The value of Level 1 and 2 scoring

When all of these level 1 and 2 observations are made and recorded for each tank of larvae at each stage, an overall picture of larval health can be derived, with higher numbers relating to healthier larvae and vice versa. With experience it becomes easy to judge the overall health of each tank of larvae and to recommend courses of action to combat the problems encountered, depending on the scores obtained.

4.5.3 Level 3 Health assessment techniques

Level 3 techniques are becoming more commonly employed in shrimp hatcheries and in laboratories servicing such hatcheries. Polymerase chain reaction (PCR) methods are used for the screening of PL and broodstock for viral diseases, as are dot-blot and other immunodiagnostic tests.

PCR tests for at least WSSV (and preferably YHV and MBV) should be conducted on all broodstock held in the quarantine facilities. At least the heavily positive individuals should be destroyed immediately. PCR checks of PL (either once before sale or twice – including early PL) should be conducted using nested protocols for the same viruses to ensure only uninfected PL are sold to the farmers.

TABLE 22
Summary of PL quality assessment using Level 3 procedures

Analysis	Observations	Qualitative determination	Score
PCR	WSSV	Negative	10
	YHV	Negative	10
	MBV	Negative	10

TABLE 23
Use of Level 1, 2 and 3 diagnostics in shrimp hatcheries

Level 1	Examination of broodstock for general health condition, sex determination, staging of ovarian development, moult staging, removal of sick/moribund individuals.
	Selection of nauplii by phototactic response, zoea/mysis stage feeding by observation of faecal strands, larval activity, PL health, activity and behaviour, stress tests.
Level 2	Examination of egg quality by microscope. Checking bacterial flora of normal or moribund animals.
	Microscopic examination of naupliar quality. Routine microscopic examination of larval condition and PL quality. Checking bacterial flora of rearing water and larvae.
Level 3	Screening of broodstock by PCR.
	Screening of nauplii and PL by PCR.

Level 3 assessments should be carried out on a statistically determined number of PL (usually 150 for a population >10 000) from each tank (in order to provide a 95 percent confidence level at 2 percent prevalence in the result) using PCR techniques for the detection of important viral pathogens. This testing must be done according to standard protocols by a competent health laboratory, following all the rules for sampling, preservation and transport of the samples. For a detailed discussion of sampling for disease detection, see OIE (2006).

The only acceptable result for any of these viral pathogens is a negative result (which scores 10 points – see Table 22), where both negative and positive controls have simultaneously given their corresponding expected results. All batches testing positive should be destroyed.

The various applications of the different diagnostic techniques in a shrimp hatchery are shown in Table 23.

4.6 QUALITY TESTING/SELECTION OF PL FOR STOCKING

Currently in India there is no universally accepted measure of PL quality and hence no baseline for comparison of different batches of PL. Different laboratories use different ways of reporting health status results so a harmonized approach to boost farmer confidence is urgently required.

The following protocol was developed by the Shrimp Biotechnology Business Unit of Mahidol University, Thailand to standardize PL quality assessment and help hatchery operators and pond farmers produce and select high quality *P. monodon* seed. The adoption of this objective protocol has resulted in a 30 percent premium being paid for PL that pass the assessment, particularly where this involves screening for WSSV. In India awareness programmes for the farmers to inform them about this quality testing system will also be required to get it universally adopted.

The PL quality assessment involves five main areas: gross examination, microscopic examination, stress test, *Vibrio* test and PCR screening. The last two tests may involve taking samples that must then be sent to a competent laboratory, as most hatcheries do not have these facilities.

All of these tests should be performed on each batch (tank) of PL 10-13, two to three days before harvesting the PL (at PL15), providing enough time to complete the PCR and bacteriological analyses so that remedial action can be taken should the PL score be too low.

Gross examination (level 1)

A preliminary examination of PL in the tank is made to assess size distribution, benthic behaviour, swimming activity, feeding and colour. Then a sample is examined more closely: looking at size (PL 15 minimum length 11–12 mm for *P. monodon*); colour (clear or dark, not red/white); activity (active, swimming with no dead); fouling; appearance; behaviour (jump when bowl is tapped); and feeding and gut fullness.

Microscopic examination (level 2)

For this closer examination, a sample of 20–30 PL are randomly selected and examined at 40X magnification. The six criteria used are scored using a standard score and are:

- Hepatopancreas (HP): full and dark with lipid vacuoles (score 10), partially dark and full with some vacuoles (score 5), light, empty, no vacuoles (score 0)
- Gut condition: full, with vacuoles and peristalsis (score 10), partially full, some vacuoles and peristalsis (score 5), empty, no vacuoles or peristalsis (score 0) (for India this analysis should include SHG syndrome)
- Fouling (protozoans, bacteria or dirt): no fouling (score 10), medium fouling (score 5), heavy fouling (score 0)
- Deformity (moulting problems, necrosis): none (score 10), slight (score 5), heavy (score 0)
- Muscle to gut ratio (muscle:gut) (in the 6th abdominal segment): muscle 75 percent of width (score 10), muscle 50–75 percent of width (score 5), muscle <50 percent of width (score 0)
- MBV (malachite green-stained smear of HP looking for occlusion bodies): none (score 10), few (score 5), many (score 0)

Scoring: The maximum score is 60 points, and the pass mark is usually set at 50 points (depending on PL availability). Any batch with more than three zero scores fails, regardless of the overall score.

Salinity stress test (level 1)

This involves an exposure of the PL to 50 percent of the ambient salinity (28–32 ppt) by taking a sample of water from the PL tank and diluting it (1:1) with clean freshwater (drinking water) in a beaker (1 litre). About 300 PL are taken from the tank, counted and placed into the beaker, and after 3 h, the PL that are still active or that move when prodded with a needle are counted and the result expressed as a percentage.

Instead of the salinity test a stress test using formalin may be used if desired. In this case a sample of 300 PL is taken from the tank, placed into a beaker with seawater (at the same temperature and salinity as the larval-rearing tank) containing 100–200 ppm formalin (0.1–0.2 ml/litre). After 30 min the PL that are still active or which move when prodded with a needle are counted and the result expressed as a percentage.

$$\text{Survival} = (\text{No. active PL}/\text{total PL in beaker})/100$$

Scoring: the PL batch passes if the survival is >75 percent and fails if below this score. Note that the PL must be at least 10 mm long (>PL8-10) to withstand these

tests, and it is better performed as close to harvest as possible, i.e. PL12-15 at >12 mm total length.

Vibrio *examination (level 2)*

This is done to check for potentially harmful *Vibrio* spp. in the PL.

A random sample of 100 PL is taken from the tank, sterilized externally by dipping in 70 percent alcohol, washed, ground and then streaked with sterile wire loops all over two replicate thiosulphate citrate bile salts (TCBS) (+1.5% NaCl) and two replicate tripticase soy agar (TSA) (+1.5% NaCl) media plates (1 loop per plate). The plates are then incubated at 30–35 °C for 18–24 h, the number of green and yellow colonies counted and the average taken for the two plates of each media type.

Scoring: The PL pass if the number of green colonies is <60/plate ($1.2x10^{13}$) and the number of yellow colonies is <80/plate ($1.6x10^{12}$) on the TCBS plates and if no luminescence is noted in the TSA media. If not within these criteria, the PL fail the test.

PCR testing (level 3)

PCR testing for WSSV can help reduce the risk of crop loss due to this disease and should therefore be conducted on each batch of PL stocked. This is now a common practice in Indian hatcheries.

A sample of 150 shrimp (preferably the weakest out of a larger batch of salinity/formalin-stressed shrimp) is taken from each tank and preserved in 90 percent alcohol. The samples are then sent to a PCR laboratory and analysed using a 2-step nested PCR technique.

Scoring: If the result is negative PL pass and if positive PL fail.

Other viruses can be tested for (i.e. YHV and MBV for *Penaeus monodon* and TSV and IHHNV for *Litopenaeus vannamei*) if the funds and equipment are available and the analyses desired.

As with larval-quality assessment, a summary table should be made of these three levels of PL quality and the points system employed (using some or all of the above indicators, depending on circumstances). This table then is used to determine which tanks of PL are selected for on-growing, which may require treatment before selection and which will be rejected. As before, experience will guide the manager in his selection of indicators to use and of a cut-off point for points scored, below which the batch of PL will be treated or rejected.

The decision to stock or not to stock a batch of PL is ultimately an assessment of risk. No fixed guidelines or standards can be provided, as this generally comes from experience, but the following guide can be used to reduce the risk of experiencing mortalities or poor growth in pond culture of *P. monodon*. In this risk analysis, the order of importance of assessment is Level 3 > Level 2 > Level 1.

4.7 PL HARVEST AND TRANSPORTATION

Harvest and transport are stressful times for PL shrimp. These processes should be done gradually and with minimal stress, and only of intermoult (hard-shell), disease-free and health-checked PL. This will ensure a good survival rate of PL on stocking into the grow-out ponds and help generate a good reputation for the hatchery.

PL ready for packing (left) and PL being acclimatized for temperature in a tank before packing

Plastic bowls are prepared on an elevated floor (foreground) for PL packing. This reduces chance of contamination however, unused bowls should also be kept on shelves rather than placed on the floor (background) [Vizag]

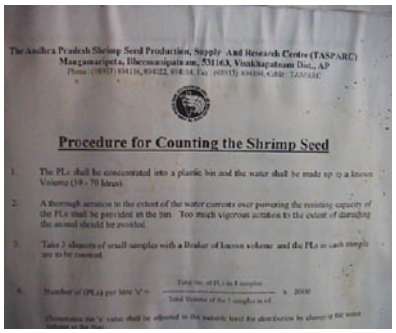
If possible the PL should be acclimated in the hatchery to the expected salinity in the on-growing farms. This is to reduce the stress on stocking the ponds, a critical point in the process that can lead to high mortalities if not done smoothly. Procedures for acclimation are currently not standardized in Indian hatcheries. Salinity adjustment can be done by adding freshwater to achieve a salinity change of <3 ppt/h from 30 to 20 ppt, but should be reduced to <1 ppt/h from 20 to 10 ppt and <0.5 ppt/h from 10 to 5 ppt. Such salinity adjustments should commence only when the PL are older than PL10 (preferably one to two days before harvest), when their gills are fully developed (look like Christmas trees) and they are able to tolerate such rapid salinity changes.

Biosecurity measures for harvesting in Indian hatcheries are currently weak. On the day of transportation, the PL should be carefully harvested, held in tanks containing clean, disinfected and filtered seawater with aeration (or preferably oxygenation) at no more than 1 million/tonne. Then the temperature should be decreased gradually (by adding bagged ice) until the desired transport temperature is reached. Decreasing the temperature from 28 –30 °C to 23 °C should take at least 30–40 min to help reduce stress. Water salinity should be the same as the water in the larval-rearing tanks (which should have already been adjusted to that found in the target ponds).

It is at this point that the PL should be counted, preferably in the presence of, and with the assistance of the buyer. This is required to estimate the survival rate of the shrimp in each tank and for accounting for the sale of the PL to the customer. Currently many Indian hatcheries omit this step or do not make good records of these details, which are required to evaluate management procedures in the hatchery.

Transportation temperature reduction is required to lower the metabolic rate of the PL so that they will use less oxygen, excrete less waste and remain calm during transportation. The temperature used and stocking density of PL will depend upon the duration of the transportation. Typically no temperature reduction is required if the hatchery is within one hour of the farm. Temperature should be reduced to 26–28 °C for transportation times of 1–3 h, 25–26 °C for 3–12 h or 23–25 °C for over 12 h.

There are two main methods for PL transportation from the hatchery to the farm. PL can either be transported free in large, aerated tanks or packed into plastic bags, usually held inside polystyrene and/or cardboard boxes. In either case these should be filled with filtered seawater (at the same salinity as in the larval tanks) chilled to the desired temperature. New, already washed activated carbon (1 g/litre) can be added to absorb any ammonia produced by the PL and tris-HCL buffer can be added at 100 mg/litre to stabilize pH. Live *Artemia* nauplii should be disinfected, washed (with freshwater) and added at 15–20/PL for each 4 h of transport to provide food and prevent cannibalism during transportation.


The Andhra Pradesh Shrimp Seed Production, Supply And Research Centre (TASPARC)

Procedure for Counting the Shrimp Seed

Good management practice also includes putting a notice like this (TASPARC-Vizag) on a wall of the respective operational area so that hatchery personnel can be aware of the operating procedure

If using tanks, the tanks and all other equipment (nets, airstones, airlines etc) should be first disinfected with 20 ppm chlorine, then washed and dried thoroughly. The transportation vehicle should also be disinfected (at least the tires and wheels) before and after entering the hatchery to prevent cross-contamination between farms and hatcheries.

During transportation the tanks should be closed with a lid to prevent loss of water and PL. Pure oxygen should be pumped into the tank slowly via airstones throughout transportation to ensure adequate oxygenation of the water. One staff member should be charged with checking the tanks frequently to ensure that everything is working as it should be.

If using plastic bags, these should be double (using two bags, one inside the other) to prevent loss due to bag breakage. These bags should be one-third filled with seawater, the PL added and then filled by bubbling pure oxygen into them. The bags are then sealed with elastic bands, placed into cardboard or ideally polystyrene boxes (better able to maintain temperature) that are taped shut and are then ready for transport. Plastic bags and cardboard cartons should be incinerated after each shipment. Polystyrene boxes however can be disinfected with chlorine and dried before reuse.

In either case stocking densities up to PL15 should range from 250–500 PL/litre (depending upon PL size and transportation time) or a maximum of 2.5 g/litre. If larger PL or juveniles are transported, a corresponding reduction in stocking density maintaining 2.5 g/litre should be used.

Efforts should be made to conduct transportation only at night, when temperatures are cooler, and in insulated trucks, thereby reducing the stress on the PL being transported. They can then be acclimated to the ponds at first light, when the temperature in the ponds is coolest and less stressful to the shrimp.

4.8 NURSERY REARING

Nursery rearing of PL shrimp can take two forms – primary and secondary nursing. Primary nursing involves transfer of young PL at about PL4-5 from the larval rearing tanks to separate PL tanks until PL15 or more before sale to the farmer. This system is used to optimize use of the larval-rearing tanks, so that each tank can run two cycles of about two weeks each per month. It also helps maintain clean culture facilities, as each tank is only stocked for a maximum of two weeks.

Secondary nursing involves the harvest of older PL at PL12-15 and transferring them to tanks or ponds (either in the hatchery or farm) and then on-growing the PL for two to three weeks before transferring to the on-growing culture ponds. This type of nursing is done to head-start the young shrimp, to enhance their fitness for stocking and minimize culture time in the ponds, thereby increasing the number of cycles per year possible.

In primary nursing the PL4-5 should be collected from the larval-rearing tanks, disinfected with 300 ppm formalin or povidone iodine dip treatment for 30 s, and then transferred to outdoor nursery tanks at 20–40 PL/litre. Feed should comprise crumbled artificial diets (200–500 µm particle size) and (preferably) enriched *Artemia* nauplii and/or biomass. During the later stages other live feed items such as clam meat can be used (carefully to avoid water quality problems) to increase the growth rate.

For the secondary nursing, the tank or pond (especially the pond) must be prepared properly before transfer to minimize stress and assure the stability and suitability of the water and sediment quality. It must also be properly

Poorly built nursery ponds with low water levels are common in some nurseries (left). Harvesting and collection methods are stressful to the PL (right)

fertilized with inorganic and/or organic fertilizers to enhance the availability of natural food. Stocking densities should be about 50 PL/m^2, and only with previously disease-checked, uninfected PL. Feeding is done with crumbled diets (500–1 000 µm), supplemental live feeds and natural production, stimulated by fertilization. Secondary nursing periods should be about two to three weeks long. Any longer than this is risky and transportation of large juvenile shrimp becomes problematic.

Currently in India these secondary nursing systems are not working well because of inappropriate pond design, poor pond preparation, poor management and lack of quality checks for the PL stocked.

Commercial nurseries are particularly risky in India as they tend not to check the quality/disease status of the PL being stocked and mix batches from different hatcheries, leading to problems with contamination. Such commercial nurseries therefore require help with their management practices to enable them to produce good quality seed for stocking the shrimp farms.

Private nurseries are generally better managed, but still require encouragement. Group nurseries may be ideal to meet the requirements of small-scale farmers. The concept of promoting collective local nurseries (where transportation times and stress can be minimized) among small self-help groups needs to be encouraged.

Rather than direct release of the PL into earthen ponds, there is also the possibility of nursing in net happas, which facilitates management of feeding and harvesting but requires more research for optimization.

4.9 TIMING OF PL STOCKING

Nationwide stocking plans for PL are required in order to ensure that hatcheries have a market for their products and that the farmers have access to PL at the time when it is most advantageous to stock their ponds. In India the existing stocking schedules drawn up in an attempt to minimize the risk of disease are only partially followed in certain areas. This has caused some difficulties in acquiring broodstock and the production of PL.

The hatchery associations should cooperate with farmer's groups or associations to plan in advance for their PL requirements. A proper study on the relationship between success rate and other related factors should be conducted and recommendations then made to the Department of Fisheries (DOF) regarding the best stocking period. Additionally MPEDA in consultation with the state governments should be responsible for suggesting appropriate stocking patterns based upon their experience.

4.10 USE OF MULTIPLE SPECIES IN SHRIMP HATCHERIES

Due to the risks involved with transmission of disease between different shrimp species, hatcheries should be dedicated to the production of only one species at a time. However, some Indian hatcheries are currently operating with two different species (*Penaeus monodon* and *Macrobrachium rosenbergii*) in the same facility. There is also the danger that if *Litopenaeus vannamei* culture becomes more widespread, a third species may be cultured concurrently.

The production of these two species in the same hatcheries can be permitted, but only in different modules and in compliance with the required COC, to minimize cost, ensure biosecurity and maximize productivity. However, for exotic, imported species (like *L. vannamei*), each hatchery must be separate from all other activities.

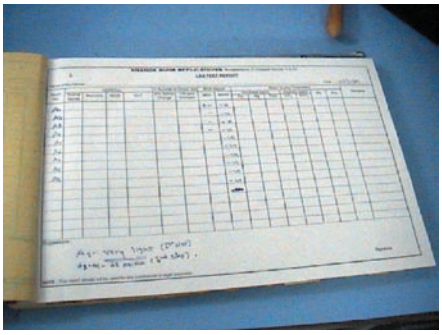
Where essential, such multi-species hatcheries should be registered as two different units within the same hatchery premises. However, the practice of culturing more than one species is not recommended, and should be phased out if possible. Meanwhile close monitoring and assessment of multi-species hatcheries are required. When developing plans for stocking ponds with the various species, the state should consider such factors as the season and the availability of quality broodstock and PL. Thus different species

may be cultured in the same facility, but in different seasons as dictated by the demand.

4.11 DOCUMENTATION AND RECORD KEEPING

For reasons of biosecurity and good management of shrimp hatcheries, a comprehensive system of documentation and record keeping should be established. This should include indications of daily shrimp numbers, larval health, treatments/chemicals used, water quality and other relevant information for each tank stocked. This will help determine the cause of any problems and any remedial actions required.

Extensive records should be kept daily and by cycle according to the information in the recording sheets presented as Annexes 4 to 9.



Some hatcheries have proper data recording sheets, but their actual use is questionable, as most hatcheries do not complete them