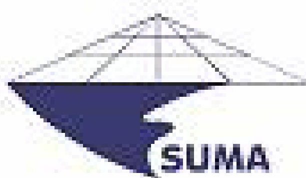

Better Management Practices (BMP) Manual for Black Tiger Shrimp (*Penaeus monodon*) Hatcheries in Viet Nam

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1 Background

The production of black tiger shrimp (*Penaeus monodon*) seed has been conducted in Vietnam for nearly 20 years. Currently, there are more than 5,000 hatcheries throughout Vietnam which produced a total of 26 billion PL in 2004. The two provinces of Khanh Hoa and Ca Mau are considered the major seed production centres. To date, hatcheries have been able to supply the demands of the commercial shrimp farms but variable seed quality, and the prevalence of diseases has resulted in huge damages to both the hatchery and grow-out sectors.

In order to provide practical and effective technical guidance for shrimp hatchery management, it is necessary to establish a set of Better Management Practices (BMPs) which underpin an effective hatchery production system. These include the presence of essential infrastructure, the development of the maintenance of biosecurity, the provision of adequate amounts of clean water, the responsible use of chemicals, correct feeding practices, and the assurance of the health status of stocks through in-house and laboratory testing.

This document is not a complete manual on the management of *P. monodon* hatcheries, but rather, it concentrates on the implementation of BMPs for the hatchery covering all of the critical stages and processes in the production cycle, which are currently believed to be causing problems in Vietnamese hatcheries.

These BMPs have been written for Vietnamese hatchery managers. They should be given to all personnel and a meeting should be held to introduce the protocols and explain the need for, and contents of the BMPs. This is a good opportunity to clearly identify and explain any points that generate doubts or that may be misinterpreted, and to get practical input from the hatchery staff. As new information becomes available, it will be necessary to update or modify the BMPs, and any changes must be communicated to all personnel.

Some of the BMPs discussed are essential (in black text) whilst others are desirable, but more difficult to implement **(in blue text).** To ease implementation, the hatchery manager should focus on the BMPs that are essential first, and on the desirable practices only when the essential practices have been implemented. Each BMP is discussed with regard to its ease of implementation and level of importance for optimal functioning of the hatchery.

Validation of many of these BMPs has been conducted at 6 pilot hatcheries, 3 in Khanh Hoa and 3 in Ca Mau for the past year under the project “Reducing risk of aquatic animal disease outbreaks” implemented through a collaboration of the Ministry of Fisheries, the Danida-funded SUMA (Support to Brackish Water and Marine Aquaculture) component and the Network of Aquaculture Centres in Asia-Pacific (NACA).

Although preliminary results have been inconsistent (due largely to ineffective application of the BMPs), where successful, considerable numbers of large, high quality seed have been produced, which have been sold at a premium of 30-40% over the price of PL produced using the traditional techniques. Thus, their potential has been clearly established.

2 BMPs for General Hatchery Management

2.1 Infrastructure Requirements

Shrimp hatcheries should be designed (or modified, in the case of existing hatcheries) to ensure biosecurity, efficiency, cost-effectiveness and should implement Better Management Practices (BMPs) aimed at producing large numbers of high quality nauplius and postlarvae (PL). The infrastructure requirements for successful biosecurity and management of the hatchery operation will be discussed in the relevant BMPs, however, there are a few basic guidelines.

- A well-designed shrimp hatchery will consist of physically separate facilities for quarantine, maturation, spawning, hatching, larval rearing, indoor and outdoor algal culture (where applicable), and for *Artemia* preparation and hatching (See Fig 1)

Figure 1: A well designed and laid out shrimp hatchery

- Larger hatcheries may have separate units within each of these categories, which should be run like mini-hatcheries for reasons of biosecurity (See Fig 1). This should include attempts to stock the entire hatchery (or at least the individual units) as quickly as possible in order to reduce problems with internal contamination
- Supporting infrastructure is also required for the handling of water (facilities for abstraction, filtration, storage, disinfection, aeration, temperature adjustment and distribution), larval laboratories, feed laboratories (for analysis and preparation) and storage facilities, maintenance areas, packing areas for nauplii and PL, offices, storerooms and staff living quarters and facilities
- **In existing hatcheries with no physical separation, effective isolation may also be achieved through the construction of barriers and implementation of process and product flow controls**
- **If possible, the hatchery facility should have a wall or fence around the periphery of the property, with enough height to stop the entrance of animals and unauthorized persons. This will help to reduce the risk of pathogen introduction and increase security**

2.2 Water Quality and Treatment

Water for the hatchery should be filtered and treated to prevent entry of disease carrying organisms and any pathogens present in the source water, and provide oceanic quality seawater to the larval shrimp. A number of steps may be used to achieve this including: initial

filtering through subsand well points, sand filters (gravity or pressure), and/or mesh bag filters into the first reservoir or settling tank. Following settlement, the water should usually be disinfected by chlorination (and sometimes potassium permanganate). Where possible, it should then be filtered again with a cartridge/bag filter and finally disinfected using ultraviolet light (UV) and/or ozone. The use of activated carbon filters, the addition of ethylene diamine tetra acetic acid (EDTA) and temperature and salinity regulation may also be features of the water supply system

- More specific water treatment procedures to be used for each phase of maturation and larval rearing are detailed in the appropriate sections
- **Each functional unit of the hatchery system should have the appropriate water treatment systems and, where necessary, should be isolated from the water supply for other areas. Separate recirculation systems may be used for part or the entire hatchery to reduce water usage and further enhance biosecurity, especially in high-risk areas**
- **Ensure that all water discharged from the facility is free from pathogens, particularly that known (or suspected) to be contaminated, for example, water originating from the quarantine areas. Discharge water should be held temporarily and treated with hypochlorite solution (>20 ppm active chlorine for not less than 60 min) or another effective disinfectant prior to discharge. This is particularly crucial where the water is to be discharged to the same location as the abstraction point**

2.2.1 Sedimentation/sand filtration of inlet water

Sedimentation and/or sand filtration tanks/units are required where the quality of the seawater brought to the facility is poor, particularly where high levels of suspended solids are present (i.e. in the Ca Mau area). Removal of these solids will help enhance the quality of the seawater, facilitate disinfection by chlorine and reduce the level of fouling and disease organisms in the water for use in the hatchery.

- Before the water is brought into the facility, it should be checked for salinity to determine whether it is of suitable quality. Normally the highest salinity obtainable (up to 33-34 ppt) is optimum, **whilst salinity as low as 29-30 ppt is acceptable.** The highest salinity is usually found at the time of high (especially spring) tides, so if possible water should be pumped only at this time. **If water of >29ppt salinity is unavailable at the hatchery location, obtaining seawater by tanker from areas with higher salinity should be considered**
- The seawater obtained is pumped into settlement tanks (See Figs 2 & 3) and allowed to sit undisturbed for 1-3 days until all the suspended material has settled to the bottom. The water can then be pumped to a separate tank for chlorination (see section 2.2.2)

Figures 2 & 3: Seawater settlement tanks

- **Sometimes it is necessary to add 0.5-2 ppm of potassium permanganate ($KMnO_4$) to the settlement tank to aid settlement and disinfection. Whether this is required depends upon the quality of the seawater brought into the facility and personal experience**
- Alternatively, the water can be passed directly through sand filters, either large gravity-flow filters (See Fig 4), or pressurized sand filters (See fig 5), before passing to reservoir tanks for chlorination

Figure 4: Gravity sand filter

Figure 5: pressurized sand filters

- In either case, the tank used for sedimentation/sand filtration must be separated from the tank used for chlorination. If the same tank is used (even if not aerated) the high organic matter content of the sedimentation tank will decrease the effectivity of the chlorine
- **Concerns of hatchery managers that they do not have enough water storage capacity, may be resolved by chlorinating the water for 12-24 hours and then dechlorinating with sodium thiosulphate (see section 2.2.2), so that the water is ready for use in only one day.** The typical current procedure (to wait 3 days with aeration until the chlorine is gone) means that the reservoir tanks need 3 days for chlorination. Therefore, using a treatment protocol of only one day, allows for the use of reservoirs/chlorination tanks of $^{1}/_{3}$ of the size.

2.2.2 Disinfection of inlet seawater using chlorine

Incoming seawater should be disinfected prior to use to minimize the chances of viral, bacterial, fungal, microsporidian or protozoal diseases in the environment causing disease problems in the hatchery. The commonest and best chemical treatment for such disinfections is the use of chlorine in the reservoir tanks.

- Chlorine can be a powder (calcium hypochlorite – usually 60-70% active ingredient), a liquid (sodium hypochlorite – usually 7-10% active ingredient) or tablets (sodium

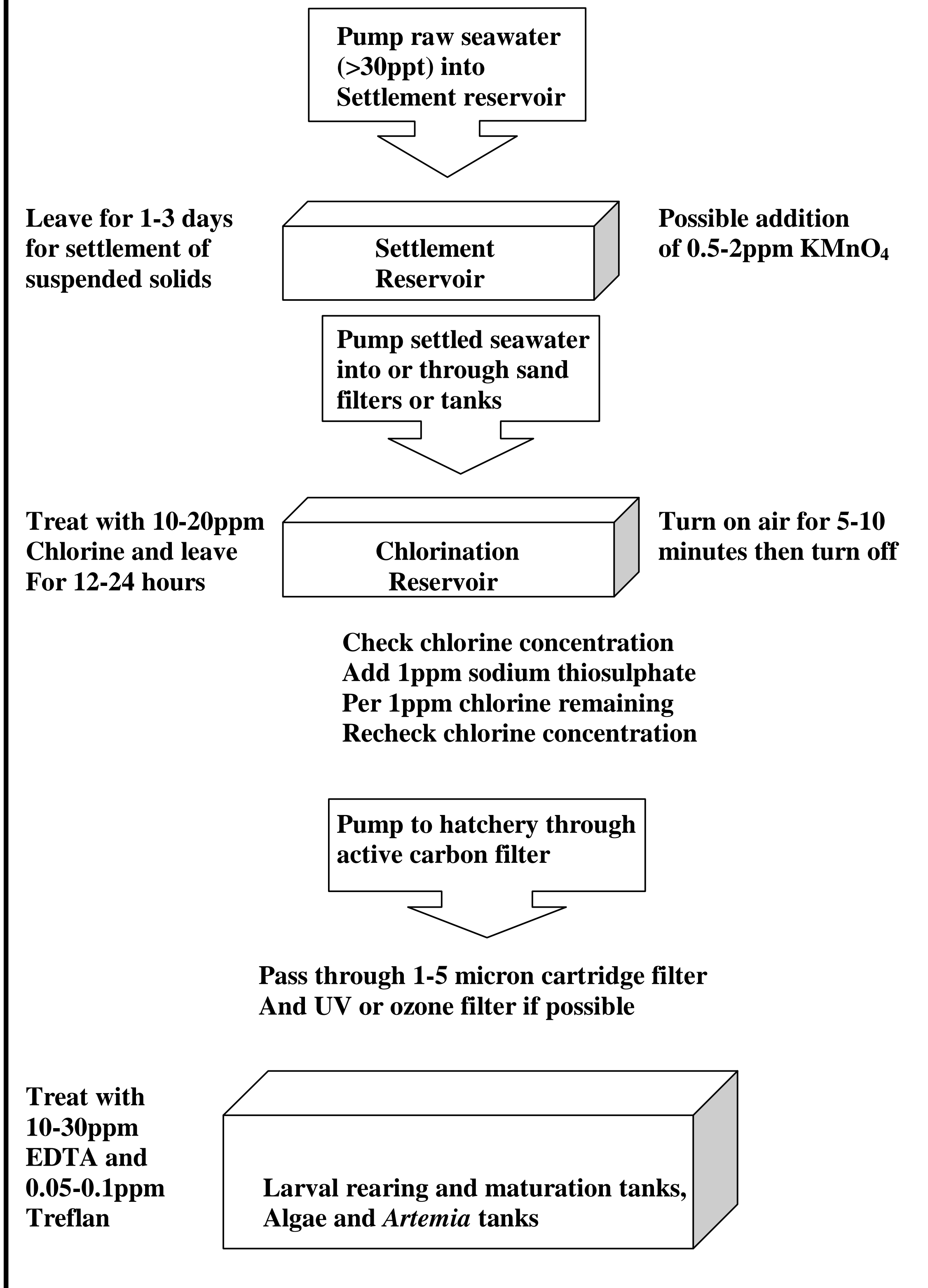
dichloroisocyanurate – usually >90% active ingredient). All these forms of chlorine are effective and can be used depending upon price and availability

- Normally, a level of active chlorine in the water of 10-20 ppm for 12-24 hours is sufficient to kill most pathogens. **If the pH of the water is >7.5, the water can first be acidified with hydrochloric acid until pH 7.5 is reached. This will improve the killing power of the chlorine**
- Chlorination is achieved by first filling the reservoir tanks with **(preferably filtered)** seawater. For an active chlorine concentration of 10ppm: add 15 g of 65% calcium hypochlorite powder (dissolved first in water), or 100ml of 10 % sodium hypochlorite (liquid bleach), or 10-11g of 90% chlorine tablets per m^3 (1,000 litres) of water. Turn on the aeration for 5-10 minutes until the chlorine is fully mixed, then turn it off and let the tank stand for 12-24 hours
- The aeration is turned off to maintain the chlorine concentration in the water for a long time, so it is able to kill any pathogens. Maintaining high aeration from the beginning releases the chlorine into the atmosphere, hence reducing its killing ability and may account for the ineffectiveness of current protocols
- After 12-24 hours, turn on the aeration system, measure the chlorine concentration with a swimming pool chlorine test kit (5 drops of ortho-toluidine liquid in 5 ml water sample) and compare the deepness of the yellow colour developed with the colour comparison charts of the test kit (See Fig 6)

Figure 6: Use of chlorine test kit

- Then add sodium thiosulphate crystals dissolved first in water at the rate of 1 ppm (1 g/m^3) for every 1 ppm of chlorine left in solution. Wait 10 minutes and measure the concentration of chlorine again. If no yellow colour whatsoever develops, the water is ready. If it is still yellow, add another 1 ppm of sodium thiosulphate and recheck. Continue doing this until there is no yellow colour on retesting
- **It is a good idea to pass all water through an activated carbon filter before use to ensure that no chlorine byproducts or other dissolved organics remain. Activated carbon can be housed in a filter or a filter bag on the inflow into the maturation and larval rearing tanks**
- The flow and processing of inlet seawater to the hatchery facilities is shown in Figure 7

Figure 7: Seawater inlet process and treatment



2.3 Facility Preparation

Effective tank, pipework and general hatchery preparation protocols should be used prior to each cycle/use to ensure that diseases affecting one cycle are not transmitted to the next. *Vibrio Sp.* bacteria, viruses, fungi, microsporidians and protozoans are able to survive and multiply from small founder populations, which may escape disinfection within the bare concrete of hatchery or reservoir tanks or within un-disinfected facilities, pipes and other equipment.

- Separate the broodstock and larval rearing facilities (and equipment and staff) from all other areas of the hatchery. Provide separate lines for water and air so that each facility can be totally disinfected, cleaned and dried between cycles, independently of the other units
- **Wherever possible, use tank liners or epoxy paint (make sure the concrete is completely dry before painting) for maturation, spawning and hatching and larvae rearing tanks to enhance the efficacy of tank and facility disinfection protocols between cycles/uses (See Figures 8-11)**

Figures 8 & 9: Epoxy painted Broodstock maturation systems

Figures 10 & 11: Epoxy painted larval rearing tanks

- At the end of each use, the broodstock and larval rearing tanks must be washed, scrubbed and washed again to remove all debris from the last cycle. The water and air pipes should also have a 10% solution of hydrochloric acid (HCl) (100 ml of concentrated HCL in 1 litre of water) pumped through them from start to finish and left there for 12-24 hours before flushing through with clean fresh or seawater. The entire facility should then be left to dry (preferably in direct sunlight) for >5-7 days to kill any disease organisms. (Note: always add concentrated HCl to water, not water to acid, to prevent explosions)
- Immediately before the start of the next cycle, the tanks should be washed and scrubbed with a 10% Hydrochloric acid (HCL) solution (100 ml of concentrated HCL

in 1 litre of water) and then washed again with clean fresh or seawater prior to filling with clean, disinfected, **(preferably filtered)** seawater

- Do not use formalin to disinfect maturation or larval rearing tanks since any residues are highly toxic to larval shrimp
- Each broodstock or larval rearing tank should be filled immediately prior to stocking, checked for chlorine residues (and treated with sodium thiosulphate if required) and then 10-30 ppm EDTA and 0.05-0.1ppm Treflan added prior to stocking
- **Where possible, use water filters (for example active carbon, 5 and 1 micron cartridge and UV filtration) in the broodstock and larval rearing facilities to maintain optimum water quality, especially for spawning, hatching and nauplius holding tanks**

2.4 Separation and Disinfection of Equipment for Each Tank, Hands and Feet

Diseases which can affect one tank of larvae can be easily spread to other tanks through contamination of hands or equipment, if they are used for more than one tank. Therefore all equipment should be maintained separate, with one set for each tank. Procedures for disinfection of hands and feet should be strictly followed.

- A 5-20 litre bucket containing a solution of 100ppm PVP povidone iodine should be hung above or placed on the side of each larval rearing or broodstock tank and a 0.5-1 litre glass beaker or glass (for checking larval health and feeding) kept in each bucket to maintain sterility. The iodine solution should be changed daily for a new solution. Each tank should also have its own mesh nets as required for catching and/or checking larval or broodstock shrimp quality. This equipment should be reserved for use in that one tank only (See Fig 12).

Figure 12: Individual disinfection buckets and equipment hung above larval rearing tanks

- The entrance to each section of the hatchery (each larval rearing, broodstock, algae, *Artemia* and/or water treatment unit) should have shallow bowls or trays placed there and filled with solutions of either 200 ppm povidone PVP iodine, 50-100 ppm chlorine or 500ppm potassium permanganate to disinfect the feet/boots of each person entering the facilities (See Fig 13)

Figure 13: Foot bath containing potassium permanganate

- Wash bottles containing 100ppm povidone PVP iodine solution (or 70% alcohol) must be placed at the entrance to each room in the hatchery, so that hands can be disinfected before entering each separate unit

2.5 Documentation and Record Keeping

Establish a comprehensive system of documentation and record keeping, indicating 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 action required.

Keep extensive and thorough notes daily according to the information in the recording sheets shown in Appendix 1.

3 BMPs for Broodstock Management

The foundation of a successful hatchery is the use of high quality broodstock. In Vietnam currently, there is a complete dependence on the capture of wild broodstock from the sea. In order to minimize stress, damage, mortality, and infection of broodstock with pathogenic diseases, the collection, holding, preparation, transportation, maturation and spawning of these wild broodstock should be done as carefully and efficiently as possible.

3.1 General Guidelines for Collectors and Suppliers

There are a number of basic principles which should be followed in order to obtain high quality broodstock which will perform well in the hatchery and produce high revenue for both the broodstock suppliers and the maturation/hatchery units.

- Minimize handling of broodstock at all times
- All of the required equipment (tanks, buckets, air lines, airstones, nets etc) must be disinfected carefully (washed in 20ppm chlorine) before and after each use. Polyethylene plastic bags must be used only once
- Make and keep records of the entire process so they can be checked if problems occur
- **Where possible, measure and maintain saturated oxygen levels (>6ppm). Check using an oxygen meter or test kit**
- **Where possible, the sea water used throughout the whole process of collection and transportation should be filtered and sterilized using UV light or an ozone machine**
- The broodstock should be held in groups as small as possible**, and where possible, they should be held individually, at least until their disease status is checked (especially for MBV and WSSV viruses)**

3.2 Broodstock Collection

In order to obtain strong, healthy shrimp and sell them to the maturation/hatcheries, broodstock collectors must maximize their efficiency in catching the shrimp and bringing them back to port.

- **Attempt to catch broodstock shrimp from clean, deep water (30-60m) as far as possible from the influence of the coast**
- **Preferably utilize gill and trap nets, rather than trawls to minimize damage to the shrimp**
- Which ever type of net is used, retrieve and check them frequently to reduce stressing and damaging the shrimp
- Quickly transfer collected shrimp to tanks that are well and continuously aerated, with continuous or frequent water exchange and that are maintained at <29°C, out of direct sunlight
- Use clean, high salinity seawater, **preferably obtained from the area where the shrimp are caught and filtered (to <5 microns using a cartridge filter) before use**

- Do not keep collected broodstock in overcrowded tanks for prolonged periods prior to selection, holding and transport

3.3 Broodstock Selection

Hatchery success depends to a large extent on the quality of broodstock selected for maturation. Every effort should be made to ensure that only large, productive, healthy, disease-free shrimp are selected (See Fig 14).

Figure 14: Large, healthy broodstock female *Penaeus monodon*

- The correct size of broodstock must be selected for maturation and spawning so that they will be ready and able to provide large numbers of healthy seed. **Although difficult, wherever possible, female broodstock should be chosen which have a total length of >28 cm and weigh in excess of 217 g, to give a ratio of at least 7.5g/cm (See Table 1).** If female broodstock are selected with a lower ratio, they may not be in optimal spawning condition, but may be all that is available.
- **Males of at lest 21 cm should be selected, which should weigh >70g.**

Table 1: The relationship between total length and weight of *P. monodon* broodstock.

Total length (cm)	Weight (g)	Ratio (g/cm)
19	52	2.7
20	60	3.0
21	70	3.3
22	82	3.7
23	97	4.2
24	119	5.0
25	142	5.7
26	167	6.4

27	192	7.1
28	217	7.8
29	242	8.3
30	267	8.9
31	292	9.4
32	323	10.0
33	359	10.9
34	398	11.7
35	438	12.5

- A gross examination of each broodstock shrimp should be done to ensure that they are outwardly healthy, have a good, bright color (not red or with black spots), a clean body and gills, intact appendages and eyes, and are undamaged
- **Where possible, each broodstock should be checked to ensure that it is free from disease (for at least MBV and WSSV). To do this, a piece of pleopod (or telson) is cut from each shrimp (the place where the cut was made is then disinfected with pure liquid povidone PVP iodine) and preserved in 90% alcohol (note: do not use coloured alcohol) in a small bottle or tube. This is then sent to a PCR laboratory to check for WSSV. MBV (and HPV if possible) can also be checked from this sample by PCR, or a piece of faeces from each broodstock is put onto a microscope slide, a drop of malachite green stain added and then squashed and examined under a high power (x400) microscope. MBV and HPV viral occlusion bodies, where present will appear as shown in Figs 15 and 16. Consider discarding all positive shrimp, and at least all heavily infected shrimp. If possible, only those broodstock free from WSSV, MBV and HPV should be chosen.**

Figure 15: MBV

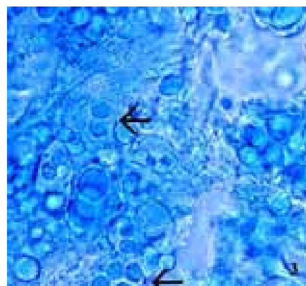
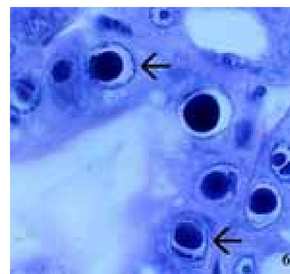


Figure 16: HPV



3.4 Broodstock Preparation for Transport

Before broodstock are transported to their destination hatchery, they must be prepared so that they will have the best chance of arriving alive and in good condition, with minimum stress.

- Plan and reconfirm in advance all connections and handling procedures to minimize transportation duration
- Gradually reduce the temperature in the broodstock holding tanks to the desired transportation temperature **(18-28°C, depending upon duration of transportation),** no quicker than 1°C every 10 minutes (i.e. to decrease from 30°c to 20°C should take

100 minutes). This is accomplished by putting ice sealed inside plastic bags into the water holding the broodstock until the desired temperature is reached

- **If the broodstock require feeding during holding and prior to transportation, then the feed should be mixed with vitamin C (2g/kg) and either paprika (2g/kg) or astaxanthin (0.1g/kg), plus an acceptable probiotic formula to help reduce stress and bacterial levels**

3.5 Broodstock Transportation

Correct transportation of broodstock is critical to the provision of high quality broodstock that will survive the stress of transportation and be able to produce healthy seed in the hatchery maturation system.

- Do not feed the broodstock for 12 hours prior to shipment and only transport the ones with hard shells (as the moulting ones will die)
- Maintain dissolved oxygen levels >5ppm by filling each bag with $^{1}/_{3}$ chilled, filtered seawater and $^{2}/_{3}$ pure oxygen, bubbled into the water
- Put the broodstock shrimp in double polyethylene plastic bags into polystyrene boxes and maintain the water temperature at 18-22°C (putting ice into the boxes where required) for transport times >6 hours (See Fig 17). Refill the bags with pure oxygen during shipping if the transport time exceeds 24 hours. Avoid direct sunlight at all times

Figure 17: Broodstock shrimp in polystyrene boxes during transportation

- Handle the boxed shrimp with extreme care and avoid bumping or dropping them
- **If possible, place a rubber tube over the rostrum of the shrimp to stop it from puncturing the bag during transportation**
- **If possible, only transport during the cooler time of day (i.e. at night) and minimize transportation times as much as possible**
- **If possible, transport each broodstock individually with a maximum of 2 per bag, ensuring that they are stocked at <500g of shrimp per 10 litres of water**

- **Add 10ppm EDTA (to chelate heavy metals and reduce bacteria), 1g/l activated carbon (to control ammonia and nitrite) and if possible, 10ppm tris HCL buffer (to stabilize pH)**

3.6 Broodstock Acclimation

Once the broodstock holding facilities have been prepared correctly (See section 2.1) and the broodstock arrive at the hatchery, they should be slowly acclimated to the water quality conditions in order to avoid stress and maximize survival.

- **Where possible, incorporate a quarantine unit in which new animals are held individually until tested and found to be free from pathogenic diseases**
- The broodstock quarantine and holding area must be separated from the other hatchery units if possible
- Prepare the quarantine tanks one day before arrival to match the expected water quality (temperature, salinity and pH) of that in the transportation containers. Where required, to cool the water use bagged ice, to decrease salinity use clean freshwater, and to decrease pH use dilute hydrochloric acid (HCl)
- Float the still-closed bags in the tanks for 30 minutes, then open the bags and slowly bubble pure oxygen into the water
- Gradually fill the bags with water from the tank for 30-60min to slowly acclimate the broodstock to the hatchery water
- **Gently take each broodstock from its bag and immerse it in a bath of 100 ppm (1 ml/10 litres) potassium permanganate ($KMnO_4$) or liquid povidone (PVP) iodine for 30-60 seconds and then carefully release the shrimp into the receiving aerated, but static water tank**
- Offer high quality feed to demand immediately as they may be hungry
- Gradually increase the temperature in the receiving tanks to match the ambient temperature at a rate of not more than $1^{o}C$/hour
- The salinity in the water of the receiving tanks should be at least 30ppt, and any changes should be made at a maximum of 1 ppt every 10 minutes
- If shrimp arrive healthy, but begin dying after a few days, they may have high levels of bacteria in the haemolymph – after confirmation, this can sometimes be reduced using 5-7 daily applications of suitable probiotics
- Regularly monitor the health of the gills and if excessive fouling by algae or filamentous bacteria is found - treat in an aerated bath with 0.1 ppm of copper control (based on copper sulphate, $CuSO_4$), or if epicommensal protozoans are found - treat in a one hour aerated bath treatment with 30-50 ppm formalin. This is done by reducing water level to 20% of maximum, turning up the aeration, adding the desired concentration of formalin (or copper) depending on the volume of water treated, leaving for one hour, and then refilling and flushing the tank, and hence diluting the treatment chemical
- Any shrimp that have serious black melanized lesions on the body, large areas of white muscle, or are bright red in colour are probably sick and should be discarded immediately before they infect the others

3.7 Broodstock Holding, Disease Checking and Feeding

Once in the hatchery, the broodstock to be used should be disease checked and those selected should be kept in a stress free environment with oceanic water quality and fed adequate quantities of high quality fresh and dry feeds so that they are able to produce high quality seed.

- On arrival at the hatchery, place the broodstock into individual holding tanks and keep them there at least until their disease status can be checked. **Try to obtain a health certificate for each broodstock, confirming that it is negative for at least MBV and WSSV.**
- **Where possible (and where this has not been done before), each individual should be checked for disease by a competent disease diagnosis laboratory with PCR capabilities. One pleopod (or a small piece of telson) from each broodstock should be cut off and preserved in 90% absolute alcohol (90 ml of absolute alcohol plus 10 ml of drinking water). This sample should be sent to a PCR laboratory for viral disease diagnosis for WSSV, MBV (and preferably also BMNV). A drop of PVP iodine solution should be placed on the area where the pleopod was removed from the broodstock shrimp, before returning it to the tank. If possible, any heavily infected broodstock should be rejected and replaced.**
- **Faeces from each individual broodstock should also be collected, placed in separate plastic bottles in seawater and sent to a competent laboratory for analysis with malachite green and H & E - stained scrapes for MBV, HPV and BMNV. If possible, any infected broodstock should be rejected and replaced.**
- **If possible, keep each broodstock in separate polystyrene-lines tanks throughout their lifetimes in the hatchery to facilitate temperature control (maintain at 28-29°C) and prevent disease spread**
- **Try to limit the stocking density of the broodstock in the maturation tanks to 2-3 individuals/m^2 for *P. monodon*, to maintain water quality and encourage spawning**
- Water should be exchanged at 200-300% per day in these tanks (preferably on a flow-through, rather than rapid change basis), permitting adequate feeding rates, whilst maintaining optimum and stable water quality (particularly temperature).
- Full strength seawater salinity should be maintained throughout, which might require importation of clean seawater by truck/boat if local water salinity is too low (especially during the rainy season)
- As for all water for the hatchery, if the incoming water has high bacterial loads, it should be chlorinated (>10ppm for 12-24h) and dechlorinated with 1ppm sodium thiosulphate for every 1ppm of residual chlorine prior to use
- Maintain water quality parameters as follows: temperature 28-29°C, salinity 30-35ppt, pH 7.5-8.5, and NH_3 ammonia and NO_2 nitrate at < 0.1ppm
- Add10-30 ppm EDTA to the reservoirs, or directly to the tanks to chelate heavy metals and reduce bacterial contamination. Suitable probiotics can be added, but only after dechlorination
- Feed shrimp with fresh, high quality feed including polychaete worms, squid, bivalve molluscs, krill or enriched adult *Artemia*, and dry broodstock diets as in Table 2

Table 2: Feeding regime for *P. monodon* broodstock

Diet	Time	Polychaete worm	Mollusc Mussel,clam,oyster	Squid	Krill or *Artemia*	Pellet
1	0.00				2%	
2	3.00			3%		
3	6.00	4%				
4	9.00		3%			1%
5	12.00				2%	
6	15.00			3%		
7	18.00	4%				
8	21.00		3%			1%

Note: Numbers are based on % of shrimp wet weight/feed

- Hermit crabs (live or dead) should not be used since they may be carriers of viral pathogens
- **A thick paste comprising vitamins A (0.2g/kg), C (2g/kg) and E (0.2g/kg) and paprika (2g/kg) or astaxanthin (0.1g/kg) should be made with a little water and mixed thoroughly with the squid, bivalve molluscs and/or pellet diet just before feeding, to increase vitamin and pigment levels in the broodstock and nauplii**
- The feed should be provided at 25-26% of the fresh body weight of the shrimp, divided into 6-8 feeds per day (See Table 2)
- Remove by net or siphon any uneaten feed before the next feeding time to maintain clean tanks (See Fig 18)

Figure 18: Removal of faeces and uneaten food from maturation tank

- It is essential to keep records of all of the above activities so that they can be inspected for irregularities when problems are encountered (See section 2.5)

3.8 Broodstock Spawning and Hatching

Broodstock must be maintained, spawned and hatched individually so that any infected broodstock cannot infect the others in the facility. Spawning and hatching techniques must be used which promote production of high quality, disease free eggs and nauplii.

- Unless the female broodstock are already mature and mated (gravid), they will need to be ablated to stimulate egg production and spawning

- Wait for at least five days before ablation of females, so that the shrimp have fully recovered from the transportation stress
- Only ablate intermoult (with fully hard shell) females (held for a short time during the process in a bucket of chilled, 20-25°C seawater to reduce stress) (See Fig 19)

Figure 19: Eyestalk ablation of female broodstock

- The eye can be either cut with hot pincers or tied with string or cut off with scissors or a sharp knife.
- Disinfect the area around the cut eye with pure liquid povidone (PVP) iodine solution after ablation.
- Wait one week to ablate pre-moult or immediately post-moult females, so that they are strong enough to withstand ablation stress
- 3-7 days after ablation, the females are typically ready for their first spawning
- **Try to spawn females and hatch batches of eggs individually to assess how many eggs have been produced by each female and reduce disease transmission between batches**
- When each female is ready to spawn (at about 5pm with females possessing stage 4 ovaries), place them into separate (1/female) >500 litre (but, the bigger the better) spawning buckets with lids or covered tanks (See Figs 20 & 21)

Figures 20 & 21: Broodstock spawning/hatching tanks

- **If possible, the water used for spawning and hatching should be filtered through active carbon, cartridge filters (1-5 micron) and preferably UV (See Figs 22 & 23).**

Figures 22 & 23: Cartridge and UV filtration systems for spawning/hatching tanks

- The spawning tanks should be partially filled with clean seawater with 10-30ppm EDTA and 0.05-0.1 ppm treflan added.
- Allow the females to release their eggs into the spawning tank. Immediately after spawning (usually between 7-12pm), remove the female and place her back into her (cleaned, washed, scrubbed and rinsed) maturation tank. Then siphon out any faeces released by the females as this could be contaminated with diseases (particularly MBV)
- At about midnight to 1.00 am (1-5 hours after spawning) harvest the eggs, disinfect them as in Appendix 2, and transfer to separate (1/female) 100-200 litre egg hatching tanks filled with clean seawater and 5-30ppm EDTA and 0.05-0.1 ppm treflan (See Figs 20 & 21)
- The hatching tank should have very slight (or no) aeration until the nauplius are hatched, and then the aeration should be increased
- **Keep hatching tanks illuminated to increase hatching rate. If a single spawn shows a low hatching rate (<40%), consider discarding this unhealthy batch. Only healthy nauplii, attracted to light (with the aeration turned off) should be harvested and stocked into larval rearing tanks**
- Allow the nauplii to hatch, then at about midday of the day following spawning, harvest the good quality nauplii that come to the light (See Figs 24 & 25) and disinfect the stage 3-4 nauplii as in Appendix 2

Figures 24 & 25: Good quality nauplii

- Check that the temperature and salinity are the same as in the larval rearing tanks and if not, slowly adjust these parameters until they coincide. The nauplii are then ready to be transferred to the larval rearing tanks

- **Preferably, each female broodstock should be spawned a maximum of 3 times before discarding in order to maintain high quality nauplii. The females should not be remated (unless the female spawns less than 3 times before the first moult)**
- Keep records of which female spawns and how many times to know which females have mated and how frequently and thus when they should be discarded (See section 2.5).

4 BMPs for Larval Rearing Management

4.1 Larval Rearing Tank Stocking and Water Exchange

To optimize water quality and reduce disease and stress levels for the growing larvae, it is important to stock the correct number of larvae and exchange sufficient water to maintain optimum water quality conditions throughout the larval rearing process.

- Before stocking nauplii into larval rearing tanks, the high quality, disinfected and washed nauplii should be counted by averaging the number from at least 3 small samples from the nauplius holding tanks. They should then be acclimated by flow-through of water from the larval rearing tank until temperature and salinity levels are equalized
- The nauplii should be stocked at a density between 100 and 150 nauplii per litre (100-150,000/m^3), assuming a full larval rearing tank (even though it may only be 50-75% full at stocking)
- **Attempt to stock the entire hatchery, or at least each unit of tanks within 3-4 days to maximize biosecurity**
- The larval rearing tanks should be filled to 50-75% of capacity with clean, disinfected, filtered seawater at 30-35ppt and 28-30°C. No water should be exchanged, but only slowly added during the delicate 4-6 day zoea stages (length of zoeal stage depends on temperature). Water is added daily until the tank is full by early mysis
- Water should be exchanged at 10-30% per day through the 4-6 day mysis period, 30-50% per day from PL1-5 and at >50% per day from PL6 until harvest at PL15. If any disease or water quality problems occur, water exchange rates should be increased
- Water temperature should be maintained stable at 28-30°C day and night, throughout the larval rearing period, whilst salinity should be maintained at 30-35 ppt until acclimation for pond conditions from PL8-10 onwards (once the gills have fully formed) if required
- Water quality parameters of temperature, salinity, pH (optimum 7.8-8.2), ammonia (optimum <0.1 ppm NH_3) and nitrite (optimum <0.1 ppm NO_2) should be monitored daily in each tank and, together with other relevant information, recorded on data sheets to ensure optimal conditions are maintained throughout the larval rearing period (see section 2.5)
- Uneaten food and faeces (seen using a light on the tank floor) may need to be siphoned from the bottom of the tanks periodically (although use of a good probiotic will minimize this requirement). This should be done by turning off the air and allowing the larvae to come to the surface of the tank. Debris from the bottom of the tank should be siphoned into a net and the contents put into a bucket to separate and return any larvae siphoned from the tank

4.2 General Health Assessment

Routine assessments of shrimp health are an important component of BMPs to ensure that any potential problems are recognized early and solutions employed to rectify the underlying causes, and thereby increase productivity.

- Assessment of larval condition is one of the most important activities carried out in the hatchery. The assessment is usually done in the morning, and decisions on water exchange, feeding and other management activities made so that action can be taken in the afternoon. The larvae in each tank should be inspected at least twice/day. Initially, a visual inspection of the larvae, the condition of the water in the rearing tank and feed consumption is made. A sample of larvae can be taken with a beaker and inspected with the naked eye or with a magnifying lense (See Fig 26)

Figure 26: Examination of larval shrimp with a magnifying lense

- Observations are made on the larval stage, health, activity, behaviour and abundance of feed and faeces in the water and the shrimp body
- The same, or a separate sample of larvae, should also be taken quickly to the laboratory for a more detailed microscopic examination. This will provide information on the stage, condition, feeding and digestion and presence of any disease or physical deformity. This information is recorded on data sheets as shown in section 2.5
- **Samples may also be sent to a PCR laboratory once (2-3 days before harvest) or twice (at nauplius or PL5 also) during the cycle for screening for viral diseases**
- The type of observations that are made can be categorized into three levels (See Table 3). They provide a simple and convenient separation based on the complexity of the techniques used

Table 3: Diagnostic level descriptions adapted for use in shrimp hatchery systems

Level 1	Observation of animal and environment. Examination based on gross features. i.e. Health examination of broodstock, sex determination, moult and ovarian development staging, removal of sick/moribund individuals, selection of nauplii by phototactic response, zoea/mysis stage feeding activity by observation of faecal strands, larval activity, postlarval health, activity and behavior, stress testing
Level 2	More detailed examination using light microscopy and squash mounts, with and without staining, and basic bacteriology i.e. Checking bacterial flora of shrimp, feed and water, microscopic examination of egg/ nauplius quality, routine microscopic examination of larval and postlarval quality
Level 3	Use of more complex methods such as molecular techniques and immunodiagnostics (e.g. PCR) i.e. Screening of broodstock, nauplii and postlarvae by PCR

Level 1 Health assessment techniques

Level 1 techniques are commonly employed in most hatcheries. Detailed examination of large numbers of larvae is not practical and hatchery staff frequently use Level 1 techniques to get a preliminary feel for the health status of larvae and to prioritize more detailed examination. Level 1 observations are frequently sufficient to make a decision about the fate of a hatchery tank or batch of larvae.

Selection of nauplii, for example, generally includes a decision based on phototactic response without the need for a more detailed microscopic examination. If a batch of nauplii shows poor phototaxis and weak swimming behavior, it should be rejected. Likewise, a severe case of white body disease in early PL, should be countered by destroying the larvae in that tank through chlorine disinfection before discharge to prevent transmission of the disease to other larvae.

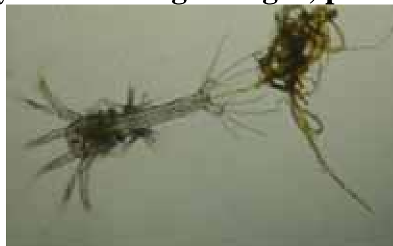
Level 1 observations include:

- ***Swimming activity*** The swimming activity of the larvae changes dramatically but predictably through the larval cycle. Zoea stages swim rapidly and consistently forwards, usually in circles, filter feeding on phytoplankton. Mysis swim backwards with intermittent flicks of their tails, maintaining themselves in the water column and feeding visually on phyto- and zooplankton. PL swim rapidly and consistently forward, searching for food whilst being maintained in the water column by strong aeration. Generally, the more larvae swimming actively, the better their quality (See Fig 27)

Figure 27: Actively swimming PLs

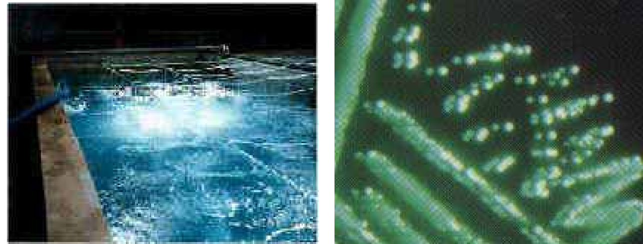
- ***Phototaxis*** Zoea stage larvae should swim strongly towards light. To test this, a sample of larvae is placed in a glass or beaker next to a light source and their movement observed. Mysis and PL do not show such attraction to light
- ***Faecal string*** During the zoeal stages, when the zoea are feeding almost exclusively on algae, long faecal strings can be seen projecting from the anus and loose in the water column. When most of the larvae have these long, continuous strings all along the digestive tube, through their bodies and continuing outside, they are considered well fed. When few larvae have them, the larvae are either being underfed or suffering from poor water quality and/or disease (See Fig 28)

Figure 28: Healthy zoea feeding on algae, producing faecal strings



- ***Luminescence*** This factor is often observed directly in the larval rearing tank in total darkness (See Fig 29). Luminescence is generally due to the presence of luminescent bacteria such as *Vibrio harveyi* (See Fig 30) If luminescence is present, it signifies high concentrations of potentially pathogenic *Vibrios* and action must be taken such as applying probiotics and/or changing water until the luminescence is gone. If treatment fails, badly affected tanks should be quickly chlorinated and drained to prevent transfer of the infection and mass mortalities

Figures 29 & 30: Luminescent bacteria in a larval tank and on a TCBS plate



- ***White body disease*** White body disease is commonly found in Vietnamese shrimp hatcheries, particularly in the dry season (March-July), a period of low tidal ranges. It typically manifests in early PL (PL3-5) stages, but can also affect younger larvae. There appear to be two forms of the disease (which may have separate causes and therefore be different diseases). The first form displays necrosis and whitening of the hepatopancreas and mid-gut, with white spots in the cephalothorax or a white line from the head to the tail. This disease causes rapid and heavy mortality. The second form shows a whitening of the tail where it bends in the 3rd abdominal segment, which gradually spreads throughout the entire body until causing death, and the body to split into two pieces. This form shows lower mortality. The water also appears to go reddish in colour, with white strands in it. The exact causes of this disease(s) are unknown, but may be related to the presence of microsporidian (fungal) parasites and/or viral disease such as Baculoviral Midgut gland Necrosis Virus (BMNV). Antibiotics are nearly always ineffective and the tank must be destroyed rapidly before the disease is transferred to neighboring tanks. Prevention through selection of disease free broodstock, proper water disinfection and treatment, tank cleaning and the use of probiotics instead of antibiotics is recommended (See section 4.6)
- ***Stage homogeneity*** This indicates the uniformity of larval stages in a tank. Most of the larvae should be within one moult stage of each other. Many stages in a single tank indicates a problem (such as disease or poor water quality) needing attention
- ***Intestinal contents*** Intestinal contents can be observed by eye in older larval stages. The intestine is visible as a dark line from the hepatopancreas in the larva's head that is easily observed in larvae held in a glass beaker. This is useful as a guide to larval feeding and feed availability. If the intestines of most larvae are not full and dark they are probably being underfed or are diseased and remedial action is required

Level 2 Health assessment techniques

Level 2 techniques should also be used in the decision-making process in shrimp hatchery management. BMP hatcheries should have a microscope that is used to make more detailed examinations of larval condition. Level 2 techniques are based on microscopic examination and squash mounts, if necessary, of a randomly taken sample of at least 20 larvae per tank (more for larger tanks). Special attention is paid to the state of the hepatopancreas and

intestinal contents, necrosis and deformity of limbs, fouling organisms and the presence of baculovirus in the faeces or hepatopancreas of older larvae.

BMP hatcheries should routinely (daily) employ basic bacteriology to identify possible pathogens when the larvae become weak or sick and determine sources of infection (i.e. algae, *Artemia* or incoming water). If they do not have these facilities, samples should be taken and sent to a competent laboratory for analysis. This information may then be used to make a decision on how the tank should be treated or whether it should be discarded.

- ***Condition of the hepatopancreas and gut contents:*** The condition of the hepatopancreas gives an indicates larval feeding and digestion. It is observed using a wet mount of a sample of larvae on a microscope slide at a magnification of 100-400X. In healthy larvae showing active feeding and digestion, the hepatopancreas and midgut will be full of small digestive or "lipid" vacuoles, and strong peristalsis will be seen in the intestine (See Figs 31, 32 & 33). If the hepatopancreas appears empty or pale, without lipid vacuoles, then the larvae are either underfed and/or diseased and require treatment

Figures 31, 32 & 33: Zoea and PL with full hepatopancreas and intestine

- ***Necrosis:*** Necrosis of the larval body and limbs, which is an indication of cannibalism or bacterial infection, can be observed by microscope under low power (See Fig 34). Necrosis should be absent. If found, it might signify underfeeding or poor water quality, leading to increased bacterial concentrations and improvements to the water quality should be made

Figure 34: PL with necrotic pleopods

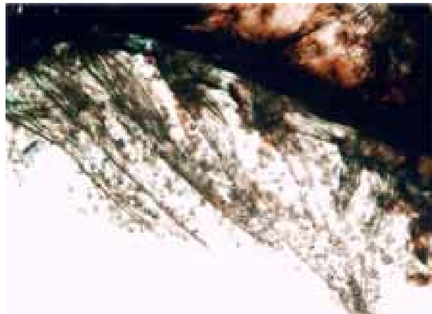
- ***Deformities:*** Deformities may indicate poor water or nauplius quality, if in the early stages, or bacterial infection, mishandling and stress later on. Typically, the fine setae on the limbs of the larvae and/or their rostrums/antennae may appear bent, broken or missing, the tail may appear bent, or the gut may terminate before the anus (See Fig 35). Typically, no remedies exist for these problems (unless due to rough handling),

and such deformed larvae will die. In severe cases, it may be preferable to discard the whole tank to prevent infection of other tanks. Deformities should be monitored and if encountered frequently on many larvae, the water quality and disease status of the tank/unit should be checked and rectified

Figure 35: PL with deformed antennae due to moulting problems

- ***Epibiont fouling:*** The larvae may become host to a range of fouling organisms ranging from bacteria and fungi through to protozoans. These will typically attach to the exoskeleton on the head and body, and particularly around the gills or legs of the larvae (See Fig 36). Where the infections are light, the next moult may remove the fouling without further problems, but in severe cases, the fouling will persist, indicating poor water quality and necessitating action, such as the application of 20-30ppm of formalin (with high aeration) for one hour, followed by a large water exchange

Figure 36: Severe fouling of PL pleopods with protozoan parasites



- ***Baculovirus:*** Baculoviruses can usually be detected in whole or squashed (stained with malachite green for *Monodon baculovirus, MBV*) preparations of hepatopancreas or faecal strands from larger-sized larvae, using a microscope (400X) to spot the characteristic viral occlusion bodies (See Figs 15 & 16). The expression of bacculoviruses can often be improved by reductions in levels of stress (i.e. improving water quality). Emphasis should be placed on prevention, which entails the selection of uninfected broodstock and disinfection of eggs and nauplii (See Appendix 2), together with proper disinfection and treatment of incoming seawater (See section 2.2.2)
- **Bolitas:** Bolitas is a syndrome involving the detachment of epithelial cells from the intestine and hepatopancreas, which appear as small spheres within the digestive tract. It is believed to be caused by bacteria and can be fatal. Some success in preventing bolitas has been achieved by rapid stocking of the hatchery (within three to four days), use of probiotics, and good health and feeding management practices

The value of Level 1 and 2 diagnosis

When all of these level 1 and 2 observations are made and recorded (see section 2.5) for each tank of larvae at each stage, an overall picture of larval health can be derived. With experience, it becomes easy to judge the health of each tank of larvae and to implement remedial measures.

Level 3 Health assessment techniques

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

4.3 Larval Feeding Regime

Larval growth and survival, and the water quality of the larval rearing tanks depend to a large extent on the quality and quantity of food offered to the larvae. Optimization of feeding regimes based on live feeds helps maintain good water quality, whilst promoting fast growth and high survival of the larvae and hence optimal production from the hatchery.

- The feeding regime used in BMP hatcheries should be based on the use of live/preserved algae for zoea and mysis (See section 4.4), frozen/dead *Artemia* nauplii (either umbrella stage, or nauplii killed or stunned by freezing) only for mysis stages (since mysis have difficulty catching live *Artemia* nauplii) and live *Artemia* nauplii for PL stages (see section 4.5)
- Live diets require supplementation with artificial dry or liquid diets to achieve optimum production of larvae. Dose rates for artificial diets should be based on observations of the larval feeding habits and water quality, manufacturer's recommendations and experience. Take care not to overfeed with formulated diets, as this may lead to water quality problems and fouling of the larvae. Algae dose rates are shown in section 4.4 and Table 6. *Artemia* dose rates are calculated based on the use of about 6 kg of *Artemia* cysts/million PL produced, whilst trying to always maintain 3-5 Artemia nauplii/ml in the larval rearing tanks (See section 4.5)
- For zoea, **the best feed (if available) is clean live algae (*Chaetoceros, Thalassiosira* or *Skeletonema Sp.*), maintained at 80-130,000 cells/ml.** Alternatively, dried *Spirulina* and liquid or powdered/microencapsulated dry diets (10-80 microns in size) can be fed every 2-4h if live algae are not available. Zoea should be checked frequently (multiple time per day) to see if they have long faecal trails and full digestive tracts (See Figs 28, 37 & 38), which indicate the zoea are healthy and feeding.

Figures 37 & 38: Healthy zoea feeding on algae and artificial diets

- Formulated diets should be fed to zoea alternately to the addition of live algae, up to 6 times per day each over a 24 hour period, to satiation. If the zoea maintain long trails of faeces, then they are being fed enough. Care should be taken to ensure that overfeeding does not occur. This can be checked by examination of the amount of food in the water and the gut of the larvae, and the amount of faeces and uneaten food on the tank bottom, which should be siphoned out daily
- Disinfected *Artemia* at umbrella or first instar nauplius stage and killed by freezing should be fed to mysis stage larvae, whilst live, first instar *Artemia* nauplii should be fed to postlarval stage shrimp (See section 4.5)
- For mysis, similar dry or liquid diets of 50-150 micron sized particles should be fed every 2-4 hours to demand, which is judged by observation of the quantities of food in the larvae and in the water (See Figure 39 & Table 4)

Figure 39: Healthy mysis feeding on algae, *Artemia* and artificial diets

Table 4: Feeding regime for zoea and mysis stage larvae

Day	Stage	Time											
		0:00	2:00	4:00	6:00	8:00	10:00	12:00	14:00	16:00	18:00	20:00	22:00
1	N/Z1					A		A	F1	A	F1	A	F1
2	Z1	A	F1	A	F1	A	F1	A	F1	A	F1	A	F1
3	Z1/Z2	A	F1	A	F1	A	F1	A	F1	A	F1	A	F1
4	Z2	A	F1	A	F1	A	F1	A	F1	A	F1	A	F1
5	Z3	A	F1	A	F1	A	F1	A	F1	A	F1	A	F1
6	M1	A/D	F2	A	F2/D	A	F2	A/D	F2	A	F2/D	A	F2
7	M2	A/D	F2	A	F2/D	A	F2	A/D	F2	A	F2/D	A	F2
8	M3	A/D	F2	A	F2/D	A	F2	A/D	F2	A	F2/D	A	F2
9	M3/PL	A/D	F2	A	F2/D	A	F2	A/D	F2	A	F2/D	A	F2

Note:
A = Algae (Live or preserved *Chaetoceros, Thalassiosira or Skeletonema Sp.* algae)
F1 = Formulated feed (dry/liquid feed/spirulina etc) 10-80 microns
F2 = Formulated feed (dry/liquid feed/spirulina etc) 50-150 microns
D = Dead (frozen) *Artemia* nauplii

For PL, a variety of similar dry, liquid and crumbled flake diets of 200-300 micron sized particles for PL1-8 and 300-500 microns from PL9-15, should be fed. This feeding is in the same manner, little and often, once every 4 hours (6 times/day) over each 24 hour period, alternating with up to 6 feedings per day of newly-hatched, live *Artemia* nauplii (See Fig 40 & Table 5).

Figure 40: Healthy PL feeding on algae, *Artemia* and artificial diets

Table 5: Feeding regime for postlarval stage larvae

Day	Stage	Time											
		0:00	2:00	4:00	6:00	8:00	10:00	12:00	14:00	16:00	18:00	20:00	22:00
10	PL1	L	F3	L	F3	L	F3	L	F3	L	F3	L	F3
11	PL1	L	F3	L	F3	L	F3	L	F3	L	F3	L	F3
12	PL3	L	F3	L	F3	L	F3	L	F3	L	F3	L	F3
13	PL4	L	F3	L	F3	L	F3	L	F3	L	F3	L	F3
14	PL5	L	F3	L	F3	L	F3	L	F3	L	F3	L	F3
15	PL6	L	F3	L	F3	L	F3	L	F3	L	F3	L	F3
16	PL7	L	F3	L	F3	L	F3	L	F3	L	F3	L	F3
17	PL8	L	F3	L	F3	L	F3	L	F3	L	F3	L	F3
18	PL9	L	F4	L	F4	L	F4	L	F4	L	F4	L	F4
19	PL10	L	F4	L	F4	L	F4	L	F4	L	F4	L	F4
20	PL11	L	F4	L	F4	L	F4	L	F4	L	F4	L	F4
21	PL12	L	F4	L	F4	L	F4	L	F4	L	F4	L	F4
22	PL13	L	F4	L	F4	L	F4	L	F4	L	F4	L	F4
23	PL14	L	F4	L	F4	L	F4	L	F4	L	F4	L	F4
24	PL15	L	F4	L	F4	L	F4	L	F4	L	F4	L	F4

Note:
L = Live *Artemia* nauplii
F3 = Formulated feed (dry/liquid feed/flake etc) 200-300 microns
F4 = Formulated feed (dry/liquid feed/flake etc) 300-500 microns

For all of these formulated diets, sufficient aeration must be maintained to keep the feed particles in suspension. Then they will always be available for consumption and do not settle to the tank bottom where they are unavailable and may lead to deterioration in water quality. Check water quality and siphon wastes daily.

4.4 Use of Live or Preserved Algae

Live (or preserved, if live are unavailable) diatom microalgae are the perfect food for early larval stage (zoea and mysis) shrimp. Not only do they offer the perfect nutrition, they also are self-suspending in the water column, enhance water quality (by absorbing ammonia, nitrite and carbon dioxide etc.), they maintain shade in the water, they

produce natural and helpful antibiotics, and they act as enrich the nutritional value of *Artemia*. The supplementation of artificial foods with live microalgae should therefore be a prerequisite of a BMP hatchery.

- **If live algae is bought as a starter culture, the species should be either *Chaetoceros Sp*., *Thalassiosira Sp*. or *Skeletonema Sp*., which can be bought from one of the RIA stations (or elsewhere) in pure (sterile) 20 litre bags or bottles. One (or more) such bags are put in a disinfected (>10ppm chlorine) algae tank and fertilized with a good microalgal fertilizer, with aeration and allowed to bloom for 2-3 days direct sunlight (See Figs 41, 42 & 43)**

Figures 41, 42 & 43: Stages in pure algal culture

- **The bloomed algae is then pumped into the larval rearing tank or filtered through a fine mesh bag and the concentrate added to the larval rearing tanks. In both cases, the final cell densities (for *Chaetoceros Sp*.) should be at 80-130,000 cells/ml for zoea and mysis (peaking at Z3), declining to 50-60,000 cells/ml during early PL stages (optional) (See Table 6)**

Table 6: Algae feeding regime for zoea, mysis and early PL stage larvae

Day	Stage	Time					
		0:00	4:00	8:00	12:00	16:00	20:00
1	N/Z1			80-100	80-100	80-100	80-100
2	Z1	80-100	80-100	80-100	80-100	80-100	80-100
3	Z1/Z2	80-100	80-100	80-100	80-100	80-100	80-100
4	Z2	100-130	100-130	100-130	100-130	100-130	100-130
5	Z3	100-130	100-130	100-130	100-130	100-130	100-130
6	M1	100	100	100	100	100	100
7	M2	100	100	100	100	100	100
8	M3	80-100	80-100	80-100	80-100	80-100	80-100
9	M3/PL	80	80	80	80	80	80
10	PL1	60-80	60-80	60-80	60-80	60-80	60-80
11	PL2	60	60	60	60	60	60
12	PL3	60	60	60	60	60	60

Note:
Numbers are in thousands of algae cells/ml to be maintained in larval rearing tank

- **If concentrated preserved algal products are to be used, they should be purchased fresh and maintained in a fridge until required. They are then added up to 6 times per day in small quantities as per manufacturer's recommendations, to maintain the desired algal cell densities as shown in Table 6.**

4.5 *Artemia* Hatching and Disinfection Procedures and Use

4.5.1 Hatching procedures

Specific *Artemia* egg hatching procedures should be used to obtain the highest number of *Artemia* nauplii from each can of cysts hatched. These techniques are necessary to produce clean *Artemia* for feeding the larvae at the lowest possible cost.

- Clean and disinfect the *Artemia* hatching tanks by washing with water, scrubbing with a chlorine-soaked brush or cloth, washing again and then filling with clean, disinfected seawater (>10ppm chlorine for >12 hours, then dechlorinated with sodium thiosulphate at 1 ppm for every 1 ppm of chlorine remaining)
- **If possible, decapsulate the *Artemia* cysts prior to hatching (See Appendix 3)**
- **To improve the hatching rate, instead of full-strength seawater, use 18-25 ppt seawater (which is made from seawater diluted with clean freshwater) at 28-30°C**
- **In order to reduce bacterial loading of the hatching tanks, either 60ppm of chloramine-T or 20ppm of suitable probiotics can be added to the tanks with the cysts**
- Add the (preferably decapsulated) *Artemia* cysts/eggs) to the conical shaped hatching tank at <2 kg cysts/m^3 of water (<2 cans of 425-450 g/500 litre tank). Place an airstone in the tank and provide full and constant aeration. **Switch on a light placed 30cm above center of each tank and leave light on constantly (day and night) (See Figs 44 & 45)**

Figures 44 & 45: *Artemia* hatching tanks

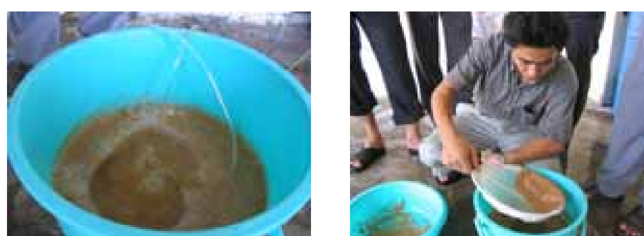
- If umbrella stage nauplii are required (i.e. for mysis stage larvae) they should be harvested after 15-18 hours, washed in running freshwater and fed immediately. If first instar *Artemia* nauplii are required, they should be harvested after about 20-24 hours when most of the cysts have hatched
- To harvest the *Artemia*, switch off the air, allow the nauplii to settle and collect the live nauplii from the bottom of the tank in a 100 micron (200 mesh) net. Wash thoroughly under clean running fresh (preferably) or seawater then disinfect as described below

4.5.2 *Artemia* nauplii disinfection techniques

Following harvesting, specific techniques should be used to disinfect the *Artemia* nauplii from viral, bacterial, fungal, microsporidian and parasitic diseases and remove unhatched cysts from the *Artemia* nauplii. This will help maintain the larval rearing tanks free from disease and organic material which serves as a nutrient source for disease organisms.

- **These same techniques should be used whether or not the *Artemia* have been decapsulated**
- **To disinfect the nauplii, the washed and de-watered nauplii are placed into a 15-20 litre bucket which is half filled with 10 litres of clean seawater.**
- **To this bucket, 125 ml of 50% hydrogen peroxide (H_2O_2) is added and the aeration turned on. The hydrogen peroxide will form bubbles and all of the unhatched cysts and debris will form foam on the surface of the water.**
- **The air is turned off after 5 minutes, the foam allowed to float and then the debris is removed using a small net (See Figs 46 & 47)**

Figures 46 & 47: Collection of cysts and floating debris from the hydrogen peroxide bucket



- Once all the debris has been removed and thrown away, pour the live nauplii into a 100 micron mesh net and wash thoroughly in running clean freshwater (preferably) or seawater
- The *Artemia* are now ready to be fed to the larvae, or can be placed into the fridge (or iced water) for later feeding. **If keeping them in the fridge, they can be stored at up to 5 million nauplii in one litre of water, without aeration, at 4°C for up to 2 days**
- The feeding rate of *Artemia* required depends on the health, consumption and stage of the larvae, but a general idea, based on the use of 6.4 kg *Artemia* cysts/million PL15 produced is shown in Table 7

Table 7: Quantity of *Artemia* to feed to each larval shrimp stage

Day	Larval Stage	kg cysts/million larvae/day
1	n	
2	z1	
3	z1/2	
4	z2	
5	z3	0.10
6	m1	0.15
7	m2	0.20
8	m3	0.25
9	p1	0.30

10	p2	0.35
11	p3	0.40
12	p4	0.45
13	p5	0.50
14	p6	0.50
15	p7	0.50
16	p8	0.50
17	p9	0.48
18	p10	0.45
19	p11	0.40
20	p12	0.35
21	p13	0.30
22	p14	0.25
Kg cysts total/million PL		**6.4**

Notes: This feeding rate is per 1 million surviving larvae at each stage
From PL9 onwards, *Artemia* use is replaced by increasing percentages of formulated feeds to acclimate the PL to pond conditions

4.6 Use of Probiotics to Replace Antibiotics

Antibiotics should not be used in a BMP hatchery. Antibiotics are problematic because: they are often ineffective for major problems, they are expensive, dangerous and illegal (for some), they can create strains of bacteria resistant to antibiotics (even for humans), they cause slow growth and low immunity in the larvae, and it is illegal to export shrimp containing antibiotic residues. The use of effective probiotics exclusively is therefore recommended and tends to work better than using antibiotics.

- **A good probiotic (high cell count [>10^9 cfu/g] of multiple bacterial strains) should be used from zoea stage 1 throughout the larval and post-larval rearing stages on a daily basis**
- **The probiotic is first weighed depending on tank size and dose rate, and then put into a bucket with seawater and aerated for 1-24 hours. It is then filtered through a 100 micron (200 mesh) net. The solid material (bran carrier) should be discarded and the liquid filtrate added to the larval rearing tanks daily (See figs 48 & 49)**

Figure 48 & 49 Probiotic hydration and filtration

- **Typical dose rates for zoea are 2-3ppm/day, for mysis are 3-4 ppm/day and for PL are 4-5 ppm/day, according to the manufacturers recommendations**
- **Wherever possible, antibiotics should never used at any phase during the larval rearing cycle**

4.7 PL Quality Testing

PL quality can be assessed according to the NACA/SUMA pamphlet “10 steps to stock ponds with good quality seed” (See Appendix 4). Alternatively, a protocol has been 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% premium being paid to PL that pass the assessment, particularly where this involves screening for WSSV.

This PL quality assessment involves 5 main areas: Gross examination, microscopic examination, stress test, V*ibrio* test and PCR screening. The last two tests may involve taking samples which 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, 2-3 days before harvesting the PL (at PL15), providing enough time to a) complete the PCR and bacteriological analyses and b) take remedial action should the PL score be too low.

4.7.1 Gross examination

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

4.7.2 Microscopic examination

For this closer examination, a sample of 20-30 PL are randomly selected and examined at 100-400X magnification. The 6 criteria used are scored using a standard score and are:

1. **Hepatopancreas (HP):** Full and dark with lipid vacuoles (score 10), partially dark and full with some vacuoles (score 5), Light, empty, no valcuoles (score 0)
2. **Gut condition:** Full, with vacuoles and peristalsis (score 10), Partially full, some vacuoles and peristalsis (score 5), Empty, no vacuoles or peristalsis (score 0)
3. **Fouling (protozoa, bacteria or dirt):** No fouling (score 10), medium fouling (score 5), heavy fouling (score 0)
4. **Deformity (moulting problems, necrosis):** None (score 10), slight (score 5), heavy (score 0)

5. **Muscle:gut ratio (in the 6th abdominal segment):** muscle 75% of width (score 10), muscle 50-75% of width (score 5), muscle <50% width (score 0)
6. **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 3 zero scores fails, regardless of the overall score.

4.7.3 Stress testing

This involves an exposure of the PL to 50% of the ambient salinity 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, placed into the beaker, and after 3 hours, the PL that are still active or which move when prodded with a needle are counted and the result counted as a percentage (See Fig 50)

Figure 50: Stress-testing PL

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

Survival (%) = (No. active PL/total PL in beaker) * 100

Scoring: the PL batch passes if the survival >75% and fails if below this score.

Note: 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 >12mm total length.

4.7.4 Vibrio examination

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

A random sample of 100 PL are taken from the tank, sterilized externally by dipping in 70% alcohol, washed, ground and then streaked with sterile wire loops, or 0.1ml pipetted, all over 2 replicate TCBS (+1.5% NaCl) and 2 replicate TSA (+1.5% NaCl) media plates (1 loop per plate). The plates are then incubated at 30-35°C for 18-24 hours and the number of green and

yellow colonies counted and the average taken for the 2 plates of each media type (See Figs 51, 52 & 53)

Figures 51, 52 & 53: Preparation and use of TCBS agar plates in bacteriology

Scoring: The PL pass if the number of green colonies is <60/plate and the number of yellow colonies is <80/plate on the TCBS plates, and if no luminescence is noted in the TSA media. If not within these criteria, the PL fail the test.

4.7.5 PCR testing

PCR testing for WSSV can help reduce the risk of crop loss due to this disease and should therefore be checked on each batch of PL stocked.

A sample of 150 shrimp (preferably the weakest out of a larger batch of salinity-stressed shrimp) are taken from each tank and preserved in 90% alcohol. The samples are then sent to a PCR laboratory and analysed using a 2-step, or nested PCR technique (See figs 54-57)

Figures 54-57: PCR testing procedures

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

Other viruses can be tested for (i.e. YHV and HPV for *P. monodon*) if the funds and equipment are available and the analyses desired.

4.8 PL Harvest and Transportation

Harvest and transport of PL should be done gradually and with minimum stress. 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.

- **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 which can lead to high mortalities if not done smoothly. Salinity adjustment can be done by adding freshwater to achieve a salinity change of <3ppt/hour from 30-20 ppt, but should be reduced to <1ppt/hour from 20-10 ppt and <0.5ppt/hour from 10-5 ppt. Such salinity adjustments should commence only when the PL are older than PL10 (preferably 2-3 days before harvest), when their gills are fully developed (look like Christmas trees) and they are able to tolerate such rapid salinity changes**
- 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 PL/M^3. Any wastes should be siphoned and 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 minutes to help reduce stress. Water salinity should be the same as the water in the larval rearing tanks (which ideally should have already been adjusted to that found in the target ponds)
- 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 25-28°C for transportation times of 1-3 hours, 23-25°C for 3-12 hours or 18-23°C for over 12 hours
- Separate all PL packaging material and equipment. Carefully disinfect hands before and after packaging of each tank to avoid spreading pathogens during harvesting and transportation
- 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 (See figs 58, 59 & 60).

Figures 58, 59 & 60: Transportation of PL from hatchery to farm

- 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 (1g/l) can be added to absorb any ammonia produced by the PL and tris HCL buffer can be added at 100mg/litre to stabilize pH. Live *Artemia* nauplii should be disinfected, washed (with fresh water) and added at 15-20/PL for each 4 hours of transport to provide food and prevent cannibalism during transportation
- If using tanks, the tanks and all other equipment (nets, airstones and airlines etc) should be first disinfected with 20 ppm chlorine, washed and dried thoroughly. The transportation vehicle should also be disinfected (at least 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. Somebody should be charged with checking the tanks frequently to ensure that everything is working
- If using plastic bags, these should be double (using 2 bags one inside the other) to prevent loss due to bag breakage. These bags should be $^{1}/_{3}$ 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), which are taped shut and are then ready for transport
- In either case, stocking densities up to PL15 should range from 500-1,200 PL/litre (depending upon PL size and transportation time) or a maximum of 2.5g/litre. If larger PL or juveniles are transported, a corresponding reduction in stocking density, maintaining this figure of 2.5g/litre should be used
- **Efforts should be made if possible to only conduct transportation at night, when temperatures are cooler, 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**

Appendix 1: Examples of recording sheets

Quarantine/Maturation tank daily data sheet

Tank No: **Sex:** **Date stocked:** **Source:** **Condition:** **Gravid:** **Weight (g):** **Length (cm):** **Date of ablation:**

Date	Time	Volume	No. males	No. females	Feed type	Feed amount (g)	Feed left over	Water exchange (%)	Temperature (^oC)	Salinity (ppt)	Notes

Spawning/Hatching tank daily data sheet

Tank No:

Date	Volume	No. females	Ovary stage	No. Source tank (ST or MT)	No. spawned	No. Eggs	No. nauplii	Temperature (^oC)	Salinity (ppt)	Transferred to LRT No.	Notes

Larval rearing tank daily data sheet

Tank No: **Date stocked:** **Nauplii stocked:** **Female No.:** **PL harvested:** **Survival (%):**

Day	Date	Larval Stage	Larval Number	Larval Health	Tank Volume (m^3)	Water Exchange (%)	Temp (^oC)	Algae			*Artemia*		Formulated feed		Notes
								Species	Cells/ml In tank	Cells/ml Fed	No/ml In tank	No/ml Fed	Type	g fed	
1		N5/Z1													
2		Z1													
3		Z1/Z2													
4		Z2													
5		Z3													
6		M1													
7		M2													
8		M3													
9		M3/PL													
10		PL1													
11		PL2													
12		PL3													
13		PL4													
14		PL5													
15		PL6													
16		PL7													
17		PL8													
18		PL9													
19		PL10													
20		PL11													
21		PL12													
22		PL13													

Larval health data sheet

Tank No: **Date:** **Average Score: (3 good, 2-3 medium, <2 poor):**

Larval Sample	Swimming Activity	Feeding	Stage Homogeneity	Deformity	Luminece	Fouling	Saete Developmt	White body disease	Phototaxis	No. Dead	Notes
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
Total											
Average											

Notes:
Scores: 3 Good, highest, most
2. Medium
1. Poor, lowest, least

PL quality testing results sheet

Tank No.: **Date:** **PL stage:** **Female No.:** **Score (>50 pass):**

1. Gross examination			
Characteristic	Comment		
Size distribution			
Size (more than 12mm)			
Swimming activity			
Feeding			
Colour			
2. Microscopic examination			
Hepatopancreas	Full, dark (10)	Medium (5)	Empty, pale (0)
Gut condition	Full, vacuoles (10)	Medium (5)	Empty (0)
Fouling	None (10)	Medium (5)	Heavy (0)
Deformity	None (10)	Medium (5)	Heavy (0)
Muscle:gut ratio	75% (10)	50-75% (5)	<50% (0)
MBV	None (10)	Few (5)	Many (0)
3. Stress test			
Freshwater (1:1)	>75% (pass)	<75% (fail)	
Formalin (100ppm)	>75% (pass)	<75% (fail)	
4. Vibrio testing			
Green	<60/plate (pass)	>60/plate (fail)	
Yellow	<80/plate (pass)	>80/plate (fail)	
Luminescence	Absent (pass)	Present (fail)	
5. PCR testing			
WSSV	Absent (pass)	Present (fail)	

Appendix 2: Washing/Disinfection Protocols for Shrimp Eggs/Nauplii

WSSV and MBV are viral pathogens that can lead to huge production losses in ponds. For this reason, increasing control over the presence of these pathogens is being implemented by NAFIQAVED and can lead to the destruction of severely infected batches.

Both WSSV and MBV can be transmitted from the broodstock to the offspring. However, when the shrimp are in the egg and nauplius stages, the pathogens are generally present only on the outside of the shrimp seed. Therefore, washing and disinfecting the outside of the eggs and nauplii can greatly reduce the risk of transmission of these pathogens from infected broodstock to their offspring.

Here we present a technique for washing and disinfection of eggs and nauplii. These procedures are cheap and simple, but effective, so should be used routinely. Their use has been shown to avoid the transmission not only of WSSV and MBV, but also of other diseases, including pathogenic *Vibrio Sp.* bacteria (such as the luminescent *V. harveyi*), that can cause poor health and/or mortality of the shrimp larvae.

You need:

- Clean and disinfected tanks/buckets
- A clean 300 micron mesh
- A clean and disinfected 50-60 micron mesh for handling eggs
- A clean and disinfected 50-100 micron mesh for handling nauplii
- A source of aeration (air pump, airline and air stones)
- Clean seawater
- Povidone iodine (10% liquid PVP or povidone iodine) or Virkon-S
- Formalin (a solution of 37% formaldehyde gas)
- EDTA
- Treflan

Treatment of eggs:

If possible, following spawning, the broodstock should be quickly removed from the spawning tanks and 1-5 hours after spawning the tanks drained slowly through a preliminary 300 micron mesh (to retain faeces and other debris) and a secondary 50-60 micron nylon net partially submerged in a tank/bucket, to retain the eggs. The 300-micron mesh net containing the faeces/debris, is then removed and disinfected (See Fig 61).

Figure 61: Harvesting eggs for washing and disinfection

The eggs (still in the **50-60 micron** net, partially submerged in a tank/bucket) should then be washed for 5 minutes with a steady, but slow current of clean seawater at the same temperature and salinity as the water in the spawning tanks (See Fig 62).

Figure 62: Washing the eggs with clean seawater

After washing, the eggs are gathered in the net and then dipped into an aerated bath of 50 ppm povidone iodine solution (0.5 ml in 10 liters of water) for one minute (See Figs 63, 64 & 65)

Figures 63, 64 & 65: Disinfecting eggs with povidone iodine

Finally, they are washed once again for 5 minutes with a steady, but slow current of clean seawater, this time at the same temperature and salinity as the water in the hatching tanks (See Fig 62).

The eggs are then transferred to the hatching tanks, which are prepared with 5-30 ppm EDTA (5-30g per m^3 water) to remove heavy metals and 0.05-0.1 ppm treflan (0.05-0.1 ml per m^3 water) to kill fungi.

Treatment of nauplii:

After the eggs have hatched to nauplii they should be harvested from the hatching tank through a 50-100 micron nylon net partially submerged in a tank/bucket (See Fig 62).

Only nauplii that are attracted to the light should be collected, since these are the healthiest. The nauplii should then be washed for 5 minutes with a steady, but slow current of clean seawater at the same temperature and salinity as the water in the spawning/hatching tanks (See Fig 62).

After washing, the nauplii are gathered in the net and then dipped into an aerated bath of 100-300 ppm (1-3 ml/10 litres) of formalin for 30 seconds. They are then dipped into an aerated bath of 50 ppm (0.5 ml/10 litres) povidone iodine (or Virkon-S) solution for one minute (See Fig 66). Finally, they are washed again for 5 minutes with a steady, but slow current of clean seawater, this time at the same temperature and salinity as the water in the larval rearing tanks (See Fig 62 and Table 8).

Figure 66: Disinfecting nauplii in povidone iodine solution

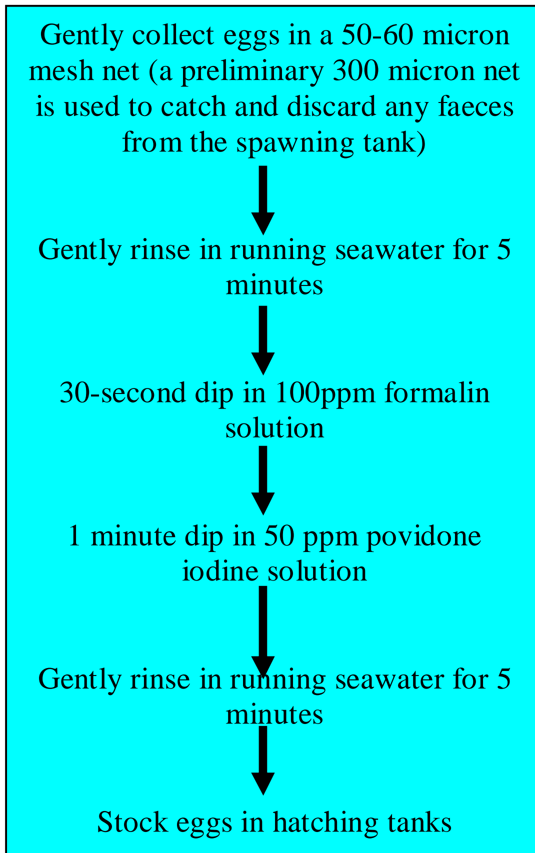
The nauplii are then already acclimated and are ready for transfer and stocking into the larval rearing tanks.

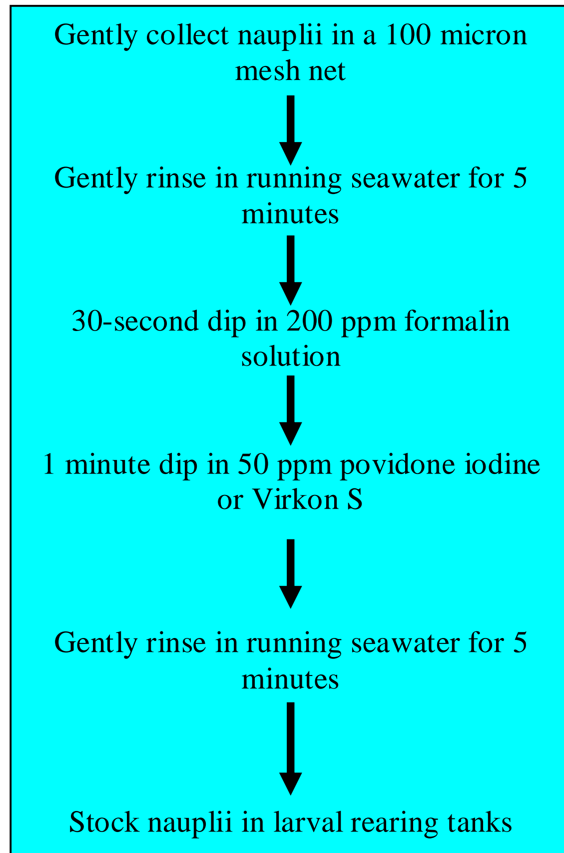
Notes:

This procedure for nauplii works best when it includes all the steps: washing, formalin and then povidone iodine disinfection and then final washing. However, if one or more of the chemicals is unavailable, the procedure should be conducted using what is available, even if it means only the washing stage, since washing alone will help greatly in reducing transmission of viruses, bacteria, fungi and debris from the broodstock to the eggs/nauplii.

Experience with BMP pilot hatcheries in Vietnam has shown that this protocol can remove *Vibrio Sp.* bacteria and help reduce WSSV and MBV viral loads from the nauplius used for stocking the larval rearing tanks.

Table 8: Washing and disinfection procedures for eggs and nauplii:





Appendix 3: Decapsulation Protocols for *Artemia* Cysts

Artemia **cysts consist of an egg surrounded by a shell or cyst. Many pathogens are present on the shell and may be introduced into the larval rearing tanks when adding *Artemia*. To avoid this, the removal of the cyst (decapsulation) and/or disinfection of the *Artemia* eggs is essential. In this appendix we present 2 methods for decapsulating *Artemia* cysts. Whichever method is used, the advantages over non-decapsulated cysts are the same: better disinfection of the cysts, leading to reduced bacterial/fungal contamination of the larval rearing tanks, and higher hatching rate, leading to better cost efficiency.**

If decapsulation is not possible, then the *Artemia* cysts should be washed in 20ppm chlorine prior to hatching and then disinfected with hydrogen peroxide and washed again after hatching (See later).

The decapsulation methods presented are intended for the decapsulation of 1 kg of *Artemia* cysts. To decapsulate a different amount of cysts modify also the amount of chemicals used.

You need:

- ***Artemia*** **cysts**
- **Clean buckets to be used only for decapsulation**
- **Two clean and disinfected 60-80 micron meshes**
- **A thermometer**
- **Freshwater**
- **Seawater**
- **Aeration**
- **Ice**
- **100g Sodium thiosulphate**
- **Salt**

For decapsulation method 1 (/kg cysts)

- **40g of Sodium Hydroxide (NaOH)**
- **4 liters of Sodium hypochlorite**

OR

For decapsulation method 2 (/kg cysts)

- **250g Calcium oxide (CaO)**
- **550g Calcium hypochlorite**

Hydration:

First open the *Artemia* cans and pour the cysts into a large plastic bucket. Add 3-4 liters of freshwater and hydrate with continuous aeration for one hour (See Fig 67).

Figure 67: Hydration of Artemia cysts prior to decapsulation

Then collect the hydrated cysts by emptying them through a 60-80 micron mesh and washing under running freshwater (See Fig 68).

Figure 68: Collecting and washing hydrated cysts

Decapsulation:

There are two methods for decapsulation – method 1 (traditional) and method 2 (longer, but cheaper).

Method 1:

Empty the net with the hydrated cysts into the bucket and add 40g of sodium hydroxide (NaOH), 4 litres of sodium hypochlorite liquid (chilled to 4°C) and 4 litres of seawater (also chilled to 4°C) per kg of cysts (See Figs 69 & 70).

Figures 69 & 70: Chilled sodium hypochlorite and seawater being added to hydrated cysts

Stir continuously for 5-8 minutes (See Fig 71), until the eggs begin to turn orange (See Fig 72). Whilst stirring, the solution will warm up. Check the temperature regularly and maintain it at 18-25°C by adding ice to the bucket.

Figures 71 & 72: Stirring the cysts during decapsulation and checking for orange colour

Method 2:

Empty the net with the hydrated cysts back into the bucket and add about 7 litres of seawater cooled with ice to a temperature of 20°C. Add calcium oxide (CaO) at the rate of 125g/kg of cysts. Stir until mixed. Then add Calcium hypochlorite at the rate of 275g/kg of cysts. Stir continuously, check the temperature regularly and maintain it at about 40°C by the addition of ice. After having stirred for about 5-8 minutes, add some more ice to decrease the temperature to 30°C. Then add some more CaO (125g/kg cysts) and calcium hypochlorite (275g/kg) and stir for another 5-8 minutes until the eggs begin to change in color from white to orange. The whole procedure will now have taken 10-16 minutes.

Stopping the reaction and washing the *Artemia* eggs:

Immediately after the eggs have turned orange, empty them into a clean 60-80 micron mesh and wash with running freshwater (See Fig73).

Figure 73: Collecting and washing decapsulated eggs

Then empty the net into another bucket containing a solution of sodium thiosulphate at 100g/kg of cysts in 1 liter of freshwater and stir with aeration for 5 minutes. This will neutralize the remaining chlorine and stop the reaction.

Stop the aeration and the decapsulated eggs will sink and any remaining cysts and debris will flat to the top, where it can be caught with a net and discarded (See Figs 74, 75 & 76).

Figures 74, 75 & 76: Collecting cysts and floating debris from thiosulphate bucket

Collect the decapsulated eggs by emptying them into a 60-80 micron mesh, wash them once again and squeeze the net to remove most of water. The eggs are then ready for being put into the *Artemia* hatching tank or for being preserved in salt (See Fig 77).

Figure 77: Decapsulated eggs in *Artemia* hatching tank

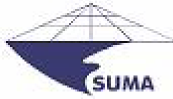
Preservation:

There are two methods for storing the decapsulated eggs if they are not required immediately. They can either be preserved by putting them in 1 liter of water containing 300g of salt, or in dry refined salt at about 300g NaCl/kg of cysts (See Fig 78).

Figure 78: preservation of decapsulated eggs in salt

The first technique keeps the eggs in good condition for about 1 week, during which time they should be used. The second technique can preserve the eggs for up to 7 weeks. If using the second technique, drain off the water produced during the salting process and store the eggs in the shade at room temperature.

Appendix 4: Leaflet on how to plan stocking with good quality seed



Reducing Risks of Aquatic Animal Disease Outbreaks

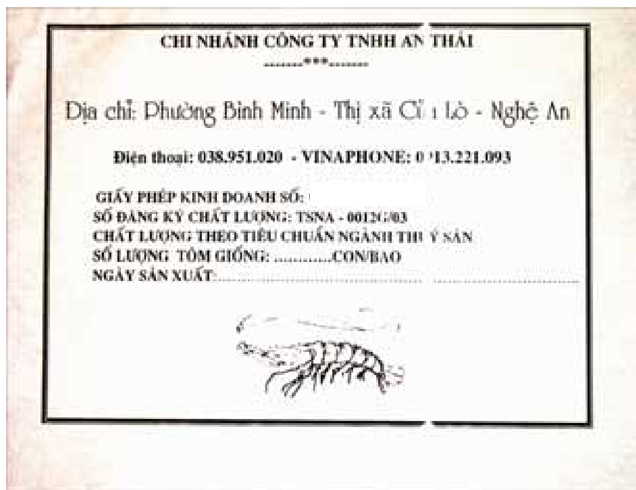
10 STEPS FOR PLANNING TO STOCK WITH GOOD QUALITY SEED

March 2004

1) Stock your pond only once per crop. Organize stocking so that all the farmers in your area stock within 3-4 days. Within one area, try to stock the seed from the same batch in neighboring ponds.

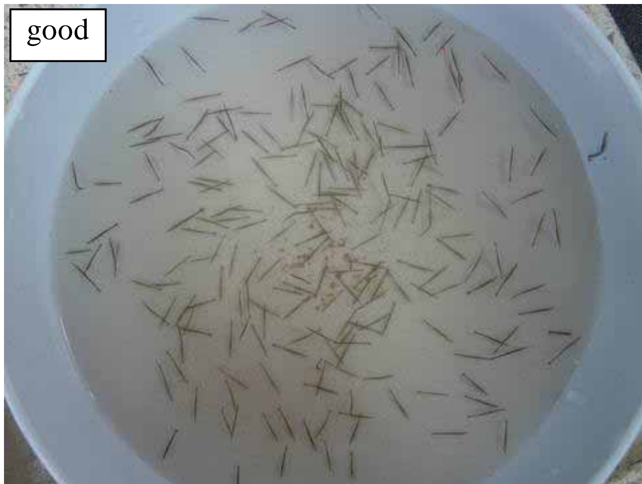
The guidelines that follow will help you to select good quality seed. If you cannot find a batch with good quality, use these guidelines to select the best batch available to you.

2) Check if the hatchery has the certificate for good postlarvae. If they do, make sure that all the bags of postlarvae that you buy have a hatchery label (see picture). Try to buy the postlarvae from a hatchery that has these labels.



3) Select seed batches with good activity. To do that, collect a sample from the bottom of the tank. Put the postlarvae in a large bowl and stir the water rapidly. If most of the postlarvae concentrate in the center, it means that they are weak and the batch is poor.

4) Select seed batches with large postlarvae of about the same size. Postlarvae 15 should be about 12mm in total length. If they are smaller, they may not be ready for stocking. If the size difference is large, some postlarvae may be diseased, underfed and of low quality.

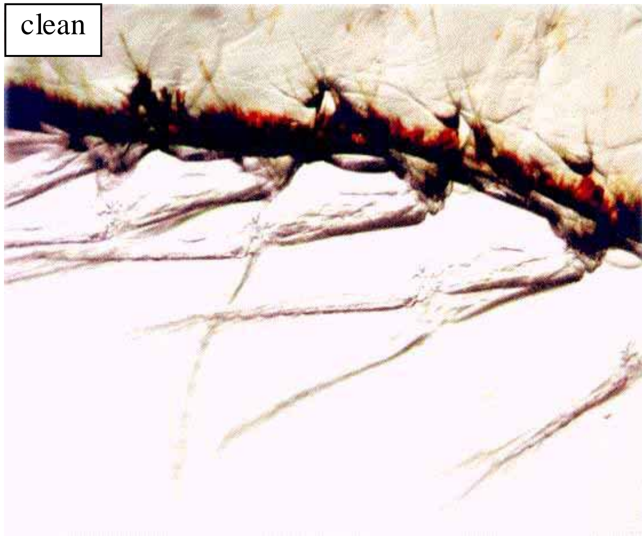


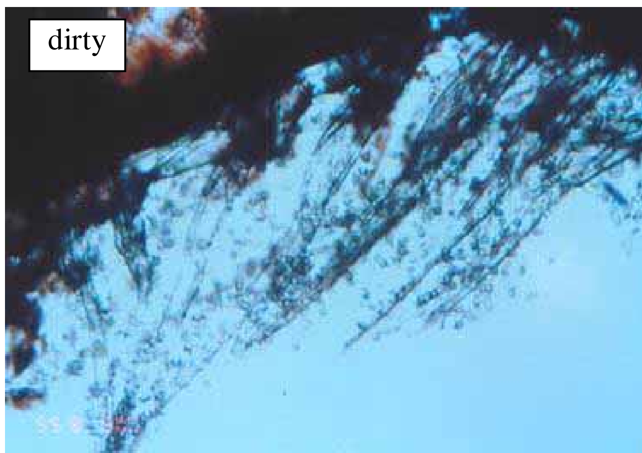
5) Take another 20-30 PL from the batch, put them in a glass half full with the water they came in, add an equal volume of freshwater and wait for one hour. If more than one quarter dies, look for a better batch.

6) If you have a magnifying lense, take 15 postlarvae from the batch and see if any of them have a bad (small and/or empty) hepato-pancreas and empty gut (see pictures). If yes look for a better batch.

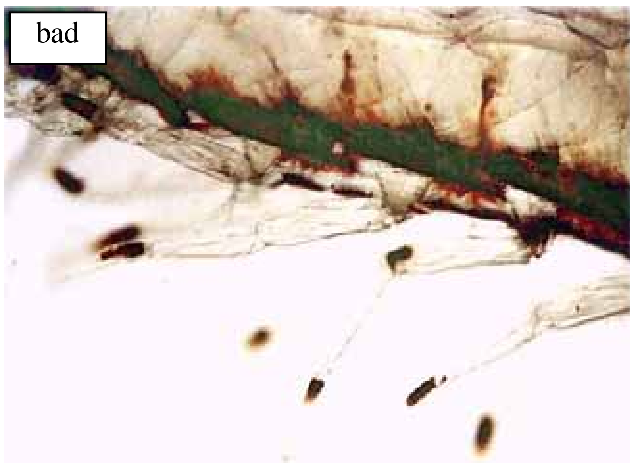
If the hatchery has a microscope ask them to help you use it to examine the postlarvae more clearly (steps 7 and 8). If there is no microscope available continue to step 9.

7) Check the same 15 postlarvae to see if any of them are dirty. If they are, look for a better batch.





8) Check the same 15 postlarvae to see if any of them have many legs damaged (broken and black) or missing. If they have, look for a better batch.



9) If possible, take another 15 postlarvae from the batch, put them into a plastic bag and give them (while they are still alive) to a laboratory that can test for a viral disease called MBV. Try to take only batches which test negative for MBV.

10) Take another 60 postlarvae from the batch and put them in a small jar with alcohol and deliver them to a PCR laboratory for WSSV testing. Try to take only batches which test negative for WSSV.

Developed by Drs Pornlerd Chanratchakool, Flavio Corsin & Matt Briggs. We acknowledge Prof. Timothy Flegel for supplying many of the pictures presented.