Hatchery Manual for the Pacific Oyster

Wilbur P. Breese
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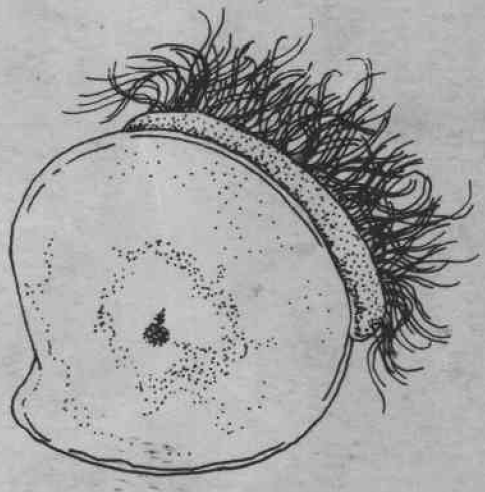

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NORTHWEST MARICULTURE LAWS: PAPERS AND PRESENTATIONS FROM A SYMPOSIUM HELD AT THE LAW CENTER, UNIVERSITY OF OREGON, EUGENE, JUNE 7, 1974. Publication no. W-74-005. (Price: $2.00) 31 pp.

Proceedings of a symposium which examined the legal implications of the growing interest in mariculture and focused discussion on the laws, new and old, that affect the establishment and operation of mariculture businesses.

NETARTS BAY CHUM SALMON HATCHERY, AN EXPERIMENT IN OCEAN RANCHING, by James E. Lannan. Publication no. H-75-001.

Describes the construction and operation of the OSU Netarts Pilot Chum Salmon Hatchery. Special attention is given to low-cost, low-maintenance designs for the weir, incubator and water system. The rationale for extensive mariculture (ocean ranching) is discussed.

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CONNIE MOREHOUSE: illustrator

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introduction

Oyster seed hatcheries are a reality. An acceptable product can be produced by known methods and techniques.

In the past, essentially all oyster seed for the West Coast Pacific oyster fishery was imported from Japan. Recent years, however, have seen increased use of domestic seed caught in Pendrill Sound, Canada, and in Willapa and Dabob Bays, Washington. Now, development of oyster seed hatcheries in Washington and California has further reduced dependence upon Japanese Pacific oyster seed.

In the near future the oyster farmers of western North America will probably no longer depend on imported Japanese seed. The chief West Coast seed source can be expected to shift—first to locally caught wild seed, next to hatchery produced wild seed, and finally to selectively-bred hatchery seed.

This manual is intended to provide guidance in the latter two phases—both of which involve artificial seed production by the emerging oyster seed industry. In addition to that rather broad objective, this manual has three more specific purposes.

First, it will present the "state of the art" in oyster hatchery techniques. Many methods presented in this manual were developed at Oregon State University's Pilot Oyster Hatchery. Virtually all have been tested there, although a few examples of techniques used elsewhere are described that have not been tested at OSU.

The second objective of this manual is to present new techniques that have not been rigorously tested anywhere. We will attempt not only to present the current status of oyster hatchery technology, but also to speculate on future directions and developments and to discuss how future advances in technology might be made.

Third and finally, the manual will describe a method for evaluating hatchery efficiency. Only through continuing appraisal of operating efficiency can hatchery operators weed out less efficient methods and further improve efficient ones. The evaluation method to be described includes consideration of the biological, engineering and economic aspects of oyster seed production.

hatchery operations, procedures and equipment

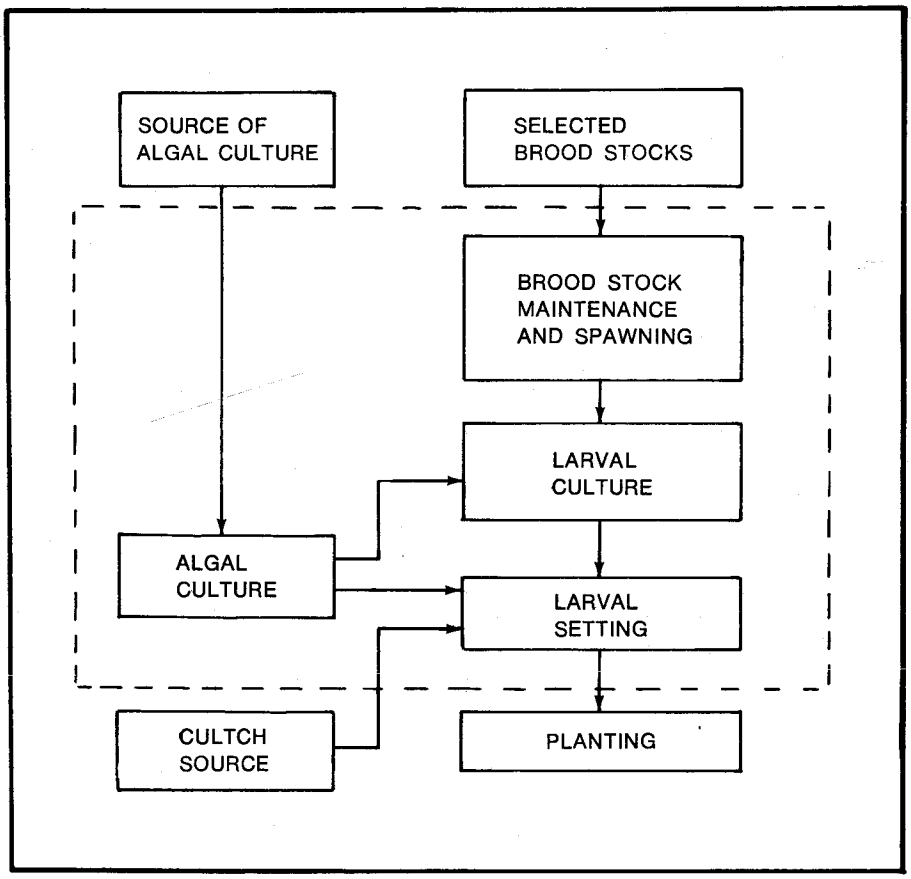

Fig. 1. General flow chart

A general flow chart of the hatchery system is shown in Fig. 1. Only the major components—broodstock maintenance and spawning, larval culture, setting and cultch preparation and algal culture—are shown. These four subsystems are essential to any oyster seed operation.

The floor plan for the pilot hatchery is shown in Fig. 2. The figure merely indicates the location of the various subsystems in the pilot hatchery; it is not intended as a guide for hatchery construction. The characteristics of each hatchery site dictate its unique floor plan. At each location the components have to fit together; they shouldn't be forced.

In practice it is not possible to separate the four hatchery subsystems. All subsystems—from broodstock maintenance and spawning to algal culture—must mesh together. The two major biological systems—larval growth and algae production—must be kept at their peaks at the same time. This synchronization is the key to a successful oyster seed hatchery.

WATER SUPPLY. Oyster seed hatcheries are similar to fish hatcheries in that water supply is of prime importance in site location. Water for the hatchery should have a salinity higher

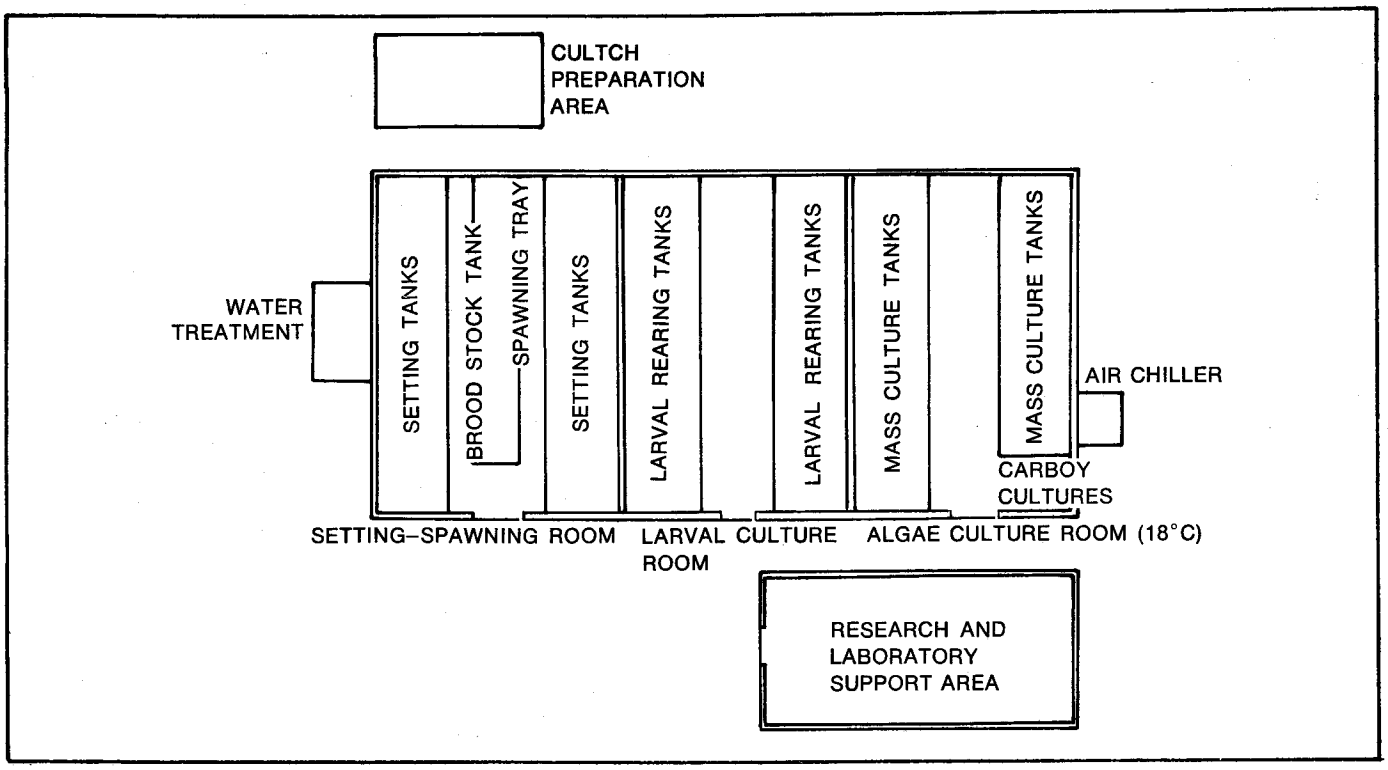

Fig. 2. Floor plan

than 20 parts per thousand. Some water storage may be necessary to provide for short periods of low ambient salinity.

Water temperatures should not exceed 20 C. Primary settling of solids is desirable and sand filtering may be necessary. Special water treatments required for various subsystems will be discussed later.

The saltwater should be transferred and stored in non-toxic plastic containers. When in doubt, questionable materials should be tested against embryonic development of the oyster following accepted bioassay procedures.*

Broodstock Maintenance and Spawning

The brood stock maintenance and spawning subsystem is diagrammed in Fig. 3. It shows the role of the adult oyster in the hatchery system. In general terms, adults are taken from the field and returned to the field, less mortalities and those sacrificed for gametes.

SELECTION OF ADULTS. In the Pacific Northwest, the Pacific oyster may be induced to spawn and viable gametes may be obtained at any time during the year. Year-round spawning is possible because the Pacific oyster normally does not completely spawn out in all Pacific Northwest estuarine areas, generally too cool to induce complete spawning.

To insure a supply of spawners, 300 or 400 oysters should be moved to cool seawater (normally an area of direct ocean influence). Spawners should possess a suitable level of "fatness," or stored glycogen. During the four to eight weeks that the adults are in conditioning trays, they receive little if any food and must rely on stored reserves.

CONDITIONING. Bi-monthly, 50 "fat" oysters are placed in the hatchery conditioning trays. These are flow-through aquaria using unfiltered seawater heated to a temperature of 18-20 C. During a four-week period the gametes in these adult oysters will mature and the adults will grow ready for induced spawning. These adults will be prime for induced spawning for the following two weeks. They may be retained an additional two weeks to assure a source of conditioned oysters in the event that problems occur with a succeeding group.

Possible problems may include a failure of the oysters to reach spawning condition or a complete spawn-out during the initial four-week conditioning period. It is always wise to have more conditioned adults on hand than are actually needed to supply gametes for the hatchery.

After two months in the hatchery, surviving adults may be returned to the oyster beds in the field. They should not be reused for at least six months. Spawned adults would not normally be reused at all for producing wild hatchery seed. In a breeding program, however, spawners should be marked and detailed records kept so that desirable brood stock can be re-spawned to improve the strain of hatchery produced seed. The records should include data about fecundity as well as larval survival and growth rates.

*Procedures to be followed in a bioassay are described in R.E. Dimick and W.P. Breese. 1965. Bay mussel embryo bioassay. Pages 165-175 in Proceedings of the 12th Pacific Northwest Industrial Waste Conference. University of Washington Press. Seattle.

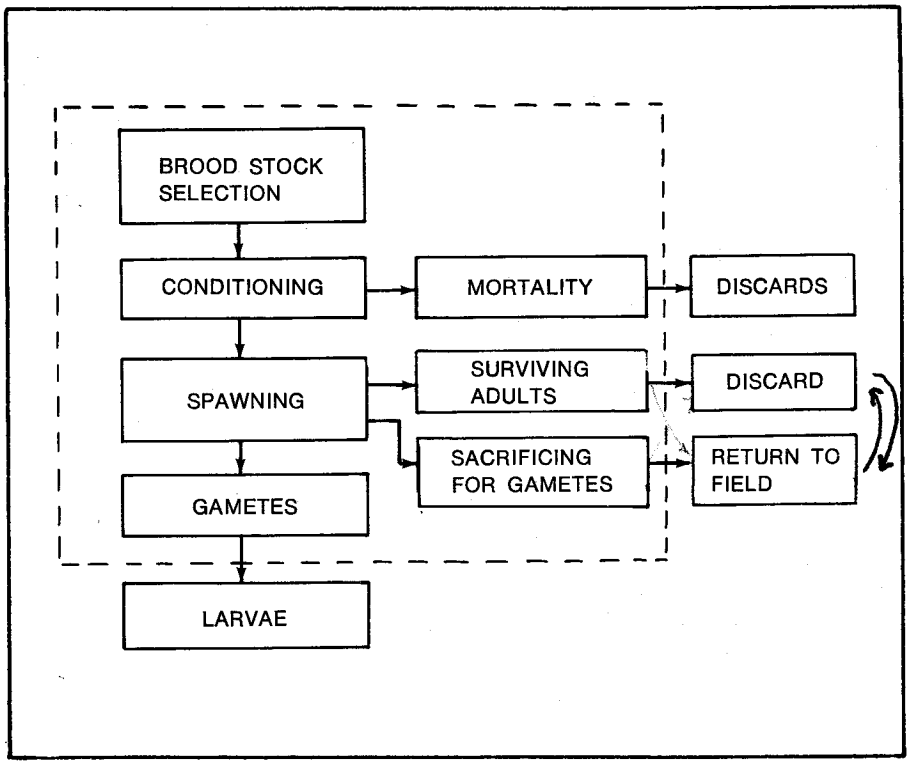

Fig. 3. Brood stock subsystem

INITIATING SPAWNING. Spawning is generally induced in conditioned oysters by manipulating water temperature. Conditioned adults are placed in aquaria with recirculating seawater. Water temperature is raised first to 25 C and then to 30 C over a half-hour period. The temperature is then allowed to fluctuate between 25 C and 30 C. This temperature manipulation may initiate spawning of one or both sexes. If spawning does not occur, the aquaria may be drained, left empty for a few minutes, and then refilled with heated seawater. Although the biological basis for it is not clear, this technique seems to stimulate pumping and occasionally spawning by the oysters.

If temperature manipulation does not initiate spawning, sex products (either eggs or sperm) should be added to the water. There appears to be a hormone released by spawning oysters that stimulates other adults to spawn. This hormone has not been identified, but it is the basis for stimulating spawning with gametes.

The sex products used to stimulate spawning can be obtained in any one of several ways. If one or more oysters spawn by temperature stimulation, they are isolated in small aquaria to collect sex products. These gametes may then be used to induce other adults to spawn. If no adults spawn in this fashion, a conditioned adult may be sacrificed to obtain gametes to stimulate other conditioned adults. Alternatively, excess gametes may be frozen and stored for future use as a spawning inducement.

If the adult oysters have been properly conditioned, these techniques nearly always induce spawning. On rare occasions when spawning does not occur, the procedure should be repeated the following day.

FERTILIZATION. Unless mating specific individuals, eggs from at least two females should be mixed. Fertilization takes place by mixing sperm and eggs in the ratio of 2-4 ml of dense sperm suspension to 4 liters of egg suspension (approximately one million eggs). Care should be taken to avoid adding too much sperm to the egg suspension. The presence of excess sperm can result in a condition known as polyspermy, which leads to abnormal embryonic development and poor survival.

The fertilized eggs should be passed through an 80 micron screen to remove excess debris. The eggs are afterwards diluted with a known volume of saltwater and a sample of known volume is withdrawn with a pipette. The eggs are counted and an estimate is made of the total egg count.

An example may make the sampling procedure clearer: An unknown number of eggs are diluted to 10 liters. After agitation to insure equal distribution of the eggs, a 1 ml sample is withdrawn and further diluted to 100 ml. This subsample is agitated and a 1 ml sample is withdrawn and put in a small dish. The eggs in this sample are counted using a dissecting microscope. Three or more samples are counted and averaged. The average count is multiplied by 1×10^6 to obtain the total number of eggs or by 100 to find the number of eggs per milliliter.

Oyster larvae may also be counted by this method. The second dilution may not be necessary. If not, multiply by 1×10^4 to obtain the total population.

After counting, the fertilized eggs should be diluted to not more than 200 eggs per milliliter and allowed to develop for 24 hours at 25 C.

After enough gametes have been collected and fertilized, the adult oysters should be placed in cold running seawater. This will usually end the spawning response, although some individuals will occasionally continue spawning until completely spent.

Larval Culture

Twenty four hours after fertilization, when held at a temperature of 25 C, the fertilized eggs will have developed into swimming, straight-hinged veligers ready to feed. These larvae now enter the larval rearing subsystem (Fig. 4) and must be provided with cultured algae.

The tanks commonly used for rearing the larvae through the free swimming stage are relatively large, at least 500 liters. Exact tank size will be dictated by the individual goals of each hatchery.

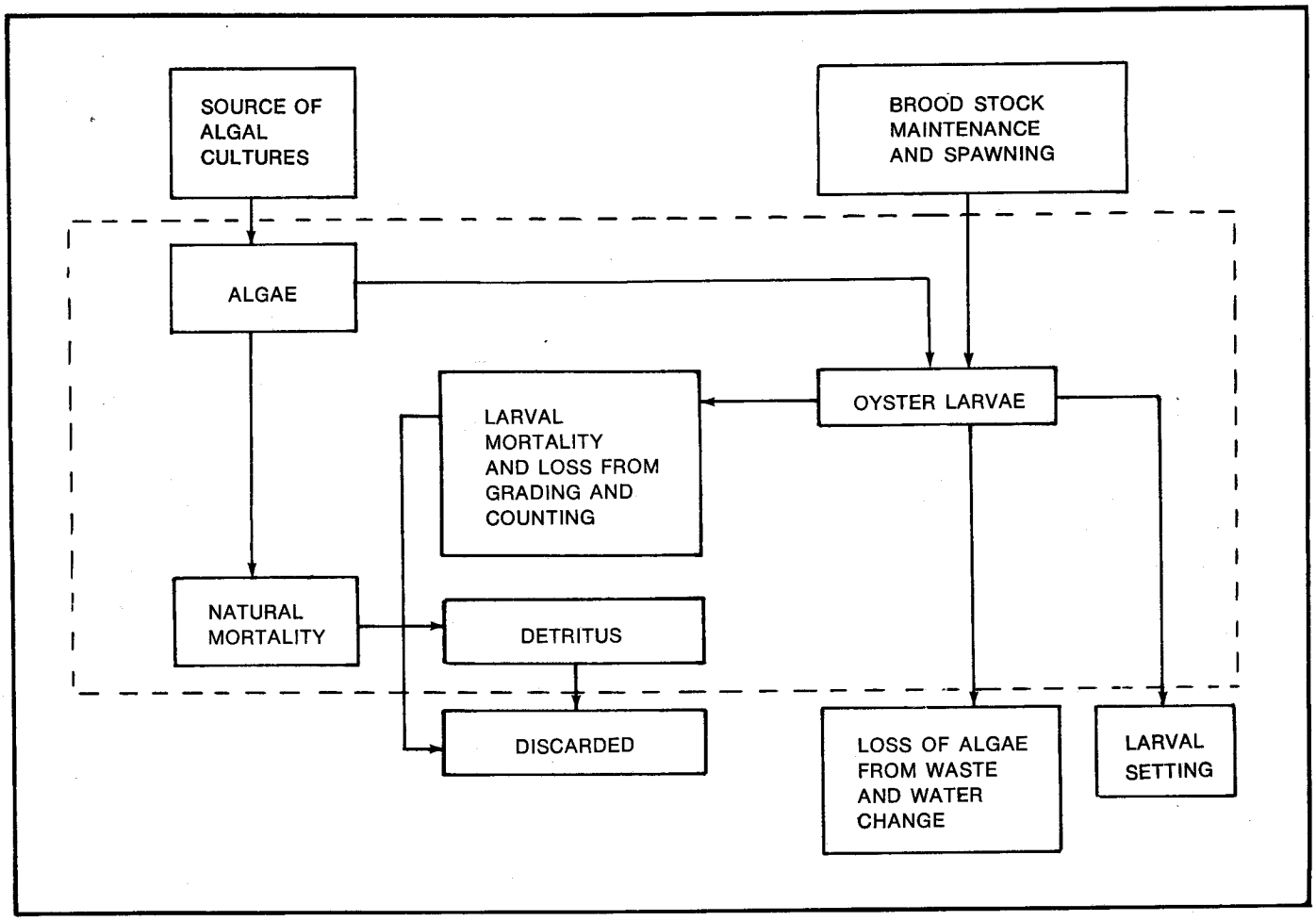

Fig. 4. Larval rearing subsystem

DAY-OLD LARVAE. The 24-hour-old veligers measure about 75 to 80 microns. They will be retained when washed onto a 40 micron screen; unfertilized eggs and malformed larvae will pass through and can be discarded. The larvae retained on the screen should be resuspended in a known volume of water, usually about 10 liters. While the water is stirred to keep the larvae suspended, a sample is drawn out with a pipette. The larvae should be counted to provide an estimate of their total numbers. The larvae are then placed in the rearing tanks at a concentration of about 10 larvae per milliliter.

Even though shelled larvae are relatively hardy, some precautions are necessary. Care should be taken that they are not left on the screen to air dry more than 30-45 minutes. They should not be left at high larval concentrations during counting for more than 30 minutes. They should not be exposed to temperatures less than 20 C nor exceeding 30 C.

WATER SUPPLY. The water used for larval rearing is sand filtered to remove organisms and particles larger than 5 microns. The filtered water is next passed through an ultraviolet sterilizing unit. The UV sterilizer should kill most of the organisms that were not removed by the filter. The temperature of the rearing water should be 25 C and the salinity should be between 25-30 parts per thousand. Rather heavy aeration should be provided so that water circulation is maintained. This provides oxygen for the larvae, drives off volatile waste products and insures the mixing necessary to bring the larvae and algae into frequent contact so feeding can proceed.

The rearing water is changed at least once each week (some hatcheries change water two or three times each week depending on local conditions). The water is changed by draining the tank through a sieve, which retains the larvae but passes through waste products, dead algae and debris. The larvae are then resuspended in clean rearing water.

During the water change, the larvae can be counted and measured. The concentrated larvae can be sampled for counting and a sub-sample may be measured with the aid of a microscope. These measurements will give information about the growth and survival of the larvae.

Some hatcheries use the water change as an opportunity to discard small, slow-growing larvae. They pass the larvae through a series of different sized screens, discard the small larvae and keep only the large, fast-growing larvae.

ANTIBIOTICS. Prophylactic

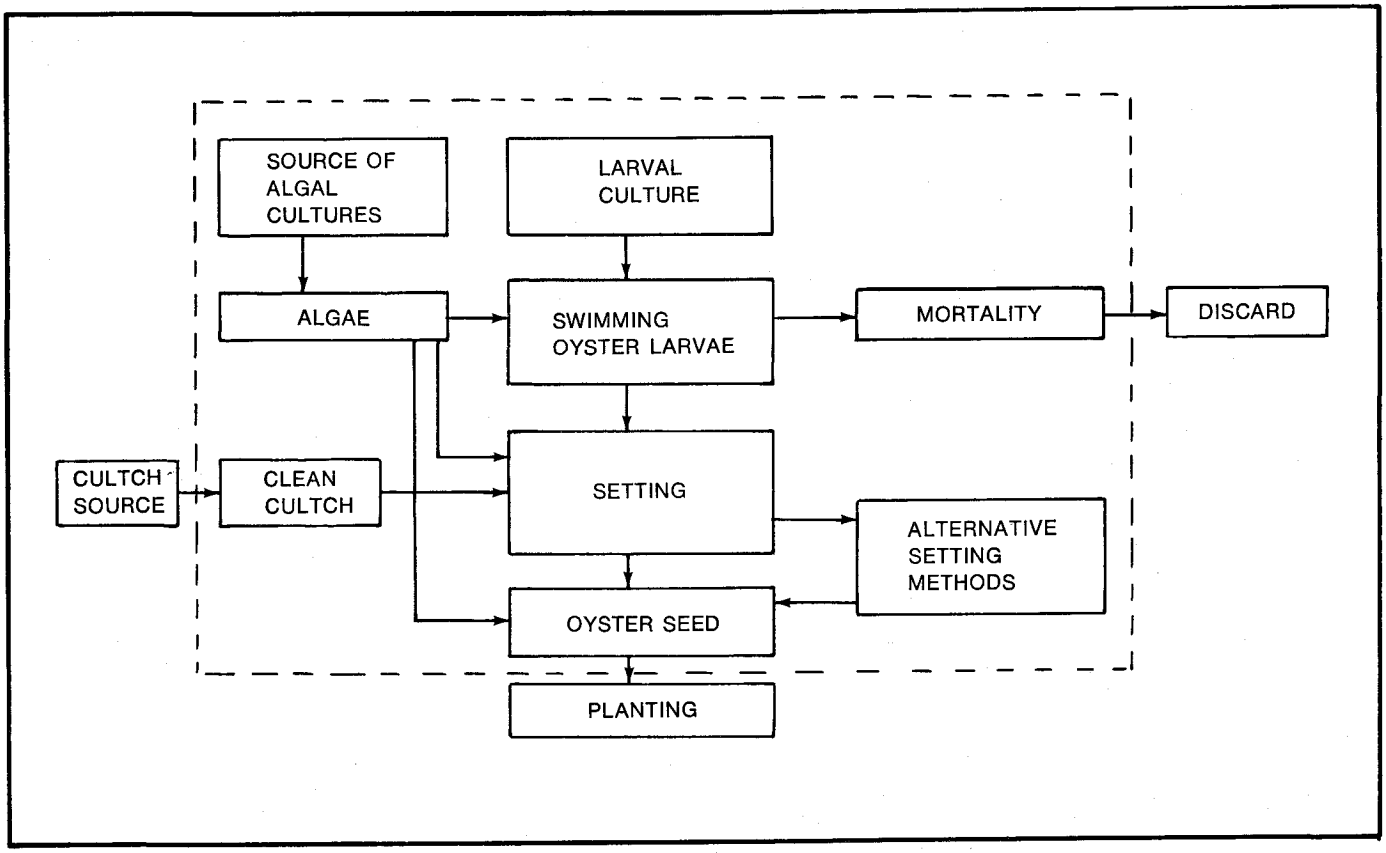

Fig. 5. Larval setting subsystem

antibiotics are occasionally added to the rearing water as a precaution. Commercial sulfamethazine (Sulmet®) may be added at a concentration of 50 parts per million. This dosage is repeated at each water change.

It should be noted that the necessity, and perhaps the desirability, of the use of antibiotics in commercial oyster hatcheries is open to debate and may be restricted by the U.S. Food and Drug Administration.

FEEDING. The straight-hinged veliger larvae are now suspended in the rearing tank and must be provided food. Cultured algae (see section on algal culture) are introduced to the rearing tank in numbers sufficient to attain a concentration of 30,000 algal cells per milliliter of water. For the first week, the larvae are fed once daily at this concentration.

Algal cell count is increased to 50,000 cells per milliliter during the second week and increased further to 80,000 cells per milliliter for the third week. Larvae are fed these algal concentrations twice a day during the second and third weeks. Experience will make it possible to use the color of the larval rearing water as an indicator of low food concentration in the rearing tanks.

PREPARATION FOR SETTING. When the larvae are 20 days old they should measure 250 to 300 microns in length and will be approaching setting size. Larvae of this size develop a distinctive pigment or "eye" spot a few days prior to setting. At this time, two or three clean oyster shells are placed near the bottom of the rearing tank. Each day, these shells are inspected for newly settled spat. When about 50 spat are observed, it is time to transfer the larvae to the setting tanks.

Larval Setting

The larval setting period begins with the attachment of the larvae to a cultch material and extends through metamorphosis from free-swimming larvae to sedentary spat (or seed) and a subsequent growth period until the spat finally leaves the hatchery as oyster seed (Fig. 5). The setting subsystem requires the input of algae, advanced larvae and cultch material.

CULTCH. Cultch is any material placed in the water to provide a substrate for larval attachment and metamorphosis. Cultch material may vary from oyster and other molluscan shells to artificially produced surfaces of plastic. Cultch must be acceptable to the larvae, free of contamination and, of course, must be non-toxic.

In the northwestern United States cultch material consists mainly of oyster shell. When oyster shell is used for hatchery cultch, it must be air cured to remove oyster tissue and fouling organisms. Then it must be thoroughly cleaned before placement in the setting tanks. The use of improperly prepared cultch will cause a poor set at best. More probably it will result in complete mortality of the larvae.

CULTCHLESS SEED. So-called cultchless seed is popular in California and France. Cultch-

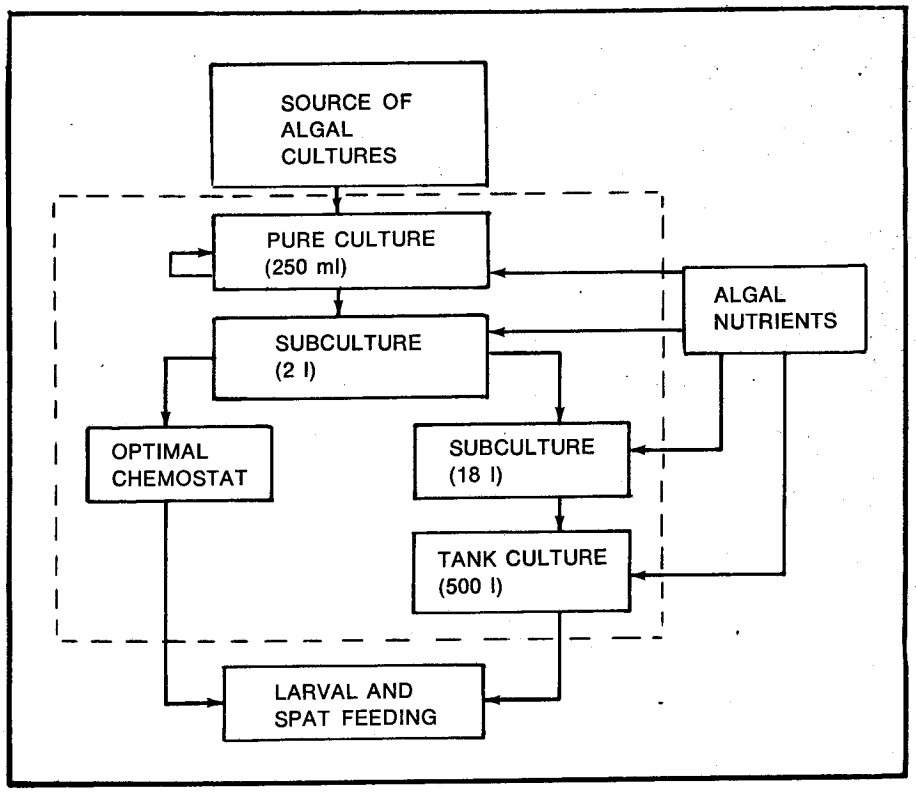

Fig. 6. Algal culture subsystem

less seed is spat that has first set normally, but has been subsequently removed from the cultch material. One method of producing cultchless seed involves using a flexible sheet of smooth plastic for cultch. Soon after setting, the seed is popped off by bending the flat plastic sheet.

An alternative method involves the use of small shell fragments for cultch so each piece has only one or two spat attached to it. Cultchless seed is cheaper to transport and, of course, virtually eliminates cultch handling in the hatchery; but it does require more effort when it is first transferred to the field. Cultchless seed requires extra care until the seed grows to about one inch in diameter.

SETTING CONDITIONS. When the larvae are transferred to the setting tanks, the desired cultch material is added. It may consist of plastic bushel baskets filled with clean oyster shell or thin sheets of plastic for cultchless seed production.

The water temperature is increased from 25 C to 30 C. Algal cell concentration is established at 80,000 cells per milliliter and maintained there by feeding algae twice a day. This feeding schedule continues until one week after setting. Feeding can then be increased to between 100,000 and 150,000 cells per milliliter per day. Other tank conditions are the same as those used in rearing the larvae.

The length of time the seed remains in the hatchery after setting depends on space availability, the destination of the seed and the time of the year. In any case, prior to any move the water temperature should be changed gradually to avoid a temperature shock to the spat. In the warmer spring and summer months, younger and smaller spat can be planted in the field sooner than would be advisable during the winter months, when the estuaries may contain cold, low salinity water.

Algal Culture

The production of large quantities of algae suitable for feeding oyster larvae is essential to any successful oyster seed hatchery. The diagram for this subsystem is found in Fig. 6.

Many species of algae are cultured as food organisms for oyster larvae. Among the most common are *Monochrysis lutheri* and *Isochrysis galbana.* Pure algal cultures of these and other species may be purchased from Indiana University in Bloomington. (For further information, contact the Department of Botany, Indiana University, Bloomington, Indiana, 47401.) To be acceptable as food, the unicellular algae must be less than 6 microns in diameter for young larvae and up to 10 microns for older larvae. The larvae must be able to ingest the algae and obtain necessary nutrients. The growth and survival rates of larvae are the most meaningful criteria for judging a particular species of algae or method of algal culture.

CULTURE TYPES. Various methods have been devised for culturing algae; three will be discussed here. Many methods produce an acceptable product, but quantity and quality often vary.

The batch method is used in the majority of oyster seed hatcheries. This technique produces large quantities of algae.

Nutrient	Formula	Stock Solution
1. sodium nitrate, granular, refined	$NaNO_3$	150 gm/l
2. sodium phosphate, monobasic, certified	$NaH_2PO_4 \cdot H_2O$	10 gm/l
3. trace metals[1]		
cupric sulfate	$CuSO_4 \cdot 5H_2O$	1.96 gm/l
zinc sulphate	$ZnSO_4 \cdot 7H_2O$	4.40 gm/l
cobalt (II) chloride	$CoCl_2 \cdot 6H_2O$	2.00 gm/l
manganese dichloride	$MnCl_2 \cdot 4H_2O$	36.00 gm/l
sodium molybdate	$Na_2MoO \cdot 2H_2O$	1.26 gm/l
4. vitamins		
biotin-crystalline		1 mg/l
vitamin B-12 crystalline		1 mg/l
thiamine hydrochloride		200 mg/l

[1] The concentrations of the trace metals given in the table are for the 5 primary solutions. The single secondary trace metal solution is made by mixing 1 ml of each primary solution plus 10 gm of ferric sequestrene and filling to 1 liter.

Table 1. Nutrient solutions in algal culture, after Matthiessen and Toner. Taken from "Possible methods of improving the shellfish industry of Martha's Vineyard, Duke's County, Massachusetts," by G. C. Matthiessen and R. C. Toner, 1966. Marine Research Foundation, Edgartown, MA.

However, since the condition and age of the cultures change daily, quality will also vary. Batch cultures, usually open to the air, are also subject to contamination, which sometimes results in poor food quality or loss of the culture.

Chemostats or continuous algal cultures, on the other hand, can produce large quantities of high quality algal cells all of basically the same age and can do so under sterile conditions. This is an ideal method but for one drawback—it usually does not work (for reasons discussed below). Research in chemostat culture is continuing, however, since its potential advantages are considerable.

The third algal culture method is dilution or "semi-continuous" culture. This technique is essentially a compromise between batch and continuous culture.

NUTRIENTS. Regardless of the culture method employed, the nutrient solutions used to feed the algae are roughly the same. Only relatively minor variations in particular ingredients are found from one laboratory to another. Table 1 lists the nutrients for the four necessary stock solutions. All solutions are prepared in convenient amounts using distilled water and then autoclaved. One milliliter of each of the four solutions is added to each liter of final culture medium.

BATCH CULTURE. The batch culture technique outlined below uses four culture vessels of different sizes: 250-ml, 2-liter, 18-liter and 500-liter. These sizes are well suited to the OSU Pilot Oyster Hatchery, but culture volume may vary considerably in commercial hatcheries—vessels may be 5,000-liter or larger.

In the pilot hatchery, the light source is a series of four-tube fluorescent units with 40-watt "cool white" tubes. For the smaller vessels the lights are positioned about 24 inches above the cultures. In the 500-liter tanks the lights are placed about 6-8 inches above the surface. These lights provide approximately 300 foot-candles of light over the surface of the shallow cultures, 550 foot-candles over the surface of the carboys and 660 foot-candles over the surface of the 500-liter tanks.

The 250-ml and 2-liter flasks are filled with 200 ml and 1.5 liters of nutrient solution, respectively, and autoclaved. Pure cultures are maintained in the 250-ml flasks on an orbital shaker table. The contents of one 250-ml flask is used to inoculate four 2-liter flasks. The 2-liter flasks are also maintained on a shaker table. We have found the shaker tables to be convenient and useful, but they are not essential and are generally not used by commercial hatcheries.

The 18-liter vessels (5-gallon carboys) are filled with about 16 liters of sand-filtered, UV-treated water along with 50 ml of combined stock nutrient solutions and the contents of one of the 2-liter flasks of algae. The carboys are placed under the light banks and vigorously aerated from a filtered air source.

The 500-liter tanks are filled with filtered seawater that is sterilized in the following manner. The seawater is first chlorinated by the addition of sodium hypochlorite (commercial strength bleach) in quantities sufficient to produce a free

chlorine concentration of about 5 parts per million. The chlorinated water is then allowed to stand at least overnight in a 500-liter storage tank. Finally, the water is dechlorinated by passing it through 2 cubic feet of activated charcoal as it flows into an algal culture tank at a rate of about 3-4 liters per minute. Two liters of combined nutrients and the contents of one or two 18-liter carboys are finally added to the culture tank. The larger the inoculum, the sooner a feeding density is reached in the algae tanks. The water is vigorously aerated, which prevents settling of the algae and rolls it up to the lighted surface.

It takes about a week at each of the four steps for the algae to reach an acceptable concentration. Cell density can usually be estimated by color in the first three sizes of culture vessels, since a certain amount of latitude is permissible. In the 500-liter tank, however, the algal density should exceed one million cells per milliliter and should be known relatively precisely. Experienced operators may be able to feed algae by estimating cell concentration in the 500-liter tank by appearance. This is not as easy as it may seem, however, and it could result either in wasteful and harmful overfeeding or in inadequate feeding.

The most reliable method for counting algae employs an electronic particle counter. Such instruments are essential for research, but are too expensive, and in fact unnecessary, for normal commercial applications. A satisfactory commercial method is based on the use of a simple photometer.

An optical density vs. cell concentration curve is developed by calibrating the photometer readings (optical density) against readings from a borrowed particle counter (cell concentration). The particle counter may be any instrument like a hemocytometer or Coulter Counter. The photometer can thereafter be used to estimate algae cell numbers rapidly and accurately by determining optical density and referring to the previously-developed curve. (See Fig. 10.)

It is wise to always have more cultures on hand than necessary for feeding. Occasionally a culture will be lost and extra inoculum will be needed to catch up. The key to a successful hatchery, again, is to keep the two biological systems—algae and oyster larvae—at their peaks at the same time.

CONTINUOUS-FLOW CULTURE. Chemostats, or continuous-flow algae culture systems, are considerably more difficult to maintain than batch culture systems. As in the batch system, the pure stock cultures to be used with the chemostats are maintained in 250-ml flasks and the inoculating culture is a 2-liter flask. The chemostat culture itself is maintained in a chamber such as a 5-gallon Pyrex carboy. Larvae are fed with algae produced by the chemostat culture and no larger cultures are needed.

The chemostat system requires that certain methods be developed and carefully applied. Aseptic methods for injecting air and for carefully metering nutrients and water into the chamber are required. A simple, reliable system for continuously removing algae from the chemostat is also necessary.

To start the chemostat system, inoculate the chamber and allow the algae to increase in density under relatively static water conditions until the desired cell density is reached. Then initiate a flow of nutrient-enriched sterile seawater such that the exchange rate equals the cell division rate as closely as possible.

This equalization should stabilize the algal population in the culture. Theoretically, cell numbers can also be controlled by limiting one of the nutrients or by regulating pH, light or temperature; but control by flow-rate regulation seems to be the most practical.

This system will produce uniform quality algae of a consistent age class in large quantities. Clearly, the main problems with this system are balancing flow with cell division and maintaining sterility. Chemostat culture currently lacks the reliability demanded by commercial application. But the advantages of producing relatively large quantities of high quality algae in a small system demand further study.

SEMI-CONTINUOUS CULTURE. The third basic system of algal culture, the semi-continuous method, involves periodic, rather than continuous, harvest and dilution. In this system the algae is cultured in a sterile chamber until a harvestable density is attained. Half (or some other suitable fraction) of the culture is siphoned off for use in larval culture. The chamber is then refilled with sterile water and nutrients. The algae in the growth chamber return to harvestable density due to the high concentration of inoculum. This system works quite well for limited production but is difficult to maintain for large scale operations.

general techniques and methods

During the course of research on bivalve hatcheries some general methods and techniques have evolved that can spell the difference between success and failure. Some of these are explained below.

Of utmost importance in day-to-day operations is attention to detail. Changes in routine procedures should only be made after they have been tested under controlled conditions. Unproven techniques may result in failures that will be difficult to trace and correct.

CONSTRUCTION MATERIALS. Avoid untested materials. Outward appearance does not reveal toxic potential. Advertised toxicity clearance by federal agencies does not have any particular meaning for applications in marine invertebrate culture. Many substances and materials which are harmless to humans are extremely toxic to oyster larvae.

The best method available for testing these materials is a controlled bioassay. Larvae are developed from the egg to the straight-hinged veliger in the presence of the material in question. Larval development in the test group is then compared with larval development in a control group raised in an aquarium which does not contain the material. Materials that have not been pretested are to be avoided at all times.

Generally preferred materials for use in oyster hatcheries are glass and plastics. Heat resistant glass and soft glass have proven to be non-toxic. Plastics vary in toxicity: cured fiberglass, plexiglass, polyethylene and polyvinyl chloride (PVC) are usually non-toxic, but should be tested by bioassay with oyster larvae. The only Tygon® tubing formulation found non-toxic is R3603. All plastic tubing should be tested. All metals are suspect and should be avoided if possible. Titanium and stainless steel 316 may be used in small amounts if absolutely necessary. If metal pump parts cannot be avoided, use stainless steel 316 or cast iron. Metal should be coated with a tested epoxy paint wherever possible.

WATER TEMPERATURE. Seawater temperature must be controlled for some hatchery activities. The conditioning of the brood oysters, for example, requires a continuous flow of seawater at 18-20 C. This temperature can be maintained by using Vycor immersion heaters with adequate thermoregulation. Quartz, Pyrex and other glass heaters have proven inadequate; metal immersion heaters are invariably toxic.

The use of heat exchangers made of heat resistant glass is another excellent way to control water temperature. The seawater can be either heated or cooled when using heat exchangers.

Temperatures of standing water in rearing tanks can be controlled either with immersion heaters or by controlling the temperature of the room and allowing the water temperature to equalize with it. When the water in open tanks is heated to temperatures above ambient air temperature, the humidity in the room will be higher than when the air is heated. Increased humidity will in turn increase the chances of mildew and corrosion.

AIR SUPPLY. Compressed air is needed in both algal- and larval-rearing subsystems. The pressure need not be high (15-20 pounds per square inch), but the air must be free of contaminants. Oil lubricated piston compressors deliver unclean air. Water lubri-

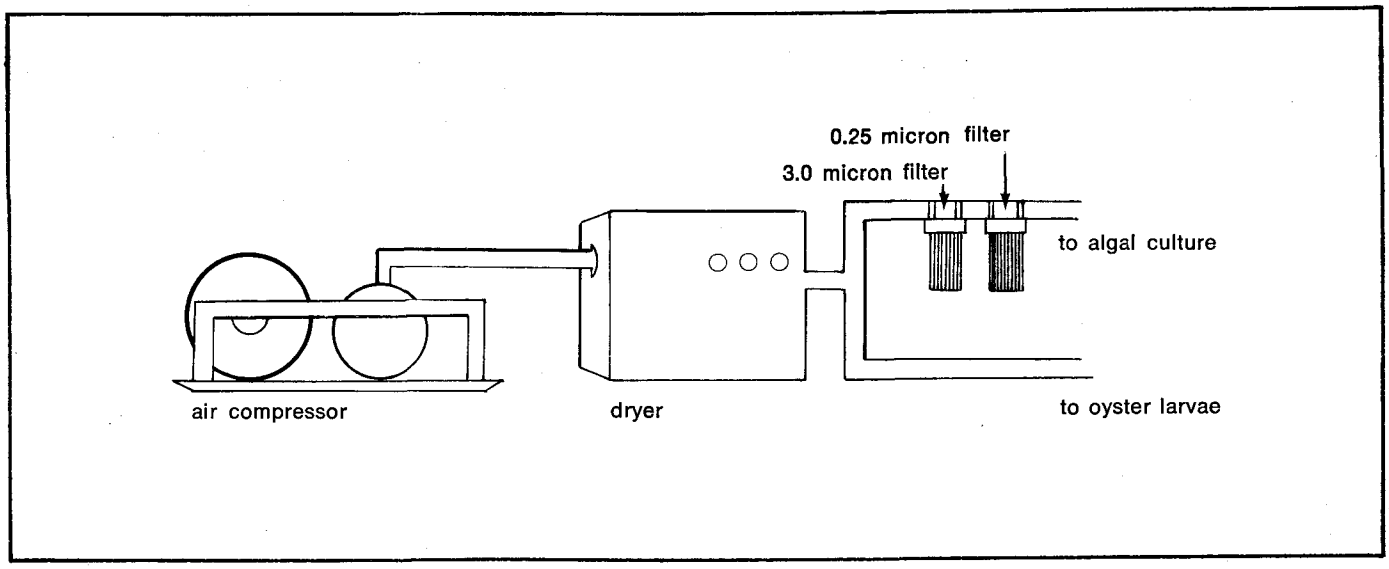

Fig. 7. Air supply system

cated compressors deliver satisfactory air but it is usually too moist.

An air dryer connected with a water lubricated compressor provides air suitable for larval rearing. This air should be filtered through a 0.25 micron filter for use in algal culture to prevent contamination from bacteria, protozoans and unwanted algae. A schematic diagram of the pilot hatchery air supply system is shown in Fig. 7.

SCREENS. Manufactured stainless steel screens with various mesh sizes can be purchased to handle larvae. Stainless steel screens are expensive, however, and serviceable screens can be made out of 4-8 inch PVC pipe.

A section of pipe 6-8 inches long is cut in two, a sheet of Nitex® plastic cloth inserted and the ends glued to reform the short section (see Fig. 8). Nitex® can be purchased in a variety of mesh sizes to construct all necessary screens.

CLEANLINESS. Cleanliness is very important in any hatchery operation. Oysters are no exception.

The worker must be clean, especially his hands. Hand preparations should not be used.

Small utensils should be sterilized in chlorine water baths. Those used in algal culture should be kept in a chlorine bath when not in use. Rearing containers should be clean and allowed to stand overnight filled with chlorinated water or at least flushed with chlorinated water. Glassware for algal culture should be autoclaved.

Sponges, brushes and other cleaning aids should be marked and used for one operation only. For instance, a sponge used to clean algal tanks should not be used to clean larval tanks. Inadvertent contamination is a common cause of culture failure.

WATER SUPPLY. Saltwater is supplied to the hatchery in PVC pipes. Raw, non-filtered seawater should be available continuously, either directly from the bay or from a large storage tank. This unfiltered water can be used for adult oysters as well as for the advanced spat.

Culture water should be sand filtered to remove organisms and particles larger than a few microns. Subsequent UV-light treatment will kill or attenuate most remaining organisms. The water can thereafter be stored for use in the larval cultures or further treated for use in algal cultures. The pilot hatchery water system is diagrammed in Fig. 9.

ESTIMATING POPULATION SIZES. Sampling to determine numbers of eggs, larvae and algae is essential to the hatchery system. It allows larval populations to be controlled in the rearing containers and algae to be fed according to plan.

To take an egg or larval sample, the solution should be agitated with a perforated plexiglass plunger. A sample of known volume is withdrawn quickly with an automatic pipette. The entire sample is counted using a compound microscope. If the numbers in the sample are too high for accurate counting, further dilution will be necessary.

Once the sample size and number of eggs or larvae are known, all necessary calculations can be made. The total population can be estimated or the dilution necessary to achieve a given concentration in an aquarium can be determined.

Algal cell numbers can best be estimated from a standard curve. The curve should plot cell numbers per milliliter against optical density (also called absorbance). The light reading is taken with a photometer. The curve should initially be established using a particle counter to enumerate subsamples of dense algal culture. Separate curves will be needed for different algal species. A sample calibration curve is shown in Fig. 10.

TIME SCHEDULE: A summary of culture densities and feeding regimins for each of the oyster larval stages is given in Table 2. The typical time schedule for a brood of larvae is shown below.

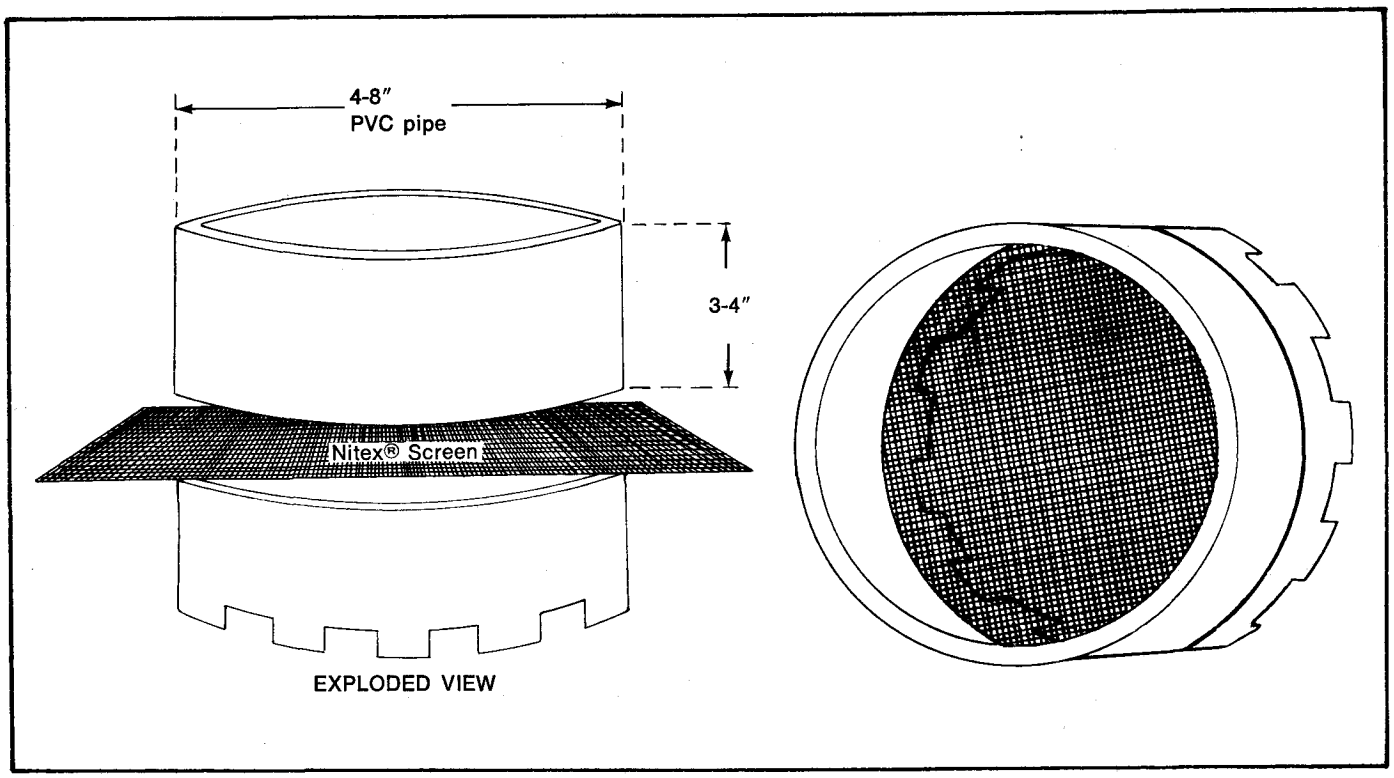

Fig. 8. Home-made screen

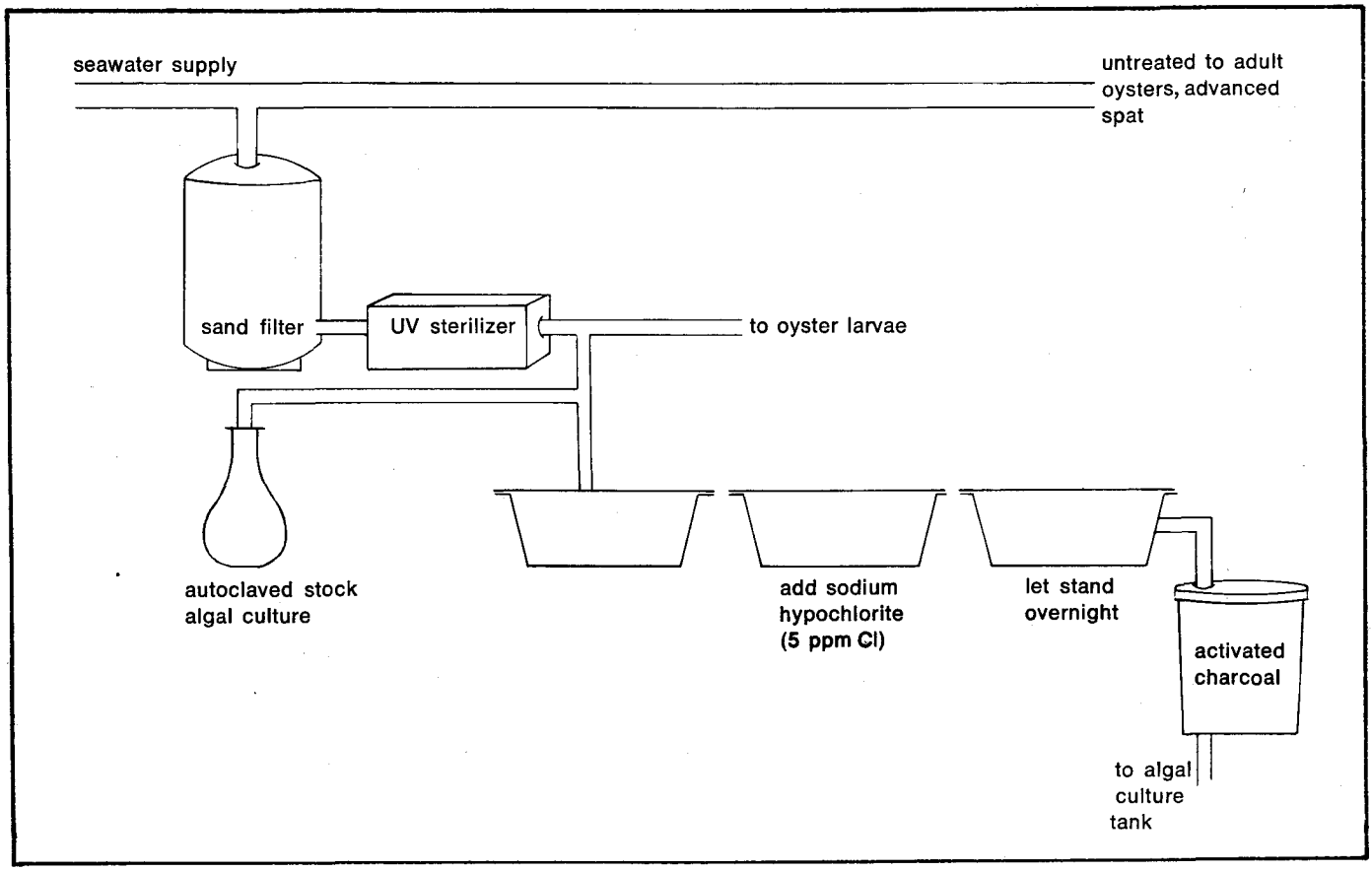

Fig. 9. Water supply

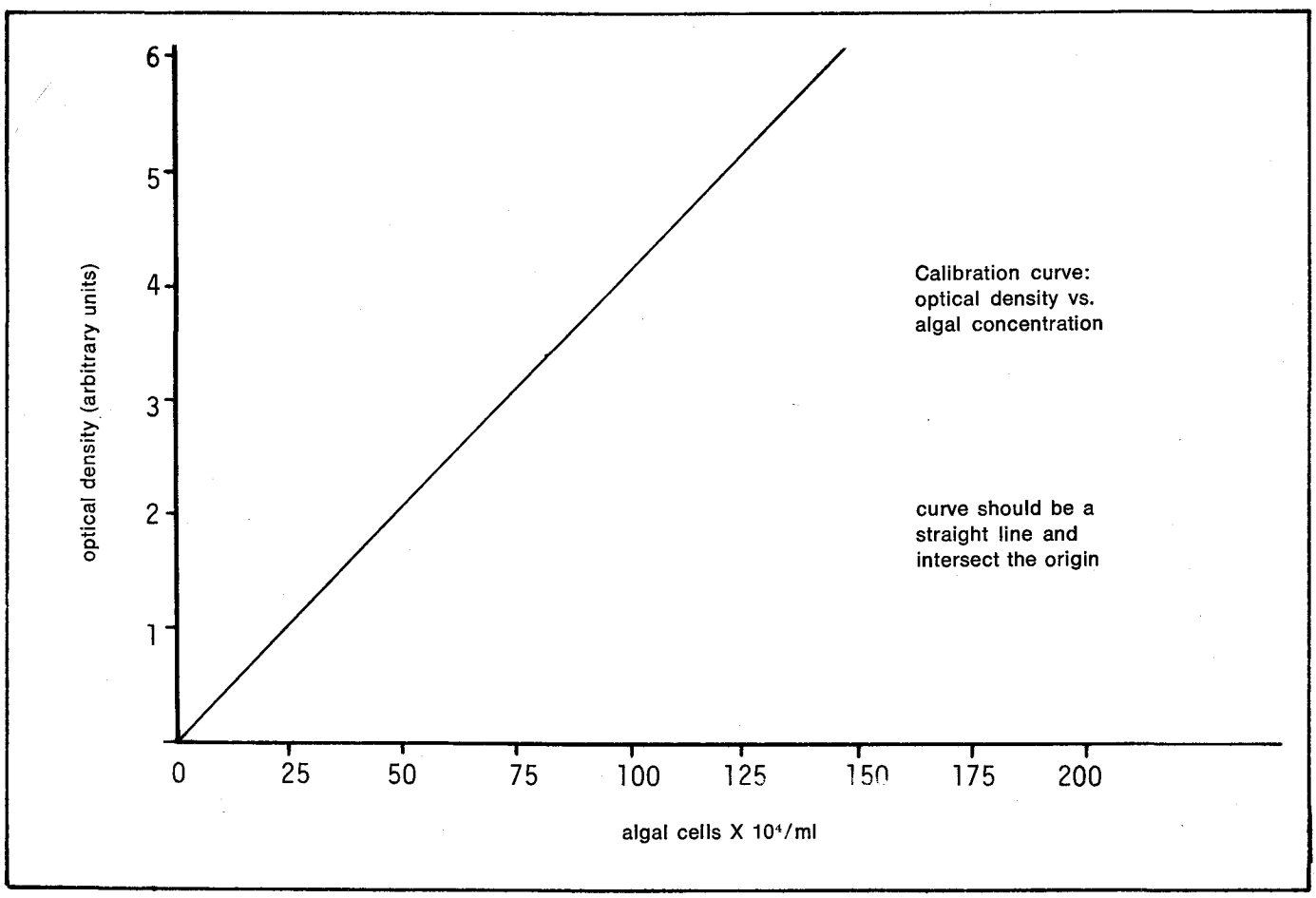

Fig. 10. Calibration curve

Day	Number in tank	Mean shell length	Screen size used
Day 1:	5.0 x 10^6	75-80 μ	40-60 μ
Day 7:	3.35 x 10^6	110-120 μ	40-80 μ
Day 14:	2.25 x 10^6	160-170 μ	80-120 μ
Day 21:	1.5 x 10^6	250-270 μ (setting begun)	120-210 μ

Table 2. Size and survival of larvae under optimum conditions.

Day 0: Fill storage tanks; chlorinate rearing tanks for larvae.

Day 1: Spawn oysters; put embryos in clean tanks.

Day 2: Examine larvae. If shelled up, screen out larvae, count and distribute to tanks. Add Sulmet® and food.

Day 2-7: Feed larvae 30,000 cells/ml each day.

Day 7: Change water on larvae. Begin feeding 50,000 cells/ml twice each day. Count larvae and record survival.

Day 14: Change water on larvae. Count and record numbers. Begin feeding 80,000 cells/ml twice each day.

Day 21: Change water on larvae. Count and record numbers. Put test cultch in tanks. Fill one setting tank for every 1 x 10^6 larvae and bring to 30 C. Have plenty of clean cultch ready.

Setting begins on test cultch: Drain out larvae, put in setting tanks, add cultch. Start new brood.

Two weeks after setting: In summer, spat may be moved to holding pond. In winter, hold the spat in hatchery until the setting tanks are needed for the next brood.

record keeping and evaluation method

An oyster hatchery, like any other business, should be constantly evaluated for best use of effort, equipment and space.

The method to be described should provide a means of optimizing biological reliability and procedural efficiency in hatchery operation and management. The key to optimization lies in periodically reviewing the biological and procedural data, identifying problem areas and making selected changes in operating routines to solve the problems. After operating according to the altered routine for a period of time, the resulting data should be reviewed to judge the effectiveness of the change. This evaluation procedure should be an integral part of the hatchery routine. Continual improvements must be made to optimize productivity.

HATCHERY ROUTINES. The evaluation operates in a cyclic manner. Periodic review encourages implementation of carefully-considered changes in the operating routine (Fig. 11). The routine should be established with a daily schedule of duties accompanied by a thorough description of each task.

Operation of the hatchery is broken down into units of work, or tasks. Each task should be defined in such a way that time expended on it can be identified exclusively with that task. A daily record is kept of times spent on each task. Table 3 shows a hypothetical breakdown of the proportion of time expended on various tasks. The table is meant only as a guide to show how a technician's time may be recorded; actual times will vary among workers and hatcheries. If excessive time is indicated, techniques should be streamlined.

BIOLOGICAL RECORDS. Biological records should be kept regarding such items as spawning success, algal culture cell densities, larval growth and survival, and setting success. Records of oyster production should start with adult selection, conditioning history, spawning, larval rearing, continue through setting and eventually include growth and survival in the field and yield upon harvest.

Consistently poor results at one stage may be caused by problems earlier in the life history. For instance, inadequate conditioning time of adult oysters may result in good spawning success as measured by numbers of eggs, but eventually lead to poor survival of the veliger larvae.

In algal production, contamination is the worst problem. Records of algal production should clearly show techniques used and any

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OYSTER CULTURE AND GENERAL OYSTER BIOLOGY

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Galtsoff, P.S. 1964. The American oyster, *Crassostrea virginica,* Gmelin. U.S. Fish & Wildlife Service, Fishery Bull. 64. Washington, DC. 480 pp.

Matthiessen, G.C. 1970. A review of oyster culture and the oyster industry in North America. Woods Hole Oceanographic Inst., Contribution 2528. Woods Hole, MA. 52 pp.

Morse, N.H. 1971. An economic study of the oyster fishery of the maritime provinces. Fish. Res. Bd. Can., Bull. 175. Ottawa. 81 pp.

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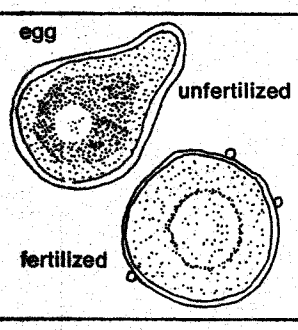
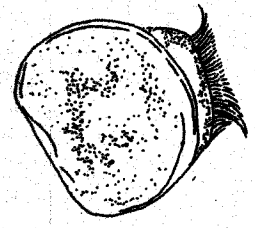
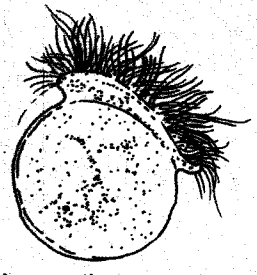
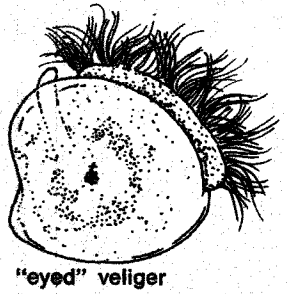
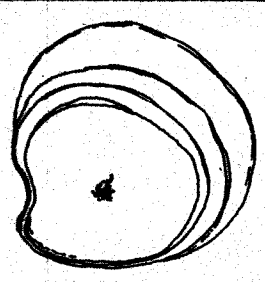
Summary of oyster larval stages, culture densities and feeding regimens.

STAGE	AGE	SIZE	DENSITY	FOOD*
egg 	0-24 hrs.	55 microns	100/ml	none
 "D" or "straight hinge" veliger	1-6 days	75-120 microns	10/ml	30,000 cells/ml once daily
 later veliger	7-14 days	130-200 microns	5-10/ml	50,000 cells/ml twice daily
 "eyed" veliger	14-21 days	200-300 microns	5/ml	80,000 cells/ml twice daily
 young spat	21 days	up to about 4 mm	——	100,000 cells/ml once daily

* Based on use of *Isochrysis galbana* or *monochrysis lutheri*