Seed Health Research & Technical Training in New Oyster Seed Rearing Practices

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Executive Summary

Remote setting of oyster larvae has been in practice for the genus *Crassostrea* for the past 40 years. Techniques for setting Pacific oysters (*Crassostrea gigas*) were pioneered by Lee Hansen, long-time owner and operator of the Whiskey Creek Shellfish Hatchery located on Netarts Bay, Oregon. Early research on methods for remote setting was provided by researchers at Oregon State University's Hatfield Marine Center working with Whiskey Creek in the early 1980's with fundamental information on optimal parameters for satisfactory production established by Henderson (1983).

For reasons not fully understood, commercial rearing of Pacific oysters is more problematic now than 20-30 years ago. Operators routinely face issues with larval mortalities at various points in their larval life history, poor survivorship to the setting stage and poor seed survival. Starting around 2007, oyster production in the Pacific Northwest has been periodically compromised for nearly all hatcheries. Changes in carbonate chemistry of seawater are recognized as a major issue impacting oyster rearing since the mid 2000's. While workarounds have been developed, several factors require additional investigation.

Refinements have been made to the fundamental setting approach that have served to optimize production. During the period of 2016-2019, Pacific Hybreed evaluated current seed rearing practices for the Pacific oyster, resulting in a full review of common methods and recommendations for standard industry best practices. Additional research into existing and new technologies, including broodstock conditioning protocols by Baywater Inc., demonstrate that changing and refining standard husbandry practices may help to alleviate the trend of declining survivorship in oyster seed.

A survey distributed by Pacific Shellfish Institute (PSI) in Washington, Oregon, California, Alaska and Hawaii in 2016-2019 characterized shared challenges faced by regional oyster growers, identifying the frequency, severity and source of hatchery-related mortality events. Environmental factors (water quality and marine pathogens) were identified as primary inhibitors to production. In response, PSI increased the number of real-time water quality monitoring devices in The Northwest Association of Networked Ocean Observing Systems (NANOOS) and deployed multimeter probes to on a rotational basis to hatcheries in North Puget Sound & Hood Canal over a three year time span. Continuous, real-time data enhances producers' understanding of water quality dynamics, allowing them to buffer and enhance seawater accordingly.

Seed mortalities are ubiquitous along the west coast, and are strongly associated with underfeeding, cold temperatures, smaller seed sizes, poor water quality, and pathogens. Investigations into hatchery mortality events over 2016-2019 identified *Vorticella spp.* ciliates and *Vibrio spp*. proliferation as a source of seed mortality, as well as bacterial contamination of algae mothers. Diagnostic exams performed by AquaTechnics over the same time period indicate common underfeeding of seed oysters, leading to death in nurseries and shortly after out planting. The high occurrence of mortality in adult Pacific oysters in Washington over the 2018-2019 summers led to demand for diagnostic testing of adults, in addition to larvae and seed oysters. Causes for adult mortalities were not frequently linked to a single source, assumed to be a combination of stressors including temperature and pathogen effects.

This report examines the environmental and logistical factors that impact seed production in hatcheries, remote set facilities, and nurseries across the west coast, and provides guidelines for standard best practices and methods to optimize production. Much remains to be learned as the production landscape for Pacific oysters appears to be changing with the environment in an era of rapid climate change.

New and Existing Setting systems: a review of methods, technologies, and standards

A number of different setting systems are in use today, including many varieties of downwell/upwellers, ranging from small hatchery-based systems to large FLoating UPwelling Systems (FLUPSY's). Below is a review of systems, and setting methods, commonly used on the west coast. This section also includes best practice recommendations and information on both novel and underutilized setting techniques.

Setting Oysters for Spat on Shell Production

Spat on shell production utilizes indoor or outdoor fiberglass supplied with filtered seawater (20 micron) and plentiful aeration. US west coast seawater tanks are commonly 3000 to 30,000 L. Seawater is pumped and heated to 20 - 25°C (and up to 28°C) and maintained in tanks for a minimum of 48 hours before draining and refilling. Static tanks must be supplied with an electric immersion heater to maintain temperatures Continuous flow through tanks are common and use banjo style screens to discharge seawater and prevent the loss of larvae. Flow through tanks eliminate the need for draining and refilling tanks at regular intervals and serve to maintain the desired temperature.

Figure 1. Pacific oyster spat on shell indicative of a heavy set. Density among oysters in a typical setting system for single oysters is far higher.

Tanks are supplied with settlement substrates, most commonly consisting of clean oyster shells in plastic mesh bags. There are typically about 300-350 oyster shells in each cultch bag. Bags are stacked into the settlement tank and allowed to condition for a day or two prior to introducing larvae. Larvae are added to the settlement tank in sufficient numbers to average between 10-30 spat per shell. The number of larvae added to the tank is dependent on the likelihood of a set and hatchery preference.

Calculating approximate stocking density: If a setting tank accommodates 100 shell bags with 300 pieces of shell per bag, the total substrate available is 30,000 shells. If the desired set is 10 spat per shell then 300,000 larvae must set on the available substrate. Settlement is highly variable after accounting for loss. If an estimated ~30% of the total larvae added set on shell, then 2 million larvae would be added to a tank. Return is highly variable and depends on the operator's experience. Larval survivorship to the spat stage may be low (<5%) but often exceeds 30% and is dependent on the quality of larvae.

Pediveliger larvae received via overnight shipment arrive cool (5-10 C) and must either be set immediately or stored in a "warm refrigerator maintained at 6-8°C. The following addresses how to **optimize settlement success** after receiving larvae:

- Allow refrigerated larvae to come to room temperature over at least 45-60 min
- Disperse room temperature larvae into adequate volume (1,000,000 larvae per 10 L volume) of 25°C seawater
- Once larvae become active, assess competence to settle (see page 7).
- Larvae will swim and/or settle onto available substrates over a 24-hour period
- Supply vigorous aeration to the tank to disperse the larvae
- Cover tanks to maintain dark to semi-shaded conditions
- Note loss from setting onto the sides and bottom of the setting tank compared to the numbers setting on the oyster shell. Settlers on tank sides and bottom will be lost from the cohort.
- Add live algae to the tank after 24-48 hours. (Many operators supply algal feed to the tank at the beginning of the process. However, this adds organic substrates to the tanks and uneaten algae may generate unwanted bacterial growth that may contribute to declining water quality.)
- Seed return is dictated by experience of the hatchery operator and the level of competence exhibited by the larvae
- Move cultch shells to beach once spat has reached a 2-4 mm size

Setting Larvae for Single Oyster Production

Typical systems for settling single oysters include trays suspended in troughs, flexible plastic sheets, down well systems, and bottles or cylinders. All of the systems are supplied with running seawater and require a supply of micro-cultch as a substrate. Ideally, shell fragments between 300-400 microns are retained for use in setting.

Setting in trays is the most straight forward approach to setting Pacific oysters. Trays are constructed of a wooden or fiberglass frame and covered with nylon mesh with a porosity of 180 microns. Trays are suspended in larger tanks or troughs that are supplied with running seawater at the appropriate temperature (20-25°C). Micro-cultch is spread as a thin layer on the bottom of each tray. Competent larvae are added to the trays and left for 2-4 days. Trays are typically covered to maintain the larvae in shaded conditions. Supplemental feeding may be added as described above for cultch based systems. Spat ideally attach to shell fragments after 24 hours. The spat are allowed to grow until they hold on a 500-micron screen (4-6 days). At that point, the spat should be rinsed using a gentle spray of filtered seawater through a 450- or 500-micron screen to remove colonized micro-cultch, unsettled/dead larvae, and additional debris. Feeding of microalgae should be initiated after 24 hours with cell counts maintained in the range of 100,000 – 150,000 cells per ml of volume. The diet should consist of a mix that includes *Isocrysis aff. galbana*, clone T-iso (*Tahitian Isocrysis*), *Pavlova lutheri* (NMFS Milford Shellfish Laboratory clone 459), *Chaetoceros calcitrans, C. gracilis, Rhodomonas salina* and other candidate species.

Stocking density for screen-based systems are based on the area devoted to micro-cultch. A simple rule of thumb is to stock larvae at no more than 100 larvae per square cm of screen area. A screen having an area of 5000 square cm could therefore be stocked with 50,000 larvae. Higher or lower stocking densities are used, though overstocking decreases success.

Setting in downwellers is a common practice. Companies surveyed by PSI indicated that upwell/downwell systems are the most common seed rearing system (utilized by 95% of survey participants). Typical systems are characterized by 4" to 30" diameter silos suspended in a trough or box supplied with filtered seawater. Most utilize three screen upwell/down well boxes. Typically, the box system has an option for upwell mode (seawater passes up through the bottom of the screen and out of the silo), or downwell mode (seawater passes through the silo exiting the bottom of the screen). Downwell mode is usually used for the initial setting phase for Pacific oysters.

The transition from downwelling to upwelling mode is dictated by the size of the oyster seed (1600 microns). 180 micron mesh is common for downwell silos. Once oysters are set and initiate shell growth, silo mesh is usually increased, first to 400-500 microns, then up to 1000+ microns to accommodate larger seed. Increasing the mesh size reduces daily maintenance requirements.

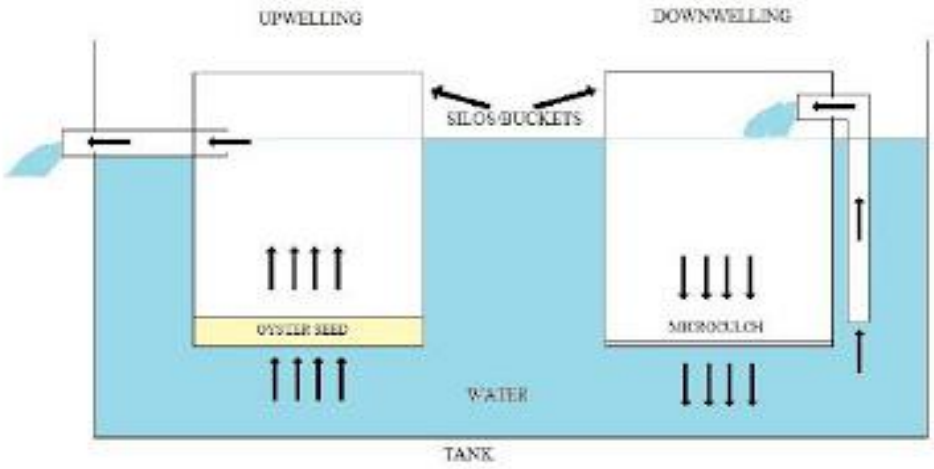

Figure 2. Diagram of simple upwelling and downwelling technology. There is a large variety of systems in use today that vary in size and capacity. All use the same principles of operation.

The stocking density of larvae for down well based systems should be about 100 pediveligers per cm^2. Each screen has a screen area of about 2000 cm^2 and should be stocked with no more than 200,000 larvae per screen, or 600,000 larvae per box. A typical return to the spat stage should be between 10-40%. Thus, a down well/upwell box system should typically return between 60,000 to 240,000 seed under satisfactory conditions. Typical success rates are rarely in excess of 40% and are often <10%.

The process for handling new oyster spat is similar to that described above for tray setting. Oysters are rinsed with saltwater and screened to remove unused micro-cultch and debris and fed cultured algae until the juveniles reach a size suitable for out planting into nursery systems (typically a size that holds on a 2000-micron screen). Rinsing of screens should occur daily, initially using seawater as source for spray downs and cleaning. Avoid freshwater rinsing until the seed are week old. At that time, a daily freshwater rinse is preferred if there is any evidence of post set mortality.

Feeding rates should adhere to similar levels recommended above for screen-based systems. In silo-based systems, the feeding rate of spat may be estimated by estimating the depletion rate for algal cells as they pass through the system. Settlement of algal cells is unavoidable. An appropriate feed rate should result in a 20% particle density reduction on exit from the system.

Setting on flexible plastic sheets is not regularly used in the Pacific Northwest. However, their use is widespread in European and Asian Pacific oyster culture practices. Flexible plastic or stretched polyethylene terephlalate sheets are suspended vertically in setting tanks and larvae added to the system as described above. In this system, oysters are allowed to settle and metamorphose onto the plastic sheets and to grow for 1-3 weeks, depending on productivity and growth rate. At that time, the sheets are flexed to enable the oyster spat to pop away from the sheet as single oysters. Oysters are then reared as single oysters in trays or upwellers as described above.

Use of epinephrine to produce single oysters is not a common practice (0% of surveyed producers cited using this method to enhance settlement or induce metamorphosis). However, single oyster production using the hormone/neurotransmitter epinephrine may be used to produce single Pacific oysters without micro-cutch. Small concentrations of epinephrine (~10^{-5} molar) for brief periods (>10 minutes) will induce the larva to bypass the cementation phase and directly initiate metamorphosis.

- Epinephrine solution can be made using epinephrine bitartrate salt: dissolve 2g of the salt into a 10ml volume of de-ionized water. Add 1 ml of that stock solution to 1000ml filtered seawater to create a 10^{-4} molar seawater solution.
- Introduce competent larvae into of epinephrine solution of 10^{-4} – 10^{-5} molar (Coon et al. 1985; Coon and Bonar 1987)
- Regularly plunge larvae over a 20-130-minute period. Best results occur in a darkened room.
- Rinse oysters and suspend in seawater following treatment.
- Any remaining swimming larvae may be returned to the larval rearing tank to be re-treated a 1-2 days later with a freshly made epinephrine seawater mix.
- The epinephrine seawater mix must be disposed responsibly.
- Oysters remaining on the bottom of the vessel (no longer swimming) may now be reared as spat utilizing a screen-based nursery system as described above.

Design and Construction of a Combined Setting and Primary Nursery System

Pacific Hybreed initiated design, construction and testing of a combined setting and primary nursery cylinder for setting and rearing Pacific oysters in 2018-2019. Several characteristics of a setting system were considered of primary importance during the design phase:

- Accommodate setting of pediveliger larvae through metamorphosis and a high percentage throughput to the juvenile stage (>2000 microns).
- Enable both setting and post set nursery operations in an upwell mode only in order to avoid issues associated with downwelling seawater.

- Inexpensive and simple to construct, easy to clean and maintain.
- Integrate well in either indoor or outdoor settings and use pumps powered by regular 110V power.
- Produce a commercial quantity of oyster seed as a solitary unit and/or produce commercial quantities in multiple units (>1,000,000 seed per cohort in multiple units).

Figure 3. Multiple combined setting/primary nursery cylinders attached to wall in Pacific Hybreed laboratory facility.

For a detailed account of the primary nursery construction and performance, visit www.pacshell.org/seed-health.asp and navigate to the Project Final Report. A full description of Pacific Hybreed's combined setting a primary nursery cylinder begins on page 22.

Assessing Larval Competency

Larvae must be determined competent to settle and metamorphose for use in all systems. Competent larvae share these features and timeline to develop to the juvenile stage (at a seawater temperature of 20-25°C):

- Shell length of 275 microns (holding on a 245-micron screen).
- Stomach of the larvae is brown in color.
- Small drops of oil (lipid) are dispersed in the tissues of the larvae and visible through the semi-transparent valve.
- The eye spot diameter is10-14 microns.
- Larvae "swarm" in the water column. Characterized by long larval trails (mucus threads secreted by the larvae).
- Larvae swim gregariously in agglutinated spiral columns just prior to settlement.
- Larvae swim to the top of the column near the water's surface and then cease swimming and fall back to the bottom before rejoining the spiral column.

- In contact with a substrate, competent larva will crawl and/or rock from side to side (often observed on a glass microscope slide) with the foot extended.
- Larvae just prior to settlement turns over onto left side and attaches to the substrate (utilizing a byssal cement).
- Once cemented to a substrate the pediveliger ceases to feed and initiates metamorphosis to the juvenile stage (24-48 hours).

Further Considerations:
Natural larval recruitment differs from artificial systems for setting and growing oysters:

1) The density of setting oysters in nature is likely far less than the100 larvae per cm^2 industry standard. Reducing the setting density for commercial operations is possible but certainly not a preferred option because hatchery and nursery space is usually limited.
2) The physical location for new spat observed in both nature (and setting tanks for spat on shell production) is usually higher in the water column, away from the benthic boundary and microhabitats characterized by a significant flow of seawater.

Possibly, the position of oyster spat, and any behaviors exhibited by pediveligers within the confines of a setting tank can help inform better design criteria for high density setting systems.

Alleviating Environmental Stressors Though Best Practices

Pacific coast shellfish farmers cite oyster seed limitation as one of the most serious constraints to industry growth. Pacific Shellfish Institute and AquaTechnics examined factors impacting seed production in U.S. west coast hatcheries, remote set facilities and nurseries, and assisted growers with analytical and diagnostic support. A summary of the issues facing growers and best practices to mitigate these challenges follows.

Seed Mortality Background
Mortalities in Pacific hatchery and remote set facilities were reported by 14/19 (78%) of survey participants, with 50% experiencing mortalities in colder weather (April and earlier) and 78% experiencing mortalities in smaller seed classes (<1 mm). The most common cause of mortality identified by 25% of producers, was environmental parameters, including low salinity, low temperatures, low pH and high turbidity. Water chemistry is dependent on tidal, diel, and seasonal patterns, and is strongly influenced by the weather. Correctly timing the draw of water into facilities will ensure juvenile oysters have enough oxygen, dissolved calcium, thermal energy, etc. When water quality conditions are poor, hatcheries adapt by heating water or providing buffering solutions to ensure the proper carbonate chemistry. Producers cited biological pathogens (specifically viruses, bacteria, and amoeba) as a secondary cause of mortality. Materials and pathogens can interfere with the setting process (most noticeably by preventing setting and/or causing mortality in the early life stage (<1mm; 8-10 days). The quality and quantity of food was observed by 37% of facilities to cause failures. (Note: histological examination indicates underfeeding as the primary causative agent in juvenile mortality). 20% of surveyed producers linked mortalities to ciliate contamination.

Histological examination of oyster seed performed by Dr. Ralph Elston shows the four most common verifiable causes of lost production:

1. ***Inadequate feeding:*** the most commonly found cause of juvenile (seed) losses or poor performance in bivalve nurseries participating in this project. The consequences of inadequate feeding are slower growth and development and higher risk of predation and disease effects, resulting in lower survival when outplanted.
2. ***Feed contamination with free-living ciliates or shellfish pathogenic bacteria:*** a common source of infection in hatchery system. High nutrient loading into algal systems can result in bacterial contamination.
3. ***Invasive Vorticella ciliates in bivalve nurseries:*** a severe problem in nurseries, particularly during the spring season. Invasive ciliates are an intermittent problem in Pacific oyster seed culture. These are distinct from typical free-living ciliates commonly observed in cultures. Differentiation and identification of the true invasive ciliate usually requires assistance provided to hatchery and nursery operators. The pathogenic ciliates are truly invasive into the body cavity of shellfish seed. The invasive ciliates are slow moving and found in the mantle and body cavity of shellfish seed. Puget Sound has invasive pathogenic ciliate specific to early post-set life stage (< 8mm).
4. ***Vibriosis in bivalve nurseries:*** Bacterial infections, caused by shellfish pathogenic vibrios, typically *Vibrio corrallyticus* (=*V. tubiashi*) were found occasionally during the 2016-2019 study but were far less common that in previous years. *Vibrio tubiashii* is an issue depending on location (can be on coast and elsewhere) and grow rapidly.

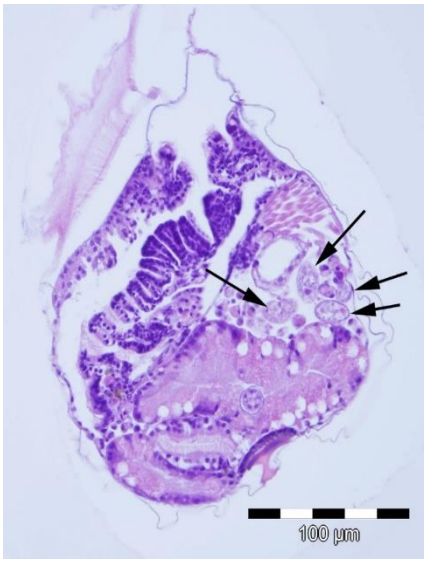

Figure 4. Invasive ciliates (black arrows) within the body cavity of a Pacific oyster seed. Histology cross section, H&E stain.

Notably, hatcheries report poor survivorship during blooms of *Cocolithophore*s and *Akashiwo sanguinea*. The evidential relationship between blooms of certain species of native phytoplankton and mortality in juvenile Pacific oysters warrants future research.

Water Quality Monitoring and Standards

The Northwest Association of Networked Ocean Observing Systems (NANOOS) online network of monitoring equipment (http://nvs.nanoos.org/ShellfishGrowers) can be accessed by seed producers and farmers to view ocean chemistry near their facilities. PSI staff served seed producers who had additional water quality monitoring needs via two deployable multimeter probes measuring depth, temperature, Chl a, pH, DO, and salinity on a monthly rotational basis at hatcheries and nurseries in Washington State. Water chemistry samples were also collected in association with the deployments to gather pH and other carbon chemistry values. The data

proved valuable to each producer by enhancing understanding of local water quality dynamics and enabling hatchery staff to buffer and enhance sea water.

The following water quality parameters are considered standard best practice:

- ***pH***: standard industry practice is to set oysters at a pH >8 (8.1-8.2 common). The desired pH can be reached by adding sodium carbonate to buffer acidic water.
- ***Temperatur*e**: the range of setting temperatures reported by survey participants ranged from 20-28. In remote set facilities, warmer water allows larvae to swim longer and eat more, and higher temperatures notably increase survivorship. However, a temperature range of 20-25°C is sufficient for seed rearing systems.
- ***Salinity***: setting at salinities ≥27 ppt was reported as preferable. Salinities below the desired threshold can be supplemented with Instant Ocean®.

Suppressing Pathogens

The most critical time to suppress bacteria is the larval to 1 mm stage. Cell culture incubators can allow companies to monitor bacterial levels on-site. Feeding larvae to rapidly surpass 8 mm threshold alleviates ciliate infection. **Probiotics** can be added to algae culture to suppress pathogens and can be useful in the context of setting.

Probiotic PO2-45, developed independently of this project, was provided to selected shellfish production companies. The probiotic was reported to be highly effective in small hatchery/nurseries systems and reduced mortality losses, while improving the efficiency of algal food production. Project participants reported similar success with use of the probiotic.

Inquiries regarding Probiotic PO2-45 should be directed to Dr. Ralph Elston at ralph@aquatechnics.com.

Proper **sanitation** can reduce bacterial loading and proliferation in systems. The following sanitation lessons and suggestions were taken primarily from the WSG 2017 remote set workshop discussion led by Dr. Ralph Elston, with additional information supplied by survey participants:

- Regularly clean and neutralize equipment. Mechanical sanitation in conjunction with UV filtration is effective in reducing pathogens.
- Use UV filtration to keep bacterial and ciliate counts low. UV filtration alone will not ensure absence of pathogens from the system.
- Balance chemicals in tanks.
- Set with filtered water. (Ultra-sanitation of water doesn’t always eliminate failures!)
- Condition cultch with either freshwater or saltwater post-set rinses. (Rinsing with seawater can last up to a week.)
- Hold and cycle water depending on the season. Summer months require more frequent water exchange.

- Maintain high flow rates and increased exchange rates through up/downwell systems. Low flows foul screens with excess algae and debris and increases the opportunity for pathogenic bacteria to colonize.
- Nicotine is toxic to larvae. Consider personal habits and cleanliness when handling larvae.
- Keeping records and maintaining consistency is a critical aspect of good sanitation.

Flow rate through upwell/downwell systems is often underestimated by hatchery operators in an effort to conserve heated seawater and/or microalgae supplied to the system. If flow over and through the screens is set too low, the screens are quickly fouled with algae and debris, increasing the opportunity for pathogenic bacteria to colonize. Partial recirculation mode will assist the conservation of both heat and food and can partially alleviate issues associated with the rate of delivery of microalgae to the oysters as well as the rate of waste removal.

A study was conducted by Pacific Hybreed to address the relationship between flow and survivorship. A flow rate of 5.49 cm^3 (the volume of seawater passing through the screen per minute per cm^2 of screen area) yielded 30% survivorship in a downwell system over 7 days. In contrast, a flow rate of 2.38 cm^3 yielded no survivors over the same time period. Both systems were cleaned daily. This work pointed to the importance of seawater throughput to accommodate what may be an intuitive need for satisfactory feed delivery and waste removal.

The issues facing a pediveliger larvae when placed onto nitex nylon screens at a density of 100 larvae per cm^2, suggests rethinking and improving current practices. Downwelling by definition subjects the larvae to an avalanche of feed and debris and is potentially an entry point for contamination by pathogenic bacteria, unless the cultures are very well maintained. Within the setting of commercial hatchery operations this is seldom the case, and alternatives should be considered.

Feeding to Optimize Health

Feeding the right quantity and quality of food is essential to survival. The general rule for feeding is 100,000 – 150,000 cells per ml of volume. Lipid content in Pacific oysters is the best metric for determining the health of larvae, and is highly correlated with survivorship. Polyunsaturated fatty acids (PUFA's) are important to the health and growth in *C. gigas* larvae. Oysters in particular need DHA and EPA as an essential part of their diet for optimal larval growth.

Algal species typically utilized in hatchery operations in the PNW include:

- *Isocrysis aff. galbana,* (clone *T-iso; Tahitian Isocrysis*)
- *Pavlova lutheri* (NMFS Milford Shellfish Laboratory clone 459)
- *Chaetoceros calcitrans*
- *C. gracilis*
- *Rhodomonas salina*

Microalgae for all these species are cultured following standard algal rearing methods and harvested during exponential growth phase. To learn more about nutritional algal species, see the following important publications: Brown et al. 1989, 1997; Tzovenis et al. 2003; Ponis et al. 2006a, 2006b.

Optimal feeding strategies for culturing Pacific oysters include a diet rich in lipids, including n-3 HUFAs (C22:6n3 and C20:5n3). T-iso and *P. lutheri* have higher levels of these than *C. calcitrans* under normal phytoculture conditions (i.e. harvesting at exponential growth phase) (Soudant et al. 1996, Brown et al. 1997).

The majority of algal culture facilities associated with bivalve hatcheries culture microalgae under full light 24/7. However, a 12:12 or 16:8 light: dark cycle could maximize DHA and EPA content (Fidalgo 1998). PUFAs may be enhanced by utilizing urea as a source for nitrogen (as opposed to nitrate supplied typically in phytoplankton nutrient mixes) and harvesting cells at early stationary phase (as opposed to exponential phase). If culture conditions for microalgae are better managed for PUFA production, including the revision of light dark protocols, this may enhance preferred long chain fatty acids in algal production.

Pacific Hybreed evaluated the potential of Reed Mariculture Shellfish Diet (SD1800) to produce Pacific oyster larvae. Reed SD1800 was selected for consideration because the manufacturer focuses on enhanced PUFA production. Prospects for using the Reed Mariculture SD1800 for rearing larval Pacific oysters are not promising based this preliminary study. The issue appears associated with either bacterial loading associated with the formulated diet or perhaps inhibitions associated with the preservative used to stabilize the feed.

Figure 5. Turner Designs hand-held fluorometer (left) and Luna II Cell Counting Slide (right).

A hand-held fluorometer (*Turner Designs 2850-000-F Handheld Little Dipper Fluorometer*) enables a simple measure of chlorophyll content in seawater. In this case, the sensor of the fluorometer is simply dipped into a tank of seawater containing microalgae and a measure of chlorophyll content attained. This could be used as a possible tool by the industry to better evaluate larval and seed nutritional conditions. Preliminary results indicate the Turner hand held fluorometer could be used as a proxy for cell counts made on the Luna II Cell Counting Slide. (A full explanation of this approach can be found in the Project Final Report at www.pacshell.org/seed-health.asp).

Broodstock Conditioning: optimizing physiology

Successful cultivation of Pacific oysters does not begin with receipt of generic pediveliger larvae to the hatchery or remote set facility. Attention to broodstock nutrition produces gametes and larvae with optimal reserves, and attention paid to larval nutrition produces larvae competent to settle and metamorphose to the juvenile stage. The following section discusses best practices for broodstock rearing and suggests techniques that may improve the quality of larvae generated during year-round production.

Nutrition and Feeding

A strong relationship exists between the health of marine larvae and the condition of the broodstock used for production. Successful production of larvae and seed depends upon adequate nutrition of broodstock oysters when production is attempted outside of the natural cycle for this species. Hatchery operators do not routinely screen oyster broodstock gametes for lipid content, nor are oyster broodstocks managed to maximize this critical biochemical component.

Oysters are typically fed a mix of hatchery cultured algae. Cultured species used for conditioning Pacific oysters typically include the following:

- *Prymnesiophyte*s (*Isocrysis* aff. *Galbana)*
- Clone *T-iso (Tahitian Isocrysis*)
- *Pavlova lutheri* (NMFS Milford Shellfish Laboratory clone 459)
- *Chaetoceros calcitrans*
- *C. gracile*
- *Tetraselmis suecica*
- *Rhodomonas salina*

To avoid underfeeding, feed oysters a mix of live algae totaling at least $2x10^9$ algal cells per oyster per day. For a tank of 100 oysters fed a typical algal culture of live algae having a cell concentration of 2.5x106 cells per ml, this equates to a feed ration of at least 800 ml per oyster per day. For 100 oysters in the tank, this equates to a minimum of 80 L per day supplied to the oysters as a maintenance ration. (If oyster broodstocks are maintained on flow through conditions, a component of the algal feed is wasted and should be accommodated by providing excess feed to the tank.)

In *C. gigas*, supplemental feeding results in increased fecundity in females. Observations for oysters fed a smaller percentage of body weight per day (often the case for oysters conditioned out of season in Pacific Northwest shellfish hatcheries) suggest that body tissues devoted to reproductive tissues are significantly less than that observed during the peak of natural reproduction.

Standard Conditioning and Recommendations

Broodstock is introduced into the hatchery for conditioning in late Fall and early winter, depending on the schedule for first spawns of the season. Oysters are generally placed into

broodstock tanks supplied with running seawater or seawater that is changed out daily, at the ambient temperature they were experiencing in the field. The temperature is ramped one degree per day until the conditioning temperature is reached (usually between 18-22°C.). Oysters are maintained at the prescribed temperature for the number of days required to produce ripe (viable) gametes based on a prescribed feeding regime and degree days.

The Muranaka and Lannan (1984) degree day calculation approach calculates conditioning time from a baseline temperature of 0°C. However, a baseline of Pacific Northwest temperatures (6-8°C) may be more appropriate. Modifying the baseline for the degree day calculation can result in hatcheries expending significantly less resources during the oyster conditioning phase. (The industry generally uses 1100 degree days to produce viable gametes for Pacific oysters.) Commonly, the industry will over ripen broodstock based on degree day assumptions, wasting resources and miss-timing optimal gamete development.

To see calculations comparing conditioning period for Muranaka and Lannan (1984) verses 8°C threshold visit www.pacshell.org/seed-health.asp and navigate to the Seed Health Project Final Report. A detailed explanation of calculating conditioning period begins on page 38.

A 6-8°C baseline allows better control over the rate of gamete development. Larval survival and vitality depend upon the optimal utilization of Pacific oysters. Many hatcheries use gametes for as long as they are present in oyster broodstocks. Ideally, hatcheries should use gametes undergoing the latter stages of oocyte growth (e.g. vitellogenesis). Hatchery operators need to recognize this distinction and potentially incorporate into their oyster conditioning protocols.

Cannuel and Beninger (2005) identify four predictors of optimal egg quality in *C. gigas* that result in healthier larvae. These include:

- Stage of gametogenesis
- Diameter or volume of the average oyster egg in the gonad
- Lipid content of the egg
- Presence or absence of gonadal atresia (reabsorbsion of eggs)

Gamete production in Pacific oysters may be significantly influenced by both increasing day length in concert with increasing temperature (Fabioux et al. 2005). The role of **photoperiod** has been largely ignored by the oyster industry. A strategy might be to expose oysters that have come in from the field during winter months immediately to a 12:12 light dark cycle while increasing the ambient seawater temperature and increasing the level of feeding. Photoperiod should then be increased 30 minutes per day so that after 10 days the photoperiod experienced by oysters emulates that observed in late spring in the Pacific Northwest (17:7 light dark cycle). This approach warrants further attention by the oyster industry.

A Revised Conditioning Protocol Based on Initial Thermal Shock

A 2017 study by Baywater, Inc. modified the Pacific oyster broodstock conditioning protocol. The study assumed that wide swings in ambient temperature and stressful conditions can also

serve to trigger rapid gametogenesis and the production of gametes. The goal was to produce gametes over a short period of time to conserve hatchery resources.

Preliminary study results show significant gamete development in temperature shocked oysters after only 14 days (compared to >35 days for the traditional conditioning method). However, a significant increase in mortality is observed in temperature shocked oysters. If oyster broodstocks are plentiful then the increased mortality using this conditioning approach may warrant its use. Also according to this study, reliance on degree day determinations may not be a reliable approach for conditioning. Pacific oysters, as oysters were ripe enough to provide fertilizable gametes after just 220 degree days exposure.

For a detailed discussion of the methods and results of the 2017 Baywater Inc. study, visit www.pacshell.org/seed-health.asp and navigate to the Seed Health Project Final Report. A full description of the conditioning protocol based on an initial thermal shock begins on page 29.

Adult Mortality: findings and resources

There was an overwhelming response by industry managers to utilize seed health funding to obtain health diagnostic data during summer adult mortalities in 2018-2019. On the U.S. west coast mortalities typically begin to occur in diploid and triploid oysters during the second year of growout, often when the oysters are nearing market size. Large-scale mortality events affected growers in all of Washington's major water bodies during the summers of 2018-2019.

Summer mortality. Mortality of Pacific oysters during summer months does not appear to be caused by any specific disease agent, with a few exceptions. Oyster herpes virus (OsHV-1) was ruled out as a cause of summer mortality in all 15 samples examined. In Pacific oysters on the west coast, nocardiosis, a bacterial infection, is responsible for occasional morbidity and mortality of this oyster during summer months but is usually absent in oysters examined for summer mortality. Thus, this bacterial infection is considered a minor cause of the summer mortality syndrome.

The cause may be related to abnormal gamete production but a detailed understanding of the cause of summer losses in Pacific oysters is lacking. A more detailed determination of the cause(s) of Pacific oyster summer mortality is a top priority for further research. "Summer mortality" remains largely unresolved.

Triploid oyster mortality. Triploid Pacific oysters were found to be more likely affected in mortality and morbidity episodes than diploid Pacific oysters. Triploid sensitivity has been previously observed (Cheney et al 2000) but the cause is unknown and is a high priority for further research and definition.

In 2018, PSI, in partnership with Wasington Sea Grant WSG and Washington Department of Fish and Wildlife (WDFW) facilitated expansion of the Sound Toxins monitoring network in Willapa Bay to include two underserved areas essential to Pacific oyster production (Bay Center and Nahcotta). This expansion was conducted in response to industry interest. Sound Toxins

traditionally focused on characterizing the three main harmful algal bloom (HAB) species known to impact human health. However, in light of the recent severe mortality events, the focus has expanded to include two plankton species suspected to cause illness and morbidity in shellfish, specifically *Akashiwo sanguinea* and *Protoceratium reticulatum.*

A **Mortality reporting** form was developed for growers to record seed and adult mortalities. To report a mortality, visit the PSI website: www.pacshell.org and follow the "Report Your Mortality" link on the homepage to a fillable PDF form. Completed forms can be emailed to PSI@pacshell.org. All sensitive information will be kept confidential.

To pursue diagnostic testing following a farm or hatchery mortality event, contact Dr. Ralph Elston at AquaTechnics. Dr. Elston's contact information can be found on page 10 of this report.

References

Brown, M., S. Jeffrey, J. Volkman and Dunstan, G. 1997. Nutritional properties of microalgae for mariculture. Aquaculture 15: 315-331.

Brown, M., S. Jeffrey and Garland, C. 1989. Nutritional aspects of microalgae used in mariculture: a literature review. CSIRO Marine Laboratories Report, 205:1-44.

Cannuel, R. and Beninger, P. 2005. Is broodstock feeding always necessary? A study using oocyte quality predictors and validators in Crassostrea gigas. Aquatic Living Resources 18: 35-43.

Cheney, D.P., Macdonald, B.F. and Elston, R.A. 2000. Summer mortality of Pacific oysters, *Crassostrea gigas* (Thunberg): Initial findings on multiple environmental stressors in Puget Sound, Washington, 1998. Journal of Shellfish Research 19(1): 353-359.

Fidalgo, J., A. Cid, E. Torres, A. Sukenik and Herrero, C. 1998. Effects of nitrogen source and growth phase on proximate biochemical composition, lipid classes and fatty acid profile of the marine microalga Isochrisis galbana, Aquaculture 166: 105-116.

Henderson, B.A. 1983. Handling and remote setting techniques for Pacific oyster larvae *Crassostrea gigas*. MS Thesis, Oregon State University. 45pp.

Ponis, E., G. Parisi, J. Le Coz, R. Robert, G. Zitteli and Tredici, M. 2006a. Effect of the culture system and culture technique on biochemical characteristics of Pavlova lutheri and its nutrition for Crassostrea gigas larvae. Aquaculture Nutrition 12; 322-329.

Ponis, E., I. Probert, B. Veron, M. Mathiey and Robert, R. 2006b. New microalgae for the Pacific oyster Crassostrea gigas larvae. Aquaculture 253: 618-627.

Muranaka, M.S and Lannan, J.E. 1984. Broodstock management of Crassostrea gigas: environmental influences on broodstock conditioning. Aquaculture 39: 217-228.

Soudant, P., M. Yanic, J. Moel, R. Robert, C. Quere, J.R. Le Coz and Samain, J.F. 1996. Effect of food fatty acid and sterol quality on Pecten maximus gonad composition and reproduction process. Aquaculture 143; 361-378.

Tzovebis, I., N. Depauw and Sorgeloos, P. 2003. Optimization of T-ISO biomass production rich in essential fatty acids. Aquaculture 216: 203-222.