Prevention and Management of Infectious Diseases in Bivalve Hatcheries

Revised and updated by Dr. Susan Laramore in March 2015
from Dr. Ralph Elston, Florida Clam Industry Workshop, Cedar Key, FL, September 2008

Diseases in the hatchery are caused by *opportunistic* pathogens or by *host-specific* pathogens. These two kinds of diseases require different management approaches. Disease causing agents, which are of regulatory consequence and considered to be of major risk for geographic movement, are referred to as "reportable" or "certifiable" diseases.

The diseases found in bivalve hatcheries are caused by bacteria that live in the marine environment that opportunistically take advantage of the high densities of larvae in the hatchery. Therefore, opportunistic diseases, such as *vibriosis*, are called *management* diseases.

Most bacterial diseases of bivalve larvae are management diseases. Viruses are host-specific pathogens, as are some parasitic diseases. Certain bacterial and fungal pathogens of larvae are host specific, resulting in an unclear distinction between opportunistic and host-specific pathogens.

Opportunistic Diseases

Opportunistic diseases are caused by bacteria or other microorganisms that exist in the marine environment. Poor management practices can allow bacteria to enter or proliferate at many sites in a hatchery, while good management can control or eliminate them. The approach to managing diseases is as follows:

1. Maintain pathogen-free algal stocks and expand cultures.
2. Maintain absence or low levels of pathogenic *vibrios* and other disease-causing microorganisms in the system (water column and surfaces) by proper water filtration, hygiene of system surfaces, and frequent water changes.
3. Isolate infected stocks and associated equipment at first sign of disease.
4. Discard infected stocks and sterilize equipment.
5. Identify source of contaminants and modify and clean system.

Bacteriological Sampling

Disease-causing bacteria can enter the hatchery system at many source points. Bacterial growth should be sampled throughout the hatchery to determine bacterial presence and extent. Samples may be taken from water, algal stocks, container surfaces, or larvae.

Bacteriological sampling can be done at several different levels of detail. It may be adequate to locate large numbers of likely problem-causing bacteria without counting bacteria. Counting bacteria is more detailed and time consuming. If needed, it is possible to count in the production hatchery or have done by an assisting laboratory.

Once bacterial “hotspots” have been located and corrective measures taken, the areas should be resampled to determine the success of the measures.

The following procedures are directed toward the detection of *vibrios*, since *vibriosis* is the best-known bacterial disease, but they can be adapted to other bacteria. The steps outlined assume that the reader has some basic knowledge of bacteriological methods. Any hatchery worker who has successfully cultivated algae in order to feed bivalve larvae will be able to perform these procedures.

Note: Dispose of used bacteriological plates carefully (by sterilization, if this is practical) to avoid contaminating your system.

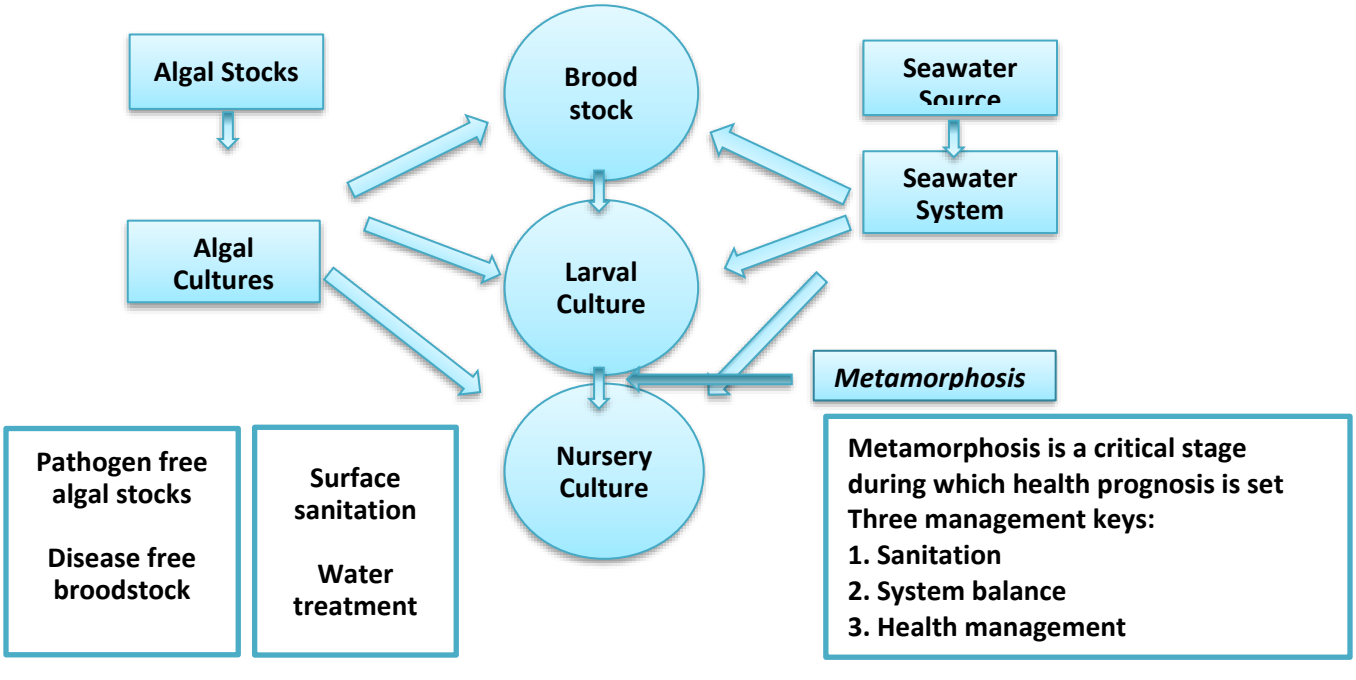
Supplies and Equipment

- Bacteriological growth medium
- Household bleach diluted 1:20 with tap water.
- Sterile plastic inoculation loops (10µL volume).
- Sterile swabs.
- Sterile spreaders.
- Pipet (100 µL volume).
- Plastic petri dishes (100 mm x 15 mm).
- Weighing balance.
- Sterile 1.5 mL microcentrifuge capped tubes.
- Sterile seawater for dilutions.
- Sharpie for labeling plates.
- Parafilm.

Taking the Samples

Bacteriological samples may be collected from several places: the seawater source, any intermediate seawater holding tanks, processed seawater as it is utilized, neutralizing solutions, algal stocks, expanded algal cultures, and larval and spat tanks. We will go through sample collection to determine the presence and relative abundance of bacteria for a qualitative analysis, not to develop actual quantitative results. A simple semi-quantitative presence or absence of *vibrios* on selective or non-selective mediums will enable you to monitor bacteriological levels in your own hatchery and allow you to determine the next diagnostic step, if needed.

Identify bacteriological problems and how to locate them by process of elimination and systematic sampling:



Schematic diagram of intensive hatchery and nursery production of molluscan shellfish with notes regarding health management. *Adapted from Elston and War (2003).*

Container Surface Samples

Using a sterile cotton swab, collect samples from a defined square (1-2 cm on a side). This must be an area which has been removed from the water but not dried. Either drain tanks down slightly or use standard sheets of fiberglass, glass, or similar material which can be removed easily from tanks for surface sampling. Dilute sample by placing the swab in a sterile microcentrifuge tube containing 1.0 mL sterile seawater or 2.0% sterile saline solution and vortexing or vigorously swirling. With a sharpie marker label and date the bottom of the TCBS agar plate. Streak the sample rinse from the swab onto a TCBS agar plate using a 0.01 mL (10µL) inoculating loop, this will give you a 1:1,000 dilution. Alternatively, remove a 100 µL sample with a sterile pipette and use a spreader to cover the agar with the sample. This will give you a 1:100 dilution. Allow plate to remain upright with the cover on to dry for a few minutes, seal plate with paraffin and then incubate plate upside down at room temperature. Examine in 24-48 hours.

Aqueous Samples

With a sharpie marking pen, draw a solid line down the middle of the outside of the bottom of a TCBS plate and label one half 10^{-1} and the other half 10^{0} along with the sample name and date. Label an empty sterile microcentrifuge tube 10^{0} and add approximately 1 mL of any aqueous solution sample (e.g. water, algal culture). Make a 1:1,000 dilution by transferring a sterile inoculating loop sample (10 µL) to 1 mL of sterile seawater in a microcentrifuge tube labeled 10^{-1}. Gently swirl the inoculating loop in the dilution tube, close the cap and flick the bottom of the tube several times to mix. Using the inoculating loop, streak a sample of the 10^{-1} dilution onto the labeled 10^{-1} half of the plate and then streak a sample of the 10^{0} dilution onto the labeled 10^{0} half of the plate. This will give you a 1:100 dilution. *It is important to streak from the most dilute sample to the least dilute sample if using the same inoculating loop.*

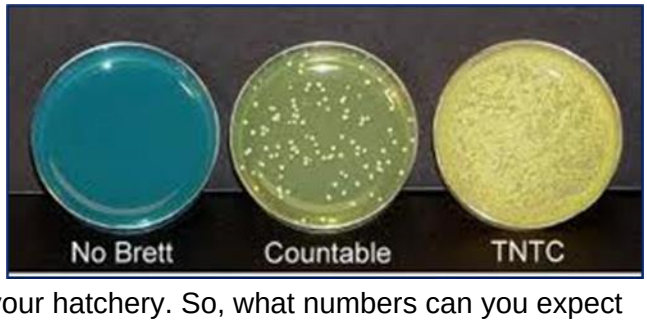
Alternatively, simply label the bottom of the plate 1:10 and remove a 100 µL sample with a sterile pipette and use a spreader to cover the agar with the sample. This will give you a 1:10 dilution. Allow plate to remain upright with the cover on to dry for a few minutes, seal plate with paraffin and then incubate plate upside down at room temperature. Examine in 24-48 hours.

Algal Stock Culture Samples

With a sharpie marker, label and date the bottom of the TCBS or marine Agar plate. Using aseptic techniques, remove a sample from an algal stock culture using a sterile 0.01 mL (10µL) inoculating loop. Using a loop, streak the sample onto a labeled TCBS and/or marine agar plate. This will give you a 1:100 dilution. Alternatively, using a 0.100 mL pipet (100µL) transfer the culture to a labeled TCBS or marine agar plate, and use a spreader to cover the agar with the sample. This will give you a 1:10 dilution. Allow plate to remain upright with the cover on to dry for a few minutes, seal plate with paraffin and then incubate plate upside down at room temperature. Examine in 24-48 hours.

Interpretation

After the 24 or 48-hour, incubation period it is time to examine the plates. Count each individual colony and multiply by the dilution factor. For example, if you count 20 total colonies and you diluted your sample 1:10 your actual count is 20x10=200 colony forming units (CFU) per ml. Make separate counts and determinations of the number of yellow and green colonies on TCBS plates.



It is not uncommon to have some level of bacteria in your hatchery. So, what numbers can you expect to see under normal conditions? Naturally, we do not want to see anything in our incoming water or in our algae cultures, however we expect to find some bacteria in tanks.

Incoming water: The number of general bacteria in your incoming water will depend on the source of that water and type of pretreatment. Well water will typically have a much lower level of environmental bacteria (and unlikely to have any pathogenic bacteria) compared to water pumped in from a body of water such as the Indian River Lagoon. Ideally you **do not want any *Vibrio* in your system water**. Monitoring your incoming water at specific points will alert you to the need to clean reservoirs, hoses, and replace or clean filters and UV bulbs.

Algae cultures: It is not uncommon to find bacterial growth (as seen on Marine agar plates) in algae cultures, particularly as the cultures are expanded. However, **any level of *vibrio* is unacceptable in algae cultures** that are being fed to larval bivalves.

Tank culture water: It is not uncommon to have counts as high as 1,000,000 CFU/mL of total bacteria (Marine agar plates). Anything over that number is unacceptable. *Vibrio* counts (yellow colonies) can be as high as 10,000 CFU/mL in healthy larval tanks. *Vibrio* counts (blue-green colonies) can be as high as 1,000 CFU/mL. Keep in mind that these are **maximum** numbers that have been reported in tanks of healthy larvae. Add a stressful situation such as high ammonia, poor quality algae, etc. and your larvae may easily succumb to *vibriosis*. Ideally yellow colony counts should be below 1,000 CFU/mL and blue-green colony counts 0.

Tanks and equipment: Bacteria adhere to surfaces. No level of *Vibrio* is acceptable on tank surfaces or equipment used to drain down larvae, including buckets, sieves and beakers. So chlorinate, rinse and scrub, scrub, scrub. Then test surfaces to see how well you scrubbed! The larvae themselves will harbor some bacteria that will be transferred to the tank culture water. Don't make it worse!

Some references:

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