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Health & Welfare

How good are your shrimp postlarvae?

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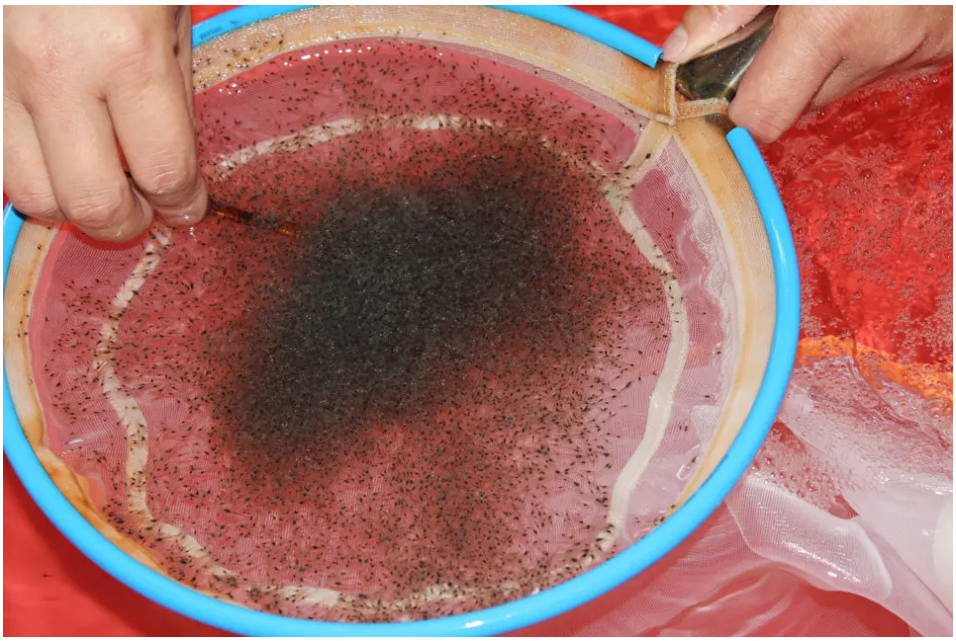
Use stress tests and PCR sampling to assess quality



Stocking only the best quality shrimp PL will have a significant effect on the production and profitability of a shrimp farm. Photo by Darryl Jory.

Stocking only the best quality shrimp postlarvae (PL) is critical to the success of a **shrimp farm** (https://www.aquaculturealliance.org/advocate/we-can-grow-better-shrimp-and-in-better-ways/). Several well-established criteria are used to assess PL quality, including the origin and hatchery reputation, visual evaluation, stress tests and various tests to detect the presence of pathogens. Strict use of PL quality assessment criteria in the evaluation and selection of PL for stocking, and a careful acclimation procedure using the best quality seedstock available are instrumental steps in the farmed shrimp production chain, and will have a significant effect on the production and profitability of a shrimp farm.

Minimizing stress is also a top priority during PL harvest at the hatchery, transportation to the farm, and through the acclimation and stocking processes, because stressed animals – when released into the grow-out environment, much more hostile and unforgiving than the hatchery – may be less likely to survive, and if they do survive may carry a handicap they will not overcome, resulting in reduced survival, growth, production and profitability.

Assessment of PL quality

Several important criteria are used when evaluating PL quality before stocking. One of these is the microscopic examination of PLs before acclimation in regards to index of gut fullness, mucus and debris on setae, opaqueness of swimmerets and tail muscle, and morphological deformities.

Healthy animals should have a complete and well-developed rostrum, well-formed and not bent; tail not curved nor cramped; well-formed eyes and eye stalks; well-formed and complete swimmerets, and an overall good physical appearance. During acclimation, other criteria to examine include assessing the swimming activity of the animals, any erratic swimming behavior, tail muscle opaqueness, presence of molts, index of gut fullness, mortalities, and frequency of cannibalism.

The following criteria to assess PL quality have long been used in the industry: age, size and size distribution, condition index (weight), activity, percent and degree of morphological deformities, presence/absence of pathogens (viruses, bacteria, fungi, protozoans and microsporidians), clean carapace (free of fouling organisms), color and chromatophore pattern, musculature (form and coloration), environmental stress resistance, previous exposure to chemicals or vaccines, nutritional history, biological origin (wild vs hatchery reared), and parental origin.

Stress tests: assessing animal hardiness

The strength or “hardiness” of PL from different hatcheries and/or batches can vary significantly, and the acclimation schedule can be tailored to the “fitness” of the PL, where stronger animals can be acclimated at a faster rate than weaker ones. Different stress tests have been used to challenge a batch of animals and determine how hardy the PL are, to decide on a suitable acclimation schedule. These tests or challenges usually involve subjecting a PL sample of 100 to 200 animals to thermal, osmotic and/or chemical (typically formalin) shock for 1 to 4 hours and “counting the survivors.”

One widely used challenge is a standardized stress test method where a sample of animals are placed in a container or tank, and the water salinity and temperature are simultaneously brought down to 20 ppt and 10 degrees-C, respectively, for four hours (a test lasting under four hours generally does not adequately account for lingering PL mortalities). A variation of this test is to use a 100 to 150 ppm formalin challenge, where a survival of 80 to 100 percent of the test animals indicate high quality PL, while survivals of 60 to 79 percent is considered acceptable, and survival rates under 60 percent justifies either rejecting the batch or holding it in the hatchery for a few more days try to improve their strength and quality.

Another variation for assessment of PL fitness is the one- or two-parameter test (temperature and/or salinity) fitness test, where 100 to 200 randomly collected PL are placed in a bucket containing 10 to 15 liters of water at 22 degrees-C and 5 ppt (two parameter test) or at hatchery ambient temperature and 0 to 1 ppt. Animals are maintained under these conditions for one hour, and survivors (animals that swim and respond normally) counted. The population is considered to have passed the test if survival rate is 80 percent or higher.

Several well-established criteria are used to assess PL quality, including visual evaluations of various characteristics. Photo by Darryl Jory.

Sampling PL for PCR assessment

The shrimp farming industry globally has been affected for three decades by periodic outbreaks of various major diseases that have significantly affected the industries in several countries, including the top producing countries. One key procedure, as part of an overall biosecure production strategy, is to stock pathogen-free PL, and the **Polymerase Chain Reaction** (https://www.aquaculturealliance.org/advocate/limited-decomposition-enhances-pcr-detection-of-ahpnd-vibrio-in-shrimp/) (PCR) test has become a major tool to assess the health status of shrimp PL before leaving the hatchery.

Following proper sampling procedures is vital to support the statistical validity of the tests to assess PL quality. Photo by Darryl Jory.

In this regard, the protocol utilized for sampling a population of shrimp for PCR analysis is important since it will affect the statistical validity of the results. If the objective is to determine the incidence, or degree of infection, in a certain population, then a random sample should be collected. But if the objective of the sampling program is to confirm the suspected presence of a targeted pathogen, then a biased sampling program would be employed in which sick, weak, or moribund animals would be intentionally selected. For random sampling programs, the minimum number of shrimp in the sample to be collected is a function of the size of the population.

A 95 percent confidence interval for a 2 percent infection rate has been adopted as the standard guideline for PCR sampling in many shrimp production facilities. So, based on statistical principles, for any larval tank or pond containing more than 100,000 animals, a minimum of 150 animals must be collected from each tank or pond. When sampling larvae for PCR analysis, the 150 larvae can be pooled and macerated, and tested as one large sample, although depending on the type of PCR equipment utilized, it may be necessary to subdivide the 150 larvae into one or more sub-samples of 30 larval per sample. In this case, make sure that all five of the sub-samples are tested.

Strict use of PL quality-assessment criteria in the evaluation and selection of PLs for stocking, and a careful acclimation procedure using the best quality seedstock available are instrumental steps in the farmed shrimp production chain, and will have a significant effect on the production and profitability of a shrimp farm. Photo by Darryl Jory.

This number of 150 PL from each larval tank constitutes the minimum sample size for screening larvae for many diseases. PL should be assayed using the proper PCR test on zoea, mysis and early PL stages, but generally more reliable results can be obtained at the larger postlarval stages.

Some critical issues to consider when interpreting PCR results are: 1. PCR detects fragments of viral DNA, not necessarily intact, viable virions. Therefore, a positive PCR result does not automatically denote the presence of infectious material; 2. Positive PCR results on certain organisms may be due to passive contamination by viral fragments; for example, in filter feeding mollusks, the intestinal tract of fish or insects, etc.; and 3. There may be different strains of the targeted pathogen, not all of which react to the same primers.

A positive PCR reaction can occur as a result of the presence of intact virus (true positive), the presence of DNA fragments complementary to the primer, and/or sample contamination. A negative PCR result can be caused by the absence of viral DNA (true negative), nucleic acid degradation, presence of PCR inhibitors, and/or poor sampling technique Because of the various potential sources of error, some producers confirm PCR results can by other diagnostic procedures like histology or *in situ* hybridization.

Perspectives

Stocking postlarvae of the highest quality possible, healthy and free of pathogens, is critical to the success of any shrimp farm. There are a number of well-established criteria that are used to assess PL quality, including its origin and hatchery reputation, visual evaluation, stress tests and various tests to detect the presence of pathogens.

The transition from hatchery conditions to those prevailing in open grow-out systems such as tanks and ponds, where water conditions can continually or unpredictably change (day/night, dry/rainy seasons over the production cycle) can be a traumatic experience for PLs unless the transition is gradual and stress is minimized by following proper acclimation procedures.

Strict use of PL quality assessment criteria in the evaluation and selection of PLs for stocking, and a careful acclimation procedure using the best quality seedstock available will be invaluable and will have a significant effect on the production and profitability of any shrimp farm, and should be standard procedures.

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