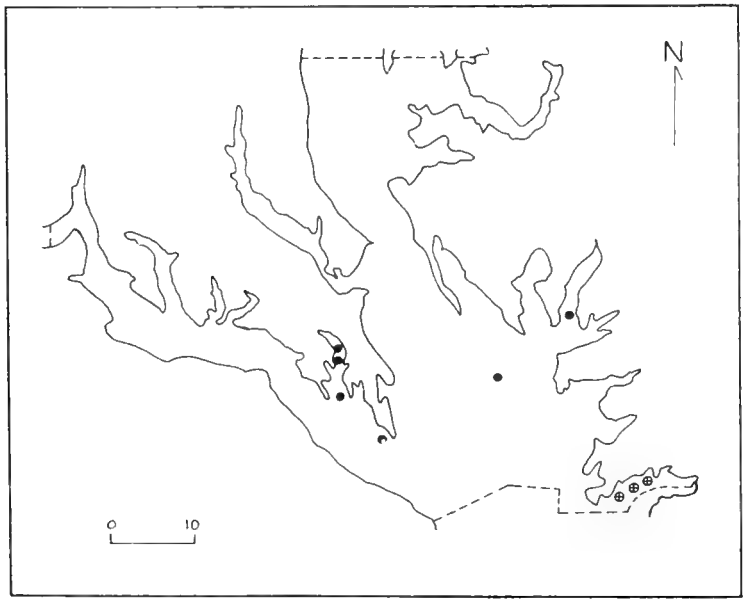

Fig. 3. Chart of portion of Maryland-Chesapeake Bay showing location of oyster bars infected with Dermocystidium marinum (Solid dots) and MSX (open circle).

oyster through the digestive diverticulum and gonad. These slides were examined first at a magnification of 150X for general orientation, for condition of the tissues, and for large parasites such as the trematode Bucephalus cuculus. The entire section was then searched at 430X for smaller microendoparasites. Those observed were then studied at 970X.

OBSERVATIONS

Of the 141 oysters collected during the spring survey of 1959 (Table 1), one contained Bucephalus cuculus McCrady (see Hopkins, 1954); five were parasitized by the sporozoan Nematopsis ostrearum (Prytherch, 1938; Sprague, 1949; Landau & Galtsoff, 1951); two showed heavy concentrations of bacteria; and no parasites were observed in the remaining 133 oysters.

The only gaping oyster (weak or dead) dredged during the fall survey of 1959, and taken on Cinder Hill bar in Holland Straits, was heavily infected with Dermocystidium marinum (Mackin, 1951; Andrews and Hewatt, 1957).

Four of the 312 oysters dredged during the spring survey of 1960 contained the ciliate Ancistrocoma sp., 4 had the flagellate Hexamita sp. (Mackin, Korringa and Hopkins, 1952), 8 were infected with B. cuculus, 71 contained spores of N. ostrearum, 3 had heavy concentrations of bacteria, and 235 contained no parasites. The two parasites not found in previous surveys of this study, Ancistrocoma sp. and Hexamita sp., with one exception, were found only in the 9 gapers collected during this period.

Twelve of the 210 oysters taken in the fall survey of 1960 contained MSX.[1] These were found among oysters dredged in Pocomoke Sound (Fig. 3) and represent 2 per cent of the total number of oysters collected at that time, 26 per cent of the oysters taken in Pocomoke Sound, and 55 per cent of the sample taken from the bar in

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[1]The term MSX refers to a sporozoan first observed by Haskin and Stauber in the tissues of Delaware Bay oysters and described as a multinucleated sphere (personal communication). It was later considered by Mackin to be a haplosporidian (personal communication; Caullery and Mesnil, 1905; Granata, 1914). MSX has been associated with extensive mortalities of oysters in Delaware and Chesapeake Bays in recent years.

Table 1. Parasitization of oysters collected in three surveys of the Maryland-Chesapeake Bay, 1959-1960

Surveys	No. oyster bars sampled	No. gapers collected	No. oysters examined histo-logically	No. of oysters parasitized by:							No. non-parasitized oysters
				MSX	Dermocys-tidium	Ancis-trocoma	Hexa-mita	Buceph-alus	Nema-topsis	Bac-teria	
Spring 1959	135		141					1	5	2	133
Spring 1960	75	9	312			4	4	8	71	3	235
Fall 1960	14		210	12	13			3	6	2	175
Totals	224*	9	663	12	13	4	4	12	82	7	543

* Represents 165 different bars.

Pocomoke Sound showing the greatest infection. Thirteen oysters, 11 from the Potomac River and 2 from the Nanticoke River, contained D. marinum (Fig. 3), 3 were infected with B. cuculus, 6 with N. ostrearum, and 2 with dense concentrations of bacteria; no parasites were observed in the remaining 175 oysters.

A small nematode was first observed in a histological section of an oyster collected on Marumsco Bar, Pocomoke Sound, during the spring survey of 1960. A similar nematode was seen in an oyster taken on Halls Bar in Tangier Sound during the same period. Four more were found in oysters from Marumsco Bar in the fall survey of 1960, one from Clay Island Bar in Fishing Bay, and one from Cornfield Bar at the mouth of the Potomac River. Nematodes were tightly coiled in tissues in the region of the digestive diverticulum, and measured approximately 75 microns in cross section.

Dense concentrations of leukocytes, scattered more or less uniformly throughout or clumped in a single area of an oyster, were seen. Likewise observed was encapsulation of some areas of infection by organized accumulations of leukocytes, apparently followed by the formation of connective tissue. Lesions like stomach or intestinal ulcers were also observed in one oyster.

Abnormal histological appearance of oyster tissues and unusual concentrations of leukocytes in some cases may be attributed to the presence of such micro-organisms as Hexamita, Ancistrocoma, Dermocystidium, MSX, or dense accumulations of bacteria.[2] In many instances, however, the histological picture is abnormal without apparent cause in the particular section under examination. This may be explained in some cases by a light infection or a localized infection in another part of the oyster. Cellular reaction may also be caused by rough handling of oysters during collection (Mackin, personal communication).

Microendoparasites found in oysters in the Maryland portion of the Chesapeake Bay to date include: Dermocystidium marinum, MSX, Nematopsis ostrearum, Hexamita sp., Ancistrocoma sp., Bucephalus cuculus, and many bacteria. MSX plasmodia observed in these studies ranged in diameter from 7 to 25 microns, and contained 2 to 32 nuclei characterized by eccentric nucleoli.

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[2]More than 80 different species of bacteria, most of which may be transients in the food, have been isolated in pure culture from oysters in the Biological Sub-Station of the Fisheries Research Board of Canada, Ellerslie, P.E.I., Canada (R. Drinnan, personal communication).

DISCUSSION AND CONCLUSIONS

Surveys of oyster bars in Chesapeake Bay have been conducted during the past 18 years by personnel of the U. S. Bureau of Commercial Fisheries, the Chesapeake Biological Laboratory, and the Maryland Department of Tidewater Fisheries, primarily to determine intensity of setting of oysters, composition and extent of productive oyster bars, and general condition of oyster meats throughout the bay, and in some cases to permit a search for oyster mortalities as indicated by the presence of fresh, empty, hinged oyster valves (Engle, 1946). The spring survey of 1959 was the first during which oysters in the upper Chesapeake Bay were collected for histological study of oyster parasites.

Because of the large number of oysters examined, we studied only one or two histological cross sections of the visceral mass of each oyster. Such a slice, seven microns in thickness, is equivalent to approximately 1/10,000 of the volume of an oyster three inches long. Thus, a light infection throughout the oyster, as well as a severe localized infection, may not have appeared in the sections chosen for study. This limitation of the method of sampling must be kept in mind in interpretation of the degree of parasitization reported here.

The importance of periodic histological examination of tissues of oysters from a given area was clearly demonstrated during and following the heavy mortality of oysters which began in Delaware Bay in 1957 (Haskin, personal communication). Microendoparasites were observed in tissues of weak and dead oysters taken during the mortality, but since no periodic collections of oyster tissues had been made in previous years, it was not possible to compare the newly observed parasites with those that formerly occurred in that area. Guided by this experience we have established a slide file which contains histological sections of oysters from all surveys of the Maryland portion of Chesapeake Bay since the spring of 1959. These sections are being used to compare parasites and abnormal histological conditions found in oysters in Maryland with those in other areas of the world.

These studies indicate that the upper Chesapeake Bay is relatively free of oyster parasites. This may be attributable to a rather high flushing rate in the upper Bay from land and river drainage and an over-all lower salinity than in the lower Bay.

Except in Pocomoke Sound, no unusual mortality of oysters was observed during the four surveys reported here; even here mortality was not high compared to Virginia mortalities. The role such micro-

organisms as Hexamita and Ancistrocoma may have played, if any, is not known. To our knowledge neither of these has caused severe mass mortalities in Maryland such as those reportedly associated with MSX in the surrounding coastal waters of New Jersey, Delaware and Virginia.

Distribution of Dermocystidium marinum infections was similar to that reported by Andrews and Hewatt (1957) for Chesapeake Bay.

MSX was known to infect oysters throughout much of the lower half, or Virginia portion, of Chesapeake Bay prior to the fall of 1960 (J. D. Andrews, personal communication). We focused special attention on the lower Maryland portion of Chesapeake Bay during this fall survey because of the proximity of these waters to those in Virginia where infections of MSX were known to occur. MSX was not observed in Pocomoke Sound prior to the fall survey of 1960; it may have invaded this area between the spring and fall surveys of that year.

ACKNOWLEDGMENTS

Acknowledgment is made to personnel of the Chesapeake Biological Laboratory and the Maryland Department of Tidewater Fisheries for their cooperation during the Chesapeake Bay surveys. I should like also to express my gratitude to Drs. H. H. Haskin and L. A. Stauber of Rutgers University for instruction in laboratory techniques and in identification of oyster parasites, and for valuable assistance throughout this work; to Drs. J. D. Andrews and J. L. Wood of the Virginia Fisheries Laboratory for help in histological methods and in identification of parasites; and to Austin Farley and Barbara Janz for preparation of histological slides of oyster tissues.

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CHEMICAL CONTROL OF THE GREEN CRAB, *CARCINUS MAENAS* (L.)

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ABSTRACT

Development of a method to protect soft-shell clam (*Mya arenaria* L.) stocks from green crab predation is considered necessary for efficient management of the New England clam fishery. The development of new organic pesticides has suggested practical and economical methods of controlling this predator. Bait fish soaked in lindane and attached to long trawl lines proved to be an effective barrier to green crabs. The barrier not only prevented crab movement into the protected area, but reduced resident crab populations. Experiments at Kittery, Maine, resulted in a 76 per cent decrease in crab catch nine weeks after the barrier was established. Catches of all traps declined during the experimental period with the greatest decrease in those traps closest to the barrier. Significant differences between trap catches inside the barrier and those outside the barrier were evident after four weekly baitings. The barrier restricts toxic bait to a predetermined area, is lethal to arthropods that feed on the bait, and is relatively inexpensive to maintain.

INTRODUCTION

The green crab, *Carcinus maenas* (L.), is the most important predator of the soft-shell clam, *Mya arenaria* L., north of Cape Cod. Low wire fences, developed to curtail crab predation (Smith, 1954, Glude, 1955), have not been entirely satisfactory and alternative methods of crab control are needed. Research at the Bureau of Commercial Fisheries Biological Laboratory, Milford, Conn., on chemicals as predator control agents has been most promising (Loosanoff, Hanks and Ganaros, 1956; Loosanoff, 1959, 1960; Shearer and MacKenzie, 1959; Loosanoff, MacKenzie and Shearer, 1960). When specific pesticides are found, there remains the problem of using these materials in the marine environment without undesirable side effects. Although many apparently excellent methods of predator control with chemicals are available (Loosanoff, 1960), further studies will be made in the hope of expanding the application of this potentially valuable management tool. This paper describes one method, developed at the Bureau of Commercial Fisheries Biological Laboratory, Boothbay Harbor, Maine, to control green crabs with a commonly used pesticide.

EXPERIMENTS IN CRAB CONTROL

Lindane is the gamma isomer of benzene hexachloride (BHC). It is by far the most toxic of the several isomers of BHC and has been widely used as an agricultural pesticide (Metcalf, 1955). Crude BHC has been used in aquatic predator control by Jordan (1955) who found that spray applications at dilutions of 6.5 ppm protected young rice seedlings from destruction by the crab Sesarma africanum. In 1953, John Hurst, Jr. (personal communication), of the Maine Department of Sea and Shore Fisheries, found that lindane was extremely toxic to marine crustacea and that concentrations as low as 1.0 ppm were lethal to green crabs within 48 hours. Bait fish, soaked in lindane, attracted crabs to the toxic substance, and appeared to offer a convenient method of handling and restricting the action of organic pesticides in the marine environment.

In 1958, preliminary experiments were conducted to test whether a band of toxic bait, interposed between a clam-producing area and the source of crab recruitment, would act as a barrier to crab predators. A small creek system (Area 1 in Fig. 1) at Wells, Maine, was chosen as a study area on the basis of its size, accessibility, and existing records of crab and clam populations. Trapping records from the previous year showed that numbers of crabs and their movements in this creek were similar to those in other creek systems at Wells. The barrier was established during the middle of June by spreading, at low tide, a band of toxic bait 30 feet wide across the mouth of the creek. Small pieces of fish (alewives) used as bait had been soaked in lindane [1] for 24 hours at the proportion of one pint pesticide to one bushel of fish, a concentration found lethal to green crabs in previous experiments. The barrier was renewed with freshly treated bait every 2 weeks. Results were evaluated from the catch of four crab traps, two inside the barrier and two outside (Area 1, Fig. 1) which were fished every week. Figure 2 shows that with no pesticide barrier the average monthly catch, based on the weekly catch of green crabs in each trap, was similar in all three areas during 1957. The data for 1958 demonstrate a definite reduction in crab abundance in the experimental area, while the two control areas again exhibited similar patterns of population increase. Lindane had a definite impact on the weekly crab catch which was always lower in the week following bait renewal than in the week preceding (Fig. 3). Subsequent sampling of crabs and clams in the creek

[1] Lindane was in the form of Isotox Spray #200 (20% gamma isomer content) obtained from the California Spray Chemical Corp., 622 State St., Springfield, Mass.

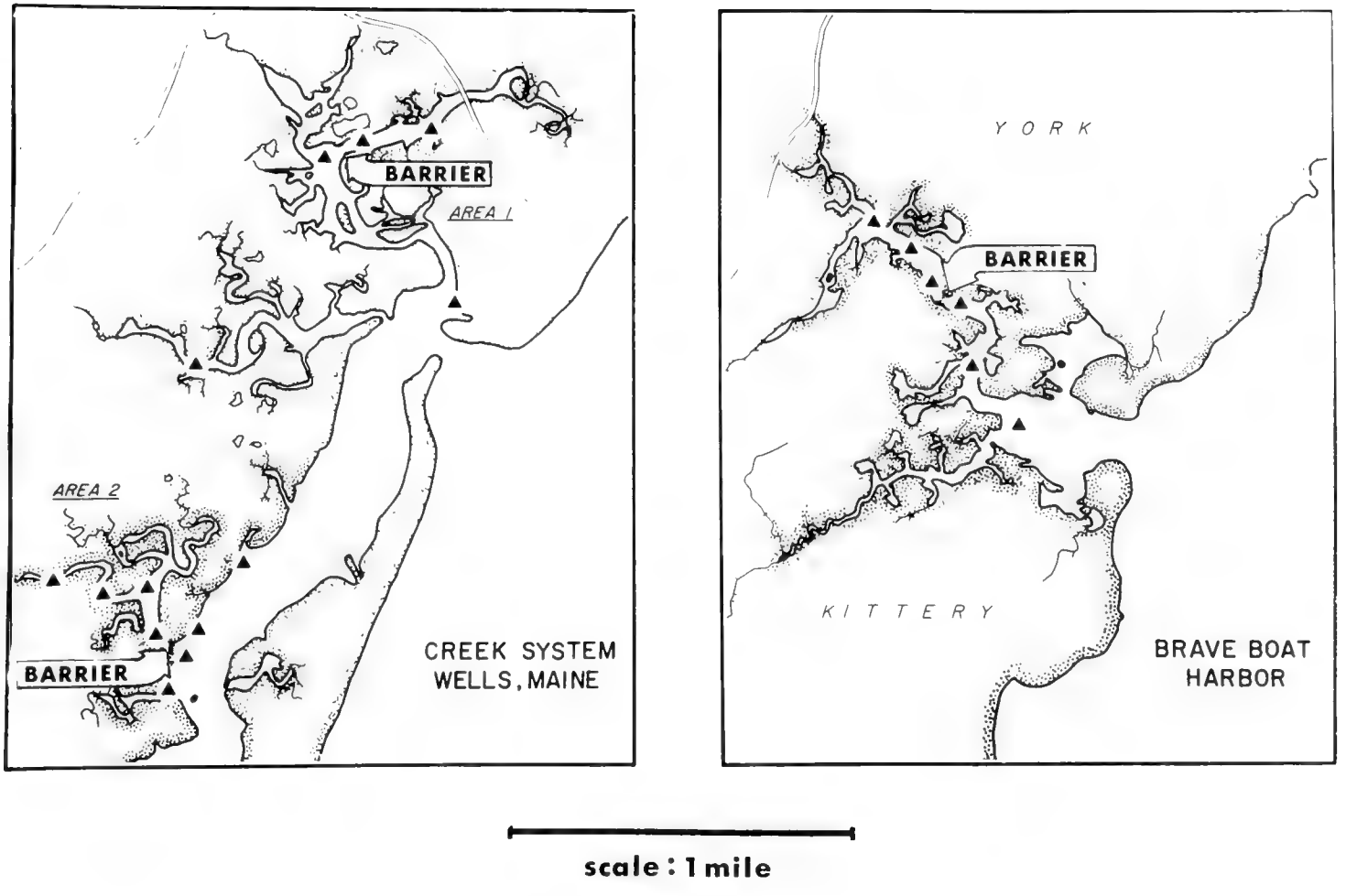

Fig. 1. Experimental areas at Wells and Kittery, Maine, showing location of traps (solid triangles) and barriers (hatched portions).

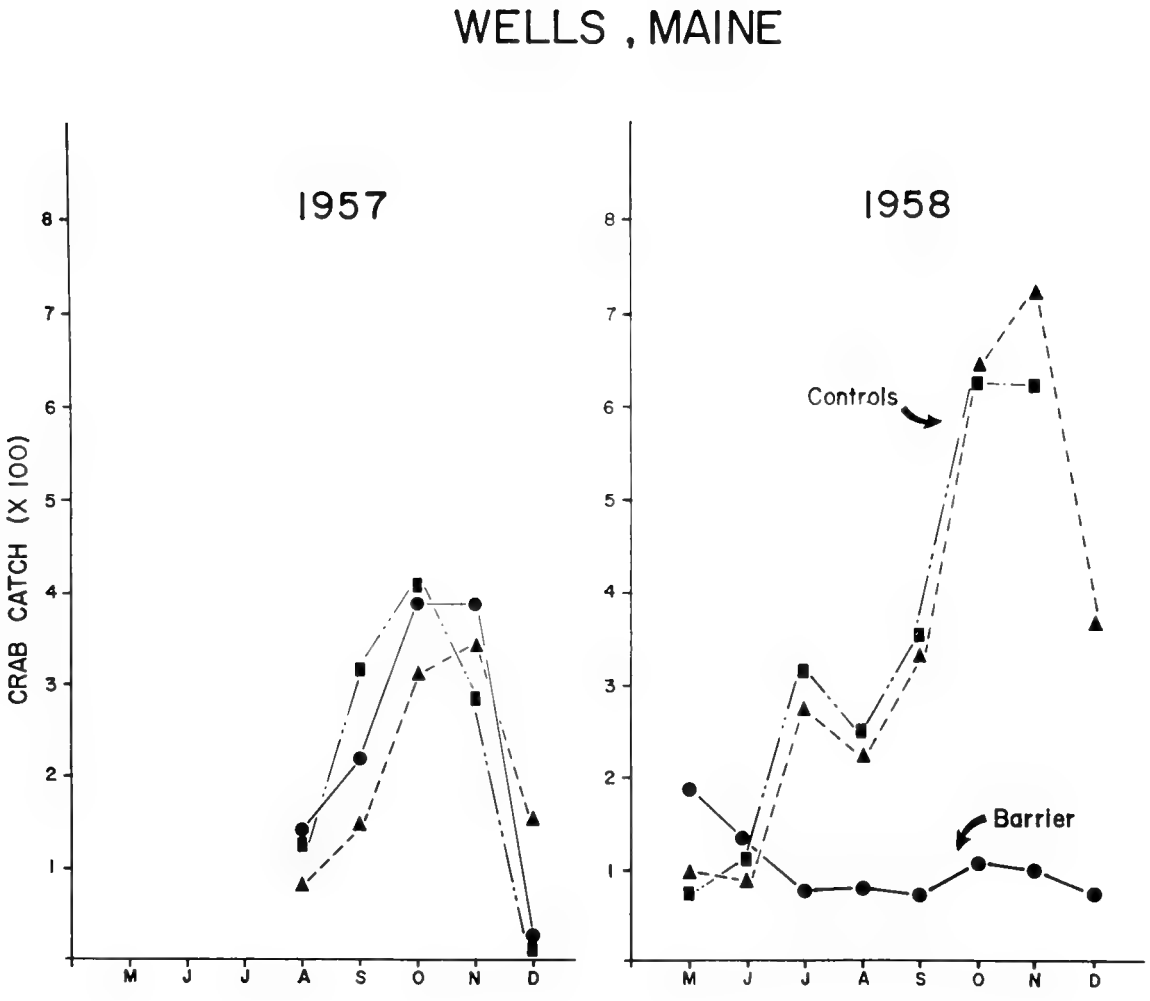

Fig. 2. Average monthly catch (in hundreds) of green crabs at Wells, Maine. Area 1 = ● , Area 2 = ▲ , and Control Area = ■ .

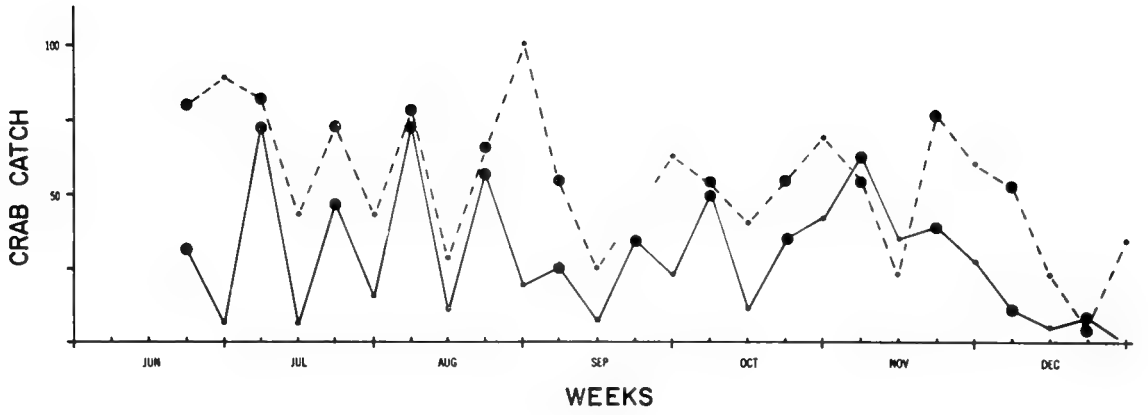

Fig. 3. The effect of lindane on the weekly green crab catch of two traps, one inside (solid line) and one outside (dashed line), near the barrier. Enlarged dots indicate dates of bait renewal.

indicated that the crab populations remained at a very low level throughout the winter. Survival of juvenile clams larger than 3 mm (a minimum size for crab predation) was much greater and in sharp contrast to other nearby sections of flats. Samples of clams in the 4 to 10 mm size range, taken in May 1959, produced means of $16/ft^2$ for the protected area and only $3/ft^2$ for the non-protected areas. Even as late as August 1959, mean numbers of juvenile clams in the same size class were $18/ft^2$ in protected flats and $1.5/ft^2$ in the other areas.

The experiment showed that the broadcast method of bait distribution was not satisfactory as bait was rapidly washed away by the strong currents thereby weakening the barrier and possibly carrying toxic bait into areas inhabited by commercially important crustacea. Bait distribution was also restricted to the period of low tide, often an inconvenience, and some training was required to spread the bait properly. These difficulties were corrected in the following experiments by using specially constructed trawl lines (Fig. 4). During the 1959 season, five trawl lines were stretched across the mouth of Pope's Creek in Wells, Maine (Area 2, Fig. 1). Each line consisted of a length of 5/16 inch diameter manilla rope, commonly referred to as "pot-warp," from which No. 14 cod hooks were suspended at 2-foot intervals. The distance between trawls was about 15 feet, and each line was weighted to hold it on the bottom. Whole fish, soaked overnight in lindane (1 pint of pesticide to 1 bushel of fish), were placed on the trawl hooks and renewed every 2 weeks. Unfortunately, the Wells crab population was

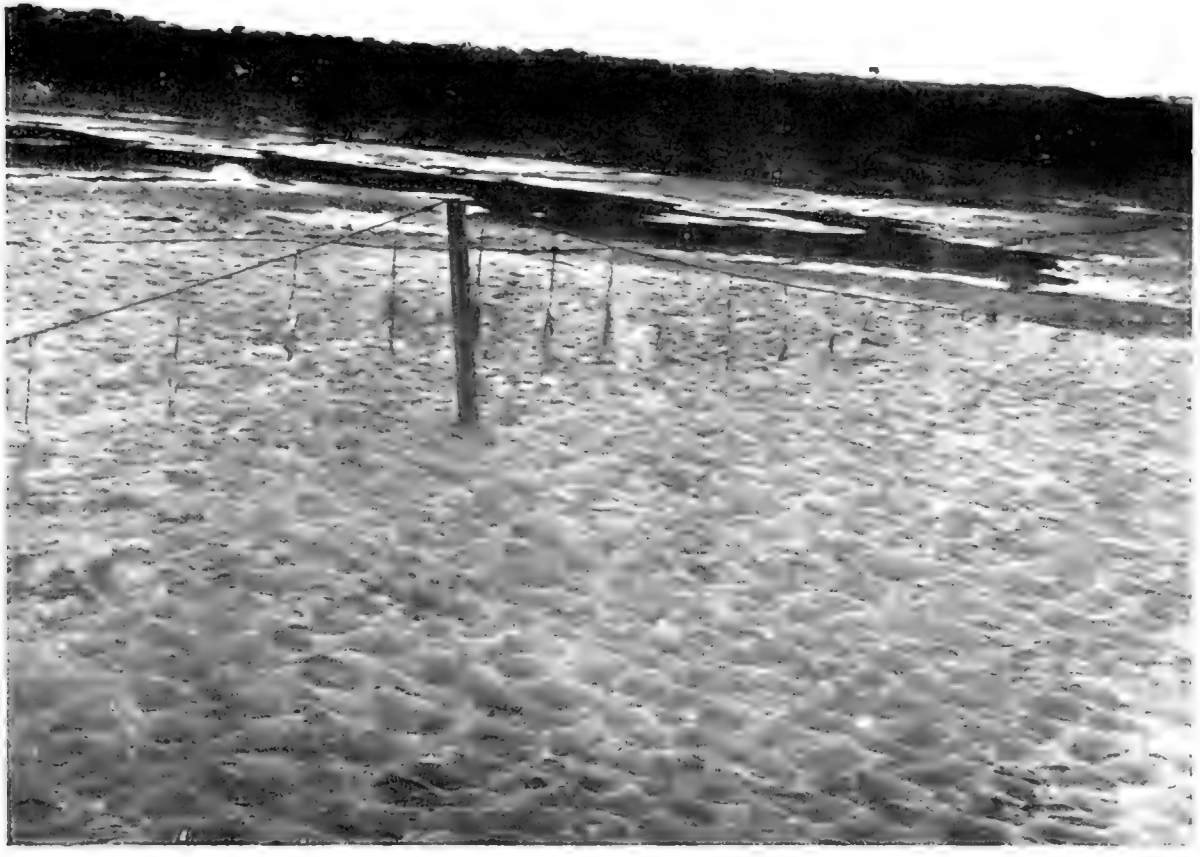

Fig. 4. Photograph of a barrier trawl line. The line has been supported on a wooden stake to provide a clearer view.

reduced in 1959 by a severe winter mortality. Trapping for green crabs was ineffective and evidence for barrier control of crab activity was unobtainable.

A concurrent and similar experiment in Brave Boat Harbor, Kittery, Maine (Fig. 1) provided data for 1959. The winter mortality had lowered the crab population, but sufficient numbers of crabs survived to provide evidence of the barrier effect. Two trawl lines were baited every week. Six traps, three inside and three outside the barrier, were fished daily. The average monthly catch for the inner traps and the outer traps is compared in Fig. 5 for 2 years, 1958 (without a barrier) and 1959 (with a barrier). The 1959 data show that both inner and outer catches declined during the trapping period. In addition, the general trend in catches with the barrier is directly reversed from the trend of catches without the barrier in the previous year. Traps closest to the barrier, both inside and outside, consistently exhibited the lowest catches, while the outermost traps maintained a higher level of catch. Student's "t" test, as applied to these data, showed that there was a significant difference at the 1 per cent level between the average catch per baiting period inside and outside, after the fourth baiting period. Fig. 6 demonstrates the impact of the lindane barrier on the entire creek system. There is a decreasing pesticide effect with increasing distance, and trap catches inside are consistently lower than catches equally distant from the barrier on the outside. This latter factor is ascribed to continuous recruitment from sources outside of the experimental area and indicates control of crab movement into the area protected by the barrier.

The project was terminated in October, prior to the period of most intense crab activity, because bait fish were unobtainable. Previous crab trapping in both Wells and Kittery indicated that the catch in the outermost trap would have shown a marked increase during this period, while those nearer to the barrier, particularly those inside, would have continued to decline. Even during the short experimental period, the average daily catch per trap inside the barrier was reduced from 44 to 14 crabs, or 76 per cent. The greatest decrease (80 per cent) occurred in the inner trap, nearest the barrier, while the least decrease (69 per cent) occurred in the outermost trap.

Clam samples, taken throughout the Brave Boat Harbor area in the winter of 1959, indicated an excellent survival of small Mya inside the barrier.

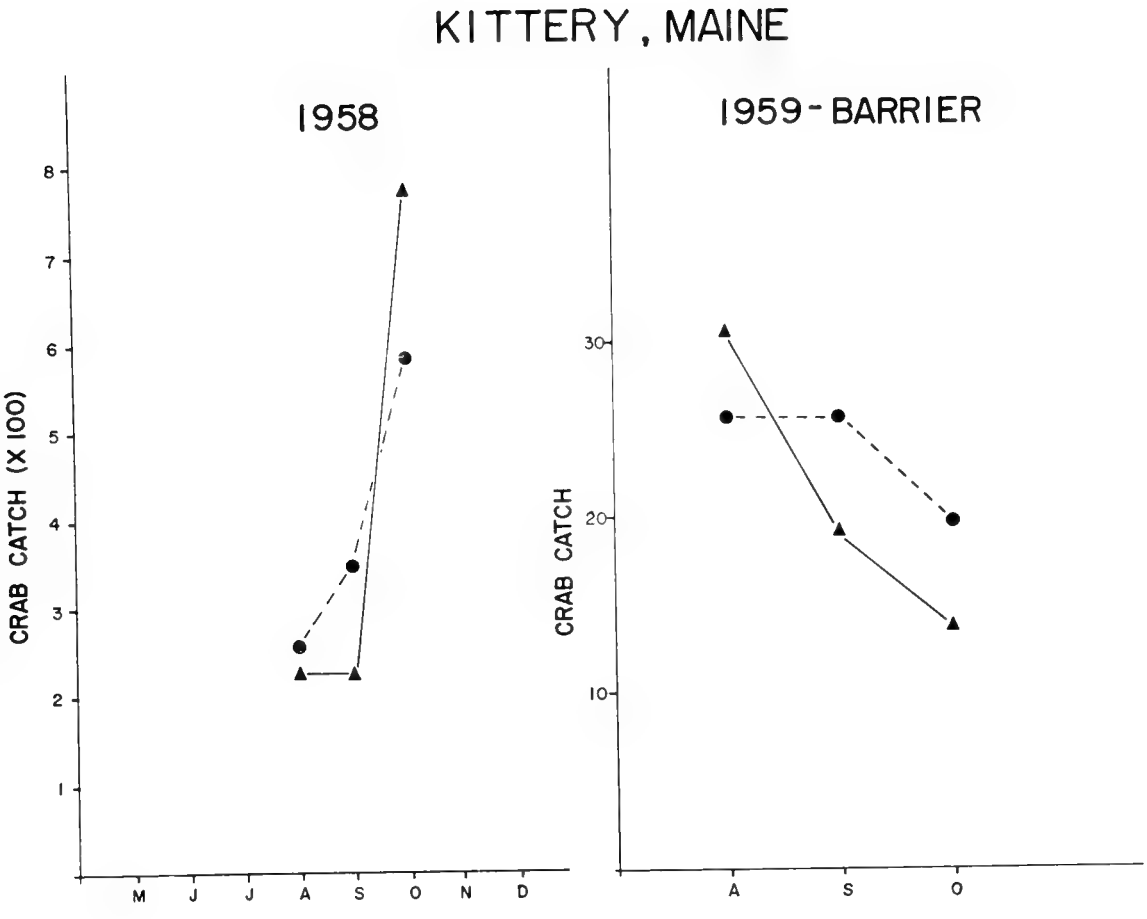

Fig. 5. Average monthly catch (data for 1958 in hundreds of crabs; for 1959 in actual numbers) of green crabs at Kittery, Maine. Inner trap (s) = ▲ ; outer trap (s) = ● . Note: the graphs are <u>not</u> drawn to the same scale.

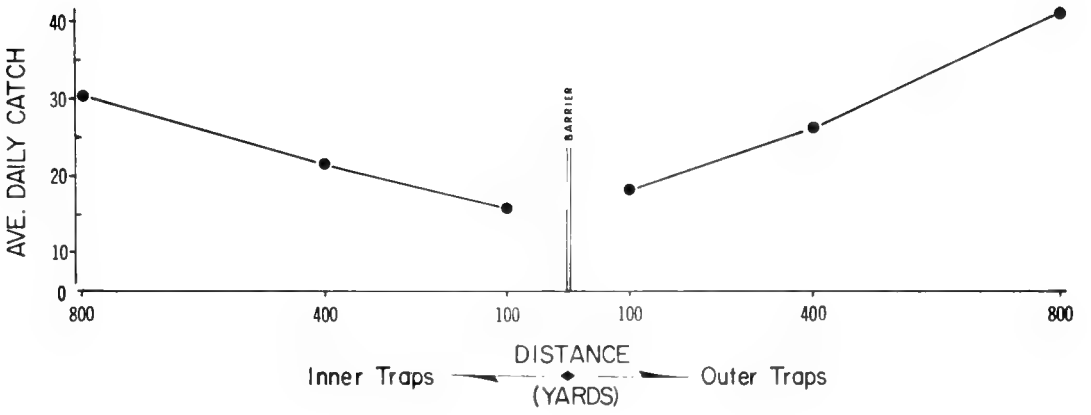

Fig. 6. Average daily catch for 1959 compared with distance from barrier site for six traps at Brave Boat Harbor, Kittery, Maine.

DISCUSSION

A predator control method must meet several requirements if it is to become a practical management tool. The method must be inexpensive, relatively safe to use in the presence of other organisms, and amenable to maintenance by unskilled labor.

Table 1 lists the items and costs of three different barriers and compares the cost per week in each area. The table does not list the cost of trawl line and hooks because these items become negligible when amortized over the entire life of the barrier. The expense of the barrier could be further reduced by use of cheaper bait and pesticide preparations.

Although lindane has been a satisfactory pesticide for research work (inexpensive, easily obtainable, specific in action, low aqueous solubility) considerable modification of the chemical part of the barrier is possible and, perhaps, desirable. Loosanoff, Hanks and Ganaros (1956) listed over 100 compounds that would kill green crabs and many other materials have become available. Lindane, however, can be a very selective toxin if used properly. Fish mortalities have been observed in relatively high dilutions of lindane (P. A. Butler, U. S. Bureau of Commercial Fisheries Biological Laboratory, Gulf Breeze, Florida, personal communication), but no fish deaths have ever been recorded in our laboratory experiments with lindane-soaked fish bait or in field experiments with the barrier. The only observed mortalities

Table 1. Cost of materials and labor for pesticide barriers

1958 Season

Wells, Maine—Upper Landing Creek, June to December.

13 baitings @ 2 bushels of fish:

Labor	13 man hours	@	$1.50/hr.	=	$19.50
Fish	26 bushels	@	1.50/bu.	=	39.00
Lindane	26 pints (3 1/4 gal.)	@	10.39/gal.	=	33.77
					$92.27

Materials only = $72.77

Cost per week: $3.55

1959 Season

Wells, Maine—Pope Creek, June to December.

12 baitings @ 3 bushels of fish:

Labor	18 man hours	@	$1.50/hr.	=	$27.00
Fish	36 bushels	@	1.50/bu.	=	54.00
Lindane	36 pints (4 1/2 gal.)	@	10.39/gal.	=	46.75
					$127.75

Materials only = $100.75

Cost per week: $5.32

Kittery, Maine—Brave Boat Harbor, August to October.

9 baitings @ 1 bushel of fish:

Labor	9 man hours	@	$1.50/hr.	=	$13.50
Fish	9 bushels	@	1.50/bu.	=	13.50
Lindane	9 pints (1 1/8 gal.)	@	10.39/gal.	=	11.69
					$38.69

Materials only = $25.19

Cost per week: $4.30

have been in sand shrimp, Crago septemspinosus, apparently susceptible to trace amounts of lindane. Chin and Allen (1957) found that lindane concentrations of 50 parts to one billion were lethal to two species of penaeid shrimp. Sand shrimp repopulate the treated area within one tidal cycle after application indicating that the observed mortalities were produced by excess lindane preparation washed from the surface of the bait fish and not from material leached out of the flesh. The low solubility of the pesticide when incorporated with the natural fish oil is advantageous because only those arthropods that feed directly on the bait and only those that are susceptible to very small concentrations of ingested lindane are affected.

Though simple to maintain after installation, the barrier must be carefully located on the basis of preliminary area surveys. The barrier method is particularly suited to the behavior of green crabs since their habitat requirements differ from those of most commercially important arthropod species. Lobsters (Homarus americanus), equally susceptible to lindane, are rarely found in the same area and the rock crab (Cancer irroratus) is seldom found in as shallow water as the green crab. Thus, by placing the barrier no deeper than the mean low water line, toxic effects on other species can be effectively eliminated. The examination of predator feeding habits and determination of local hydrographic features should also be employed in locating barrier sites.

The present data support the use of the pesticide-bait fish barrier as a practical means of crab predator control. The barrier method is applicable to many arthropod predators, where habitat requirements do not intergrade with those of commercial species or, alternatively, when species-specific pesticides can be found. With modifications, the method can possibly be utilized to control predators other than arthropods, provided they can be attracted by bait materials.

The barrier is particularly valuable where physical conditions such as tide, soil type and currents are most difficult; but it is restricted to intertidal or shallow water application. The barrier's advantage in restricting organic toxins to predetermined localities is obviously important in preventing pesticide pollution of the coastal waters.

Based on methods suggested by these studies, the pesticide-bait fish barrier is undergoing experimental trials in Maine and Washington to protect commercially important shellfish stocks.

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PESTICIDE TESTS IN THE MARINE ENVIRONMENT IN THE STATE OF WASHINGTON

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ABSTRACT

For killing ghost shrimps, Callianassa sp., that ruin oyster ground by burrowing, two combinations of chemicals were tested on small intertidal plots: furnace oil with Lindane, and orthodichlorobenzene with Sevin, both mixed with fine sand as a carrier. Good control of ghost shrimps could be obtained with materials costing $54 per acre, not including cost of application. For control of Japanese oyster drilling snails, Ocinebra japonica, one ton per acre of sand, orthodichlorobenzene and Sevin was not effective, but snails could be kept from moving into a 35 x 35-foot plot by a barrier made of one ton of gravel and sand mixed with orthodichlorobenzene and Sevin or polystream and Sevin. These methods are not recommended for use until more is learned of long-term effects on biota and possible public health risks.

The State of Washington has its fair share of pests affecting shellfish. These include Japanese oyster drills, eastern oyster drills, moon snails, ghost shrimp, starfish, at least four species of crabs, flatworms and shellworms. Some of these were introduced and some are native.

In the intertidal environment where much of Washington's oyster culture is carried on, it is often possible to reduce losses from some pests by altering cultural methods and by direct physical control. For those species that do not yield to physical control measures, chemical control could be an economical, effective means of controlling or locally eradicating serious pests, provided the treatment itself does not cause undesirable effects on associated biota, or create a public health hazard.

This report deals with the results of field tests conducted in 1960 and 1961 by the Department of Fisheries in Puget Sound on two major Pacific Coast pests: the ghost shrimp of the genus Callianassa, and the Japanese drill Ocinebra japonica.

The ghost shrimp has now riddled more than 15,000 acres of valuable oyster ground with U-shaped burrows. Oysters are unable to survive on this ground. In addition to making the ground soft and porous, the ghost shrimp also causes a continuous shifting of the top layer of substratum as the burrows are enlarged.

Callianassa have been observed carrying eggs in spring and early summer in Washington state. Upon hatching, the larvae drift freely in the water while undergoing a series of molts. The length of larval life in this area has not been determined, but it is likely settlement and burrowing begins during early summer and continues throughout the summer.

After 1958, which was an unusually warm and favorable year for a number of local invertebrates, large numbers of Callianassa became established in grounds not colonized during the previous 15 years.

The oyster drill Ocinebra japonica was carelessly introduced from Japan and became established at Samish Bay between 1902 and 1920. Drills subsequently became established in other areas through transplantation of infested oyster seed and adult oysters. The Japanese drill now infests 13 different bays of Puget Sound, comprising an estimated area of more than 8,000 acres. However, this still represents less than 15 per cent of the presently cultivated ground.

Studies of drill abundance at Liberty Bay near Poulsbo, which is considered to be one of the worst drill-infested areas in the state, indicate a population in excess of 50,000 drills per acre. Predation has been of such magnitude that, although the area is one of the best growing areas in the state, seed oysters are no longer planted on the Liberty Bay grounds. Culture is restricted to growing and fattening half-grown and adult oysters.

The development of safe, economical drill control methods would permit eventual rehabilitation of Liberty Bay, as well as other infested areas.

CONTROL EXPERIMENTS

We have thus far carried out two types of tests on intertidal areas in an effort to develop practical procedures for control of ghost shrimp and Japanese oyster drills. Ghost shrimp control experiments are designed to eradicate them from the treated area. Drill control experiments are designed to test practicability of eradication, and of barriers to exclude them from uninfested tracts.

We have essentially followed the recommendations of Dr. V. L. Loosanoff (1960) regarding chemical combinations and materials, but have modified techniques to attempt to obtain effective results in the intertidal enviroment.

All tests reported for ghost shrimp and drills have a number of features in common. Sand or gravel or a mixture of these is used as a carrier to place and hold control chemicals near the desired point of application. The chemicals are a mixture of liquids and dissolved solids. They are combined with thoroughly dried sand or gravel in a ratio by weight of 1 part chemical to 19 parts aggregate. Control chemicals include a bonding agent such as orthodichlorobenzene, polystream, or furnace oil, in which is dissolved a saturated solution of the insecticides Sevin or Lindane. Cost of dry sand or gravel is about $20 per ton. Cost of chemicals to treat one ton of aggregate at the 1:19 ratio is approximately $34.

Ghost Shrimp Tests

Several small-scale field tests were run on ghost shrimp ground during 1960 in an infested portion of Quilcene Bay, a short distance from the laboratory. These have involved variations in concentration, methods of application, and different chemicals. Initial tests were made in 8 x 8-foot plots using 15 pounds of fine sand, orthodichlorobenzene and Sevin in one plot; fine sand, furnace oil, and Lindane in the other. This application was at the ratio of 0.23 pounds of mixture per square foot.

Initial results were striking, with the effective area of kill extending, in the case of the Lindane and fuel oil, to more than ten times the actual area originally treated, while orthodichlorobenzene and Sevin affected an area about eight times the treated area. Within two weeks the ground had changed from soft, porous, shifting bottom to firm ground covered with a film of brown diatoms. Ghost shrimp were entirely eliminated from the affected area, and the ground had the appearance of a satisfactory habitat for growing seed oysters. Core samples taken inside and outside the plots immediately after and at intervals during the summer of 1960 showed no ghost shrimp present inside the two 8 x 8-foot plots or the affected areas. Sampling outside the affected areas showed no evidence of reduction in ghost shrimp population, and averaged 13 ghost shrimp per square foot.

Effect of the treatment is illustrated in Fig. 1, which shows typical sandy ghost shrimp ground at the bottom of the photograph; the dark area affected by Lindane is in the middle and right, with orthodichlorobenzene and Sevin plot in the background. Fig. 2 illustrates soft bottom of heavily infested ghost shrimp ground. Burrow holes and castings are visible in the foreground.

Fig. 1. Two pesticide treatment plots on ghost shrimp ground. Quilcene Bay, 1960.

Fig. 2. Heavily infested ghost shrimp ground in Quilcene Bay.

The condition of these plots was again observed during the spring and early summer of 1961. Due to winter storm activity, considerable shifting of ground occurred in that part of the bay, and a six-inch layer of beach sand was deposited over the top of the original furnace oil and Lindane plot and affected area. Treated sand within this 8 x 8-foot plot was trapped in a subsurface layer. Ghost shrimp became redistributed so that most of the original affected area was reinfested. However, the 8 x 8-foot treated plot inside the stakes did not become reinfested.

The orthodichlorobenzene and Sevin plot was totally destroyed as a result of the shifted ground, and became reinfested in the same degree as the surrounding ground.

Additional tests in 1960 were also made using furnace oil and Lindane mixed with dry sand on one 30 x 75-foot plot, and furnace oil alone mixed with dry sand on another plot of the same size. Eighty pounds of treated sand was spread on each plot as the incoming tide was covering the ground. At the time, this application (0.03 lb/ft^2) was considered light.

Initially, eradication of ghost shrimp in the furnace oil and Lindane plot appeared effective. A heavy kill of ghost shrimp was achieved within one-half hour after application of the chemical. Dying ghost shrimp were also observed outside the plot beyond the deposited sand. Within a week the bottom became firm and maintained the appearance of good oyster ground throughout the summer.

The ghost shrimp population in the plot treated with furnace oil and dry sand alone appeared unaffected, and active burrowing continued throughout the summer.

Wave action of the winter storms removed or dispersed the furnace oil and Lindane-treated sand, and by late spring of 1961 the ground inside the plot was again subject to extensive burrowing.

During 1960 examinations were made on effect of treatment on associated forms. Results of this in brief were that during initial treatment, shore crabs (Hemigrapsus), shrimp (Crago), and gobies within the treated area were killed. Small bentnose and eastern softshell clams (Macoma nasuta and Mya arenaria) were killed. Japanese littleneck clams (Venerupis) and sand worms (Nereis) were irritated but not killed.

Drill Control Tests

Liberty Bay was chosen as a good area for oyster drill control tests, since harvesting operations had removed the market oysters from the ground, and replanting was not scheduled until the summer of 1961.

In July 1960 a barrier plot was established, using 190 pounds of pit run gravel and sand mixture (3/4 inch minus) treated with ortho-dichlorobenzene and Sevin. This formed a barrier approximately 8 inches wide by 3 inches deep surrounding a plot 16 x 16 feet. The ground was sandy substrate with a 2-inch covering of mud. The ground inside the plot had previously been cleaned of Japanese drills, oysters, and dead shell. One bushel, consisting of several clusters of uninfested adult Pacific oysters and 25 mother shells containing 1960 Japanese oyster seed, was planted within the plot. One bushel of clustered uninfested adult Pacific oysters was also planted outside the plot.

This barrier persisted and was effective throughout the summer and autumn of 1960. During this period drills moved up to the barrier, became inflated and inactive. A few were washed over the barrier by waves, but these caused no observable mortality. The seed was protected from attacks by drills throughout the summer and autumn of 1960. Oysters placed outside the plot soon became infested with adult drills and their egg cases.

Winter storms, however, washed most of the seed oysters and some clusters of adult oysters outside the plot. Japanese drills were washed over the barrier into the plot. In addition, silt was deposited from 1/2 to 1 inch deep over the top of the barrier. Thus, by spring 1961 the barrier, for practical purposes, was no longer effective. However, even protection during the first months after seed planting was sufficient to yield normal clusters of year-old juveniles.

To attempt area control of drills, a 125 x 75-foot plot was set up in November 1960, not far from the first barrier plot, on ground from which the oysters had been harvested by dredging. Only occasional single oysters, small clusters, and dead shell remained throughout a five-acre area. Drills were still present in moderate quantities on the muddy bottom, and extremely abundant on an adjacent gravelled ridge forming the outer boundary of this bed and an end boundary of the plot.

Five hundred pounds of fine sand, orthodichlorobenzene and Sevin was spread evenly over the plot at low tide. Two days later, 183 marked drills were introduced next to a stake in the center of the

plot. Several attempts were made to examine this low ground during the winter night tides, but stormy weather prevented the plot from becoming exposed.

Determination of the effect of chemical control on marked or unmarked drills could not be made until spring. A search on 1 May 1961 yielded 47 live marked drills of an original plant of 183. The majority of these had migrated about 45 feet to the gravel ridge. No dead marked drills were found. Only a few drills, either marked or unmarked, could be found in muddy ground adjacent to the pole or under oyster shells scattered over the plot. Only one of the drills found appeared to be inflated and inactive. The treated sand was buried under a thin layer of silt.

Utilizing experience gained in initial ghost shrimp and drill control experiments, a second drill barrier experiment was set up in July 1961 at the same site as the November 1960 area control experiment. Due to the muddy bottom, the barrier was laid on top of 20-inch-wide strips of airplane tow-target netting and polyethylene sheet. Aggregate was one ton of "pit run 1 inch minus" gravel and coarse sand, placed on the strips enclosing an area 35 by 35 feet. Two of the adjacent sides contained orthodichlorobenzene and Sevin, and the other two adjacent sides were treated with polystream and Sevin. The primary purpose in this experiment was to set up a barrier judged to be heavy enough, high enough, and wide enough to remain intact and silt-free through summer and winter. The area inside the barrier was adequate to contain, at normal planting density, five cases of Japanese oyster seed. Prior to placement of the barrier, all visible oyster shells, live oysters, and Japanese drills were removed from the ground inside the barrier.

To test the effectiveness of this type of barrier for protecting seed oysters, half of a case containing 450 shells of 1961 Japanese seed was planted inside the barrier, and the other half containing a like amount of shells was planted outside the barrier, adjacent to the gravel ridge. At planting this seed averaged 12.6 spat per shell and was considered to be of average quality.

Fig. 3 shows size composition of treated pit run gravel, target netting foundation, and mud bottom.

Two weeks after placement of the barrier, the gravel stabilized into a summer condition forming a strip from 16 to 18 inches wide and 3 to 4 inches deep in the center. The top of the barrier was clean and free of silt, but inside the barrier 1/4 inch of silt had accumulated on

Fig. 3. Close view of polystream and Sevin treated ground on mud bottom at Liberty Bay, June 1961.

surfaces of the mother shell, and silt was encroaching along the base of the gravel. Five inflated and inactive drills were found inside the plot adjacent to the barrier. Drills were abundant and active on the gravel ridge outside the plot but none had so far begun to attack the control seed or move toward the barrier. Seed in both groups appeared to be growing normally.

DISCUSSION

Tests thus far conducted in Puget Sound have been deliberately kept small in order to minimize any public health hazard. They show that control of ghost shrimp and Japanese oyster drills can be feasible.

Observations during application for ghost shrimp control indicate that initial kill probably results from a portion of the chemical washing off the treated sand and diffusing through the water. Since furnace oil does not seem to kill ghost shrimp, the initial effective agent is probably insecticide. Residual effect may also occur from toxicity of orthodichlorobenzene or unhydrolyzed insecticide.

Results indicate concentrations of 0.046 lb/ft^2 (1 ton per acre or 2.24 metric tons per hectare) of mix are adequate to eliminate ghost shrimp from an area. Cost of materials is estimated at $54 per acre, not including cost of application. If the presence of these two pests precludes full utilization of valuable Pacific oyster ground, the cost of aggregate and chemicals, plus labor to apply them, is probably low enough to be economically feasible. However, for long-term use costs should be reduced to about $20 per acre annually, preferably through treatment to provide residual effect for the duration of a crop (2-3 years).

Area control of Japanese drills was not effective where an equivalent of slightly less than 1 ton per acre of sand, orthodichlorobenzene and Sevin was used. Two, three, or five tons per acre might be effective but would cost from $100 to $250 per acre for materials alone. At present it would seem that physical control such as dredging to remove drills, clusters of oysters, and loose shell, combined with subsequent establishment of a heavy barrier to protect seed growing areas, would be most practical. In this case also, long-term residual effect could reduce the annual cost of such measures and make chemical control economically feasible.

The principal problems in Washington State now are: first, since the material is known to retain toxicity for a considerable period of time, to determine whether or not dangerous residuals will be

accumulated by the oysters being grown for human consumption; and second, to determine whether the widespread application that might be expected in normal commercial practices would be detrimental to the habitat. As soon as chemical means have been perfected for detecting residuals of chlorinated benzenes and insecticides in bivalve meats, further trials must be conducted to improve practical field application methods.

CONCLUSIONS

Area control of ghost shrimp using sand and a binding compound as a carrier of appropriate chemical pesticides appears economical and practical. Application techniques need to be perfected to achieve residual effect without permanently eliminating associated harmless forms.

Control of Japanese oyster drills in the intertidal environment appears most promising using gravel treated with orthodichlorobenzene or polystream and Sevin, to form permanent barrier strips surrounding areas used exclusively for rearing oyster seed to transplant size.

Large scale experimental or commercial applications in Washington State must be deferred until clearance is obtained from Public Health authorities.

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ANNUAL CONVENTION

The 1961 Convention was held jointly with the Oyster Institute of North America and the Oyster Growers and Dealers Association of North America, Inc., on July 30—August 2, at the Emerson Hotel, Baltimore, Maryland.

Reports on studies on causes of oyster mortality again made up an important part of the meeting. There were a number of reports on the biology of mollusks other than oysters.

Cedric Lindsay reported that the Pacific Coast section of NSA would meet with the Pacific Coast Oyster Growers Association on August 25 and 26.

Plans were made for reprinting the Proceedings of past years (1930-1960) on Microcards. The Microcards will be sold by the Secretary-Treasurer at $8.00 per set.

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