especially in Vizag and Kakinada, has caused pollution along the east coast. Information on the industrial growth occurring in the coastal zone of Andhra Pradesh can be summarized as follows:

- paper mills and tanneries in Srikakulam, and Vizianagaram districts;
- steel, fertilizer, metal alloy and shipping industries in Vishakapatnam District causing hydrocarbon and heavy metal pollution with cadmium, lead, mercury, nickel, zinc and iron (See Table 13) (a detailed investigation is needed to determine the risks of sourcing shrimp broodstock from this area);
- fertilizer plants near Kakinada;
- a paper mill at Bhadrachalam;
- agricultural pesticides in the Godavari-Krishna River Delta; and
- lead and zinc mining and agricultural pesticides in Guntur.

TABLE 13
Concentrations of lead and cadmium in the seawater around India[1]

Sampling station	Sampling date	Lead (µg/litre)	Cadmium (µg/litre)
Vishakapatnam	28-01-98	164	3.38
Coringa	24-01-98	47.1	2.65
Kakinada	11-01-98	64.2	1.34
Godavari	10-01-98	72.6	3.28

[1] Source: Coastal Ocean Monitoring and Prediction System (COMAPS), Annual Report, 1997–98

The Nellore and Prakasam coast is relatively free from pollution and the possibly of sourcing more broodstock from this coast should be explored.

3.2 DOMESTICATED AND SPF/SPR/SPT BROODSTOCK

Specific Pathogen Free (SPF) shrimp are those that are maintained in highly biosecure facilities and have been routinely checked and found to be free of specified pathogens. There is no single internationally recognized SPF list although it is generally agreed that SPF shrimp must be regularly tested for and be declared free from the following pathogens:

- infectious hypodermal and haematopoietic necrosis virus (IHHNV)
- white spot syndrome virus (WSSV)
- hepatopancreatic parvo-like virus (HPV)
- Taura syndrome virus (TSV)
- yellow head virus (YHV)
- monodon baculovirus (MBV)
- microsporidians
- gregarines
- haplosporidians
- other protozoans
- metazoan parasites

Specific Pathogen Resistant (SPR) shrimp are those that are not (or are less) susceptible to infection by one or several specific pathogens, and Specific Pathogen Tolerant (SPT) shrimp are those that are intentionally bred to develop tolerance to disease caused by one or several specific pathogens. For example there are lines of commercially available *Litopenaeus vannamei* in the United States of America that are SPF and SPR, but only to Taura syndrome virus (TSV), and often only to certain strains of that virus. These shrimp are not necessarily any more resistant to other viruses (or strains of TSV) than any other shrimp. Lines of *L. stylirostris* that are resistant to IHHNV are also available.

Research in Thailand aimed at identifying the cause of the slow growth syndrome of *P. monodon* (monodon slow growth syndrome, MSGS) has indicated an increase in the viral load of apparently healthy wild spawners. It was shown that a significant number carry multiple viral infections, some of which could be passed on to their offspring and result in mortality and/or the reduced growth rate seen recently during on-growing. Recent research has also identified a new lymphoid organ virus that may be carried

by unaffected *L. vannamei*, but which causes MSGS when injected into healthy *P. monodon* (T. Flegel, pers. comm.). This highlights the danger of culturing the two shrimp species in the same location.

Due to such disease problems and the periodic low quality and shortage of wild *Penaeus monodon* broodstock, there is a need for the selective breeding and development of domesticated broodstock. The development of such alternative sources of broodstock would also help to improve maturation and spawning success and limit the high price of broodstock during the seasons of highest demand. Private operators should undertake the domestication of shrimp to supplement the programme that MPEDA plans to undertake in the Andaman and Nicobar Islands.

This process has occurred for the production of domesticated (and sometimes SPF and SPR) lines of *Litopenaeus vannamei* and *L. stylirostris* broodstock throughout the Americas and now through much of Asia (particularly in P.R.China, Thailand, Indonesia and Malaysia) with *L. vannamei*. In fact the advantages that the use of such animals offers has in the past four years led to *L. vannamei* becoming the world's most important cultured shrimp species.

Advantages of using domesticated and SPF/SPR stocks include:

- ready, year-round availability of disease-free broodstock;
- the ability to be selected for desirable traits such as fast growth rate, disease resistance and hence high survival, good FCR and increased production and productivity;
- reduced use of chemotherapeutants;
- better adaptability of domesticated shrimp to captive environments, leading to reduced stress and better mating and reproductive success; and
- increased traceability of the origin of stocks and their past performance and future potential.

To avoid potential genetic problems and associated poor growth and survival due to inbreeding, details of the different families or origins and the past performance of the domestic stocks, whether of foreign or native origin, must be obtained. It is also useful to have performance and development data for the candidate families or lines under a range of environmental conditions. The selection protocol used is also important, i.e. whether the stocks were selected from ponds or tanks with better performance or for survival following a disease outbreak, and the exact timing of the selection procedures. Some criteria that are used for phenotypic selection (usually done first at harvest size and later, when nearly ready for maturation) are: relative size and general physical appearance, absence of necrosis or other clinical or subclinical signs of disease or ill health in muscle and exoskeleton, clean pleopods, no rostral deformities and a translucent body.

Currently there are a number of programmes aimed at producing domesticated stocks of disease-free *P. monodon* broodstock; these include projects in Hawaii, Thailand and Australia.

There have been recent claims by the private sector on the commercial availability of domesticated *Penaeus monodon* in Thailand and in Hawaii, United States of America (FAO, 2005). Private, government and academic institutional cooperative development of SPF *P. monodon* broodstock domestication also began in Australia in 1997. Although significant success has been achieved, stock from the programme are not yet commercially available.

A shrimp industry consortium, Shrimp Culture Research and Development (SCRD) in Thailand, initiated similar efforts in 1999. In addition Thailand's National Science and Technology Development Agency (NSTDA), together with the National Centre for Genetic Engineering and Biotechnology (BIOTEC), have continued their previous work with *P. monodon* domestication with a US$ 4 million government grant and have already developed sixth generation animals SPF for WSSV and YHV (FAO, 2005).

Moana Technologies in Hawaii has been working on selective breeding and genetic improvement of domesticated *P. monodon* with a United States of America government grant in Hawaii since 2001. They have collected families of wild shrimp from around the world and produced large numbers of families that they are currently selecting for fast growth and testing for disease resistance. Their aim is to form joint venture partnerships with government and big businesses in each Asian country to build multiplication centres for their stocks of SPF animals by the end of this decade. The Indian Government may be able to take advantage of these developments to fast-track their own domestication efforts.

In 2002 a collaborative partnership between the Fisheries Research Development Centre (FRDC), the Australian Institute of Marine Science, Commonwealth Scientific and Industrial Research Organization (CSIRO), Queensland's Agency for Food and Fibre Sciences and three leading Australian prawn farms has approved a AUD$1.8 million, three year project aimed at domestication of *P. monodon*. Similarly in Malaysia, government and private-sector collaboration on development of *P. monodon* SPF domestication was initiated in 2003 and has advanced to F1 generation at present, with reported success.

Although some private companies claim commercial production of domesticated SPF *P. monodon* in the United States of America and Thailand, reports from successful commercial-scale production using such SPF PL are still scares. Thus in the shorter term, importation of such stocks is impossible. However, these developmental activities and achievements are sufficient to prompt the development of similar programmes by the Indian shrimp aquaculture sector aimed at improving its PL quality.

The development of SPF stocks is probably a viable long-term solution for India. The Government of India through MPEDA has already begun efforts in SPF development. Two entrepreneurs have been permitted to import 500 SPF *Litopenaeus vannamei*, while a proposal to import 10 000 *P. monodon* broodstock has been approved by the Ministry. A consultant for transfer of technology on SPF shrimp has been identified, and he has already prepared a prefeasibility report. The site for the nuclear breeding centre has also been identified on the (relatively clean) Andaman and Nicobar Islands, and hatchery facilities at the Andhra Pradesh Shrimp Seed Production and Research Centre (TASPARC) and the Orissa Shrimp Seed Production Supply and Research Centre (OSSPARC) will be testing the SPF broodstock in a commercial production environment. Later there are plans to develop broodstock multiplication centres in the Andaman and Nicobar Islands and on the mainland to meet the demand for SPF broodstock.

For this programme all possible pathogens would be included in the SPF list. The culture system is being planned to include raceways and recirculation systems and a self-contained diagnostic facility will be established in the nucleus centre. Biosecurity aspects would be taken care of with all stages, founder populations to F2 generation being continuously screened to obtain reliable SPF stock. The nuclear breeding centre with six multiplication centres are planned to produce around 60 000 brooders yearly, sufficient for up to 60 percent of the current demand.

Major elements of an SPF programme include the capture of apparently healthy wild stock from areas of low disease prevalence followed by individual primary quarantine where the shrimp can be individually screened for specific pathogens and the contaminated individuals destroyed. The shrimp are then transferred to secondary quarantine where they are reared to broodstock size while being monitored monthly by histological, microscopic and immunodiagnostic (i.e. PCR) means for all pathogens of concern (OIE-listed shrimp pathogens and any others of interest). The disease-free broodstock are then transferred to the breeding centre for production of, and genetic selection between, multiple families from different sources. Larvae are then reared in biosecure hatcheries from the selected families. Any infected and/or inferior quality stock detected through continual monitoring are immediately discarded.

BOX 1

Organization and flow of shrimp in an SPF shrimp industry

Founder Population
F1 SPF Juveniles
⊠
SPF Nuclear Breeding Centre
Produces SPF PL
⊠
HHS BS Multiplication Station
Produces HHS nauplii
⊠
HHS Larval Rearing Station
Produces HHS PL
⊠
Commodity Production
SPF stream
HHS stream
CP stream

From Lotz, 1997

An SPF shrimp culture system is composed of three streams: the Specific Pathogen Free (SPF) stream, the High Health Status (HHS) stream, and the Commodity Production (CP) stream. From the SPF stream, the operational flow strictly follows the HHS stream until they reach the CP stream and should never deviate from the pattern to maintain the sustainability of the model (Box 1).

Such a model could also be implemented in the Indian shrimp industry, where it could be gradually adopted, improved and modified in line with the changing situation and environment. Although it is costly and may require much effort, this strategy could meet the quality and quantity of PL demanded by the grow-out sector.

For development of SPR lines of broodstock *P. monodon*, the primary steps are similar to those for the SPF programme. However, a more rigorous (and higher investment) genetic selection programme utilizing a greater number of families to select for desirable traits is required.

Whichever programme is selected, the development of SPF and/or SPR lines of *P. monodon* should be regarded as a long-term investment. It requires absolute control on all aspects of culture on a continuous basis, highly trained scientific personnel, the highest standards of discipline and team work, specialized training for staff and continuous laboratory analysis.

The quarantine, hatchery and broodstock grow-out facilities must be specially designed to incorporate high technology equipment and bioassay, biosecurity and safety procedures and facilities. All of these measures are essential, since contamination of the facilities could lead to the need to destroy all the stock, wasting considerable sums of money and time.

3.2.1 Limitations of SPF shrimp

All pathogens that pose a significant threat need to be reliably diagnosed and physically excluded from the facility. However, it must be remembered that the shrimp could still be infected with a pathogen not included in the list. Also although *Vibrio* spp. bacteria can cause significant disease problems and can be reliably diagnosed, they cannot be included on an SPF list as it is impossible to physically exclude them from any facility.

It must be remembered that SPF status is not heritable. Offspring of SPF shrimp are not SPF unless they are produced and maintained at a biosecure SPF facility. Once they leave that facility, they can no longer be termed SPF and should instead be referred to as "High Health", meaning that they originate from disease-free stock but are currently of unknown disease status. Also SPF shrimp do not have innate resistance to particular pathogens and may in fact be quite susceptible to them, since they are naïve to those pathogens.

Finally it cannot be forgotten that domestication of tiger shrimp (*Penaeus monodon*) is far more difficult than working with open thylecum white shrimp (*Litopenaeus vannamei*). Since maturation of *P. monodon* in captivity is very difficult, a much longer holding period is required until they reach a viable size (12–18 months and 120 g for *P. monodon* females compared to 8–10 months and 40 g for *P. vannamei* females).

There are also significant gaps in the knowledge of their nutritional and environmental requirements for captive maturation and spawning.

3.2.2 Importation of broodstock

An intermediate-term solution may be to source wild disease-free (PCR-checked) broodstock from wherever in the world they are available. Thailand is currently attempting this with imported *P. monodon* broodstock from Africa and Australia. These animals are then held in biosecure facilities in-country, spawned and then retested before allowing their nauplii to be used in the local hatcheries. The first results from these efforts are expected shortly.

For India perhaps the best source of such high quality broodstock is from the Andaman and Nicobar Islands, which are still within Indian territorial waters but are clean areas far from shrimp-farming operations. The Government of India has given permission for a limited quantity of broodstock to be caught based on the results of their survey and the nauplii/PL produced to be transferred to the mainland. There are guidelines and limitations however designed to help protect local genetic resources and prevent contamination of the local stock.

3.3 BROODSTOCK LANDING CENTRES AND HOLDING TECHNIQUES

The major broodstock landing centres in India are as follows:

- Andhra Pradesh: Kakinada & Bhiravapalem, Machilipatnam, Nizampatnam. and Krishnapatnam; In Vishakapatnam a broodstock collection centre (BSCC) for hygienic handling and maintenance is established;
- Tamil Nadu: Pazhiar, Nagapatnam and Rameswaram;
- Orissa: Gopalpur-on-sea, Puri and Paradeep; and
- the Andaman Islands.

In the Andaman Islands, high quality broodstock is reported to be available because of the pristine nature of the sea. At present the Indian Government has recently permitted only three operators to gain licenses, with the restriction that they may only use 500 broodstock/operator/yr. The performance of the licensed operators, including their production methods, the quality of their PL and the feasibility of their operations should be assessed. In the intermediate term, the island's administrative authorities might permit additional operators, while a longer-term solution is to permit movement of broodstock in line with appropriate protocols and standards.

The All India Shrimp Hatcheries Association, Vishakapatnam in association with DOF, MPEDA, and Boat Owners has established the country's first broodstock

A specific area is provided for holding broodstock within Vizag fish landing complex. However, additional facilities such as a good water supply system, a laboratory for checking for pathogens, quarantine tanks and quality control services should also be adequately provided to manage broodstock supply and to assure the quality of broodstock supplied to hatcheries

Broodstock landing area is within the fish market at a fishing port in Kakinada, posing a threat to the quality of the broodstock supply

collection centre at Vizag fishing harbour, for the hygienic handling and maintenance of broodstock. The centre functions as a temporary holding facility maintaining broodstock for a brief period in disinfected and filtered seawater brought from hatcheries. A similar set up is being planned for other landing centres in a phased manner.

The broodstock landing and auction centres currently have many shortcomings and limitations. Most facilities are in urgent need of improvement and require better management regimes. Separate broodstock holding facilities exist only in Vizag, while all other broodstock landing stations are situated within fish markets. Even Vizag fish landing complex was built for all other fisheries activities and there is no separate jetty for broodstock landing. None of the broodstock landing stations are fully equipped with necessary facilities such as a high quality disinfected water supply system, a quarantine and holding system, a pathogen screening facility or a feed supply and feeding management system. Only oxygen tanks and plastic or fibreglass tanks for holding both sexes and maturation stages together are available. The Ministry of Agriculture and the private-sector food-processing industries should be approached through the state governments to help upgrade the landing centres or to create new facilities.

To help minimize stress, trawler operators at the landing centres should be trained in the importance of hygienic and careful handling of broodstock during catching, holding and transportation to the landing centres. Landed broodstock are often kept collectively and are pooled prior to auction, inevitably leading to contamination of previously clean broodstock via horizontal transfer of pathogens. The animals are also subject to stress caused by excessive handling and a lack of sufficient high quality seawater.

The broodstock should be held and auctioned individually in oxygenated tanks or bags chilled to <29 °C with ice to maintain biosecurity and allow inspection of individual shrimp. Broodstock should not be kept in overcrowded tanks for prolonged periods prior to transport. During holding prior to transportation, the use of high quality feeds enriched with vitamin C and astaxanthin (or paprika), and an acceptable probiotic formula help reduce stress and bacterial levels. However, the shrimp should not be fed for 12 h prior to shipment, as any faeces produced during transport will lead to poor water quality and possible infection of clean broodstock. High quality seawater should be made available with which to hold and repack the shrimp prior to transport to the maturation centres.

The holding of wild-caught broodstock from different trawlers in one container at the fish landing will result in contamination of clean broodstock

Currently, no one (suppliers, middle men or hatchery operators) is checking the captured broodstock for known pathogens at the landing port, holding area or prior to purchase. This has resulted in occasional high prevalence of viral infections in broodstock at hatcheries, compromising PL quality. Government intervention is urgently needed to help control the quality and price of broodstock.

Disease testing using commercially available rapid diagnostics kits could be conducted at the auction centres, allowing health certificates to be issued. Staining of faeces with malachite

green to detect MBV and PCR analysis for all major viral pathogens prior to shipment should be considered. Shrimp that test positive and/or show heavy infections should be discarded. Handling of shrimp during collection, holding and packing should be reduced to a minimum.

3.4 BROODSTOCK SELECTION AND TRANSPORT FROM LANDING/AUCTION CENTRES

Many of the recent problems with lack of maturation success of wild *Penaeus monodon* broodstock in Indian hatcheries are thought to be due to poor selection and transportation of the shrimp to the maturation units. Currently, broodstock selection is based only upon gross examination. Some of the criteria include:

- lack of red coloration;
- avoidance of weak and/or moribund animals;
- clear gill coloration;
- absence of black spots (necrosis) on the thelycum;
- absence of gill fouling;
- lack of obvious white spots; and
- the stage of development of the ovaries.

Although there are normally no shortages of broodstock, often quality is at a premium and the hatcheries have to select from what is available. Sometimes immature animals are kept on the boats for several days before being brought to shore, resulting in low quality. Also different stages may be pooled together for auctioning as a lot, so that individual purchasing is not possible. As individual holding allows the buyer to undertake rapid disease screening procedures, auctioning should be done on broodstock packed in individual oxygen-filled bags. In this way a premium price may be obtained for high quality disease-free broodstock, while diseased or substandard shrimp can be avoided. However, if the hatcheries adhere strictly to the screening criteria, it may be difficult to meet their demand.

In the past hatcheries sent their representatives to acquire broodstock directly from the trawlers; however since supplying broodstock became big business, middlemen have become highly influential. The recent price of broodstock in India has fluctuated between 1 000–15 000 Rupees each, while during the late 1990s the price was as high as 50 000–70 000 Rupees per gravid female. Therefore trawler operators and groups of hatchery owners have been trying to cut out the middlemen wherever possible to save costs. However, some influential people have been financing trawler operators, and in order to ensure their financing business, some middlemen have regained their former status.

Transportation times should be minimized by planning and reconfirming in advance all connections and handling procedures. Overland transport should only be done during the cooler night-time periods. Only broodstock that are in intermoult stage (hard shelled) should be transported, as animals that moult in transport will die and may kill the other shrimp packed with them. Rubber tubes should be placed over the rostra of the shrimp to avoid puncturing of plastic bags. Shrimp should be packed in individual plastic bags if possible or at low density (<500 g of shrimp/10 litres of water).

The transport bags should be filled one-third full with the cleanest seawater available (already chilled to the desired temperature), preferably using sand, cartridge and ultra-violet or ozone disinfection. Dissolved oxygen levels should be maintained at >5 ppm by filling the bags two-thirds full with pure oxygen and refilling during shipping if transport times exceed 24 h. For transport times >6 h, the water holding the shrimp should be cooled to 22–26 °C (depending on duration of transport) at the rate of 0.5°C/h to reduce the physical and metabolic activity of the shrimp.

Low temperatures in the transport bags should be maintained by enclosing the bags in polystyrene boxes and ensuring no direct sunlight contact at all times. A few grams

of new, washed, activated charcoal (1 g/litre) should be used in each transportation bag to reduce the build up of ammonia and nitrite in the bags. EDTA at 10 mg/litre can be used to chelate heavy metals and inhibit bacterial growth, while Tris HCl buffer can be added at up to 10 mg/litre to stabilize the pH of water. The boxed shrimp should be handled with extreme care and bumping or dropping of the boxes should be avoided.

3.5 BROODSTOCK UTILIZATION

The Shrimp Hatcheries Association has projected the total requirement of broodstock at about 100 000 (about 65 000 females and 35 000 males) to produce 8.5 billion PL/yr for the years 2004 and 2005. However, the stocking so far (up to May 2004) in Andhra Pradesh is only 1.08 billion PL in 32 500 ha of ponds. Many hatcheries are currently shut down because of the low demand for PL by shrimp farmers.

Nationwide there is a total of 301 hatcheries. The breakdown of hatcheries by state is as follows: Andhra Pradesh (178), Tamil Nadu/Pondi (72), Kerala (25), Orissa (10), Karnataka & Goa (7), Maharastra (6), West Bengal (2) and Gujarat (1).

There are 178 tiger shrimp hatcheries in Andhra Pradesh, all located in close proximity to fish landing centres: Vishakapatnam (31), Kakinada (72), Prakasam (25) and Nellore (50).

Currently many hatcheries depend on sourcing of gravid females to obtain nauplii, while many more buy them from the nauplii production centres. Thus broodstock utilization by the hatcheries has declined significantly over the period, especially after 1997. Although most of the hatcheries have facilities for maturation of broodstock by eyestalk abalation, only a few actually use them to produce nauplii.

3.6 BROODSTOCK QUARANTINE

Currently the quarantine facilities in Indian hatcheries (if they exist at all) are inadequate, and disposal of infected broodstock, when done, is inadequate to prevent contamination of other stocks. Also in most hatcheries the understanding of the concepts of biosecurity and quarantine is a little weak.

The broodstock quarantine facility should be completely isolated from the rest of the maturation and hatchery facilities since it is an area having a high risk for disease transmission. Isolation includes the spatial separation of the buildings as well as the separation of water/air lines. If this is not possible, the hatchery design should be altered so that there is no possibility of contamination from the quarantine or holding area into the other production areas. Particular care should be taken with waste disposal and effluent treatment. Staff working in this area should not be permitted to enter other production sections and should follow sanitary protocols at all times.

The quarantine unit should have the following characteristics:

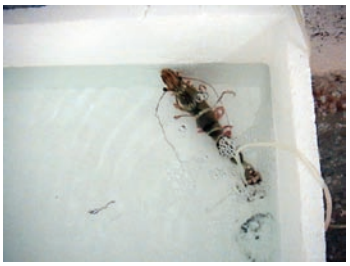
- It should be adequately isolated from all rearing and production areas to avoid any possible cross-contamination.
- It should be in an enclosed and covered building with no direct access to the outside.
- There should be means provided for disinfection of feet (footbaths containing hypochlorite solution at >50 ppm active ingredient) and hands (bottles containing povidone iodine (20 ppm and/or 70 percent alcohol) to be used upon entering and exiting the unit.
- Entrance to the quarantine area should be restricted to the personnel assigned to work exclusively in this area.
- Quarantine unit staff should enter through a dressing room where they remove their street clothes and take a shower before going to another dressing room to put on working clothes and boots. At the end of the working shift, the sequence is reversed.

- An adequate number of plastic buckets and/or similar containers should be available in the quarantine room to facilitate effective daily routine movement of shrimp in and out of the facility.
- The quarantine facility should have an independent supply of water and air with separate treatment and disinfection systems and a system for the treatment of effluents to prevent the potential escape of pathogens into the environment.
- The seawater to be used in the facility must enter a storage tank where it will be treated with hypochlorite solution (20 ppm active ingredient for not less than 30 min) before inactivating with sodium thiosulfate or vitamin C (1 ppm for every 1 ppm of residual chlorine) and strong aeration.
- All wastewater must be collected into another tank for chlorination (20 ppm for not less than 60 min) and dechlorination before release to the environment.
- All mortalities or infected animals must be incinerated or disposed of in another approved manner.
- Used plastic containers and hoses must be washed and disinfected with hypochlorite solution (20 ppm) before reuse.
- All implements used in the quarantine unit must be clearly marked and should remain in the quarantine area. Facilities for disinfection of all equipment at the end of each day should be available.

The individual sections of the quarantine area should be designated "dirty" or "clean" depending on whether they contain shrimp that are not yet screened for infection (pre-testing) or that have been passed (post-testing). Shrimp should only move one way, from the "dirty" to the "clean" sections of the quarantine facility, and all movements should be controlled to ensure no mixing between the two areas.

To avoid having to discard entire batches due to individual infection, potential broodstock should be held in isolation (unless they are SPF), at least until their disease status is ascertained, and preferably at all times in the quarantine unit. Laboratory facilities and associated expertise must be determined based on the specific needs of the hatchery. Typically, individual holding tanks of >100 litres per individual broodstock should be provided. On introduction into the quarantine unit, the broodstock must be well acclimatized, the duration of acclimatization depending upon the temperature and salinity of the transport water.

The receiving quarantine tanks should be prepared at least one day ahead of arrival to match the expected conditions in the water of the arriving shrimp. Upon arrival the water quality in the tank receiving the broodstock should be checked to ensure that it is high and that the salinity, temperature and pH are the same as that in the transportation bags. The still-closed bags are then floated in the receiving tank until the temperature inside and outside the bags is the same. Then the bags are opened and an airstone connected to a low flow of air (or preferably oxygen) inserted. The bags are gradually filled with water from the tank over a 20–60 min period. After this time, the broodstock should be taken from the bag and passed through a dip of povidone iodine solution (20 ppm), potassium permanganate (100 ppm) or formalin[1] (50–100 ppm) for 30–60 seconds and then released into the receiving tank.



Individual holding of broodstock at a hatchery in Tamil Nadu State. Such good operational practice should be promoted to enhance health management

High quality feeds to demand should be immediately offered, as the shrimp may be hungry. Over the next few hours, the temperature of the receiving tanks is gradually allowed to increase to ambient (which should be 27–29 °C) at a rate of <2 °C/h and (if required) the salinity to normalize to ambient (which should

[1] When formalin is used, avoid using the whitish sediment at the bottom of the container (paraformaldehyde), as it is highly toxic. Take absolute care and use appropriate guidelines during use of any potentially hazardous chemicals.

be 30–35 ppt) at <2 ppt/h. Handling of broodstock shrimp should be reduced to a minimum at all times and dissolved oxygen concentrations maintained at saturation. If shrimp arrive healthy but begin dying after a few days, they usually have high levels of bacteria in the haemolymph. After confirmation this can sometimes be reduced using 5–7 daily antibiotic baths (10 ppm oxytetracycline) or by feeding diets containing 1–2 ppm oxytetracycline or appropriate probiotics. The health of the gills should be monitored regularly and if excessive fouling by algae or filamentous bacteria is found, treatment in an aerated bath with 0.1 ppm of copper control (based on copper sulphate, $CuSO_4$), or if epicommensal protozoans are found, application of a one-hour aerated bath treatment with 30–50 ppm formalin is indicated. Any shrimp that have serious melanised (black) lesions on the body, large areas of white muscle or bright red coloration should be discarded immediately before they infect the others.

Water quality requirements in the quarantine system are a temperature of 27–29 °C, salinity of 29–34 ppt and a pH of 7.8–8.5, maintained by 200–300 percent of water (filtered to <20 µm) exchange daily (preferably on a flow-through rather than rapid-change basis), permitting adequate feeding of the broodstock while maintaining optimal and stable water quality. Fresh (sterilized) or pelleted feeds are fed as for the maturation systems, feeding little and often to demand so as to maintain water quality.

Prophylactic treatment of broodstock with formalin at 50–100 ppm for 30–60 min under strong aeration should be conducted before introducing the stock into the maturation/hatchery systems. Only spawners free from pathogens such as MBV and WSSV should be transferred into the maturation/hatchery systems.

3.7 BROODSTOCK HEALTH SCREENING

From the entry of the broodstock into the quarantine system onwards, appropriate standard diagnostics tests must be routinely performed (particularly prior to grouping the animals, if this is required), and actions must be taken accordingly. This could involve preliminary screening with rapid (<30 min) immunodot-style test kits for WSSV (the "shrimple" kit is available locally for Rs50[2]), examination of faeces for HPV and MBV, and general microbial and morphological testing for other pathogens.

However, more thorough testing by PCR (or dot-blot essay) for major pathogens (e.g. WSSV, YHV, IHHNV and MBV) should be done if at all possible. This is to reduce the risk of transmission of viral diseases from broodstock to larvae and to ensure that ablated females are healthy enough to survive ablation prior to maturation. Commonly in India, especially during the colder months (December–February), broodstock shrimp may be stressed and carry high loads of WSSV, MBV and other viruses. Maltreatment of such animals during collection and transport often leads to mortality on ablation in the maturation facility.

On about the third day of quarantine, one pleopod (or part of the telson) is removed from each shrimp held individually. If shrimp are held collectively, random samples should be taken from each container to evaluate the general condition of the population held in that container. Groups of ten pleopods can be analysed as one sample. Any groups that give a positive result can be discarded or, in the case of a pooled sample from animals held individually, the shrimp can then be tested on an individual basis to identify and discard only the positive individuals.

The pleopod or telson piece(s) should be preserved in 90 percent alcohol (90 ml of absolute alcohol (ethanol) plus 10 ml of distilled water) and sent to a PCR laboratory for viral diagnosis. A drop of povidone iodine solution should be placed on the area where the pleopod was removed from the broodstock shrimp before returning it to the tank.

Faeces from each broodstock shrimp should also be collected, placed in separate plastic bottles in seawater and sent to a competent laboratory for analysis with malachite

[2] US$ 1 = 44.9 IDR

green and hematoxlyn and eosin (H&E)-stained scrapes for the presence of MBV, HPV and baculovirus midgut gland necrosis virus (BMNV). Heavily infected broodstock should be destroyed using sanitary procedures such as chlorination or use of formalin at >100 ppm for 30 min, incineration or some other method (e.g. autoclaving and deep burial) that will prevent the potential spread of virus.

PCR screening at critical points (after spawning and at the naupliar stage after washing) would greatly reduce the risk of disease transfer from broodstock to larvae. If possible, PCR screening should be carried out on individual broodstock. Where numbers of broodstock are large, the tests may be carried out on pools of 10 individuals from different broodstock groups. A minimum sample of 140 animals for each group of 1 000 shrimp should be taken and divided into groups of 10 shrimp for each analysis.

Currently most Indian hatchery and farm operators do not screen for major pathogens. Of the few that do, most are concerned only with MBV and WSSV. In most cases, if broodstock or PL test (highly) positive for MBV, they are discarded without even waiting to test for WSSV. However, HPV is as easy to test for as MBV (through malachite green and H&E staining of faeces), and should be considered in any health screening programme, since it can cause serious slow growth in shrimp on-growing ponds.

So that good broodstock is not wasted, a ratings system should be used to assist in deciding whether a particular animal should be kept or discarded. However, it must be remembered that some broodstock is known to test negative by 2-step PCR for WSSV before spawning but to test positive after the stress of spawning. Therefore all broodstock should be held until after the first spawn before being checked by PCR for viral infections. Of course this is problematic with gravid females, since they are spawned very quickly after introduction to the maturation systems, leaving little time for testing. The hatchery operators also do not want to lose the first spawn from a gravid female, since it invariably produces large numbers of high quality nauplii. However, if the batch of eggs/nauplii can be maintained separately until the results of the pathogen testing (immediate analysis for WSSV and MBV) are known, then these nauplii need not be wasted.

The hatcheries should seriously consider adopting a risk assessment based approach using HACCP guidelines. Attention should also be placed on ensuring that reliable PCR results are obtained from the various PCR laboratories through intercalibration and/or harmonization operations.

The quarantine period will vary depending on the time required to complete the health screening procedure. In all cases animals should be kept under observation in the quarantine facility until all tests are completed and each shrimp's health status is known. Depending on the design of the facility and the location of the quarantine unit relative to the maturation facility, this may involve repacking the broodstock for shipment to a distant location or their movement to a separate section of the same facility using disinfected buckets with water from the maturation facility.

In either case the equipment used for the transfer should be kept separate from that used in the quarantine room and disinfected before and after transport. All equipment used in the quarantine area should remain in the quarantine area and be disinfected at the end of each day in tanks especially designated for that purpose.

Basic laboratory facilities (e.g. a microscope, some microbiological capability, etc.) will be required to carry out routine inspections of shrimp health. The addition of more complex facilities to carry out PCR tests, for example, will require the construction of dedicated facilities to avoid the possibility of contamination. The design and operation of these facilities is outside the scope of this document.

Further details on the construction and operation of a quarantine facility can be found in MAF (2001), Anon. (2002) and AQIS (2003).

3.8 BROODSTOCK MATURATION

The first step in larval production is the maturation and breeding of mature shrimp. The protocols to be adopted will depend to some extent on whether the hatchery operation is a component of a controlled breeding programme or if it is intended primarily for the production of PL for commercial pond culture. Up-to-date knowledge on maturation appears limited in most Indian hatcheries. Main weaknesses are in the areas of broodstock collection and transportation systems; lack of broodstock screening to weed out diseased shrimp that may die following ablation; knowledge on responses to chemicals, water quality and pollution and in analytical problem solving.

Depending on this distinction, the maturation system will be designed either to maximize the production of nauplii for commercial production of PL or to allow for maximum control over mating and genetic crosses. Although it is possible to control mating in a conventional maturation unit, good control of individual parents requires unisex culture and artificial insemination, with larval culture and nursery systems designed for a large number of batches with relatively few larvae per batch. This presents operational challenges very different from a typical commercial hatchery or nursery system (Jahncke *et al.*, 2002).

The maturation building must have supporting infrastructure and must be large enough to accommodate the number of broodstock to be held. The factors to consider in designing the facility are the level of naupliar production required, the stocking density and sex ratio of the broodstock to be used, the estimated spawning rate of the females, the estimated hatching rate, the estimated number of eggs and nauplii per female and the production system (batch or continuous) employed.

Selected disease-free and acclimated shrimp broodstock should be held in the maturation area for at least four days before ablation, so that they will have fully recovered from the transportation stress. Only intermoult (with fully hard shell) shrimp should be ablated (held for a short time during the process in a bucket of chilled seawater). After ablation The area around the cut eye should be disinfected with 200 ppm povidone iodine solution. Wait one week to ablate pre-moult or immediately post-moult females. Ablation can be conducted either by tying or pinching the eye and then squeezing out its contents or through cauterization with hot pincers with the aim of causing minimal stress.

The females for ablation should be above 100 g (preferably 120 g) in size to ensure good numbers of high quality eggs and nauplii. Males can be any size above a minimum of 70 g. The ablated female shrimp are stocked in the maturation tanks along with unablated males at a density of 4–5 individuals/m^2. Stocking of females and males at a ratio of 1.5–2:1 ensures best mating success.

Light intensity should be maintained low and the ablated shrimp should not be disturbed by the movement of personnel near the maturation tanks. The maturation room should be equipped with a system to control photoperiod at about 10–12 h dark and 12–14 h light, the light level gradually changing between the two over a period of 1–2 h. Access to the maturation room should be restricted; noise (particularly loud or intermittent noise), movement and other disturbances should be kept to a minimum.

The maturation room should have round (preferably) or square tanks that are dark-coloured, smooth-sided, and of at least 200 litres (0.4 m^2 area) for individual holding (preferably) or approximately 5 m in diameter (20 m^2 area) for communal holding. Currently in India only one hatchery in Tamil Nadu and none in Andhra Pradesh use individual broodstock holding. At the stocking densities employed, a 5 m diameter tank can accommodate 50–60 females and 30–40 males. It is important also to consider the biomass in weight rather than the numbers of broodstock per square meter that can be held in the tank without causing deterioration of the water quality through the feed used. A biomass/unit area of 0.2–0.3 kg/m^2 is recommended.

The environmental conditions in the maturation room must be closely monitored, controlled and recorded. Cleanliness of tanks and good water quality must be maintained as stable as possible in the maturation tanks. The broodstock should preferably be held with flow-through (new and/or recycled) water exchange of a total of 250–300 percent per day (although 100–150 percent can be sufficient if wastes are removed promptly) and a continuous but not too vigorous air supply. Water depth is generally around 0.5–0.7 m. Water temperatures are usually controlled to be maintained in the range of 28–29 °C. Temperatures higher than 29 °C will lead to deterioration in sperm quality and should be avoided wherever possible. Salinity should be maintained at 30–35 ppt and pH at 8.0–8.2. Ammonia and nitrite nitrogen levels should be maintained through water exchange at <0.1 ppm at all times.

The use of recycled water systems for broodstock maturation systems has gained prominence in other parts of the world and may be considered. Recycled water systems using 50–100 percent new water and up to 200 percent recycled water per day are being used in maturation systems in other countries. Such systems ensure stable water quality and allow high feeding rates that increase the nutritional status and naupliar quality of the broodstock. Such systems range from simple sand and mechanical filtration (with frequent backwashing) to more complex systems incorporating biological and mechanical filtration, protein skimming (foam fractionation) for organic matter removal and cartridge, activated carbon and UV filtration.

Due to the high feeding rates employed, the maturation tanks require daily siphoning of uneaten food, faeces and moults. The siphon consists of two parts, a PVC tube and a hose. Each maturation tank should have its own PVC tube, but the hose may be used for all tanks. The hose should be rinsed with clean treated water before each tank is siphoned.

Debris and waste siphoned from the tanks can be collected in a mesh bag placed at the end of the hose and incinerated after the cleaning operation. At the end of the working day, the hose should be washed and remain immersed inside a tank of calcium hypochlorite solution (20 ppm).

Intermittent scrubbing of tank walls and bottoms must also be undertaken if there is an excessive build-up of algae or other sedentary organisms, including protozoan fouling organisms. This can often be achieved through lowering water levels in the tank without removing the broodstock, but occasionally requires the transfer of broodstock to new tanks. It is a good idea to leave at least one tank empty for such procedures, which can then be programmed on a regular basis. Care must be taken during these cleaning exercises that the broodstock are manipulated as little as possible, as excessive disruption of mature brooders will interfere with their spawning rhythms.

Separate utensils/handling equipment should be used for each tank and must be cleaned and disinfected prior to each use. They should be maintained in recipient(s) containing povidone iodine and/or hypochlorite solutions (20 ppm active ingredient).

If any bacterial, protozoan or other fouling problem arises with the broodstock, they can be given bath treatments with formalin (30–40 ppm for 60 min with high aeration), which should improve conditions as long as suitable water exchanges can be maintained.

To avoid deterioration of naupliar quality, ablated females should typically be retired from the maturation unit after a maximum period of one month or three spawns, depending on the feeding regime used and health of the spawners. This usually requires that females be identified individually by tagging or some other method. The hatchery should try not to remate (unless the female spawns less than three times before the first moult) or spawn the females more than three times. Records of which female spawns and how many times must therefore be kept to know which females have mated and how frequently. Additionally, the fecundity, spawning rate (number of spawns per female) and length of time that the broodstock are kept in maturation should be

monitored. Attempt to keep a record of all of the above activities so that they can be inspected for irregularities when problems are encountered with the broodstock.

3.9 BROODSTOCK NUTRITION

The feed preparation area should be adjacent to, but separated from the maturation room. It should be equipped with all feed preparation utensils (knives, spoons, bowls/buckets, cutting surfaces, mixers, pelletisers etc.) and a refrigerator and a freezer to store food items. Feed preparation should be carried out using hygienic standards. Utensils must be kept clean, washed before use with povidone iodine solution (20 ppm) and rinsed with clean water.

A good diet and feeding protocol for broodstock are key factors in the production of good-quality nauplii. The optimal diet should be supplied to the broodstock shrimp in an appropriate manner to help maintain the nutritional status and fitness of the broodstock and nauplii while reducing the risk of disease transmission and problems with deteriorating water quality. Currently there is no quality assurance or quality/disease checking of the feeds used for broodstock maturation in India.

Feed fresh high quality feeds comprising live polychaete bloodworms (*Glycera* sp.) (10–12 percent/d), plus fresh squid (*Loligo* sp.) (6–10 percent/d), plus live but deshelled bivalve molluscs (either oysters, mussels (*Perna viridis*) or clams (*Meritrix* sp.) (4–8 percent/d), at a total of 20–30 percent of wet body weight/d. The exact quantity of feed given should be adjusted frequently based on the consumption rate of each tank. The feeding should continue until only a very small amount of uneaten food remains in the tank a couple of hours after each feeding.

Frozen adult *Artemia* biomass and krill are other alternatives. Emphasis should be placed on feeds offering similar polyunsaturated fatty acid (PUFAs such as arachidonic, eicosopentaenoic and decasohexaenoic acid) profiles to that of the shrimp themselves.

When using fresh feeds, efforts must be made to ensure that the material is as fresh as possible. Live feeds should be washed in clean treated seawater before use. To ensure that fresh feed is not a biosecurity risk, a certificate should be requested at the time of purchase stating that the feed is free of the viruses TSV, WSSV and YHV by PCR analysis. Live (or dead) crabs should never be given to the broodstock since they may be carriers of viral diseases. Alternatively the feeds may be sterilized or pasteurized (recommended) to inactivate any virus, as long as this does not affect their acceptability or nutritional quality. Ideally different types of frozen feeds should be stored in separate freezers.

Fresh feeds need to be chopped to a size suitable for ingestion by the broodstock and washed with clean water and weighed prior to feeding. These feeds should be offered to the broodstock throughout the day and night at least six times per day (two feedings of each diet per day), as in Table 14.

A paste of a vitamin mixture (particularly vitamins A, C and E) and paprika or astaxanthin can be made with water and mixed thoroughly with the squid or the bivalve molluscs just before feeding to increase vitamin and pigment levels in the broodstock and nauplii. Alternatively artificial/formulated feeds with vitamin, mineral, pigment (astaxanthin or paprika), immunostimulant and PUFA supplements may also be offered to ensure good egg quality over multiple spawns. Several commercial companies produce artificial feeds to supplement the fresh feeds used in maturation, although none yet serve as full replacements. Dry or moist diets can also be economically cold-extruded (using a pelletiser or an extruder) on site using regular shrimp feeds ground to powder and incorporating the various additives mentioned above plus a binder such as alginate or gelatine. However, wild broodstock

Blood worms, unchecked for any pathogen, seen in a plastic container at a backyard hatchery

TABLE 14
Feeding regime (% of wet weight) for *Penaeus monodon* broodstock

Feed	Time					
	00.00	04.00	08.00	12.00	16.00	20.00
Live polychaete worms	5–6%			5–6%		
Fresh squid		3–5%			3–5%	
Live bivalve molluscs			2–4%			2–4%
Dry diet			1–1.5%			1–1.5%

often prove reluctant to eat dry feeds and must be acclimated to them very gradually over time. Therefore dry feeds should be fed little and often, two to three times per day (up to 2–3 percent of shrimp wet body weight/d) to ensure that they are completely consumed.

As with all management practices with broodstock, changes to feeding regimes, types, quantities and times should be minimized as much as possible to limit stressing the animals. Hence stocks of all feed ingredients or types used should be maintained at all times.

In order to maintain water quality, high rates of water exchange (200–300 percent/d, preferably flow-through) should be used. This will still not amount to much water if the animals are held in small individual containers. Excess feed should be removed by siphon or net after alternate feedings.

3.10 BROODSTOCK SPAWNING

Broodstock spawning should take place in a dedicated room separated from the maturation area in order to keep the spawning area clean and to be able to carry out daily washing and disinfection of tanks without disturbing the broodstock. The spawning room should have sufficient and appropriate infrastructure for the level of naupliar production required.

Broodstock should be maintained, spawned and hatched individually so that one infected broodstock cannot infect the others in the facility. Spawning and hatching techniques should be aimed at promoting production of high quality, disease free eggs and nauplii. Individual spawning will reduce the risk of the vertical (or false vertical) transfer of diseases from the female broodstock to the eggs/nauplii. It has been shown that the tissues exuded during spawning and faeces can contain high levels of some viruses (IHHNV, HPV, baculovirus penaei (BP), MBV etc.) and bacteria, and exposure to these pathogens can result in the infection of uninfected females and the eggs produced during collective spawning.

Spawning tanks can be of any size ranging from 300–500 litres (for individual) and up to 5–8 tonnes (for collective) spawning. They can be made of a range of materials but are preferably black-coloured plastic or fibreglass or black epoxy-painted concrete. If collective spawning must be carried out, the number of females per tank should be as low as possible to limit the number of females exposed to potential infection (i.e. one female to 300–500 litres of water).

Spawning tanks may be flat bottomed, but if they are slightly conical or at least angled to the outlet, it allows easier and less damaging harvesting of all the eggs. They should allow the harvest of the eggs in such a way that they can be subjected to washing and disinfection after collection.

Water purification steps should be taken for spawning tank water so that the water quality is as good as possible. This will typically include passage through activated carbon (for removal of dissolved organics), cartridge filtration to <1 µm for suspended solids, followed by UV-light treatment to kill pathogens. Water quality should be maintained with a temperature of 28–32 °C and salinity of 30–35 ppt, as in the maturation tanks. EDTA is often added to the spawning tank water as a heavy metal

chelating agent at a dose rate dependant on the heavy metal loadings of the location, but typically 5–30 ppm. Laboratory reagent (LR) grade EDTA or preferably liquid Versene EDTA should be used, along with 0.05–0.1 ppm of treflan to kill fungi.

To help maintain optimal water quality, feed should not be provided in the spawning tanks. Aeration can be provided prior to introduction of the spawners, but should be removed thereafter to avoid the spawners bumping into the airlines during spawning and aborting the process.

As a general principle broodstock should be handled only when necessary to avoid undue stress. Excessive chasing of individual shrimp should be avoided. When holding the broodstock, grasp it firmly with the abdomen bent so that the uropods and telson are tucked between the walking legs to minimize flexing and the risk of dropping the shrimp. Avoid keeping the broodstock out of water for extended periods. For example, when transferring females to the spawning tank, they should be held as described while maintaining them underwater in beakers or buckets containing clean water.

Sourcing of gravid females should be done in the late afternoon/early evening (as soon as night falls) or at the most suitable time dictated by the photoperiod employed. When sourcing, use a strong, preferably waterproof, flashlight to see which of the females in the tank are gravid (those with the most highly developed, or stage IV ovaries). When a gravid female is found, a scoopnet is used to capture it as gently as possible and bring it to the side. The female is then inspected to see if there is a spermatophore inside the thelycum. If the spermatophore is present, the female should be disinfected with a formalin dip of 100 ppm for 3 min before being placed individually into the spawning tank. If there is no spermatophore present, the female is replaced in the maturation tank for remating.

The females should then be left in peace to release their eggs. Immediately after spawning, the female should be caught, dipped in povidone iodine (20 ppm) for 30 seconds and then replaced into her (cleaned, washed, scrubbed and rinsed) maturation tank. Then any faeces released by the females should be carefully siphoned out as this could be contaminated with pathogens. At about midnight (2–5 h after spawning) the eggs should be harvested and disinfected. A suitable system for harvesting the eggs, excluding broodstock faeces and ovarian tissues (using a prefilter made from 300–500 µm mesh, for example) is required. The eggs should be collected into a receptacle (about 20 litre volume, depending on the number of eggs) with a large, mostly submerged mesh of <100 µm pore size in order to retain them without damage. The 300 µm net containing any faeces/debris is then removed and disinfected. Once harvested the eggs should be washed and disinfected according to the protocols shown in Section 3.13. Following collection, washing and disinfection, the eggs can then be transferred to (preferably) separate egg hatching tanks in the hatching unit.

Egg and sperm counts should be made to determine egg production and fertilization. As a guide, the quantity of eggs spawned per female should be in the range of 200 000–400 000 eggs for females of 90–150 g body weight, and up to 450 000–1 000 000 eggs for 160–300 g females. To ensure good fertilization, sperm should be observed and quantified regularly through sperm counts using a high-powered light microscope.

The quality of the eggs should be assessed within 2 h after spawning when it will be easier to identify the fertilized and unfertilized eggs. If the quality of eggs is very poor, it is advisable to discard the eggs. The fertilization rate should be at least 50 percent and is more typically >75 percent. Where fertilization rates fall below 40–50 percent, consideration should be given to discarding the entire batch and investigations begun to determine the cause of the problem. A count of the number of eggs should also be made at this stage to allow an estimation of the hatching rate.

Specific protocols have to be followed during spawning for protecting the eggs from viruses such as WSSV, MBV and HPV and *Vibrio* sp. bacteria being passively transmitted from the female broodstock. Treating the females with any chemotherapeutic agent at

this stage might not turn out to be a viable option as the stress that would be imposed due to the presence of the chemical might affect the spawning. Therefore it is essential to maintain the animals in high quality filtered and treated water for successful spawning. Any faeces from the broodstock must be removed quickly and both the eggs and nauplii should be washed and disinfected as detailed later.

The testing of spent spawners by PCR for WSSV is recommended and should be compulsory for subsequent spawnings. MBV should be checked both before and after spawning by malachite green staining and microscopic examination of faecal matter.

3.11 EGG HATCHING

Egg hatching should take place in a clean, isolated room, away from the maturation and spawning areas to avoid contamination. Hatching tanks should be 200–500 litres in volume for individual hatching (preferable) or up to 1 000 litres for communal hatching and ideally should be stocked with up to one million eggs/tonne (1 000/litre). The hatching tanks usually have pronounced conical bottoms to allow good water circulation and aeration and easy harvesting, but can be flat.

Water quality should be maintained at 29–32 °C and 32–35 ppt salinity for optimal hatching. Laboratory reagent grade (LR) EDTA (10–30 ppm) and Treflan (0.05 –0.1 ppm) are usually added to the water in the hatching tanks for the same reasons as for spawning. The tank is provided with no or very slight aeration until the nauplii hatch, whereupon it is added/increased. If no aeration is provided to the eggs, periodic agitation can be accomplished by slowly stirring the water in the hatching tanks with perforated paddles to prevent the eggs from piling up on the tank floor. Currently in Indian hatcheries, very poor record keeping; failure to properly count and document spawning, fertilization and hatching rates; inappropriate use of chemicals; inadequate egg stirring and poor hygiene are all problems commonly encountered.

3.12 NAUPLIUS SELECTION

The nauplii should appear approximately 8 h after stocking the eggs. After this point (typically after 12–15 h, at about midday of the day following spawning), the aeration is stopped in order to harvest the nauplii (typically at stage 3–4).

As the nauplii display strong positive phototaxis, healthy nauplii can be harvested using a light to attract them to the water surface. To accomplish this, a dark cover or lid having a small hole cut in its centre is placed over the tank and a light bulb is suspended above the hole. The healthy nauplii are allowed to aggregate below this hole over a period of 20–30 min (while the eggs sink to the bottom) and can then be collected by bucket, siphon or <100 µm net from the water surface into a separate 10–30 litre bucket or nauplii collector in which they can be washed and disinfected.

The unhatched eggs and weaker nauplii that remain in the hatching tank are then chlorinated and discarded and the tank cleaned and disinfected. The spawning and hatching tanks are washed daily with calcium (or sodium) hypochlorite solution (30 ppm active ingredient) and rinsed with abundant treated water before being refilled. Discarding the weaker nauplii reduces the percentage of weak and deformed nauplii. A deformity rate of <5 percent is generally considered acceptable. An estimate is made of the naupliar condition using the extent of the positive phototaxis. To carry out this test, a sample of larvae is placed in a translucent container next to a light source and the displacement of the animals is observed. If 95 percent or more of the larvae move strongly towards the light, the batch is good; it is intermediate if 70 percent or more respond and poor if less than 70 percent move towards the light. Poor batches may be discarded, depending upon the selection criteria of each hatchery.

Also at this stage the nauplii should be counted and if the hatching rate is less than 40–50 percent, indicating poor quality, they should be destroyed by chlorination at 500–1 000 ppm calcium hypochlorite.

FIGURE 6
Schematic flow diagram of washing and disinfection procedures for eggs and nauplii

EGGS

As a preliminary step, use a 300 µm net to collect and discard any faeces from the spawning tank
↓
Gently collect eggs in a 50 µm mesh net
↓
Gently rinse in running seawater for 5–10 min
↓
Dip for 1 min in 50 ppm povidone- iodine solution
↓
Gently rinse in running seawater for 5–10 min
↓
Stock eggs in hatching tanks

NAUPLII

Gently collect nauplii in a 50–100 µm mesh net
↓
Gently rinse in running seawater for 5–10 min
↓
Dip for 30 sec in 100–300 ppm formalin solution or 60 ppm chloramine-T
↓
Dip for 1 min in 50–100 ppm povidone-iodine
↓
Gently rinse in running seawater for 5–10 min
↓
Stock nauplii in larval-rearing tanks

3.13 EGG/NAUPLIUS DISINFECTION AND WASHING

There is considerable confusion and inconsistency in the protocols used for disinfection of eggs and nauplii in Indian hatcheries. Eggs and nauplii must be washed and disinfected appropriately to prevent the vertical (or false vertical) transmission of viral (i.e. MBV, WSSV, BMNV), bacterial (*Vibrio* spp.), fungal, microsporidian and other diseases from lightly infected broodstock to eggs and nauplii. Suitable techniques for eggs and nauplii are described below and a schematic flow diagram is given in Figure 6.

A specially designed washing container (foreground) is used for washing both nauplii and Artemia *in hatcheries.*

3.13.1 Eggs

Following their harvest from the spawning tanks, the eggs (still in the <100 µm net, partially submerged in a tank or bucket) should be washed for 5–10 min with a steady but slow current of clean seawater at the same temperature and salinity as the water in the spawning tanks.

After washing the eggs should be gathered in the net and then dipped into an aerated bath of 50 ppm povidone iodine solution for 1 min. Finally they are washed once again for 5–10 min with a steady but slow current of clean seawater, this time at the same temperature and salinity as the water in the egg-hatching tanks. Treflan may also be added at 0.05–0.1 ppm to combat fungal infections. This disinfection will help to reduce the risk of disease transmission.

The eggs are then transferred to the hatching tanks, which are prepared with 5–30 ppm EDTA and 0.05–0.1 ppm Treflan to remove heavy metals and fungi, respectively.

3.13.2 Nauplii

After the eggs have hatched to nauplii and allowed to reach nauplius stage 3-4 (at about midday of the day following spawning), they should be harvested from the hatching

tank using a <100 µm nylon net partially submerged in a tank or bucket. Only nauplii that are attracted to the light at the top of the hatching tank should be collected, since these are the healthy ones.

The nauplii should then be washed for 5–10 min with a steady but slow current of clean seawater at the same temperature and salinity as the water in the spawning/hatching tanks. After washing the nauplii should be gathered in the net and then dipped into an aerated bath of 100–300 ppm (0.1–0.3 ml/litre) formalin for 30 seconds or 60 ppm (0.06 g/litre) chloramine-T for 0.5–1 min. They should then be dipped into an aerated bath of 50–100 ppm (0.05–0.1 ml/litre) povidone-iodine solution for 1 min. Finally, they should be washed again for 5–10 min 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. The nauplii are then already acclimated and are ready for transfer and stocking into the larval-rearing tanks.

This procedure for nauplii works best when it includes all the steps: washing, formalin treatment 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.

3.14 HOLDING AND DISEASE TESTING OF NAUPLII

Once the healthy nauplii have been harvested, washed and disinfected they should be held in holding tanks (preferably one per batch of nauplii) so that they can be checked for disease and be acclimated to the conditions in the larval-rearing tanks. The tanks should be 20–30 litres in volume so that they can contain one batch of nauplii (300 000–500 000 nauplii at up to 25 000/litre). These tanks can be static water, but are preferably able to have flow-through exchange of high quality filtered and treated seawater to maintain optimal naupliar quality and reduce the chances of contamination. Constant illumination should be provided.

During this holding phase, a sample of the nauplii should be examined by eye and using a compound microscope to check their quality. Additionally (if possible) all lots of nauplii should be tested for WSSV by PCR before transfer to the larval-rearing tanks. WSSV-positive nauplii should be rejected and destroyed by chlorinating at 500–1 000 ppm. Only WSSV-negative nauplii should be used for stocking the larval-rearing tanks.

Once the nauplii are accepted for stocking, the temperature and salinity in the holding tanks should be checked to ensure that they are the same as in the larval-rearing tanks and if not, these parameters should be slowly adjusted until they coincide by flushing water of the same quality through the holding tanks. The nauplii are then ready to be transferred to the larval-rearing tanks.

3.15 TRANSPORTATION OF NAUPLII

If the larval-rearing tanks are in the same location as the broodstock facilities, then nauplii can be transferred directly from the holding tanks to the larval-culture unit, either within the holding tanks/buckets or preferably by first transferring them to plastic bags and then carrying the bags, inside buckets, to the larval-rearing tanks.

If the larval-rearing tanks are in a distant location, then the nauplii must be packed in double plastic bags, one-third-filled with filtered, treated seawater from the holding facility and then filled up with pure oxygen. The plastic bags can be of any size, but should be stocked with a maximum of 30 000 nauplii/litre at up to one third of the bag volume, which is then filled with oxygen before sealing the bags with elastic bands. The sealed bags are then put into cardboard boxes or (preferably) insulated polystyrene foam boxes to maintain temperature and reduce stress during transportation.

No temperature adjustment is required for transportation times of 1–3 h, but if the duration will be longer, the nauplii should be chilled to 24–26 °C (depending on duration) to minimize stress and prevent them from metamorphosing into zoea before arrival at the hatchery. The maximum time available for transfer is 24 h (if the nauplii are packed at N2-3 stage) or 12 h (at N4-5 stage). Metamorphosis to zoea 1 larval stage should occur roughly 48 h after first hatching, or sometime during the evening/night of the second day after hatching.

If possible the transport vehicle should first be disinfected before entering the hatchery facilities. After unpacking the nauplii, the packing material must be incinerated.