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High-Density Rearing of Oyster Larvae in Flow-Through Systems

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Since the oyster life cycle became known in the late 1800s, researchers have studied the artificial rearing of oyster larvae. Hatchery development in the U.S. began along the east coast, where consumer demand for the eastern oyster *(Crassostrea virginica)* and cyclical oyster fishery production stoked interest in commercial oyster culture. Hatcheries originally started as small operations producing a few million larvae for vertically integrated oyster companies seeking to provide consistent supply during fishery failures. In the 1980s, remote setting techniques (i.e., shipment of larvae wrapped in damp paper toweling to other locations for setting on cultch) were developed along the west coast. These techniques for the Pacific oyster *(Crassostrea gigas)* allowed a division of labor between the hatchery and the grower. Now there are hatcheries that specialize in larval rearing; their refined techniques produce large volumes of larvae.

Recent advances in grow-out techniques have substantially increased demand for hatchery-based seed production. Hatcheries have responded by increasing the scale of larval production, either by using larger volume larval rearing tanks (>8,000 gallons) at lower stocking densities or by increasing stocking densities using flow-through larval culture systems.

Comparison of static and flow-through larval culture

Static culture

In static systems, tanks are filled with filtered seawater, continuously aerated, and drained every other day for tank cleaning and larval assessment. Then they are refilled with filtered seawater to help maintain clean growing conditions within the tanks. Larvae are stocked at recommended densities of 1 to 15 larvae per ml (depending on size) and fed live microalgae, cultured at the hatchery, at recommended densities of 10,000 to 100,000 cells per ml of larval culture water once or twice per day. Typically, the stocking density decreases as larvae grow from D-stage (about 70 microns in size) to pediveliger stage (about 320 microns in size, i.e., diploid eastern oysters), while daily larval feeding increases in both algal cell count per ml and frequency, depending on hatchery management. Static culturing has both advantages and disadvantages.

Advantages

- Larvae cultured at lower densities
- Less seawater volume required
- Easier hatchery management

Disadvantages

- Greater tank size and numbers needed
- Lower production per square foot of space

Flow-through culture

Bivalve larvae are also cultured in flow-through seawater systems, where tanks are supplied with filtered seawater at a constant flow rate, continuously aerated, and drained and refilled every day to maintain clean growing conditions in the tanks. Larvae may be stocked at recommended densities of 50 to 200 larvae per ml and fed live microalgae at similar densities as in static culture, but there is a constant flow of algae. Flow-through culturing also has both advantages and disadvantages.

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Advantages

- Smaller tanks and numbers needed
- Continuous flow flushes wastes
- Continuous algal food supply
- Greater floor space efficiency

Disadvantages

- More risk of brood contamination by culturing larvae at high densities
- Greater water flow and filtration requirements
- Greater algal culture requirements
- Culture management can be more difficult

Flow-through system components

Flow-through larval rearing systems have the same fundamental components as static culture systems. Components include tanks, pumps, and plumbing, but also may include flow meters for seawater and algal delivery. Flow-through systems include screens to retain larvae that allow waste to be constantly flushed from the culture tanks but can also allow unconsumed algal food to escape. A seawater supply or head tank is commonly used to supply filtered seawater to the flow-through system and may be used for mixing algal rations with the seawater.

The following discussion describes a flow-through larval rearing system requiring 32 feet by 4 feet (9.75 m by 1.2 m) of hatchery floor space for eight 105-gallon (400-L) conical tanks, using stocking densities of up to 100 larvae per ml of culture water for the production of up to 320 million eyed oyster larvae per month. Other sites, facilities, and culture situations will likely result in different scenarios.

Tank size and shape

Because larval wastes are continuously flushed from the culture tanks, smaller tanks may be used for rearing larvae at higher densities. Although conical tanks are typically seen in many applications, large, flat-bottomed tanks may also be used. Conical tanks are usually 79 gallons (300 L) or 105 gallons (400 L) in volume and aerated from the bottom to keep larvae in continuous suspension (Fig. 1).

Screen construction and function

Small circular screens, referred to as "banjo" screens, of appropriate mesh size are submerged near the water surface of the larval culture tank and connected unglued to the seawater discharge plumbing built into the tank side wall (Fig. 2). The central placement of the screen allows rising aeration to help keep the screen clear of larvae.

The screen frame size can vary depending on the tank size and seawater exchange rate. For example, the frame size for a 105-gallon (400-L) conical tank can be built from 8-inch-diameter thin-walled (sewer) PVC pipe with Nitex® screen of appropriate mesh size mounted to the perimeter with epoxy resin (Fig. 3). Appropriate screen mesh sizes are 48, 60, 75, and 100 microns (for diploid eastern oysters); therefore, at least one banjo screen of each mesh size should be fabricated for each tank of the flow-through culture system. Constructing the banjo screens so they can be taken apart for easy cleaning and disinfection is useful; the screen frame halves are held together by a custom-made rubber band cut from 1/16-inch-thick, general purpose grade, butyl rubber sheet.

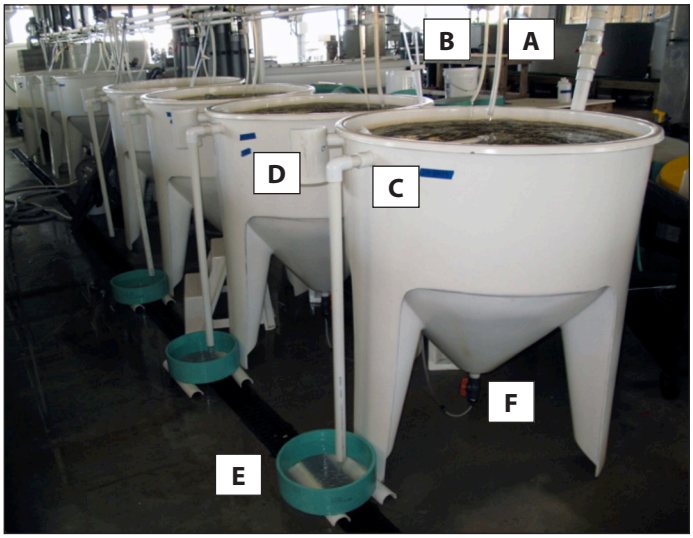

Figure 1. An eight (400-L) tank flow-through system. Each tank includes: A = incoming seawater tube; B = incoming algal tube; C = seawater discharge; D = 6-inch-diameter overflow trap; E = 12-inch-diameter floor trap; and F = incoming air and drain valve.

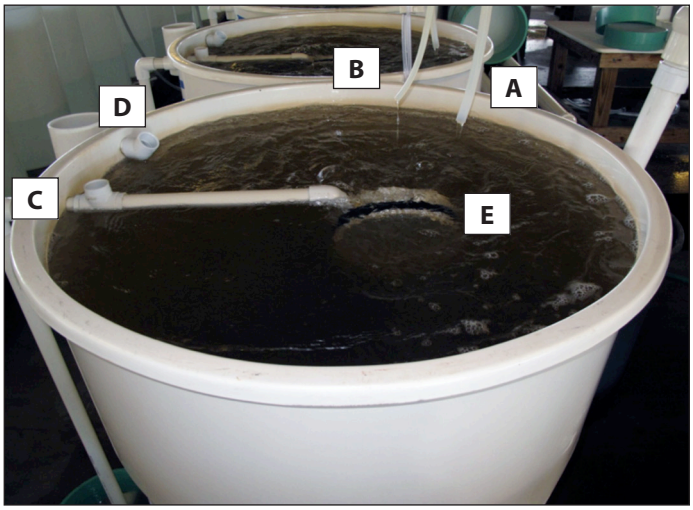

Figure 2. Top view of culture tank, including: A = incoming seawater tube; B = incoming algal tube; C = seawater discharge with overflow "T"; D = overflow trap; and E = submerged banjo screen.

Figure 3. Banjo screens made of 8-inch-diameter thin-walled (sewer) PVC pipe. Screens are made in two pieces (left) to take apart for easy cleaning and held together with a custom rubber band (right). The seawater discharge pipe is made of 1-inch-diameter PVC pipe mounted flush with the screen wall's interior using epoxy resin. A 45-degree PVC elbow is used to connect the screen to the tank's seawater discharge pipe.

To prevent larval loss, overflow screens or traps are important for capturing escaping larvae if the banjo screen clogs or fails. Figure 1 depicts two overflow traps on each tank, one plumbed beneath the lip of the tank and another raised above the floor receiving tank water discharge. These traps should be the same mesh size as the banjo screen or smaller. Floor traps and larval grading screens can be built from various diameter pipe, such as 12-inch-diameter thin-walled (sewer) PVC pipe. A trap screen plumbed through the tank side wall near the lip can be made from 6-inch-diameter thin-walled (sewer) PVC pipe to serve as a redundant overflow trap.

Pumps and plumbing

Air, seawater, and algae are delivered to the culture tanks via manifolds constructed from various diameter PVC pipe. Centrifugal pumps are commonly used and magnetic drive impellers offer advantages over others when cavitation may be an issue. A bypass valve plumbed before the manifold to return water drawn from a storage tank allows for simple water pressure and volume control. Pumps and plumbing are sized to supply adequate flow rates to achieve multiple tank water volume exchanges per day, depending on preference (Fig. 4). For example, the system depicted in Figure 1 uses a ¾ horsepower (hp), magnetic drive centrifugal pump to supply seawater from a 750-gallon (2,835-L) storage tank and a ⅓ horsepower regenerative blower for tank aeration, with a properly sized manifold bleed-off valve.

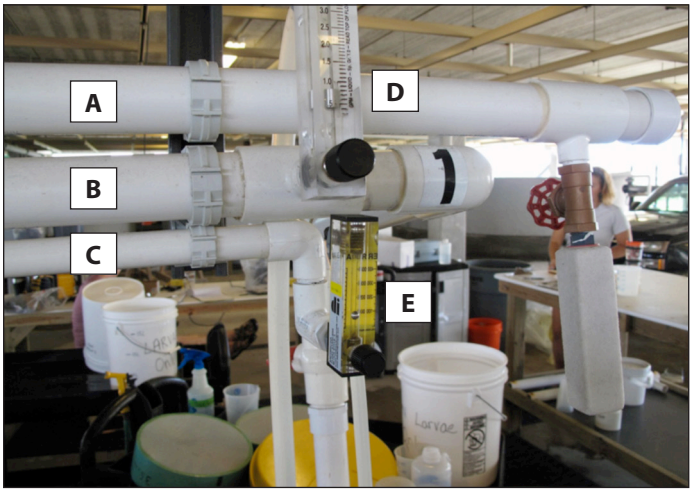

Figure 4. System manifolds and flow meters, including: A = air manifold (1.5-inch-diameter PVC pipe); B = seawater manifold (1.5-inch-diameter PVC pipe); C = algal manifold (¾-inch-diameter PVC pipe); D = seawater flow meter; and E = algal flow meter. Tubing is used to deliver seawater and algae from the flow meters to the tank surface.

System operation

Water management

Seawater filtration is important for all hatchery operations. Since banjo screens can be prone to clogging, it is important to have adequate filtration to keep large zooplankton that may inhabit ambient waters from entering or blooming in the hatchery. There are many methods for filtering incoming seawater, including settling tanks, sand filters, and/or cartridge filters (e.g., 1-micron nominal filtration).

Flow-through systems can use large volumes of filtered seawater. As an example, the system depicted in Figure 1 uses approximately 8,000 gallons (30,240 L) of seawater per day during normal operation. This can be a challenge in estuarine environments where freshwater introduced from nearby runoff, streams, or rivers can affect salinity. Large capacity seawater storage, therefore, can be very useful for water management. It provides time for additional filtration and allows for salinity adjustment and acclimation. Two 8,000-gallon (30,240-L) seawater storage tanks are used alternately for the system depicted in Figure 1, allowing daily cleaning and additional filtration before use.

Flow rate control

Flow-through systems can be managed by using adjustable flow meters for seawater and algal delivery to the larval rearing tanks (Fig. 4). These should be constructed of non-corrosive materials (e.g., acrylic, stainless steel, etc.) for saltwater applications. Flow meters should be sized with the appropriate scale for suitable application and performance. Tables 1 and 2 provide examples of required seawater and algal flow rates to the culture tanks.

Stocking density

Fertilized eggs should be stocked in a separate static incubation tank at 100 per ml of culture water until eggs hatch and have developed into D-stage larvae, usually after 48 hours of culture at water temperatures of 77 to 82 °F (25 to 27.8 °C). D-stage larvae should be maintained in

static cultures at appropriate stocking densities (e.g., 10 per ml) and fed until they are retained on an appropriate sized screen. For example, larvae retained on a 60-micron screen will be used to stock a tank with a smaller banjo screen (e.g., 48-micron mesh) for the flow-through larval system.

As with static systems, the grading of larvae by screening is important to keep larval size more uniform within each tank, eliminate slow growing larvae, rinse larvae of debris, and harvest larvae that are ready to set. Table 1 shows approximate stocking densities per tank following the grading of larvae every other day of culture.

Algal feeding rate

To successfully maintain high-density larval cultures in flow-through systems, an algal production system is required. Many hatcheries use continuous algal production systems (e.g., bag, tubular fence, or spiral) that can produce the required volumes of algae at appropriate cell counts. Table 2 suggests the algal requirements for a flow-through larval culture system based on a continuous algal bag system (Fig. 5) producing about 26 gallons (100 L) of algae per bag per day at 2 and 2.4 million cells per ml, using flow meters for seawater and algal delivery (Fig. 3). An algal cell count from the larval tanks should be made daily to ensure that the background algal level is maintained at approximately 40,000 cells per ml for younger larvae and 80,000 cells per ml for older larvae; the sample can be taken at the tank overflow opening (Fig. 2). Algal cell counts can be determined by using a hemocytometer, typically used to count blood cells in laboratories, to count a 1-ml sample of algae.

Figure 5. A typical installation of a 28-bag continuous algal bag production system.

Table 1. Examples of larval grading screen size, minimum larval length, tank volume, total larval stocking density per ml, total larvae per tank, water flow rate, and tank water exchanges per day for a flow-through larval production system with 105-gallon conical tanks.

Screen size (microns)	Minimum larval length (microns)	Tank volume (gallons)	Stocking density (no./ml)	Total larvae per tank (millions)	Water flow (gpm)	Tank water exchanges per day
48	70	105	200	80	0.8	11
100	145	105	130	52	1.2	16
180	260	105	90	36	1.4	19
225	320	105	75	30	1.5	21

Table 2. Examples of approximate algal requirements for larval tanks, including banjo screen size (mesh), water flow rate to tank, algal cells per ml of water to larval tanks, and algal flow per minute to larval tanks based on two cell densities. Note that this is only a guide to the algae flow. Actual needs of the larvae will vary with water temperature, health of the brood, etc. The number of background algal cells in the larvae tanks should be estimated daily so the number can be maintained at approximately 40,000 cells per ml for younger larvae and 80,000 cells per ml for older larvae.

Screen size (microns)	Water flow (gpm)	Algal density of water to larval tank (cells/ml)	Algal flow per minute per 105-gallon tank (ml)	
			2,000,000 cells/ml	2,400,000 cells/ml
48	0.8	40,000	60	50
100	1.2	80,000	182	150
180	1.4	100,000	266	220
225	1.5	120,000	342	285

System management

Larval growth, performance, and competence

Larval growth in flow-through systems can be comparable to that in static cultures. It can also be less. The seawater and algal flow rates shown in Tables 1 and 2 are a guide for operation of a similar system depicted in Figures 1 through 5. Larval brood performance (i.e., growth, narrow size range, and survival) is always the best parameter to use for system management. Daily microscopic examination of larvae is very important and another key to success. Larval size, color, movement, gut and velum condition, and vitality are important criteria to observe. For example, as eastern oyster larvae grow larger, they

should grow darker in color, appearing on the collection screens as pink to tan for D-stage larvae (60 microns in size), brown as mid-sized larvae (150 to 180 microns), and black as pediveligers (320 microns). Such coloration will be very apparent because the culture tank water appears darker as the larvae reach setting size. Once larvae are eyed, they should be graded daily to remove pediveligers that are ready to set. The harvest screen size is critical for removing pediveligers before they set on the tank walls. Because larvae growth and performance can be specific to each hatchery, the manager must decide on the best harvest screen mesh size for the site's growing conditions. For diploid eastern oyster larvae cultured in seawater temperatures of 77 to 82 °F (25 to 27.8 °C) and salinities of 15 to 25 parts per thousand, the pediveliger stage should be reached in 10 to 12 days and larvae harvested with a 210- to 225-micron mesh harvest screen, dependent on individual hatchery management. The challenge is to return undersized larvae to the culture tank for additional growth and have very little larval setting on the culture tank, banjo screen, and/or plumbing by the following day.

Maintenance

Cleanliness is key to successful hatchery production, and even more so for flow-through larval culture. The high larval stocking densities are prone to bacterial contamination, which can quickly lead to high larval mortality. It then becomes nearly impossible to grade dead from live larvae of equal size, since the smaller tank water volume and the bottom aeration do not allow dead larvae to sink to the tank bottom for removal during cleaning, as happens in static culture. This is the greatest concern in managing flow-through larval rearing systems: Dead larvae feed greater microbial contamination, so regular maintenance and sanitary practices are very important.

It is important that the seawater and algal delivery plumbing to the tanks be designed for easy cleaning. These pipes should be drained and flushed daily with fresh water and disinfected weekly by pumping a weak bleach solution (approximately 0.01%) through the seawater and algal delivery components, followed by seawater flushing, to keep the flow meters and pipe interiors clean. Flushing and disinfection can be accomplished while larvae are being cultured by temporarily coupling additional lengths of tubing to the seawater and algal delivery tubes at each tank for discharging onto the floor away from the larval cultures in the tank. In this way the manifolds and flow meters can be serviced without draining the tanks they supply.

Storage containers for cultured algae also should be designed and managed to reduce contamination and allow regular cleaning and disinfection. Small-volume, dual storage tanks are useful because they can be cleaned alternately. Each tank should be the appropriate size to supply algae to the flow-through system for a 24-hour period so that one tank can be in use while the other is cleaned and filled with fresh algae.

Smaller mesh (<60-micron) banjo screens are more prone to clogging, usually from algal mucilage delivered from the algal system. Banjo screen performance should be monitored regularly throughout the day and the screens removed and rinsed as often as needed, especially in the evening to avoid overnight clogging.

Recordkeeping

Recordkeeping is a standard practice for all bivalve hatcheries and is not unique to flow-through systems. Apart from daily maintenance (i.e., tank cleaning, seawater and algal line flushing), it is important to record algal production parameters and larval brood performance when managing a flow-through system. Daily algal records should include cell count per ml and production volume, in addition to the usual recordkeeping for algal production (i.e., salinity, temperature, pH, flow rate, etc.). Larval production records should include spawning date, egg stocking number, daily service date, seawater temperature and salinity, larval size and count, banjo and grading screen sizes used, seawater flow rate, algal flow rate, and background algal cell count.

Investment and operating costs

Flow-through systems are valuable in that they allow greater larval production in smaller tanks using less hatchery floor space. The investment and operating costs for these systems are similar to those for static culture and a full accounting will not be made here. Examples of approximate investment costs (estimated during 2014) unique to flow-through systems include: 105-gallon (400-L) conical tank ($800 each); flow meters ($50 to $80 each); banjo screens ($25 each without screen); 750-gallon seawater storage tank ($500); system pump ($135); blower ($800); and plumbing ($200). Plan about 2 days of labor for one person to fabricate the system, excluding screen construction. Operating costs unique to flow-through systems include additional daily labor to:

- drain, clean, and refill culture tanks;
- rinse and restock larvae;
- flush seawater and algal plumbing and flow meters;
- count algal cells; and
- check and clean banjo screens.

However, these costs are not additional to total hatchery costs if spent to replace a static culture system.

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Recommended reading

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USDA United States Department of Agriculture National Institute of Food and Agriculture

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