EXPERIMENTS ON THE LARVAL CULTURE OF THE NATIVE OYSTER, *Ostrea lurida* CARPENTER

by

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INTRODUCTION

The text of this thesis is an account of experiments concerned with artificially rearing the larvae of the Native Oyster, *Ostrea lurida* Carpenter. The work was carried out during the summer months of 1947 at the Yaquina Bay Fisheries Laboratory. The experiments herein described are only those concerned with some of the physical and mechanical aspects of the problem and were conducted for the purpose of laying a groundsel of feasible methods of operation which would prove expedient to future efforts in this field of study. The work was conducted in the laboratory exercising control over some of the physical conditions ecologically related to the species under consideration.

Artificially propagating the larvae of the Native Oyster will undoubtedly prove to be an indispensable factor in the permanent restoration of the beds of this species in Yaquina Bay, Oregon, Dimick (1946). This attitude has arisen by reason of the fact that failures in the successful natural spatfalls of the Native Oyster in recent years far outnumber successes

in practically every oyster producing area of the Pacific Coast. It does not seem unreasonable to assume that this condition may have always existed and is not difficult to surmise if one recalls the classic principle of Darwin, i. e., biotic forms, with even the lowest breeding potential, produce more offspring than can survive, if the population is to remain numerically stationary, Dobzhansky (1941). In the Native Oyster there are about 250,000 to 300,000 larvae produced per spawning by the average adult in female stage, Hopkins (1937), and since this species continues to bear young for several years, it is difficult to eschew the speculation that the natural limit to their populations may well be exercised in specific years in the form of complete loss of the young of that season.

HISTORICAL

The reader will not be burdened with a prolixity of historical material concerned with propagation and conservation of oysters, however, it is believed that pertinent citations should be given in order that the reader may be cognizant of the present stage of development of this phase of the oyster industry.

In the United States, the first attempts at artificial oyster propagation came in 1879 by Brooks who was able to artificially fertilize and develop the eggs of the Eastern Oyster, O. virginica Gmelin, but the many efforts to rear the larvae, by this and subsequent investigators, have resulted in failure, Galtsoff et al (1930).

The larvae of O. edulis Linnaeus, were successfully reared in large, outdoor, concrete tanks having a capacity of 20,000 gallons. In his attempt to determine reasons for success or failure, the investigator gave primary attention to the food organisms present and could not draw a definite conclusion due to the lack of control in such voluminous containers, Cole (1936).

From a survey of the literature, it would appear that the greatest success enjoyed by investigators was that of English scientists working with the larvae of the European Oyster, O. edulis Linnaeus. Here again, we find the focus of attention upon the food organisms and in this work, pure cultures of unicellular algal forms were fed to the larvae. That organism which was administered with greatest success was a naked flagellate from the Class Chrysophyceae, measuring about 3 to 5 microns in diameter. It appeared that mixtures

of more than one species had the highest nutritional value, Bruce et al (1940). This work was carried out in the laboratory and was based on the very prevalent hypothesis that the limiting factor in artificial propagation is one of nutrition. These workers were unable to duplicate their success in more recent attempts.

It is understood that investigators in Japan have recently perfected methods of rearing the larvae of the Japanese Oyster, O. gigas Thunberg, and have enjoyed hearty success, Wood (1948). This publication has been issued only recently however, and the explanation of methods used have not yet become available to local personnel.

Other measures of conserving and restoring the resources of oyster beds have been resorted to but with limited or no success. As early as October, 1766, The Assembly of East Greenwich, Rhode Island, passed an "Act for the Preservation of Oysters," Galtsoff et al (1930), and since that time many other legislative measures have been taken in an effort to effect their conservation. In recognition of the fact that legislation alone was not a sufficient supplement to nature in perpetuating oyster beds, attempts were made to transplant adult oysters as early as 1810 in the State

of New Jersey, and in 1855, shells for catching oyster spat were planted in Long Island Sound, Galtsoff et al (1930). Since that time, many and varied are the methods initiated for collection of the natural spatfall. Only two of the multitude of references to verify the last statement are: Bonnot (1937); Elsey (1933). Methods of adequately anticipating the time and intensity of the spatfalls have also been given diligent attention. It must here be realized, however, that these methods satisfy the demand for oyster seed only when the ecological conditions of the water are such that the larvae can successfully complete their development and that if the natural conditions are unfavorable, these measures are valueless.

IMPORTANCE

That the project of producing oyster seed is one of merit and warrants considerable attention, can best be demonstrated by the fact that even with sustained markets, the oyster industry, in most parts of the world, has steadily declined. Typical of this decline of Eastern and Native Oysters is that shown from 1937 to 1940 when production fell from 95,600,000 pounds to 89,400,000 pounds, the decline being attributed to the lack of sufficient seed stock, Galtsoff (1943). Other

literature indicates that no remedies have been instituted which can cause a cessation of this production failure. All three of the states of the Pacific Coast of the United States have potential waters for the production of the Native Oyster if seed stock were available, Galtsoff (1929). In all three of these states, the production of the Native Oyster has declined steadily since the turn of the century, Galtsoff (1943). In the territorial waters of the entire United States, there are 1,428,500 acres of potential oyster producing area, and production can be greatly increased over much of this area if and when adequate stocks of seed become available.

OBJECTIVE

It is hoped that the progress of the investigations at the Yaquina Bay Fisheries Laboratory will ultimately result in the accumulation of facts and techniques which will enable those interested to produce in quantity seed stock of the Native Oyster.

PROCEDURE AND METHODS

Identification

Experiments concerned with the rearing of the Native Oyster larvae during the course of this work

were given separate identities by affixing Roman numerical designations and the various mechanical apparatus for their conduct were identified by designations of Arabic numerals.

Rearing Containers

Initially, it is deemed advisable to render a discussion of the mechanical arrangements which were employed in the culture experiments and to identify each with its numerical designation in order that the reader may properly orient himself as to the methods used in each experiment.

In all of the efforts at rearing the larvae, the containers used were rectangular aquaria constructed of plate glass and having a frame of one-half inch angle iron which was welded at the corners. They were of six gallon capacity and the dimensions of each were 18 inches long by 10 inches wide by 9 3/4 inches high and the top was partly covered with a pane of glass. The glass of the sides and bottom was sealed to the frame with non-hardening aquarium cement which was recommended by the manufacturer as being suitable for marine use. In all experiments, the aquaria were placed on a laboratory bench three feet high which was lighted by north windows and which received some

direct sunlight from west windows in the late afternoon. The tanks were screened from direct sunlight during later experiments as described in that portion of the text devoted to their conduct. The water in the aquaria was kept in circulation and was aerated by Thiberg aerators with stone air breakers at the termini of the air tubes. The pane of glass in one end of each aquarium was marked with wax pencil in such a manner that graduations of two liters were shown.

Effecting Water Change

Bruce et al (1940) working with O. edulis Linnaeus in England described the use of bolting cloth screens on outlets for negotiating a change of water in the culture tanks, and comment was made concerning the frequent cleaning and maintenance that was required in order to maintain functional conditions. Prytherch, working with O. virginica Gmelin, used sand for the retention of oyster larvae in changing water in rearing vessels. He used wooden containers, the floor of which was cut to form an opening, and over this aperture was placed asbestos for holding the sand in place. Galtsoff et al (1937) describe a sand and charcoal filter for cleansing seawater and

removing undesirable organic matter therefrom. This filter involves the use of siphons, the intakes of which are constructed on the principle of a funnel and are placed below the surface of the filtering agent. The use of the siphon eliminates the necessity of using culture vessels which have a bottom through which an orifice could be cut to permit the removal of the water in such a fashion that it could pass through the sand as it leaves the container. The citations given above imparted a thought, which, with some modifications, evolved into what is believed to be a very satisfactory method for retaining the oyster larvae in culture vessels while changing the water in the containers.

The sand used in the bottom of the culture aquaria was taken from the beach at Fogarty, Oregon, during June of 1947. It was of a type used locally in concrete construction and was collected above mean high tide where it had been deposited by extreme winter tides. This sand was dried and the larger pebbles and debris were removed by sifting through a wire screen of 17 meshes per inch. The sand was then washed thoroughly with fresh water and fine particles of floating organic matter were skimmed off with cheesecloth. The sand was then placed in each

aquarium to a depth of about 1½ to 2 inches. Funnels for the intake of the siphons were made from bottles, having a diameter of three or five inches. Removal of the bottom portion of the bottle was accomplished by the following process: A piece of ordinary cotton string was soaked in alcohol and wrapped around the bottle three or four times at the level at which the division was desired; the string was then ignited and allowed to burn for several seconds; while still burning briskly, it was thrust into a jet of cold water. A clean break will usually occur just above the level around which the string is placed. By this method, funnels of the desired type and length may be had at a minimum of cost and effort.

Placing the open end of the funneled siphon intake on the bottom of the aquarium and filling around it with the sand as described in the preceding paragraph, gives approximately 170 square inches of sand surface through which the water may pass to be drawn off by the siphon. This allows for rapid rate of removal of the water without creating undue pressure on a localized drain site which would be the result if one were to use merely a screened outlet or a similar device.

Apparatus Arrangements

Apparatus No. 1, Figure 1, was set up using aquarium, and sand as previously described. The siphon funnel was made from a bottle having a diameter of five inches. Including the neck of the bottle, a section five inches in length was used. The siphon funnel was placed near the end of the aquarium and the neck was stoppered with a cork which was bored to accommodate a glass tube having an inside diameter of one-quarter of an inch. This section of glass tubing extended upward about six inches and then was bent in such a fashion that it would extend downward outside the aquarium (i. e., two angles of 90 degrees, both in the same plane). To the end of this glass tube was attached a rubber tube which extended downward about two feet. The siphon was started by orally diminishing the pressure at the end of the tube and then a small siphon clamp was fixed to the rubber portion of the line. This apparatus did not prove satisfactory for removing the water from the tank since activity of the water rushing up into the funnel was such that the sand was carried upward with the water, the sand being of such light weight that it could not maintain its position against such a rapid draught of water.

By closing the clamp on the rubber portion of the siphon tube, the flow was diminished to a point where the sand suffered no such disturbance. This was not satisfactory, however, since the water could not be removed with sufficient rapidity to effect a water change without considerable expenditure of time. For replacing the water, a beaker of 1000 ml. capacity was placed in the end of the aquarium opposite the siphon and the water was poured into this. Use of the beaker prevented disturbing the sand which would result if the water were poured directly into the tank.

Apparatus No. 2 was essentially the same as No. 1 except that the neck of the funnel just below the stopper was filled with cotton. The cotton served to hold the sand in place, but reduced the flow of water to such an extent that this method was considered a failure.

Apparatus No. 3, Figure 2, followed the same principle as that of No. 1, except that a bottle having a three inch diameter was used for the funnel. From this bottle, only the bottom was removed and this resulted in a funnel about 10 inches in height. By leaving the entire walls of the bottle intact, it was possible to have the stopper and glass tube

connection above the surface of the water and any leakage around the cork would not result in a loss of larvae. Prior to placing the funnel in the tank, a layer of poplin cloth was placed over the mouth and was bound in place with cotton string. The funnel with its cloth cover was then seasoned in sea water for about 48 hours prior to use in a larvae tank. By placing this cloth over the mouth of the funnel, the sand was not allowed to enter the funnel and was held in place quite advantageously.

Subsequent arrangements of equipment injected various features into the work and that apparatus which appeared the most satisfactory as far as convenience and ease of maintenance, with a large number of aquaria running concurrently, was modified as follows: Use of funnels as described for apparatus No. 3 was continued; however, instead of the rubber tube with affixed screw clamp for controlling the siphon, only about one foot of rubber tubing was used and to this was attached a straight glass tube about two feet in length. To the edge of the aquarium was fixed a small nickle-plated hook upon which the two foot section of glass tubing was depended with its terminus above the water level of the aquarium. When it was desired to drain the aquarium, the glass

tube was removed from the hook and lowered to the laboratory drain, thus the flow of the siphon (which was initially created in the manner earlier described) started. After the desired quantity of water had been removed, the glass tube was replaced on the hook. This eliminated the use of the stop clamps which with frequent contacts with sea water became corroded and difficult to manipulate. Instead of 1000 ml. beakers for receiving water poured into the aquarium, a beaker of 250 ml. capacity was substituted. This was innovated because a smaller quantity of water was isolated from the rest of the tank when the water level was down during the process of water change, thus reducing the number of larvae that were temporarily stranded in water without circulatory movement, Figure 3.

In the material concerning the experiments, the arrangement referred to as apparatus No. 4 was a set-up devised to give a more or less continuous water change. In this apparatus, each aquarium had two siphons and these terminated in jars which were full of water. The tops of the jars were at the level of the water of the tank and provided a means whereby the water level in the aquarium could be maintained constant even with water running into the aquarium in a steady flow. The jars were placed so that their

overflow would be caught and conducted to the drain from the laboratory. Water was fed into the aquarium from two, five-gallon carboys which were placed about two feet above the aquarium. Siphons conducted the water into the aquarium, and these were adjusted by screw clamps until the water going into the tank was in equilibrium with that leaving the tank.

Water Supply

Water used in the experiments was collected and treated in essentially the same manner on all occasions. It will be noted that a brief mention is made as to the physical conditions of the water at the time of collection and any remarks considered pertinent are included in the discussion of the experiments. Water was collected from the bay near the site of the laboratory in five gallon glass containers. In the collection of the water, care was exercised to prevent the entrance of surface material into the collecting jars since the presence of oil or other such contamination would have a detrimental effect on the reliability of the results of the experiments. After collection, temperature and salinity readings were taken by means of a centigrade thermometer and a hydrometer respectively. If the temperature of the water

collected was radically different than that contained in the experimental tank under consideration, the water collected was allowed to stand in the laboratory for a period of time until the temperature approximated that of the water containing the larvae, after which time the water change was effected. The water was then strained through No. 20 bolting silk to remove debris and larger plant and animal forms. It is understood that this last mentioned procedure probably deprived the water of many organisms which were potential food for the larvae but election to follow the method was made on the basis of the multitude of competitive forms which were removed by so doing.

In all of the experiments, the water was maintained at a level of 20 liters. In some experiments a change of 10 liters was made each day and in others a change of 12 liters. Notation as to the amount replaced is made in the discussion of each experiment.

In replacing the water in the aquaria, undue splashing of the water was avoided and those larvae caught on the sides of the tank, by the receding film of the water removed, were washed back into the water by means of a pipette.

Experimental Larvae

Hopkins (1937) gives the following biological data concerning reproduction and early development of the Native Oyster. This is a larviparous species, i. e., the eggs are not shed into the water as in the case of the Japanese or Eastern oysters, but rather are held in the mantle cavity of the parent until fertilization has been achieved and a certain portion of the larval period passed. Fertilization of the eggs of the Native Oyster is achieved by the female pumping water over the ripened eggs, the water bearing spermatozoa previously released by the males. The gametes are thus brought together and fertilization results. The eggs of this species are approximately 105 microns in diameter at the time of their release from the gonads to the mantle cavity, and after fertilization they are retained for a period of about 10 days. During this period, embryonic development has proceeded to the stage termed "straight-hinge veliger" larvae. At the end of this period, the larvae are about 180 microns in length. Since the adult female will bear upwards of about 250,000 young, one is readily able to determine whether or not a particular individual is bearing larvae by merely opening the valves and examining with the

naked eye. "White-sick" and "gray-sick" are terms applied to oysters that are bearing young, the difference in the two terms implying the relative stage of development of the larvae. Since the larvae are white in color in early stages and grow progressively darker as development progresses, one can, by microscopic examination of a few representatives, soon learn the approximate age of the larvae by the depth of their coloration. It must be understood, of course, that the larvae be observed as groups of many individuals in order to make such differentiation. This species is hermaphroditic and protandric but in the above discussion of its reproduction, for the sake of brevity, references to sexual stages are given as merely male or female.

Further discussion of the biology of the adult of this species will not be presented since it is not the purpose of this work to review that subject. The above data was presented in order that the reader might gain a knowledge of the early phases of development in this species and thus understand the manner of collection of larval subjects for the conduct of the rearing experiments.

The oysters from which the larvae were obtained were removed from oyster beds in the vicinity of

Winant, Oregon, on Yaquina Bay. They were collected whenever the need for experimental larvae arose and were transported to the laboratory in a burlap bag. After their arrival at the laboratory, they were thoroughly scrubbed with a coarse fiber bristled brush and washed off in sea water. The scrubbing removed most of the large animal and plant forms and any silt that had accumulated on the surface of the valves. After the oysters were thus washed, the larger specimens were selected and opened. In the experiments, young were removed from adults which ranged from three to five years of age. Only the larvae of deepest tone of coloration were selected for experimental purposes, and of these a representative sample was isolated and the mean size of its members was determined by microscopical measurement.

From the results of sampling, it was verified that oysters of the size used could reasonably be expected to contain approximately 250,000 larvae, and it is believed that this is sufficiently accurate for our purposes. The number of larvae used in each experiment was arrived at by this means, and therefore in all cases it can be considered only as an approximation. When an oyster was opened and found to contain larvae of deep coloration, the larvae were

washed into a finger bowl containing about 250 ml. of strained sea water. By using a medicine dropper, with water from the finger bowl, the larvae could be flushed from around the mantle and gills of the oyster and allowed to drain into the bowl. Experiments which were started on the same date were conducted with larvae from one or more oysters, the larvae being mixed thoroughly prior to their introduction to the experimental tanks. By taking the larvae from two or more parent oysters and mixing them, it is reasonable to assume that the potentialities of the larvae in the various tanks to which they are subsequently introduced are approximately the same. It is recognized that heredity may have a profound effect on the viability of the larvae, and this is suggested by Bruce et al (1940). Although controlling the male parentage under the methods used in this research is out of the realm of possibility, the matroclinous characters of larvae used in different concurrent experiments can reasonably be expected to approximate each other. Larvae thus highly concentrated in numbers in the finger bowls were measured into each experimental aquarium by means of a pipette, thereby getting approximately the same number of specimens into each tank.

Consideration of Variables

The following accounts the variables which were given attention during the summer's work. For purposes of clarity, the discussion of each experiment is given individual attention. To facilitate the work, however, the actual collection of data was done in the manner of a diurnal log. By this method, a chronological sequence could be maintained and recording of some of the data could be made without duplication for each of the experiments currently under consideration, i. e., conditions of water collected at one time and used in more than one experiment.

In recording the progress of each experiment, the following conditions were given attention: Weather (such as clear, cloudy, or partly cloudy); wind direction and velocity (such as calm, mild, or strong) at the time the water was collected; general turbidity of water collected; amount of bottom detritus in the water collected; temperature; salinity; stage of tide at the time of water collection; abundance of larvae in the aquaria; mean temperatures of the water in the aquaria; and remarks as to condition of larvae and any other information which, at the time, appeared significant. By a color comparator method the pH of the water in the water was taken on occasions.

In the collection of the original data the variables mentioned in the preceding paragraph were made of record. However, after careful consideration of the results, it was found that not even a tentative conclusion could be arrived at in the case of some of these factors. In light of these findings, it is deemed unnecessary to burden the reader with excess verbiage concerned with material which is apparently of little consequence. In the category of factors affecting the results of the experiments to no determinable degree were: Weather and wind at the time of water collections; general turbidity of water collected and amount of detritus coming from the substrate in the water collected. The last mentioned condition correlated with the stage of tide to such a degree that it can be stated that the water collected at the low stages of water had a correspondingly higher quantity of bottom detritus and that collected at the high stages of tide contained relatively very little detritus. This was caused by the type of substrate found on the shoreline adjacent to the laboratory. The pH of the water in the aquaria remained very constant and was therefore not considered to be a vital factor.

Temperatures in the aquaria during the course of the experiments ranged from a low of 16 degrees C to 21 degrees C, the water having reached 22 degrees C in a very few extreme occasions. It was found that minimum temperatures could be taken between the hours of 6:00 A.M. and 9:00 A.M., and the maximum temperatures could be expected between the hours of 5:00 P.M. and 8:00 P.M. During the investigation, the mean temperature of the tanks could be reasonably stated to be about 19 degrees C. It is believed that the temperature of the various experimental tanks was sufficiently consistent to eliminate this factor from entering into the conclusions drawn.

EXPERIMENTS CONDUCTED

Experiment I, Trial of Apparatus

This experiment was started June 17, 1947. Approximately 250,000 larvae were introduced to this tank. It was concluded at the end of 20 days, the last living larvae having been observed July 7, 1947. Apparatus No. 1 was used for the first two days of the operation, dut due to the loss of larvae through the siphon, No. 2 was devised and used for the balance of the experiment. In this experiment, the aquarium

was placed on the laboratory work bench before north illumination but during the afternoon. Direct sunlight fell on the side of the tank for a period of about two hours. Twelve liters of water were drawn from this tank and replaced with bay water each day. The water introduced to the tank was taken from the bay at low tide on 11 collections, mid-rising tide on four occasions, mid-ebbing tide on one occasion, and at high tide on four occasions. The temperature of the water collections ranged from 14 degrees C to 18 degrees C, and had a mean temperature of 17.2 degrees C. The salinity was not taken during the first seven days of this experiment but during the last 13 days the salinity ranged from 24.2 p. p. mille. to 31.2 p. p. mille. and the mean salinity of these water collections was 27.5 p. p. mille. On the 14th day of the experiment, it was noted that the larvae were considerably diminished in numbers, and from this date to the conclusion, the remainder of the larvae died out steadily. By the 15th day of the investigation, growth of filamentous algae on the surface of the sand and on the walls of the aquarium had become quite heavy. During the remainder of the operation of this experimental tank, the growth of filamentous algae became progressively heavier.

Samples of this algae were removed and it was found that large numbers of larvae had apparently become entangled in the filaments. Larvae were measured from time to time during this experiment but no growth was ever noted. At the conclusion of this experiment it was decided that a reduction of the amount of light on the tank would be necessary to suppress the rank growth of filamentous algal forms.

Experiment II, Trial of Apparatus

This experiment was started June 21, 1947. Approximately 250,000 larvae were introduced to this tank. This experiment was concluded at the end of 16 days, the last living larvae having been observed on July 7, 1947. Apparatus No. 3 was used during the entire experiment. The aquarium for this experiment was placed in circumstances similar to that in Experiment I except that greater care was taken to exclude the direct rays of the afternoon sun by means of a cloth shade over the west side of the tank. Twelve liters of water were changed in this tank each day during the period this experiment was in operation. The water placed into this tank was collected at low tide on eight occasions, mid-rising tide on four occasions, mid-ebbing tide on one occasion, and at high

tide on four occasions. The temperature of the water collections ranged from 13 degrees C to 19 degrees C and the mean was 17.2 degrees C. The salinity of the water was not determined during the first three days of the experiment. In the remaining 14 days of the experiment the salinity ranged from 24.2 p. p. mille. to 31.9 p. p. mille., and the mean salinity was 27.3 p. p. mille. In this experiment, as in Experiment I, on the 14th day a very noticeable diminution in numbers of larvae was recorded. There was a luxuriant growth of filamentous algae on the surface of the sand and the walls of the tank in the later days of this experiment. Its effects were considered to be the same as those recorded in Experiment I. No growth of the larvae could be established during the course of this experiment. From this work, no additional conclusions were drawn except that the modifications of the mechanical apparatus were considered to be an improvement. Data from only two experiments could not be considered comprehensive enough to be conclusive.

Experiment III, Consideration of Tide

This experiment was started June 30, 1947. Approximately 125,000 larvae were introduced to this

tank. This experiment was concluded at the end of 11 days, the last larvae having been observed July 11, 1947. Apparatus No. 3 was used during the entire experiment. The location of this aquarium in the laboratory was similar to those of previous experiments. This tank was screened with muslin cloth to exclude the direct sunlight of the late afternoons. Twelve liters of water were changed in this tank each day during the period covered by the experiment. The water placed into this tank each day was collected at low tide on all occasions. The temperature of the water collections ranged from 17 degrees C to 19 degrees C, and had a mean of 18 degrees C. The salinity of the water collected and introduced to the tank ranged from 23.4 p. p. mille. to 27.8 p. p. mille. and had a mean of 26.2 p. p. mille. The larvae decreased steadily in numbers in this tank and there was no certain time when a sudden change in the population level was noted. Filamentous algae were abundant and heavy by the time the experiment was terminated. This experiment and Experiment IV were run concurrently and under the same conditions as far as could be determined, excepting for tidal stage of water collected. The reader will please refer to

the conclusions of IV concerning both of these experiments.

Experiment IV, Consideration of Tide

This experiment was started June 30, 1947. Approximately 125,000 larvae were introduced to this tank. This experiment was run concurrently with Experiment III, the larvae being of the same parentage in each of the two. Laboratory circumstances of the experiments were made to approximate each other as nearly as possible. Water introduced to this tank was collected at high tide on all occasions, whereas that of III was collected at low tide. This experiment was concluded at the end of 19 days, the last larvae having been observed on July 19, 1947. At the time of collection, the water introduced to the tanks ranged in temperature from 12 degrees C to 19 degrees C, and had a mean of 15.9 degrees C. The salinity of the water collections ranged from 27.7 p. p. mille. to 33.7 p. p. mille., and had a mean of 31.8 p. p. mille. The first apparent reduction in the numbers of larvae in the tank was noted July 13, 1947. From this date to the conclusion of the experiment there was a steady depletion in the population of larvae. As in the case of the other experiments undertaken in the course of

this study, larvae were removed from time to time and examined with an ocular micrometer to determine whether or not shell growth had taken place. There was an admittedly small sample taken on all occasions (only 10 to 20 larvae), therefore one cannot definitely conclude that growth did or did not take place. In this experiment, examinations were made to determine shell size on July 11, 1947 and on July 17, 1949, and considerable growth was demonstrated by most of the larvae in the samples. Filamentous algae grew heavily in this tank as in previous experiments and was in noticeably greater abundance than in Experiment III when examined at the conclusion of the latter. Although the evidence presented by Experiments III and IV is admittedly meagre, due to the short period of time that was to be available for the study, it was elected to collect water for most of the future experiments at the higher stages of tide. It is assumed that the high tide water contained less toxic substances or was richer in food materials than that collected at low tide. The salinity of the water used in both of these experiments is considered to be within the range tolerated by the larvae and therefore it is not considered to be the determining factor

concerned with the different degrees of success of the two experiments.

Experiment V, Consideration of Light and Water Quantity

This experiment was started July 3, 1947. Approximately 170,000 larvae were introduced to this tank. Apparatus No. 4 was devised for the conduct of this experiment. The tank for this work was placed near a shaded east window of the laboratory and received light of much lower intensity than did the tanks in former experiments. This was arranged in order to minimize the growth of filamentous algae that was previously found undesirable. Efforts concerned with this experiment were discontinued on July 14, 1947, the last living larvae having been observed on that date. Forty liters of water were collected each day and placed into the carboys from which the flow was directed into the aquarium. Water used in this experiment was collected at low tide on two occasions, mid-rising tide on three occasions, and at high tide on five occasions. The temperature of the water collected ranged from 13.0 degrees C to 19.5 degrees C and had a mean of 17.0 degrees C. The salinity of this water ranged from 25.2 p. p. mille. to 33.6 p. p. mille. and had a mean of 28.6 p. p. mille. This

experiment was a decided failure from the aspect of positive results, the larvae having expired entirely at the end of 10 days. Fewer larvae were introduced at the outset of this experiment, therefore there should have been a greater amount of food per individual. There was also a greater quantity of water changed than in previous experiments which should have increased the amount of food in the tank. There was practically no growth of filamentous algae in this tank, due, it is presumed, to the reduction in the amount of light striking the water. No growth was observed among the larvae examined from this tank. The failure of this effort contributed to the decision to conduct future experiments with water collected at high tide. This tank was given the greatest protection from light of any of the experiments thus far conducted and though the undesirable algae was held to a minimum growth, the larvae also failed earlier than in other efforts.

Experiments VI, VII, and VIII, Light Considerations

These experiments were started on July 16, 1947. They were run concurrently throughout and their purpose was to establish, if possible, the intensity of light that would be the most conducive to the success

of the larval development. Approximately 80,000 larvae were used in each of these three tanks and these were taken from the same adult oyster. Apparatus No. 3 was used for the conduct of these experiments.

In VI the tank was completely darkened by means of black building paper which was cut to fit the panes of the aquarium and was held in place by means of straight brass pins. This tank was opened only during the time that water change was made and at certain times in the evening when an examination was made to determine the level of the larval population.

In VII the tank was darkened in the manner described for VI except that one end of the aquarium was left without a covering of the black paper.

Tank VIII was darkened in a like manner except that one end and one side were left free of the paper.

Ten liters of water were withdrawn from each tank each day and replaced with freshly collected bay water. The water used in these three tanks was collected at the same time and in the same containers each day. Collections of water were made at high tide on all occasions. The temperature of the water when collected ranged from 13 degrees C to 20 degrees C, and the mean of the temperatures was 16.3 degrees C. The salinity of the water collections ranged from

29.5 p. p. mille. to 33.7 p. p. mille. and had a mean salinity of 31.4 p. p. mille. At the end of seven days, there was considerable difference in the number of larvae in the three tanks. By this time the larvae were practically gone from the water in tank VI and were definitely reduced in numbers in tank VII. Tank VIII contained a substantial number of larvae at the end of the first week that the experiment was under consideration. During the second week the larvae in tank VI disappeared entirely and those in VII became greatly reduced in numbers. The experiment was concluded at the end of 14 days and at this time a few larvae remained in VIII but all had died in tank VII. There was no growth of filamentous algae in any of these tanks, and only in VIII did a very thin layer of small algal forms appear on the surface of the sand. It was concluded from this experiment that proper light conditions were definite factors in contributing to the success or failure of larval development. It was decided that further attempts at reducing the light to control algal forms would need be carried out in such a manner that the reduction in intensity would not be so radical as in these experiments.

From the explanation of the preceding experiments, it can be seen that efforts at maintaining the proper intensity of light on the experimental tanks were unsuccessful. With the greater amounts of light, the undesired algal forms grew luxuriantly, and with the decrease in the amount of light, the failure of larvae was quickly realized. Although positive proof that the filamentous algae offered an impediment to the development of the larvae has not been demonstrated, it is considered highly probable that these forms are mechanically detrimental to the normal development of the larval oysters.

In consideration of the findings derived from these last three experiments, it was decided that further experiments would be conducted in such a manner that the light would be excluded from one side and one end of each aquarium. The two panes chosen for screening were those which faced the greatest sources of light, i. e. the north and west. Subsequent experiments were conducted with the installation of black paper screens over the two mentioned aquarium walls. These were affixed in the manner previously described. In addition to these screens, a layer of black paper was placed over the windows adjacent to the aquaria,

thus excluding all direct light from sources outside the laboratory.

Experiment IX, Food Content of Water

This experiment was started August 4, 1947. Approximately 50,000 larvae collected from two adult oysters were introduced to this tank. Apparatus No. 3 with its final modifications was used in the conduct of this experiment. In this experiment, it was hoped that determination could be made as to the length of time that larvae would survive without change of water. The initial filling of the tank was with water collected at high tide. This water had a salinity of 33.0 p. p. mille. and the temperature at the time of collection was 16 degrees C. The water was strained in the manner described earlier in this writing and was introduced to the tank. Temperature range throughout the course of the experiment was that given earlier in this writing. The last living larvae in this tank were observed August 16, 1947. Some of them having lived, it is seen, for a period of 12 days. Examination of the larvae from time to time indicated that no growth had taken place. The salinity of the water dropped to 31.5 p. p. mille. during the course of the experiment. At the conclusion of the experiment a

strong odor of deterioration was noted in the sand in the bottom of the tank. The pH of the water was 7.5 and no conclusion was arrived at concerning the effect that the deterioration of organic matter might have had on the viability of the larvae.

Experiment X, Consideration of Tide

This experiment was started August 4, 1947. Approximately 50,000 larvae were introduced to this tank. Apparatus No. 3 with the final mentioned modifications was used in the conduct of this experiment. These larvae were from the same adult as those used in Experiment IX. This experiment was run in an attempt to collect more data concerning the influence of the stage of tide upon the longevity of the larvae. Tanks X and XI were conducted under parallel conditions with the exception of stage of tide at the time of water collection. Experiment X was concluded at the end of 12 days, the last living larvae having been observed in this tank August 16, 1947. For this experiment the water was collected at low tide on all occasions. The temperature of the water of these collections ranged from 17 degrees C to 19 degrees C and had a mean of 17.7 degrees C. The salinity of these collections ranged from 26.9 p. p. mille. to

30.6 p. p. mille. and had a mean of 29.2 p. p. mille. Twelve liters of water were changed each day during the course of the experiment. For conclusions concerning this experiment, see those given for the succeeding work.

Experiment XI, Consideration of Tide

This experiment XI was started August 4, 1947. Approximately 50,000 larvae were introduced to this tank. The apparatus and laboratory arrangements used in the conduct of this experiment were the same as those for Experiments IX and X. In this tank twelve liters of water were withdrawn and refreshed daily, the water being collected at high tide on all occasions. The temperature of the water collections ranged from 13 degrees C to 18 degrees C and had a mean of 15.8 degrees C. The salinity of the collections of water ranged from 31.2 p. p. mille. to 33.0 p. p. mille. and had a mean of 32.0 p. p. mille. This tank was maintained for the same period of time as that in Experiment X and the larval population declined at the same rate, as nearly as could be determined, in both tanks. With reference to conclusions drawn from the conduct of these two experiments, it would appear that differences in the qualities of

the water with reference to tidal stages was negligible and that the water collected at low tide contained the same potentialities and/or limitations as that collected at the higher stages of tide. It was concluded from the short duration of the life of the larvae in these tanks that the light falling on them was insufficient for their success. Great care was exercised in maintaining the same amount of light on the tanks in Experiments IX to XIII and it is believed that this factor was the one responsible for the almost coincidental failure of the larvae in these efforts. That a toxic substance was the cause of the failure of the larvae was considered but this was discounted as improbable since the decline of the populations in the tanks followed the same pattern as that which took place in the tank completely darkened in an earlier experiment. Further evidence that toxic substances were not responsible for the failures mentioned is that experiments commenced during the conduct of Experiments IX to XIII passed through the dates on which the latter failed and without appreciable decimation of larvae. The water for all experiments under concurrent consideration was collected and handled in the same manner and at the same times.

Experiment XII, Food Content of Water

This experiment was started August 4, 1947. Approximately 50,000 larvae were used in this experimental tank. The apparatus and arrangements were the same as those mentioned in the preceding paragraph. Twelve liters of water were changed in this tank each day during its consideration. Water used in this experiment was collected at high tide from the closed end of a mud-flat inlet. Water thus collected was that which had been forced over the mud by the rising tidal movements. The temperature of the water collections ranged from 16 degrees C to 26 degrees C and had a mean of 21.1 degrees C. The salinity of these collections ranged from 28.4 p. p. mille. to 32.5 p. p. mille. and had a mean of 29.8 p. p. mille. This experiment was concluded 12 days after the introduction of larvae, the last living individuals having been observed August 16, 1947. It appeared from the conduct of this experiment that this source of water was of no greater value than the source previously used, at least under the prevailing laboratory conditions. The decline of the larval population in this tank followed much the same pattern as that of the other experiments currently under consideration.

Experiment XIII, Food Content of Water

This experiment was started August 4, 1947. Approximately 50,000 larvae were introduced to this tank. The apparatus and equipment were the same as those used in the preceding four experiments. Twelve liters of water were changed in this tank each day that it was under study. The water that was placed into this tank was not strained to remove the larger forms as was done in all previously conducted experiments. The water was introduced to the tank in the condition that it was collected. This experiment was run for 10 days and at the end of that time the larvae were all dead. The water for the conduct of this experiment was collected at high tide on all occasions. The temperature of the water collected ranged from 13 degrees C to 18 degrees C and had a mean of 16.2 degrees C. The salinity of these collections of water ranged from 31.2 p. p. mille. to 33.2 p. p. mille. and had a mean of 32.0 p. p. mille. Early in the conduct of this experiment the appearance of large crustacean forms was noted. These doubtlessly contributed to the competition for food which would be keen even under circumstances prevailing in previous experiments. The disappearance of the

larvae from the water followed the same attritional pattern that has been noted in all experiments. It appeared that nothing was to be gained by using water as it came from the bay without straining it through bolting cloth. It was concluded that no further attempts should be made without straining the water prior to use.

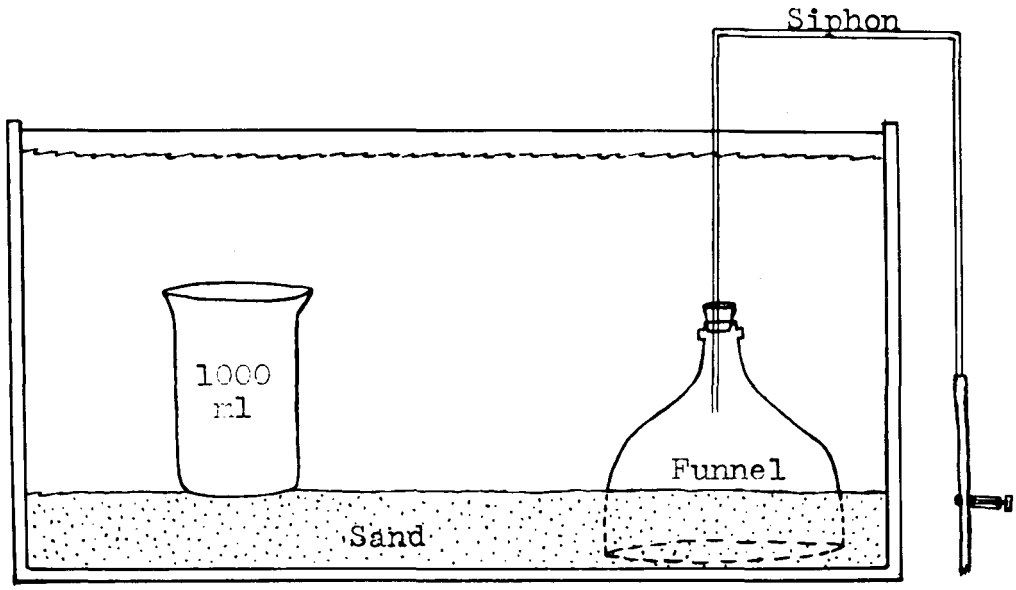

Fig. 1. Apparatus No. 1.

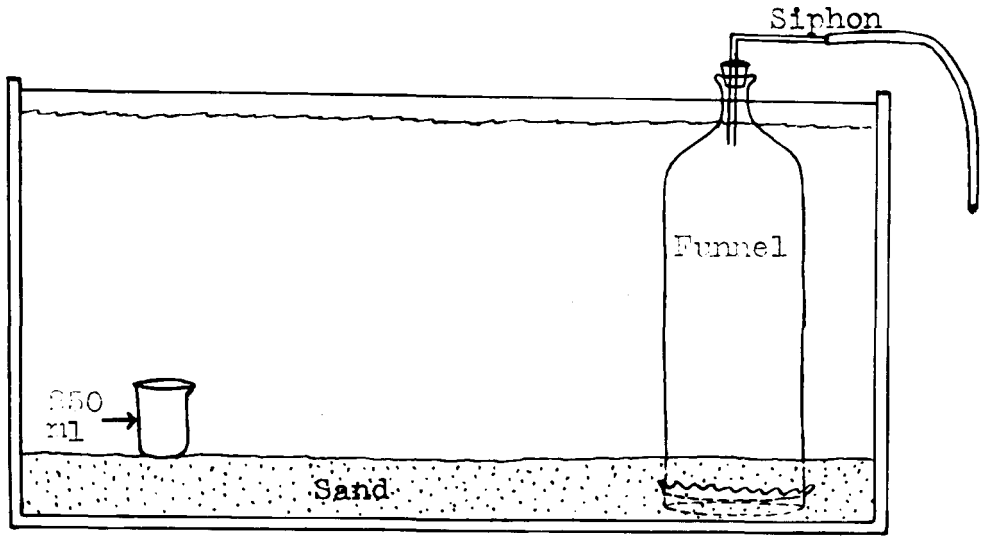

Fig. 2. Apparatus No. 3.

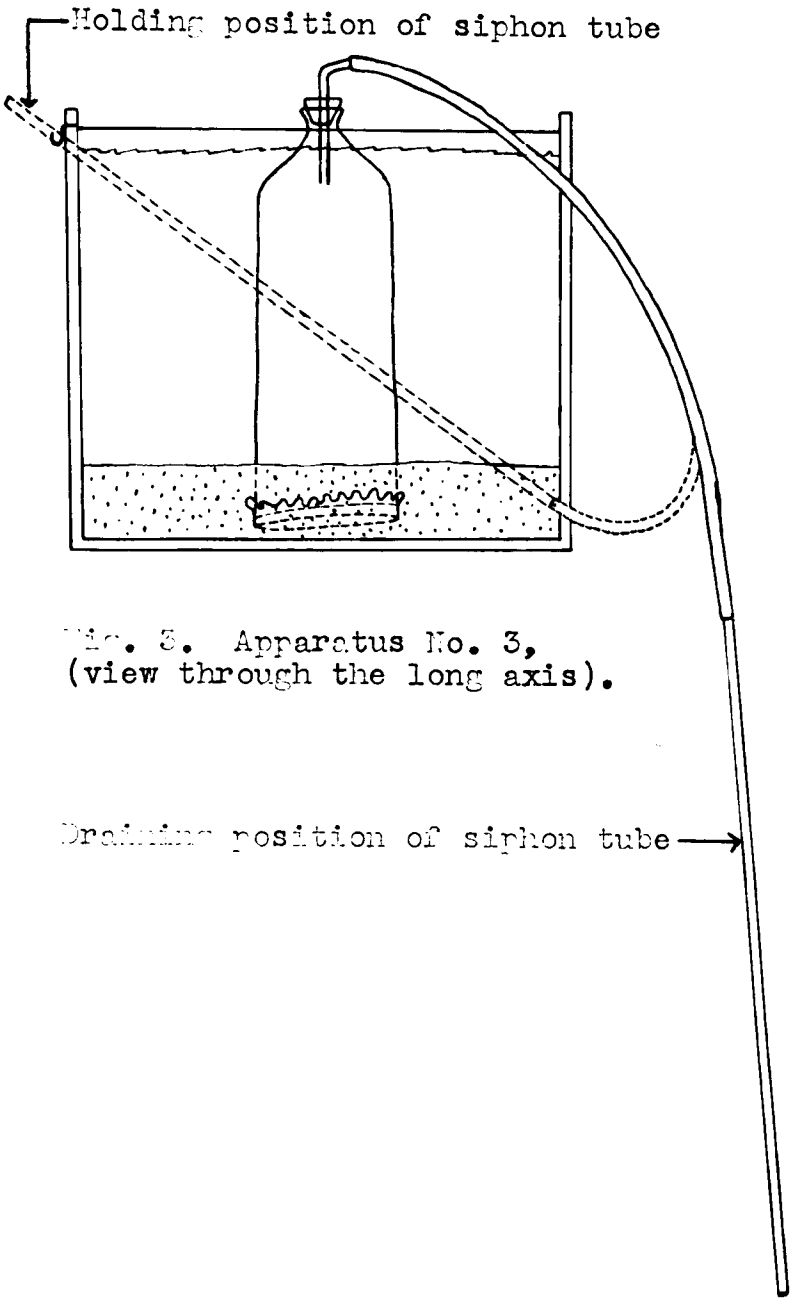

Fig. 3. Apparatus No. 3,
(view through the long axis).

CONCLUSIONS

1. Apparatus No. 3, with innovations to the procedure as are seen fit, will be a satisfactory mechanical arrangement for future rearing experiments.

2. Future experiments should give primary consideration to the illumination of the culture vessels.

3. Rearing studies should be conducted under more natural conditions of illumination, i. e. using a greater portion of the shorter wave lengths of the solar spectrum and eliminating more of the longer wave lengths from contact with the culture vessels.

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