REARING OF THE NATIVE OYSTER LARVAE,
OSTREA LURIDA CARP., IN
CONCRETE AND WOODEN TANKS
UNDER CONTROLLED CONDITIONS

by

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INTRODUCTION

The attempted larval rearing of the native oyster, Ostrea lurida Carp., in concrete and wooden tanks, under controlled conditions, was undertaken at the Yaquina Bay Fisheries Laboratory during the period of June, 1952, to May, 1953. All of the studies were conducted in tanks with a capacity of approximately 250 gallons.

A preliminary investigation of rearing the native oyster larvae in small containers under controlled conditions was made by Robert W. Morris in 1947 and 1948. The first limited success in rearing oyster larvae to the setting stage was obtained by Eugene Haydu in 1948, 1949, and 1950 (unpublished reports). Similar studies, based on rearing larvae of the native oyster under controlled laboratory conditions, were made during the same period of June, 1952, to May, 1953. These experiments were conducted in containers with a maximum capacity of 12 gallons. Data from these studies are included in a companion thesis by Wilbur Breese (1, pp.1-48).

The purposes of the studies at the Yaquina Bay Fisheries Laboratory, in 1952 and 1953, were to duplicate the rearing process developed by Eugene Haydu, to improve the

procedures if possible, and to find a simple and economical method of producing seed oysters that would result in an adequate stock for commercial operations. At the present time, the production of the native oyster is of minor importance in Yaquina Bay. The limiting factor to commercial production of the native oyster in Yaquina and other Oregon bays is, in part, the lack of adequate spatfalls from year to year.

REVIEW OF PREVIOUS WORK

Sporadic attempts have been made to obtain large quantities of spat, or young, recently settled oysters, by the artificial propagation of oyster larvae in tanks. Only in a few cases has it been possible to claim success. Limited success in the rearing of oyster larvae has been attained in England with the European oyster, Ostrea edulis; in Japan with the Japanese or Pacific oyster, Ostrea gigas; and with the native oyster, Ostrea lurida, at the Milford Laboratory in Connecticut.

Larval Rearing of the European Oyster, Ostrea edulis L.

In England, the works of H. A. Cole (1936) and E. Hughes (1940) are cases of successful rearing of oyster larvae in large tanks (9, p.69).

Since the works of Cole and Hughes were practically identical, a brief description of Hughes' work will be given as it is the most recent. During 1939, a water tight tank with brick walls and a concrete floor was put into operation on the River Yealm in England. The capacity of the tank was 32,000 gallons. On June 1, 1939, 200 four-year old European oysters, Ostrea edulis, were placed in single layers on slatted frames on the floor of the tank. The European oyster has a larviparous type of

reproduction. In order to create food organisms for the
oysters, three medium sized crab, minced with sand, were
fed on alternate days. Twenty-two days later, free-swim-
ming larvae were noted for the first time. The feeding
of crab was continued at the same rate throughout the
free-swimming period of the larvae. On July 5, settle-
ments of spat were noted on shells which were placed in
the tank soon after larvae were noticed. Additional
cultch, in the form of limed tile (tile dipped in cement)
was placed into the tank. A later examination of the tile
showed heavy concentrations of spat. The temperature
range from the time of introducing the adults into the
tank to the time larvae were noted was 59° to 68° F.
(6, pp.543-546). As in Cole's work, little attention was
paid to water changes and temperature control; the empha-
sis was placed on development of food organisms.

Larval Rearing of the Pacific Oyster, Ostrea gigas (Thun-
berg).

The most recent work involving the use of concrete
tanks is that of Takeo (1950). The tanks used in these
experiments are located at the Onagawa Fisheries Labora-
tory, Japan. In capacity, they ranged from 19,000 liters
to 2,500 liters. In this case, larvae of the Japanese
oyster, Ostrea gigas, were obtained by artificial

fertilization. The larvae were kept in the laboratory
until they had reached the veliger stage, a free-swimming
stage, and then placed in the tanks (9, pp.71-74).
"Records of breeding experiments in the 1949 season re-
vealed that, under the scheme applied in rather small out-
door tanks, fairly high percentage of larvae reached full
grown stage and rather uniform settling of spat was ob-
tained." (9, p.86) From the results of the 1949 experi-
ments, and other experiments of preceding years conducted
in Japan, it was shown that artificial production of oys-
ter spat was possible by feeding Monas sp. (9, p.54)
"Essential factors for tank culture were a continuous sup-
ply of suitable food organisms and the maintenance of
favorable water conditions for larval growths." (9, p.70)
As for the partial successes and complete failures in
tank rearing of oyster larvae, Takeo states: "It is due to
the difficulties in controlling the quality and quantity
of food organisms, and in managing the condition of the
tank water." (9, p.54)

Larval Rearing of the Native Oyster, Ostrea lurida.

In 1949, larvae of the native oyster were success-
fully reared to the setting stage in aquaria at the Mil-
ford Laboratory in Connecticut.

To obtain the larvae, spawning of adult oysters was

induced in aquaria of warm, aerated, filtered seawater. The water was changed daily through fine screens. The temperature throughout the experiments ranged between 15° C. (59° F.) and 18° C. (64° F.) (2, p.111).

Food of the larvae consists of a culture of green phytoplankton, consisting mostly of a species of Chlorella. Daily feedings were made.

Swarming in the laboratory occurred in seven to nine days. The size of the larvae at swarming varied. Some measured 169 to 185 microns, and others measured only 158 microns at the time of their release.

Different temperatures did not affect the growth rate of the larvae to a great extent, but various temperatures did influence the setting of the larvae. Larvae kept at a temperature of 19° C. (66° F.) to 22° C. (72° F.) appeared to make normal growth and setting occurred when the larvae measured about 300 microns. With a temperature range of 16° C. (61° F.) to 18.5° C. (65° F.), the larvae showed considerable growth, reaching a size of 275 to 290 microns in length. Some larvae lived for 30 days but no setting occurred. A lower temperature of 14° C. (57° F.) to 16° C. (61° F.) seemed to affect both the growing rate and the longevity of the larvae. Some larvae lived for 20 days, but only reached a size of about 220 microns.

LIFE HISTORY OF THE NATIVE OYSTER

The native oyster, _Ostrea lurida_, is found in various locations along the Pacific Coast of North America. It ranges from Queen Charlotte Sound, British Columbia, southward to San Diego Bay, California. "In Oregon, this species is found in Yaquina Bay, Lincoln County, and in extremely limited numbers in Netarts Bay, Tillamook County." (3, p.41)

A market size of 1½ to 2 inches is reached by the native oyster in three to five years (Figure 1). It can be readily identified from the exotic species of oysters found on the Pacific Coast by its small size, and a clearly outlined, uncolored muscle scar on the inner surface of the valve (3, p.42).

The native oyster is a protandric hermaphrodite, reaching sexual maturity at the age of one year as a male, and for the rest of its life is alternately female and male. It has a larviparous type of reproduction, in which the young are retained in a brood chamber for a short period.

Spawning in Yaquina Bay may begin as early as April and continues until August. A few gravid oysters have been found as late as September. The usual spawning period is from late May into early August, with the
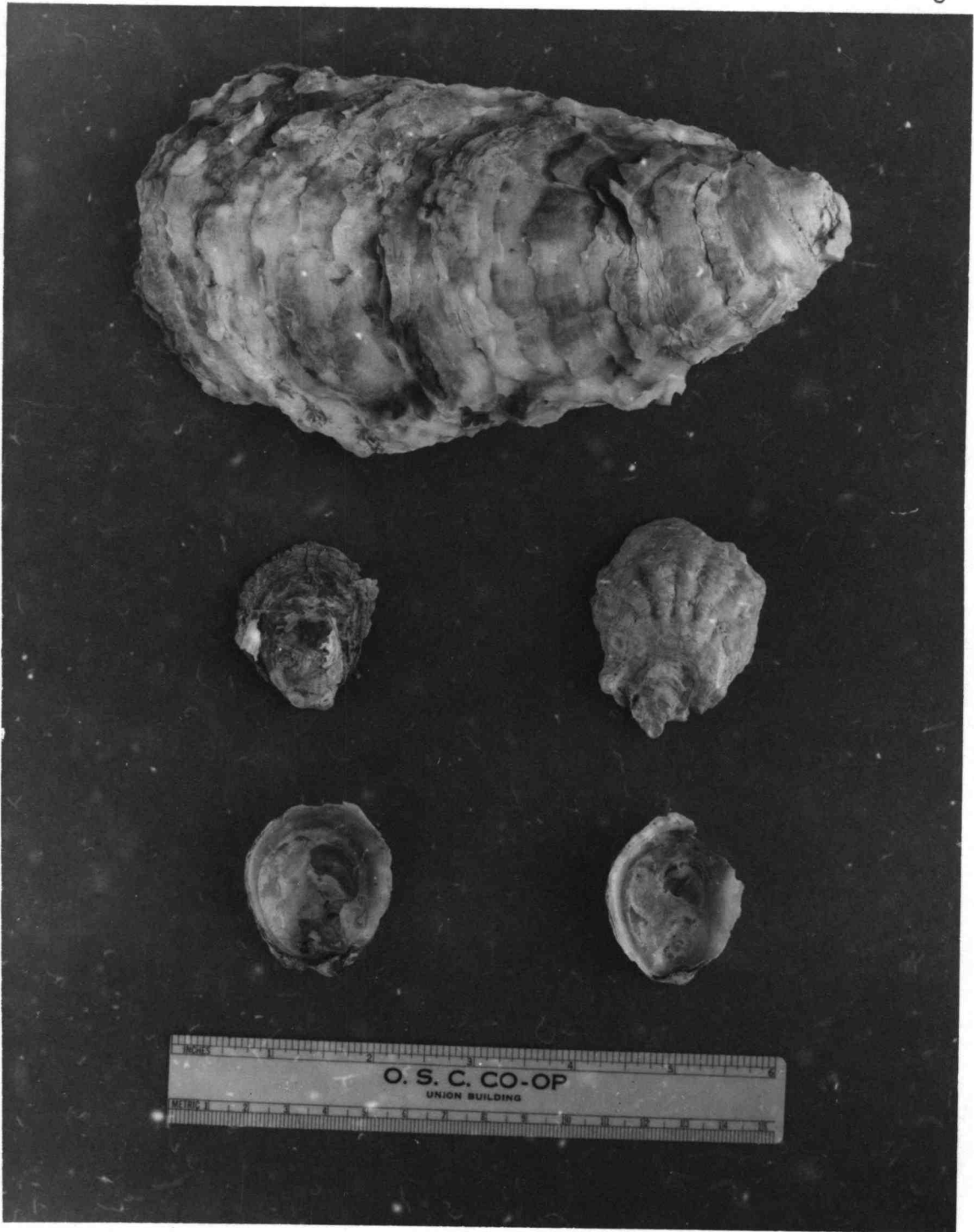
Figure 1. Market size of native oysters of Yaquina Bay compared with larger market size of Pacific oyster (top of picture)

greatest intensity of spawning from the latter part of
May to the middle of July (3, pp.45-48). Spawning con-
sists of the discharge of eggs or sperm balls from the
gonad. The eggs pass from the exhalent chamber through
the gill ostia, and into the inhalent chamber (3, p.43).
(Sperm clusters pass from the gonads to the exhalent
chamber, where they are washed out with the water pumped
by the gills.) Sperm clusters consist of 250 to 2,000 or
more sperms. When in contact with seawater, the matrix
enclosing the sperm disintegrates to release the sperm
into the sea water (5, p.457).

The eggs are fertilized by sperm brought into the
inhalent chamber with water pumped by the gills. During
the period of development, the eggs are retained in the
upper end of the mantle or branchial chamber next to the
gills and labial palps (5, p.457).

According to Hopkins, who studied this species in
Puget Sound, development of the eggs following fertiliza-
tion proceeds as follows: "one day, blastullae; two days,
gastrulae; three days, trochophores; four days, first
conchiferous larvae with incomplete valves; five days,
straight-hinge veliger larvae completely enclosed by
valves 110 to 120 microns long; ten days, veliger larvae
with valves 180 to 195 microns long." (5, p.501)

Upon leaving the gonad, the eggs measure 100 to 105 microns in diameter (5, p.468). Development in the brood chamber following fertilization proceeds at a slow rate until the fifth day when the larval shells become complete and the larvae are in the straight hinge stage (the dorsal border of the valves is straight). Further growth in the length of valves progresses at the rate of about 12 microns per day. On the average, ten days is required for development from fertilized egg to larva of 180 to 185 microns (5, p.570).

Various terms are used to describe the stage of maturity of the oyster larvae within the adult oyster. "White sick" refers to an oyster carrying white embryos or larvae, "greysick", if the larvae are grey, and "blacksick" when of a very dark color (8, p.41). The coloration of the larvae is from pigment which forms in the tissues. Blacksick larvae are usually the larger, but coloration is not a true indication of size (5, pp.469-470).

The term "spawning" has been used to designate the release of the eggs and sperm balls from the gonad. "Swarming" refers to the final release of the larvae from the brood chamber to the water. At the time of swarming, the larvae usually measure 180 to 185 microns (5, p.470). The average number of larvae per brood is between 250,000 and 300,000 (5, p.459).

After swarming, the larva is a free swimming organism
for a period of about 30 days. If the conditions are
satisfactory, a growth of approximately five microns per
day is made by the larvae. During this free swimming
period, the larvae reach an average size of 320 microns
(5, pp.470-471). Development of the larvae from the free
swimming stage to the full grown stage is shown in Figure
2.

Upon completion of the free swimming period, the
larva seeks a suitable substrate for attachment. The
process of attachment is called "setting". Objects such
as rocks, logs, or shell provide places for attachment.
It is to this cultch that the larva cements itself by its
left valve. Following attachment, the "spat" undergoes a
morphological change and begins a sedentary mode of life.

Since the oyster can no longer move about, it must
depend on the water to transport food to it. During feed-
ing, the action of cilia on the gills creates a flow of
water through the inhalent chamber. As the water passes
over the gills, certain cilia select suitable food parti-
cles which are engulfed in mucous, and passed by another
set of cilia toward the mouth. Food may consist of dia-
toms, peridinians, microscopic animals, bacteria, and
fragments of plants.

A
160 x 149

B
165 x 147

C
189 x 163

D
204 x 185

E
223 x 200

F
232 x 205

G
241 x 214

H
254 x 219

I
277 x 241

J
295 x 260

K
312 x 295
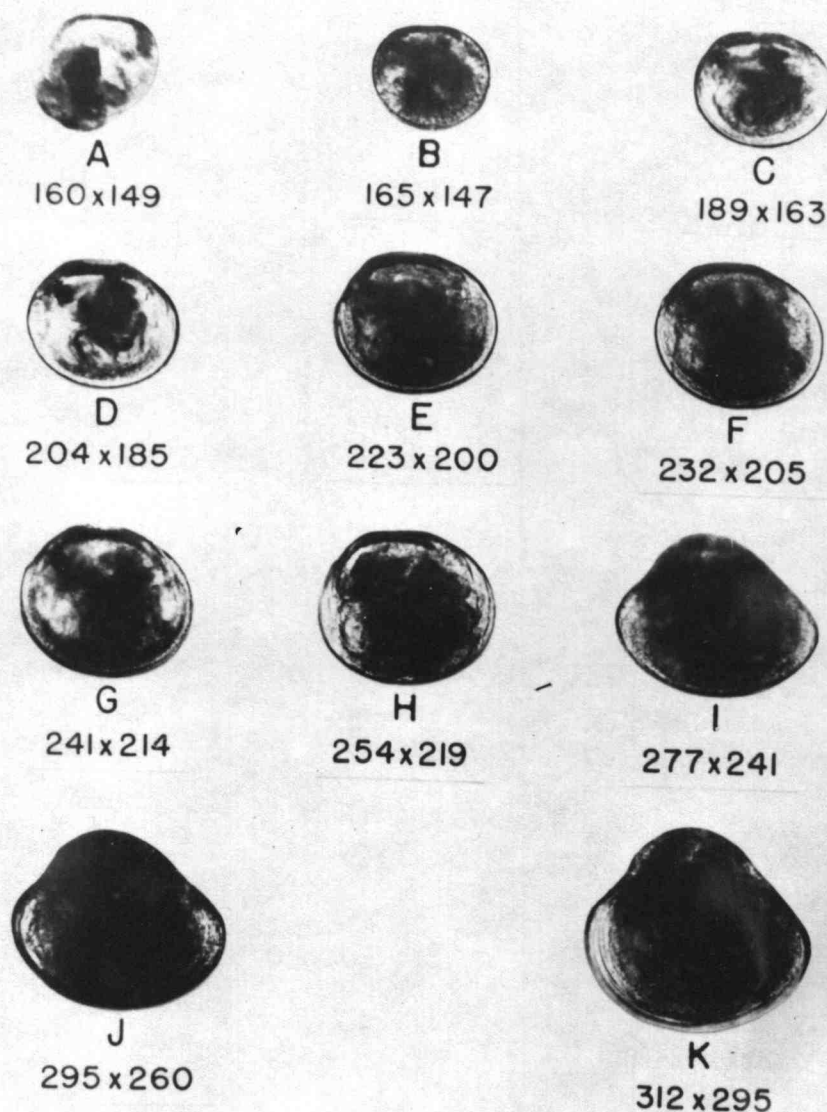
OSTREA LURIDA

X 112
Figure 2

Stages in the development of the larvae from the straight
hinge veliger stage to full grown larvae of setting size.
(Permission for use of photograph from Dr. V. L. Loosan-
off, U. S. Fish and Wildlife Service, Milford, Connecti-
cut. Picture taken by Harry C. Davis. Picture is not to
be published, mimeographed, or prepared for general dis-
tribution until published by Davis.)

DESCRIPTION OF EQUIPMENT

Concrete Tank with Temperature Control

At the time Eugene Haydu was assistant biologist at the Yaquina Bay Fisheries Laboratory, two concrete, steel reinforced tanks were constructed. A coil heater was imbedded in the floor of one tank; the other tank was to be used as a control tank. Both tanks were housed in an unheated, wooden frame building adjacent to the laboratory proper.

The outer dimensions of the temperature controlled tank are six feet long, three feet, two inches high, and four feet wide. The walls are four inches thick. Inner dimensions are five feet, four inches in length, three feet, four inches in width, and the depth at one end is two feet, one inch, and at the other end two feet, two and one-half inches. The bottom of the tank has a slope of 2.34 per cent. Side, end, and plan views, with dimensions, are shown in Figure 3. Drainage of the tank is controlled by a two-inch valve. The drainage pipe empties directly into the bay. The capacity of the tank, within six inches of the top, is approximately 950 liters. A photograph of the temperature controlled tank is shown in Figure 4.

Side View

End View

Plan View
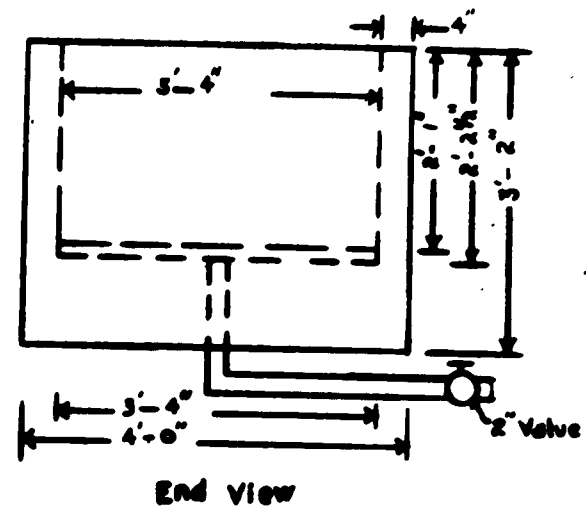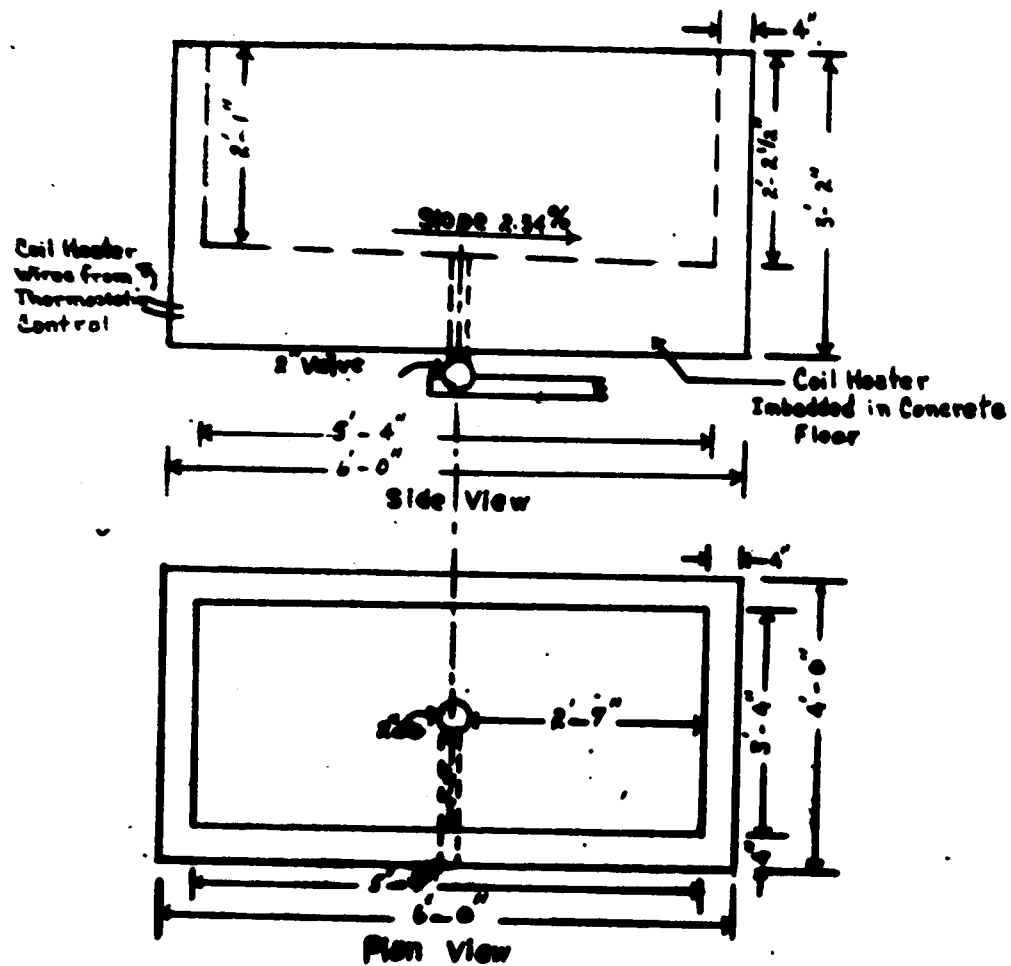
TEMPERATURE CONTROLLED
CONCRETE TANK
Scale 1/2" = 1'
Fig. 3

Figure 4

Temperature Controlled Concrete Tank

Heating the water in the tank is provided for by a 250 volt (alternating current) thermostat controlled coil heater with a heating range of 0° - 250° F. The heater is imbedded in the cement floor of the tank between alternating layers of sand and lead plate.

Following completion of the tank, it was found that it was not completely water tight. It was then necessary to put another layer of concrete on the floor of the tank. This resulted in the heater being covered with approximately 10 inches of material. Further treatment to make the tank water tight consisted of painting the inner surface with a clear, plastic base liquid called Tygon.

During the summer of 1952, no difficulty was experienced in maintaining an even temperature of 68 to 70 degrees F. in the tank. Attempts to bring water in the tank to desired rearing temperatures during the winter of 1953 failed. The highest temperature that could be maintained was about 55° F. Too much material covering the heater and possible damaging effects by water seepage to the coils of the heater may have been the causes of the improper functioning of this heated tank.

This problem of heat control during the winter has now been eliminated. Recently, a ceiling and walls have been put inside the building which houses these tanks. The walls and ceiling were well insulated. Heat is

provided by a portable 220 volt thermostatically con-
trolled electric heater. This arrangement does not only
do away with a serious handicap, but permits expansion of
facilities needed at the Yaquina Bay Fisheries Laboratory
in order to conduct increased numbers of experiments with
rearing of larvae during the winter months.

Concrete Control Tank

The concrete control tank has the same outer dimen-
sions, approximately, as the temperature controlled
heated tank. Its inner dimensions are five feet, four
inches in length, three feet, four inches in width, and
two feet, seven inches in depth. The approximate capa-
city of this tank filled within six inches of the top is
1019 liters. Side, end, and plan views of this tank are
shown in Figure 5.

Wooden Tank

The circular wooden tank in use at the laboratory was
obtained from the National Tank Company of Portland, Ore-
gon. It was assembled during the summer of 1952. The
tank is four feet high, has a diameter of four feet and a
capacity of about 1000 liters. It is made of Douglas Fir
staves about five inches wide and one and one-half inches
thick, and is held together by bands of steel rods,

Side View

Plan View

End View   Section A-A'
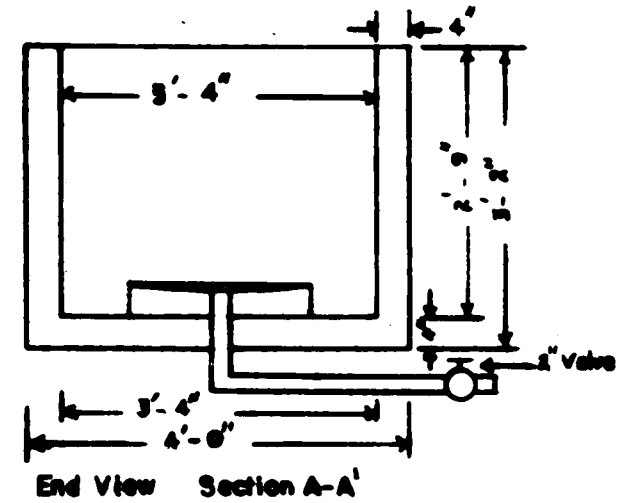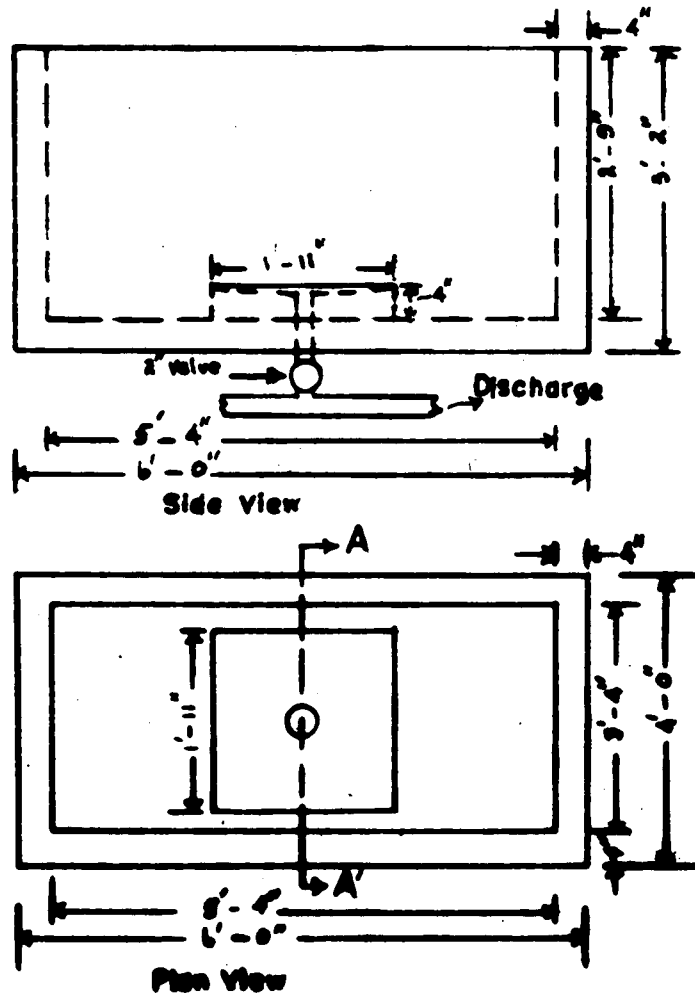
CONCRETE CONTROL TANK

Scale 1/2" = 1'

Fig. 5
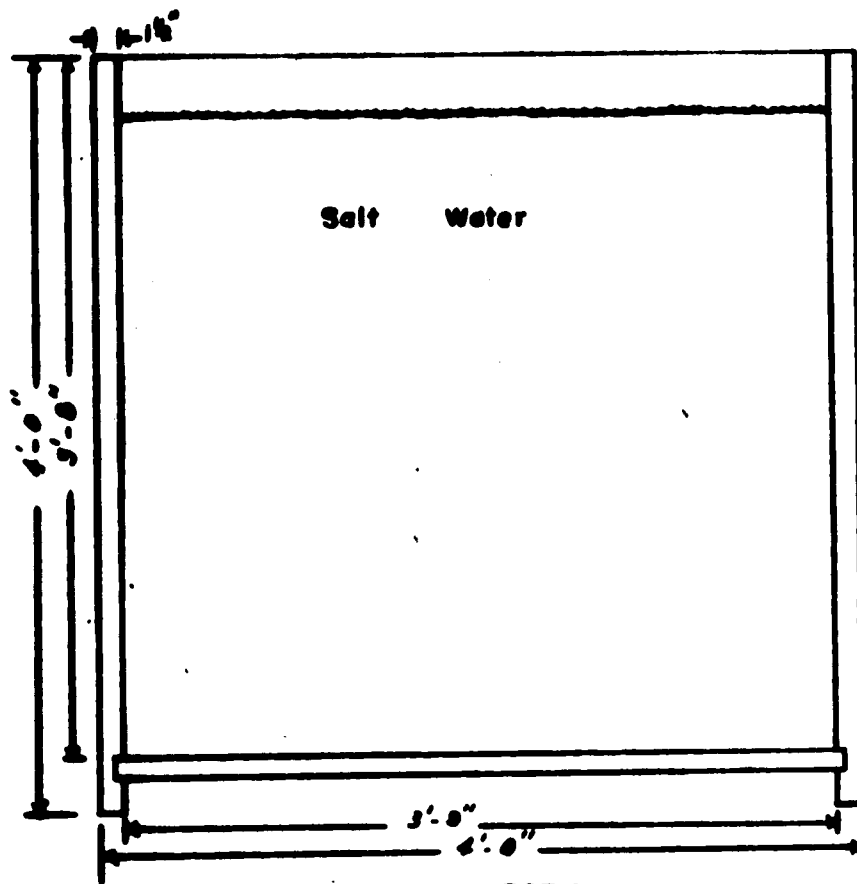
CROSS SECTION
250 Gal. WOOD TANK Fig.6
Scale 1"= 1'

Figure 6

Figure 6.

Rendering Concrete Tanks Innocuous to Larvae.

One of the main problems encountered before the
smaller concrete tanks at the Onagawa Fisheries Laboratory
in Japan could be used for the larval culture of Ostrea
gigas was the elimination of alkaline diffusion from the
tank. The Japanese oyster larvae never showed very good
growth in waters with the pH above 8.5. It took four
years of conditioning before the tanks were ready for use
(9, pp.70-72).

A similar problem of excessive alkaline diffusion
presented itself when the two concrete tanks were made at
the Yaquina Bay Fisheries Laboratory. Since Haydu placed
the tanks into satisfactory operation for oyster larvae in
a short time after construction, an answer to an inquiry
as to procedure for rendering the tanks harmless to
larvae states: "In reply to your question concerning
treatment of the concrete tanks for rendering them in-
nocuous to oyster larvae, the following was done: a. De-
termined alkalinity (as p.p.m. CO_3); b. Added an equiva-
lent amount of H_2SO_4 (in p.p.m.) to the concrete tanks
which were filled with fresh (not sea-water) water. They
were allowed to soak for about one week. During this
time, I checked the pH daily. When it increased above

7.5, I drained the water, renewed the fresh water, and
gave it the same acid treatment for another week. The
tanks were then filled with sea water and were ready for
use. However, I changed the sea water every few days for
the next couple of weeks as a precaution against further
leaching of alkali from the tanks."

Filter Apparatus

As a means of filtering the sea water used in the
concrete tanks, a circular tank of about 130 gallons ca-
pacity was used. The filter apparatus employed to elimi-
nate undesirable organisms from the bay water was a modi-
fication of that first used by Robert Morris (10, p.35).

At the bottom of the tank, a layer of glass wool was
used to keep the sand from escaping. A 12-inch layer of
beach sand was placed over the glass wool. Above the
sand, a perforated stainless steel plate was placed to
break the fall of the water, which was pumped directly
into the filter from the bay. A cross section of the
filter ready for actual use is shown in Figure 7.

This type of filter, although removing larger or-
ganisms sufficiently, did not prove to be entirely satis-
factory. The rate at which the water was filtered was too
slow, requiring from two to three days to fill one con-
crete tank. During the winter, the filter was again put

Intake

2' - 6"

Salt   Water Circular
        Tank

3' - 7"

4' - 5"

Perforated
Plate

Beach Sand

Glass
Wool

2' - 5"

Outlet

2"
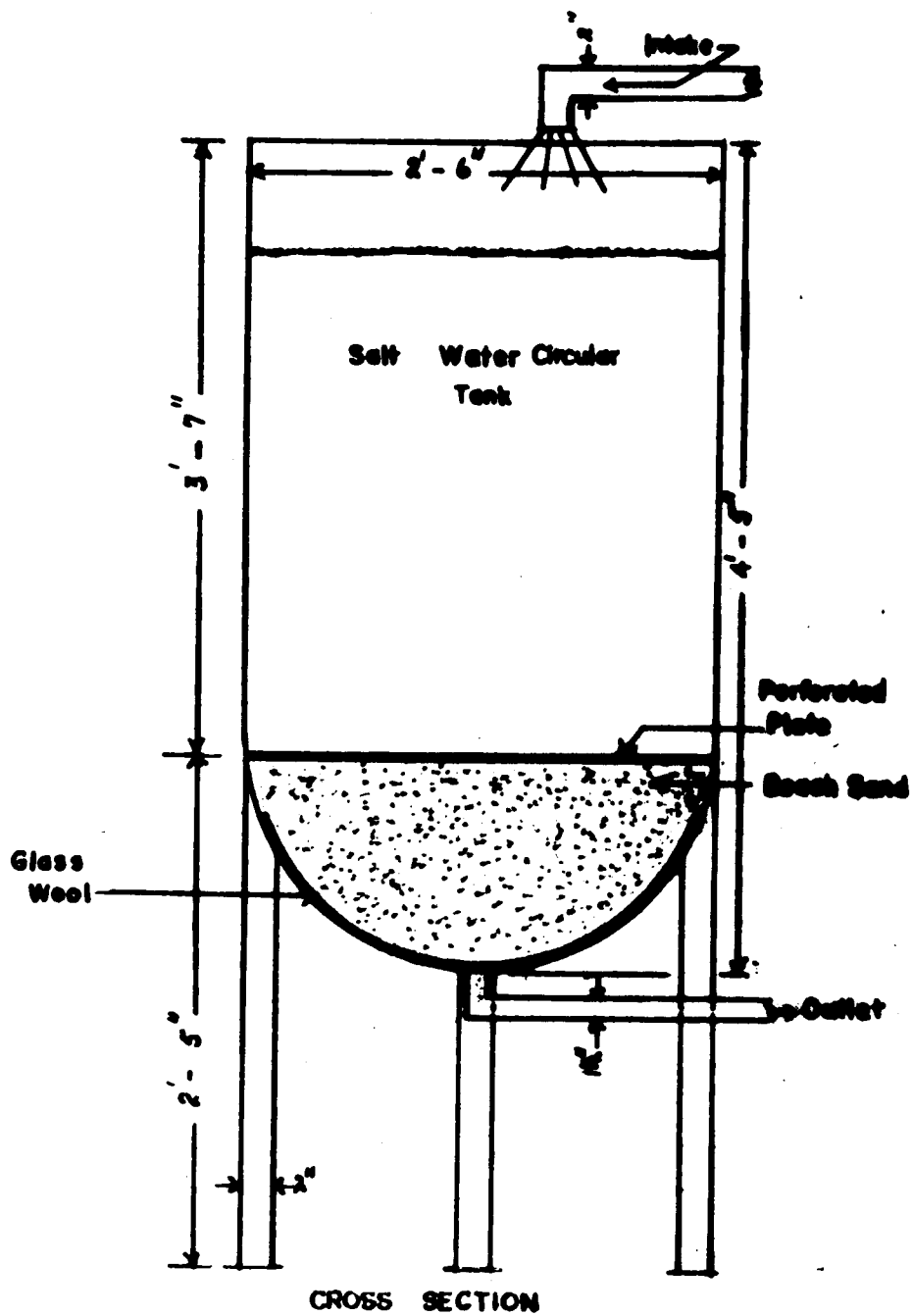
CROSS   SECTION

130 Gal. SALT WATER FILTER

Scale 1"= 1'

Fig. 7

use and the water from the filter was a deep rust color. Apparently, the inside jacket had rusted to quite an extent due to its contact with the salt water. It was believed that such a concentration of rust in the water might be harmful to oyster larvae, so the filter was discarded.

The filtered sea water employed in the wooden tank was obtained by a type of filter apparatus designed by Robert Morris (10, p.35). The original filter was recently modified so that greater quantities of water could be obtained in a shorter period of time. This same type of filter has now replaced the tank filter used to obtain water for the experimental concrete tanks.

Since this vacuum type apparatus is described in detail in the companion thesis by Wilbur Breese, only a brief outline of its operation will be given here. Salt water was brought into the laboratory room by a plastic line. A regulated flow of water was directed into a 12-gallon earthenware crock which had from two to three inches of beach sand in the bottom. Imbedded in this sand was a quart bottle without a bottom. The bottle was partly filled with glass wool to keep the sand from passing out of the crock. Glass tubing inserted through a rubber stopper was placed into the neck of the bottle, and was connected to a number of 5-gallon jars which were

connected to each other by glass and rubber tubing.
Vacuum in the bottles was obtained by attaching a water
jet laboratory vacuum pump to a water line. Vacuum cre-
ated in the bottles maintained a suction which pulled the
water through the filter and into the bottles.

Salt Water Supply

Formerly, salt water used in the concrete tanks was
pumped directly into the tank filter. Water used to sup-
ply the wooden tank in the laboratory was brought into
the laboratory by a plastic hose leading from the wooden
salt water storage tanks. These tanks are adjacent to
the building housing the concrete tanks, Figure 8. These
storage tanks will now supply water for use in the con-
crete tanks.

The salt water was pumped directly from the bay by a
stainless steel, electric water pump. Stainless steel,
one and one-half inch pipe, extending from the pump to
about 40 feet out into the bay serves as the intake for
the pump. The water from the pump passes through a one-
inch rubber hose which can be directed to either of two
storage tanks.

Figure 8

Salt Water Storage Tanks

METHODS OF OBTAINING LARVAE

Source of Larvae

All of the larvae of the native oyster, used in the studies at the Yaquina Bay Fisheries Laboratory, were obtained from mature oysters tonged at Lewis Flats, located at Oysterville.

The beds from which the oysters were tonged are usually covered with 10 to 20 feet of water at low tide. Oyster tongs were used to obtain the oysters lying on the bottom. This method of gathering oysters is usually referred to as "tonging". A pair of oyster tongs can be readily visualized if one would place two garden rakes together with the teeth interlocking. A bolt through both handles, placed well below center, would allow that part of the rake having the teeth to open when the handles were spread apart. Pressure against the handles would cause the teeth to come together to form a sort of basket to hold any object caught above them.

Swarming.

After the adult oysters were brought back to the laboratory, the shells were scraped relatively free of barnacles and other organisms which usually are found on oysters. Then the oysters were thoroughly cleaned with

water to remove any further organisms which would be detrimental to the larvae.

Following cleaning, 24 oysters were placed in 5-gallon wide-mouthed jars containing 15 to 18 liters of filtered salt water. These jars were kept in the laboratory where the temperature could be maintained at about 70 degrees F. Swarming usually occurred in one to two days during the summer months. Upon swarming, the larvae together with the water were siphoned into another jar. Larvae, under laboratory conditions, appear to have the action of settling to the bottom of the container in about 12 hours following swarming, and then swimming up again in about 24 hours. In order to insure having active larvae, they were allowed to stay in the jars for 48 hours.

Induced Winter Spawning

Spawning of the native oyster in mid-winter can be accomplished by keeping the oysters in sea water at room temperature. Repeated success was attained at the Washington State Oyster Laboratory at Gig Harbor, Washington, in inducing the native oyster, _Ostrea lurida_, to release larvae in mid-winter by keeping them in warm sea water. Feeding of the adult oysters does not appear to be necessary in order for gonads to develop. In his work with

the American oyster, Ostrea virginica, Loosanoff found
that the gonad development of oysters kept for one month
at a temperature of about 25° C. (77° F.) during the
winter compared favorably with oysters developing their
gonads in the summer under natural conditions (7, p.125).

On December 12, 1952, three dozen adult native oys-
ters from Lewis Flats were brought into the laboratory
and placed in 5-gallon wide-mouthed jars. Each jar con-
tained 15 to 18 liters of filtered sea water. Twelve
oysters were placed in each jar. The water in the jars
was kept at a temperature of 70 degrees F. No food was
given to the oysters. The water was constantly aerated
by a Thiberg Aerator. About once every week to ten days,
the water was changed. Probably some food was made avail-
able to the oysters through these water changes.

In six to eight weeks after the adult oysters were
brought into the laboratory, several swarmings of oyster
larvae occurred. On one occasion following swarming, the
majority of the larvae were dead. The first swarming of
larvae from oysters brought into the laboratory in Decem-
ber occurred on March 4, 1953.

Measurement of Larvae by Volume

Following the removal of the oyster larvae from the
adults, an estimate as to the number of larvae per liter

was made. The water was gently stirred in order to obtain a fairly even distribution of larvae. With a pipette having a large opening, a 10 ml. sample was obtained. The sample was divided equally in 10 small dishes. A count of the larvae was made with the aid of a hand lens or dissecting microscope. The total number of this sample was multiplied by 100 to obtain the approximate number of larvae per liter.

Measurement of Larvae in Length

The first procedure in measuring larvae was to inactivate them with a five per cent solution of urethane. Following this, the larvae were placed under the low power objective of the microscope, and measured with a micrometer type of ocular. The greatest shell distance parallel to the hinge was usually measured. All measurements were recorded in microns.

FOOD OF LARVAE

The materials which make up the food of the free swimming native oyster larvae in its natural habitat is not definitely known. It is thought to consist of mostly micro-organisms. In size, any food particles of less than nine microns probably can be readily utilized by the oyster larvae.

A series of studies, terminating in 1951, were conducted at the Yaquina Bay Fisheries Laboratory. They were based on the use of the flagellate, Bodo lens (O. F. Muller), as food for the larvae of the native oyster. Feeding of larvae was observed under low and high power of a microscope (10, p.56). Bodo lens was seen to be entangled in the mucous used by the larvae in feeding. No intact Bodo lens were observed in the gut of the larvae. Stained particles thought to be nuclei were observed. It is apparent that Bodo lens can be readily ingested, but whether or not this flagellate can be assimilated by the oyster larvae is not definitely known (10, pp.111-112).

In recent work with rearing of the native oyster larvae at the laboratory, Bodo lens was used as food for the larvae. It is assumed that this flagellate is utilized as food by the larvae.

Bodo lens is a minute, colorless, unarmored

flagellate having two flagella, and a rather plastic form
(10, p.37). This species can be readily identified by
its possession of one or two posterior vacuoles. In the
other species, the vacuoles are anterior in position
(10, pp.18-19).

In order to be certain that the cultures now in use
at the laboratory contained Bodo lens, a sample was
brought to Corvallis. With the assistance of Mr. Eugene
Wellman, bacteriologist at the Corvallis Clinic, speci-
mens were fixed in a Shaudinn's Solution and stained with
Wright's stain. Observation of the stained specimen under
the high power objective of a microscope revealed the
vacuoles to be in a posterior position which complies with
the characteristic used to identify Bodo lens.

CULTURE OF LARVAL FOOD

Culture Medium

A suitable medium for culturing <u>Bodo</u> <u>lens</u> was first developed by Robert W. Morris (10, p.21). The medium now used at the Yaquina Bay Fisheries Laboratory has been slightly modified. In place of one liter of salt water having a salinity of 17 parts per thousand, 500 ml. of fresh water and 500 ml. of salt water of various salinity are substituted. The components of the present medium are as follows:

> 500 ml. of fresh water
> 500 ml. of salt water
> 1.50 grams of starch
> 0.15 grams of potassium nitrate
> 0.30 grams of sodium phosphate

Medium used for the culture of <u>Bodo</u> <u>lens</u> is made up as follows: 500 ml. of fresh water and 500 ml. of salt water are poured into a 1500 ml. beaker. The water is heated and allowed to boil for a few minutes to destroy any large organisms which may prey on the <u>Bodo</u> <u>lens</u>. Next the dry ingredients are added. As a precaution against burns, the dry ingredients should be added slowly. A rapid introduction of the dry material causes violent boiling. The mixture should be stirred for a short time to dissolve all materials and then allowed to cool.

Obtaining a Pure Culture

After the medium has cooled, about 200 ml. is poured
into 250 ml. Erlenmeyer flasks. The medium is inoculated
with two to three ml. of salt water directly from the bay.
This culture is then allowed to stand at room temperature
for three to five days. During this period, the culture
should be examined. If large unwanted organisms are
noted, re-inoculate new medium until the desired, pure
culture of Bodo lens is obtained. Once the pure culture
is available, re-inoculate succeeding medium from this
pure culture. The culture should be examined frequently
as large organisms can be introduced to the pure culture
accidentally.

Growth Rate

Bodo lens, as well as many other protozoa, reproduce
by binary fission, which is the division of an individual
into two parts. The number of individuals produced in a
certain length of time varies considerably.

In order to establish an idea of how many Bodo lens
per cc. would determine if the culture was good or poor,
an arbitrary scale was employed. A 200 ml. sample of a
culture containing 1,500,000 or more individuals per cubic
centimeter was considered very good; one with 320,000 to
500,000 per cc., good; one with 100,000 to 200,000 per

cc., fair; one with 50,000 to 75,000 per cc., poor. The determination of the approximate number per cc. was based on an actual count of two samples of culture. Eugene Wellman, bacteriologist, made the actual count with the use of the Neufield hemocytometer. The culture considered good contained 321,000 Bodo lens per cc., and that one considered very good had 1,040,000 per cc.

In a period of three to five days following inoculation, a poor to good culture may be expected. From six to eight days of growth, a culture may range from fair to good. Good to an occasional very good culture can be expected in nine to thirteen days. A very good culture usually appears in 14 to 15 days. It appears that in a 200 ml. sample, Bodo lens reach their reproductive peak in about 15 days. After this time, there is a gradual decline in numbers. The time in which all individuals will disappear from a culture is not known, but cultures have been kept in the laboratory for a period of 27 days.

Usually, the medium to be inoculated is divided among six 250 ml. Erlenmeyer flasks. Each sample is inoculated from the same culture and with the same amount. No matter how long the period of growth, seldom will the rate of reproduction in each flask be the same. The cause is unknown.

Factors Affecting Growth

Temperature. A fortuitous circumstance during the winter of 1952-1953 accidentally disclosed the limiting effects of high temperatures on growth of the larval food organisms. The shelf in which the cultures were normally placed proved to be too small to hold the necessary cultures required for feeding. Consequently, flasks containing the cultures were placed at various levels throughout the temperature controlled room.

Examination of the cultures showed those nearer the ceiling had fewer numbers of _Bodo lens_. Six series of six each of 250 ml. Erlenmeyer flasks were filled with 200 ml. of medium, inoculated, and placed at various levels in the room. In those cultures nearest the ceiling, the temperature was 89.6° F. No reproduction occurred at this temperature. In fact, no trace of the original inoculation could be found. Cultures with a temperature of 87.8° Fahrenheit showed very little reproduction upon examination. A temperature of 87.8° F. appears to be the maximum temperature at which _Bodo lens_ can survive. Those cultures having temperatures of 75° F., 73° F., 63.5° F. and 63° F. showed good to very good concentrations of _Bodo lens_. The minimum temperature at which _Bodo lens_ will reproduce has not yet been determined.

Salinity. As previously mentioned, 500 ml. of salt
water is a part of the medium used for culturing Bodo
lens. At irregular intervals throughout the winter, the
salinity of the bay water was checked and recorded. It
was noticed that periods of heavy rains greatly lowered
the salinity of the bay. In order to determine the in-
fluence of various known salinities on the growth of Bodo
lens, culture medium was made up of fresh water and of
salt water with various known salinity. Six 200 ml.
samples were obtained from medium made up, in part, of
fresh water and salt water with salinities of 5, 14, and
26 parts per thousand. All samples were inoculated. Al-
lowing a period of five days for growth, all six cultures
made of each of the various types of media were examined
under the low power objective of a microscope. Cultures
of the medium made with fresh water showed no reproduc-
tion or trace of Bodo lens. That culture made from salt
water with a salinity of five parts per thousand showed
very poor growth. Those cultures made with salinities of
14 parts per thousand and 26 parts per thousand showed a
good growth of Bodo lens.

Cultures made in the summer from medium with salt
water having a salinity of 34 parts per thousand did not
indicate that such a high salinity would hamper the nor-
mal reproduction rate of Bodo lens.

EXPERIMENTAL REARING OF OYSTER LARVAE IN
HEATED CONCRETE TANK

First Experimental Rearing.

Prior to the introduction of oyster larvae to the heated concrete tank, the walls and bottom were thoroughly scrubbed with brush and water. The drain of the tank was covered with a perforated piece of stainless steel screen, and a sheet of glass wool was placed over the screen. A layer of sand two to three inches thick was spread over the entire bottom of the tank. The screen kept the glass wool from plugging the drain, due to the weight of the sand, and the glass wool prevented the loss of sand by drainage. This arrangement permitted drainage of the water without loss of oyster larvae. To prevent the force of the filtered water from disrupting the sand level on the bottom, the flow of water was directed into a glass jar which was placed on the layer of sand. The tank was then filled with approximately 963 liters of filtered sea water, and heating of the water was started. A thermostat maintained the water at a fairly constant temperature.

On June 6, 1952, approximately 96,300 larvae with an average size of 166.8 microns were introduced into the tank. The temperature at this time was 62° F., the

salinity 31.4 parts per thousand, and the pH of the water
was 8.0. Feeding of the tank with 400 cc. of culture of
Bodo lens was started on June 21, 1952. On the following
day, 150 cc. more of the culture was added to the tank.
Thereafter, 200 cc. of culture was put into the tank at
irregular intervals. The number of feedings increased as
the larvae became larger.

Temperature was maintained as near to 70° F. as pos-
sible, but the temperature ranged from 65° to 70° F. The
average temperature throughout the experiment was 67.7° F.

The salt water in the tank was changed once in the
course of the experiment. The salinity of the new water
was 31.5 parts per thousand; at the time that the experi-
ment was concluded, the salinity had increased to 33.4
parts per thousand. This increase appears to be caused by
evaporation of the water.

Larvae were measured on the fourth day after being
placed in the tank. At this time, the maximum size found
was 207.7 microns, the minimum 183 microns, and the ave-
rage size was 198 microns. The average size was that of
ten larvae selected at random. The maximum and minimum
size was that of the largest and smallest larvae in that
sample. On July 5, 1952, the average size of larvae
measured was 228.8 microns and maximum size was 250.8 mi-
crons. The rate of growth during this 15-day period was

4.2 microns per day. This corresponds very closely to the average growth of five microns per day made by oyster larvae during a 30-day free-swimming period in their natural habitat.

On July 6, 1952, cultch in the form of shells of the Japanese oyster, Ostrea gigas, were placed in the tank. The shells were drilled and strung, ten shells to a string, on nylon leader material. Six such strings served as cultch in the tank. On July 8, 1952, 18 days after oyster larvae were placed in the tank, some setting of larvae, now spat, were noticed on the cultch. The spat were not measured, but since a number of larvae were measured two days previously, it was assumed that size of the spat was about 250 microns.

There are varied opinions concerning the size of newly set spat of the native oyster. Some spat found on shells had a length of 255 microns, and other spat artificially propagated had a length of 320 microns. Under natural conditions, larvae usually have a length of near 320 microns at setting (5, pp.470-471). In previous studies at Yaquina Bay Fisheries Laboratory, larvae were reared, under artificial conditions, to the setting stage. Spat measuring 233 microns were found on cultch on the 21st day (10, p.84).

The usual procedure in recent studies at the Yaquina Bay Laboratory when larvae of 250 microns were found, was to introduce cultch. At the end of 21 days, if no larvae measure 250 microns, cultch was placed in the tank regardless of the size of the larvae.

Following the initial setting observed on July 8, 1952, a spat count of the under surface of the cultch was made on July 12, 1952. Since the old spat were circled with a pencil, newly settled spat could be readily counted. There was a fair increase in the number of spat. On July 15, 1952, the last spat count was made. This count again showed a slight increase in the number of spat. The actual number of spat on the under surface of the cultch or string #1 was 50; string #2, 34; string #3, 69; string #4, 67; string #5, 79; and string #6, 85. The total number of spat counted on all cultch was 384. About an equal number of spat were found on the glass bowl which had been in the tank since the beginning of the experiment. Figure 9 shows the bowl with a few remaining spat. Spat of the native oyster are characteristically found most abundantly, in nature, on the under surface of shells and other forms of cultch (4, p.86). In this spat count, little attention was paid to the top surface of the shell. When the experiment was concluded on July 17, 1952, the
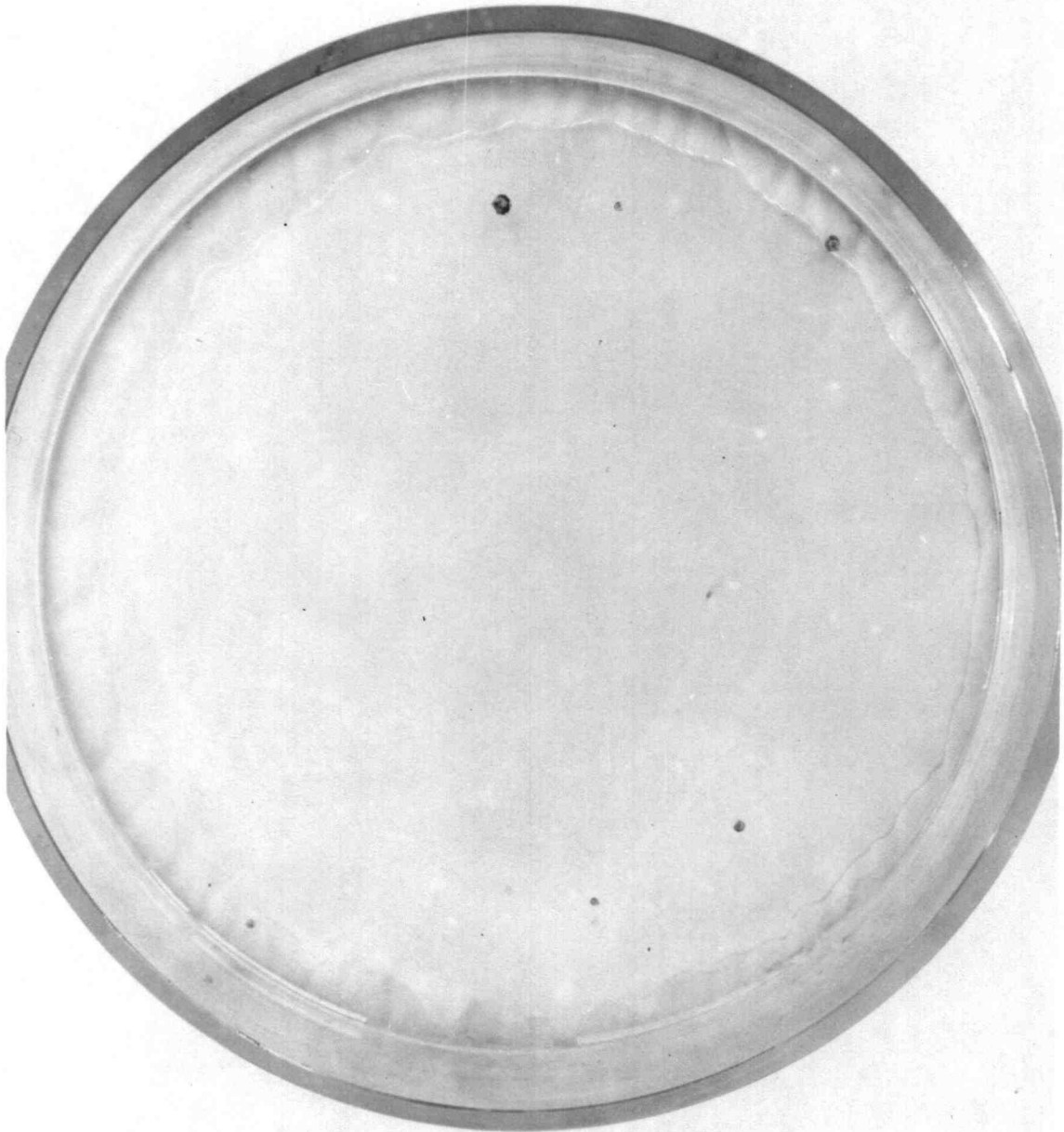
Figure 9.
Glass bowl with remainder of spat which set in July, 1952.

cultch, together with the spat, were placed in one of the salt water storage tanks. The glass bowl was kept in a crock in the laboratory. Examination of the cultch several months later revealed that about the same number of spat were in the depressions on the top surface as were found on the flat, smoother, lower surface of the shells. The reason that the spat on the top surface were overlooked during the first examination was that they were very small, not numerous, and well hidden in crevices in the shell. In many instances, in later experiments, spat have been found to be as numerous on the top surface of the shell and, on several occasions, to be more numerous than those spat found on the lower surface of the cultch.

In concluding this experiment, the primary purpose of the studies under way at the Yaquina Bay Laboratory, that of rearing native oyster larvae to the setting stage in cement tanks, has been accomplished. A start toward the goal of obtaining spat on a commercial basis has been established. Data of this first experiment in the heated concrete tank is presented in Table I.

Second Experimental Rearing

Following the conclusion of experiment #2, the water was drained out of the tank, the old sand in the bottom

TABLE I

DATA OF FIRST EXPERIMENT IN HEATED CONCRETE TANK

1952 Date	Size of Larvae in Microns	pH	Salinity in parts per thousand	Temperature Degrees F.	Amount of Food Culture in cc.	Water Change
6/20	Ave.166.8	8.0	31.4	62		
6/21				66 69	400	
6/23				74	150	
6/24	Max.207.7 Min.183 Ave.128	8.0	31.4	71 74		
6/25				70		
6/26				68	200	X
6/27					200	
6/28				68		
6/29				68		
6/30	Max.229 Min.208 Ave.217			68	200	
7/1				72		
7/2			31.5	72	200	
7/3				70		
7/4				68	200	
7/5	Min.204.2 Max.250.8 Ave.228.8			70	200	
7/6 (cultch added)				72	200	

TABLE I (Continued)

1952 Date	Size of Larvae in Microns	pH	Salinity in parts per thousand	Temperature Degrees F.	Amount of Food Culture in cc.	Water Change
7/7				70		
7/8 *				72	200	
7/9				72.5		
7/10				70	200	
7/11				70		
7/12				72	200	
7/13				70		
7/14				70	200	
7/16				70	200	
7/17**			33.4	70.5		

* Some setting of larvae on cultch

** Discontinued Experiment

was removed, and the tank was scrubbed with brush and water. A layer of fresh sand was placed in the bottom. On July 17, 1952, filtered sea water was started into the tank, and the heating unit in the tank was put into operation.

On July 18, 1952, an estimated total of 482,000 native oyster larvae were placed into the water. The average size of these larvae was 174.9 microns. The temperature at this time was 68° F., the salinity 33.4 parts per thousand, and pH of the water was 8.0. Feeding of the tank with 1000 cc. of culture of Bodo lens was started on July 19, 1952, and continued throughout the experiment at two to three day intervals.

As in the first experiment, the temperature fluctuated to some extent. The temperature ranged from 68° to 75° F.; the average temperature throughout the experiment was 70.5° F.

On July 22, 1952, the fourth day after the larvae were placed into the tank, a sample of ten larvae was measured. The maximum size was 183.9 microns, the minimum size was 176.8 microns, and the average size was 178.6 microns.

Examination of the water for living larvae on July 26, 1952, proved to be fruitless. A further check on the 28th failed to disclose any larvae. In ten days

time, all of the larvae had disappeared. In this time,
the original salinity of 33.4 parts per thousand had in-
creased to 34.2 parts per thousand. Such a high salinity
is very near that of ocean water. Since it is known that
oysters do not thrive in normal ocean water, it is as-
sumed that the excessive salinity of the tank water,
reached through evaporation, was responsible for the
mortality of the larvae.

In concluding this experiment, it was suspected
that the cause of failure was excessive salinity. Data
from this experiment is presented in Table II.

Third Experimental Rearing

At the time that the third and final experiment in
the heated concrete tank was started, bay salinity at
Yaquina was approximately that of the ocean, 34.0 parts
per thousand. With the decision to retain the water of
experiment #2, 190 liters of fresh water were added. This
reduced the salinity from 30.2 to 26.4 parts per thousand.

Since salt water with a salinity range of 28 to 32
parts per thousand was frequently needed in the various
experiments under way at the laboratory, a search for a
quick and accurate method of reducing salinity was made.

A series of tests using 1000 ml. samples of salt

TABLE II

DATA OF SECOND EXPERIMENT IN HEATED CONCRETE TANK

Date 1952	Size of Larvae in Microns	pH	Salinity in parts per thousand	Temperature Degrees F.	Amount of Food Culture in cc.	Water Change
7/18	Ave.174.2	8	33.4			
7/19				68	1000	
7/20				70.5		
7/21			34.1	75	1000	
7/22	Max.183.9 Min.176.8 Ave.178.6			68		
7/23				70	1000	
7/24				74		
7/25			34.1	70	1000	
7/26*			34.2	69	1000	
7/27				72	1000	
7/28*						

* Failed to find larvae

* Further check failed to disclose any larvae

water was made on July 25, 1952. The salinity of these
samples ranged from 34 parts per thousand to 21 parts per
thousand. The temperature ranged between 72° F. to 73.4°
F.; the average temperature throughout the experiment was
72.7° F. In each case, the salinity of the 1000 ml. of
salt water was first determined. By constantly checking
the salinity, fresh water was added until the desired
lower salinity was reached. In all tests, the salinity
was lowered one part per thousand. The amount of fresh
water required to lower the salinity varied greatly. No
satisfactory results were obtained from this series of
tests.

With the assistance of W. P. Breese, a ratio and
proportion formula for reducing salinity was formed.
The known salinity was divided by the salinity desired.
This number was multiplied by 1000, then subtracted from
1000. The resulting number was multiplied by the number
of liters in the container. This answer gave the amount
of fresh water, in ml., that should be added to the salt
water. The formula was correct to one part per thousand
for reducing salinity within 20 to 34 parts per thousand.
Whether or not the formula was workable for a higher or
lower range is not known.

Salinity was determined by using three specific
gravity hydrometers with graduated calibration of

1.000 - 1.012, 1.011 - 1.021, and 1.020 - 1.031. A hydrometer jar holding about a 500 ml. sample of salt water was used to obtain readings on the hydrometer. Correction factors of plus or minus, varying with the temperature, and the final salinity according to the reading, were obtained from the tables in The Manual of Tide Observations. Special Publication No. 196, U. S. Department of Commerce U. S. Coast and Geodetic Survey, 1941. A standard centigrade thermometer was used to record temperatures.

Following the reduction of the salinity in the heat controlled tank, approximately 482,000 larvae were placed into the rearing water on July 29, 1952. The average size of these larvae was 172.3 microns. Water temperature was 70.5° F., and the salinity was 26.4 parts per thousand.

Feeding of the larvae was started on July 21, 1952, and was continued at irregular intervals until the larvae had reached an average size of 250.9 microns on August 25, 1952. Beginning on July 27, 1952, the larvae were fed daily. Each feeding consisted of 1000 cc. of culture containing Bodo lens.

The temperature at the start of the experiment was 70.5° F. It ranged from 68 to 74° F. The average temperature throughout the experiment was 71.0° F.

There was no great increase in the salinity of the water. Salinity at the beginning of the experiment was 26.4 parts per thousand; at the end of the experiment the salinity was 28.2 parts per thousand.

On the seventh day of the experiment, a sample of ten larvae were measured. The maximum size found was 228.8 microns, the minimum, 183.9 microns, and the average 207 microns. The average growth of the individual larvae per day for this period was five microns. On August 18, 1952, an equal number of larvae were measured. The growth for this group in 13 days was less than two microns per day. The final measurement of larvae took place on August 25, 1952. The average size of ten larvae was 250.4 microns, the maximum size 282.5 microns, and the minimum 221.7 microns. The growth rate per day from July 30, 1952, to August 25, 1952, a period of 27 days, was 2.8 microns. All of the growth rates were based on the measurements of the average size of the larvae.

Cultch was added to the water on August 25, 1952. No spat were seen until September 9, 1952. Very few spat were found on the cultch. The experiment was terminated on September 9, 1952.

The results of this experiment were not very satisfying. Extremely few spat were found. A free-swimming period of 43 days before any setting occurred was very

unusual, as was the slow rate of growth during a 27-day
period. Perhaps these unusual occurrences can be attri-
buted to the lack of water change during the course of
the experiment. The fresh water used to dilute the
salinity may have had some effect on the larvae. Data
on this experiment is presented in Table III.

TABLE III

DATA OF THIRD EXPERIMENT IN HEATED CONCRETE TANK

Date 1952	Size of Larvae in Microns	pH	Salinity in parts per thousand	Temperature Degrees F.	Amount of Food Culture in cc.	Water Change
7/28 *			26.4	67		
7/29**			26.8	69.8	1000	
7/30	Ave.172.3					
7/31				70	1000	
8/1				74		
8/2				70	1000	
8/4			27.4	73	1000	
8/5	Max.228.8 Min.183.9 Ave.207					
8/6				70	1000	
8/7			27.6	73		
8/8				70	1000	
8/10			27.4	73	1000	
8/12			27.8	68	1000	
8/13				74		
8/14			27.4	76		
8/15				70		
8/16				68	1000	

TABLE III (Continued)

Date 1952	Size of Larvae in Microns	pH	Salinity in parts per thousand	Temperature Degrees F.	Amount of Food Culture in cc.	Water Change
8/18	Max.256 Min.205 Ave.232.2					
8/21			27.8	70	1000	
8/23			28	72	1000	
8/25***	Max.282.5 Min.221.7 Ave.250.4			68	1000	
8/27			28	71.6	1000	
8/29				72	1000	
8/31			28.2	74	1000	
9/2				70	1000	
9/4				70	1000	
9/6				74	1000	
9/8				70	1000	
9/9****						

*   Added 190 liters of fresh water to reduce salinity
     from 34.2 to 26.0 parts per thousand.
**   New larvae in tank 500 per liter.
*** Added cultch.
**** Checked cultch - very few larvae set.

EXPERIMENTAL LARVAL REARING IN CONCRETE CONTROL TANK

First Experimental Rearing

Before placing oyster larvae into the concrete control tank, it was thoroughly cleaned with brush and water. As in the heated tank, the drain was covered with a piece of stainless steel screen, and a layer of glass wool was placed over the screen. The drain is not flush with the bottom of the tank. It is boxed in a layer of concrete 23 inches square and four inches high, having a concave depression. Sand was placed over the glass wool up to the level of this "box". This arrangement, as in the heated concrete tank, permitted draining of the water without loss of oyster larvae. The tank was filled with approximately 1019 liters of filtered sea water.

On July 17, 1952, approximately 100,000 native oyster larvae with an average size of 164.6 microns were placed in the water of the control tank. The temperature was 60° F., the salinity at this time was 32.4 parts per thousand. Feeding of the tank with 400 cc. of a culture of Bodo lens was started on the following day.

In this first experiment in the control tank, the water was changed twice. The first change was made on July 25, 1952, eight days after the larvae were introduced into the tank. Salinity at this time was 31.9

parts per thousand. The second and last water change was made on August 7, 1952, twelve days after the first water change was made. Salinity was not recorded at the time of this water change.

The temperature of the water followed closely that of the atmosphere. A range of 57 to 68° F. was recorded. The average temperature throughout the experiment was 59.1° F.

Measurements of a sample of ten larvae were first made on July 24, 1952. The rate of growth during this seven-day period was 2.1 microns per day. The average size was 178.6 microns, the minimum, 158.4 microns, and the maximum size was 184.6 microns. The next sample of ten larvae showed better growth for a same period of seven days. An average growth of 5.3 microns per day was made. A final measurement of the dwindling numbers of oyster larvae was made on August 8, 1952. The average size of this group of ten was 196 microns, the minimum 158 microns, and the maximum was 201 microns. In this seven-day period, a growth of 2.7 microns per day was noted. There was quite a contrast in the sizes of the larvae between those measured on August 1, 1952, and those measured on August 8, 1952. This probably was caused by obtaining various sizes of larvae in the sample, or by the deaths of the larger larvae. No

attempt was made to determine an average growth rate per day of the larvae throughout the course of the experiment.

Feeding of the larvae began on the day after larvae were placed in the tank. The first two feedings of 400 cc. of culture medium were made within five days of each other. On the sixth day, 150 cc. of culture was added to the tank at various intervals. Throughout the experiment, feeding of 200 ml. of culture containing Bodo lens were made. A few actively swimming larvae were seen on the 21st day after they were placed into the tank. Within the next eight days, the remaining larvae gradually diminished in numbers until none could be found in the water. The experiment was concluded August 16, 1952.

At this early stage of the studies at the laboratory, conducted in cement tanks, the time of placing cultch into the water of the control tank was determined by the size of the larvae. Cultch was put into the water when the maximum size of the oyster larvae reached 250 microns. Since no larvae reached that size, no cultch was added to the water of the concrete control tank.

During the course of this experiment much rust appeared on the surface of the inner walls of the control tank. This was apparently caused by the rusting of the steel reinforcing rods imbedded in the walls of the tank.

By the end of the experiment the water in the tank had a faint rust color. This accumulation of rust in the water may have been harmful to the larvae in some unknown way. Data of this experiment is presented in Table IV.

Second Experimental Rearing

With the ending of the first experiment, the tank was drained and cleaned. Refilling of the tank with filtered salt water was started. When the tank was filled to the desired level, an estimated 509,500 larvae were placed into the tank. Since this was the time of high salinity in the bay, the tank salinity was 33.4 parts per thousand. The temperature was 58° F. At the same time that larvae were placed into the tank, 1000 cc. of a culture of _Bodo lens_ was poured into the rearing water. The average size of these oyster larvae was 176.6 microns.

For the first twelve days of the experiment, the salinity was maintained at 33.4 to 33.2 parts per thousand. The variation in the salinity may be due to the slight degree of variation in the different thermometers used to check temperatures of the water. On August 29, 1952, the salinity was thought to be excessive, and therefore was reduced with tap water to 28.6 parts per thousand. The salinity at the end of the experiment had increased to 29.8 parts per thousand.

TABLE IV

DATA OF FIRST ATTEMPTED LARVAL REARING IN CONCRETE CONTROL TANK

Date 1952	Size of Larvae in Microns	pH	Salinity in parts per thousand	Temperature Degrees F.	Amount of Food cc.	Water Change
6/17	164.65			60		
6/18				60	400	
6/19				50		
6/21				57		
6/22				57	400	
6/23				59	150	
6/24	Max. 184.6 Min. 158.4 Ave. 178.6	8.5	32.4	59		
6/25						X
6/26			31.9	60	200	
6/27				60	200	
6/28				60		
6/29				60		
6/30				60	200	
7/1	Max. 239 Min. 191 Ave. 216			60		
7/2			31.9	60	200	
7/3				60		
7/4				60	200	

TABLE IV (Continued)

Date 1952	Size of Larvae in Microns	pH	Salinity in parts per thousand	Temperature Degrees F.	Amount of Food cc.	Water Change
7/5				60	200	
7/6				60	200	
7/7				64		
7/8	Max. 201 Min. 188 Ave. 196*			68	200	
7/10				60	200	
7/11				59		
7/12				60	200	
7/13				62		
7/14				64	200	
7/16 **						

* Larvae not as numerous
** Discontinued experiment.

There was little variation in temperature during this experiment. The temperature ranged from 58 to 61° F. The average temperature throughout the experiment was 59.5° F.

Feeding of the larvae began with the introduction of the larvae into the tank. The original feeding of 1000 cc. of culture medium was continued in equal amounts throughout the experiment. Food was poured into the water at intervals of two to three days.

On August 22, 1952, the first measurement of a sample of ten larvae was made. The average size was 181 microns, the minimum 175 microns, and the maximum size was 186.5 microns. Very little growth was made in this five-day period. The next measurement of the larvae was made on August 26, 1952. The average size of this group of larvae was 187.6 microns, the minimum 176 microns, the maximum size was 201.5 microns. Again little growth resulted in this four-day period. The final measurement of the less numerous larvae was made on September 8, 1952. As in the first experiment, there was a marked "decrease" in size. The average size was 184.8 microns, the minimum 170 microns, and the maximum 198 microns. Thinning of the number of larvae became apparent about the time that the experiment had reached its fourteenth day. At the time that the last measurement was made, it was difficult

to obtain a sample of ten larvae. The experiment was carried on throughout the latter part of August and up to September 8, 1952, because few larvae were available for another experiment as natural swarming had almost ceased, and it was thought that perhaps a few larvae had escaped detection.

The failure of the larvae to make sufficient growth to reach the setting stage was believed to be caused by the diffusion of rust into the water, and that the tap water from a galvanized pipe line, in some unknown way, was harmful to the larvae. The low temperature range may also have been partly responsible for the failure of this experiment since a difference in temperature of 11° F. existed between the water of the heated tank and that of the control tank through the entire course of the experiments conducted in the concrete tanks.

Data of this experiment are presented in Table V.

TABLE V

DATA OF SECOND ATTEMPTED LARVAL REARING IN
CONCRETE CONTROL TANK

Date 1952	Size of Larvae in Microns	pH	Salinity in parts per thousand	Temperature Degrees F.	Amount of Food cc.
7/17	Ave. 176.		33.4	58	1000
7/19				60	1000
7/20				60	
7/21			33.3	60.8	1000
7/22	Max. 186.5 Min. 175 Ave. 181			60	
7/23				59	1000
7/24				60	
7/25			33.2	62.6	1000
7/26	Max. 201.5 Min. 176 Ave. 187.6			61	
7/27				60	1000
7/29	*		33.7 28.6	66 60	1000
7/30			29.	60	
7/31				60	1000
8/1				60	
8/2				60	1000
8/4			29.3	60	1000

TABLE V (Continued)

Date 1952	Size of Larvae in Microns	pH	Salinity in parts per thousand	Temperature Degrees F.	Amount of Food cc.
8/5	Max. 198 Min. 170 Ave. 184.8				
8/6				60.5	1000
8/7			29.4	64	
8/8				60	1000
8/10			29.5	60	1000
8/12			29.8	60	1000
8/13				60	
8/14			29.5	62	
8/15				60	
8/16				60	1000
8/18				60	1000
8/21			29.4	60	1000
8/23			29.5	61	1000
8/25				60	1000
8/27			29.7	60	1000
8/29				60	1000
8/31			29.8	60	1000
9/2				60	1000
9/4				60	1000

TABLE V (Continued)

Date 1952	Size of Larvae in Microns	pH	Salinity in parts per thousand	Temperature Degrees F.	Amount of Food cc.
9/6				59	1000
9/8				60	1000

* Salinity reduced by addition of tap water.

EXPERIMENTAL LARVAL REARING IN WOODEN TANK

By the time midsummer had arrived, it was decided that further attempts to rear native oyster larvae in the concrete control tank would be useless until some way of preventing the seepage of rust into the water could be found. As it was desired to conduct as many experiments as possible while the natural swarming of oyster larvae was in progress, a means of obtaining a large container with a similar capacity to the concrete tank was sought. Acting upon the suggestion of Professor R. E. Dimick, a Douglas fir, circular, wooden tank was requisitioned.

First Experimental Rearing

When the tank arrived, it was assembled in the temperature-controlled room in the laboratory on August 8, 1952. The tank was then filled with fresh water and permitted to soak and swell for three days. On August 11, 1952, the fresh water was drained and the tank was filled with filtered sea water. The salinity of the sea water was reduced from 33.3 to 27.7 parts per thousand with fresh water from a plastic line.

On August 12, 1952, approximately 400,000 oyster larvae were placed into the rearing water of the wooden tank. The average size of the larvae was 173.5 microns.

A water temperature of 70° F. was recorded. On the following day, August 13, 1952, the temperature was 72° F. The pH of the water was 7.4. At 4:30 p.m. of the same day, the larvae had greatly decreased in numbers. On August 14, 1952, the majority of the larvae were on or near the bottom of the tank. The temperature remained at 72° F. At 3:30 p.m. on August 15, 1952, no living larvae could be found. In a period of three days, all of the larvae had died. Throughout this short experiment, the initial pH reading of 7.8 dropped to 6.73. A further check on August 16, 1952, of the pH of the water revealed a difference in pH from top to bottom. A pH of 6.73 was recorded for the bottom layer of water, and a pH of 6.95 for the top layer. The salinity of the water, in this short time, did not vary.

From the fluctuation of the pH of the water and the rapid loss of the larvae it is apparent that some substance or substances were leaching out of the wood and that in some unknown way was lethal to the oyster larvae.

Second Experimental Rearing

Following the rearing failure of the first experiment, the wooden tank was drained on August 18, 1952. On August 20, 1952, the tank was filled with sea water. Approximately 400,000 native oyster larvae were placed into

the water on this same day. The salinity of the water
was 33.4 parts per thousand. The pH was 7.6. A temperature of 70° F. was recorded. At this time, no measurement of larvae was made as it was assumed that the tank
might still be toxic to larvae. As in the first trial,
the larvae began thinning in numbers on the day following
their introduction into the tank. On August 22, 1952,
larvae were less evident in numbers, and during August 23,
1952, no living larvae were found. The pH of the water
did not fluctuate as greatly as in the first experiment.
It ranged between 7.6 and 7.5. The temperature remained
about 70° F. throughout the experiment.

With the termination of the second experiment on
August 23, 1952, it was assumed that the unknown toxic
substances were still leaching out of the wood of the
tank. It was believed that a period of "tempering", in
which water would be maintained in the tank at all times,
would leach out the substances which were being harmful
to oyster larvae.

Third Experimental Rearing

During the time that the wooden tank was undergoing
tempering, it was kept full of fresh water. The water
was changed occasionally. The period of tempering was
from the latter part of August, 1952, to the end of

February, 1953, an approximate time of six months.
During this course of conditioning, young silver salmon
were, at times, held in the tank without suffering any
ill effects. The lack of oyster larvae prevented making
any tests of the tank in order to determine whether it
was still toxic to oyster larvae.

Through induced spawning, larvae were swarmed in the
laboratory on March 4, 1953. The water was drained and
the tank was filled with filtered sea water having a
salinity of 31.0 parts per thousand. On March 5, 1953,
approximately 400,000 larvae were placed into the water
of the tank. The average size of a sample of ten larvae
was 157.5 microns. The temperature of the water was
64.4° F.

Feeding of the larvae began on the same day they
were introduced into the tank. A 1000 ml. of a culture
of Bodo lens was poured into the water of the tank at ir-
regular intervals throughout the experiment. The lapse
of time between feedings was never greater than three
days. Altogether, a total of 15,000 ml. of culture
medium was poured into the water of the tank.

The salinity from the beginning of the experiment to
the end did not vary greatly. The salinity was usually
at 31.0 parts per thousand. The one recording of sa-
linity of 30.4 parts per thousand was probably caused by

the different temperature readings obtained by using a different thermometer. Larvae were no longer visible on April 2, 1953. On April 5, 1953, the tank was drained and refilled with filtered salt water. No water change was made during the course of this experiment.

On March 11, 1953, a sample of ten larvae were obtained and measured. The average size was 167.2 microns, the maximum size was 173.4 microns, and the minimum size was 160.2 microns. During this six-day period, a growth of about two microns was made by the larvae. Eleven days after the experiment had been started, another group of larvae was measured. These showed a fair growth of about three microns per day. On March 20, 1953, larvae were again measured for growth. The average size was 228.4 microns, the minimum size 186.6 microns, and the average size was 197.1 microns. Based on the average size of 197.1 microns recorded on the twentieth of March and the average size of 190.0 microns recorded on the sixteenth of March, there seems to be very little growth of the larvae. The small sample of such a large number of larvae cannot give a true indication of how much growth is made per day by the larvae, but it does indicate that the larvae are making some progress toward reaching the setting stage. The final recording of size of larvae was made on March 24, 1953. The average size

was 213 microns, the minimum 196.2 microns, and the average size was 202.4 microns. This measurement was made when the larvae had been in the tank for 19 days. The estimated growth of the average size larvae during this period was 2.5 microns per day.

On the 21st day, March 26, 1953, after the larvae were placed into the tank, cultch was added. On March 27, 1953, it appeared that the larvae were thinning in numbers. An examination of the cultch on April 2, 1953, revealed that a few larvae had settled as spat on the cultch. A number of spat were found on a glass rod which had been in the tank throughout the experiment. Apparently, many larvae settled on the walls of the tank. A few spat were found in scrapings taken from the sides of the tank.

It is apparent that the cause of the failure to rear larvae to the setting stage during the first two experiments was due to the leaching of toxic substances from the wood to the water. In order to eliminate those toxic substances, a period of conditioning of the tank seemed to be necessary. Data of this experiment is presented in Table VI.

TABLE VI

DATA OF EXPERIMENTAL LARVAL REARING IN
WOODEN TANK WITH NO WATER CHANGE

Date 1953	Size of Larvae in Microns	Salinity in parts per thousand	Temperature Degrees F.	cc. Amount of Food
3/5	Ave. 157.5	31.0	65	1000
3/8			67	1000
3/9		30.4	70	1000
3/11	Max. 173.4 Min. 160.2 Ave. 167.2			
3/13				1000
3/15				1000
3/16	Max. 206.8 Min. 172.5 Ave. 190.1 *			
3/18		28.9	70	1000
3/20	Max. 228.4 Min. 186.6 Ave. 197.1 **			
3/21		31.0		
3/22				1000
3/24	Max. 213 Min. 196.2 Ave. 202.4			1000
3/26				1000
3/27	***			
3/29		31.0	67	1000

TABLE VI (Continued)

Date 1953	Size of Larvae in Microns	Salinity in parts per thousand	Temperature Degrees F.	Amount of Food cc.
3/31				1000
4/2		**** 31.0	67	1000
4/5	Experiment concluded			

*   Larvae numerous

**   Larvae plentiful

***   Larvae thinning

**** No larvae visible. Fair set.

SUMMARY

1. Experimental rearing of native oyster larvae was conducted under controlled conditions in concrete and wooden tanks at the Yaquina Bay Fisheries Laboratory in 1952-1953.

2. A limited success in rearing oyster larvae to the setting stage was attained in the heated concrete and wooden tanks.

3. High salinities of 33 to 34 parts per thousand appear to be inimical to native oyster larvae.

4. Of the experiments conducted in the concrete control tank, none were successful. The low temperature of about 59° F. may have been one of the most important factors causing failure.

5. Experimental larval rearing in the wooden tank revealed that a period of conditioning is necessary in order to render the tank harmless to oyster larvae.

6. Adult native oysters may be induced to spawn in six to eight weeks during the winter months when kept in sea water at room temperature.

7. The growth of larval food, _Bodo lens_, is inimically affected by temperatures exceeding 87.8° F., and by salinity of less than five parts per thousand.

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