AN ABSTRACT OF THE THESIS OF

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Date thesis is presented: April 30, 1953

Title Rearing of the Native Pacific Coast Oyster Larvae, Ostrea lurida Carp., under Controlled Laboratory Conditions

Abstract approved _____
(Major Professor)

This thesis presents the techniques employed at the Yaquina Bay Fisheries Laboratory during 1952, in the rearing of the native Pacific Coast oyster, Ostrea lurida Carp., in small containers. The decline of the oyster fishery in Yaquina Bay, Oregon, has been attributed in part to the lack of adequate spat-falls. Investigations were begun in 1947 to devise methods of artificially rearing oyster larvae under controlled conditions which might later be extended in producing seed oysters in adequate numbers for the commercial production of the native oyster.

Adult oysters from which larvae were obtained for the rearing tests were tonged from the native oyster beds near Oysterville on Yaquina Bay. The larvae were taken from the adult oyster by two methods. The adult oysters were placed in five-gallon wide-mouth jars and the temperature raised to 20° C. until natural swarming occurred, or the adult oysters were opened manually and the larvae were taken from the gravid oysters. These methods apply during the natural spawning season of the native oyster which occurs in late spring and summer.

The rearing containers used were five-gallon wide-mouth jars and 12-gallon crocks. The larger containers gave better results. A filtering device was installed in each container to accomplish the water change without loss of larvae. A rearing temperature of 18 to 20° C. was maintained by controlling the room temperatures. All salt water used in rearing experiments was sand filtered to remove organisms that may prey on the larvae or take food in competition with them.

Larval food consisted of plankton which measures less than 9 microns. These organisms were cultured in small containers and 25 ml. of the media was introduced into the rearing containers every 48 hours. Rearing containers with various larval concentrations received the same amount of media and the results showed no indications of over or under feeding.

A salinity range of 25 to 32 parts per thousand yielded satisfactory results. The upper and lower limits were not determined. When the bay salinity exceeded 34 parts per thousand, the salinity was reduced by adding fresh water. This resulted in larval mortality. The reason for this mortality was not determined but it is possible that the fresh water used was toxic.

The water in the rearing containers was changed every other day in the early experiments. A less frequent water change was tried which yielded satisfactory results. A water change once or twice during the larval free-swimming period appears to be adequate.

The larval growth rate was measured and a daily growth of 4 to 5 microns is considered satisfactory. The rate of growth appears to be a reliable indication of the condition of the larvae.

The larvae set out on a cultch after a 15 to 25 day free-swimming period. Setting began when the larvae measured about 260 microns. The spat received the same care as the free-swimming larvae and considerable

shell growth was evident.

During the winter months, induced spawning was investigated. Adult oysters were taken from the bay and held in the laboratory at a temperature of 18 to 20° C. The water was changed occasionally and media was added. Some spawning occurred after the oysters were held for 6 to 8 weeks. The larvae thus obtained were successfully reared to the setting stage.

REARING OF THE NATIVE PACIFIC COAST OYSTER LARVAE, *Ostrea lurida* Carp., UNDER CONTROLLED LABORATORY CONDITIONS

by

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A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1953

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ACKNOWLEDGMENT

Grateful acknowledgment is given Professor Roland E. Dimick for his help and guidance. Mr. Robert W. Morris, Mr. Gerald H. Watson, Mr. Charles E. Warren, Mr. Eugene P. Haydu and others who have studied oyster larval rearing at the laboratory contributed much to the success of this project. Mr. Nicholas Pasquale, who is writing a companion thesis, deserves much credit and again proved the adage that two heads are better than one. Also, my wife, Mrs. Katherine Breese, rates honorable mention for her help and indulgence.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

REARING OF THE NATIVE PACIFIC COAST OYSTER LARVAE, *Ostrea lurida* Carp., UNDER CONTROLLED LABORATORY CONDITIONS

INTRODUCTION

This report presents the larval rearing techniques employed with the native oyster, *Ostrea lurida* Carp., at the Yaquina Bay Fisheries Laboratory, during the period from March 1952 to March 1953. The previous studies made at the laboratory by Robert W. Morris in 1948 (17, pp.1-46) and Eugene P. Haydu in 1950 and 1951 (unpublished reports) were employed and extended during the summer of 1952.

The rearing of native oyster larvae under controlled conditions was undertaken as a basis for developing techniques which might eventually result in large scale or commercial production of seed oysters. Native oyster production in Yaquina Bay, Oregon, has declined to a position of minor importance. One of the main limiting factors has probably been the lack of adequate spat-falls from year to year, thus resulting in insufficient seed oysters necessary for a sustained yield in commercial operations. Consequently, the oyster larval rearing studies have been underway at the Yaquina Bay Fisheries Laboratory for several years and Haydu first raised oyster larvae to the setting stage during 1950. With his resignation in March 1952 as assistant biologist of the Oregon Agricultural Experiment Station, it was thought

advisable to duplicate his methods and to improve further the techniques under controlled laboratory conditions.

A brief description of the native oyster, Ostrea lurida Carp., is desirable at this time. It is a small oyster, figure 1, which is found along the Pacific coast of North America in various locations from Queen Charlotte Islands to Tijuana, Mexico. It measures from 2 to 3 inches in length when mature. Since the oyster in the female phase retains the eggs at spawning, it falls into a group of oysters termed larviporous. This means that during spawning the eggs pass into the inhalent chamber of the oyster and are fertilized there by sperm drawn in with the salt water. The sperm are expelled into the water by the oyster in the male phase. The larvae undergo development in the inhalent chamber until they are well into the straight-hinged stage. If the adult oyster is opened during the early stages of larval development, the larvae appear white when viewed at the concentration found. This stage is given the term "whitesick". "Greysick" is the name given to a more advanced stage of larval development and "blacksick" is the term for the most advanced larval stage found within the mantle cavity of the adult oyster. At this later stage of development, the concentrated larvae appear somewhat black if viewed by the naked eye. The larvae now measure from 145 to 170 microns in length,

Figure 1. Adult oyster shells of Ostrea lurida Carp.

measured parallel to the hinge. Hopkins (12, p.459) reports that the larvae measure from 160 to 170 microns at this stage of development. The smaller measurements found at the Yaquina Bay Fisheries Laboratory may be influenced by measuring aborted larvae or an error in the calibration of the micrometer eyepiece. Since the calibrations employed may not have been exactly accurate, measurements will be made on oyster larvae during the summer of 1953. If it is found that a correction factor is necessary the larval measurements as given in this report will be adjusted before a final report is made on the larval rearing techniques of Ostrea lurida.

At this stage of development the larvae, which are about 9 to 10 days old (11, p.556) are released from the mantle cavity into the water. This action is termed swarming and should not be confused with spawning which occurs about 9 to 10 days earlier. After swarming the larvae are free-swimming for about 30 days. During this period the larvae grow to a length of from 260 to 320 microns and, at this time, they will come to rest if a suitable hard surface is available. This action is termed setting. Soon after setting the larvae go through a metamorphosis and become adapted to a sedentary life. At this period they are termed oyster spat. Seed oysters are advanced spat of suitable size in transplanting operations.

Figure 2 shows various stages of larval development.

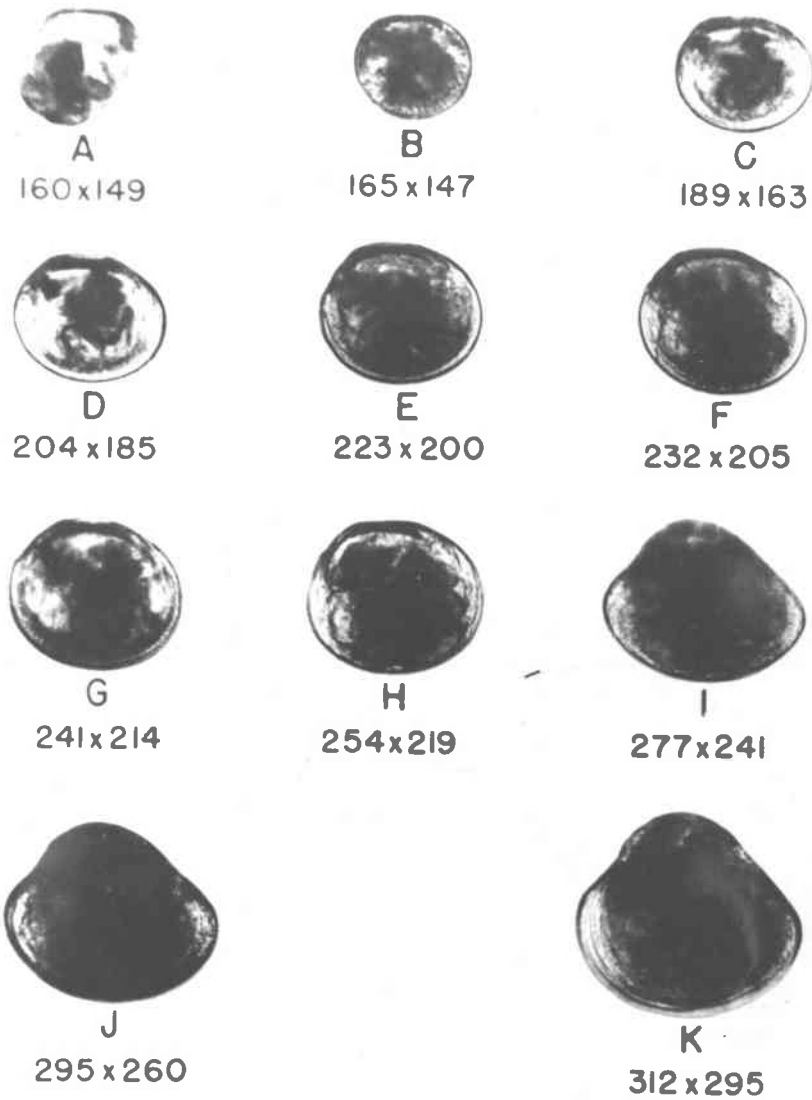

Figure 2. Microphotographs of larvae of Ostrea lurida. (Photographed by Dr. Harry C. Davis. Reproduced here with permission obtained from Dr. V. L. Loosanoff. Photograph is not for general distribution until published under proper authorship by H. C. Davis.)

The measurements shown under the larvae are in microns. Permission to use the picture was obtained from Dr. V. L. Loosanoff (16), United States Fish and Wildlife Service, Milford, Connecticut. The picture was taken by Dr. Harry C. Davis and is not to be published, mimeographed or otherwise prepared for general distribution until published under his authorship.

Rearing of oyster larvae under artificial conditions is not a new undertaking. It has been investigated in England, Japan, United States and other countries (2, pp. 337-374)(3, pp.1-28)(6, pp.125-127)(7, pp.264-265)(9, pp. 371-372)(13, p.79)(14, pp.543-547)(15, pp.69-86). These experiments were conducted with several species of oysters and varying degrees of success and failure were experienced.

At the Fisheries Laboratory on Yaquina Bay, Robert W. Morris (17, pp.11-16) developed a formula for media used to culture larval food organisms and the filtering device for changing water in rearing containers without loss of larvae. As previously mentioned, Eugene P. Haydu had some success in rearing the larvae of Ostrea lurida to the setting stage and for a year after the setting stage.

The following account is written in a procedure style so that the report may be used as a guide in similar oyster larval rearing attempts. This is followed by a summary including some of the aspects of larval rearing that need further investigation.

OBTAINING LARVAE

Of primary importance in rearing techniques is the obtaining of larvae so as to have an adequate supply throughout the experiments. A concentration of adult oysters should be close at hand. The oysters that are available for use at the Oregon State College Fisheries Laboratory are obtained from the natural beds upbay from Yaquina, Oregon. This natural oyster bed, known as Lewis Flats, is located opposite the place known as Oysterville, near the middle of the bay. The adult oysters are found in 8 to 10 feet of water at normal low tide, and they are gathered at slack tide because the water is shallowest at this time and there is only a slight current. Oyster tongs are used from a boat to bring the oysters to the surface. Oyster tongs resemble two long handled rakes fastened together near the rake ends and operating similar to pliers.

After the oysters are brought aboard the boat, they are separated from the mud and debris. They are then taken to the laboratory and thoroughly cleaned externally. Barnacles and other organisms growing on the shells are removed by scraping and the oysters growing in clusters are broken apart. Fresh water is then used to wash the outside of the oysters. The reason for this operation is to remove the dead and decaying as well as the living

organisms from the oysters. This will help prevent future contamination in holding containers and will remove organisms that may prey on the larvae or take food in competition with the larvae.

At this stage of operations there are two methods of obtaining the larvae from the adult oyster. One is manually taking larvae from gravid oysters and the other is obtaining larvae through normal swarming in the laboratory. The first method is limited to the period of natural swarming. It consists of opening oysters until an adequate supply of larvae is found. If the oysters were tonged between June 1 and August 15, one could usually expect 1 to 17 per cent of the adult oysters to have larvae in the inhalent chamber, with the higher percentage occurring in June and early July. The larvae range from the "whitesick" to the "blacksick" stage of development. Table 1 presents a record of gravid oysters tonged from Yaquina Bay from June 12 to September 15, 1952. Periodic samples of oysters were opened and examined for larvae. During the early part of the spawning season a sample consisted of 100 adult oysters and when the percentage of gravid oysters decreased, the sample was increased to over 200 oysters. In some years natural spawning may occur earlier, as was found by Mr. Dimick (5, pp.45-48). He found some spawning in April and up to 16 per cent in May with larvae that had developed valves.

TABLE 1

NUMBERS OF GRAVID AND NON-GRAVID NATIVE OYSTERS
SAMPLED FROM YAQUINA BAY DURING THE SUMMER OF 1952

Date	Number of Oysters	Condition of Larvae			Total oysters with larvae	Per cent Gravid
		"Whitesick"	"Greysick"	"Blacksick"		
June 12	100	4	3	0	7	7
June 20	100	4	2	11	17	17
June 27	100	14	3	0	17	17
July 3	100	7	0	2	9	9
July 10	100	4	0	7	11	11
July 24	100	1	2	2	5	5
July 31	100	1	0	6	7	7
Aug. 9	128				1	0.78
Aug. 11	200	1	0	0	1	0.5
Aug. 26	215				3	1.4
Sept. 9	205	0	0	0	0	0.0
Sept.15	200	1	0	0	1	0.5

Larvae from the so-called "blacksick" oysters are washed with salt water into a Petri dish with an eye-dropper. The larvae are then placed in a large glass container having 15 to 18 liters of filtered sea water at a temperature of 20 to 22° Centigrade. Some of the larvae will settle down in about twelve hours but usually they will be swimming up again in about 24 hours. Because of this larval action, it is best to leave them in the jar for about 48 hours before using them in rearing experiments.

Larvae obtained by this method may be premature but this has not proved undesirable. A uniform size or development group can be obtained with this method as larvae obtained from the same oyster are all the same size and stage of development (12, p.459).

The other method of obtaining larvae is by natural swarming. About 24 clean oysters are placed in a glass jar containing 15 to 18 liters of filtered salt water. The water temperature should be held at 20 to 21° Centigrade. If any of the oysters are "blacksick", swarming will take place within a day or two. The adult oysters should be removed soon after swarming. This is necessary as adult oysters will eat larvae in solution. Also, one of the adult oysters may have been injured in the cleaning process. If one of the oysters dies and starts to decay in the jar with the larvae, the larvae will usually die.

The larvae thus obtained will usually be of proper size. A single jar may contain different-sized larvae as swarming frequently occurs from two or more oysters. This method of obtaining larvae from adult oysters can be used best during June and July.

Five-gallon wide-mouth glass jars, figure 3, are used for swarming and holding larvae. These jars are satisfactory as the larvae can be readily observed. Larvae can be held in the jars for about a week without food or a water change, but should be used as soon as practicable. After a week, the larvae start thinning out in numbers. During the summer months larvae are easily obtained and should be collected fresh for each new experiment.

PREPARING THE CROCKS FOR CULTURING LARVAE

Crocks with a 10 to 12 gallon capacity, figure 4, are satisfactory for experimental rearing of oyster larvae. Smaller containers such as the five-gallon wide-mouth jars were tried but proved unsatisfactory. Only a few of the larvae reared in these smaller containers reached the setting stage and the results in general were not satisfactory. In the smaller containers the limiting conditions are probably more pronounced as any inimical condition affects a greater part of the whole in a smaller volume of water. For large scale production, larger containers will

Figure 3. Five-gallon wide-mouth jars used in oyster larvae swarming and holding procedures.

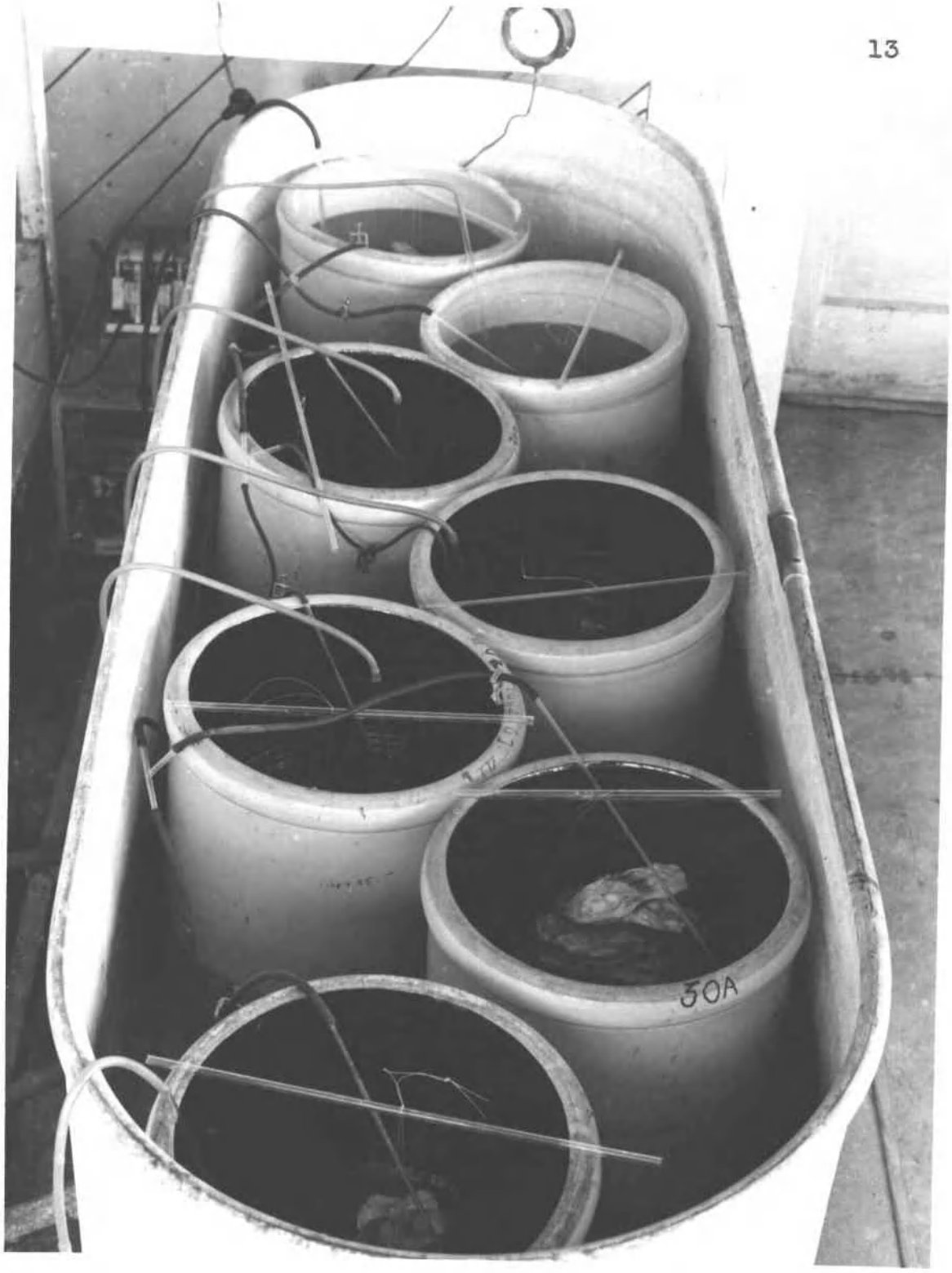

Figure 4. Twelve-gallon crocks in water bath tank used in rearing of oyster larvae.

have to be used to grow oyster seed in quantity. Concrete and wooden tanks of about 250-gallon capacity are being tried for large-scale production of oyster larvae and will be reported on by Nicholas Pasquale in a thesis entitled Rearing of the Native Oyster Larvae, Ostrea lurida Carp., in Concrete and Wooden Tanks Under Controlled Conditions.

Before using the rearing containers, the crocks should be thoroughly cleaned and should not contain paint, tar or other foreign matter that might be toxic to larvae. The crocks should then be filled to a depth of four inches with coarse sand. The sand is used as a filter in changing the water and should not be too fine. Most beach sand is too fine. Suitable sand is found at the mouth of Fogarty Creek, which is located a mile or two north of Depoe Bay.

The water change apparatus is next put into the rearing crock. A quart bottle such as a common beer bottle is used, but the bottom must be removed. This can be done by tying a gasoline soaked string around the bottle near the bottom and then lighting it. When it is heated put the bottle quickly into cold water and the bottom should break off. A handful of glass wool is then placed in the bottle and the bottle is inserted in an upright position down into the sand. A rubber stopper which has a glass tube through it is placed in the bottle. A piece of rubber tubing is fitted to the glass tubing and the rubber tubing extends

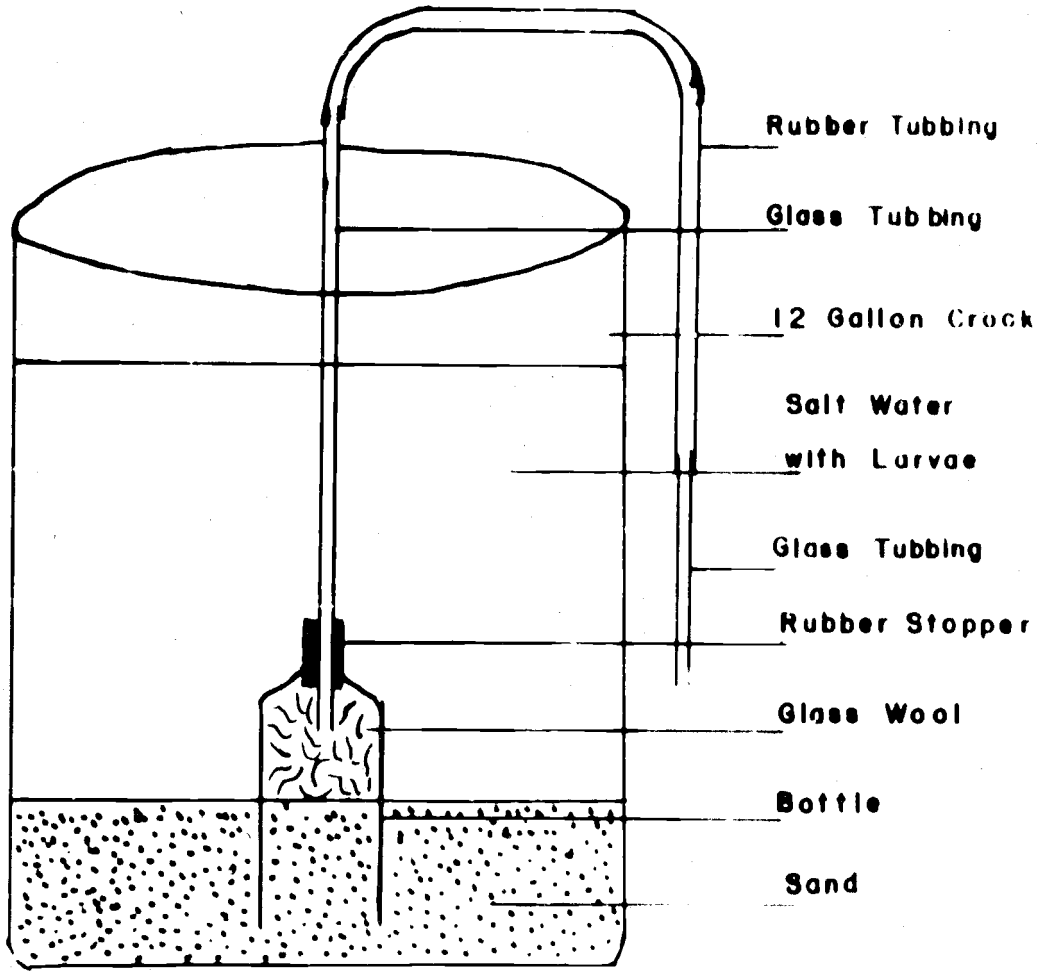

Figure 5. Diagrammatic drawing of water change apparatus.

out of the crock and down the outside nearly to the bottom. To accomplish the water change a suction can be applied to the rubber tubing and the water siphoned. With this arrangement the water must pass down into the sand and up through the bottle thus preventing the loss of larvae. The glass wool in the bottle prevents the sand from being siphoned out. A diagrammatic drawing of the water change apparatus is shown in figure 5.

After the sand is in the crocks and the water changing apparatus is in place, the culture water can be put in the crocks. Filtered salt water should be used and it should be brought to the rearing temperature of approximately 20 to 21 degrees Centigrade before the larvae are introduced. The salinity of the water should be recorded to determine if it falls within the desired limits. These limits will be discussed later. The salinity can be determined with the aid of three precision specific gravity hydrometers with ranges of 1.000-1.011, 1.010-1.021 and 1.020-1.031. Very satisfactory hydrometers were obtained from The Emil Greiner Company, New York City, New York. With these hydrometers the specific gravity can be read to four decimal places, which is necessary in determining accurate salinities. This degree of accuracy is not possible with most ordinary hydrometers. A specific gravity reading of the water is taken and the temperature noted. A table is consulted for the correction factor due to temperature and

the corrected reading is then converted to salinity in parts per thousand of salts. The tables employed are found in Manual of Tide Observations Special Publication No. 196, United States Department of Commerce, United States Coast and Geodetic Survey, 1941.

The next step is the introduction of the larvae. The number of larvae per liter desired should be determined. Setting has been obtained with concentrations of from 25 larvae per liter to 500 larvae per liter. A concentration of only 25 larvae per liter did not appear to make efficient use of the equipment as would be the case in higher concentrations. Concentrations of 300 to 500 larvae per liter have proven satisfactory and as yet the greatest practical density has not been ascertained. Haydu (unpublished reports) was of the opinion that 50 oyster larvae per liter of water represented optimum larval density in the rearing crocks. Larval concentration will be further discussed in the section on larval food.

The method of determining the number of larvae per liter is as follows. Select a jar containing a high concentration of larvae. Stir the contents slightly to get an even distribution of larvae in the jar. Draw off a 10 milliliter sample with a pipette that has a large terminal opening. Divide this sample equally into ten small dishes and count the larvae with the aid of a hand lens or a dissecting microscope. Multiply the number in the

samples by 100 and the answer will be the approximate number of larvae per liter in the jar. The count should be made just before introducing the larvae into the rearing crocks. Measure the amount of water in the rearing crocks that is above the sand and introduce the number of larvae necessary to obtain the desired larval density.

At this time the larvae should be measured so that at later dates the growth rate may be determined. Take a small beaker of water from the jar containing the original supply of larvae. With an eye-dropper remove ten larvae and put them on a clean glass slide. Add a drop or two of 5 per cent urothane to inactivate the larvae. Place the slide under a compound microscope, using low power, and measure the larvae with a micrometer eyepiece. The measurements are made of the greatest shell distance parallel to the hinge of the larvae. Record the measurements in microns as that is the unit most commonly used. If the larvae were obtained from two or more adult oysters, the average measurement should be recorded. The counting and measuring of the larvae are approximations but they have proven adequate.

The final step in preparing the rearing crocks is to bubble air slowly through the water in the crocks. This is accomplished by a Thiberg aerator which will supply three or four crocks or by a larger air pump with one or two cylinders. The larger pump will supply air to ten to

twenty crocks. The air is delivered to the crocks by glass tubing, rubber tubing and various 3 and 4 way connections. The supply of air to each crock is controlled by a small clamp. Glass tubing is used to introduce the air into the crocks and the tubing terminates 6 to 8 inches below the surface of the water. The air is allowed to bubble into the crocks and no attempt is made to break up the bubbles as would be the case if stone air breakers were used. A slight current is created by the air bubbles, and this circulation of the water may help in distributing food organisms and larvae throughout the rearing crocks. Air bubbling may not be an essential technique in rearing larvae, but it appears desirable.

The crocks should be protected from direct sunlight. When the water in the crocks receive too much light, a dense growth of algae usually occurs which may entangle the larvae and is generally undesirable. A weak or subdued light did not prove detrimental.

CARING FOR THE LARVAE

After the larvae are introduced into the crocks, the close attention given the larvae throughout the growing period is of the utmost importance. The various factors to be considered in caring for the larvae will now be discussed, and it should be emphasized that they are all

vital to the welfare and growth of the larvae. Even with great care, some groups of larvae did not survive to the setting stages and in such cases the causes of failure have been difficult to explain.

Temperature Control

A water temperature of about 20 degrees Centigrade appears to be ideal for the growth of Ostrea lurida larvae. Haydu found the optimum rearing temperature range to be from 18 to 20 degrees and observations during the 1952 season substantiate this finding with the upper limit being more ideal. Larvae reared at this temperature complete the free-swimming period in about 21 to 25 days which is 9 to 5 days sooner than the expected duration of the free-swimming larval period in nature. Emersion heaters employed in water baths were used to control the water temperature but resulted in undesirable temperature fluctuations. This was due to wide fluctuations in air temperature encountered in rearing room. During the cool nights the water temperature in the crocks would fall and conversely high daytime air temperatures resulted in increased rearing temperature. Also, many small heating units with thermostatic controls were required.

A controlled room temperature is the most satisfactory method of maintaining the desired water temperature. The ceiling of the laboratory was insulated and a thermostat

controlled electric wall heater was installed. This arrangement kept the air temperature of the room fairly constant. The water temperature remained constantly at a degree or two below the air temperature throughout a 24-hour period. Indications to date were that temperatures higher than 21 degrees Centigrade were unfavorable but results were far from conclusive. A crock was placed high in the rearing room, resulting in a water temperature of 23.5 degrees Centigrade (74 degrees Fahrenheit). On two occasions larvae died within 24 hours, and on two other occasions larvae died within 12 days. Larvae in crocks on a lower level having a water temperature of 20.5° Centigrade (68 degrees Fahrenheit) lived and reached the spat stage. A lower temperature appeared to slow down the growth of the larvae (4, p.111).

Water Change

A water change in the rearing containers is in reality only a partial water change as a little over half of the water is siphoned off and filtered water is added to restore the previous water level. By the method previously mentioned the water is siphoned off without loss of larvae. The introduced water should be sand filtered and should be approximately the same temperature as the water in the rearing crocks. As explained later the water is warmed by letting it stand in the heated rearing room. There is,

however, no evidence thus far of a sudden temperature change having an adverse effect on the larvae, but such may be possible. The warmed water may contain more organisms as will be explained in the section on food.

Most of the rearing experiments were carried out by changing water every 48 hours with satisfactory results. A more frequent water change appears unnecessary. Recent indications are that one or two water changes during the entire free-swimming period of the larvae may be sufficient (15, p.72). The data presented in table 2 were obtained from five experiments started on August 27, 1952 to determine the effect of a less frequent water change. In all five experiments the salinity of the rearing water was controlled at 30 parts per thousand. The effects of the fresh water used in controlling the salinity is in question and may be the cause that no setting occurred. The apparent reduction in the size of some of the larval samples may have been due to the small numbers of larvae measured or to the larger larvae dying due to the fresh water used in reducing the salinity. The larvae used in all five experiments measured 176.9 microns at the beginning of the experiments and were taken from the same jar. Other conditions were similar.

In experiment 30A there was no water change during the 17 days the experiment was conducted. At the end of the period the larval size averaged 215 microns. Experiment 30B

TABLE 2

DATA FROM FIVE REARING EXPERIMENTS STARTED ON AUGUST 27, 1952 TO DETERMINE THE EFFECTS OF A LESS FREQUENT WATER CHANGE

Experiment number	Date larvae examined	Average larval size in microns	Frequency of water change
30 A	8-27-52	176.9	none
30 A	9- 6-52	208.0	none
30 A	9-12-52	215.0	none
30 B	8-27-52	176.9	2 day interval
30 B	9- 6-52	224.4	2 day interval
30 B	9-12-52	*220.0	2 day interval
30 C	8-27-52	176.9	4 day interval
30 C	9- 6-52	219.8	4 day interval
30 C	9-12-52	*208.6	4 day interval
30 D	8-27-52	176.9	8 day interval
30 D	9- 6-52	222.6	8 day interval
30 D	9-12-52	*221.2	8 day interval
30 E	8-27-52	176.9	12 day interval
30 E	9- 6-52	210.0	12 day interval
30 E	9-12-52	*208.0	12 day interval

* The apparent reduction in size of the larvae may have been due to the small sample measured or to the larger larvae dying due to fresh water used in reducing the salinity.

had 8 water changes at 24 hour intervals which resulted in an average larval size of 220 microns. The water was changed 4 times in Experiment 30C at 4 day intervals. This resulted in an average larval size of 208.6 microns. In Experiment 30D the water was changed twice at 8 day intervals and the average larval size reached 221.2

microns. The fifth experiment, 30E, had one water change at the 12th day and the average size of the larvae was 208 microns. Considering the size of the sample measured, there was no great size difference at the conclusion of the experiments. The average size for the 5 experiments was 214.5 microns. It is indicated that the frequency of water change had little effect on the growth of the larvae. As the natural spawning season of the adult oyster was about over, further experiments with frequency of water change were not conducted.

The rearing water used was always filtered to remove undesirable organisms that may prey on the larvae or compete with the larvae for food. Salt water was pumped from the bay into a wooden storage tank, figure 6, by means of a stainless steel centrifugal pump, figure 7. The intake consists of a stainless steel pipe with a foot valve. A large rubber hose carries the salt water from the pump to the storage tank. From the tank the water is carried by a plastic pipe and plastic hose to the laboratory rearing room. The salt water is then passed through a sand filter similar to the water change apparatus. Suction is obtained by the use of a Venturi pump through which water is continually circulated by a small centrifugal water pump, figure 8. The filtered water is collected in 15 five-gallon carboys which are incorporated into the suction line, figure 9. The filtered water is allowed to

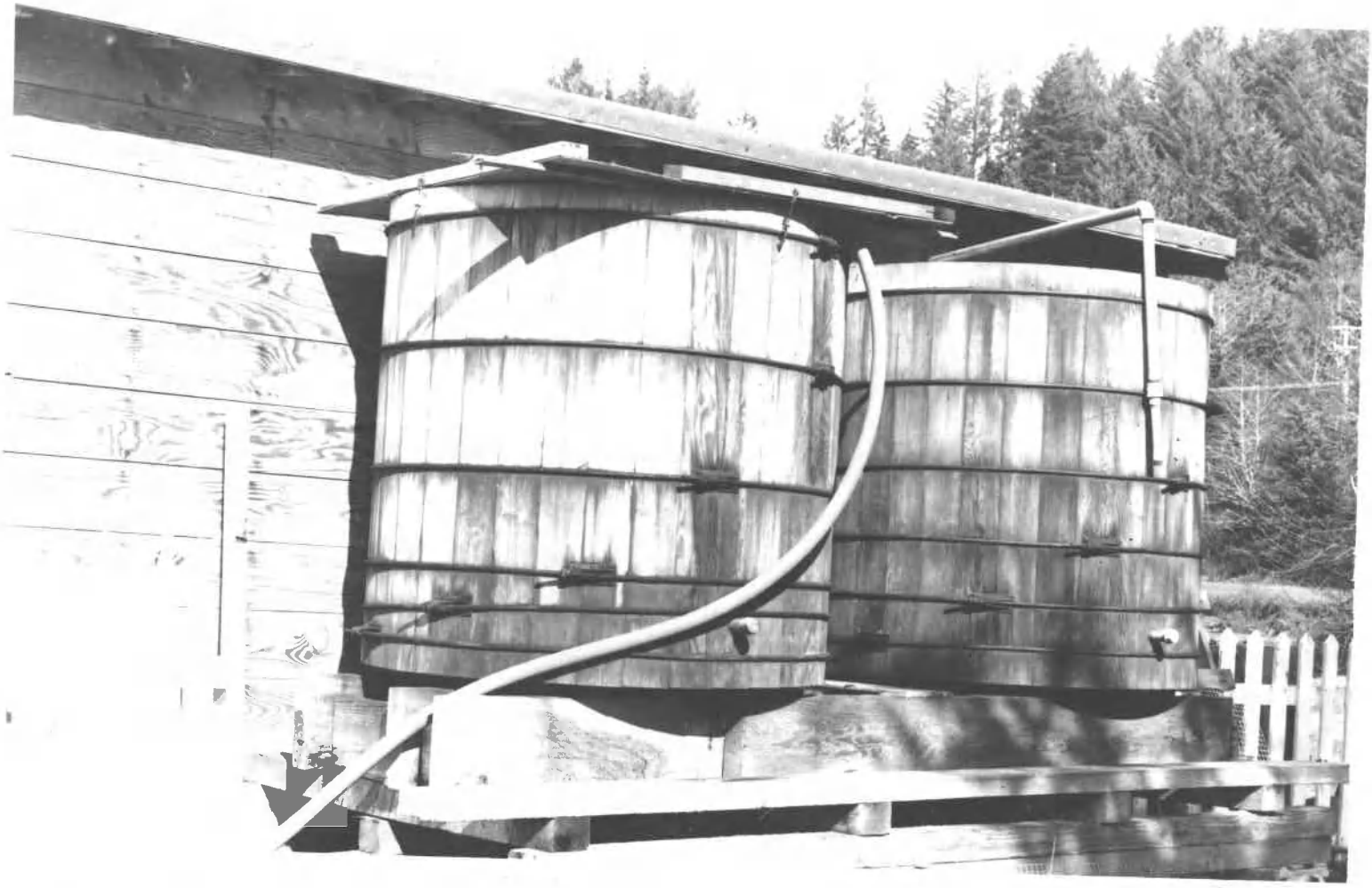

Figure 6. Wooden tanks for storing salt water, capacity of each about 550 gallons.

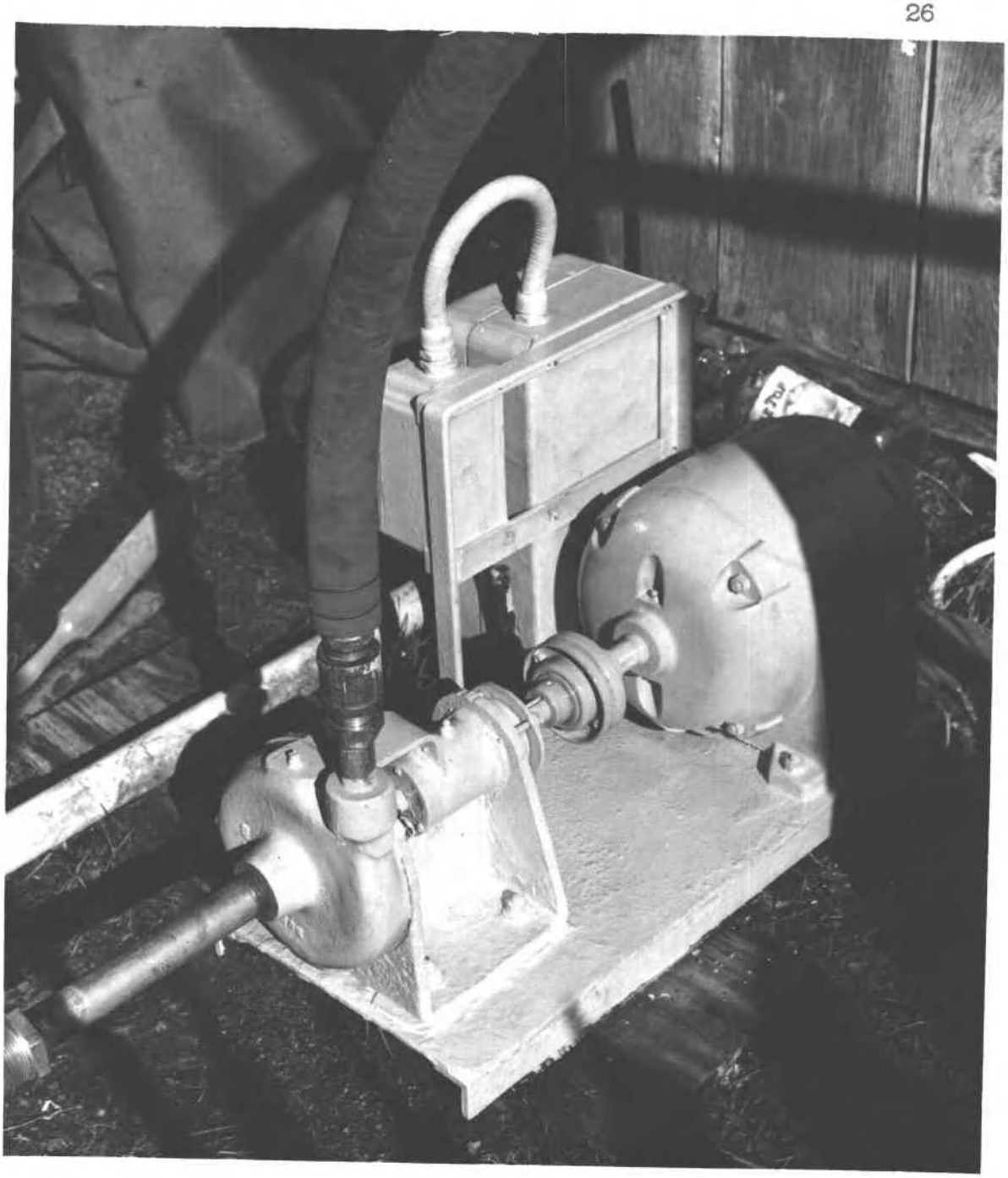

Figure 7. Stainless steel centrifugal pump for pumping salt water from the bay into the storage tanks.

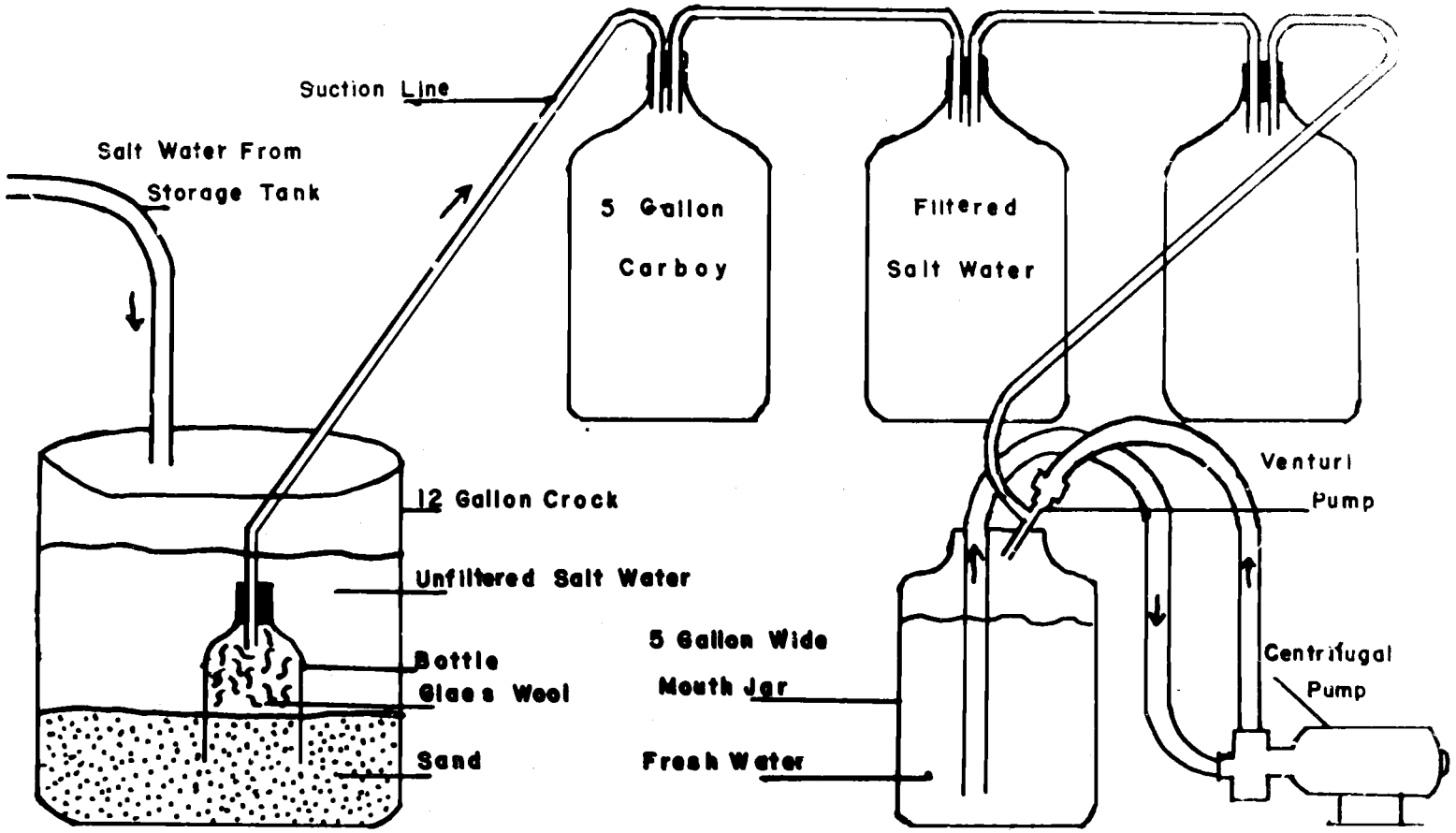

Figure 8. Diagrammatic frawing of filtering apparatus.

Figure 9. Picture inside the laboratory rearing room showing a portion of the filtering apparatus.

stand until reaching room temperature.

Food

Feeding animals as small as oyster larvae presented many problems. Theoretically the food organisms should measure 7 to 9 microns or less in order to be ingested by the larvae and in addition they must be acceptable to the larvae. The media in which these food organisms are cultured must not be toxic to the larvae. A non-toxic media was developed by Robert W. Morris at the laboratory in 1949. The modified formula for the media is:

500 milliliters salt water (not filtered)
500 milliliters fresh water
1.5 grams starch, soluble, purif. powder
.15 grams potassium nitrate
.3 grams disodium phosphate

The fresh and salt water are mixed into a 1500 ml. beaker and heated to the boiling point. Add the starch, potassium nitrate, and disodium phosphate and stir. Allow the mixture to cool and pour 100 ml. of the media into several 250 ml. Erlenmeyer flasks. Innoculate each flask with 2 to 3 ml. of salt water obtained from the bay. The media should be kept at a temperature of 18 to 20 degrees Centigrade; this temperature can be maintained by use of an incubator or by leaving the media in the heat-controlled rearing room. Examine the media, after 3 to 5 days, under the low power objective of a microscope. Only media containing the desired culture should be used.

Reinnoculations of media should be from a culture that is 3 to 5 days old and shows a good growth. Several innoculations may be made before a desirable culture is obtained that contains no large undesirable organisms. The density of the food organisms in the culture may be determined with a hemocytometer. Approximately 4 to 5 million food organisms per milliliter is considered a good culture.

No attempt was made in the 1952 operations to identify the food organisms. Bodo lens (O.F.Muller), a naked flagellate protozoa which measures 7-9 microns, was identified by Warren at the laboratory in 1950 (15, p.18). This organism was used as a gauge to ascertain the desirable sizes of the food organisms. The only selection made was to keep only organisms that were as large or smaller than Bodo lens. Thus the cultured larvae food consisted of microscopic protozoa and bacteria in the main.

The rearing crocks were supplied every other day with cultured food organisms immediately following water change. A feeding consisted of 25 ml. of culture media regardless of the number of larvae per liter. After introduction into the crocks, the food organisms may reproduce rapidly before they are eaten by the larvae, thus the food potential is greater than that supplied by media alone. No growth or mortality differences were noted between the crocks containing 25 larvae per liter and the crocks

containing 500 larvae per liter. Tables 3 and 4 present data from two different series of experiments. The experiments in both series were supplied with 25 ml. of culture media every other day. The rearing temperatures were maintained between 18 and 20 degrees Centigrade and the water was changed every 24 hours.

Table 3 presents data obtained from seven experiments which were started June 17, 1952 to ascertain the maximum concentration of larvae per liter under the conditions stated above. The larvae measured 144.3 microns at the time of introduction into the rearing crocks. In all seven experiments setting occurred with concentrations of 50 to 350 larvae per liter. No attempt was made to count the total set but the experiments with greater concentrations of larvae appeared to have the most set. On July 15, 1952 five more experiments were started with still higher larval concentrations. The data from these experiments are presented in table 4. The larvae used in these experiments measured 174.7 microns at the time of introduction into the crocks. The concentrations of larvae were from 300 to 550 larvae per liter. Setting occurred in all but the experiment with 450 larvae per liter. It is believed that the maximum concentration of larvae per liter was not reached in these trials. In all twelve experiments the same amount of culture media was introduced and the results indicate no differences that can be attributed to amount of

TABLE 3

DATA OBTAINED FROM SEVEN REARING EXPERIMENTS STARTED JUNE 17, 1952 REGARDING FEEDINGS HAVING VARIATIONS IN LARVAL CONCENTRATION

Experiment number	Date	Average larval size	*Set	Larvae/liter
X106	6-17	144.3		50
"	6-23	203.4		
"	7-1	240.0		
"	7-8		6	
X107	6-17	144.3		100
"	6-23	209.4		
"	7-1	283.0		
"	7-8		37	
X108	6-17	144.3		150
"	6-23	209.0		
"	7-1	227.0		
"	7-10		1	
X109	6-17	144.3		200
"	6-23	192.3		
"	7-1	224.0		
"	7-10		2	
X110	6-17	144.3		250
"	6-23	199.3		
"	7-1	258.0		
"	7-10		16	
X111	6-17	144.3		300
"	6-23	202.4		
"	7-1	260.0		
"	7-10		72	
X112	6-17	144.3		350
"	6-23	228.0		
"	7-1	268.0		
"	7-10		69	

* No attempt was made to count the total set. The count was made on the under side of the cultch.

TABLE 4

DATA OBTAINED FROM FIVE REARING EXPERIMENTS STARTED JULY 15, 1952 REGARDING FEEDINGS HAVING VARIATIONS IN LARVAL CONCENTRATION

Experiment number	Date	Average larval size	Salinity	*Set	Larvae per liter
1X106	7-15	174.7	32.4		300
"	7-21	193.9	32.8		
"	7-24	222.6	32.9		
"	7-30	229.7	32.4		
"	8-5	229.0	32.9		
"	8-10		33.0	12	
1X108	7-15	174.7	32.4		400
"	7-21	203.9	32.8		
"	7-26	214.4	32.9		
"	7-30	222.8	32.4		
"	8-5	270.1	32.9		
"	8-10		32.9	10	
1X109	7-15	174.7	32.4		450
"	7-21	200.6	32.8		
"	7-26	209.9	32.8		
"	7-30	218.2	32.4		
"	8-5	243.9	32.9		
1X110	7-15	174.7	32.4		500
"	7-21	203.1	32.8		
"	7-26	215.2	32.9		
"	8-5	262.0	32.9		
"	8-10		33.0	17	
1X111	7-15	174.7	32.4		550
"	7-21	193.4	32.8		
"	7-26	214.4	32.9		
"	8-5	243.3	32.9		
"	8-10		32.9	1	

* No attempt was made to count the total set. The count was made on the under side of the cultch.

culture media introduced.

Overfeeding of culture media may prove undesirable. This does not refer to the quality of the culture, only to the quantity of the media. Media will differ from time to time as to the amount of food organisms present and as yet no mortality has been attributed to too rich a culture. However, mortality was noted on one occasion when a large quantity of media was fed. On August 8, 1952 one liter of media was added to a 5-gallon jar containing 17 liters of rearing water. On August 9 all the larvae were dead. This may have resulted from too high a concentration of media which was harmful to free-swimming larvae.

There are other possible food sources. The salt water used in the water change is held at rearing temperature at least 36 hours before it is used. During this time there is a possibility that the food organisms present in the filtered water may reproduce, supplying an unknown amount of food. Also, the sand in the rearing crocks may contribute organic material which may favor the growth of food organisms.

Growth Determination

During the course of the experiments it is desirable to know if the larvae are growing and, if so, the rate of growth. Every few days remove ten larvae and measure them by the method previously described. Record the measurements

and after three or four measurements are taken, a trend may be determined. Due to the small number of larvae in the sample usually measured, no growth or even a reduction in size may sometimes be indicated. Usually this apparent condition will be offset when the next measurements are taken. The allover measurements will give the growth rate. A growth of 4 to 5 microns a day is excellent.

Growth rate and general rearing condition of the larvae can also be determined by gross observations. After viewing a number of larvae during the course of an experiment, the various stages of development can be easily recognized. The development of the umbo will show if a satisfactory rate of growth is being maintained, figure 2. The color of the larvae is also an indication of its condition. If the color of the internal organs have a yellowish-brown appearance, the larvae are healthy.

Salinity Range

The ideal salinity range for rearing larvae was not determined but a salinity varying from 25 to 32 parts per thousand yielded satisfactory results. During late spring and early summer, salt water from the bay was used without altering the salinity. Toward the end of July the bay water increased in salinity to 33 parts per thousand and slightly above. As it was thought that a lower salt concentration was more ideal for larvae, a method was

devised for lowering the salinity.

First, measure the amount of salt water to be altered and determine its salinity. Then divide the desired salinity into the known salinity. Subtract 1.0 from the result and multiply the remainder by the number of liters of salt water to be altered. The final answer will equal the number of liters of fresh water which must be added to the salt water to obtain the desired salinity. For example, if the volume is 30 liters of salt water with a salinity of 34.0 parts per thousand and a salinity of 30 parts per thousand is desired, divide 30 into 34, which is 1.133. Subtract 1.0, which leaves .133. Multiply .133 by 30 (the number of liters of salt water) and the answer is 3.99, which is the number of liters of fresh water to add to 30 liters of salt water with a salinity of 34 parts per thousand to obtain a salinity of 30 parts per thousand.

Rearing experiments were conducted in water having controlled salinities at various levels from 20 to 30 parts per thousand. In these experiments all went well until the larvae approached the setting stage of development. Some reduction in numbers of larvae was noted in the lower salinities. Then, for no apparent reason, the larvae thinned out in numbers, and little, if any, setting was observed. It is possible that the fresh water used in the dilution was unsuitable for larval rearing in that it may have been toxic or inhibited setting.

Table 5 presents data from 6 experiments which were started July 15, 1952. The water was changed every other day and 25 ml. of culture media was supplied after each water change. Rearing temperatures were held between 18 and 20 degrees Centigrade. In all experiments the larval concentration was 350 larvae per liter and the larvae were from a common stock which measured 174.7 microns. The salinity at the beginning of the experiments was 32.4 parts per thousand. During the course of the experiments, the salinity rose to what was thought was too high a salt concentration. Reductions in salinity were made as noted in table 5. The fresh water used in the reduction was from a galvanized pipe line. Although some setting occurred, as noted, it was thought that reducing the salinity had an undesirable effect.

There was a possibility that the fresh water from the galvanized pipe was toxic. In an attempt to prove or disprove this, six experiments were conducted in which the dilutions were made from fresh water conducted through a plastic hose. Table 6 presents the data from these experiments which were started August 17, 1952. The rearing temperatures were held at 18 to 20° Centigrade. The water was changed every 48 hours after which 25 ml. of culture media were supplied each rearing crock. The larvae were from a common stock which measured 179.3 microns. The larval concentration was 500 per liter. Reduction in

TABLE 5

DATA OBTAINED FROM SIX EXPERIMENTS STARTED JULY 15, 1952 IN WHICH REARINGS WERE CONDUCTED UNDER REDUCED SALINITIES

Experiment number	Date	Salinity	*Set	Average larval size
1X100	7-15	32.4		174.7
"	7-21	32.8		202.7
"	7-26	33 reduced to 22.5		210.3
"	7-30	22.7		210.3
"	8-2	22.6		221.1
"	8-5	22.7	0	223.8
1X105	7-15	32.4		174.7
"	7-21	32.8		197.4
"	7-26	33.0		213.0
"	8-3	25.8 reduced		
"	8-5	26.3		251.2
"	8-10		1	
1X107	7-15	32.4		174.7
"	7-21	32.8		186.6
"	7-26	32.9 reduced to 26.0		217.2
"	7-30	26.1		236.5
"	8-2	25.9		234.5
"	8-5	26.0		223.8
"	8-10		2	
1X112	7-15	32.4		174.7
"	7-21	32.8		208.5
"	7-26	32.9 reduced to 29.0		201.0
"	7-30	29.0		216.5
"	8-5	29.0		198.8
"	8-10		1	
1X115	7-15	32.4		174.7
"	7-21	32.8		196.2
"	7-26	33.0		211.6
"	7-31	32.4 reduced to 26.1		
"	8-5	26.5		239.3
"	8-10		3	

TABLE 5--continued

Experiment number	Date	Salinity	*Set	Average larval size
1X116	7-15	32.4		174.7
"	7-21	32.8		195.9
"	7-26	33.0		193.9
"	7-31	32.4	reduced to 25.1	
"	8-5	26.3		207.7

* No attempt was made to count the total set. The count was made on the under side of the cultch.

salinity was made before the introduction of the larvae and before each water change. Salinities were controlled at various levels from 20 parts per thousand to bay water salinity, which was 34.1 parts per thousand. In experiment 33C, which was supposed to be a control, bay water was used. It ranged from 34 parts per thousand to 32.9 parts per thousand. This is believed to be too high a salinity. In the other five experiments the salinities were 20, 22, 25, 27 and 30 parts per thousand. The number of the experiments corresponding to the controlled salinity. In all 6 experiments, growth was poor and no setting occurred. Controlling salinities doesn't seem feasible at this time but it is possible that the poor results were caused by some other undetermined factor.

TABLE 6

DATA OBTAINED FROM SIX EXPERIMENTS STARTED AUGUST 17, 1952 IN WHICH REARINGS WERE CONDUCTED UNDER REDUCED SALINITIES

Experiment number	Date	*Salinity	Average larval size
33C	8-17	34.1	179.3
"	8-27	34.0	196.2
"	8-31	33.7	203.7
"	9-8	32.9	208.3
30	8-17	30.2	179.3
"	8-25	29.9	230.5
27	8-17	27.1	179.3
"	8-25	27.2	194.0
"	8-31	27.1	201.3
"	9-8	26.9	255.6
25	8-17	25.4	179.3
"	8-25	25.6	185.1
"	8-31	25.2	194.0
"	9-8	25.4	201.6
22	8-17	22.0	179.3
"	8-25	22.4	176.0
"	8-31	22.4	187.8
"	9-8	22.2	203.7
20	8-17	20.0	179.3
"	8-25	20.9	182.6
"	8-31	19.9	197.1
"	9-8	20.1	203.1

* The salinity was reduced by adding fresh water conducted to the laboratory through a plastic hose. No larvae setting was noted.

SETTING THE LARVAE

When free-swimming larvae grow to about 280 microns in the rearing containers, they come to rest on a solid substrate and go through a metamorphosis adapting their internal structure to a sedentary life. Shell that is used as a hard surface for the larvae to attach is called cultch. Shell of the Pacific oyster, Ostrea gigas, was used because it was easily obtained and fairly large in size. A large quantity of old shell is found on the bank of Yaquina Bay near the place called Oysterville.

The shell was washed, wire brushed, and boiled before being introduced into the crocks with the larvae. This cleaning was done to prevent contamination. After each shell was clean, two holes were drilled through it. Nylon leader material (which was apparently non-toxic to larvae) was used to string the cultch and suspend it in the crocks. The lower end of the cultch should come close to the bottom when suspended in the crock. The shell should be strung with the smooth side down as most of the setting has been reported as occurring on the underside (10, pp.82-87).

The introduction of the cultch is very important; it shouldn't be put into the crocks too soon as it may lose its ability to catch larvae with time. This is due to the organic growth occurring on the shell. The cultch should be introduced when the largest larvae measure about 250

microns. If none of the larvae measures 250 microns at the end of 21 days, the cultch should be introduced as setting will probably occur before 30 days, regardless of size.

After the cultch has been in the crocks 48 hours, it can be removed for a short time and examined for set. This can be done with a hand lens or under a dissecting microscope. Examination is made on the smooth side of the cultch as the larvae there are easily seen. The cultch should be returned to the rearing crock as soon as possible. When setting is observed, the cultch should not be removed again until setting is complete. Also, the water change should be discontinued during the setting period. When the larvae find suitable places, they cement themselves down and should not be disturbed at this particular time.

The cultch should be left in the crocks until the setting period is complete. This can be determined by examining the water in the crocks for the presence or absence of free-swimming larvae. Take a beaker of water from the crock and hold it up to the light. If larvae are present they can be observed swimming. This should be done several times on consecutive days and, if no larvae is observed, setting is complete. The cultch can then be removed and put in a crock or other containers for growing the spat, figure 10.

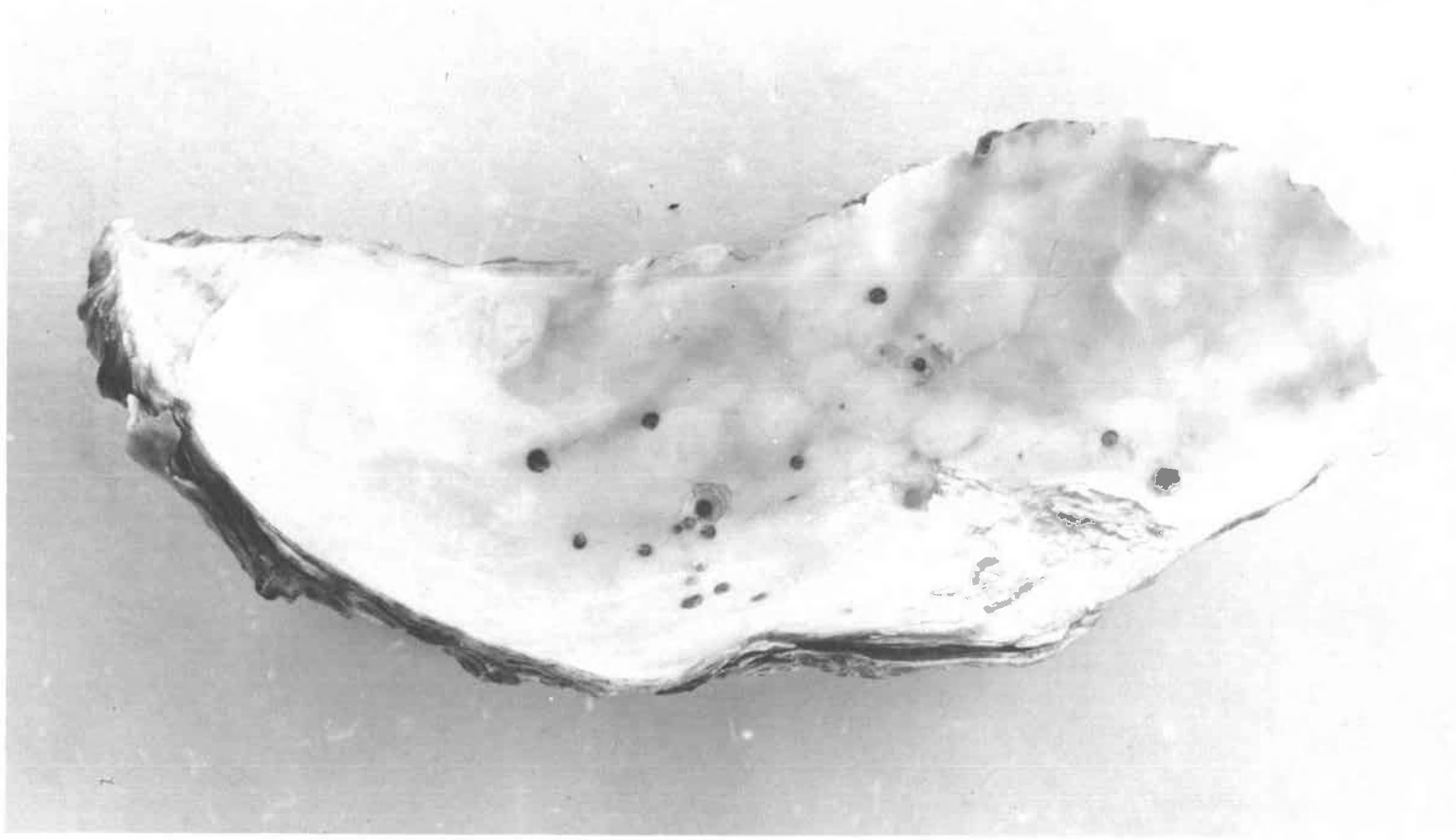

Figure 10. Spat of Ostrea lurida on shell of the Pacific oyster.

INDUCED SPAWNING

The above method was used successfully for rearing larvae obtained during the regular spawning season of the native oyster in 1952. Larvae may also be obtained by induced spawning during the winter months. This technique is not perfected as yet but some success was attained during the winter of 1952-1953. Table 7 presents the data on induced spawning. The adult oysters were held during winter months in five-gallon jars at a temperature of about 20 degrees Centigrade. Air was bubbled through the water. Some cultured food media was added at irregular intervals. Twelve adult oysters were put in each jar and swarming occurred 6 weeks to 2 months after their introduction. Some of the swarming was premature and larvae that do not swim up should not be used in rearing experiments.

It is believed that larvae obtained by induced spawning can be reared to the setting stage by the method described above. At present, rearing experiments are being conducted with induced spawned larvae.

SUMMARY

Larvae of Ostrea lurida were successfully reared in small containers under laboratory conditions at the Yaquina Bay Laboratory during the summer of 1952. Emphasis of this year's investigations was placed on demonstrating

TABLE 7

DATA REGARDING INDUCED SPAWNING OBTAINED DURING THE WINTER OF 1952-1953

Experiment number	Date started	Date of swarming	Average size in microns at time of swarming
1	12-5-52	1-18-53	larvae dead
"	"	1-19-53	156.6
"	"	2-12-53	178.6
1a	12-5-52	2-25-53	153.5
2	1-12-53	2-27-53	127.7

that larvae could be grown successfully throughout the free-swimming stage until ready to spat. Although small numbers of spat were obtained, 1953 experiments will be directed toward developing and perfecting techniques for obtaining a greater degree of spatting than has thus far been obtained.

The rearing procedure described in this paper includes the collecting and care of the adult oysters used in obtaining oyster larvae for rearing purposes, the mechanics of preparing the rearing containers to receive the larvae, introduction and care of the larvae during the free-swimming period, and setting the larvae and some information on induced spawning.

A workable salinity range was found to be from 25 to

32 parts per thousand, but the upper and lower limits were not determined. If the results of further experiments show the necessity for reducing the salinity in late summer, experiments should be conducted with regard to the amount of reduction necessary. Also, the fresh water used in lowering the salinity should be tested for toxicity to oyster larvae.

No attempt was made to determine the degree or amount of setting per unit of area of cultch. This should be done with experiments designed to increase the per cent of set. As Paul Bonnot (1, p.227) found that 3 or 4 spat per square inch were all that could be expected to grow to market size. The spat should be treated the same as the larvae until considerable growth is evident. The seed oysters can then be transplanted to suitable growing grounds.

The upper and lower temperature limits should also be investigated. In fact, each of the major factors affecting oyster larvae would furnish material for a paper such as this.

LITERATURE CITED

1. Bonnot, Paul. Setting and survival of spat of the Olympia oyster, Ostrea lurida, on upper and lower horizontal surfaces. California Fish and Game 23:225-228. 1937.

2. Bruce, J.R., Margery Knight and Mary Parke. The rearing of oyster larvae on an algal diet. Journal of the marine biological association of the United Kingdom 24:337-374. 1940.

3. Cole, H.A. Experiments in the breeding of oysters, Ostrea edulis, in tanks with special reference to the food of the larvae and spat. Ministry of agriculture and fisheries, Fisheries investigation, Series 2, 4:1-28. 1936.

4. Davis, H.E. On cultivation of larvae of Ostrea lurida. Anatomical record 105:111. 1949.

5. Dimick, Roland E., George England and Jay B. Long. Native oyster investigations of Yaquina Bay, Oregon, progress report 2, covering the period July 4, 1939 to September 30, 1941. 153 p. Corvallis, Oregon agriculture experiment station, 1941.

6. Douglas, Wilson P. Oyster rearing on the river Yealm. Journal of the marine biological association of the United Kingdom 25:125-127. 1941.

7. Dover, Cedric. Oyster culture in Malaya. Nature (London) 124:264-265. 1929.

8. Fasten, Nathen. The Yaquina oyster beds of Oregon. The American naturalist 65:434-468. 1931.

9. Galtsoff, Paul S. and R.O. Smith. Stimulation of spawning and cross-fertilization between American and Japanese oysters. Science 76:371-372. 1932.

10. Hopkins, A.E. Attachment of the Olympia oyster, Ostrea lurida, to plane surfaces. Ecology 16:82-87. 1935.

11. Hopkins, A.E. Ecological observations on spawning and early larval development in the Olympia oyster, (Ostrea lurida). Ecology 17:551-566.1936.

12. Hopkins, A.E. Experimental observations on spawning, larval development, and setting in the Olympia oyster, Ostrea lurida. Bulletin of the bureau of fisheries 48:439-505. 1937.

13. Hori, J. and D. Kusakabe. On the artificial culture of oyster larvae (2). Journal of the Imperial Fisheries Institute 23:79. 1927.

14. Hughes, Edward. The breeding of oysters in tanks. Journal of the marine biological association of the United Kingdom 24:543-547. 1940.

15. Imai, Takeo and others. Artificial breeding of oysters in tanks. Tohoku journal of agricultural research 1:69-86. 1950.

16. Loosanoff, V.L. Letter to W.P. Breese, Oregon State College Fisheries Laboratory. (from) United States department of the interior, fish and wildlife service, Milford, Connecticut. October 14, 1952.

17. Morris, Robert W. Experiments on the larval culture of the native oyster, Ostrea lurida Carpenter. Thesis Oregon State College library. 46p. 1948.

18. Warren, Charles Edward. The flagellate Bodo lens (O.F. Muller) as food for larvae of the native Pacific coast oyster. Thesis Oregon State College library. 1950.