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REMOTE SETTING AND NURSERY CULTURE FOR SHELLFISH GROWERS

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WORKSHOP RECORD

Remote Setting and Nursery Culture for Shellfish Growers

Workshop Record
February 19, 1991 • Olympia, Washington

Organizers
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Kenneth K. Chew

Sponsors
Washington Sea Grant Marine Advisory Services
in cooperation with
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About the Workshop

Pacific oyster growers in the Pacific Northwest largely depend on seed produced in hatcheries to provide them with the stock to produce a crop. As Lee Hanson points out in his historical account, the first domestic oyster hatcheries on the West Coast were a great boon to the industry.

Now the advent of remote setting and nursery culture of oysters and other bivalves has again propelled the industry a great step forward. Seed producers and growers no longer have to ship seed on heavy cultch to the growout site; now just the tiny larvae—millions of them wrapped in wet gauze the size of a baseball—can make the journey, saving shipping and maintenance costs. As a result, however, the setting and nursery activities that once took place at a centralized hatchery are now dispersed about the countryside and are conducted by individual growers rather than hatchery operators.

Although seed production is no longer a major problem on the West Coast, securing the right quantity and the best quality of larvae for remote setting can still be critical. Furthermore, continued proper handling through the sensitive nursery stages is critical to ensure that adequate numbers of juveniles survive for growout to market.

During the past several years, the need has grown to have a special meeting to bring together shellfish growers, regulators, and scientists to address the problems of survival of oyster and clam seed through the nursery period prior to placement into growout facilities or natural shellfish beds. The attendance at this workshop would definitely indicate that there is great interest in the subject.

We have organized this workshop so that we can help each other by sharing current information and views about how best to conduct these activities. Commercial growers will relate some of their many experiences "in the trenches," and researchers will present some promising recent results that should determine how the industry evolves in the future.

The accompanying diagram outlines how the practices of remote setting and nursery culture fit into the basic process of bivalve culture as conducted in the Pacific Northwest. In oyster culture, remote setting intervenes at the life cycle stage when larvae are "competent," or ready to settle, attach to cultch, and metamorphose to become "seed" or "spat." This is the pediveliger or "eyed" stage at which the larvae can be safely removed from the culture medium for up to a week and shipped from the hatchery to the grower. The culture of clams, mussels, and geoducks is patterned after this sequence of steps.

The papers on setting procedures discuss recommendations, approaches, and parameters for growers who order and receive eyed oyster and Manila clam larvae and plan to set them successfully at their sites. Ordering larvae correctly, choosing an appropriate site, and providing the proper water temperatures, food supplies, and substrates are critical to maximizing the survival of the seed.

In our second group of papers, commercial growers and hatchery operators share their experiences with the next phase of the culture process, the nursery. This is a stage at which the oyster seed are given extra care to maximize their growth rates and survival before being transferred to their eventual growout site.

There are many approaches to nursery culture, including the use of devices such as "upwellers" and "downwellers" for providing a proper substrate and an adequate flow of clean water and food past the seed. This section also includes short presentations on challenges that face workers at all stages of the bivalve culture process: preventing and treating disease, and obtaining or producing an adequate and appropriate supply of microalgal food.

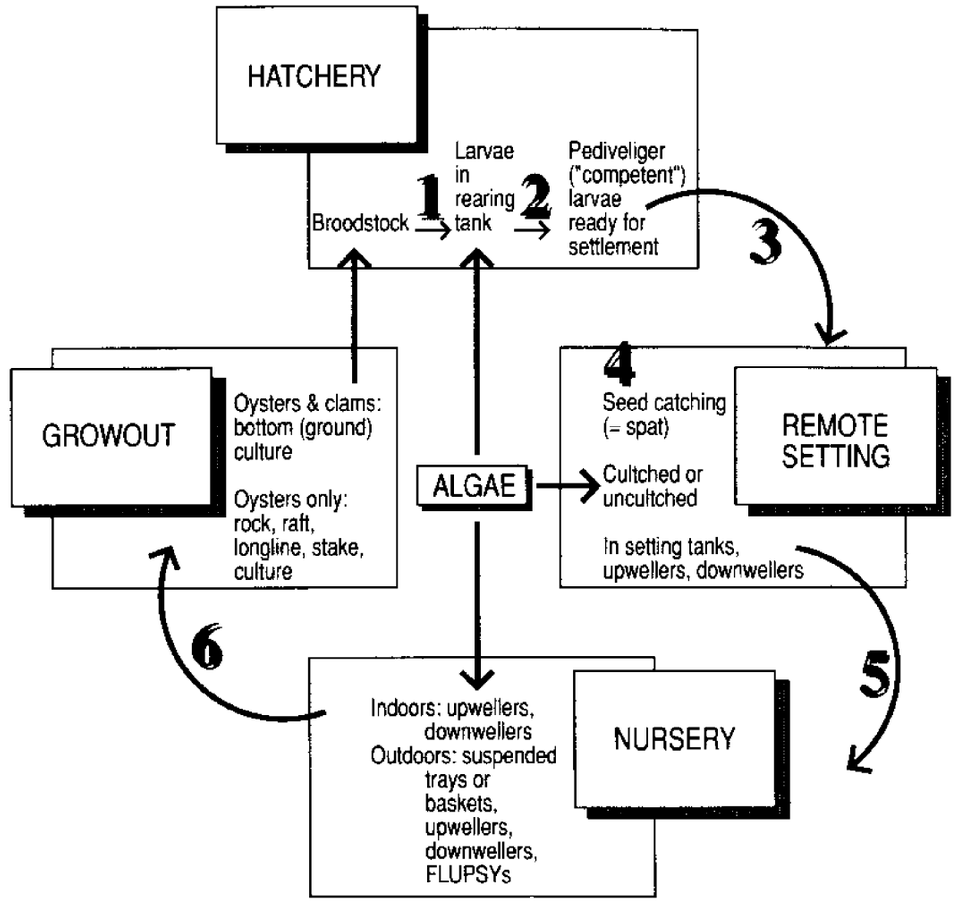
In the final section, four authors present their research on how bivalve seed behave and respond during the nursery stage, and on some experimental nursery practices that may hold promise for expanding the ability to culture Manila clams and geoducks.

We organized this workshop to promote communication and understanding within the rapidly evolving bivalve culture industry. We appreciate the support of the Pacific Coast Oyster Growers Association and Washington Department of Fisheries in working with Washington Sea Grant Marine Advisory Services and the University of Washington School of Fisheries to organize the workshop. We hope that this record will serve as a useful reference work for growers, researchers, and students, and will also help advance shellfish culture in the Pacific Northwest.

Terry Y. Nosho, Aquaculture Specialist, Washington Sea Grant Marine Advisory Program

Kenneth K. Chew, School of Fisheries, University of Washington

The Commercial Bivalve Production Cycle



R. Strickland, V. Loe, Washington Sea Grant Program

1. Larvae are spawned from conditioned broodstock in the hatchery, and are grown in rearing tanks provided with cultured microalgal feed.

2. Larvae mature to the pediveliger (or, in oysters, "eyed") stage, at which point they may safely be strained out of the rearing tank and transported. Larvae at this stage are "competent," meaning that they are ready to settle and attach to a substrate to form "seed" when conditions are right.

3. In preparation for remote setting, larvae are packed with ice and will remain viable for up to a week during shipping to the grower's site.

4. For setting at the remote site, larvae are furnished the proper salinity, temperature, and microalgal food conditions in a setting tank, where they set onto a suitable substrate. Oyster larvae usually set onto shell, called "cultch." Alternatively, they may be induced to settle without attaching to shell, thereby becoming "cultchless" seed. Cultchless seed is used for producing single oysters for the half shell market. Clam larvae typically settle on sand of a particular range of grain size placed in tanks.

5. Seed is transferred to a nursery environment to feed and grow larger before being planted in the field. The nursery may consist of natural seed grounds, trays, or "upwellers" or "downwellers"—tanks holding sand through which clean water flows continuously upward or downward, providing natural or cultured algal food.

6. At the proper time of year and upon reaching an adequate size, seed is transferred to growout facilities—ground culture or some form of suspension culture—to reach market size.

Remote Setting: A Brief History

Approximately twenty-five years ago, two businessmen started Pacific Mariculture, the first shellfish hatchery on the West Coast. William Budge and Charles Black contacted Victor Loosanoff, who encouraged them to investigate the possibility of producing oyster seed for both U.S. coasts. The hatchery they built on the California coast south of San Francisco produced cultched seed. This new seed on shell from hatcheries was not readily accepted by Pacific oysters growers. Their techniques were already firmly established, and they preferred to use naturally caught seed or seed from Japan.

In the early 1970s, Coast Oyster Company and Dave McMillin of Olympia Oyster Company accepted a shipment of mature oyster larvae for settlement in their own tanks away from the hatchery. All indications were that these trials were successful, but the product was withdrawn from the market for one reason or another.

During this same period, Dick Wilson (Bay Center Mariculture) and the Lummi Indians of Bellingham started hatcheries in Washington that were more in tune with the needs of the industry. The hatcheries in the state of Washington remain open. A second hatchery in California, International Shellfish, was built in the 1970s to produce cultchless oyster seed only. Oregon State University became active in research to develop hatchery technology.

In 1973 two more shellfish hatcheries were started in Washington: Sea Farms at Poulsbo and Coast Oyster Company at Nahcotta. The problems realized by both hatcheries were too complex. Sea Farms moved to Brinnon, then to South Point. Coast Oyster moved to Quilcene. During the same period, the Lummis constructed a new facility and are considered by some as having begun a trend to place emphasis on larval rearing. Sea Farms, somewhat adrift and with limited seed customers, began to ship larvae to Bay Center Mariculture and to Coast Oyster.

Vance Lipovsky, the shellfish hatchery manager of Coast Oyster, discovered by accident that larvae could be left drying on screens for hours and still live. The technicians who left the larvae on the screens may not have survived, but from their error came a new way to ship larvae. It soon became clear that a hatchery could be located anywhere. A small container holding 50 million larvae weighs about five pounds and can be shipped anywhere in the world.

In the late seventies several hatcheries either closed or moved. Pacific Mariculture sold its hatchery in California and became Pigeon Point Shellfish, which later shut down its operation. International Shellfish of California closed, and so did Sea Farms. But others started up. In Mexico, a new and large facility at Kino in the state of Sonora was built to replace an old structure. The two Jones brothers continued to enlarge their facility on Lasqueti Island in British Columbia. The Webbs built a hatchery at Friday Harbor in Washington, and Whiskey Creek Oyster Farm was started in Oregon.

The Whiskey Creek hatchery was started with only one goal in mind: to sell larvae ready to metamorphose and attach. Two Oregon State University faculty members, Wilbur Breeze and John Faudskar, staked their reputations on a conviction that transporting larvae was economically and biologically sound. Out of this effort two new phrases were coined, "eyed larvae" and "remote setting." The first large shipments of

eyed larvae were made to Bay Center Mariculture, where Dick Wilson coaxed and demanded a product that gave his farm an alternative to seeking a wild set.

The past twenty-five years have been exciting but not without some pain. New investigations are going on in genetics, as well as in high-density rearing, feeding, and alternative shellfish growout. We are now a maturing industry.

Lee Hanson, Whiskey Creek Oyster Farm, Tillamook, Oregon

Setting Procedures

Ordering, Shipping, and Handling Larvae: View from the Hatchery

Jim Donaldson, Coast Oyster Co., Quilcene, Washington

Commercial hatcheries have achieved tremendous success with the Pacific oyster on the west coast of the United States. They now provide the vast majority of all oyster seed that is planted commercially every year.

One very important advance in seeding has been the development of remote setting of larvae. This is the shipment of larvae to sites that are remote from the hatchery, for settlement and metamorphosis to produce seed. Remote setting has become standard practice and is now the envy of the world, as far as molluscan aquaculture is concerned. Oysters, clams, and scallops can now be produced successfully by hatcheries at any time of the year and shipped as larvae anywhere in the world. For financial reasons most hatcheries operate and have larvae available only from March through September. Unless special arrangements are made, larvae are produced and shipped only during these times.

Ordering

When ordering larvae from a commercial hatchery for remote setting, one must consider the amount of time it takes in a hatchery from the arrival of appropriate broodstock to the point at which the resulting larvae are competent; that is, they have achieved the right size and stage of development for setting and metamorphosis. Winter and early spring larvae orders require broodstock that is conditioned for about two months for most species, with a decrease in the conditioning time required as temperatures warm in the summer. Midsummer conditioning time for the Pacific oyster in the state of Washington is generally less than a week. Pacific oyster larvae require 15–20 days of rearing to achieve competence for setting, regardless of the time of the year. Therefore, the amount of time required in a hatchery from the start of broodstock conditioning to a stage of fully competent eyed larvae will vary from at least one month to almost three months.

Because hatcheries produce a variety of species, hatchery production plans must be laid out months ahead of time to be able to adjust for the seasonality and variety of species. Generally speaking, a three-month lead time is not unreasonable early in the year and one month by summer. Of course, as we all know, most customers prefer receiving larvae during the months of June, July, and August, so scheduling will always be difficult during those times. To ensure that a customer receives his order as requested, any large order (more than 10 million larvae) should provide for as much lead time as possible.

Determining the Competency of Larvae for Setting

Hatchery biologists must determine the competence of larvae for setting while the larvae are still in the rearing tanks. This is a crucial step. If the larvae are removed too early and placed in a setting system before they are ready, they will set poorly and

maybe not at all. In most setting systems it is very difficult to rear larvae. On the other hand, if they are removed from the rearing system too late, they may have already set on the rearing tank and may not be recoverable, or they may have dropped to the bottom of the tank where they may come in contact with undesirable bacteria and protozoans, reducing their quality.

Physical Characteristics

A number of physical characteristics are evident for all molluscan larvae when they have achieved competency. The larval shell dimensions and eyespots are the easiest to measure and the most important. A list by species which are commonly grown in this area follows. Keep in mind that there will be some variation with location and method of culture.

Larval shell size (screen minimum for grading):

- Pacific oysters—300 μ (230 μ screen)
- Manila clams—230 μ (160 μ screen)
- European oysters—280 μ (210 μ screen)
- Kumamoto oysters—340 μ (250 μ screen)
- Rock scallops—220 μ
- Japanese scallops—260 μ

Eyespot size:

- Pacific oysters—12–14 μ
- Manila clams—no visible eyespot
- European oysters—20–25 μ
- Kumamoto oysters—15–20 μ
- Scallops—10 μ

The development of the foot, at the larval pediveliger stage, is another crucial characteristic that appears at the time of setting. For all species of oysters the foot appears only a few days prior to full competency, then soon disappears at metamorphosis. In a sampling of oysters taken from a tank for microscopic examination, the larvae should alternately swim and crawl on the slide if they are ready. Often they swim with their foot protruding. For clams the foot will appear much earlier than competency—at 180 μ for Manila clams, for example. This has led many biologists to assume wrongly that these clams are ready to set, when in fact they should be held for many more days in the rearing system. Another complication with determining competency for Manila clams is their lack of eyespots.

There are a few other, less important physical characteristics which must be noted. The development of the gill rudiments in oysters is an obvious feature which becomes apparent at about the same time as the development of the other more obvious features such as the foot and the eyespots. Larval color must be a dark brown for all oysters; however, this varies with the location and rearing method. Clams tend to be a much lighter brown and often have a yellow-brown tint. Color can also be a direct result of the type of food and the time of feeding just prior to sampling. For these reasons color is not so important a character as the others.

The appearance of the velum is an important indicator of larval health. Diseases caused by bacteria and viruses and probably many other stresses can manifest themselves as blisters or irregular formation of the outer tissues of the velum. Usually, velum problems cause the larvae to sink to the tank bottom, which only aggravates their condition. All larvae should be active and vigorous when they are sampled from the rearing tank.

Preparation, Storage, and Shipping of Larvae

All molluscan larvae appear to be very hardy physically. They can be drained from the tanks with siphons onto screens that collect them while letting the water pass through. Usually the screen is placed in a water bath while the tank is draining to relieve stress from pressure, which can be considerable when the tank is ten feet tall. The larvae are then graded through a series of screens by a vigorous washing with seawater from a hose. No physical damage has ever been evident from this technique.

Once the larvae have been separated by size they can be bundled on a piece of screening material, blotted with a piece of paper towel to remove excess moisture, and weighed for a count of the larvae in the group. As an example, competent Pacific oyster larvae weigh 15–16 grams per million. This was determined by doing numerous counts and calibrating the counts with actual wet weights.

After the larvae are weighed and separated into groups for remote setting, they can be wrapped in a wet paper towel, put into a plastic bag, and either refrigerated at about 5° C for storage or packaged for shipping. When packaged for shipping, the larvae should be placed into an insulated box containing gel ice so that the larvae do not come into direct contact with the ice. There should be enough ice in the container that it does not melt completely within the shipping time. Generally, it should reach its destination within 24 hours to ensure the best success with the larvae. However, a 48-hour transit time has been found to be short enough, as long as the contents of the package are cool and not spoiled when examined on arrival.

The question of how long larvae can be stored has been investigated by a number of researchers using Pacific oysters. Barbara Carlson, in her Master's thesis from Oregon State University in 1982, found that storage of eyed larvae for 1–6 days at 5° C appeared to increase the setting percentage. Bruce Henderson, in his Master's thesis in 1983, also from OSU, found a slight increase in percent set over storage time, but he thought it was the result of a faster rate of setting. He concluded that storage did not impair the ability of larvae to set, but neither did it increase the setting percentage. Kevin Joe, working at the Bodega Marine Lab in 1984, found that larvae stored for up to eight days at 3°–7° C showed no ill effect on their ability to set. After eight days, however, the percent setting declined drastically.

In experiments at Coast in 1983, we found that storage up to a week did not affect the percent set. However, velar loss occurred after only a few days and severely hampered the distribution of those larvae. It is important to realize that these were all lab scale experiments. When the same tests were done on a commercial scale, the poor distribution of those larvae made the resulting seed unacceptable even though the total number of larvae setting was acceptable. As with a number of experiments done in the laboratory, the results must be proven as the experiments are scaled up. For this reason I recommend that larvae be set as soon as they are received to get the best results.

Once larvae are received, preparing them for remote setting and handling them in the setting system are familiar techniques and can be reviewed by the hatchery personnel when larvae are ordered or as the setting process is taking place. It is important to notify the hatchery where the larvae were purchased as soon as possible if larvae are received in poor condition or resulting sets are not satisfactory.

Remote Setting of Pacific Oyster Larvae

Vance Lipovsky, Aqua Business Management, Royston, British Columbia

Remote setting of Pacific oyster larvae is a fairly straightforward procedure: you obtain eyed larvae, put them into a tank, and set them on a substrate. Recoveries generally run 10%–80% per set. The actual percentage set, however, is difficult to determine because the hatcheries usually ship an overage of larvae. For example, you may order one million larvae but receive two million.

Hatchery work has not changed much over the past twenty years. One reason is that larvae price has essentially remained unchanged. Hatcheries, however, have been able to lower production costs.

Shipment

Larvae selected for shipment are removed from rearing tanks, chilled with gel packs, transported from hatcheries to remote nursery sites, and placed in setting tanks. This stressful period usually requires 3 days, and the larvae survive only because they revert to anaerobic respiration.

Once in the setting tank, the larvae should begin swimming and extending their foot and velum. Setting begins when the larvae crawl on the cultch. If larvae leave the shell and begin swimming again, they will not set.

Setting Requirements

Salinity. 30 ppt seems best, in a possible range of 20–35 ppt.

Temperature. 25°–27° C seems best, in a range of 15°–30° C.

Days in storage. 2–8 days is all right. Setting drops off in 10 days or more in storage.

Feeding. Hatcheries feed larvae at a rate of about 50,000 cells/animal/day. Larvae don't need food *while* they set; however, they must have food *after* they set. Without it at that point, they will not grow or survive. Growth will be low to moderate if spat are fed at 25,000 cells/animal/day, and it will be high if the feeding rate is 50,000–75,000 cells/animal/day.

Determining the number of larvae received. It is not always true that 16 grams equals one million larvae. Sometimes larvae can weigh 18–19 grams/million. The relationship will be more or less constant, however, if you get larvae from the same hatchery.

Densities (pieces of shell and number of larvae). I recommend putting 200,000 larvae per 1,000 pieces of shell into the setting tank. This will result in 20–30 spat per shell.

Tank time. In my experience, larvae are held for 3 days in the tank and 40% of the shells have a count of 30 spat per shell. If larvae are held 6 days, twice as many tanks are needed.

Covering setting tanks will help hold the heat, but seed in uncovered tanks will darken and tolerate the sun better.

Aeration is difficult to evaluate because of the many variables involved. Gentle aeration may provide for spat distribution.

Larval size. We do not know why some larvae are larger than others. Size may be influenced by hatchery technology. Size alone may not be that critical. For example, 280-micron larvae may set well, but larvae from another hatchery may have to reach 340 microns before they set.

Improving Oyster Spat Survival in the Setting Tank

Paul S. Williams, School of Fisheries, University of Washington

Mortality rates of oyster spat (*Crassostrea gigas*) are highest during and immediately after setting and diminish rapidly over the next few weeks. Thus, setting tank procedures and the treatment of spat when they are first transferred to the beach are critical areas where spat survival can be increased.

The main study presented here was designed to investigate the relative merits of holding spat in the setting tanks for different lengths of time. In addition, the results of two additional studies will be reported: an evaluation of the methods used to estimate spat survival in the main experiment, and a comparison of practices designed to protect spat from the effects of exposure in the nursery.

Holding Time in the Setting Tank

Many of the parameters for successful remote setting have been established and are available in several publications, such as the *Remote Setting Manual* by Bruce and Gordon Jones. In a manual published by the British Columbia Ministry of Agriculture and Fisheries, the authors (Bill Roland and Tom Broadley) suggest 3–5 days in setting tanks or possibly longer depending on a variety of factors including environmental conditions, availability of food, and production decisions. Oyster growers report using lengths of time in the setting tanks of 3 days to 2 weeks depending on the time of year. One strategy is to keep spat in the tanks for longer periods of time in early spring and allow the water temperature to lower gradually to ambient bay temperature before putting the spat out in the bay. It is not clear whether this practice succeeds because the spat become acclimated to the cold bay water or because the spat are larger when they are transferred to the beach.

The length of time spat should be kept in the setting tanks to achieve highest survival depends primarily on how long it takes oysters to go through metamorphosis and start adding layers of shell. The rate of shell formation depends mainly on the water temperature, the quality and quantity of food available to the oysters, and how ready the eyed larvae are to set. These factors can vary considerably among locations and seed batches.

The transition from larvae to spat is actually a two-step process: settlement and metamorphosis. When larvae are fully developed and ready to set, they swim to a solid surface, then crawl around to determine if it is a suitable place to remain the rest of their lives. If it is, they will attach by secreting a small amount of cement; if it is not, they resume swimming and searching. Recent research by Dale Bonar and others indicates that the larvae are attracted to settle on a substrate by the presence of ammonia, a byproduct of the bacteria that grow on the substrate. Soaking the cultch for 24–48 hours before adding the eyed larvae allows a film of bacteria and algae to grow (bioaccumulation) and has been shown to increase setting success. The initial attachment with an adhesive secretion is weak, and at that stage larvae can easily be knocked off.

When spat have completed the second stage, metamorphosis, and have started to grow and lay down shell, the larvae will have a much better chance to survive transfer and life on the beach. This stage can be seen with a little magnification and is a good way to judge when it is safe to transfer them to the beach.

Counting spat

Counting spat on oyster shell during the period of highest mortality, just after setting when they are 0.5 mm. or less can be very inaccurate because many of the spat set in the cracks and crevices of the shell and are not noticeable until a week or more of growth has occurred. To overcome this problem in the study, we used 2 x 4 inch clay tiles for cultch. Tiles permit a much more accurate count of very small spat than is possible for spat set on shell. By making the tiles out of white clay with no impurities I was able to count spat without magnification or eye strain only 3 days after the eyed larvae were put in the setting tanks. However, as with other artificial clutch (for example, french tubes), setting and survival rates do not appear to be as high on the tiles as on oyster shell cultch. Therefore, tiles should be used not as a regular procedure but only to study the relative differences in survival between one treatment and another.

Experiment

In the main experiment the effect on survival of 3, 6, and 9 days in the setting tanks was estimated at different levels of exposure, at different locations in the Hood Canal area, and at different times during the setting season. In each run of the experiment, spat were set on tiles in a commercial setting operation. The spat on each tile were counted, and each tile was tagged so that the spat mortality rate could be calculated for the duration of each 2-month experiment. Ten replicates were used for each treatment (combination of time in the setting tank, exposure level, and location) used in each run of the experiment. The spat were (1) placed on long lines and hung one foot below the surface for the submerged treatments and (2) strung one foot above the beach supported by rebar stakes at the specified tidal height for each of the beach treatments.

Results

The main finding of this study was that in each month, each location, and each tidal height, survival of oyster spat was significantly greater for spat kept in the setting tanks for 6 days rather than 3 days. Other factors, especially exposure, were found to have severe effects on spat survival. Analysis of some of the individual runs of the experiment illustrates some of the patterns of mortality that can occur after spat are transferred to the beach.

In the May–June run, we held groups of larvae in the setting tanks for 3, 6, and 9 days. We found no significant differences in survival between the 9-day spat and the 6-day spat, but the 3-day spat had significantly lower survival. Because holding the larvae for 9 days appeared to confer no advantage over holding for 6 days—and it is uneconomical to keep spat in the tank any longer than necessary—the 9-day setting-tank treatment was eliminated from subsequent trials.

The May-June run was conducted when the water temperatures had risen to temperatures much higher than the temperature in early spring. Therefore, this observation should not be interpreted as implying that the practice of keeping spat in the tanks for longer periods in early spring is not effective. On the contrary, it may be a very good strategy to increase survival during seasons of cold water. It is a question worthy of research.

From the data taken in July at Tarboo Bay the pattern emerges that larvae held for 6 days in the tanks had a higher survival rate than those held for only 3 days. Also, it is apparent that spat on the long lines under the float had greater survival than those on the beach at the +2 ft. tide level. The mortality on the beach is due primarily to the exposure. Clearly, spat that are held only 3 days in the setting tanks and then are subjected to the beach treatment suffer a double disadvantage. This group was put on the beach on a very hot day during a low tide cycle. This illustrates how much survival of spat can depend on timing of placement out on the beach in relation to tidal cycles, weather, and time in the setting tanks. The group that stayed in the tank another 3 days missed most of that exposure. The tiles that were put under the float after 3 days in the setting tanks had 34% survival after 23 days, the same survival as the 6-day-in-the-tank, +2 tidal height group. This suggests that 3 days in the tank during a period of fast growth followed by total submersion in the bay may be an effective alternative nursery strategy if tank time is at a premium. However, the best survival was for the 6-day-in-the-tank group placed under a float: in that group, 52% survived.

Shell Versus Tile

A separate experiment was run to find out just how much lower survival was on tiles than on shell. In this experiment spat on ten shells from the same set that the tiles were set on in July were carefully counted then alternated with tiles on a longline at the +2 foot tide level. Survival was quite a bit lower on tiles than on shell: 60 days after setting, survival was 31% for spat set on tiles and 47% for those set on shell.

Exposure

Exposure is a mayor cause of mortality of oyster spat during the first few months after they are put on the beach. In July, another experiment was conducted to measure the effectiveness of practices used by some growers to protect small spat once they are removed from the setting tanks. Shells and tiles that had 6-day-old spat on them were counted and tagged. Ten of each were put on a short line which was held in the center of a standard black mesh bag and the bag filled with spat on shell. The bags were placed under three different conditions: one in a group which was sprinkled with water, one in the center of a group of six bags on the beach with no sprinkling, and the last hung under a float. After 60 days the tagged tiles and shells in each bag were recounted and the percent survival was calculated.

Not surprisingly, there was a statistically significant difference between the bags that received some form of protection and those that did not. The bag under the float had the best survival (shell 81%, tile 57%) and had much faster growth than the bags on the beach. Spat that were sprinkled with water had surviyal of 79% on shell and 53% on tile. Spat on the beach with no sprinkling had survivals on 63% on shell and 43% on tile

The experiment was run after the hottest weather and lowest tides had passed. Had I run it in mid-June, the results would probably have been much more dramatic. This is an area in which research could yield substantial reductions in mortality of oyster spat, and it is the type of research that an oyster grower could accomplish fairly easily and quickly.

Recommendations

Let's get back to the original question. How long should remote setters keep spat in the setting tanks? I have shown that 60 days after setting (July and August), 33% of spat kept in the setting tanks for 6 days were still alive compared with only 24% of spat kept in the tanks for 3 days. The setting was done at the Rock Point facility at Tarboo Bay, which is a fairly unusual location in that it has warm water much of the summer and plenty of cultured algae for the spat in the setting tanks but a lower algal density in the bay water than the South Sound. Spat grow faster in some sites than in others, but even in an area where fast growth occurs, removing spat from the tanks after just three days may lead to considerable losses if they have not completed metamorphosis by that time.

One word of caution. In warm water, oysters have high growth rates and therefore consume considerable quantities of food. If they are held in tanks without adequate food, they will starve.

For individual remote setters, the decision of how long to keep spat in the setting tanks is really an economic decision. On the one hand, eyed larvae are very expensive and if spat are put out in the bay too fast, survival will be decreased. On the other hand, the longer the spat remain in the setting tanks, the more costly it is. They require food and maintenance, and their continued presence in the tanks can result in lost production. Ideally, remote setters should determine the optimum time in the setting tank in their own location, and then balance the economic costs incurred by lost production against the gains achieved through higher survival.

Cultchless Oyster Production and Enhanced Setting with Epinephrine and L-dopa

Dale B. Bonar, Pacific Rim Mariculture, Port Townsend, Washington

Throughout the history of oyster culture, attempts have been made to increase the natural catch. In the last century, as the combined effects of overharvesting and environmental degradation depressed harvests, aquacultural efforts intensified and increasing attention was focused on factors, both physical and biological, that enhance or suppress setting (Table 1).

Table 1. Physical and biological factors influencing oyster setting

Physical	Biological
Light/shade	Prefouled surfaces
Orientation	Gregariousness
Surface texture	Adult tissues extracts
Water flow	Shell matrix proteins
Temperature	Shellfish glycogen powder
Salinity	Mantle cavity fluids
Surface tension	Iodinated organic molecules
Copper ions	Neurotransmitter mimetics

Refs: Galtsoff, 1964; Crisp, 1974; Hidu et al., 1978; Andrews, 1979; Burke, 1983; Coon et al., 1985.

In the last twenty years, growers and researchers have identified specific chemicals that enhance settlement behavior and/or metamorphosis of bivalves and can be used in nursery or field situations. The information presented in this talk outlines the practical application of laboratory-based studies we have done on the neurophysiological control of settlement and metamorphosis in *Crassostrea gigas* and *Crassostrea virginica*. For more information on the procedures and the underlying physiological principles, refer to Coon and Bonar (1986) and Bonar et al. (1990). Most of this work has been performed in conjunction with Coast Oyster Company in Quilcene, Washington, and with St. Georges Oyster Company in Piney Point, Maryland.

Settlement and Metamorphosis

Following a period of larval growth and development, oyster larvae reach a stage at which they become "competent," or capable of permanently attaching to an appropriate substratum and metamorphosing into the juvenile form. The process of setting (sometimes called spatfall) involves two distinct phases (Figure 1). The first is a searching phase, during which the larvae exhibit a stereotyped swimming-crawling behavior, apparently testing the desirability of potential settlement sites. If a satisfactory site is found, the larvae glue themselves irreversibly to the surface and undergo metamorphosis, a process that involves loss of the foot and velum (the larval swimming and feeding structure) and the rapid development of gills and new spat shell.

Examination of the nervous control of these processes has shown that two different neural pathways are naturally involved in setting (Figure 2; see Bonar et al. 1990 for review). The first phase, settlement behavior, is controlled by a pathway that relies on the neurotransmitter dopamine, which is a derivative of L-dopa. The second phase, metamorphosis, is controlled by a neural pathway that relies on epinephrine or norepinephrine (adrenalin or noradrenalin, respectively). In the natural environment, a larva that has completed the settlement phase and has received the proper environmental cues (texture, light, bacterial surfaces) releases one of these neurotransmitters (probably norepinephrine) within its body to trigger the subsequent metamorphosis.

When solutions of these neurotransmitters are added to containers of competent larvae (in either the laboratory or the hatchery) we can trigger either settling behavior or metamorphosis, or both, on demand. Figure 3 shows the results of experiments testing the inductive effects of different concentrations of L-dopa, epinephrine, and

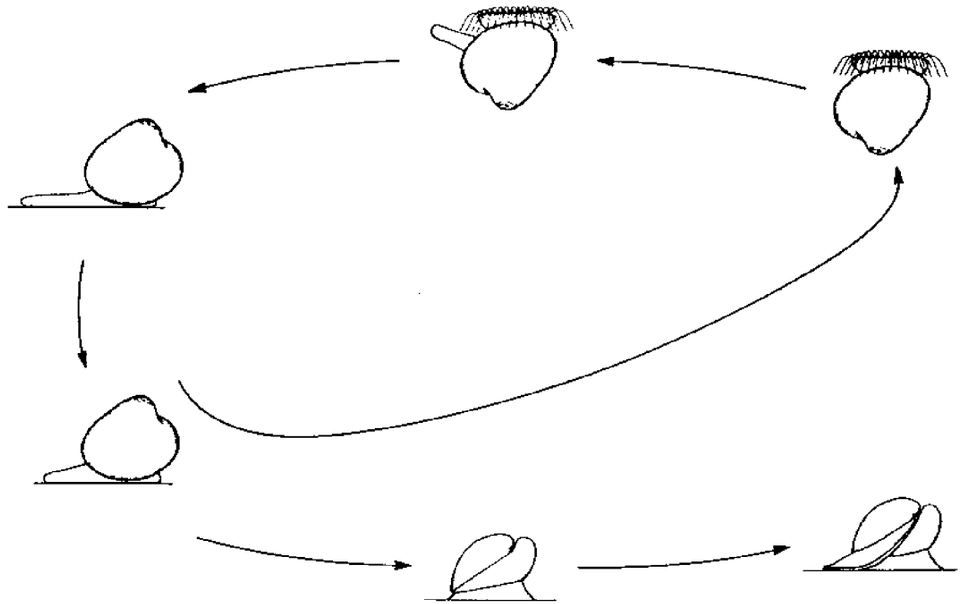


Figure 1. Oyster larva in searching and settling phases.

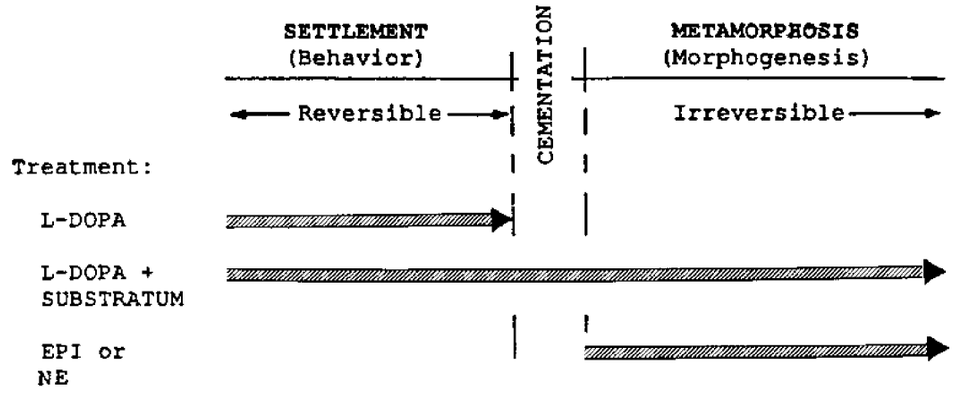


Figure 2. Differential effects of inducers (L-dopa, epinephrine [EPI], and norepinephrine [NE]) on oyster settlement (phase 1) and metamorphosis (phase 2). From Bonar et al. 1990.

norepinephrine on the metamorphosis of Pacific oyster larvae. The effective treatment range for these inducers is approximately 5 x 10^{-6} molar to 5 x 10^{-3} molar (about 1–1,000 ppm), although concentrations above 10^{-4} molar can produce toxic effects in prolonged exposures, especially with L-dopa. The optimal treatment dosage is 10–20 ppm, which produces maximal effect in minimal time without toxic effects.

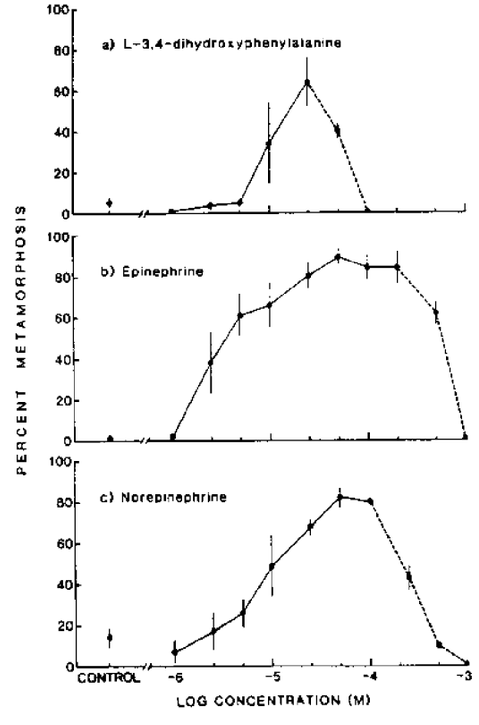


Figure 3. Inductive effects of L-dopa, epinephrine, and norepinephrine on metamorphosis of Pacific oyster larvae.

These treatment regimes were developed for use with healthy, competent *C. gigas* and *C. virginica* larvae, but they have also been used successfully with *C. rivularis* and *Ostrea edulis*, as well as with mussels, Manila clams, and East Coast hard clams. Use of these inducers with other species may require some adjustment of dosage levels and treatment times to optimize results.

Enhanced Setting on Cultch Using L-dopa

Although dopamine is the neurotransmitter within oyster larvae that controls settlement behavior, this drug has very little effect when added as a solution to a container of competent larvae. If, however, we add L-dopa (the chemical precursor to dopamine), behavior is triggered rapidly. It turns out that oyster larvae, like human beings, cannot absorb dopamine into the nervous system. (The existence of this so-called "blood-brain barrier" is the reason that patients with Parkinson's disease are treated with L-dopa; it is readily absorbed into the nervous system and then converted by nerve cells into the dopamine that they really need.) Adding L-dopa to oyster larvae ultimately results in an increase of dopamine in their nervous systems, resulting in settlement behavior. Exposure of competent oyster larvae to a 10^{-4} molar solution of L-dopa for 10–30 minutes triggers very active settlement behavior and results in 20%–40% increases in the numbers of larvae which set on cultch. This procedure is most effective on batches of larvae that are not setting well.

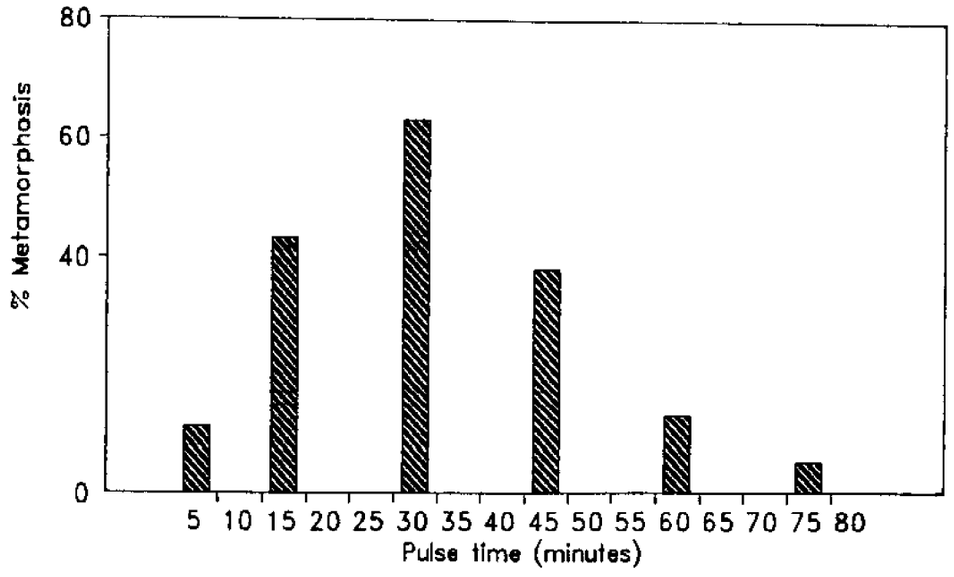


Figure 4. Metamorphosis of Pacific oyster larvae as affected by prolonged exposure to 10^{-4} molar solution of L-dopa.

The stepwise procedure for using L-dopa to enhance setting on cultch (shell or other artificial substrate) is presented as Appendix A. Larvae should be observed carefully during use of this inducer. Healthy, actively swimming larvae will begin to exhibit a typical searching behavior (alternately swimming and then gliding or crawling on the bottom of the container or on any surface with which they are in contact) with the foot extended (see Figure 1). With the naked eye, you should be able to see larvae moving actively about the bottom of the container within ten minutes of exposure to L-dopa. If a microscope is available, check to see that a large proportion of the larvae are swimming or crawling with foot extended. Within 30 minutes of exposure to L-dopa, disperse these larvae into the tank of cultch material (shells, french tubes, cemented wire, whatever cultch you prefer). Exposure of larvae to the 10^{-4} molar solution of L-dopa for more than 30 minutes can result in decreased setting due to toxic effects of prolonged exposure (Figure 4). You do not need to wash the larvae free of the L-dopa before adding them to your settling tank, since the dilution will be sufficient to prevent any toxic effects.

If larvae do not respond within 30 minutes of beginning the treatment, they may not be competent. Collect the larvae on an appropriate mesh screen, rinse them well with fresh seawater, and return them to culture for another day or two before treating them again.

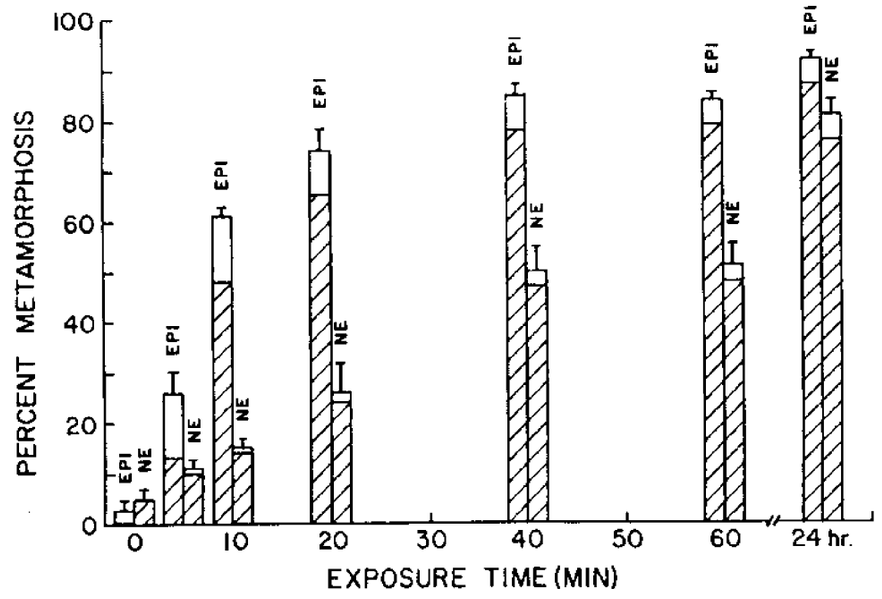


Figure 5. Percentage of Pacific oyster larvae induced to metamorphose as a function of length of exposure to epinephrine (EPI) or norepinephrine (NE). Controls are designated as exposure time = 0. Filled bars denote unattached spat; open bars denote attached spat. Data represent the means of triplicate determinations; error bars represent the standard error of the mean.

Producing Cultchless Spat with Epinephrine

Exposure of competent oyster larvae to a 10^{-4} molar solution of epinephrine (or norepinephrine) for between 30 minutes and 24 hours results in the rapid induction of metamorphosis without the normally preceding behavioral phase (Figure 5). Swimming larvae simply drop to the bottom of the container and undergo metamorphosis without cementing themselves to the surface. Cultchless spat produced in this manner are entirely normal and will develop and grow just like cultched spat or cultchless spat produced by other means (e.g., setting on shell fragments, plastic sheets).

The stepwise procedure for producing cultchless spat with epinephrine treatment is presented as Appendix B. As with the L-dopa treatments described above, it is important that larvae be competent to metamorphose. Be sure the larvae have well-developed eyespots; test their ability to set by hanging some shell in the rearing tank.

The most important consideration in using epinephrine is to be certain the solution you use is fresh. Once in solution, epinephrine begins to undergo a chemical reaction in which it forms a pinkish-orange product. This product, called adrenochrome, actually inhibits the inductive action of unmodified epinephrine. In distilled water, this reaction happens very slowly (days to weeks), but in sea water (or in tap water containing a lot of dissolved chemicals) it can occur rapidly (minutes to hours). Consequently, we make up a 10X concentrated stock solution in distilled or deionized water and keep it refrigerated. In this form it will last at least a week (probably much longer, but it's cheap enough not to take chances). For use, we then dilute the amount we need with nine or more volumes of sea water, mix well, and immediately add the larvae. After an hour or so, this mixture will become noticeably pink.

After several more hours, it can become striking red-orange in color. The larvae will not be harmed by this solution, even if left for 24 hours. As long as they had been exposed to fresh, unmodified solution for 30–60 minutes, they will metamorphose. If you were to add more larvae to this solution after the chemical conversion of epinephrine is well under way, these additional larvae would not be induced to metamorphose. This inhibition of induction can occur within 30 minutes, even before a distinct pink coloration becomes noticeable.

Appendix A: Enhancement of Oyster Setting by Treatment with L-dopa

L-dopa can be obtained from Sigma Chemical Company, St. Louis, Missouri (phone 1-800-325-3010). We use L-dihydroxyphenylalanine (Stock #D9628, $6.90 per gram), but DL-dihydroxyphenylalanine (Stock #D9503, $6.70 per 5 grams) will work as well.

1. Be sure the larvae are healthy and competent. When significant numbers of larvae have set on test shells placed in the rearing tank, they are ready.

2. Make up a 10^{-3} molar **stock** solution (10X) of L-dopa as follows: Mix 0.2 gram dopa powder into 1 liter of distilled or deionized water (good spring water will suffice for short-term use) and mix well until dissolved. Keep refrigerated (good for at least a week). Discard if any brown or black discoloration develops.

3. Make up the **working** solution as follows: Dilute 1 part stock solution with 9 parts clean, filtered, aerated seawater (e.g., 100 milliliters stock to 900 milliliters seawater).

Use the working solution (i.e., add the larvae) as soon as possible. Over time, L-dopa polymerizes to form melanin pigment, which aggregates into dark particulate flakes in the container. If you see particulates form, make up a fresh solution. If larvae are in the solution, rinse them very well.

4. Add larvae (up to 500,000 per liter) to the *fresh* working solution, swirl gently, and let sit for 10–30 minutes. Within 5–10 minutes the larvae should be actively swimming or crawling in the bottom of the container.

Prolonged exposures to L-dopa can be toxic to larvae. Don't leave larvae in the solution more than 45 minutes maximum. If you can't immediately add the treated larvae to your tanks of cultch, dilute them severalfold with fresh seawater or collect them on a screen and rinse them into fresh seawater to hold them until you can add them to the cultch.

5. Since L-dopa treatment promotes such active settlement behavior, it is helpful to supply gentle agitation to your setting tank to assure good larval distribution. Bubblers on the bottom of the tank work well.

Appendix B: Cultchless Oyster Larvae Production by Epinephrine Treatment

Epinephrine can be obtained from Sigma Chemical Company, St. Louis, Missouri (phone 1-800-325-3010). We use epinephrine-HCl (Stock #E7386, $11 per gram), but epinephrine bitartrate will work as well.

1. Be sure the larva are healthy and competent. When significant numbers of larvae have set on test shells placed in the rearing tank, they are ready.

2. Make up a 10X **stock** solution (10^{-3} molar in 0.005 molar HCl) of epinephrine as follows: Mix 0.2 gram of epinephrine-HCl powder into 1 liter of distilled or deionized water (good spring water will suffice for short-term use) and add 0.4 milliliter of concentrated hydrochloric acid. Keep refrigerated (good for at least a week). Discard if any pink coloration develops.

3. To make the **working** solution, dilute 1 part stock solution with 9 parts clean, filtered, aerated seawater (e.g., 100 milliliters stock to 900 milliliters seawater). Larvae should be added as soon as possible after preparing the working solution.

4. Add larvae (up to 500,000 per liter) to the *fresh* working solution, swirl gently, and let sit for 1–24 hours. If the larvae are to be treated for more than a couple of hours, gently aerate the water or place larvae in a large shallow dish for good air exchange. Keep the temperature below 25° C and cover the container to prevent evaporation. Few, if any, larvae will be swimming. Within a few hours the solution will turn pinkish-orange. This won't hurt the larvae.

5. If significant numbers of swimming larvae remain at the end of the treatment period, collect them by decanting the solution through a Nitex screen and save them for treatment again in another day or two (they may not have been competent). Rinse the nonswimming larvae with several changes of fresh seawater and transfer them to whatever rearing devices you use (floating Nitex screens, shallow trays, upwellers) and begin feeding them within 24 hours of treatment.

6. Gentle daily agitation will prevent the spat from sticking to their container or each other as they grow.

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Methods for Setting Manila Clam Larvae

Richard L. Poole, Sound Seafarms, Lummi Island, Washington

This presentation outlines some methods that have been used to set Manila clams. The methods have not been fully tested and may represent a one-time use or personal communication and experience. The techniques presented are not the ultimate and only way to produce clams. Some methods may be discarded as better ones are developed. The presentation also assumes that the grower is familiar with problems of toxicity of various materials and containers and with water quality problems.

Parameters

Natural parameters for production of natural Manila clam sets have been presented by Chew, Nosho, and others and are generally as follows:

Temperature: 18°–25° C
Salinity: 24–32 ppt*
Substrate: gravel/sand/mud
Circulation: wave/current

These parameters are duplicated in the hatchery and at remote setting sites. Site selection for a successful setting operation requires time and analysis. This presentation will not go into the requirements for siting of a hatchery culture operation. A recent publication of the British Columbia Ministry of Agriculture and Fisheries, *A Manual for Producing Oyster Seed by Remote Setting* by William Roland and Thomas Broadley (1990), provides guidelines for siting a setting operation for oysters. They can also be applied to clam culture.

Larval Production

It is necessary to know something about larval production in order to make wise decisions about setting. Rearing temperatures and larval history are important to the success of setting. The hatchery controls spawning conditions and may produce larvae throughout the year. The larval period in the hatchery is 7–15 days, during which time the larvae grow from 90 microns to a maximum of 235 microns, ranging from 7 to 15 microns of growth per day. Healthy larvae should grow an average of 10 microns per day. The rearing temperature is 20°–25° C, with the best results at 21°–23° C, although larvae can be reared at temperatures as low as 19° C and as high as 28° C.

During this phase, the larvae are fed mixed diets of T-Iso (Tahitian *Isochrysis* spp.), CC *(Chaetoceros calcitrans),* 3H *(Thalassiosira pseudonana),* and Chg *(Chaetoceros gracilis).* Concentrations of algal cells are generally less than 100,000 cells per milliliter in the rearing tanks. Algae cultures in the exponential growth phase are used to prevent excessive bacterial contamination. Water is changed every three days with fresh filtered seawater at the desired temperature. During the later stages, water is changed daily to catch the larvae at the setting stage.

At each change, the larvae are screened onto Nitex nylon sieves with the appropriate screen size. Samples are examined on the microscope for size, growth, and disease.

* French investigators consider summer salinities of 30–38 ppt and winter salinities of 25–30 ppt suitable for clam culture (Flassch 1990).

Small larvae are discarded and the larger larvae replaced in the tanks. When the larvae are big enough to be caught on a 120-micron screen, they are 160–235 microns (average 200 microns), the foot is evident, and some are sticking to the sides and bottom of the tank. Then they are screened through a larger sieve to remove debris. The smallest larvae exhibiting a foot are approximately 170 microns. Loosanoff (1963) reports the smallest metamorphosed larvae at 175 microns, or about the same as reported for *Mercenaria mercenaria.* He also reports that the majority of the larvae metamorphose when they are between 200 and 220 microns in length, but some larger larvae may still be swimming. Larval density is 1–3 per milliliter in the larval rearing tanks. If the larvae are not crawling adequately, they are returned to the tank to rear for another 12–36 hours or more. Samples are taken daily to determine the status of the larvae. At this time, feed includes T-Iso, CC, 3H, and Chg. *Skeletonema* can also be used during the entire larval period (Flassch 1990).

Setting

If adequate food and temperature are maintained, larvae may be ready to set after 7 days in the rearing tank, although 10–12 days is more common. The ready-to-set and newly set larvae with developed foot and functional velum are called pediveligers. At this time they alternately swim and crawl on the sides and bottom of the tank. It is not known exactly what triggers metamorphosis, but certain conditions (temperature, water flow, food, density, and chemicals, for example) stimulate the larvae to begin to lose the velum, rely on the foot for locomotion, and develop gills.

The temperature for setting can range from 18° to 28° C. We have had best results at 21°–23° C. The Washington Department of Fisheries reports setting at 18° C. Canadian Benthic sets at 28° C and allows the temperature to drop rapidly to 19° C (Lindsey 1991, personal communication). Canadian Benthic also reports that epinephrine works well to induce metamorphosis. It does not, however, yield good results in terms of survival and growth rate, and Canadian Benthic has discontinued it use (personal communication 1991).

Density of the larvae appears to have a significant effect on the consistent recovery of larvae. While good sets are not impossible with heavy densities, it appear that densities up to 200 per square centimeter provide sets more consistently and densities greater than that will cause outbreaks of bacteria and protozoa. At the density of 200 per square centimeter, the 4-foot ring can carry up to 2,200,000. We have set 5–6 million (500 per square centimeter) in the four-foot rings with mixed results. Density may vary with flow rate and the ability to keep the clams clean.

Larvae in the hatchery that are growing at good rates will fail to metamorphose and grow further if they meet unfavorable conditions in the setting process. Crowding on the screens causes food to collect on the larvae and foul the velum. Screen fouling with excess food also causes the screen to overflow and results in the loss of larvae.

Feeding rate and food type must therefore be chosen carefully. We have found that *Chaetoceros mullerii* will foul the screen quickly. Feeding with a mix of CC, T-Iso, Nanno, and 3H produces better results. Pulse feeding and the use of ambient water also produce good results. Larvae produced late in the summer and grown in flowing filtered water from the seapond produced excellent larvae with a minimum of supplemental feeding. The supplemental feed, however, appears to be necessary to produce consistent results.

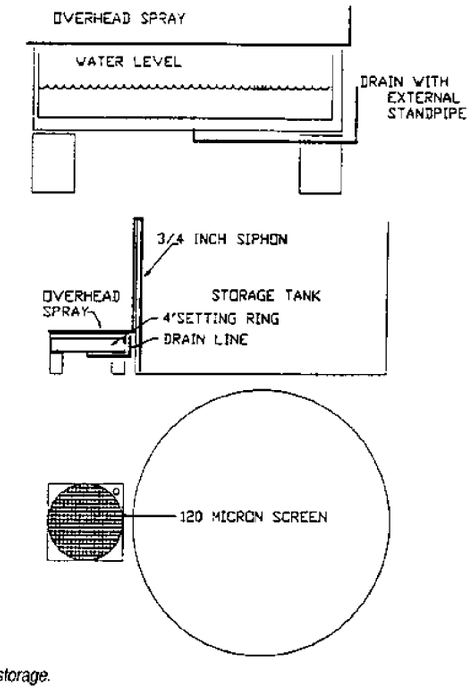

Figure 1. Setting rings with adjacent storage.

Flowing water is important, therefore, for cleaning the larvae. In the wild, flowing water apparently also provides a stimulus to set. Obstructions, contours, and natural changes in the water direction and flow create eddies and backflows where the larvae are comfortable. Natural sets apparently depend on adequate flows for survival. Figure 1 shows a 4-foot setting ring with water storage for a single pass flow-through situation. In the hatchery, the amount of flow provided is often dependent on the ability of the screen to pass the water without overflowing. The flow can be enhanced by depth of the screen and screen cleaning rates. Sometimes screens must be cleaned twice a day to prevent fouling and overflows.

Handling of Pediveliger Larvae

Running seawater at various temperatures is required for producing plantigrade clams. It is provided in a variety of ways: downwelling and upwelling systems (or a combination of the two), over a substrate, and in a series of settling tanks.

Water tables

Pediveliger larvae are placed in water tables and the water is changed at least twice daily. The larvae are screened and put back onto the water table after each change (Jones and Jones 1990). This routine can be continued for several days until they set and are placed in flowing seawater. Then the juvenile Manila clams should be cleaned at least every two to three weeks.

Tanks

As an alternative to using tables, larvae can be left in the tanks to set on the sides and bottom of the tank. However, we have not found this to be a good method. The clams tend to cluster on the bottom, where they are rapidly invaded by protozoa and bacteria due to the lack of circulation. We were successful only once using this method with Manila clams. In one small group of 500,000 in a 1,000-liter tank (4' diameter x 3' deep) the larvae were left for 4–5 days and later withdrawn with good results. They had settled on the bottom and grown rapidly. A bloom was evident in the tank and provided adequate food.

Downwellers

A downweller is a system in which the water flows down through the screen. The setting process is generally started with a downwell flow. Water can be sprayed from an overhead pipe or run directly into the system. In any case, the water circulation should be such that no dead spots are present. Trays are removed from the system and rinsed every day with fresh water to control protozoa. The whole system is drained and the water replaced on a regular basis depending on the size of the animals; systems containing smaller animals (which are more susceptible to contamination and disease) are changed more often, perhaps daily.

Containers of various materials including PVC plastic, Plexiglas, Fiberglas, and resin-coated wood are screened with Nitex screen cloth, which is usually secured to the containers with finish coat resin. Screen size may be 115–130 microns. Dimensions of the settling trays may vary from 18 to 48 inches in diameter, and from 4 to 24 inches deep. Long troughs 2' x 30' x 6" deep are also used (Figure 2). Water is circulated over

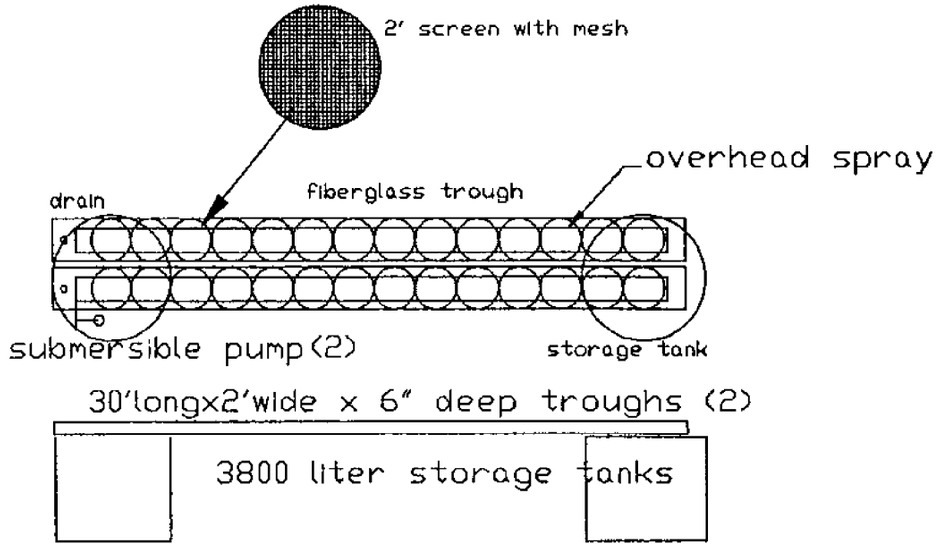

Figure 2. Downwell setting system using 2' diameter Fiberglas rings with Nitex screen cloth. Water is pumped with Little Giant submersible pumps.

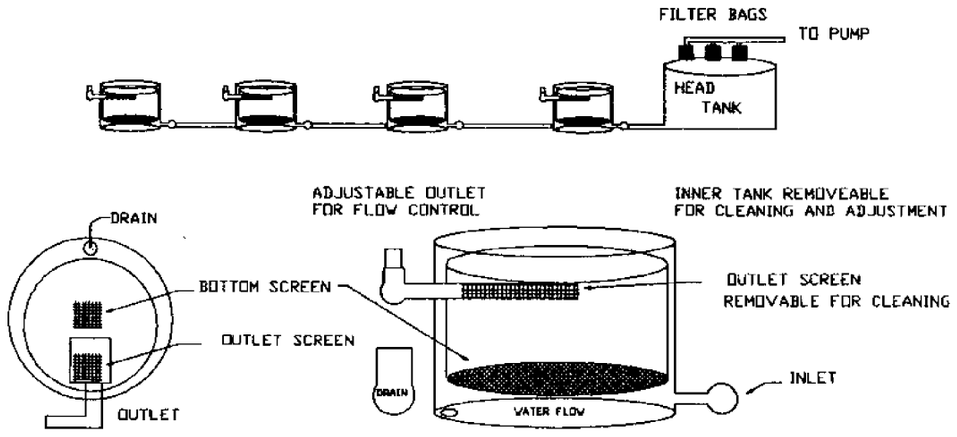

Figure 3. Upwell/downwell system commonly used on the Pacific coast. Addition of a water source above the tanks provides a downwell. Flow can be reversed when the clams are large enough. A stand pipe in the drain hole provides level control.

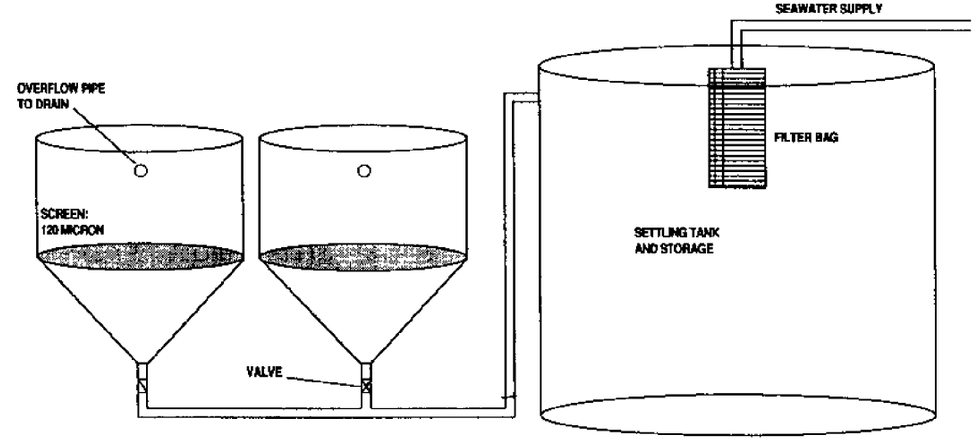

Figure 4. Cone-shaped tanks for setting and rearing clams and oysters.

the screens in a variety of ways, from sprays to heavy flows, at the rate of 1 liter per minute or more (Figures 1–4).

The small mesh screens are subject to fouling by food and larvae and can easily overflow and lose the larvae. Chg is especially troublesome in this respect because of the very long spines (75 microns) that are attached to the cells.

Acceptable per-square-foot density in the smaller 2' setting rings and trays is 185,000 per square foot, or 580,000 larvae. We have set more than 1.5 million larvae in the 2' rings.

Circulation appears to be better in the smaller rings. Depth of water in the rings also influences the circulation, with shallow water providing the best turnover rates.

At the time of settling, the larvae are especially vulnerable to fouling and bacterial contamination. They must be cleaned daily and sometimes twice a day. Examination of the larvae several times a day will show the condition of the larvae and determine if they are growing. Many of the systems recirculate water and add make-up water to the system on a regular basis. This may tend to concentrate the metabolites and bacteria if the systems do not remove and control metabolites and bacteria. Some of the systems are shown in the figures. The flow used may vary according to screen size and percent open area of the screen.

Upwellers

A system in which water flows up through the screen and out through a screened outlet near the top of the container is called an upweller. Food is carried up to the clams on the screen, and feces and bacteria are carried away with the flow. As the setting progresses, the flows are changed from downwell to upwell to reduce bacterial and food contamination problems when the larvae no longer swim up and most of them begin to lose the velum. The question of larval size and time to change from downwell to upwell differs from investigator to investigator. We have found that, to achieve maximum survival, switching should be done as quickly as possible, as soon as most of the larvae have stopped swimming.

If the larvae have not metamorphosed in 3–5 days, serious mortality usually results. If there are large numbers of larvae, bacterial contamination causes the larval

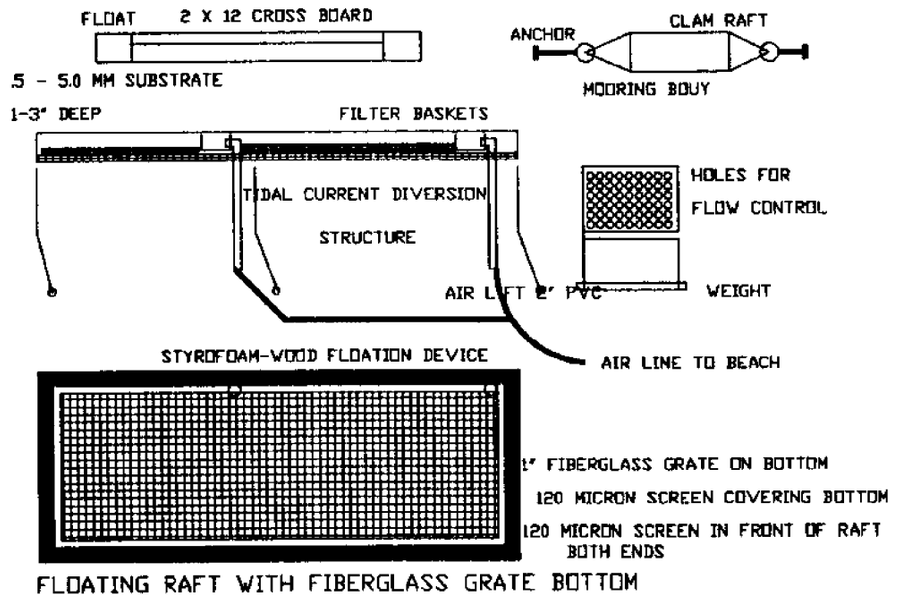

Figure 5. Floating clam rearing system filled with washed sand.

mass, when exposed, to stink. Examination of the larvae reveals that they are fouled with food and have large protozoan and bacterial infestations. This situation can be the result of overfeeding, improper temperature or pH, lack of cleaning, poor circulation, overcrowding, or bacterial contamination from food. The setting rings should be considered an extension of the larval process and should provide all the necesssary items for further development and growth.

Substrate

Is a substrate necessary in setting clam larvae? In France, larvae are set without substrate and are later transferred to substrate or to bags. In the Kumamoto prefecture of Japan in 1978, beach composition was analyzed in those areas that had the best settlement of clam larvae. Investigators concluded that the larvae preferred to settle in fine sand (0.5–1.0 mm grain size), even though 98 percent of the substrate was medium or coarser sand (up to 2.5 mm).

Experiments show survival of juvenile clams in fine silica sand (0.15–1.0 mm) to be approximately the same as without substrate. Early setting of larvae does not require the use of substrate, and the results using substrate are mixed. Systems using substrate are shown in Figure 5. Setting-size larvae are placed in the floating raft and allowed to develop to planting size.

The difficulty lies in separating the clams from the substrate. The smaller sand is easier to remove since the clams outgrow the substrate and are separated by sieves. More time is required to remove the larger substrate unless it is removed before the clams reach or after they exceed the size of the substrate particles. In our experiments, bits of oyster shell were added to the substrate, but we were unable to determine if there was any effect. When clams reach 300–350 microns, Canadian Benthic (1988) removes them from 700-micron substrate and places them in 19° C upwellers.

Some investigators believe that a substrate is beneficial because it provides more area for grazing and reduces the concentrations of bacteria and food. However, we found that good growth can be accomplished without it.

Appendix A describes an experiment we conducted with substrate.

Care of the Systems

In any system cleanliness is important and must be maintained on a regular basis in order to ensure the survival of the pediveliger larvae. Tanks and equipment are washed with a weak chlorine water and rinsed with warm or hot fresh water. If water is standing, the addition of sodium thiosulphate in the rinse will ensure that the system is free of chlorine.

Water used in the system is filtered through a filter bag and run through a UV system if it is available. Good quality water will not require the use of the ultraviolet light, but it is good insurance.

Water can be heated with electric submersion heaters (quartz or titanium) or with a heat exchanger run on propane, electricity, or oil. What kind of heater to use depends on the amount of water that is being heated. Temperature can be maintained in the system with an electric submersion heater that is temperature controlled.

A final consideration is the consistency of larvae from the hatchery and the factors that cause quality to vary: larval growth, food during larval stages, shipping, and handling by the recipient. The hatchery should provide the grower with information on and history of the larvae. When they arrive, larvae should be dark reddish brown and

have no odor. The hatchery operator has no control over the larvae once they leave the hatchery; he relies on the ability of the setter. Hatcheries realize the tender nature of the product and are willing to work with growers to ensure successful sets.

It is strongly recommended that the supplying hatcheries be contacted several months prior to the setting to determine if they can supply larvae and algal food supplements for the setting program. Appendix B is a list of clam larvae hatcheries in the Pacific Northwest.

Shipping and Handling

If the seed is being shipped outside the state, it is recommended that an application for the transfer be made before shipping. Usual carriers include UPS, Federal Express, Greyhound, and air cargo carriers.

There are no studies of the hardiness of Manila clam larvae as there are for oyster larvae. The same procedures are used; however, it appears the clam larvae are not so hardy. They are less able than oyster larvae to withstand lengthy storage and other adverse conditions. This is expected since clams settle into more protected resting places than oyster larvae do. The grower must be ready to place the larvae immediately into the setting situation in order to obtain the best survival.

The quantity of larvae to order for the setting season is based on the desired production of seed clams. The percentage set has not been fully determined and may vary widely with each batch of larvae. In oysters, the set has been estimated at 20 percent on pipe and 55 percent on shell; however, the survival after a short period of time is far less and the ultimate return is less. Estimates by Roland and Broadley (1990) are 7.5 percent for shell and 1–6 percent on pipe. Castagna and Kraeuter (1981) state that survival for *Mercenaria mercenaria* from egg to field is usually less than 1 percent. One should not expect to obtain more than 100,000 seed clams from about 10 million eggs. As stated previously, the hatchery removes the smaller larvae from the eggs very quickly. The oyster situation, however, shows the survival to still be small.

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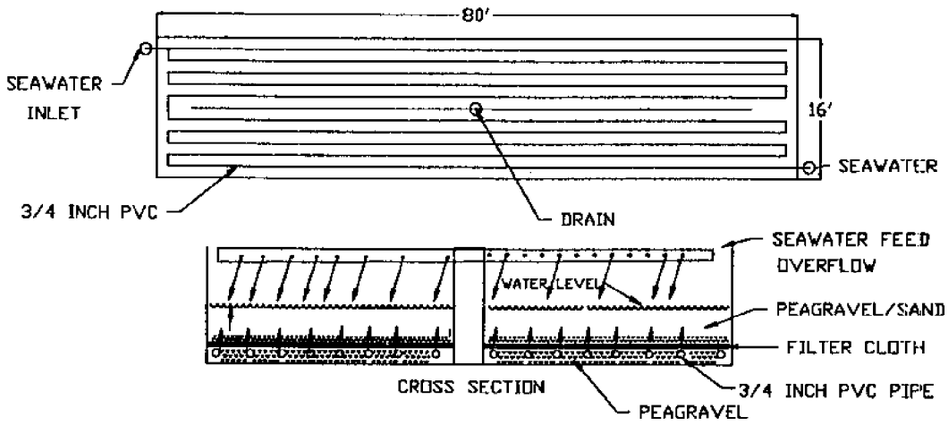


Figure 6. Setting and rearing of Manila clams in salmon raceways, with upwell and overflow.

Appendix A: Substrate Experiment

A major experiment with substrate was conducted in raceways 80 feet long, 8 feet wide, and 4 feet deep in 1988 and 1989 (Figure 6). The system is approximately the same as that used by the Washington Department of Fisheries for geoduck culture.

Plastic pipes 3/4 inch in diameter with 1/8-inch holes every 12 inches were placed in the bottom of the raceway and pea gravel was added to slightly cover the pipes. Plastic filter cloth covered the pipes and pea gravel, and the entire raceway was covered with an additional 2-inch layer of pea gravel. Silica sand was sprinkled over the entire area but did not cover the pea gravel in most areas. A 2-inch manifold was formed at the end of the pipes to feed raw seawater to the bottom of the raceway and allow it to percolate up through the pea gravel and sand. Water also flowed from an overhead pipe at each end of the raceway to provide flows from both under and over the clams. Approximately 50 percent of the water came from each source. The water flow from the bottom pipes mixed with the sand and made holes in the concrete bottoms 3/4 inch wide by 1 inch deep under some holes in the pipe that were drilled straight down at the bottom. Holes should be pointed away from the bottom to prevent erosion to the concrete.

On July 4, 1988, 100 million pediveligers were added to the raceway after the water had been running for 24 hours. A screen was placed on the outlet to catch larvae that had not settled, and once or twice a day the larvae were taken from the screen and returned to the raceway away from the outlet. In three days, there were no longer any larvae taken from the screen. Unfiltered seawater from the aquaculture pond ran continuously at approximately 100 gpm from a 15 hp cast iron pump.

Supplemental feed was provided from adjacent 40-foot x 4-foot circular concrete ponds. Water filtered through a 25-micron GAF bag was placed in the concrete tank and allowed to bloom. Aeration was provided by a blower near the surface of the tank. Approximately 5 pounds of nitrate fertilizer was added. In 2–3 days the pond was green to brown. No attempt was made to identify the algae, and it was fed as is. The raceway was cleaned regularly with fresh water one to two times a week.

The raceway water supply was interrupted by the fierce February 1989 storm, which cut electrical power for five days. Flow was not resumed until nearly two weeks later. The raceway had frozen over and did not thaw for several days following the end of the storm. Flow resumed and the raceway was not cleaned until late spring. In later June and early July the contents of the raceway were sorted on a small vibrating screen. It was estimated that more than 10 million clams were taken from the system. They ranged in size from 500 microns to 12 mm with the majority in the range of 1.5–3.0 mm.

The second year of the experiment resulted in zero survival in the system.

Appendix B: Sources of Larvae in Pacific Northwest

Sound Seafarms, Inc.
P.O. Box 100
Lummi Island, WA 98262
Dick Poole (206) 647-6261

Whiskey Creek Hatchery
2905 Bayshore Road
Tillamook, OR 97141
Lee Hanson (503) 842-8365

Coast Oyster Company
P.O. Box 327
Quilcene, WA 98376
Jim Donaldson (206) 765-3474

Westcott Bay Sea Farms
4071 Westcott Drive
Friday Harbor, WA 98250
Bill & Doree Webb (206) 378–2489

Shellfish Nurseries: Commercial Approaches

Overview and Strategies for Handling and Planting Seed

Bill Dewey, Rock Point Oyster Company, Bow, Washington

Once we have set our larvae, whether they be clams or oysters, cultched or cultchless, we typically need to hold them in some sort of nursery environment prior to planting.

Lee Wiegardt, of Wiegardt Brothers, Inc., who has been setting eyed larvae for cultched oyster seed for as long as anyone, will discuss what he does with seed after it is set. Mark Billington, the hatchery manager from Westcott Bay Sea Farms, will review nursery techniques for cultchless oysters. Paul Taylor, of Taylor United, will describe nursery techniques for Manila clam seed. Dr. Ralph Elston, of Battelle Northwest, will talk about the diseases we see in the hatcheries and nurseries and what we can do to reduce risk.

At the clam culture workshop I attended last fall in British Columbia, Dr. Robert Reid presented an intriguing talk about pedal feeding by post-set Manila clams. It may change the way many of us handle our clam larvae. Dr. Reid will shed some light on the issue of whether to upwell or downwell our juvenile clams. I think this is fascinating work. I wonder if any of the hatchery managers in the audience—Jim Donaldson, Ed Brown, Lee Hanson, Ted Kuiper, Dick Poole—have seen this green-up at 600 microns to support Dr. Reid's observations. I know that the early period for post-set clams is a particularly difficult time to keep them alive.

The speakers here today are experts whom Terry Nosho has selected to address particular topics. They are not, however, the only experts here. Many of you have been setting eyed larvae or working with clams for a number of years. I am sure you all have learned a few tricks that would help others.

For example, let me share this. When we set eyed larvae we have a technique that we feel helps get an even distribution of spat per shell. We put the larvae in the tank and turn the air on full for 45–60 minutes and really boil the tank and distribute the larvae. Then we shut off the air for 18–24 hours or until we no longer see swimmers. This air-off period allows the larvae to swim about and find an appropriate place to cement on. If the air is left on, it creates eddies and currents the larvae can't swim against, and shells in those areas will be blank.

I hope you feel comfortable enough in this formal environment to open up and share some tricks that have worked well for you—if not before the group, then among yourselves. It is really a tremendous opportunity.

Shellfish Production: Some Do's and Don'ts

Ted Kuiper, Kuiper Mariculture, Inc., Bayside, California

Kuiper Mariculture, Inc., has been producing oyster and clam seedlings for commercial sale since 1980 and bay mussel seed since 1986. We supply more than a hundred farms in the Pacific Northwest, Mexico, and Europe with about 150 million seed per year. Our niche in the shellfish industry is to supply small seed (5–30 mm) that is suitable for further nursery rearing by growout companies. Most of the farms we supply have their own nurseries, either for cultch setting or, in the case of single seed, for adding growth to the seed we supply in order to reach a size for final growout. Since many of you in the audience are experienced in nurseries, the focus of my talk is to share some of our experiences, both good and bad, describing the development of our nursery techniques over the last eleven years.

Setting the Larvae

Larval Setting of Manila Clams

We purchase all the larvae we set at our two on-land nursery sites. Our major supplier is Lee Hanson, of Whiskey Creek Oyster Farm, who has made a strong effort over the last eleven years to help us learn the do's and don'ts of Manila clam setting.

The following applies to our site which has a salinity during the setting season (April to October) of 20–33 ppt and an ambient temperature of 12°–24° C.

1. Manila larvae do poorly in low salinity—say, less than 20 ppt—so we delay our first sets of the season until we want to gamble on the heavy rain being over for the year. Our main setting site is located at an upper estuarine location that floods regularly. Our experience has been favorable from mid-April to early October. By mid-October the salinity may still be suitable, but the post-set die at our site at around 15 ppt.

2. Setting temperature does not seem to make a great deal of difference on a "hot" group of larvae (competent to metamorphose). We prefer the low side of around 22–24° C. Our experience is that at high temperature—say around 28°–30° C—the post-set larvae do not grow any faster, and they seem to have more bacteria problems.

3. Age of larvae and size seem to be important beyond the most obvious. Newly post-set seed (1–7 days post-set) does not travel well for us; that is to say, we specifically request swimmers rather than larvae that are going through metamorphosis at time of harvest from the hatchery. We prefer larvae that are a little green (not quite fully developed) to those that are very hot (fully developed and competent). The size of the larvae that are ready to set has made a difference to us in survival up to 500-micron sieve. Larvae in the 155-micron sieve size or larger seem to be the most durable in the setting system.

4. Handling of the larvae during set has the expected ramifications, but also we have found that we could discontinue the use of daily freshwater rinses that we used eight or ten years ago and now use saltwater rinse until the seed reaches a 185-micron sieve size.

5. Although it is more expensive, we credit most of our setting (and on-land nursery) success to use of an open system that does not reuse any water. Our treatment process consists solely of #20 silica sand filtration for the setting room. We used 10-micron cotton cartridge filters for many years with mixed results and discontinued any ultrafiltration after 1985.

6. Algae quality is a major consideration for most hatcheries and nurseries. We use mostly raw water in our setting system, with a capacity of 50 million larvae per shot. Algae concentration is judged by color; if we are low we use monoculture algae paste we purchase from Coast Oyster Company or Whiskey Creek Oyster Farm. Occasionally, we culture natural food (bag-filtered to 10 microns) as a supplement during July and August, the lean months of natural algae production

7. Setting is done in downwells with no substrate and no chemical inducement. In setting, great larvae yield superior results. I look for evidence of newly dead, stressed larvae on arrival. Presence of protozoans consuming newly dead larvae is a good giveaway. A hot group of clam larvae will swim vigorously for several hours after arrival, swarming, and probing about with their foot. A lethargic group of spiraling larvae with debris-clogged velums will yield poor results no matter how great a setting system you have. Fortunately for the industry, Lee Hanson is a superior larva culturist who spots any trouble before shipment, thus making a high percentage of sets successful.

8. Embolism from centrifugal pumps and water heating (gas release as temperature increases) has always been a scourge for us. We vigorously aerate the setting flow through water to de-gas the system. If you are among those opting to do remote clam setting in tanks, you most likely will not observe this problem due to greater surface.

Pacific Oyster Setting for Single Seed

The remarks regarding Manila clam larvae setting also apply to Pacific oysters. However, some particular comments may be helpful.

1. We use only finely ground, sun-dried Pacific oyster shell for single setting. We have had no success in applying the very important research results surrounding inducement with epinephrine. As you know, Pacifics will set on just about anything. Every seed hatchery in the world thinks its substrate—whether dolomite, silica sand, ground scallop shell, Mylar sheeting, or a multitude of other materials—is the best. We use what works for us, but I am sure that all the others work just as well.

2. Freshwater rinses are fine all the way through the setting system.

3. The less the newly metamorphosed seed is sieved, the better. The first sieve we use is 315 microns, and we try not to sieve again until the seed reaches 3,400 microns. From 315 to 1,800 microns we split densities frequently to reduce competition.

4. We use a setting temperature of 24°–26° C.

5. Setting density is about half that used for Manila clams.

6. The same comments hold true about vigorous larvae yielding superior sets.

7. We have observed that Pacific larvae packed very cold (say, arriving at a box temperature in the 30° F range) never seem to recover well from the trip. Larvae usually do quite well if they are packed cool (say, arriving with a box temperature under 70° F) and are not stressed in transit with very hot summer temperatures. This year we are going to attempt through gratuity to have the Fedex driver deliver our boxes first on his run.

Remote Setting of Mussel Larvae

Comments on oysters and clams apply to mussels as well. Mussel set best in the low temperature range, 17°–22° C. Mussel will set on anything to the "last man." The literature states that the southern bay mussel, *Mytilus galloprovincialis*, needs exposure to macroalgae, *Polysiphonia*, or eel grass to set. We have tried those methods, but our experience has been that competent larvae will set on anything. We use the same downwells as with oysters and clams and grow them up singly (actually clumped) without substrate to 0.5 mm–3.0 mm in upwells.

I have no special observations regarding mussels, only that you have to "step on them to kill them." They are extremely hardy and we have never set a poor group from the hatchery since we started with mussels in 1986. Because they set "to the last man" with nearly 100% recovery from the setting system to the initial phases of the nursery (upwells), we charge for mussels one-third the price we charge for the other species we culture.

A small trick I will mention I learned from George Trevelyan: a household paint brush works to remove seed from the setting trays with low mortality. We prefer to use freshwater spray (low pressure, high volume) to dislodge newly set seed from the setting trays. The tough ones get the paint brush treatment.

Our view is that Charlie Stephens's NCRI grant on remote setting of mussels is a worthwhile project and should be funded. I suspect that many growers will find that they can remote set *M. galloprovincialis* more simply than Pacifics. We do not see much future for us in the single mussel seed business, because once a grower figures out how easy they are, mussel seed will be very easy to produce at the grower's farm. In the meantime we continue to produce 30–40 million 0.5–25.0 mm mussel seed annually.

Seed Culture after Settlement

Manila Clams from Post-Set to 1,410 Sieve

After seed is removed from the setting shed it is loaded onto a 155-micron or, if large enough, a 185-micron upwell. For the first week after transplanting the seed from the setting room to upwells, care is taken not to wash the new seed with freshwater.

A few words about our experiences with upwells. We started with forced (gravity fed with a 1-foot pressure gradient) "updrafts" using raw water in 1978 at Humboldt Bay. We were successful in rearing seed from 1,180 sieve to 1,800 sieve, but the pressure gradient, combined with embolism from centrifugal pumps, led to gas bubble problems with the seed. We used aeration and baffle tubes to cut down on gas supersaturation. Air bubbles would build up under the "updraft" screen and block the flow; occasionally, bursting bubbles would push seed out of the water and onto the ground. This was an all-around poor system. A gradual change was made from 1978 to 1980 to plastic 18" pipe as updrafts and a constant "no-head" gravity system by 1981.

We started using 1-hp submersible sewage pumps in 1982 and immediately found a significant increase in our production capabilities. For clams, oysters, and mussels we now operate 72 tanks (six upwell systems of 12 tanks each) at Arcata and 70 tanks (seven 10-tank systems) at South Bay, each with its own pump, duplicate intake, and plumbing. For the raw water systems we found that "fluidizing" was very important, while "fountaining" (refers to too much water) is detrimental to all sizes of clams up to 1,410 sieve size (2–3 mm). In these upwells we now produce 75–100 million seed at

1,410 sieve size each year for sale or planting into nursery trays suspended from rafts. My view is that based on pumping cost, space, density,and market price, clam seed cannot be grown in upwells on land over the 1,410 sieve size (150,000–240,000 per liter). I have no doubt that several hatcheries are growing Manilas past this size using cultured algae, but our view is that most nurseries will have a lower-cost product by growing the seed in less energy-intensive systems after 1,410's.

Fouling is a problem in the post-set to 1,410 sieve size. Manilas are subject to external shell colonization by *Vorticella*, a colonial protozoan that multiplies rapidly in an organically rich system. Large numbers of smelt die in the vicinity of our pump intakes after spawning each spring, providing a great decomposing substrate for this protozoan. If *Vorticella* is not controlled it can take over a group of clams in one day. The protozoan is not pathogenic (infectious); our observations are that the colonies smother the seed and also attract bacteria. To control:

1. Change duplicate intake lines at least every ten days.
2. Wash seed daily with freshwater (one week post-set).
3. Soak seed in freshwater for 5–10 minutes daily.
4. Air dry upwells after use, and rotate stock onto new clean upwells as needed but no less frequently than every week. This is why we have a nonstop upwell building program. For the 142 upwells that are run daily (just like milking cows) we need at least 200 upwell screens in the 18"–20" sizes from 155 to 1,320 microns.
5. As a last ditch effort to take out the colonials, the seed can be completely moved to a new environment with different temperature and salinity. In our case, a change from North Bay to South Bay may be a change in salinity from 20 ppt to 34 ppt and a temperature change from 24° C to 15° C. The seed usually does fine and the colonials are zapped. A more radical treatment is 24-hour air drying in the shade; it usually kills the colonials but also about 50% of the very small seed.

The biggest difficulty with very small clam seed at our site is radically low salinity from unexpected flooding. In 1978–82 we put our best effort into growing 1,180–1,320 sieve seed into the winter. I was amazed at how rapidly the shell degraded (actually eroded in a few days) to the point that tissue was exposed and the animal was still alive. Low salinity (say, below 15 ppt) in conjunction with low pH (don't ask me how low) zapped the seed with shell erosion in a few days. To overcome this problem we restricted the North Bay site to culturing mussels during the low-salinity periods. The South Bay site, with salinities seldom below 17 ppt, does not manifest shell problems.

Another annoyance is incrusting by diatoms, such as *Navicula,* which leads to slow growth in post-set to 1,410 seed (after 1,410's the seed tends to laugh it off). The diatoms can be controlled by selecting high salinity and low temperature sites (more oceanic), by shading from direct light, and by daily freshwater soaks. I have seen this problem at other commercial nursery sites, although we don't speak of it in polite conversation.

Single Pacific Oyster Post-Set to 3,400 Sieve

Our experience has been very favorable for Pacifics from post-set to 3,400 sieve in upwells. They grow very rapidly at high density provided the systems are kept fluidized with a minimum of fountaining. Fouling from colonial protozoans and diatoms must be controlled to have good seed growth.

We have moved completely away from screening (sorting) Pacifics until they reach 3,400 due to our experience with stress-related loss from sieving at small size. Our strategy in the last nine years has been to split the density of seed frequently as it grows rather than break bills by sorting frequently.

The comments about clams pertain to oysters as well. Doubles are a problem if the shell is not prepared correctly or the setting density is too high. We have few answers for this little bit of hell.

Mussel Seed from Post-set to 3,400 Sieve

As mentioned under the setting section above, mussels are extremely hearty. Fouling by colonials is a laughable problem to mussel seed. Diatoms are equally of no concern, and salinity is not worth talking about except to say that mussels are remarkably well adapted to upper estuarine conditions. Within one week's time we have had very small post-set mussels in 0 ppt, up to 15 ppt, back down to 0 ppt, and then back to 33 ppt. For several weeks in a bad flood in 1987 we had mussel seed at 0 ppt, and the little devils even grew well.

There are some problems with upwell rearing of mussels. They byss (anchor) strongly to the bottom screen, attempting to clog off every bit of water flow. This creates more cleaning work daily, but even when they are successful and clog what appears to be that very last bit of water flow they don't die or even stop growing. They are cultured at half the density of oyster or clams due to the clogging problem, but this is a small price to pay for such a hearty critter.

Later Nursery Culture 2–3 mm Up

Pacific Oysters

I believe that nearly all of the hundred or more farms we sell single Pacific oyster seed to have their own "tried and true" nursery techniques that work well for them. Most receive seed from us at 1/4" sieve or 3/8" sieve for loading into Norplex 1/8" or 1/4" bags for placement on intertidal racks for nursery rearing to a size that can be sorted at 30+ mm for final growout—again mostly on racks.

Intertidal Nurseries for 1/4" and 3/8" Sieve Seed

The intertidal rack we use at Humboldt Bay for some of our nursery rearing of Pacific seed is 6'8" in length. It holds three 1/4" bags, each with two 1/8" bags inserts for use with 1/4" sieve seed loaded at 0.5–1.0 liter of seed per 1/8" bag; alternatively, it can also hold four 3/16" bags with 1/4" sieve seed or three 1/4" bags with 1,000 3/8" sieve per bag. The purpose of this intertidal nursery is to push the seed quickly up to 30+ mm or larger, to go into the 1/2" bag for final growout. Since we do not have a growout we use the intertidal nursery on +3 foot ground to hold seed through the winter or as a summer nursery to supplement the subtidal raft nursery.

Raft Nursery for 3–5 mm to 30 mm Seed

Raft nurseries for Pacific seed are very common on the North American west coast and also in Europe. Several types are used in Washington, Alaska, and British Columbia including air-lift upwells, single-point mooring upwells, long lines with trays, pumped float upwells, and paddlewheel upwells. How well these systems work depends on the site's built-in benefits (e.g., availability of electricity) or limitations (e.g., no floating structures permitted).

We have our own "limitations" at Humboldt Bay, so for thirteen years we have used floating rafts with square 22.5" trays made of wood or plastic. Pacific seed is loaded into the trays from the upwells at 3,400 sieve or larger at the rate of 5,000–8,000 per tray, and the seed is sorted every 1–14 days to harvest 1/4" sieve seed for sale or replanted for growout to 3/8", 1/2", or 3/4" sieve size. Seed is grown the year around in the rafts at three selected sites that are suitable for raft mooring.

Some observations that might be helpful:

1. Heavy densities will generally lead to poor results. The commonest questions we field from new growers are related to density. New growers should purchase more bags than they feel are necessary based on the recommended loading density, so that they can "split" the density of their seed rather than sort if they are short of time.

2. It is an unfortunate fact that Pacific seed in many subtidal locations will grow faster than the grower can tend to it. Planning for sufficient space for bags or trays and for time to adequately farm the seed is especially important for the new grower. Overcrowding and neglect, it cannot be said too often, will lead to poor yield. The most recent sorting machine that we have adapted for our use allows us to sort more than 50 bags per hour at the 1/2" sieve size. We keep telling ourselves that we have enough space to meet our production goals, but unfortunately we are frequently reentering the permit process to develop new sites.

3. Relying on one site for both nursery and growout may not always be correct. We have found that each of our three nursery sites (358, 56, and 10 acres) has radically different growing conditions which we try to use to our greatest benefit.

4. Some growers believe that sorting is counterproductive because of the stress on the seed and the time involved. That makes very good sense if the new grower has sufficient space to "split" the seed frequently rather than taking the time to sort.

5. Something you all know but which can't be said often enough for the new grower, is: "Always plan for the worst"—whether it be tide, weather, wind, market conditions, vandalism, breakdowns, or predation. I recall working for a nursery-growout company that planned to sell its oysters on a particular date. Because of poor "fatness" of the adult oysters, the market backed out. Forty rafts sank under their own weight in 30 feet of water.

Manila Clams

Manila Clams 2–3 mm to 15 mm

The purpose of the clam seed nursery after seed reaches 2–3 mm (1,410 sieve) is to grow the seed to a size suitable for bottom culture under netting (6–8 mm) or inside bottom clam bags at 15 mm.

Intertidal Rack Nursery Culture

We do not use our intertidal racks for clams because the seed grows about 50% faster in rafts. Racks are used by some Washington growers planting 6–8 mm inside a bag at 5,000–10,000 for growout to 15 mm. Growers that use this nursery technique have told me that the only drawback is that the racks must be moved very low in the intertidal during winter to avoid mortality from freezing. Most growers that use this style try to have all their seed up past 15 mm by winter and have it in the ground before the first hard freeze.

Intertidal Stack Tray Nursery Culture

This technique, developed by Don and Judy Rogers at Olympia Clam Company on Eld Inlet, is widely used in Washington to rear 2–3 mm up to 15 mm for bag or bottom planting. I have not used stacked plastic trays successfully on our mud bottoms (they tip and sink in mud), but with their permission I will describe the method as I understand it. Clam seed at 2–3 mm is loaded into four window-screen-lined plastic trays at the rate of 15,000 (or more) per tray. Empty trays are added top and bottom to form a lid and a base. The whole stack is banded (or roped) together and placed on a suitable gravel bottom with a weight to hold the stack in place. During the winter the stacks are moved very low in the intertidal zone to avoid freezing.

Raft Culture for Manila Clam Seed

The same rafts and trays that were described for oyster seed are used for Manila clam seed. Manilas at the 2–3 mm size are planted at a density of 15,000–20,000 in the 22.5" square trays. We farm about 20 million 6–8 mm seed per season using this style, which is very low tech. Several of the higher tech systems such as pumped floating upwells I am sure work just as well.

Some points for the new grower:

1. Manila seed that is grown without benefit of substrate is subject to "shell erosion" from low salinity. Just because you see Manilas growing in the bottom of creek beds does not mean that seed will survive suspended in areas of low salinity for extended periods.

2. Our view is that Manilas can be grown in trays up to 20–25 mm without substrate, but after that size they should go into the ground. Heather Rogers wrote a terrific student paper on the pluses and minuses of growing Manilas without substrate to market size (Terry Nosho has a copy).

3. Trays need to be serviced at least once a month to remove newly set crabs, starfish, or flatworms. We hand-screen 6–8 mm or 12–15 mm clam seed from these trays. As yet, we have not developed a mechanical sorter that does not stress clam seed, although many hard clam nurseries on the East Coast are using Kason machines.

Bay Mussels

Most growers that nursery bay mussels are catching and using wild seed. Wild seed is not widely available on the West Coast; only a few sites, such as Penn Cove, have reliable annual sets. We have been producing bay mussel seed to supplement the natural set. Most growers take the seed from us at 0.5–3.0 mm and reattach the single seed to their preferred substrate for further nursery rearing and final growout. We have been happiest with copolymer netting at a density of 0.5–3.0 mm at 2,000–4,000 per linear foot of material. Density is split by two-thirds after 30–60 days; then the seed is grown out to 3/4", at which size it is suitable for intertidal culture on racks in bags.

Some notes for the new grower:

1. After the seed is reattached to substrate, we found best results with hanging the strings vertically in a vigorous flow through tank for one week or longer. Drop-off was greatly reduced by allowing the seed to byss strongly before replanting the string in a sink float in the open bay.

2. High densities are better than low densities. Fouling will quickly take over a low-density set.

3. Because of heavy perch predation, the best results were with a net-enclosed sink float.

4. Bay mussels are more tolerant of low salinity than clams or oysters of a similar size.

Planning and Management

New growers that want to establish nurseries should think about the cost of nursery culture to farm 2–3 mm seed to, say, 9–14 mm versus direct purchase of 9–14 mm seed. Costs to consider are for rafts, trays, labor, mortality from neglect, vandalism, or weather damage—and risk. As for setting larvae versus purchasing seed at 2–3 mm, costs to consider are electricity, upwells, setting facilities—and risk.

Growers that want to set their own clam larvae should decide well in advance of the appropriate setting season. They must give the commercial hatchery an advance order so the hatchery can ship larvae during the optimum period. For example, if the grower hopes to have clam seed off the racks at 15 mm for bottom planting before the December "hard freeze," the larvae need to be set no later than May 1. Taking small clam seed through the winter that was set very late can be costly and discouraging.

The most difficult aspect of operating a commercial seed nursery is production management. Every seed produced has a production cost. Seed available for sale at the wrong time of the year with no market can be financially disastrous. Not having enough of the right size seed during periods of peak demand can be equally disastrous. Growers can help the commercial seed nursery farmer by making orders several months in advance; if you want seed in the spring, place your order with the seed nursery farmer in the early fall.

Shellfish Health Management and Maintenance

Ralph Elston, Battelle Marine Sciences Laboratory, Sequim, Washington

Hatchery and nursery diseases can cause mortality and reduce production, growth rate, and product quality. Disease effects can be insidious since the result is not always obvious as a distinct mortality episode. Often, poor management results in poor health of animals. Such effects are easily remedied by correcting the management procedures.

Following is a brief mention of specific infectious diseases and the locations at which they occur.*

Hatchery

Vibriosis. A "management" disease. It can enter and be amplified through algal stocks, seawater, or broodstock.

Oyster velar virus disease (OVVD) of Pacific oysters. A manageable disease transmitted from the broodstock.

Diseases caused by parasites such as the geoduck larval amoeba.

* Editor's note: Those wishing more information may be interested in Dr. Elston's book, *Mollusc Diseases: Guide for the Shellfish Farmer*, published in 1989 by Washington Sea Grant.

Setting

Setting or metamorphosis is a critical stage in which feeding temporarily is interrupted and anatomical reorganization begins. Condition of larvae prior to metamorphosis (fat reserves) directly determines the proportion of larvae which successfully make it through setting. At setting, the bivalves are faced with a new set of microbiological hazards. Control of the microbes may be a means to increase success of setting.

Nursery

Losses of juvenile bivalves are apparently very high, but the reason for the high mortality is poorly understood. This is a subject that should receive further attention.

Hinge ligament disease is a particularly insidious disease that can cause high mortality and loss of growth. It results from infection with a common marine bacterium.

Occasional parasitic infections can cause severe losses in juvenile bivalves, e.g., in *Ostrea edulis.*

Controlling the Spread of Mollusc Diseases

Serious potential losses of farmed and naturally occurring shellfish can be avoided if new and virulent diseases are not introduced to our culture areas. Avoiding the introduction of such new pathogens, pests, and predators is the objective of local and regional government regulations. Such regulations must be balanced so that they do not unnecessarily impede the ability of the shellfish industry to function. Washington state is now taking a leading role in establishing such regulations first within the state followed by regional cooperation for the control of shellfish diseases.

Rewrite of WAC-220-77-040 under way with shellfish industry participation.

Cooperative approach with members of Pacific Marine Fisheries Commission.

Procedures for "routine" movements within "West Coast Commerce Area," procedures for new species introductions, and various operational guidelines.

Cultched Oyster Seed in Willapa Bay

Lee Wiegardt, Wiegardt Bros., Inc., Ocean Park Washington

Our company began remote setting work in 1981 with information provided by Willie Breese, at Oregon State University. We are catching seed about the same as then. My comments will relate to growing oysters in Willapa Bay, which can be very different from growing oysters in Puget Sound.

Seed Count

A high number of seed per shell may be overrated. People try to get 20–30 spat per shell, but they used to buy Japanese seed with 15 per shell. Realistically, you can grow 2 oysters per shell. However, an average of 2.5 spat surviving on each shell will result in a doubling of profits.

Seed in itself is worth nothing to oyster growers. It has value when it grows up and is harvested. The cost of seed is about 25% of the sales price based on a $14 per gallon shellstock price.

The price of larvae has remained constant and averages about $0.10 per 100. We could pay twice the amount, $0.20 per 100, if 5 spat per shell would survive.

Oystermen are counters. A microscope is needed to do this properly. We look at the larvae, count the set, count the spat right out of the tank, put them on the beach for 3–4 weeks, and count them again. As far as counts per shell go, we try to get 30 at set and look for 14 at plant. If Korean shell is used, we try to get 8 spat per shell at planting time.

When to Plant

What do you do with seed if it is 90° F outside? I say leave it in the tank or put it into water somewhere. We put seed into water within one hour to keep down dessication. Everything we set from April through September is put on the beach near extreme low tide. The seed will quickly accumulate scum, which helps protect the seed against dessication.

In March, spat should be kept in the tank for 11 days before committing them to the wild. In Willapa Bay, planting should begin in April to take advantage of June growth. Experience has shown that seed planted in June will be almost a year behind seed planted in April.

Planting Cultched Seed

Mark rows in a five-acre tract with stakes spaced 25 feet apart. Fan cultch while shoveling from one side of a barge that travels up and down between rows of stakes. The next day, go out and scatter by hand to get a more even distribution. Do this as soon as possible because mud will get on the cultch. We spread conventional cultch at 300 bags per acre. Korean cultch can be spread at about 110–120 bags per acre. After two to three years, we get a yield from 2,000 to 3,000 bushels per acre.

Cultchless Oyster Seed: Equipment, Systems, and Servicing

Mark Billington, Westcott Bay Sea Farms, Friday Harbor, Washington

Westcott Bay Sea Farms produces cultchless oyster seed in its hatchery by inducing eyed larvae to set and metamorphose on oyster shell particles. The spat remain in the hatchery in recirculating seawater systems for six to eight weeks at 20° C with supplemental algae. At 2–3 mm the seed oysters are transferred to floating nursery rafts in Westcott Bay. Based on a design by John Bayes (Seasalter Shellfish, U.K.), the rafts are 12 feet by 22 feet, with a central fiber glass channel, 1.5 feet by 20 feet, to which 20 screened barrels can be connected. A submersible motor with a propeller (Flygt Corp., Germany) mounted in one end of the channel evacuates water from the channel, forcing water to upwell through the bed of oysters in the barrels.

At 2–3 mm, as many as 100,000 oysters can be stocked per barrel. Densities are reduced to 30,000–50,000 per barrel at the 9–12 mm size. The advantage of this floating nursery is that it keeps large numbers of seed in an easily accessible situation while utilizing high water flow to take advantage of natural algae in the bay.

At 9–12 mm, the seed oysters are "planted" in plastic nursery trays. Imported from Mexico, the trays measure 22.75 by 22.75 by 3.25 inches. Trays are stacked 15

high into "modules" with an empty tray as a base and a top lid. Modules are secured with ropes from the base to the top and are hung off long lines in the bay.

The modules are usually deployed between June and September as the seed in the floating nursery reaches 9–12 mm. The modules are loaded at 500–700 oysters per tray and are brought in for servicing every six months. The seed is removed, graded, and redeployed at reduced densities. Oysters larger than 35 mm are moved to lantern nets. By servicing the modules at six-month intervals, predators can be removeed before they are large enough to pose a threat to the seed and fouling is kept to a minimum, although excessively dense mussel sets may certainly provide competition for food.

Survival through this system varies depending on particular groups of oysters and weather variations, but typically 60–70 percent of the seed reaches 35 mm within 18 months.

Considerations for a cultchless nursery: site evaluation; type of equipment (floating raft, trays, modules); initial loading density; service interval; control of fouling; reducing tray density as oysters grow.

Seed Floats and Other Culture Methods for Clams

Paul Taylor, Taylor United, Shelton, Washington

Here are some methods I have experimented with in culturing clams. I have not used raceways or grown clams directly on the bottom of the tanks. Growers have to assess their own needs and site before venturing into a clam nursery, but the following assessment may help them.

Seed Floats

A seed float is a floating raceway dependent on the tidal currents for flow. The nursery area is a plywood floor that is covered with an inch of sand or crushed shell. When setting, clam larvae are placed in the float and a Nitex gate is put across the end to make sure the clams settle out in the float. After a week or 10 days, the gates should be removed. These are best started in April or May when there is a full growing season ahead.

Construction of seed floats is quite simple. They are made with 2" x 12" boards and 3/4" plywood. I put a 2" x 4" board on edge at each end to prevent the material from washing out. I also put 3/8" plywood on the top to prevent macroalgae from growing and reducing flow through the float.

The seed float method is very inexpensive when it works. The main problem is its inconsistent results.

Advantages —Very low maintenance. Can seed the clams when the tide is in. No mechanical things to break down Long-term costs can be very low. Can put any size of seed clams into system.

Disadvantages —Very inconsistent and unpredictable. Must have fairly sheltered water. If you want to sell, it's difficult to strain sand from clams. When planting out of float, clam sizes vary. Hard to get accurate numbers of seed. Difficult to get clam setters at the correct time. Difficult to tell how well clams have survived for at least a month.

Downwellers

We use downwellers for clams that are just at set size (140–160 microns). We place 5–8 million into each 4-foot tray; which is screened with a 118 screen. Water is then sprayed into the top of the tray. The incoming water on top of the screen forces the water down through the Nitex screen, making a downwell.

The downweller is used for a very small portion of the clam's life. The clam is screened on a 224-micron screen and then is at a size that is more easily handled on upwelling screens.

Advantages —Can control the water, temperature, and flow. Can inject feed into the water. Time of year is not critical. Can screen off faster-growing clams.

Disadvantages —Daily washing. Relies on mechanical means (pump) for water supply.

Updrafts

Updrafts are low-flow upwellers. They have a mesh screen that varies between 200 and 1,000 microns on which the clams rest. Water is brought in under the screen and taken off above the screen where the clams are held, thus creating an upflow.

Updrafts get clams through a critical size range for us, but we are now doing experiments to determine whether updrafts or upwellers are more efficient.

Advantages —Lower flow than an upweller, so it is more favorable to heat or feed. Provides means of taking clams through a difficult size range. Easy to get at seed to observe or screen.

Disadvantages —Different sizes of seed need different flows. Densities per updraft are limited. Requires daily cleaning. Slow growth.

Upwellers

Upwellers will take a variety of clam sizes, and the product can be grown very intensively. A header tank is used to maintain a constant water level. Pipes then disperse the water to a box upweller or tank upwells. Tank upwellers are good with small volumes, but box upwellers help to bring down costs.

I have grown clams in upwellers in a variety of places. The microalgae in the water are very important, so choosing the site for an upweller system is critical. Also, areas that have heavy siltation are very hard to deal with.

Advantages —Can adjust flow of each individual upwell. Will grow a variety of sizes. Can grow very intensively. Easy to observe.

Disadvantages —Have to depend on natural food. Must clean filter bags and screens daily. Relies on mechanical delivery (pump).

Floating Upwell System (FLUPSY)

With the floating upwell system (nicknamed FLUPSY), siting is also very important. It consists of six or twelve upwell boxes or bays that hook into a center channel. Water is exhausted from the channel by electrically driven propellers, creating an upwelling action.

Advantages —Very efficient in energy costs. Easy to clean. Can grow large volumes. Very rapid growth when conditions are right.

Disadvantages —Must have correct site. Exposed to the elements in the bay. Relies on electricity. Up-front costs are substantial. Will not take seed under 1,800 microns.

Taylor United is planning to increase its use of FLUPSY for clams in the future, particularly if we can use smaller screen sizes. We will be experimenting with screens this summer.

Nestier or Mexican Tray

This system consists of lining a stackable tray with Nitex, then putting seed in each tray and strapping 6–8 trays together. These can be floated or secured directly to the beach.

Advantages —Deal with large or small volumes. Low maintenance. Doesn't rely on pumps or props.

Disadvantages —Hard to make a visual check. Difficult to secure trays to bottom. Lining can come out of trays. Problems with smaller screens and siltation. Slower growth depending on density.

This system is very feasible for a small grower because it doesn't require daily checking. This can be a problem, though, because it does need some monitoring. The finer the Nitex lining the tray, the more siltation and more flow restriction there is.

Seed Bags

A seed bag is made of 1/8" tubular Vexar cut into three-foot sections and a PVC pipe ripped and slid over the end and secured with ladder or zip ties. The bags are then secured to the bottom on a beach that does not shift.

This method works well for wintering seed that is too small to plant in the fall or for the grower that wants to cut cost by ordering smaller seed, then growing it up larger to increase survival when planting onto the ground.

Advantages —Very low maintenance. Can handle large or small volumes.

Disadvantages —Seed must be 4 mm to put into bag. Can view only when tide is out. Cannot grow very intensively.

Development and Use of a Recirculating System for Clams

Don Dahman, Dahman Shellfish, Shelton, Washington

Our nursery is located in Totten Inlet in south Puget Sound. It is primarily a family operation that my mother and father and I run. In June of 1988 we grew one million clams in a 2,600-gallon tank. We held them for 13 weeks at 80° F. Over that 13 weeks, we cycled the water once. We fed them 11 liters of Diet A algae paste from Coast Oyster Company. When we drained the tank we estimated we had 500,000 clams 2–5 millimeters in size.

That fall through the spring of 1989 we collected all of the information we could find on closed systems. It wasn't much. In June of 1989 we made the decision to build a small nursery. We hired a consultant and got all the figures. We started the permit process and construction in July. Nearly a year later (on May 15, 1990) we brought in the first animals.

Our goals for this facility were to build a small nursery with a minimum operating cost, an energy cost of $700 or less, and the labor of one man. Our actual energy costs have been around $500 this winter.

Our building has 1,750 square feet. It has four separate systems: the setting trays, the nursery system, the algae system, and the FLUPSY.

The setting system consists of two 135-gallon trays. Each tray holds three setting rings 4 feet in diameter, with the capacity to hold 10 million clams per ring. We spent most of 1990 perfecting our setting system. The water for the setting system is drawn out of the main header tank at 75° F. We bring off the byssed larvae at 180 microns and move them to an upwell box with a 165-micron screen. We move them from the building to the FLUPSY when they reach 1,800 microns.

The closed system for the upwellers consists of a 2,600-gallon header tank with a 15,000-watt heater. From there the water goes to an 800-gallon header tank where it is drawn off for five upwell boxes. It is metered through a manifold in the upwell box to control the flow through the upwell tray. This water overflows into another 800 gallon header tank and is drawn from this into five more upwell boxes. The overflow from these boxes is collected and pumped back over two biopanels and back into the the large header tank. The biopanels have Algasize growing on them. This is Mother Nature's filter.

The algae system consists of a line of flasks, 5-gallon carboys, eight 75-gallon tubes, and eight 750-gallon tanks. We grow Chagra and 3H. The algae are fed at a continuous drip into the two small header tanks. This system has been fairly successful for us so far. We have only had one real problem. In late June of 1990 we lost a considerable number of animals due to shell erosion. The problem wasn't really solved until November. We found the solution to be quite simple. We had to monitor our pH closer and add calcium and magnesium.

Our FLUPSY consists of six boxes 3' x 3' x 4'. They are quite large upwellers. We grow our animals here until they reach 7–8 millimeters.

When we started we did not have the knowledge necessary to run a shellfish nursery. I would like to acknowledge the help given to us by Dick Poole, Lee Hanson, Chris Langdon, Ralph Elston, Vance Lipovsky, Jim Donaldson, and Ed Jones and Jan Lemons from Taylor United, and many others in the industry.

Optional Algal Feed Sources

Christine B. Edwards, Coast Oyster Company, Quilcene, Washington

There are various techniques used to grow live algae, including batch culture, bag culture, continuous or semi-continuous flow-through, and pond systems.

Coast Oyster Company utilizes the batch culture method to feed oyster broodstock, larvae, and setters. The species *Thalassiosira pseudonana* (clone designation: University of Washington; 3H) is used to feed the newly set spat.

The rate of feed is based on the algal density, clearing of the tank, and spat growth. The formula used to compute the amount to feed is (desired cells per milliliter) x (number of liters in the tank) divided by the algae density cells per milliliter.

Dick Steele of Rock Point Oyster Company has a live algae system for his setting station at Dabob Bay, Quilcene, Washington.

Preserved Diets

Our 3H is centrifuged into a paste and preserved as Diet I or added with dried *Tetraselmis suecica* (Cell Systems) to make Diet A. The preserved algae has a shelf-life of one year if kept at 35° F. We recommend Diet I for remote set feedings and Diet A for broodstock. Diet A can also be fed to day-3 or older seed; for example, 29 grams of Diet I blended with seawater will result in 100,000 cells per milliliter for a 1,000-liter tank.

Fresh Paste

This is another option for remote set feedings. From our experience, paste will keep for two weeks in a standard refrigerator. Our paste, which is preserved, has an average of 11–15 billion cells per gram. Paste is mixed with seawater before using.

Bruce and Gordon Jones of Innovative Aquaculture Products, Ltd., Skerry Bay, Lasqueti Island, British Columbia, can be contacted regarding this product. Dick Wilson of Bay Center Mariculture, Bay Center, Washington, feeds fresh paste to his larvae and spat. All of his paste production is for his own use.

Dried Algae

Concentrated algae can be freeze-dried or air-dried. While we have not had much success using dried algae exclusively, more research is needed in this area.

Our source for dried *Tetraselmis suecica* was Cell Systems of England, which has gone out of business. While Diet A will not be available in 1992 from Coast Oyster Company, we intend to pursue other blends for future diets. Joseph Weissman, Microbial Products, Inc., Vacaville, California, has a dried algal product.

In conclusion, our observations show that live algae is the best feed for spat. While many growers are not equipped to grow their own live algae, the diets, fresh paste, and dried algae are options of feed to reduce mortality of freshly set larvae, and provide nutrients in low natural algal production areas.

Producing Algal Feeds at Remote Stations: Summary

Vance Lipovsky, Aqua Business Management, Royston, B.C.

Review

Filter incoming seawater with 50-micron filter bag.
Adjust pH by adding carbon dioxide.
Basic techniques: starter bottles (try to keep free of bacteria); aeration (to keep algae suspended and add CO_2); culture tanks.

Algae Growth Cycle

Stage 1 – Beginning stationary phase; put algae in.
Stage 2 – Growth period; culture grows exponentially; can bring up diatoms twice as fast as flagellates.
Stage 3 – Growth of the culture reaches a peak.
Stage 4 – Culture goes into stationary phase.
Stage 5 – Death.

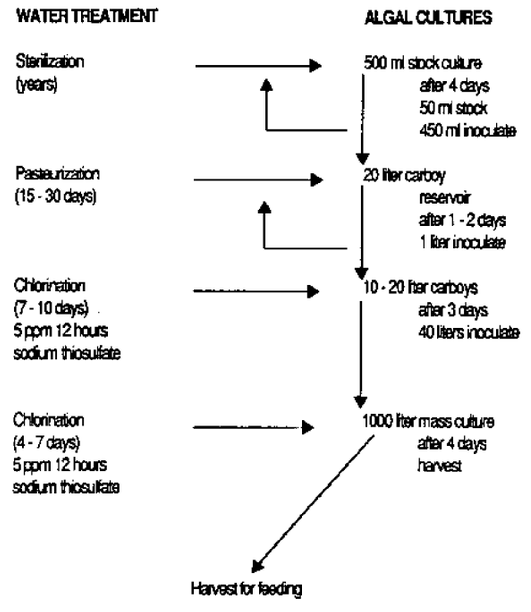
Factors Influencing Growth

Temperature. 15°–20° C; can go up to 25° C.
Light. Fluorescent bulbs or high-intensity lights.
Nutrients. Sodium nitrate; sodium phosphate; sodium silicate (for diatoms; do not need if growing *Isochrysis* or *Monochrysis);* trace metals; vitamins.
pH range. 7.5 – 8.2
Salinity range. 20–30 ppt; may get faster growth of the culture at lower salinities, but this is not crucial at remote stations.

Algal Growing Methods

Culture system. Batch
Algal species. Recommend diatoms at setting stations; 3-H or *Chaetoceros calcitrans.*
Water treatment
Sterilization. Pressure cooker will do.
Pasteurization. Warm to 180° F and leave overnight.
Chlorination. 2–5 ppm. Neutralize the next day with sodium thiosulfate; bottles and culture tanks.
Filtration. 5-micron filter bags.
Aeration. Air blower to circulate culture.
Nutrients. Can dissolve with hot (140° F) tap water.
Culture vessels. 500- or 1,000-milliliter flasks; 20-liter carboys; 1,000-liter tank.

Flow Chart of Production



Research on Shellfish Nursery Rearing

Feeding Behavior of Early Juvenile Shellfish, with Emphasis on the Manila Clam

R. G. B. Reid, Biology Department, University of Victoria, Victoria, British Columbia, Canada

In many species of bivalve molluscs there is a distinctive postmetamorphic alteration of feeding behavior. The filter feeding mechanisms of the pediveliger are lost at metamorphosis, and there is a pause before the gills develop sufficiently to allow the resumption of filtration. The delay may be no more than a few hours in oysters, the most widely maricultured bivalve type. However, in several commercially significant species the delay may last for a few days to a few weeks, and in failing to recognize this fact some standard nursery practices may contribute to setting and post-setting mortalities.

During the time gap in filter feeding, the early juvenile survives by pedal feeding. Therefore, a comprehensive understanding of bivalve pedal feeding should be valuable in designing appropriate feeding regimes for early juveniles and in stimulating research into species whose early development and feeding behavior are little understood.

A few species of relatively large bivalves are known to use the foot for nutritional purposes. Several of these are exceptional cases, such as carnivores and bivalves that possess sulfide-oxidizing symbiosis (Reid et al., submitted). Two others are interesting: the fresh-water bivalve *Corbicula fluminea*, whose pedal feeding contributes to its notoriously opportunistic distribution; and the tropical, coral-sand clam *Fimbria fimbriata*. Furthermore, pedal feeding has been observed in several genera of short-lived, small bivalves such as *Mysella, Musculus,* and *Miodontiscus* (Reid et al., submitted). In the latter, small adult size limits the filtration area of the gills, and during the course of evolution ctenidial paedomorphosis has simplified the gills to a form reminiscent of the early juvenile. The foot, therefore, is required as a supplementary feeding organ. This, however, rather than being a recent adaptation to paedomorphosis, seems to reflect a primitive condition that is found in many juvenile bivalves.

Observations of a variety of pedal feeding adult and juvenile forms (King 1986; Reid et al., submitted) indicate that some of the following behavioral modes may be expected in other juvenile bivalves. They are shown in Figure 1, with drawings labeled by letters that correspond to the following descriptions.

a. Ciliary pedal feeding during locomotion. While the bivalve is moving over the substrate, the cilia of the foot collect deposit particles that have been disturbed and resuspended. These particles are passed by a pedal ciliary tract towards the mouth and may be mechanically processed by the labial palps. This is found in scallop, giant clam, geoduck, and Manila clam juveniles.

b. Selective pedal probing. In a sedentary mode, sometimes while the bivalve is byssally attached to the substrate, the foot probes randomly. If particulate deposit matter is encountered, it sticks to the mucous-coated tip of the foot. Some of these particles are passed by cilia to the mouth, but the foot also retracts regularly and the

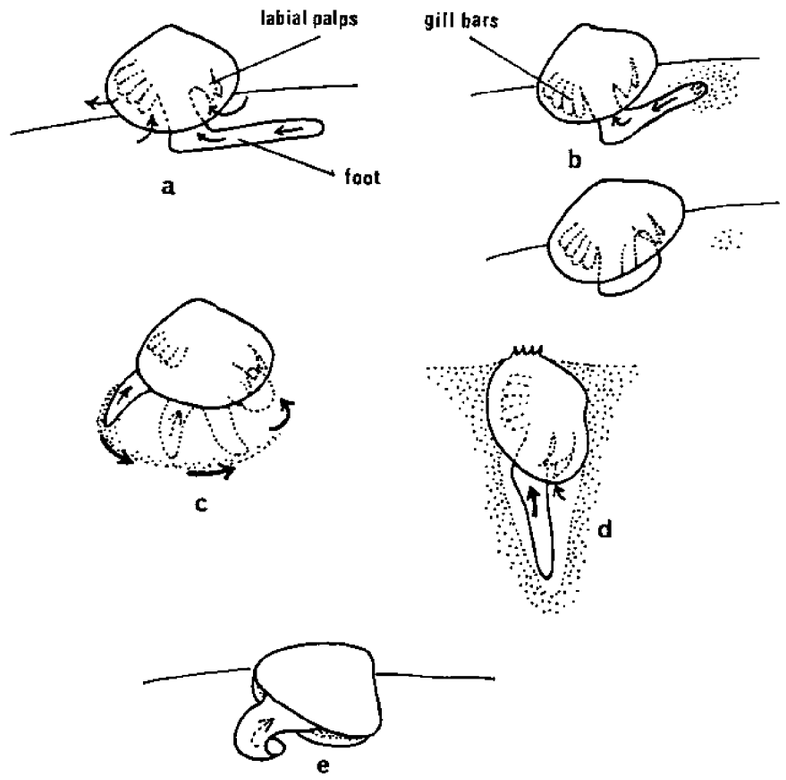


Figure 1. Pedal feeding behavior of juvenile bivalves: (a) ciliary pedal feeding during locomotion; (b) selective pedal probing; (c) stereotyped anteroposterior pedal-palp feeding; (d) interstitial suspension feeding; (e) pedal deposit feeding on a hard surface.

particle-laden tip is brought into contact with the mouth. The animal may continue to repeat this until it has cleared a 360° range. This is typically found in *Patinopecten yessoensis* (Ó Foighil et al. 1990; Reid et al., submitted).

c. Stereotyped postero-anterior pedal-palp feeding. The bivalve lies on its side, protracts the foot posteriorly, sweeps the foot anteriorly, and then retracts it. Meanwhile, the pedal cilia collect deposit particles and pass them to the mouth. When the foot is retracted some particulate matter is brushed off at the mantle margins and rejected, but the rest is formed into a bolus by the labial palps and then swallowed. This behavior is distinct from locomotion and digging cycles. It has been observed in geoduck, giant clam, and Manila clam juveniles, but it is not a frequent behavior of the last.

d. Interstitial suspension feeding. While buried at the sediment surface, the foot may probe down to form a cavity that contains water with resuspended deposit particles that are collected by the pedal cilia. This occurs in fresh water pea clams, as well as in *Fimbria* and *Geloina*, but possibly is used by some bivalve juveniles.

e. Pedal deposit feeding on a hard surface. This may be peculiar to *Corbicula*, the only genus in which it has been observed. The animal lies on its side on the

surface and the cilia on the underside only of the foot convey deposit or biofilm particles into the mantle cavity.

Manila Clam Juvenile Feeding Behavior

Manila clam juveniles move actively through a variety of substrates while the foot, during locomotion and probing behavior, captures deposit food particles and conducts them by ciliary activity to the labial palps where they are consolidated and ingested. The stereotyped postero-anterior pedal feeding mode that has been reported for *Panope, Tridacna,* and *Mysella* (King 1986; Reid et al. 1990; Reid 1990) is also occasionally observed. I estimate that suspension feeding does not effectively begin until the gills have adequately developed, about three weeks after metamorphosis, or at the size of 600 µm. This I am currently attempting to confirm in more detail, but anecdotal evidence exists to the effect that juveniles begin to "green-up" noticeably at about that size under nursery conditions in some Washington state hatcheries (Doug Thompson, Washington State Department of Fisheries, pers. comm.).

Redesign of Early Juvenile Nursery Conditions

Postmetamorphic pedal feeding has been shown to be significant in the following aquacultured genera: *Panope, Tapes, Patinopecten, Crassadoma,* and possibly *Tridacna*. It follows from an understanding of the various pedal feeding processes that normal nursery practice for the culture of early bivalve juveniles, namely the use of an upwelling system with the provision of suspended, mixed algae, is inappropriate. If pedal feeding requires access to deposited food particles a downwelling system is indicated, and food should be supplied in a form that will deposit readily.

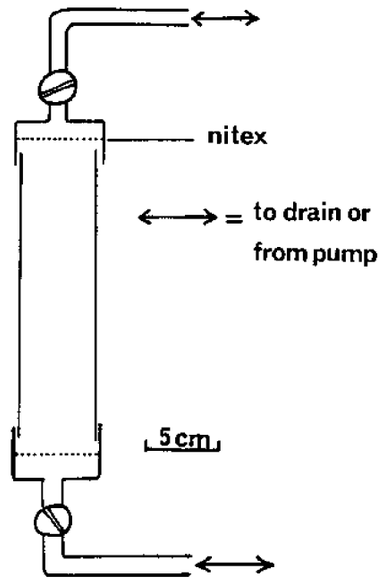


Figure 2. Reversible-flow chamber designed to test upwelling versus downwelling regimes for pedal-feeding pediveligers.

To test this hypothesis with reference to Manila clams we designed reversible-flow chambers in which we could vary feeding conditions, substrate conditions, and irrigation regimes (Figure 2). These were constructed of 2" PVC pipe with push-on caps at each end. Into the caps were set Nitex screens. [The "we" refers to the author, with the cooperation of Kelley Bartlett, Geoff Lindsay of Canadian Benthic, Bamfield Marine Station, and funding from B.C. Science Council.]

Initially we tested a variety of substrates such as fine sand, coarse sand, finely crushed shell, coarse shell, and combinations thereof, along with an epinephrine setting stimulus. These were used in upwelling regimes only. While the results of the initial experiments are not directly relevant to the central problem, it is worth noting that while epinephrine was an effective setting stimulus we doubt that the resulting metamorphosis of noncompetent pediveligers is of any long-term value. A mixture of sand (75 µm) and finely crushed shell (125 µm) was the most effective substrate for setting. However, in chambers that had no substrate and no epinephrine treatment, survival rates were almost as good as the best alternative combinations. Therefore, we simplified our subsequent tests by omitting substrate and epinephrine.

Our next experiment required replumbing so that different irrigation regimes could be tested in each reversible-flow chamber. A pulse of mixed algal cells in an available concentration of 1 million cells per milliliter was administered twice daily. The chambers were rinsed daily with clean sea water. The following regimes were tested over 24 days:

1. Eight days of continuous downwelling followed by continuous upwelling for 16 days.
2. Downwelling continuously for 24 days.
3. Four days of continuous downwelling followed by 20 days of downwelling with one hour of upwelling per day.
4. Eight days downwelling, then alternating 12-hour periods of upwelling and downwelling for 16 days.
5. Replicate of regime #3 with substrate added as an additional variable (1:1 75 µm sand and 125 µm fine shell).

The results suggest that a continuous downwelling regime is superior to a continuous upwelling regime, even for juveniles as large as 1 mm. The addition of one hour of upwelling per day (#3) seems to have improved the system of clearing debris, but a longer upwelling (#4: 12 hours per day) was more effective for this purpose. The latter produced survival rates >53% including setting. Initial indications are that the growth rates are best in a downwelling regime with substantial periodic upwelling: growth was x3 that in the traditional continuous upwelling regime. Most of our systems crashed at some time into the second phase of the experiment (after 12 days). This was probably due to the accumulation of debris including *Gonyaulax* and consequent bacterial growth.

Further research into juvenile Manila clams in the coming 1991 season will include fine-tuning the upwelling phase of the alternating flow system, and upscaling and simplifying the reversible-flow chamber for commercial use. We will also undertake experiments with feeding regimes, and study juvenile morphogenesis at the TEM and SEM (transmitting and scanning electron microscopy) levels.

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Recent Research on Nursery Practices for Geoducks and Mussels

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The previous speakers have presented several aspects of nurserying of bivalve juveniles of oysters and hardshell clams. I would like to briefly discuss nursery methods currently used for two other types of bivalves, geoducks and mussels.

At the Point Whitney Shellfish Hatchery near Brinnon, Washington, we work primarily with geoducks. We use a sand substrate nursery system which was designed in 1986 and 1987 by a former manager at this hatchery, Ron Zebal. Inspiration for this nursery type came in part from some nursery experiments with geoducks that Ted Kuiper conducted at his facility in California. Further experiments by Mr. Zebal at Point Whitney in 1986 led to the construction of our raceway nurseries.

Each of our nurseries consists of a tank in which a grid of perforated PVC pipe is laid on the bottom. This pipe is overlain by gravel, then a layer of landscaping fabric, and finally a layer of sand. Above the sand is some more plumbing which introduces flow into the surface water. We introduce newly metamorphosing geoducks onto the surface of the sand. Initially the only water flowing is a gentle upward flow from the bottom pipes through the gravel, fabric, and sand. As the animals get larger, we increase the flow from the bottom pipes until optimum flow—the point just before the sand starts to tumble. At one week we also begin a gentle surface flow. We gradually increase this flow as the geoducks get larger. Maximum surface flow for geoducks is just before the sand begins to form ripple marks.

Our sand substrate nursery provides an ideal environment for juvenile geoducks. Geoducks, as Dr. Reid pointed out, are pedal palp feeders for the first several weeks of their juvenile state. The sand substrate gives them ideal foraging surface area. We think that optimum loading in the nursery is about 5,000 9-mm animals per square foot. From initial operations at our nursery at Point Whitney we observed a maximum of 12 percent survival from metamorphosis to 9 mm. However, in 1990, in a pilot nursery in south Puget Sound, we observed 30 percent survival, with a resulting density of 15,000 animals per square foot. We will be testing this system further this season.

Mussels are a well-established global aquaculture species. Mussels grown in Penn Cove are served in restaurants throughout the Northwest. The species of mussel that grows nearly everywhere in the Puget Sound basin is *Mytilis trossulus*. With such an abundance of mussels attaching to everything, you might wonder about the need for a hatchery to produce them.

Unfortunately, this species, when grown in south Puget Sound, has a propensity to spawn in late May or early June and die en masse shortly after, or just before reaching market size. In some experiments during the mid-1980s mussel growers found that the species of mussel common to the southern California coast, *Mytilis galloprovincialis*, neither spawns nor dies prematurely in Puget Sound.

Whiskey Creek hatchery in Oregon and Taylor United hatchery in Washington have produced *M. galloprovincialis* seed in the past few years. The larvae metamorphose and set nicely on rope or twine, but one of the problems with mussel juveniles is transmigration off the setting substrate and onto tank walls or other surfaces. One proposal being explored currently is the use of setting collectors consisting of Japanese onion sacks filled with twine or rope. The purpose of the sack is to discourage transmigration. The Japanese use a similar technique to collect scallop seed.

Geoducks *(Panope abrupta)* in a Sand Substrate Nursery

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The geoduck industry in Washington state harvests about five million pounds a year from Puget Sound. To increase the quantity of future harvestable geoducks in low recruitment areas, the Washington Department of Fisheries (WDF) facility at Point Whitney now produces 10 mm geoduck seed for reseeding commercially harvested plots. Although hatchery techniques yield consistent numbers of healthy geoduck larvae, survival and quality of juveniles during the nursery state have been unpredictable.

Postlarval geoducks are mobile, and have been shown in recent histological studies to feed with their active feet on benthic diatoms, detritus, and bacteria from the sand surface for several weeks after the onset of metamorphosis. At the WDF nursery sites, newly metamorphosed geoducks are placed in sand substrate raceways that pump bay water as the only source of food. Given inconsistent survival and growth of the juveniles in the nursery system, experiments during 1989 and 1990 tested modifications of the nursery environment in nursery microcosms. We have tested sand grain size, planting density, site effects, and food sources for effects on postlarval geoduck survival and growth.

Experiments during 1990 survival and growth of plantigrade geoducks raised with wild algae were compared with survival and growth of geoducks grown in filtered sea water with added food. Temperature of the filtered water was adjusted daily to stay

within 1° C of the Point Whitney pond water. Dried *Tetraselmis suecica* was added either in a slurry to the substrate or suspended gradually in the water column, and compared with treatments of suspended cultured algae *(Chaetoceros muelleri)* and unfed controls. Preliminary results show significant differences in growth between treatments with food available to the substrate and those with food added only to the water column.

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Effects of Substrate Modification on the Growth, Survival, and Recruitment of Manila Clams *(Venerupis japonica* Deshayes)

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In recent years, statewide production of the Manila clam has increased so greatly that Washington is now the largest producer and exporter of Manila clams in the United States. Despite its rapid growth, the industry cannot meet market demand. Many of the optimal areas in Puget Sound are already under cultivation. Increasing the amount of habitat by substrate modification is one method of increasing clam production.

At Bywater Bay, Hood Canal, a site was constructed to test whether adding crushed oyster shell to a previously gravelled plot would increase the growth and natural recruitment of Manila clams. The purpose of adding crushed oyster shell was to neutralize the sediment pH, counter the effects of gravel compaction, and increase the surface area for settlement of clam larvae. Macroalgae were removed from the surface of eighteen 2 x 4 meter plots and three replicates of the following treatments were established: (1) control, no substrate alteration; (2) no substrate alteration with 5,000 seed clam added; (3) substrate rototilled to a 10 cm depth; (4) substrate rototilled to a 10 cm depth with 5,000 clam seed added; (5) a 5 cm layer of crushed oyster shell rototilled to a 10 cm depth; (6) a 5 cm layer of crushed oyster shell rototilled to a 10 cm depth with 5,000 clam seed added. Rototilling a 5 cm layer of crushed oyster shell to 10 cm gives a 50:50 ratio of shell to existing gravel.

Half of each plot was covered with 7 x 14 mm predator exclusion netting. At three-month intervals, five core samples on each half of the plot were randomly sampled to measure the rate of natural recruitment as well as growth and survival of the planted clam seed.

Initial results have indicated that survival is higher on the half of the plots covered with predator netting. Survival among the treatments is similar. The plot with crushed oyster shell had the fastest growth rate for planted Manila clams, while the control had the slowest.

Results from this study will be combined with other new clam culture techniques to revise Washington Sea Grant's popular but out-of-print *Guide to Manila Clam Aquaculture in Puget Sound.*

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Twenty-five years ago the establishment of the first oyster hatcheries on the West Coast represented a major breakthrough for the shellfish industry, for it meant that oyster growers would no longer have to depend on foreign and erratic wild sources for their seed.

The advent of remote setting and nursery culture of oysters and other bivalves has again propelled the industry a great step forward. Seed producers and growers no longer have to ship seed on heavy cultch to the growout site; now just the tiny larvae—millions of them wrapped in wet gauze and occupying a space no bigger than a baseball—can make the journey, saving shipping and maintenance costs. As a result, however, the setting and nursery activities that once took place at a centralized hatchery are now dispersed about the countryside and are conducted by individual growers rather than hatchery operators.

In February 1991, Washington Sea Grant organized a one-day workshop to bring together shellfish growers and scientists to discuss remote setting and nursery procedures prior to growout of the shellfish. The record of this workshop, it is hoped, will serve as a useful reference work for growers, researchers, and students, and help advance shellfish culture in the Pacific Northwest.

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