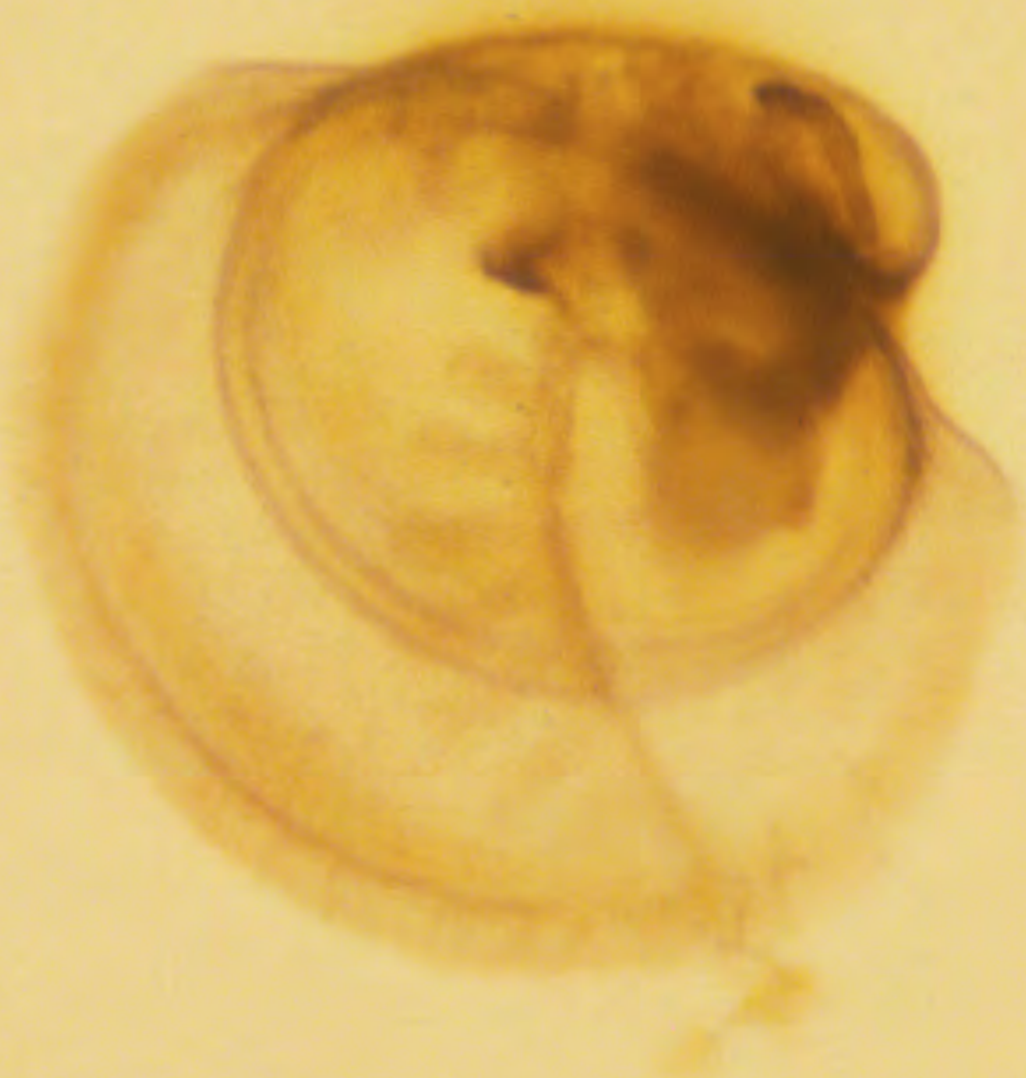
USING REMOTE SETTING TO PRODUCE SEED OYSTERS IN LOUISIANA AND THE GULF COASTAL REGION



By Brian R. Callam and John Supan, Ph.D.

LOUISIANA SEA GRANT COLLEGE PROGRAM AT LSU
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On the Cover: A two-day old spat at 100X magnification. Note the newly formed shell growth from the larval shell; a sign of successful metamorphosis.

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THE LOUISIANA SEA GRANT COLLEGE PROGRAM

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ACKNOWLEDGMENTS

Many people unselfishly shared their ideas and methods for this manual, which is an update of the original 1991 version. The research and field demonstrations in Louisiana were conducted in cooperation with Jordan Bradford and the late Tony Venterella of Oyster Farms, Inc., Hopedale, La. Their interest in transferring remote setting technology to Louisiana initiated the Oyster Culture Demonstration Project, conducted from 1987-1992. Their advice, financial and logistical support and friendship made this project possible and are greatly appreciated.

The successful larval and algal culture methods used at the Grand Isle hatchery of Gulf Shellfish Farms of Louisiana were in part a result of training by the late Lee Hanson at his Whiskey Creek Oyster Hatchery in Netarts, Ore. Hanson was a co-owner of the Grand Isle facility from 1990-1993. His knowledge of hatchery management is stilled admired and his assistance, hospitality and friendship are fondly remembered.

Thanks and appreciation are extended to Elizabeth Coleman, Chuck Wilson, Lee Hanson, Jordan Bradford and Dan Coulon for their reviews and comments on this manual and to Ken Roberts for his review and assistance with the cost analysis section of the 1991 version.

Financial support was provided by the Louisiana Sea Grant College Program and the Louisiana Cooperative Extension Service. The manual was edited designed, and produced by the Louisiana Sea Grant Office of Communications.

Figure 3 and 4 are from *Advances in the Remote Setting of Oyster Larvae*, Jones and Jones, 1988, published by the Aquaculture Association of British Columbia. Thanks are extended to the Aquaculture Association of British Columbia for its kind permission to use these figures.

Finally, the 1991 publication was dedicated to the memory of the late Warren Mermilliod, marine advisory agent with the Louisiana Cooperative Extension Service in St. Bernard Parish, whose assistance as co-investigator during the early phases of the project was just one example of his dedication to the Louisiana seafood industry and is fondly remembered. This 2018 version is dedicated to the memory of the late Ron Becker, associate director, Louisiana Sea Grant College Program, whose support of hatchery-based oyster research and outreach is alive and well today.

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PREFACE

The 1991 manual was the result of an oyster culture demonstration project sponsored by the Louisiana Sea Grant College Program and the Louisiana Cooperative Extension Service during 1987-92. The project was a response to the lack of consistent supplies of seed oysters from Louisiana's public reefs for bedding on private leases. The manual was an effort to help Louisiana oyster-farmers produce their own seed oysters.

The information here was gathered from industry manuals published and distributed in the Pacific Northwest and also generated by research and field demonstrations in Louisiana from 1990-2017. Those publications include the following.

Methods for Setting Hatchery Produce Oyster Larvae, by Gordon and Bruce Jones, Innovative Aquaculture Products, Ltd., Lasqueti Island, British Columbia, Canada. Published in 1983 by the marine Resources Branch, Ministry of the Environment of the Province of British Columbia (Information Report No. 4).

Advances in the Remote Setting of Oyster Larvae, by Gordon and Bruce Jones. Co-published and distributed in 1988 by the Aquaculture Association of British Columbia (5331 Hammond Bay Road, Nanaimo, B. C. V9S 5N7).

A Manual for Producing Oyster Seed by Remote Setting, by William G. Roland and Thomas A. Broadley. Produced in 1990 by the Extension Systems Branch, B. C. Ministry of Agriculture and Fisheries (808 Douglas St. Victoria, B. C. V8W 2Z7).

If you are interested in the tank designs and clutch handling methods used in the Pacific Northwest, you may wish to read these publications. Keep in mind, however, that although the technique of setting oyster larvae is basically the same, the Pacific and Gulf regions have different oyster species, climate and seawater temperatures. If you need assistance in using this manual, contact your local marine extension agent in the Louisiana Sea Grant Program.

INTRODUCTION

Oyster production in Louisiana has ranged from a low of 4.8 million pounds in 1966 to 15 million pounds in 2009 and has averaged 11.1 million pounds annually. Approximately 80 percent of Louisiana's oyster harvest has traditionally been taken from private leases. Although production since the early 1980s has been well above the long-range average, much of this increase can be traced to increasing effort. Issuance of oyster dredging licenses by the Louisiana Department of Wildlife and Fisheries has remained well over 1,000 per year since 1985. Although privately leased acreage has risen to approximately 400,000 acres, production from private grounds has shown very little growth, with declining production per acre.

For over 100 years, the Louisiana oyster industry has relied on the state's public oyster reefs for a supply of seed oysters. There are many reasons for the decline in the productivity of these reefs, including saltwater intrusion with its resulting marsh loss, natural oyster predation and disease and increased harvesting pressure. Lack of consistent seed supply for oyster framing has been a significant detriment to the economic development of the state's oyster industry.

During the 1970s, the Pacific Northwest also experienced a similar problem with seed supply. However, research and development by university and industry members in the region has given the individual oyster framer the ability to produce seed oysters using ready-to-set or "eyed" larvae (as known as pediveligers) from oyster hatcheries.

Larvae are microscopic, free-swimming oysters that develop from eggs and sperm spawned by adult oysters. Oyster larvae have a velum (similar to a tiny tongue with hair-like structures that beat back and forth) which propels them through the water and gathers food. The larvae begin to grow their shells within the first 24 hours. During a 10- to 15-day period, the larvae swim and feed on microalgae (microscopic, free-swimming plants). As they grow, the shell changes from a straight hinge (similar to a clam) to a typical adult oyster shape. Near the end of their swimming period, the larvae develop an eye-spot (seen as a black dot) on their shells and grow a *foot* that allows them to crawl. During the last 48 hours, they search for a place to set, or cement, themselves by crawling on whatever surface they find suitable. After setting, they then change from a swimming existence to a sedentary one; their velum and foot are absorbed and other internal organs change.

In the wild, larvae swim up and down in the water and are moved by tides and currents, but in the hatchery, they are raised in large tanks using filtered seawater. Commercial hatcheries commonly use several tanks that can each hold well over 8,000 gallons of seawater for high-volume larval production.

Larvae are small and are measured in micrometers (commonly called *microns*, of which there are approximately 25,000 microns to an inch). Larvae begin as fertilized eggs measuring 40-50 microns in diameter and grow to 285-350 microns when they finally set. At that size, the *eyed*-larvae can be seen, without magnification, swimming inside a white bucket, looking like fine grains of brown-black sand.

Remote setting methods allow the grower to utilize a hatchery without the expense, special abilities or location needed to operate one. In the Pacific Northwest and East coast, for example, large commercial, high-volume oyster hatcheries now produce billions of larvae for sale to growers.

Remote setting of the Pacific oyster (*Crassostrea gigas*) was first investigated in 1972 through laboratory studies by D. S. Lund, at Oregon State University (OSU), and commercially by W. W. Budge. Larval handling and remote setting was further refined by West Coast oyster growers and B. A. Henderson, also of OSU. Prior to these efforts, oyster hatcheries were not economically feasible because of cultch (the material on which oyster larvae will set) handling and grow-out problems. Remote setting techniques helped hatcheries become viable by allowing a division of labor between the hatchery operator and the oyster farmer and enabled the hatchery to concentrate on high-volume larval production. Successful commercial remote setting methods have been well documented from the Pacific Northwest and East coast.

The general process of remote setting is as follows:

> Hatchery-raised larvae are wrapped in moist nylon cloth or coffee filter and paper toweling then shipped to the grower in an insulated container at 41°F (5°C). Upon arrival, the larvae are suspended in a covered tank of filtered seawater at about 86°F (30°C), containing washed and aged cultch. Oyster shells are generally used as cultch, held in plastic-mesh bags (shellbags), wire mesh or stainless steel cages. The larvae are introduced at a density of 100 per shell, anticipating a 20 to 50 percent successful attachment to the cultch during the setting period of two to three days. Setting activity and spat condition are verified by microscopic examination prior to planting on leased water bottom. When and where to plant are very important decisions, since the resulting spat can be lost to crab and oyster drill predation (Figure 1).

Since larvae can close their shells like adult oysters, they can be stored in the refrigerator. Researchers found that they have a shelf life of up to seven days, so long as they are kept moist and at the proper temperature, but long storage times are not recommended. Larvae should be delivered from the hatchery overnight and set as soon as possible.

The remainder of this manual will discuss important points that need to be considered before larvae are set.

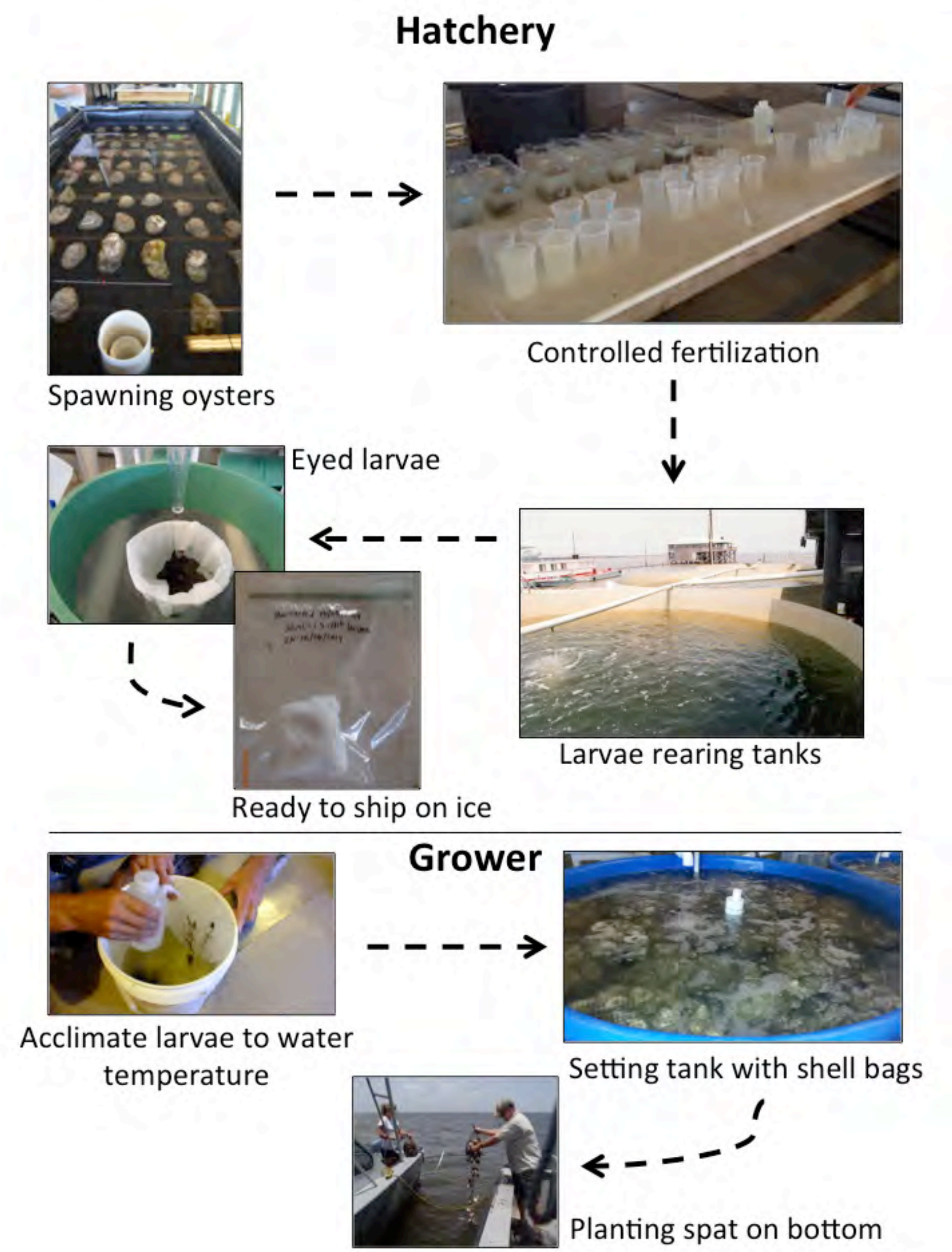


Figure 1. Oyster production from start to finish using hatchery-raised larvae and remote setting.

CHAPTER 1

CHOOSING A REMOTE SETTING SITE AND GROW-OUT AREAS

Water quality is the most important factor in choosing a site. **Consistently good water quality can mean the difference between having regular successes or failures.**

Setting Site

A common-sense approach to determining water quality is by looking at the activities in the watershed or drainage area of the bay or bayou under consideration. Herbicides and pesticides are lethal to larvae at strong doses. Check to see if local road maintenance efforts include the use of herbicides to control ditch weeds and if mosquito fogging occurs close by. Runoff from boat yards can cause problems for setting operations, especially from antifouling paints. Bilge water, which usually contains oil, fuel and hydraulic fluids, can cause problems if pumped into setting tanks.

Very high tides over the marsh can result in brown-colored *dead* water, caused by decaying marsh grass. Failed larval sets are likely when such water is used.

Check the salinity (salt content) of the water you are considering. This can be done with an inexpensive hydrometer (see Appendix 1) or, for a slightly greater investment, a refractometer (Figure 2). Larvae do not set well in water that is too fresh. The salinity should be above 10 parts per thousand.

Other factors to consider include:

- access to reliable electric power
- ease of getting to the site for providing surveillance, monitoring, maintenance and delivery of larvae from the hatchery
- ability to transport cultch to the site and seeded cultch to the grow-out area
- ease in pumping water from below the surface to avoid surface pollutants and rainwater and off the bottom to ensure that the water does not contain sediment

Grow-out Sites

Grow-out areas should be chosen the same way a lease for bedding oysters would be chosen. Oysters grow faster in areas with swift-moving currents because more food is available. This is important if placing stacked containers or shellbags of spat onto the lease. When placed in slack water, the growth of spat located in the inner areas of containers or shellbags will be less than that of spat in the outside areas. Stunted seed oysters do not grow quickly when bedded.

Bottom type is also important if the cultch is to be placed on the bottom. A bottom firm enough to walk on without sinking is the minimum requirement for placing pallets of containers or shellbags. If containers or shellbags are going to be thrown directly on the bottom, a hard reef is necessary to prevent burial.

Other factors to consider include:

- location close to the setting site, to avoid drying out the freshly set spat and to reduce transportation costs

- use of the site during rough weather

- water deep enough to keep the spat submerged

- water quality

- theft

Figure 2. Tools with which salinity (salt content) may be estimated: hydrometer (top) and refractometer (bottom).

CHAPTER 2

REMOTE SETTING SYSTEM CONSTRUCTION AND PREPARATION

The system needed for setting larvae includes a tank, air-lines, a blower, a cover, and a seawater pump and filter.

Tank Design, Construction and Preparation

Setting larvae in a tank containing cultch and seawater provides the oyster grower complete control over seed production, as opposed to releasing the eyed-larvae into the wild over a reef or planting the cultch and expecting a wild set. The tank can be simple and of any shape. The cultch and water should not be more than four feet deep to allow complete penetration by the larvae.

The tank volume depends on the amount of seed you want to produce from each batch. Larger tanks are more efficient and economical for high volume production per unit of effort and construction costs. Larger tanks require a great deal of structural support because increased water weight creates greater stress. Smaller tanks can be made much more simply and cheaply.

A tank 10 x 8 x 4 cubic feet holds 300 shellbags, which are approximately three feet long and hold about 1 cubic foot or 215 oyster shells. About 1.3 million spat can be produced from each set with a tank this size if approximately 100 larvae per shell are added and 20 percent of the larvae set - an acceptable setting success rate. Figures 3, 4 and 5 illustrate examples of tank designs.

Tanks can be made of other materials as well. Cement is acceptable if the surface is smooth. Metal tanks are not usually used because most metals are toxic to the larvae, tend to rust or are too expensive. Commercially available polyethelene tanks (i.e., poly tanks) are relatively cheap and feasible if rough handling is avoided during mechanized cultch loading and unloading.

Most setting tank interiors are light in color. Keep in mind that larvae like to set on darkened surfaces.

The tank should have at least a two-inch drain. **Draining and cleaning are easier if the tank drains from the bottom**.

After construction, the tank should be thoroughly soaked to leach out toxic chemicals from the construction materials. This is especially necessary for fiberglass, gelcoat, painted material and cement. Completely fill the tank with water and soak for at least two days. After draining, repeat the process twice more. Rinse the tank with water between soakings. Exposing the tank to direct sunlight during leaching will also help. Fiberglass, gelcoat and paints should leave no odor in an empty tank once leached. Cement tanks are well leached when the pH is neutral (close to 7).

A steam cleaner can be used to quickly leach fiberglass. The nozzle is fixed toward the center of a covered tank and the tank is steamed for six hours. This method should avoid burning a hole in the fiberglass and make the surface very hot (Jones and Jones, 1988). Afterwards, the tank should be rinsed until there is no remaining fiberglass smell.

A tank cover is necessary to keep rain out and to darken the inside of the tank during setting. Since eyed-larvae swim away from light, a cover helps in obtaining an evenly distributed set on the cultch by darkening the water column. In Louisiana, a tightly fitted tarp works well.

Aeration

Air is bubbled through the tank during setting to help keep the larvae evenly distributed. Plastic pipes with small holes (1/32 inch to 1/8 inch) drilled about every foot, laid evenly across the tank's bottom, work well. The air-lines should be designed to allow easy removal and cleaning (see Figure 4 and 5).

Air can be supplied by any means so long as it is oil-free. Compressors and pumps can be used, but blowers work best (i.e., high volume, low pressure). Aquaculture or aquarium specific blowers can be purchased through the Internet (Figure 6). Connect the blower's outlet to the tank's air pipe system. A valve may be placed between the blower and tank to control the airflow and bleed off excess air. It is also necessary to locate any air source higher than the water level in the tank so that the pump will not fill with water when it is turned off or when the power goes off.

Seawater Pumping and Filtration

Remote setting systems do not have critical pumping requirements. Ideally, a pump should be chosen to match the total dynamic head of any system, which includes the type and length of the plumbing, number and type of fittings and the height to which the water is being pumped. The pump size depends on how fast you want to fill the tank because once the tank is filled, the pump can be turned off. Radial (centrifugal) pumps with plastic, cast iron or brass impellers are commonly used. Swimming pool pumps with plastic impellers and intake baskets to catch debris work well with seawater. Locate the pump near the water to eliminate impeller cavitation. Keep silt from fouling the cultch inside the tank by locating the pump's intake off the bottom and away from boat wheel wash in shallow water.

Seawater should be filtered before it enters the tank to remove most of the larger microscopic plankton that eat or compete with the larvae. Filter bags (rated for 50 microns) are effective enough for remote setting, easy to use and economical (Figure 7). They can be purchased from most aquaculture supply companies. Commercial swimming pool sand filters work well too but are more expensive and difficult to maintain (e.g., interior fouling).

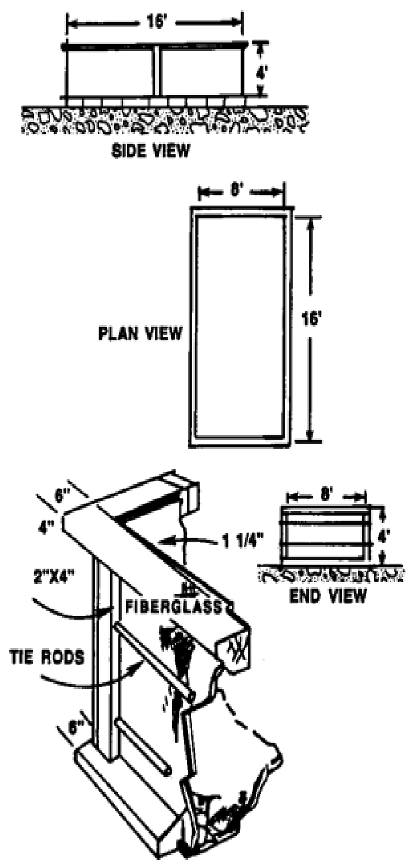


Figure 3. Plywood and fiberglass tank design.

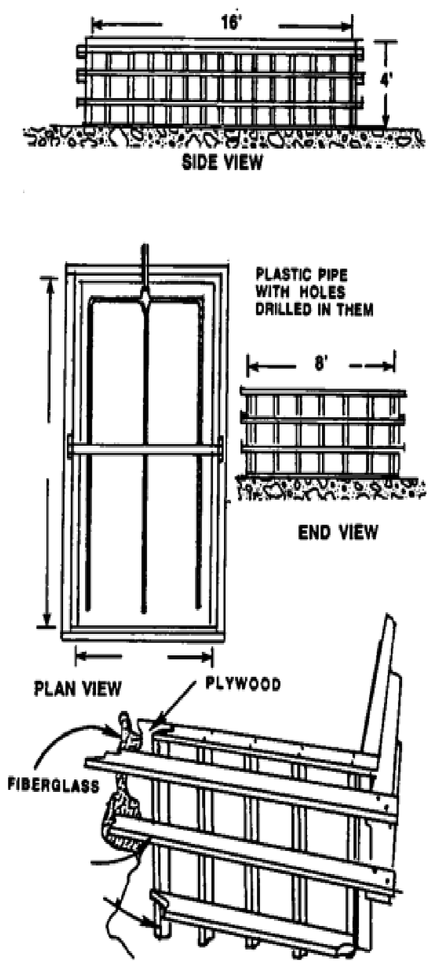


Figure 4. A setting tank design. Note that 2 x 4s and 2 x 6s are used instead of tie rods, as in Figure 3 (from Jones and Jones, 1988).

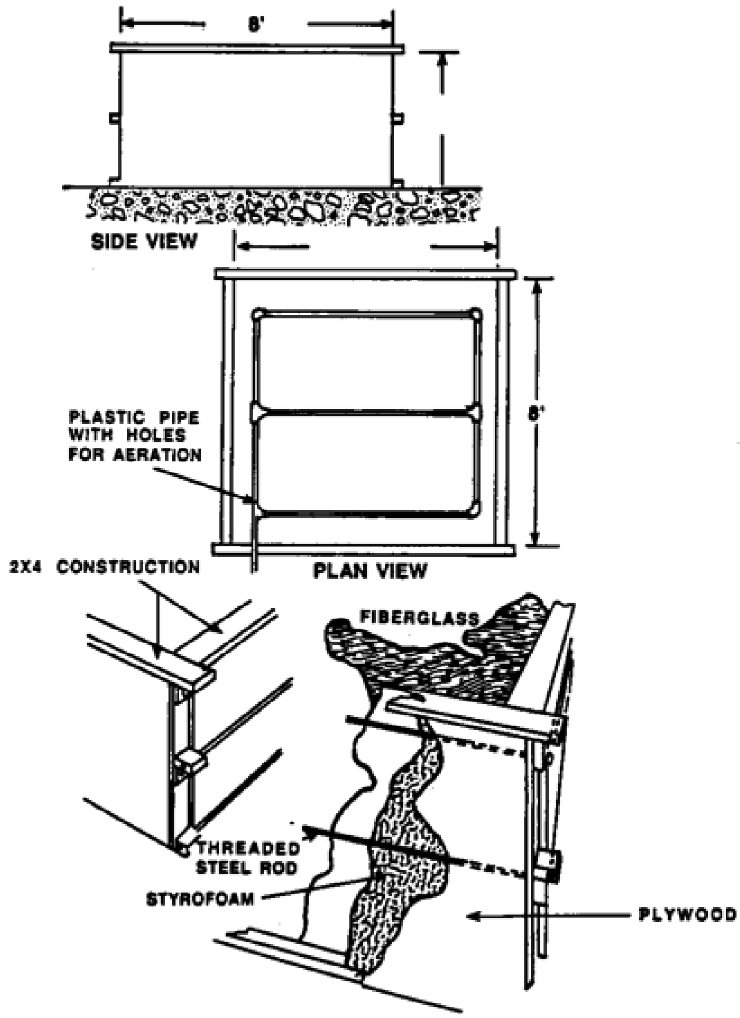


Figure 5. An insulated tank design. Insulation is particularly important if the seawater is heated, to keep heating costs low (from Jones and Jones, 1988).

Figure 6. An aquaculture-specific air blower.

Figure 7. Filter bag that fits over the setting tank source of seawater.

CHAPTER 3

CULTCH PREPARTION AND HANDLING

The majority of the labor required for seed oyster production using remote setting techniques involves cultch preparation and handling. Cultch mush be washed free of grit, chips and dirt; the tanks must be loaded and unloaded and the cultch bedded on the lease.

Cultch Preparation

Clean oyster shells (cultch) are important for obtaining a good set on a wild reef; remote setting is no different. Depending on the cultch type used, preparation may include the following:

Aging: Shucked shells should be aged at least six months to one year before using in a remote setting tank. Meat remaining on fresh-shucked shells will quickly *sour* the water in the setting tank, which will kill larvae and spat. Weathering the shells (i.e., piling them up outside) will help remove the remaining meat and fouling organisms (e.g., barnacles, mussels, sea grasses,and jelly-like animals).

Grading: Grading shells helps obtain a uniform size by removing smaller shells and fragments. Motorized tumblers and shakers can be used, which can also remove much of the powder and grit from the air-dried shells.

Washing: Washing is very important because dirt and grit will keep the larvae from setting on the cultch. Washing can be as simple as rinsing the cultch off with water from a hose or it can be part of a mechanized process that grades, washes and containerizes the cultch.

Leaching: If plastic, rubber, fiberglass, cement or other artificial cultch materials are used, leaching is important for removing toxic residues that may remain from manufacturing. Soaking the material in water is a common practice, but fouling may be a problem if it is soaked in seawater. A leaching period of four to eight months is recommended for plastic clutches (Roland and Broadley, 1990), however, the water climate in the Gulf region will quicken the process. Cement or cement-coated cultch must be soaked to neutralize the pH (i.e., a pH of 7) because cement mix is very alkaline.

Cultch Handling

Oyster shell is the cultch most used for remote setting and is usually handled in shellbags averaging approximately 40 pounds each. The bag material (a roll of 13-inch lie-flat heavy-duty shellbag material or tubing with 9/16-inch mesh) is available from most aquaculture supply companies (Figure 8). The material is cut at the desired length (typically three to six feet), knotted at one end, slipped over a three-foot length of eight-inch PVC pipe (thin-walled) and filled with shells. Some operators have designed bag-filling tables with built-in lengths of PVC pipe over which the bags may be fitted. Cultch is then loaded onto the table and pushed into the pipes holding the shellbags (Figure 9). The pipe is then removed and the shellbag is tied shut. Generally, shellbags are stacked on wooden shipping pallets for forklift handling.

Front-end loaders can be used to dump shells into a machine that grades and washes shells (Figure 10) and then sends them into a hopper. Laborers cut and knot the shellbag material, slip the bag onto the loading pipe(s) at the bottom of the hopper, remove the bagged shells, knot the bag again and stack it on a pallet.

Smaller operators simply load shellbags using a hand-held hopper next to a shell pile, then wash with a fire hose (Figure 11). Sometimes a table-loader may be used.

Wire mesh cages or stainless-steel cages may also be used to hold shell in setting tanks allowing for other mechanized handling techniques of cultch material and resulting spat (Figure 12).

Note: Sunlight will break down plastic over long periods of time, so bagging fresh-shucked shells to be washed a year later may result in brittle shellbags that break or tear easily when handled.

Tank Loading and Unloading

Shellbags or other containers can be loaded into and out of the tanks by hand, but shellbags stacked on pallets are also used in setting tanks. Conveyors, front-end loaders and various hoists are used for loading and unloading tanks.

Other Cultch Types

Clam shells, limestone and crushed concrete will tightly pack within the 13-inch lie-flat bagging or other containers and may not allow larvae to penetrate for a good, even set. Past research has shown that such cultch should not be contained more than nine inches thick to allow the aerated water to move the larvae through the cultch mass.

Crushed shell, sold in feed stores as chicken scratch, can be used to produce single oysters and small clusters. The cultch can be spread onto the bottom of shallow setting tanks (like soft crab shedding tanks). After setting, the cultch must be handled gently to reduce crushing the freshly set spat, preferably with a water hose. See Chapter 4: Cultchless Setting System Construction and Preparation for more details.

Artificial cultch, such as *French tubes* and cement-coated materials require special handling as well. Some of these materials are used in other regions with good success. For information on handling these cultch types, consult the West Coast setting publication mentioned in the first part of this manual.

Figure 8. Roll of shellbag material (top) and shellbags cut, filled and ready for handling (bottom).

Figure 9. Shell bags being filled at a table designed to aid in filling. Bags are slipped over the PVC pipe affixed to the table; cultch is then loaded onto the top of the table and pushed into the openings filling the bags.

Figure 10. Oyster size graders that can be used to grade and wash seed simultaneously. Seed oysters or cultch materials are loaded into the grader as the drum rotates and water is sprayed into the rotating drum washing shells and allowing oysters/shells to fall through appropriate sized holes in the drum for grading.

Figure 11. Debris being washed off of oyster shells using a seawater fire hose.

Figure 12. Cylindrical vinyl-coated wire mesh cage (top) with shock cord closures for drop-out bottom. Rectangular vinyl-coated cultch cage (bottom).

CHAPTER 4

CULTCHLESS SETTING SYSTEM CONSTRUCTION AND PREPARATION

Cultchless oyster setting is one alternative to setting oyster larvae on cultch (whole oyster shells) and produces single-seed oysters rather than clumps of oysters typical of setting on whole oyster shells. Single-seed oysters are mainly produced for use in containerized culture practices (e.g., cages or bags). Single oysters lack much of the protection from predation that forming clumps or reefs provide. Containerization is mainly for protection from predators and to improve growth rates and quality control of hatchery-produced oysters. Using containers also allows growers to manipulate growing conditions to maximize yield.

Setting Material

Though often called cultchless setting, oyster larvae are set on what is called *microcultch*. Microcultch is made of very small pieces of cultch material (usually sand particle size of crushed oyster shell but can be made from any material typically used as cultch). Crushed oyster shell can be purchased from farm supply stores or aquaculture supply companies, usually in 50-pound bags. To make mostly single seed, the microcultch should be graded to approximately 250 microns in size.

There are two ways of using microcultch: 1) simply spreading the microcultch on the bottom of the tank and 2) holding the microcultch in setting containers. Spreading microcultch on the bottom of the setting tank is simple and easy to do, however care must be taken when collecting freshly set spat from the tank because these spat are extremely fragile and can be crushed when sweeping up the microcultch material after a set. In such cases, water should be used to collect the spatted cultch. Holding microcultch in containers allows for more careful handling but requires either constructing or purchasing setting containers.

Tank Design, Construction and Preparation

Cultchless spat are often set in shallow tanks or troughs with a large reservoir to provide enough water exchange to the spat. Spat are held within the tank in setting containers with mesh bottoms. These setting containers are made of untreated wood (typically cedar or cypress) or PVC that has been well leached of toxins.

The operating principal of a cultchless setting system is that setting containers (trays or cylinders) act as downwellers (meaning water flows down through the containers) delivering food and water to freshly set spat. Water flows into each tray from the reservoir delivering food and water and is then returned to the reservoir through a standpipe at the end of the trough that can be fitted to maintain desired water depth (Figures 13 and 14).

The tank size depends on the amount of seed you want to produce from each batch. Unlike tanks for setting larvae on cultch, the size of the tanks for cultchless setting depends on how many and what size setting trays/cylinders are being used. Tank depth depends on the setting container design. Microcultch and larvae set in these setting containers do not need to be in more than a couple inches of seawater. If the containers are too deep the larvae may set on the containers rather than the microcultch.

A tank 8’ x 4’ x 1’ can hold 5 setting trays (32” x 16” x 6”), in which approximately five million larvae can be set.

Tank construction and materials follow the same guidelines set out in Chapter 2. Briefly, tanks can be made of fiberglass, concrete, polyethylene, etc. and should be thoroughly soaked to leach out toxic chemicals from construction materials. The tank should have a drain that is at least two inches. Draining and cleaning are easier if the tank drains from the bottom.

Setting Containers

Setting containers in a cultchless spat production system require a mesh bottom to retain the microcultch and larvae while simultaneously allowing water to pass through. A setting container can be as simple as a square or rectangle of wood with mesh tacked to the bottom or more complex by gluing mesh to cut cylinders of PVC (Figure 15).

The mesh used to retain microcultch and larvae is a critical component of the system. Eyed-larvae are graded to retain on mesh screens measured at 212 microns or greater. This means that the mesh screen used to retain microcultch and setting larvae should be slightly smaller than this to ensure no larvae are washed through the screen and into the reservoir tank (any larvae that do so are essentially wasted). Mesh screens rated as 150 microns are generally used in the construction of setting containers.

The size of the setting container depends on how many larvae are going to be set at a time. A container that is too large for the amount of larvae setting may result in wasting too much microcultch and one that is too small with result in larvae setting on each other (forming clumps) rather than as singles. A simple formula can aid in determining the setting container size based on setting no more than 1,500 larvae per square inch of setting container. This ensures larvae have enough room to set as singles without wasting space (Appendix 4).

Aeration

Aeration demands are dependent on the method of water delivery to spat (i.e., downwelled to setting containers or microcultch spread along bottom of tank). If microcultch is simply spread along the bottom of the tank, air can be bubbled through the tank via a similar setup as described in Chapter 2. If water is delivered to the spat through downwellers, then aeration occurs through the method of water delivery.

Water can be delivered to spat by either being dripped into the setting containers or through airlifts. Airlifts can also aerate water as it is delivered to the setting containers. Simple airlifts can be built using PVC pipe. A hole at the elbow piece is drilled large enough to insert the flexible hose. An air-stone, small enough to fit inside the PVC, is attached to the hose and pulled up into the pipe (Figure 16). Air-stones must be completely inside the pipe or water will not be lifted into the setting container. Airlifts are then connected to a manifold attached to a blower.

Water can also be delivered to setting containers through another method. Water from the reservoir tank can be pumped through a manifold running across the top-center of the tank with flexible rubber hosing inserted along the length of pipe then directs the water into setting containers. It is important to have the hosing suspended above the water, so the water can aerate as it drips into the setting container.

Filtration

Cultchless setting systems operate under the same filtration requirements as cultch-based remote setting systems (see Chapter 2). The pump size depends on how fast you want to fill the tank because once the tank is filled the pump can be turned off. Radial (centrifugal) pumps and swimming pool pumps work well with seawater. Locate the pump near the water to eliminate impeller cavitation. Keep silt from fouling the tank by locating the pump intake off the bottom and away from boat wheel wash in shallow water. When using setting containers with mesh bottoms, filtration becomes ever more important because the mesh can foul rapidly.


Figure 13. Setting tank. A blower is connected to a manifold that runs along the length of the tank. Flexible rubber tubing connects the manifold to the airlifts attached to setting trays. Water is recirculated from a reservoir by a sump and discharged through a hose positioned at the end opposite the stand-pipe.


Figure 14. A 35-gallon trash can acts as a reservoir for a setting tank. It is placed under the standpipe drain. Water is then pumped from the reservoir back to the setting tank by a submerged pump and hose.


Figure 15. Wooden (cypress) setting tray with mesh bottom (measuring 32” x 16” x 6”).

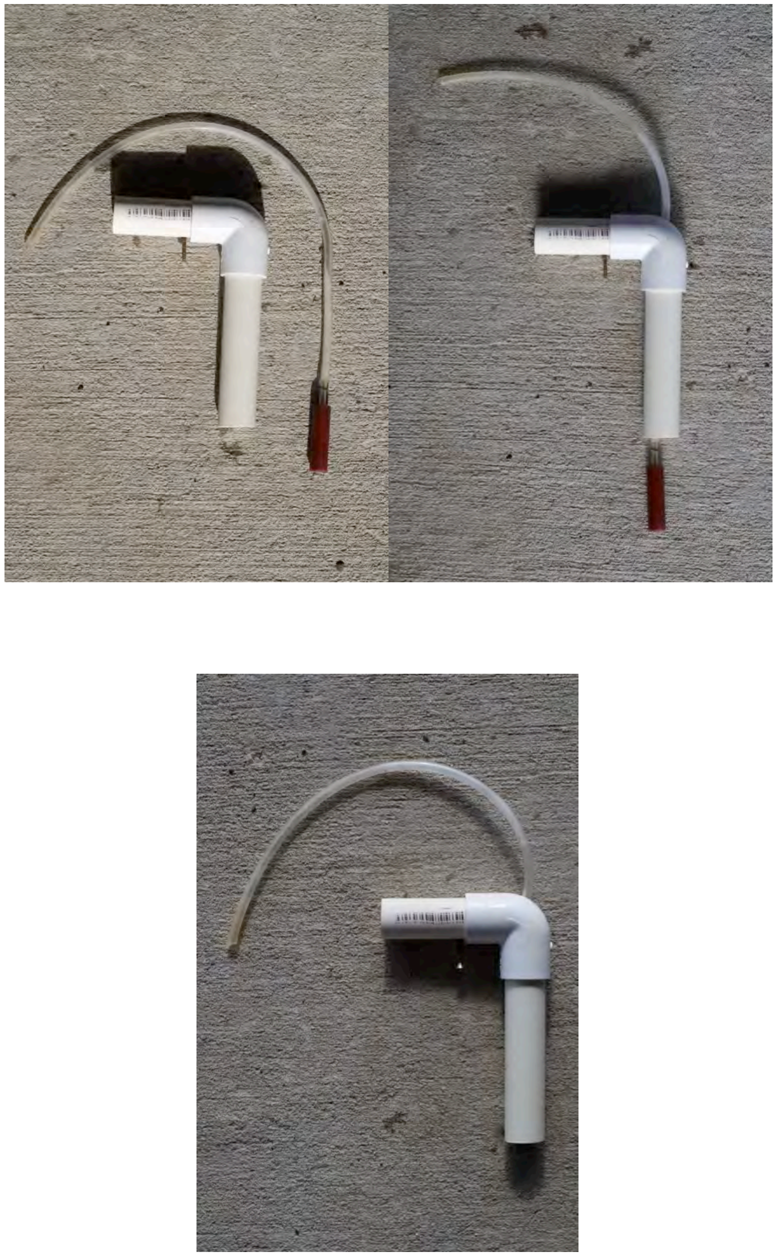


Figure 16. An airlift. ‘L’ shaped airlift with flexible air-hose and air-stone (top left). The air-hose is inserted into the airlift through a hole in the elbow and the air-stone attached to the hose (top right). The air-stone has to be pulled up into the airlift for water to be lifted (bottom).

CHAPTER 5

ORDERING, EVALUATING, AND HANDLING LARVAE

It is important to know the number of larvae needed for each set. Knowing how to tell good larvae from poor larvae (evaluation) and how to handle the larvae are also skills that must be developed.

Ordering Larvae

To know how much larvae is needed, a basic recipe can be used. Each grower can improve or change his recipe after evaluating seed production results to decide whether to use more or less larvae during later sets. Oyster hatcheries can customize an order by making individual quantities of larvae within an order, so the order does not have to be split up for each setting tank. Assuming good larval quality and that everything is done correctly, the following calculations are a good start.

Setting Tank: Average setting success is 20 to 30 percent. Adding 100 larvae per oyster shell to the tank may produce on the average approximately 20 to 30 spat per shell. Therefore, a tank that is 10 x 8 x 4 cubic feet containing 300 shellbags averaging 215 shells per bag should receive approximately 6.5 million larvae, resulting in approximately 1.3 to 1.9 million spat.

Setting Container: Setting success within setting containers is roughly equivalent to setting on whole oyster shells (20 to 30 percent). Adding 1,500 larvae per square inch of setting container area may produce approximately 200 to 300 spat. Therefore, a setting container that is 30 x 18 inches should receive approximately one million larvae, resulting in approximately 200,000 to 300,000 spat.

Grow-out: Of the 20 spat per shell bedded, three market oysters may be achieved, or a 15 percent survival rate. Most Louisiana oyster farmers have years of experience in bedding seed oysters, so individual results may be better. In general, 3 percent of the larvae added to the setting tank should reach market-size. Grow-out of cultchless seed involves containers (e.g., cages or bags) and is not covered in this manual.

Evaluating Larvae:

Larvae are usually shipped in a styrofoam cooler containing the ball(s) of larvae wrapped in nylon cloth or coffee filter and moist paper toweling with frozen gel packs. The cooler contents should be 40º-50ºF (4º-10ºC) and the gel pack still partially frozen. There should be no *fishy* odor.

The moist toweling should be removed and the individually wrapped larvae balls allowed to reach room temperature prior to placing in a clean bucket containing setting tank water. This will allow the larvae to acclimate or get used to the setting tank water conditions, especially salinity. A 15 to 30-minute acclimation period is recommended to reduce stress on the larvae and ensure that the larvae swim when added to the setting tank (Roland and Broadley, 1990). After the larvae begin to move in the bucket, do not wait long, or the larvae will begin setting on the bucket.

The wrapped balls of moist larvae can be weighed to keep track of the accuracy of each order. Weigh the wrapping again after the larvae have been removed and subtract the cloth weight from the previous total. A scale sold at local hunting supply stores for weighing gunpowder is suitable.

Buying a microscope to look at larvae and freshly set spat is a good investment (see Appendix 2). During microscopic examination, good-quality larvae can be determined by size, eye-spot development and movement.

Size: Individual larvae should be approximately 285-350 microns from hinge (umbo) to bill (see Figure 17).

Eye-spot: This should be darkly colored and 15-17 microns in diameter (see Figure 17).

Movement: Most larvae should be actively swimming with an extended velum (swimming organ). Swimming may not occur, however, after extended refrigeration storage (>3 days). Crawling with the extended foot should be noticeable. Give the larvae time to acclimate to the temperature and salinity of the water if no movement is noticed immediately. A cracked shell and a fouled velum are signs of poor handling and raising practices. Some larvae may swim together in strands of mucus, and if this is the case, add more water to the container.

Handling Larvae

It is important that once the larvae arrive from the hatchery they are either refrigerated or used immediately. Heated or dried larvae result in failed sets. *The larvae are fragile*. Do not squeeze or drop the larval ball and handle it gently. Though the larvae look like sand, they must not be treated as such.

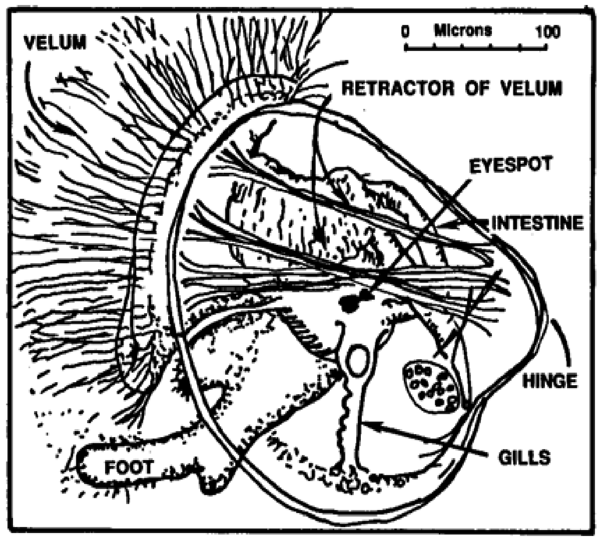


Figure 11. An oyster larvae (from Jones and Jones, 1988).

Figure 17. An oyster larva (from Jones and Jones, 1988).

CHAPTER 6

SETTING METHODS

The actual setting of the larvae is relatively easy and very similar for setting on cultch or microcultch, but certain methods should be followed.

Cultch and Microcultch Soaking

A 24-hour soaking period is recommended before the larvae are added for both cultch and microcultch. It has been said that soaking the cultch in the tank before adding the larvae *gets the land smell off*. Research has found that it may put a *sea smell* on the cultch. During soaking, a film that attracts larvae is formed by naturally occurring bacteria, originally called LST because they were found growing in a larval setting tank. The *scent*, or chemical cues, that the bacteria expel stimulate the larvae to search, by crawling with their foot, and cement themselves to the filmed surface (Weiner et al., 1989).

Microcultch needs to be rinsed thoroughly with seawater but not be soaked because when the water depth is only several inches to cover the microcultch, larvae have little choice in where they set. This is different from setting on whole oyster shells because in that case larvae have many more surfaces to set on.

Water Change

It is recommended that the water be changed after the soaking period so that fresh food can be provided before the larvae are added. However, successful sets have been obtained without doing so.

Aeration

Constant aeration is generally used, giving the water a light rolling, but not boiling action. In British Columbia, a fairly high aeration (a good roll but not boiling) for a half hour after the larvae were added produced a more even set than constant aeration. Constant aeration may have produced water circulation patterns that caused the larvae to collect in certain areas. There was no difference in the distribution of spat in the upper, middle and lower portions of the setting tank with the shorter aeration period (Roland and Broadley, 1990).

Larval Distribution

Even sets start with evenly adding the larvae to the tank or setting containers. The simplest way is to gently mix the larvae in a plastic bucket of tank water and pour them evenly into the tank or setting containers.

Some growers ladle the larvae into the tank or use a plastic sprinkling can. Others use a spoon on a pole. Make sure the aerator is on when adding the larvae.

Water Heating

A setting tank water temperature of 77º to 86ºF (25º to 30ºC) is recommended. Successful remote setting has been conducted in Louisiana with local seawater temperatures as high as 91ºF (33ºC).

If setting is going to be attempted during the early spring and fall in the Gulf region, tanks should be insulated, and heaters should be used to maintain the water temperature near 86ºF. During October 1990, a cold front passed through southeastern Louisiana, resulting in a setting tank water temperature drop from 82º to 65ºF (28º to 18ºC) within 36 hours. The remote setting attempts during and after that temperature drop resulted in failed sets. Those sets could have been saved had an electric immersion heater been available. An insulated tank would have helped as well.

Keep in mind that many metals are toxic to larvae. Incoloy 800 or titanium stainless steel is recommended for metal heating elements in direct contact with the setting tank water. Twenty-five-amp quartz-sheathed electric immersion heaters are used in the Northwest (Figures 18).

Tank Cover

Covering the tank will help obtain an even set, since “eyed” larvae tend to swim away from light. A tarp works well. Heated tanks should have insulated lids.

Setting Time

Allow 48 to 72 hours for the larvae to set after they have been added.

Feeding the larvae during setting is not necessary. After the setting time (i.e., after the set has been verified using a microscope), raw seawater should be pumped into the tank in a “flow-through” manner to provide fresh food for the new spat. The greater the water flow the better. Leaving the freshly set spat in the tank without enough food or in a dry condition for too long will cause high mortality.

When setting spat on microcultch (creating cultchless, single seed oysters), food needs to be added to the reservoir tank after the 48-hour setting time. Simple algal paste that can be purchased from aquaculture supply companies can be used (Figure 19). Or, the spatted cultch can be moved to an appropriate nearshore nursery system receiving wild food from local seawater (not covered in this manual).

Salinity

Salinity in the setting tank should be above 10 parts per thousand (often abbreviated as ppt), according to previous remote setting attempts in Louisiana. Setting efforts at lower salinities have proved unsuccessful.

If the local seawater at the remote setting site becomes too fresh at times, artificial sea salts, available from aquarium shops and aquaculture supply companies, can be added to increase the salinity and improve remote setting success. How much to add can be determined with a hydrometer (see Appendix 1).

Maintenance Between Sets

After the tank has been drained and unloaded, the tank interior (and setting containers if used) should be cleaned to remove any remaining grit and freshly set spat from the tank walls and bottom. Rubbing the interior with a nylon abrasive pad works well, and these are commercially available with plastic swivel-heads and long handles or poles to save your back. A stiff floor broom should work also. After rinsing the tank, check to see if the spat have been removed by wiping some of the surface with your bare hand. Freshly set spat look and feel like coarse sandpaper. Failure to scrub the tank between sets will result in more troublesome spat removal later on.

The filter bags are made of polypropylene-felt material and need to be cleaned soon after use. Turn them inside out and hose them out thoroughly and allow them to air-dry. They can also be cleaned in an old washing machine without detergent or bleach.

Figure 18. Small electric titanium immersion heater available from online retailers. Larger ones would be used for larger tanks.

Figure 19. Bottles of algal concentrate available from online mariculture retailers.

CHAPTER 7

EVALUATING THE SET AND KEEPING RECORDS

It is very important to check the set, especially before the tank is drained and the cultch is removed from the tank. Much can be learned from failed and successful sets with good evaluation and record keeping.

Checking for Swimming Larvae

Evaluating the set begins by counting swimming larvae and spat. A one-cup sample of water should be taken from the setting tank at intervals of 1, 24 and 48 hours after the larvae have been added. Pour the sample into a clear glass container and hold it up to or above a light and you should be able to see and count any larvae in the sample. You may also use a microscope or magnifying glass (at least 10X magnification). Use the one-cup measure each time and take all three samples from the same tank location so that accurate comparisons can be made between samples and sets.

Obviously, there should be fewer swimming larvae over time. A fast decrease in the number of swimming larvae found in the samples means that the larvae were ready to set and the cultch, tank and water conditions were right. If five or more larvae are still found swimming in the one-cup sample after 48 hours, the larvae were not ready to set, the cultch was not suitable or the tank conditions were not right and a longer setting time is needed. When the water gets too cold (e.g., at night in the late spring or early fall in an uninsulated tank), live larvae may stop swimming, though the internal organs can be seen moving under a microscope (Appendix 2).

Checking for larvae for single speed production is slightly different because the larvae are held in significantly smaller volumes of water within the setting container. Instead of using a one-cup sample, larvae can be checked by carefully collecting water using an eye dropper from inside the setting container. Checks should be done at 1, 24 and 48 hours after larvae have been added, and as with setting on cultch, fewer swimming larvae should be visible as time progresses.

Checking for Spat Set

Counting spat is very important and simple, once you get an eye for seeing freshly set spat (Figure 20, 21). Air-drying the cultch sample before counting makes it easier to see the tiny spat. When a larva successfully completes metamorphosis (changes) to become a spat, a new edge of shell will have grown onto the cultch from the bill of the larval shell with the hinge pointing slightly upward. A hand-held tally counter helps to keep track of counts.

A new shell should be hung or placed in the tank when the larvae are added and replace with another clean, new shell after 24 hours. This will give you a spat count for the first and second 24-hour periods of the setting time. These can be hung overboard for later reference. There should be a decreasing number of spat on these shells over time. The new shells should also be placed in the same section of the tank each time for accurate comparison.

As the setting tank is being unloaded, check some of the cultch from different sections of the tank to determine the evenness of the set. If shells are being used, count at least six shells from each section of the tank, such as the middle, side and corner. Be sure to take shells from the top, middle, and bottom layers of the cultch.

Accurate data collection and record keeping can help determine how good and poor seed production occurs. Thorough counting and record keeping will make future setting easier and provide better results. Setting results can vary for many reasons (i.e. different individuals, water sources, setting techniques, nursery and grow-out locations, seasons, larval sources, cultch types, etc.). Appendix 3 contains data sheets for remote setting. Copy them and use them regularly.

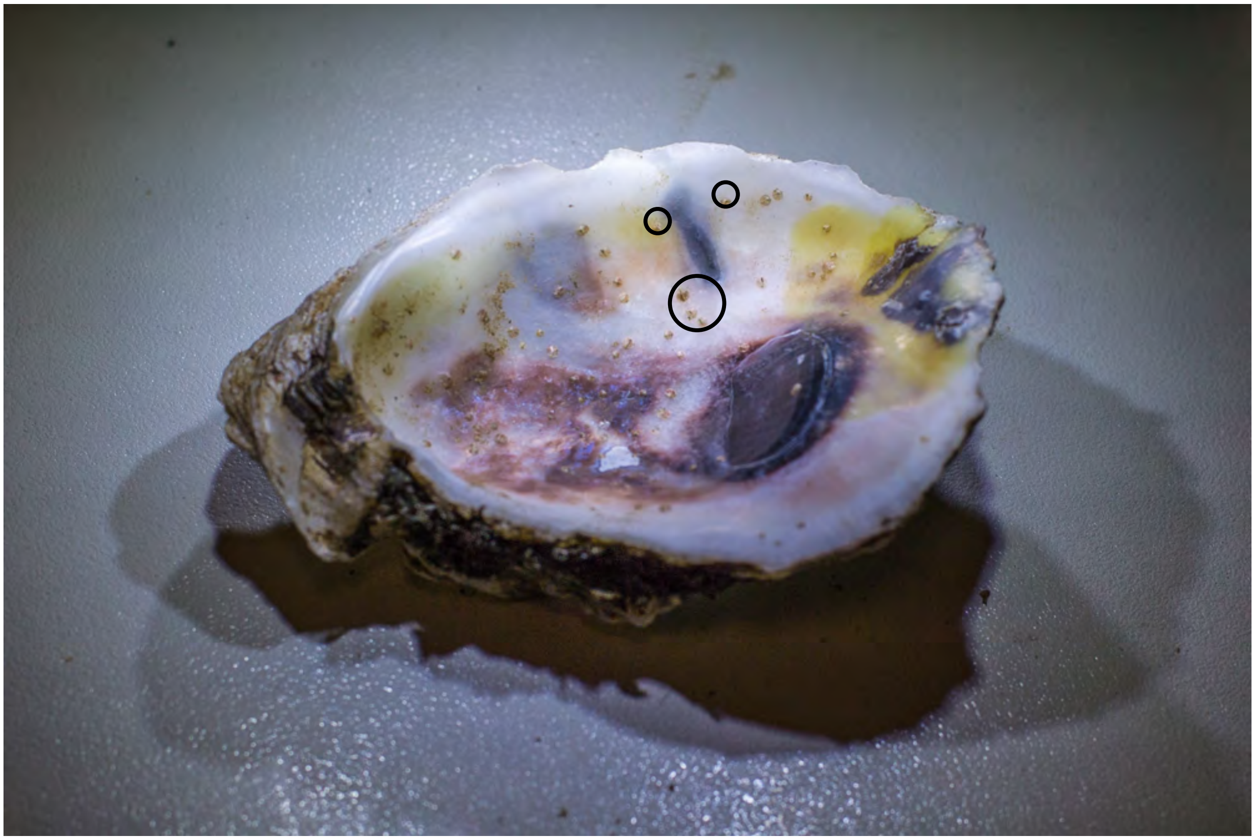


Figure 20. Newly set spat on an oyster shell. Several spat are circled for easier identification.

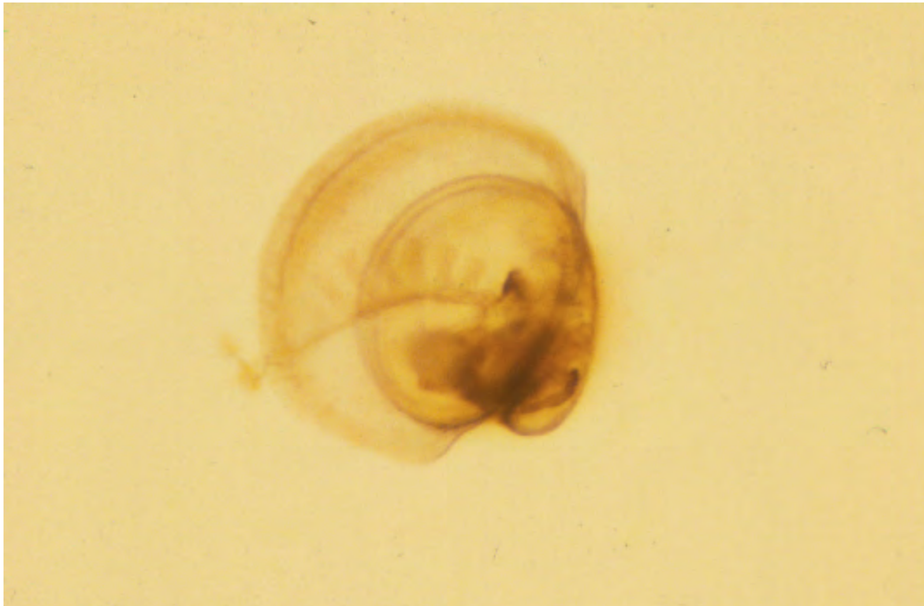


Figure 21. A 2-day old spat at 100X magnification. Note the newly formed shell growth from the larval shell; a sign of successful metamorphosis.

CHAPTER 8

EVALUATING SEED GROWTH AND SURVIVAL, AND DETERMINING WHEN TO PLANT

Spat growth of up to one inch (25 millimeters) within 30 days was common in Louisiana during 1987-90 shellbag-nursery trials in salinities ranging from 11 to 18 ppt. Research conducted in Maryland, however, showed that planting freshly set spat-on-shell obtains similar results to planting larger seed.

While the cultch is being emptied, it is important to evaluate some of the shells from different shellbags or containers to obtain an accurate average of seed production. Average number of spat per shell, the number dead and alive (percent survival) and average size are valuable information for evaluating the effectiveness of remote setting efforts and its cost. Such information will help individual oyster farmers improve their techniques and allow them to customize production, depending on the conditions encountered.

Determining when to empty the cultch containers for bedding onto the lease bottom is up to the individual oyster farmer. During summer nursery trials in Louisiana, spat began to grow through the shellbag mesh material within 40 days. This should be avoided, because high mortality will result from damaging the thin-shelled seed during bedding.

Damaged seed is more likely to attract crabs and other predators to the bedding site. Crab predation can be quite heavy when bedding is done during the summer months; however, the larger the seed size, the fewer predation problems. Crab predation can be reduced by harvesting crabs from the bedding area using baited crab traps. Black drum prefer single oysters and have difficulty feeding on clusters of oysters. Spat smaller than 1/4 inch are especially vulnerable to mud crabs.

Flatworms (i.e. the oyster leach, *Styloccus* spp.) (Figure 22) are a minor predator of spat on oyster reefs, since crabs and fish may prey them upon when they crawl from one spat to another. However, during Louisiana shellbag-nursery trials, flatworms caused up to 33 percent spat mortality. The flatworms appear to be protected by the mesh covering as well. Overnight air drying and concentrated saltwater dips are effective in treating the cultch to reduce flatworm predation. The simplest treatment is timing, which is important if flatworms become a problem. Bedding the seed before too many losses occur exposes the flatworms to their natural predators. Keep in mind, however, that early seed bedding to reduce flatworm predation must be weighed against possible crab predation later.

Planting the Seed

Since most oyster farmers have generations of experience with bedding oysters, little needs to be mentioned here. The characteristics of seed produced by remote setting are different from those of seed dredged from oyster reefs. Seed produced by remote setting usually are not as cupped and is more thinly shelled. The advantage of this technology is the large quantity of seed produced. Fewer boat loads of such seed may be needed. Seed survival obviously depends on whether the seed is mishandled before bedding, the bottom type, salinity of the bedding area and predators. Smaller seed obviously needs a longer grow-out period.

Figure 22. An oyster leach or flatworm in a glass ash tray. Note the size relative to the quarter-sized coin.

CHAPTER 9

THE COST OF REMOTE SETTING

The total cost of seed production using remote setting depends on the individual oyster farmer; the kind of equipment and labor available; the farmer's experience and bedding ground conditions. Since the costs are likely to be variable, the farmer should take great care in his own estimates. A more detailed cost accounting follows as a guide (Tables 1 and 2).

Estimates for Table 1 are based on standard setting tank designs found in this manual. The tank interior is made of fiberglass for waterproofing and for providing a strong surface against wear from repeated loading and unloading of shells. The fiberglass surface is painted with epoxy paint to eliminate a reaction of the water with the styrene in the resin. Using wood glue and good carpentry, three or four coats (two to three gallons) of epoxy paint may be good enough as a tank interior coating.

Two filter bags are used at a time during tank filling (Figure 7). Since the bags may be worn by repeated cleaning, two extra are included in the cost.

Estimates for Table 2 are calculated from remote setting experiences in Louisiana. The labor costs include bagging the shells, loading and unloading the tank, and planting the spat. Boat fuel is not included. A 1,500-foot roll of bag material costs about $75 (delivered). Generally, a one cubic foot bag of seed averaging 20 spat per shell cost about $6. Note that Table 2 included a depreciation charge for the setting system. The grower should realize that this is not an out-of-pocket expense but is an allowable deduction for income tax purposes.

A 20 percent setting success rate is guaranteed by most commercial hatcheries.

Each set from a tank is bedded on an acre. Other growers may do their planting differently. A 3 percent return of market-size oysters from the amount of larvae added to the tank may be attainable. Higher setting rates and returns may be attainable with good setting, nursery, and bedding techniques, water quality, evaluation and record keeping.

TABLE 1. INVESTMENT NEEDS FOR A SINGLE TANK REMOTE SETTING SYSTEM*

Materials and equipment for an 8’ x 8’ x 4’ setting tank:

6 sheets of 3/4” exterior-grade plywood	$120.00
6-8’ 2x4s (treated)	$24.00
16-8’ 2x6s (treated)	$104.00
1 gal. preservative (for tank exterior)	$13.00
fiberglass materials:	$244.00
16 yds of 1.5 oz. mat @ 5.00/yd.	$80.00
6 gal. resin & hardener	$150.00
1 gal. acetone	$14.00
1 gal. white epoxy paint (for tank interior)	$30.00
1/2” PVC pipe & fittings for air lines	$11.00
4-50 micron-rated filter bags & 2 support discs	$57.00
1-1/2” PVC plumbing for water pump & bag support discs	**$5.00
1-13’ 8” thin-walled PVC pipe (for making shellbags)	***$38.00
tank cover (plastic tarp)	$15.00
hardware	$30.00
air pump	***$290.00
water pump	***$135.00
Optional:	
microscope (1-100x & 1-30x pocket scopes)	$120.00
hand tally counter	$12.00
Total	**$1,248.00**

*Heaters and tank insulation not included
**Plumbing costs depend on how far the tank and/or pump is from the water source
***Can be used with additional tanks

TABLE 2. APPROXIMATE COSTS FOR SETTING 240 SHELLBAGS PER SET

Depreciation of setting system with options (Table 1)*	
Electricity for air and water pumps	$5.00
Eyed-larvae at $300/million x 6 million	$1800.00
Larval shipping	$48.00
Shellbags: $0.95/bag x 240	$229.00
9.5 cubic yds. Of oyster shell @ $16.50/yd.	(**$157.00)
6'mesh material/bag @ $0.05/ft x 240	(**$75.00)
Labor:	***$540.00
3-men each earning $60/day ($7.25/hr) for 3 days	
Total	**$2,802.00**
Cost/bag averaging 250 shells/bag and 20 spat/shell	$6.17

*The entire investment was treated as 5-year property under IRS Modified Accelerated Cost Recovery System (MACRS). Depreciation charges vary year to year under this system. The depreciation charge in the table reflects the setting system in its third year. The yearly depreciation is $219.
**Shucked shell prices range from $14-$19 (delivered).
***1,500' roll.
****Labor includes making shellbags, loading and unloading tank and placing bags of spat in nursery area. Include an extra work day ($150) for removal from the nursery area to grow-out area. Management and security are not charged here.

APPENDIX 1

HOW TO MEASURE SALINITY WITH A HYDROMETER

Whether you farm oysters, make soft crabs, or own a saltwater aquarium, knowing the salinity of seawater (the amount of salt present in the water) can be valuable information. This can be obtained inexpensively with a hydrometer.

A hydrometer is a precisely weighted, sealed glass tube that measures the density of a liquid by how high or how low it floats. A numbered scale is located inside the stem to obtain a reading of the density, also known as the specific gravity. The density changes with temperature and with differences in the amount of salt in the water.

Hydrometers are made specifically for various liquids, such as syrups, alcohol and battery acid, so make sure that the one you use for measuring salinity is specific for seawater or brine. Hydrometers for seawater can also be purchased with thermometers built inside. They can be purchased at aquarium shops and aquaculture supply companies.

To help in reading the hydrometer, try using it in bucket of water. You could also use a short length of three-inch PVC pipe, with a cap on one end, filled with sea water.

Most hydrometers are built to be used at a temperature of 59ºF (15ºC), so when the seawater temperature is different, the observed reading on the scale must be converted to the actual temperature.

Table 1 (below) can be used to estimate the salinity based on water temperature.

CONVERTING DENSITY TO SALINITY

Density		Salinity		
(sp. gravity)	59°F	70°F	80°F	90°F
1.000	0	2	4	6
1.002	4	5	7	9
1.004	6	8	9	11
1.006	9	10	12	14
1.008	11	13	15	17
1.010	14	15	17	20
1.012	17	18	20	22
1.014	19	21	23	25
1.016	22	24	25	28
1.018	25	26	28	30
1.020	27	29	31	33
1.022	30	32	33	36
1.024	33	34	36	
1.026	35	36		
1.028	38			

APPENDIX 2

BUYING AND USING A MICROSCOPE

Buying a microscope to look at larvae and freshly set spat is a good idea and investment. A poor larval shipment can be determined beforehand, reducing the possibility of a failed set and the resulting wasted labor. Cultch should also be checked to make sure you have a good set before unloading a tank.

Microscopes generally use a combination of lenses to magnify. Scopes usually have a 10 power (10x) eyepiece and different lenses (objectives) below the eyepiece that can be switched for different magnification. Some cheaper models have interchangeable eyepieces and one lens below them. Whichever is used, keep in mind that it is the combination of the two that determines the magnification or power (x). For instance, a 10x eyepiece with a 10x objective will magnify 100 times (100x). With a 3x objective, it magnifies 30x. A knob is turned for focusing.

Buying a microscope to look at larvae and freshly set spat need not be too expensive, but like everything else, you get what you pay for. The more you spend, the better the field of view (i.e., the circle you view may be the size of a jar lid as opposed to a dime). There may be two eyepieces as well. Such options make it easier on your eyes, especially if you are going to look in the scope for long periods of time.

The least expensive are pocket scopes with one or two eyepieces (biscopes) having 100x or 30x magnification (sold as separate scopes). These have a battery-operated light and a vinyl carrying case ($20-$50). Scopes used for students in high school and college have more than one lens and are more expensive ($250-$800). These are sold as compound scopes with magnification in the high range (100x-400x) and stereo (two eyepieces or oculars) dissecting scopes for close handwork with low to midrange magnification (10x-40x). Used scopes are available. If you are going to invest that much money, buy or build a carrying case as well.

To check larvae closely (i.e., to see internal organs moving to verify that nonmoving larvae are dead or alive), 100x is needed. You will be able to see the extended foot as well, meaning that the larvae are ready to set.

If you want to measure the larvae, a scale (micrometer) is needed. The scale is marked from 0 to 100 and is made from clear plastic or glass. With expensive scopes, the scale is placed in the removable eyepiece, so when you are looking in the scope, the 0 to 100 scale is in view over what you are measuring.

Less expensive scopes have eyepieces fixed in place, so if you want to measure larvae, you can place the scale down in the sample that you are observing or lay it on top of the sample as a cover slip. Inexpensive, hand-held pocket scopes can be used to measure larvae by placing the clear scale (sold as a cover slip) onto the bottom of a clear glass ashtray with a little seawater and a few larvae. The larvae can then be evaluated for velum and foot movement and measured as they swim over the scale. If they are moving too fast, some fresh water or a capful of alcohol (gin or vodka) will stop their swimming. Too much liquid in the ashtray will make measuring troublesome. If you need to push the larvae around while looking at them to get them situated over the scale, make a probe with a straight pin inserted diagonally through an eraser on a pencil.

Freshly set spat can be easily seen at 30x. A 10x magnification will allow faster shell examination by covering more area while looking for spat, then changing to 30x to get a closer look. Use the probe to push grit aside. Larvae that have successfully set will have grown a new edge of shell from the bill onto the cultch with the hinge pointing slightly upward. Freshly set larvae are hard to see on wet cultch, because of the glare from the light. Air-drying the cultch first helps.

Appendix 3

SETTING CHECK LIST

PREPARATION

Date and Time or Comment

______________ Larvae ordered from hatchery and transport arranged
______________ Setting tank preparation completed (cleaned and washed)
______________ Heating and aeration systems tested
______________ Cultch preparation completed (aged and cleaned)
______________ Tank loaded and filled with water for conditioning
______________ Test cultch hung in tank
Amount and type of cultch in each set tank
___________________ Tank A ______________________ Tank B
___________________ Tank C ______________________ Tank D
______________ Conditioning water dumped and filled with heated water

LARVAE INSPECITON

______________ Larvae arrival time and date
______________ Temperature of larvae in ice chest
______________ Color and smell
______________ Moisture of bundle of larvae
______________ Average size of larvae
______________ Food activity
______________ Loose velums

LARVAE INSPECTION (in bucket)

______________ Number of larvae per ml in bucket
______________ Total number of larvae (number per ml x volume)
______________ Mucus production (stringing)
______________ Behavior (drop to bottom, stick to bucket, swimming, etc.)

SETTING

______________ Time and date larvae put in tank
Number of larvae in tanks
__________________ Tank A ____________________ Tank B
__________________ Tank C ____________________ Tank D
______________ Bay water visibility
______________ Salinity and temperature
______________ Aeration and frequency and duration
______________ Temperature at start of set

______________ Number of larvae swimming after 1 hour in 1 cup

DAY 2

______________ Temperature in morning
______________ Number on test cultch
______________ New test cultch added
______________ Number of larvae swimming in 1 cup
______________ Food added? (amount)
______________ Water change? (amount)
______________ Temperature at end of day

DAY 3

______________ Temperature in morning
______________ Number on test cultch
______________ New test cultch added
______________ Number of larvae swimming in 1 cup
______________ Food added? (amount)
______________ Water change? (amount)

POST-SET AND PLANTING

______________ Date and time of removal from tank
______________ Air temperature and weather conditions
______________ Length of time out of water
______________ Tide foot level or depth
______________ Location of cultch for nursery
______________ Tide foot level or depth

SPAT COUNTS PER CULTCH PIECE

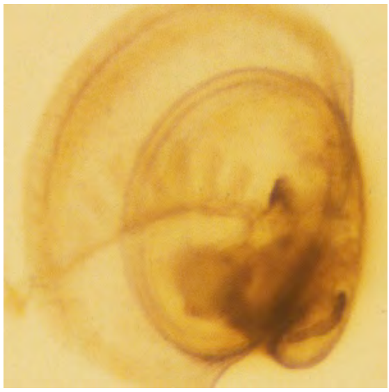
	TANK A	TANK B	TANK C	TANK D
AT REMOVAL	___________	___________	___________	___________
	___________	___________	___________	___________
	___________	___________	___________	___________
AFTER 2 WEEKS	___________	___________	___________	___________
	___________	___________	___________	___________
	___________	___________	___________	___________
AFTER 1 MONTH	___________	___________	___________	___________
	___________	___________	___________	___________
	___________	___________	___________	___________

APPENDIX 4

HOW TO DETERMINE SETTING CONTAINER LARVAL DENSITY

- Larvae are between 300-350 microns at setting
- Spat are removed from setting containers when they catch on a 500-micron screen → Length of spat around 700 microns or 0.03 inches (The spat basically double in size)
- The size at which the spat catch on the 500-micron screen needs to be determined, so when we stock larvae they have enough space to grow

700 microns or 0.03in



700 microns or 0.03in

- Changing the oyster into a square, the area is $0.0049cm^2$
- To calculate a stocking density, find the area of the desired setting container in inches and divide by the area of a freshly-struck spat ($0.0009in^2$)
    - This is approximately the number of larvae that should be set in the container
    - Being conservative with stocking densities is recommended to reduce the number of larvae that set on each other
    - If spat are transferred quickly to a nursery, setting containers can be stocked from 1 to 1.5 times the number determined by setting area

First calculate the number larvae per container based on area:

Area of downwellers (in square inches) / $0.0009in^2$ = ___________

For less conservative density, multiply the estimated larvae per container by 1.5:

(Max larvae per container) ___________ x 1.5 = ___________

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