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Improving *Penaeus monodon* hatchery practices

Manual based on experience in India

Cover photo:
Penaeus monodon hatchery in Vizag, India. Courtesy Dr. G. Subbarao

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Preparation of this document

Responding to a request made by the Government of India, a Technical Cooperation Programme (TCP) project was structured, with the view to improve the capacity of the State of Andhra Pradesh to better manage the shrimp aquaculture sector, with special reference to controlling diseases and managing health. The TCP, besides assisting the Department of Fisheries (DOF) of the State Government of Andhra Pradesh in managing shrimp health, also assisted in creating national capacity for emergency preparedness, empowering rural farmers by providing tools for the self-management of farming systems, improving the quality of hatchery-produced postlarvae and establishing overall better management practices for the shrimp aquaculture sector. It was felt that this multidisciplinary approach is required to obtain positive and permanent results.

This publication, "Improving *Penaeus monodon* hatchery practices. Manual based on experience in India" is one of several outputs of the TCP. It reviews the status of broodstock, hatcheries, postlarval production, health and opportunities for improving hatchery biosecurity and larval quality of tiger shrimp (*Penaeus monodon*). The publication also provides technical protocols and guidelines for improving hatchery biosecurity and larval and postlarval quality.

In preparing Section 3.6 (Broodstock quarantine), we have drawn extensively on material previously published in FAO Fisheries Technical Paper No. 450, *Health management and biosecurity maintenance in white shrimp* (Penaeus vannamei) *hatcheries in Latin America* (FAO, 2003).

Abstract

The successful farming of tiger shrimp (*Penaeus monodon*) in India is mainly due to the existence of some 300 hatcheries whose capacity to produce 12 000 million postlarvae (PL) annually has provided an assured supply of seed. However, the sustainability of the sector is still hampered by many problems, foremost among these being a reliance on wild-caught broodstock whose supply is limited both in quantity and in seasonal availability and that are often infected with pathogens. The current low quality of hatchery produced PL due to infection with white spot syndrome virus (WSSV) and other pathogens entering the hatcheries via infected broodstock, contaminated intake water or other sources due to poor hatchery management practices, including inadequate biosecurity, is a major obstacle to achieving sustainable shrimp aquaculture in India and the Asia-Pacific region. Considering the major contribution of the tiger shrimp to global shrimp production and the economic losses resulting from disease outbreaks, it is essential that the shrimp-farming sector invest in good management practices for the production of healthy and quality seed.

This document reviews the current state of the Indian shrimp hatchery industry and provides detailed guidance and protocols for improving the productivity, health management, biosecurity and sustainability of the sector. Following a brief review of shrimp hatchery development in India, the major requirements for hatchery production are discussed under the headings: infrastructure, facility maintenance, inlet water quality and treatment, wastewater treatment, biosecurity, standard operating procedures (SOPS), the Hazard Analysis Critical Control Point (HACCP) approach, chemical use during the hatchery production process and health assessment. Pre-spawning procedures covered include the use of wild, domesticated and specific pathogen free/specific pathogen resistant (SPF/SPR) broodstock; broodstock landing centres and holding techniques; broodstock selection, transport, utilization, quarantine, health screening, maturation, nutrition and spawning; egg hatching; nauplius selection; egg/nauplius disinfection and washing and holding, disease testing and transportation of nauplii. Post-spawning procedures covered include: larval-rearing unit preparation, larval rearing/health management, larval nutrition and feed management, important larval diseases, general assessment of larval condition, quality testing/selection of PL for stocking, PL harvest and transportation, nursery rearing, timing of PL stocking, use of multiple species in shrimp hatcheries, and documentation and record keeping. Information on the use of chemicals in shrimp hatcheries and examples of various forms for hatchery record keeping are included as Annexes.

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Foreword

The rapid development of shrimp farming in India is largely due to the setting up of a large number of hatcheries and the resulting availability of an assured supply of seed. Presently about 300 hatcheries are in operation with an annual capacity to produce about 12 000 million postlarvae (PL). In India wild-caught broodstock is the only source of shrimp seed. Studies indicate that about a quarter of wild-caught shrimp spawners are infected with white spot syndrome virus (WSSV). Furthermore the continuous exploitation of shrimp resources has brought about a scarcity of brooders, and their availability is also not uniform throughout the year. Viral-disease monitoring is an area of growing importance and biosecurity is also a serious concern for hatcheries, and thus protocols to address these concerns are urgently needed. Considering the major contribution of the tiger shrimp (*Penaeus monodon*) to global shrimp production and the economic losses resulting from disease outbreaks, it is essential that the Indian shrimp-farming sector invest in good management practices for the production of healthy and quality seed.

The FAO TCP/IND/2902 (A) project entitled "Health Management of Shrimp Aquaculture in Andhra Pradesh" is a result of a request made by the Government of India for assistance in building capacity to improve health management capabilities in shrimp farming in Andhra Pradesh. The TCP *inter alia* was aimed at providing tools to improve the quality of hatchery-produced PL through better health management and adoption of biosecurity measures at the farm and hatchery levels. The current low quality of hatchery–produced PL is considered a major obstacle to achieving sustainable shrimp aquaculture in the region.

The TCP benefited from close collaboration with other national and regional development agencies active in the field of aquaculture such as the Network of Aquaculture Centres in Asia-Pacific (NACA), the Aquaculture Authority (now Coastal Aquaculture Authority) and the Marine Product Export Development Authority (MPEDA). The TCP activities were conducted in collaboration with members of the private sector involved in hatchery production and the grow out of shrimp in Andhra Pradesh. This collaboration and cooperation between state agencies, regional and international agencies and the private sector not only improved the efficiency of implementation of project activities but also increased and expanded the size of the target groups and beneficiaries of the project.

This publication "Improving *Penaeus monodon* hatchery practices. Manual based on experience in India" is a major output of the TCP, based on strong consultation and collaboration between farmers, hatchery operators, scientists, state extentionists and several key experts in the field of shrimp hatchery production. We believe that this publication will be a milestone reference for shrimp hatchery operators and shrimp farmers in India and anyone interested in tiger shrimp farming globally. We commend and congratulate everyone involved in producing this document.

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Acknowledgements

The production of this manual was made possible thanks to the assistance of many people engaged in shrimp hatchery management and aquaculture (see Annex 1). In particular, major contributions were made by Drs Win Latt, Mathew Briggs and Rohana Subasinghe. Technical editing was done by Dr J. Richard Arthur. Mr José Luis Castilla Civit is acknowledged for layout design.

All other pictures, except cover page pictures are courtesy Dr Win Latt.

Mr P. Krishnaiah, Commissioner of Fisheries, Andhra Pradesh State Government is acknowledged for his leadership in the TCP project, which made this manual possible.

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Contents

Abbreviations and acronyms

ACC	Aquaculture Certification Council Inc.
BAP	Best Aquaculture Practices
BIOTEC	National Centre for Genetic Engineering and Biotechnology (Thailand)
BKC	benzalkonium chloride
BMNV	baculovirus midgut gland necrosis virus
BMP	Better Management Practice
BP	baculovirus penaei
BSCC	Broodstock Collection Centre
CAA	Coastal Aquaculture Authority
CCP	Critical Control Point
CIBA	Central Institute of Brackishwater Aquaculture
CMFRI	Central Marine Fishery Research Institute
COC	Code of Conduct
COP	Code of Practice
CSIRO	Commonwealth Scientific and Industrial Research Organization (Australia)
DOF	Department of Fisheries
EDTA	ethylene diamine tetraacetic acid
FAO	Food and Agriculture Organization of the United Nations
FCR	feed conversion ratio
FRDC	Fisheries Research Development Centre
HACCP	Hazard Analysis Critical Control Point
HH	high health
HPV	Hepatopancreatic parvo-like virus
HUFA	highly unsaturated fatty acid
IHHNV	infectious hypodermal and haematopoietic necrosis virus
LR	laboratory grade reagent
LRT	larval rearing tank
MAF	Ministry of Agriculture and Forestry
MBV	Monodon baculovirus
MPEDA	Marine Product Export Development Authority
NACA	Network of Aquaculture Centres in Asia-Pacific
NSTDA	National Science and Technology Development Agency (Thailand)
OIE	World Organisation for Animal Health
OSSPARC	Orissa Shrimp Seed Production Supply and Research Centre
PCR	polymerase chain reaction
PL	postlarva, postlarvae (plural form) or postlarval

PUFA	polyunsaturated fatty acid
PVC	polyvinyl chloride
SIFT	State Institute of Fishery Technology
SOP	Standard Operating Procedure
SPF	specific pathogen free
SPR	specific pathogen resistant
SPT	specific pathogen tolerant
TASPARC	Andhra Pradesh Shrimp Seed Production and Research Centre
TCBS	thiosulphate citrate bile salts
TSA	trypticase soy agar
TSV	Taura syndrome virus
UV	ultra violet
WSSV	white spot syndrome virus
YHV	yellow head virus

1. Introduction

Indian farmed shrimp production increased from about 30 000 tonnes in 1990 to around 115 000 tonnes during 2002–2003. This development underwent rapid growth between 1990 and 1995, when it reached 97 500 tonnes. The area under culture, which was 65 000 ha in 1990, expanded to about 152 000 ha in 2002–2003. The coastal State of Andhra Pradesh witnessed the maximum growth in shrimp farming, followed by Tamil Nadu. In the wake of this development, the sector also generated a large demand for shrimp postlarvae (PL), which could not be served from the hatcheries existing at that time in the country. The importation of PL from other Asian countries and poor management of the broodstock, the hatcheries and also the farms led to the outbreak of White Spot Syndrome Virus (WSSV) in 1994. The virus spread very rapidly, and the economic losses caused by mortalities were estimated at over US$ 200 million during 1999–2000.

Since 1994, WSSV has continuously affected the shrimp farms, and the lack of action plans to combat the disease has led to cross-contamination of farms in proximity. Many of the farmers in Andhra Pradesh with smallholdings of between 1 and 1.5 ha do not have the means to identify or manage the disease. This led to successive crop failures and economic hardships. The lack of alternative forms of aquaculture to utilize the shrimp ponds has further aggravated the problem.

India currently has approximately 154 000 ha of brackishwater land being used for shrimp culture. In 2004 Indian brackishwater shrimp production was 112 780 tonnes. Although India has significant potential for aquaculture development, of the 1 190 900 ha of land available for shrimp aquaculture, the current area under culture is about 155 000 ha and the average productivity is less than 0.75 tonnes/ha/yr (Table 1).

1.1 SHRIMP HATCHERY DEVELOPMENT IN INDIA

The number of shrimp hatcheries in India has increased rapidly since the late 1980s. There are now approximately 300 hatcheries, mostly in Andhra Pradesh State, with an average production capacity of 33 million postlarvae (PL) per year (see Table 2 and Figure 1). The total production of PL in India has increased with this hatchery development to approximately 10 billion per year in 2002–2003, requiring up to an estimated 200 000 broodstock per year (see Figure 2). This document will review the

TABLE 1
Analysis of shrimp culture potential, usage, and production in the maritime states in India

Maritime State	Potential area available (ha)	Area under culture (ha)	Production (tonnes)	Productivity (tonnes/ha/yr)
Andhra Pradesh	150 000	69 640	53 124	0.76
West Bengal	405 000	49 925	29 714	0.60
Orissa	31 600	12 116	12 390	1.02
Kerala	65 000	14 029	6 461	0.46
Tamil Nadu & Pondicherry	56 800	3 214	6 070	1.91
Karnataka	8 000	3 085	1 830	0.59
Gujarat	376 000	1 013	1 510	1.49
Goa	18 500	963	700	0.73
Maharashtra	80 000	615	981	1.60
Total	1 190 900	154 600	112 780	0.73

current state of the Indian shrimp hatchery sector and provide guidance and protocols for improving the productivity, health management, biosecurity and sustainability of the industry.

TABLE 2
Number and production capacity of shrimp hatcheries in India by State

State	*Penaeus monodon*		*Macrobrachium* sp.		Total	
	Number	Capacity (x 10^6)	Number	Capacity (x 10^6)	Number	Capacity (x 10^6)
Andhra Pradesh	148	7 882	43	1 453	191	9 335
West Bengal	2	100	9	66	11	166
Orissa	13	455	2	20	15	475
Kerala	22	484	7	53	29	537
Tamil Nadu & Pondicherry	73	2 863	8	215	81	3 078
Karnataka	13	301	0	0	13	301
Gujarat	2	45	0	0	2	45
Goa	1	20	0	0	1	20
Maharashtra	6	325	2	20	8	345
Total	280	12 475	71	1 827	351	14 302

FIGURE 1
Development of shrimp hatcheries in India

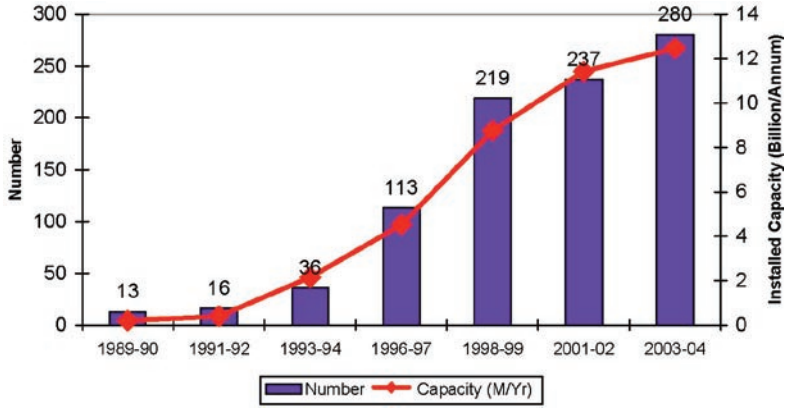
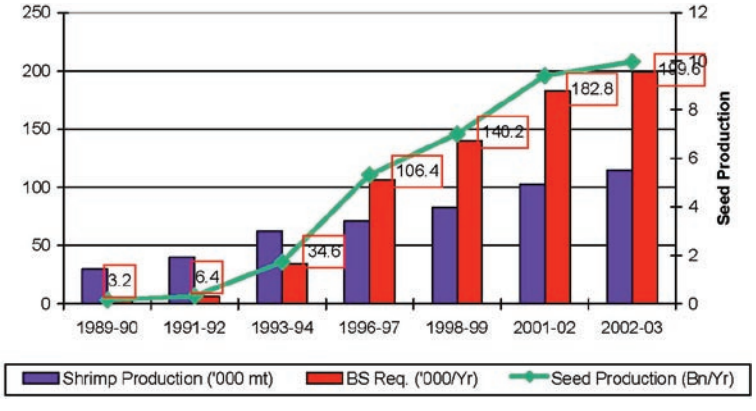


FIGURE 2
Shrimp production, seed production and broodstock requirements for India



2. Major requirements for effective hatchery production

In order to provide practical and effective technical guidance for shrimp hatchery management, it is first necessary to review the basic requirements for an effective hatchery production system. The following are the most important requirements:

- essential infrastructure;
- facility maintenance;
- inlet water quality and treatment;
- wastewater treatment;
- maintenance of biosecurity;
- development of Standard Operating Procedures (SOPs);
- consideration of the Hazard Analysis Critical Control Point (HACCP) approach;
- responsible use of chemicals; and
- assessment of health status of stocks through laboratory testing.

These components are discussed in detail in the sections that follow.

2.1 INFRASTRUCTURE

Hatcheries should be designed (or modified, in the case of existing hatcheries) to ensure good biosecurity, efficiency and cost-effectiveness and should implement Standard Operating Procedures (SOPs) in order to maintain productivity of large numbers of high quality postlarvae (PL). The infrastructure requirements for successful biosecurity and management of the hatchery operation will be discussed in the relevant sections of this document.

Many of the existing hatcheries now have infrastructural problems such as:

- inappropriate tank siting or design leading to high energy waste and high chance of contamination;
- low degree of design flexibility (so that they are difficult to modify); and
- unavailability of operating system security (i.e. a lack of alarms for water, air etc.).

A well-designed shrimp hatchery will consist of separate facilities for quarantine, maturation, spawning, hatching, larval and PL rearing, indoor and outdoor algal culture (where applicable), hatching of *Artemia* and feed preparation. Larger hatcheries may have separate units within each of these categories that should be run like mini-hatcheries for reasons of biosecurity. 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.

Additionally there will be supporting infrastructure for the handling of water (facilities for abstraction, filtration, storage, disinfection, aeration, conditioning and distribution), laboratories for disease diagnosis/bacteriology, as well as areas for maintenance, packing of nauplii and PL, offices, storerooms and staff living quarters and facilities.

The physical separation or isolation of the different production facilities is a feature of good hatchery design and should be incorporated into the construction of new hatcheries. In existing hatcheries with no physical separation between facilities, effective isolation may also be achieved through the construction of barriers and the implementation of process and product flow controls. If possible the hatchery facility should have a wall or fence around its periphery with enough height to stop the entrance

Some hatcheries have good laboratory facilities for polymerase chain reaction (PCR) diagnostics, water quality and microbiology, although day-to-day management system may not reflect the existence of such facilities

of animals and unauthorized persons. This will reduce the risk of pathogen introduction by this route, as well as increase overall security. Each operational unit should have sufficient area and perimeter to permit free passage and convenient working conditions.

The quarantine of all broodstock to be introduced into the hatchery is an essential biosecurity measure. Before introduction into the production system, the broodstock must be held in quarantine and screened for subclinical viral infections (i.e. by PCR). Many hatcheries in India are now equipped with their own PCR machines, while the others should collect and send samples to reputable external laboratories. Broodstock infected with serious untreatable diseases should be immediately destroyed and only animals negative for important pathogens

Harvest basin (below, left) is shared for four larval-rearing tanks (LRTs) and a drainage canal collects wastewater from several LRTs (below, right) before discharging into the main drainage line. This weak design is common in most hatcheries throughout the world and should be corrected by constructing a separate harvest basin for each tank before its wastewater flows into the drainage canal. This increases initial cost and requires more floor area for a hatchery but reduces the risk of disease being spread from infected tanks

such as white spot syndrome virus (WSSV) and monodon baculovirus (MBV) should be transferred to the maturation unit.

Harvest basins should not be installed in main drainage lines, as they may cause cross-contamination through water from one culture tank to the larvae being harvested. There should be a separate harvest basin/area for each culture tank before its connection to the main drainage canal. The elevation of the main drainage level should be lower than subdrainage carrying wastewater from each culture tank so that the wastewater cannot flow back and cause contamination.

2.2 FACILITY MAINTENANCE

It is not enough to have a well-built or well-modified hatchery facility. To achieve consistent production of high quality larvae, the production facilities must be maintained in optimal condition. Currently facility maintenance is not standardized in Indian hatcheries and is often quite rudimentary.

Lack of hygiene, systematic storage and maintenance is common in many hatcheries

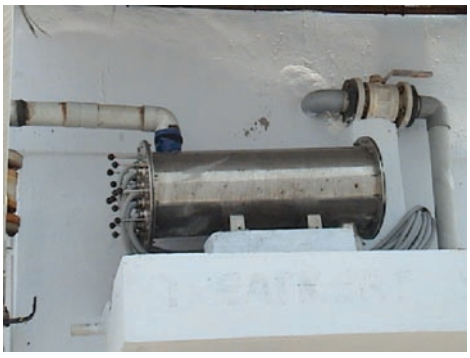
An example of an unhygienic, biologically insecure situation caused by improper management

Facilities must be maintained so as to optimize the conditions for the growth, survival and health of the shrimp broodstock, larvae and PL, minimizing the risks of disease outbreaks. In order to facilitate this, a set of protocols must be drawn up by the hatchery management as part of the SOPs and followed strictly by all staff members at all times. The hatchery's SOPs should include procedures for a sanitary dry out following each production cycle (for larval rearing) or at least every three to four months (for maturation facilities), with a minimum dry period following cleaning of seven days. This will help prevent the transmission of disease agents from one cycle to the next. Such maintenance will include (but not be limited to) the following:

Recirculating sand filters (RSF) are properly maintained at some hatcheries

2.2.1 Maintenance of machinery

Generators, water pumps, air blowers and water filtration equipment, including ultra-violet (UV) treatment systems, should be installed depending on the capacity of the hatchery. Regular inspection and servicing of all equipment is essential, including periodic changing of filters for blower inlets and backwashing and/or routine changing of the media in the filtration equipment. The generator, gas-driven pumps and blower rooms should be positioned at a sufficient distance from each other so as to avoid excessive noise and prevent the blower taking in exhaust from the machinery.



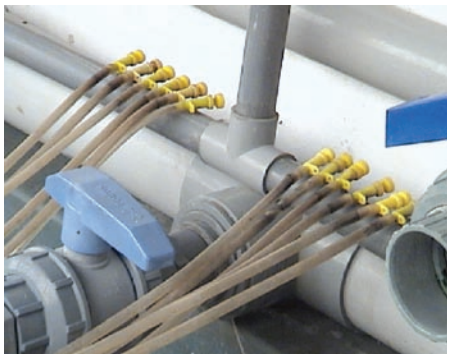
Wipers are installed to clean the UV lamp at some hatcheries

2.2.2 Regular cleaning and disinfection water, aeration and drainage pipelines

The water and air pipelines are potentially a major source of pathogen entry (particularly luminous vibrios) in the hatchery, both during and between production cycles.

Care should be taken while installing the plumbing to have the proper gradient to avoid stagnation of water in the pipelines. Pipelines and accessories should be

Filter bags for the air blower are kept clean



Air distribution pipes and diffusers should be cleaned, disinfected regularly and replaced when the pipes become contaminated (below, Tamil Nadu) The dark coloration at the connection and valve indicates the presence of dirt in the air supply and also inadequate cleaning of the air supply system

periodically checked for leakage and repaired as necessary. Assessment of biofilm formation inside the pipes should be done and remedial action taken if excessive. If possible two sets of pipes should be installed so their use can be rotated; one can be disinfected while the other is in use.

Pipelines should be periodically cleaned (at least following every cycle) by filling with a disinfectant solution, leaving for 24 h, flushing with clean water and then leaving to dry. Suitable disinfectants include chlorine (500 ppm), muriatic acid (10 percent), potassium permanganate ($KMnO_4$, 20 ppm), formalin (200 ppm) or hydrogen peroxide (20 ppm). Airline pipes should be fumigated with formalin and/or alcohol in the same way. It is also useful to install UV lights around the air pump intakes to disinfect the air before its entry into the hatchery.

The pipes drawing water from the sea by sub-sand well points or direct intake should be backwashed to the sea after every cycle with chlorine at 500 ppm or 10 percent hydrochloric acid (HCl) solution. The pipes should be filled and the disinfectant solution left to stand for 24 h before flushing with clean water and drying.

The drainage pipes carrying the wastewater away from the facility should be of a suitable diameter to drain water and avoid backflow. Regular cleaning and disinfection of drainage pipes and canals should be done as for the inlet water pipes.

2.2.3 Maintenance of tanks

To prevent the transmission of disease between tanks and cycles, all tanks and equipment should be thoroughly cleaned on a regular basis, cleaned and disinfected after use, and cleaned and disinfected again before starting a new production cycle. At this time, any problems with the tanks such as leaks should be addressed.

Tanks used for broodstock spawning, egg hatching and holding of nauplii and PL should be thoroughly cleaned after each use. The procedures used for cleaning and disinfection are basically the same for all tanks and equipment. They include scrubbing with clean water and detergent to loosen all dirt and debris, disinfecting with hypochlorite solution (20–30 ppm active ingredient) and/or a 10 percent solution of muriatic acid (pH 2–3), rinsing with abundant clean water to remove all traces of chlorine and/or acid, and then drying. The walls of tanks may also be wiped down with muriatic acid. Outdoor tanks and small tanks can be sterilized by sun drying. The following points should be considered:

- Tanks should be washed and disinfected at the end of every production cycle.
- All hatchery equipment should be regularly cleaned and disinfected.
- Concrete tanks painted with food-grade marine epoxy paint or plastic-lined tanks with rounded corners are easier to clean and maintain than bare cement tanks.

Larval-rearing tanks at some hatcheries are not painted with epoxy so cleaning is difficult

- After harvesting the larvae from a larval-rearing tank, the tank and all of its equipment should be disinfected. Similarly once all of the tanks in a room have been harvested, the room itself and all its equipment should be disinfected.
- Tanks can be filled to the maximum level and hypochlorite solution added to achieve a minimum concentration of 20–30 ppm active ingredient. After 48 h the tanks can be drained and should be kept dry (preferably with direct sunlight) until the next cycle starts.
- All equipment and other material used in the room (filters, hoses, beakers, water/air lines etc.) can be placed in one of the tanks containing hypochlorite solution after first cleaning with a 10 percent muriatic acid solution.
- Broodstock maturation tanks and all associated equipment should be cleaned and disinfected following a typical operational period of two to four months.
- Water pipes, air lines, air stones etc. should be washed on a monthly basis (or during dry out) with the same chlorine concentration and/or a 10 percent solution of muriatic acid (pH 2–3) by pumping from a central tank.
- All hatchery buildings (floors and walls) should be periodically (once per cycle is recommended) disinfected.
- All other equipment should be thoroughly cleaned and stored between cycles.
- Before stocking tanks for a new cycle, they should once again be washed with detergent, rinsed with clean water, wiped down with 10 percent muriatic acid and once more rinsed with treated water before filling.
- Disinfection procedures may require adjustment according to the special needs of the facility.

These tanks are painted with epoxy and well maintained but their corners should be rounded to allow easy cleaning and efficient disinfecting during preparation

Eliminating sources of contamination should be based on strict compliance with SOPs by hatchery personnel

Tanks and apparatus are cleaned, disinfected and placed in order but some items should be stored in a secure room

Appropriate safety measures must be taken when handling the chemicals used for disinfection. Procedures regarding chemical usage and storage, wearing of protective gear etc. should be included in the hatchery's SOPs.

Recommended products, concentrations and frequencies for the disinfection of various hatchery items are also given in OIE (2006).

2.2.4 Maintenance of filters (slow sand, rapid, cartridge, UV/Ozone)

All the filters and filter components should be washed and disinfected and replaced periodically. Slow sand filters should be backwashed (if possible) regularly and the media removed, washed and/or replaced after every cycle.

Rapid (pressurized) sand, diatomaceous earth (DE) and activated carbon filters should be backwashed before each use and at least twice each day (or as required based on the suspended solids loading of the incoming water) for a sufficient length of time to assure the cleaning of the filter. Being able to open the filters to check for channeling and thorough backwashing is an advantage. At the beginning of each production cycle, the sand must be replaced by clean sand that has been previously washed with sodium hypochlorite solution at 20-ppm active ingredient or 10 percent muriatic acid

Repeated use of cartridge filters should be justified based on the total suspended solids (TSS) of the water, volume of flow passed, and quality and condition of the filter. A condition like this is not safe to use for filtration

solution (pH 2–3). The filter media should be removed, washed and disinfected (and possibly replaced, as in the case of activated carbon) after every cycle.

For cartridge filters two sets of filtering elements must be available and these sets should be exchanged every day. Used filters are washed and disinfected in a solution of calcium (sodium) hypochlorite at 10 ppm active ingredient or 10 percent muriatic acid solution for 1 h. Some filter materials are sensitive to muriatic acid and thus care must be taken when this disinfectant is used. The filters are then rinsed with abundant treated water, dipped in a solution of 10 ppm sodium thiosulfate to neutralize chlorine (if used) and then allowed to dry in the sun. Two or more new sets of filters should be used for each hatchery cycle, depending upon the suspended solids loading of the seawater and the flow volume passing through the filters.

The recommended final size of filtration depends on the uses of the water as shown in Table 3.

Periodic assessment of the efficiency of ultra-violet (UV) bulbs should be made by maintaining records of hours of operation. Most high quality UV bulbs have a 40 percent reduction in efficiency after six months and hence require replacement. To assure efficiency, bacterial numbers before and after UV treatment should be checked routinely. Routine changing of prefiltration cartridges and regular cleaning and wiping of the crystal tubes containing the UV bulbs should be done to enhance UV filter efficiency.

Any alarm system for water levels should be checked and maintained in fully operational condition.

To prevent cross-contamination between different areas of the hatchery, separate recirculation systems should be used for each area. Water recirculation systems are the most efficient systems for broodstock maturation, as they reduce the need for water replacement and residual water discharge. Recirculation systems help maintain stable physical and chemical parameters in the water and also help concentrate mating hormones in maturation, as well as providing better biosecurity.

If recirculation of seawater is required for any area of the hatchery, additional water treatment unit(s) may be required to reduce waste loading and maintain optimal water condition. A typical water treatment unit may comprise mechanical filtration to remove settlable and suspended materials, activated carbon filtration to absorb organic wastes and therapeutic drug residues and biological filtration to reduce ammonia and nitrite. However, the exact requirements will vary depending on the area of the hatchery where it will be used and the percentages of water to be changed and recycled. There are many types of biofilters, all of which incorporate living elements (denitrifying bacteria) that must be cultivated or "spiked" (additional biological material added to the filter to accelerate the acclimation process) prior to use, so that their effects are optimized at all stages of the cycle. All types of filtration systems require periodic cleaning in a way that does not reduce their efficiency.

Water distribution from the reservoir to the various areas of the hatchery should be designed in a way that each area can be disinfected without compromising the other areas. In this way regularly scheduled disinfections can be accomplished at times appropriate to each area

TABLE 3
Recommended water filtration standards and water temperatures for different hatchery needs

Water use	Filter size (µm)	Temperature (°C)
Maturation	<15	28–29
Hatchery	<5	28–32
Spawning & hatching	0.5–1.0	28–30
Algal culture (indoor/pure)	0.5	18–24

and cross-contamination between areas can be avoided. Temperature and salinity regulation may vary between different sectors and is facilitated by well-designed distribution systems. In addition each area has specific filtration requirements that can be established prior to point of use, appropriate to each area of the hatchery. Pumps, pipes and filtration equipment should be sized so that maximum expected water exchange rates can be maintained to ensure that optimal conditions are met at all times.

2.3 INLET WATER QUALITY AND TREATMENT

2.3.1 Quality of intake water and treatment options

One of the major problems experienced in Indian shrimp hatcheries is poor quality intake water resulting in poor larval survival and overall production. This poor water quality is caused by the discharge of effluents by industries and urban areas and the clustering of hatchery systems, which leads to competition for water resources. Since most hatcheries are run as open systems, regular intake of seawater and release of effluents leads to water quality deterioration. Treatment of the effluent before discharge and the use of recirculation systems are the most viable options at this juncture, but are still little practiced in India, suggesting that inlet water quality will remain a significant problem. A survey of the Indian hatchery operators revealed a generally poor understanding of water quality management.

Water quality for shrimp hatcheries encompasses the sum total of the physical, chemical and biological factors of the oceanic waters that support healthy larval development. Regular analysis of water quality helps prediction of the level of production that could be attained under existing conditions.

Typical inlet water treatment currently involves mechanical separation of the suspended particles by filtration, chlorination and dechlorination, and storage under hygienic conditions. However, at the typical level of chlorine (10–20 ppm) currently used for disinfecting seawater, total elimination of pathogenic organisms is difficult to accomplish. Many disease organisms are able to remain domant for a short period and multiply later on at commencement of larval rearing. This has been the scenario in all hatcheries in India where *Vibrio* bacteria populations are found to increase exponentially from nauplii to PL, suggesting that chlorination alone is insufficient to eradicate pathogens from the water supply.

Under certain circumstances chlorination (and/or dechlorination using sodium thiosulphate) may have undesirable residual effects on the water quality, with the production of chloramines that may be toxic to the shrimp (particularly at the egg and naupliar stages) and precipitates of heavy metals. It is therefore sometimes impossible or inadvisable to use chlorination.

Because of this, additional (or only) sand filtration, then microfiltration, followed by ozonation and/or UV irradiation may be warranted, provided they are implemented with adequate care. UV irradiation must reach >30 000 mws/cm^2 in the incoming water flow, while the ozone content in water must be more than 0.5 µg/ml for 10 min for effective disinfection from viruses (including WSSV), bacteria, fungi and protozoa. A standardized programme should include monitoring the total bacterial and *Vibrio* counts immediately after the treatment and 72 h later to ensure complete disinfection.

Among the chemical factors to be considered under the water quality regimen, ammonia (NH_3) (< 0.1 ppm), nitrite (NO_2) (< 0.1 ppm) and nitrate (NO_3) (< 10 ppm) are the most important. No chemical method is available to attain this quality, and it is better to use biological nitrification or probiotics if these pollutants are present. Only a few Indian hatcheries currently monitor inlet water quality and when they do, it is usually limited to just temperature and salinity, and occasionally bacteriology. Each hatchery should also have (or have access to, via private-sector or governmental services) disease and water quality control laboratories to monitor the source water

TABLE 4
Ideal range for water quality parameters in maturation/hatchery facilities

Parameter	Ideal range
Salinity	29–34 ppt
PH	7.8–8.2
Temperature	28–32 °C
Oxygen	> 4 ppm
Heavy metals/pesticides	minimal level
Iron	<1 ppm
Ammonia (NH_3)	<0.1 ppm
Nitrite (NO_2)	< 0.1 ppm
Nitrate (NO_3)	<10 ppm
Hydrogen sulphide (H_2S)	<0.003 ppm

for water and microbiological quality. Currently such access is severely limited. To date no serious effort has been undertaken to understand the level of heavy metals, pesticides and dissolved organic matter in the intake waters of Indian hatcheries. The ideal range for the water quality parameters of hatchery intake water is shown in Table 4.

2.3.2 Inlet water treatment protocol

Currently although most hatcheries in India do treat their source water, treatment procedures, capacity and water treatment management systems are largely substandard. Also the water intakes of some hatcheries are located quite close to the effluent discharge of other hatcheries. Most hatcheries do not use source water quality monitoring results as a baseline for their water treatment system design, methods and application dose rates. If they do so, only two parameters, salinity and bacterial loading, are used for treatment dose rate calculations and no assessment of treatment efficiency is conducted.

Source water for the hatchery should be filtered and treated to prevent entry of disease vectors and any pathogens that may be present. This may be achieved by initial filtering through sub-sand well points, sand filters (gravity or pressure) or mesh-bag filters into the first reservoir or settling tank. Following settlement and primary disinfection by chlorination (and sometimes potassium permanganate), the water should be filtered again with a finer (1–5 µm cartridge) filter and then disinfected using ultraviolet light (UV) and/or ozone (where possible). The use of activated carbon filters, the addition of ethylene diamine tetraacetic acid (EDTA) and temperature and salinity regulation should also be features of the water supply system.

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 (e.g. quarantine areas). Separate recirculation systems may be used in critical areas or throughout the entire hatchery to reduce water usage and further enhance biosecurity, especially in high risk areas.

More specific water treatment procedures to be used for each phase of maturation and larval rearing are detailed in the appropriate sections.

Water intakes of some commercial hatcheries and nauplius centres are located close to the effluent discharge of others

2.3.3 Seawater intake

Before the water is brought into the facility, it should be checked for salinity and other water quality parameters (as in Table 4) to determine whether it is of suitable quality. Records of water quality analysis prior to abstraction should be maintained for future reference.

Normally the highest salinity obtainable (up to 33–34 ppt) is optimum, while salinity as low as 29–30 ppt is acceptable. Seawater of the best quality and 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 >29 ppt salinity is unavailable at the hatchery location, obtaining seawater by tanker from areas with higher salinity should be considered.

If possible the hatchery should use sub-sand abstraction points (either vertical or horizontal) in sandy intertidal areas, installed as low as possible on the beach, close to the limit of the low spring tides. If placed in this position, water should be available at all times apart from the lowest of low tides. The sand surrounding such points will act as a pre-filter for the water being drawn into the hatchery. However, this is site specific since sub-sand points cannot be used in muddy or rocky areas, where direct intake is preferred.

The sub-sand points comprise a series or gallery of drilled (or slotted) PVC pipes connected to the water intake pipe leading to the water pumps. These perforated pipes should be surrounded by 250-µm mesh screens and then placed into the sand and covered with gravel/rocks and then fine sand. The depth will be site specific but should not be so deep as to limit pumping capacity or enter unsuitable strata.

Direct intakes should be used in non-sandy areas or where the substrate is very dirty or contaminated. Such intakes comprise perforated pipes covered in 250–500 µm mesh (and possibly additional filtration media) and staked firmly to the seabed. The seawater is abstracted from a set height above the seafloor such that water will be available as constantly as possible without drawing in dirty/contaminated water from the seafloor.

2.3.4 Sedimentation/sand filtration of inlet water

Sedimentation and/or sand filtration tanks are required where the quality of the seawater brought to the facility is poor, particularly where high levels of suspended solids are present. 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.

The seawater is pumped into reservoir tanks and allowed to sit undisturbed until all the suspended material has settled to the bottom. The water can then be pumped to a separate tank for chlorination. 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 or not 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 backwashable sand filters (either large gravity-flow filters, or pressurized sand filters) before passing to reservoir tanks for chlorination.

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 render the use of chlorine ineffective.

Gravity flow or slow sand filters consist of one to three chambers filled with various sizes of gravel, coarse and fine sand and charcoal, in that order, before ending in a temporary reservoir. Pressurized (swimming-pool type) sand filters consist of a plastic/fibreglass shell containing gravel or coarse sand and fine sand, and valves for maintenance of the filter. The water is pumped directly through such filters on the way

to the reservoir tanks. Such filters have a small footprint and are very easy to backwash and clean, but may be more expensive than the slow-sand type.

The ideal size for these water storage reservoir tanks is about 30–50 percent of hatchery tank capacity. This should provide sufficient water for all the daily operations required in the hatchery.

2.3.5 Disinfection of inlet water using chlorine

Incoming water used in shrimp hatcheries should be disinfected prior to use to minimize the chance of viral, bacterial, fungal and protozoan diseases entering and causing disease problems in the hatchery. The commonest and best chemical treatment for such disinfection is the use of chlorine in the chlorination tanks.

Chlorine can be bought either as a powder (calcium hypochlorite, usually 60–70 percent active ingredient), liquid (sodium hypochlorite, usually 7–10 percent active ingredient) or as tablets (sodium dichloroisocyanurate, usually >90 percent active ingredient). Any of these forms of chlorine is 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 h is sufficient to kill most viruses, bacteria and fungi.

Chlorination is achieved by first filling the reservoir tanks with (preferably) filtered seawater. For an active chlorine concentration of 10 ppm add 15 g of 65 percent calcium hypochlorite powder (dissolved first in water), 100 ml of 10 percent sodium hypochlorite (liquid bleach) or 10–11 g of 90 percent chlorine tablets per tonne (1 000 litres) of water. Turn on the aeration for 5–10 min until the chlorine is fully mixed into the seawater, then turn off the aeration and let the tank stand for 12–24 h.

The reason for turning off the aeration is to maintain the chlorine concentration in the water for a long period of time so that it has the chance to kill any pathogenic organisms. 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 used in India for chlorine disinfection of incoming seawater.

After the 12–24 h time period, turn on the aeration system again to dechlorinate the water and measure the chlorine level with a swimming pool chlorine test kit (5 drops of ortho-toluidine liquid in 5 ml of water sample). Then compare the deepness of the yellow colour developed with the colour comparison charts that come with the test kit. Dechlorinating by vigorous aeration under strong sunlight requires only a short period of time. A chart or whiteboard must be provided giving the date and time of treatments and the results of these tests signed by the person who is responsible for the water treatment.

If chlorination and dechlorination is carried out in a roofed tank, a high level of chlorine residue may be present, as aeration alone is responsible for removing the chlorine. In this case add sodium thiosulphate (or vitamin C) crystals dissolved first in water at the rate of 1 ppm (1 g/tonne) for every 1 ppm of chlorine left in solution. Wait for 10 min with constant aeration and measure the concentration of chlorine again. If no yellow colour whatsoever develops, the water is ready for immediate use. If there is still yellow colour present, add another 1 ppm of sodium thiosulphate and recheck. Continue doing this until there is no yellow colour on retesting. Excess use of sodium thiosulphate to remove residual chlorine may cause larval deformity and thus should be avoided if possible.

Some hatcheries have found that chlorination may be undesirable for maturation systems, possibly due to either chlorine or sodium thiosulphate residuals. In some circumstances and/or where necessary, use of ultrafiltration including fine cartridge and UV or ozone filters may be preferable for the water intended for use in maturation systems.

It is a good idea to pass all water through an activated carbon filter before use for maturation or larval rearing to ensure that no chlorine byproducts or other dissolved organics are in the water supplied. This activated carbon can be housed in a filter or a filter bag on the inflow into the larval-rearing or broodstock tanks. The activated carbon media must be replaced at least every three weeks as it gets consumed and cannot be practically recharged.

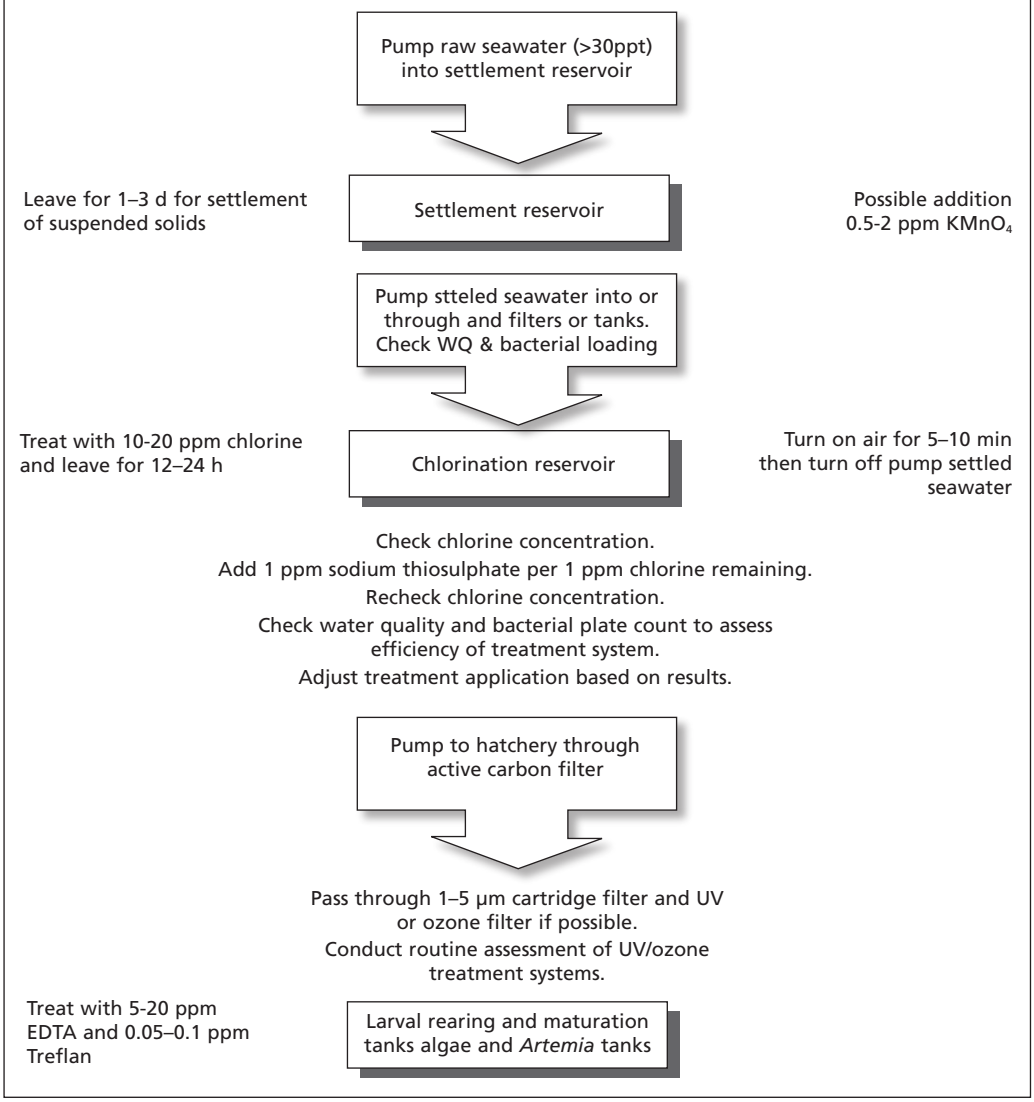
The flow and processing of inlet seawater to the hatchery facilities are shown in Figure 5.

Effluent discharge into the open sea adjacent to a hatchery without any treatment increases risks of disease

2.4 WASTEWATER TREATMENT

Recently in India and elsewhere, discharge of hatchery wastes has become a hot topic. Proper treatment and disposal of hatchery discharge will help ensure sustainability of the industry, reduce disease problems within the hatcheries and help avoid conflicts over water use with other industries and users.

FIGURE 5
Hatchery seawater inintake and treatment process



Currently only a very few hatcheries employ wastewater treatment systems before discharging into the open environment. Waste disposal areas or facilities for all types of hatchery wastes are absent from most of the hatcheries. Wastewater is neither monitored nor analysed before or after treatment in most of the hatcheries. In the case of mortality due to disease, dead animals are disinfected with chemicals and disposed of either within the hatchery compound, at designated secure places on land outside the hatchery compound, into the sea far from the hatchery operation or into the sea close to the hatchery operation. No standards are evident and this is an area for concern.

A well-run hatchery must ensure that all water discharged from the facility is free from pathogens. Hatcheries should aim at 0 percent contamination of their discharge. Wastewater from each facility will be released into special concrete or lined sedimentation tanks. From there it overflows into treatment tanks where the water will be chlorinated and dechlorinated through aeration. All water discharged from the hatchery, particularly that known or suspected to be contaminated (for example, water originating from the quarantine areas) should be held temporarily and treated with hypochlorite solution (>20 ppm active chlorine for >60 min or 50 ppm for >30 min) or another effective disinfectant and then well aerated (to dechlorinate) prior to discharge. This is particularly crucial where the water is to be discharged to the same location as the intake point. Such discharge close to the intake point should be avoided if possible.

If facilities for the effective treatment of wastewaters are not included in the current hatchery design, efforts should be made to redesign the system with this objective. In view of the large volume of wastewater discharged from hatcheries, recirculation systems should be evaluated for cost efficiency.

Construction of wastewater discharge and treatment systems is site specific. However, in general it is essential to ensure that drainage pipes, canals and treatment tanks are of adequate capacity to handle the maximum predicted flow of discharge water (taking into account the residence time in the treatment tank). Thus problems with water-logging, backflow and inadequate treatment can be avoided. The use of infiltration pits depends upon the soil, the water table and the relative height of the hatchery above groundwater level. If infiltration pits are impractical, direct connection to the receiving body of water can be made through long drainage canals, following treatment.

There are a number of water quality parameters that must be monitored in the discharge in order to comply with the general standards and to prevent polluting the environment surrounding the hatchery and its neighbours. There are some standards available, generally set by aquaculture certification initiatives. For example Table 5 shows the effluent standards for aquaculture hatchery operations in the United States of America set by the Aquaculture Certification Council. Although these standards may not be suitable for Indian hatchery operations, they may serve as a guideline for specific Indian legislation, which should be considered promptly. Additionally, although they mainly include physico-chemical parameters, there are other parameters such as bacterial and viral loads and chlorine and other disinfectant concentrations that should be considered together with flow rates to give total discharge levels rather than just concentrations.

TABLE 5
Aquaculture Certification Council Hatchery effluent standards [1]

Variable (units/frequency)[2]	Initial value	Final value
PH (standard units – M)	6.0–9.5	6.0–9.5
Total suspended solids (mg/litre – Q)	<100	<50
Soluble phosphorus (mg/litre – M)	<0.5	<0.3
Total ammonia-nitrogen (mg/litre – M)	<5	<3
5-day biological oxygen demand (mg/litre – Q)	<50	<30
Dissolved oxygen (mg/litre – M)	>4	>5

[1] Initial value is present recommendation, final value is to be complied with within five years.
[2] M = monthly, Q = quarterly checking.

Hatchery personnel must be careful not to create more problems than solutions with the treatment of effluents, since some chemicals such as chlorine, formalin, iodine, virucides, antibiotics etc. may also create problems if they are not first eliminated or allowed to dissipate prior to discharge. Use of such disinfectants must therefore be carefully controlled and excessive use avoided. Toilet wastes should not be discharged into any waterbody without treatment, which should be separated from the treatment of hatchery wastes.

Apart from discharge water, the hatchery will also produce solid wastes that also require proper disposal according to local regulations and laws. All potentially hazardous materials should be properly labeled and stored within the hatchery and disposed of by suitable means, i.e. incineration.

Shrimp stock (whether broodstock or larvae) that have become infected or died should also be disposed of properly so as to not contaminate the immediate environment with pathogens. This should involve suitable chemical disinfection (i.e. with chlorine at >50 ppm for 1 h) of the sick or dead shrimp, often within their own tanks, and removal and incineration of treated dead shrimp, before discharging the treated water into the drainage system.

2.5 BIOSECURITY

Biosecurity has been defined as "…sets of practices that will reduce the probability of a pathogen introduction and its subsequent spread from one place to another…" (Lotz 1997). Biosecurity protocols are intended to maintain the "security" of a facility with respect to certain disease-causing organisms that may not already be present in a particular system. Biosecurity encompasses policy, regulatory and programme frameworks (including instruments and activities) in response to managing risks associated with diseases.

The basic elements of a biosecurity programme include the physical, chemical and biological methods necessary to protect the hatchery from the consequences of all diseases that represent a high risk. Effective biosecurity requires attention to a range of factors, some disease specific, some not, ranging from purely technical factors to aspects of management and economics. The SOPs presented in this manual are designed to enhance biosecurity. Various levels and strategies for biosecurity may be employed depending on the hatchery facility, the diseases of concern and the level of perceived risk. The appropriate level of biosecurity to be applied will generally be a function of ease of implementation and cost relative to the impact of the disease on the production operations. Responsible hatchery operation must also consider the potential risk of disease introduction into the natural environment and its effects on neighbouring aquaculture operations and the natural fauna.

There are three components to practicing biosecurity in an aquaculture facility. These are:

- prevention – protection of the cultured/managed organisms from the harmful biological effects of undesirable organisms (especially pathogens) and the protection of humans and ecosystems from the adverse affects of the introduced culture system and its targeted and non-targeted organisms;
- control – control of the culture system, the movement of organisms and risk-related activities, and monitoring and recording of actions taken; and
- contingency planning – planning for all possible eventualities.

There are also two categories of biosecurity issues in shrimp hatcheries. These are:

- internal – concerning the introduction and transfer of pathogens within the facility; and
- external – concerning the introduction and transfer of pathogens from outside sources to the facility or vice versa.

Within aquaculture facilities, if diseases do occur, there are several options including:

- treatment – application of methods that reduce the effects of the diseases;
- containment – restriction of the diseases from spreading to other tanks/facilities; and
- elimination – elimination of the diseases from the vicinity.

Implementation of a biosecurity programme for a shrimp hatchery should include the following elements:

- use of disease-free and healthy shrimp stocks;
- use of quarantine areas for all incoming stock;
- analysis of all incoming stock for disease (i.e. through PCR or other immunodiagnostic technology);
- treatment of all incoming water sources to eliminate pathogens;
- sterilization and maintenance of clean equipment and materials;
- use of personal hygiene measures including washing of hands, feet and clothing;
- knowledge of potential pathogens, the sources of risk and methods for their control and/or eradication;
- development and use of stocks that are resistant to specific pathogens (SPR);
- maintenance of optimal environmental conditions within all phases of the facility; and
- application of immune enhancers and probiotics in order to enhance the ability of the stock to resist or tolerate diseases.

2.5.1 Personal sanitation and hygiene

Diseases that affect one tank of larvae or broodstock can easily spread to other tanks through contamination on the hands of hatchery staff or on equipment, if used for more than one tank. Therefore all equipment should be separately maintained, with one set for each tank, and wash bottles containing iodine or alcohol solution should be strategically placed for hand disinfection between visits to different tanks. Footbaths containing iodine, potassium permanganate or chlorine should also be placed at the entrance to each separate section of the hatchery to prevent transmission of diseases by foot. Separate colour coding can be used for utensils used in each section in the hatchery.

A 5–20 litre bucket containing a solution of 100 ppm 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 replaced daily with 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.

The entrance to each section of the hatchery (larval-rearing, broodstock, *Artemia*-culture and water treatment facilities) should have shallow buckets or trays placed there and filled with 200 ppm povidone iodine solution or 50–100 ppm chlorine solution to disinfect the footwear of each person entering the facility.

Wash bottles containing 20 ppm povidone iodine solution (or 70 percent alcohol) should be placed at the entrance to each culture facility in the hatchery so that hands can be disinfected before entering each separate facility. This should always be done.

2.6 STANDARD OPERATING PROCEDURES (SOPS)

Each hatchery should develop its own set of Standard Operating Procedures (SOPs). The SOPs is a comprehensive document outlining the control protocols for each stage or process of the production cycle occurring in the hatchery. The document should include details of all of the critical control points (CCP) and describe how to perform each task to control the associated risk. Once the protocol for hatchery operation is documented, the SOPs should be given to all personnel and a copy should be available

for all workers in an accessible place (dining room, meeting room etc.). A meeting should be held to introduce the protocol and explain the need for and contents of the SOPs. 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. All workers should sign a document indicating that they have read and understood the SOPs and that they will comply with all requirements.

All job descriptions of hatchery management and staff should include a clause related to following the SOPs and the disciplinary consequences of failure to comply.

As new information becomes available, it will be necessary to update or modify the SOPs, and any changes must be communicated to all personnel. Any updated version of the SOPs should have the date of the modification and a clear statement that the new version supersedes all previous versions.

It is advisable to have a group of people with higher technical training or experience who can supervise and train workers in the execution of each step of the SOPs. This point is of fundamental importance, as the workers may not understand either the standards required or the risks of non-compliance to the success of the hatchery. These technical personnel must organize meetings with the workers for each department to explain and discuss the importance of the execution of the SOPs.

Training in biosecurity maintenance should be an important component of the hatchery process. The biosecurity risk posed by each area of the hatchery should be determined. Different areas of the hatchery may be classified according to the level of risk of disease introduction or transfer. Weirich *et al.* (in press) used this system to describe four classifications:

- quarantine areas where a pathogen of concern is potentially present or suspected;
- high sensitivity areas requiring minimum exposure to avoid potential pathogen introduction or transfer;
- medium-sensitivity areas with lower risk of pathogen introduction or transfer; and
- low-sensitivity areas in which pathogen introduction or transfer is unlikely.

These classifications can be modified if required and the changes reflected in an updated version of the SOPs. Specific protocols and restrictions may be adopted for each of these biosecurity levels to prevent pathogen entry or transfer.

The document *Aquaculture Development* (FAO, 1997), part of the *FAO Technical Guidelines for Responsible Fisheries* series supporting the *Code of Conduct for Responsible Fisheries* (FAO, 1995) outlines a number of areas where SOPs should be developed. These will be specific for each type of facility and should include the following areas:

- responsible aquaculture management practices;
- improved selection and use of feeds, additions and fertilizers;
- safe, effective and minimal use of therapeutants, drugs, hormones and other chemicals;
- effective operation and health management promotion;
- regulated use of chemical inputs;
- disposal of wastes;
- food safety of aquaculture products;
- establishment of appropriate mechanisms for the collection and dissemination of information; and
- appropriate procedures for broodstock selection and the production of eggs and larvae.

This manual will suggest SOPs for each of these areas, suitable for *Penaeus monodon* hatcheries. However, each hatchery may modify the SOPs to suit their own conditions and situations without compromising the concept and objective of the SOPs. In addition an effective monitoring system with quick reporting and prompt necessary

action systems must be employed to cover all areas of the hatchery and HACCP principles must be effectively employed.

2.7 HAZARD ANALYSIS CRITICAL CONTROL POINT (HACCP) APPROACH

Development and implementation of biosecurity protocols can be made easier by a Hazard Analysis Critical Control Point (HACCP) approach. The HACCP approach is a preventive risk management system based upon a hazard analysis and has been widely used to identify and control risks to human health in food-processing systems. Critical limits are set at critical control points (CCPs) in the system where controls must be applied to prevent, eliminate or reduce a hazard. Monitoring and corrective actions are then implemented (Weirich *et al*. in press). HACCP principles have been applied as a risk management tool to control viral pathogens at shrimp research and production facilities (Jahncke *et al*., 2001).

2.7.1 Seven steps in applying the HACCP principles

Application of HACCP principles includes:

- performing a systematic hazards analysis;
- determining critical control points;
- establishing critical limits;
- determining appropriate corrective measures;
- establishing monitoring procedures;
- developing verification procedures; and
- designing record keeping systems.

HACCP analysis should be applied to shrimp production, with particular emphasis on reducing or preventing disease risks. Maximum biosecurity in shrimp production facilities can be achieved through the isolation of breeding, hatchery and production phases (Jahncke *et al.*, 2001, 2002). Good facility design with a high degree of isolation can help to reduce the risk of pathogen transfer from broodstock to their offspring. The critical control points (CCP) identified for the maturation and hatchery stages of shrimp production are the shrimp, the feeds and the water. Other potential risks to be covered by the implementation of SOPs and HACCP are disease vectors (human and animal), facilities and equipment.

A flow diagram should be created for the hatchery facility detailing all operations and the movement of shrimp and larvae through the production system. For each operation from broodstock receipt through maturation, larval rearing and where applicable, nursery, all potential hazards, impacts on larval health and quality, and points of entry of pathogens should be identified. Following this systematic hazard analysis, CCPs should be identified. For each CCP critical limits must be established and where these limits are exceeded, appropriate corrective actions determined. A system to monitor the CCPs must be established along with a good system of documentation and recording.

For different areas such as quarantine, maturation, hatchery, algal culture, *Artemia* production etc., it is necessary to identify CCPs. The following stages can be considered as CCPs, although these may not be the only ones and they can vary from one location to another:

- facility entrance – control and restrictions at entrance for operational workers, administrative employees, vehicles and other disease vectors to prevent transfer of infections from other hatcheries and the environment at large;
- water treatment – all the water used in production units must be appropriately (stage dependant) treated (chlorine, ozone, filtration, UV, etc.) to kill pathogens and their hosts;
- maturation – quarantine of incoming broodstock; checking and disinfection of fresh feed; cleaning of tanks and water/air lines; and disinfection of broodstock, eggs, nauplii and equipment;

- hatchery – regular dry-out periods; cleaning and disinfection of buildings, tanks, filters, water and airlines and equipment; quality control and disinfection of fresh feeds; separation of working materials for each room and each tank;
- algae – restricted entrance of personnel to algal laboratory and tank facilities; equipment, water and air disinfection; sanitation and quality control of algae and chemicals used; and
- *Artemia* – cyst disinfection, nauplii disinfection, tank and equipment cleaning and sanitation.

Hatchery workers must be restricted to their specific area of work and should not be able to move freely to areas not assigned to them. One practical way to manage this is to provide different colour uniforms for each area. This will allow quick identification of people in areas where they are not allowed.

The SOPs should address risks due to staff whose duties require them to pass through areas of the hatchery with different biosecurity classifications. For example, communication between staff working in different areas can be maintained while limiting movement between different areas of the hatchery by providing a central area where staff can meet to discuss and plan work schedules, and by communicating by intercom system, radios, text messaging, mobile phones or a local area network (LAN) for the computer systems.

All staff must take adequate sanitary precautions when entering and leaving a production unit. Rubber boots must be worn by staff when in the production areas. The production units (hatchery, maturation, algal culture, *Artemia* etc.) must have one entrance/exit to avoid unnecessary through traffic. The entrance must have a footbath with a solution of calcium (or sodium) hypochlorite with a final concentration not less than 50 ppm active ingredient. This disinfectant solution must be replaced when necessary. Next to the entrance door, each room must have a bowl with a solution of povidone iodine at 20 ppm and/or 70 percent alcohol, and personnel must wash their hands in the solution(s) when entering or leaving the room.

Special care must be taken with personal and shrimp transport vehicles because they may have visited other hatcheries or shrimp farms before arrival. All vehicles must pass through a wheel bath with dimensions such as to assure complete washing of the wheels. The wheel bath must be regularly filled with an effective disinfectant solution (such as sodium (calcium) hypochlorite at >100 ppm active ingredient).

The entry of potential disease vectors into the hatchery facility must be controlled. Some shrimp viruses are found in a range of terrestrial animals, such as insects and birds (Lightner, 1996; Lightner *et al*., 1997, Garza *et al.,* 1997). While it is not possible to control all potential animal vectors, their entry can be minimized by the use of physical barriers such as fencing. Wire nets or mesh can be used to exclude birds and insects while aquatic animals can be excluded by ensuring that there are no direct means of entry from open-water sources, especially via inlet pipes and drainage channels. All water entering the facility should be filtered and disinfected, and all drainage channels should be screened and/or covered, where possible, to prevent the entry and establishment of wild aquatic animals.

2.8 CHEMICAL USE DURING THE HATCHERY PRODUCTION PROCESS

Chemicals must be used responsibly during the hatchery production process. Chemicals (e.g. disinfectants, drugs, antibiotics, hormones etc.) have many uses in the hatchery production process, where they may increase production efficiency and reduce the waste of other resources. They are often essential components in such routine activities as tank, pipe and facility disinfection; water quality management; transportation of broodstock, nauplii and PL; feed formulation; manipulation and enhancement of reproduction; growth promotion; disease treatment and general health management.

Chemical use must be minimized and where essential, must be done in a responsible manner. Many chemicals are banned or restricted under Indian law. A discussion on the problems with antibiotic use and possible replacements is included in the section on larval rearing/health management (Section 4.2). Proper sanitation, hygiene and disinfection protocols; the use of modular, all-in/all-out facility designs and the use of probiotics in place of antibiotics may also help reduce the use of medicinal chemicals. In most cases chemicals should be used as a last resort; prevention is invariably cheaper and more effective than attempting chemical cures.

Many chemicals also pose potential risks to human health, other aquatic and terrestrial production systems and the natural environment. These include:

- risks to human health, such as dangers to aquaculture workers posed by the handling of feed additives, therapeutants, hormones, disinfectants and vaccines; the risk of developing strains of pathogens that are resistant to antibiotics used in human medicine; and the dangers to consumers posed by ingestion of aquaculture products containing unacceptably high levels of chemical residue;
- risks to production systems for other domesticated species, such as through the development of drug-resistant bacteria that may cause disease in livestock;
- risks to the environment, such as the effects of aquaculture chemicals on water and sediment quality (nutrient enrichment, loading with organic matter etc.), natural aquatic communities (toxicity, disturbance of community structure and resultant impacts on biodiversity) and effects on micro-organisms (alteration of microbial communities); and
- risks to marketing of the final products, since very low concentrations of some antibiotics (0.03 ppb for chloramphenicol and nitrofurans) are tested for in all shipments of shrimp imported into the United States of America and the European Union. If these banned antibiotics are found, the shipment is either returned to the point of origin or destroyed, resulting in significant losses for the exporters and the export potential and reputation of the exporting country.

It is essential that only qualified and adequately trained hatchery personnel be permitted to handle chemicals, that the chemical to be used for a particular situation is the most appropriate for the job and that it is used in the correct manner (e.g. amount, duration and treatment conditions).

Before chemicals are used, management should always consider if other, more environmentally friendly interventions might be equally effective. Effective and safe use and storage of chemicals should be an integral component of the hatchery's SOPs. A detailed review of the use of chemicals in shrimp culture, and in other aquaculture systems, can be found in Arthur *et al.* (2000).

The World Organisation for Animal Health (formerly the Office International des Épizooties, OIE), in its *Manual of Diagnostics Tests for Aquatic Animals* (OIE 2006) provides acceptable and recommended dosages of various chemicals and disinfectants to be used in shrimp aquaculture.

Annex II, Part A provides a summary of the chemicals mentioned in this document and how they are used in hatchery production of *Litopenaeus vannamei* in Latin America as given in FAO (2005). Although some of the dosages (concentrations and exposure times) provided in Annex II are slightly different from those given by OIE (2006), they have been found more effective in *L. vannamei* hatchery production in Latin America. These protocols have been discussed built consensus among the experts who participated in producing this document (FAO, 2005). Similar dosages will probably be effective for *Penaeus monodon* hatcheries in India and elsewhere.

2.9 HEALTH ASSESSMENT

Routine health assessments should be a component of good hatchery management. The health assessment techniques described below for use in shrimp hatcheries are

divided into three categories (levels) based on past experience gained from aquatic animal health management activities in Asia. The system was developed to measure the diagnostic capability required to diagnose diseases of aquatic animals, and thus the techniques commonly employed in shrimp hatcheries can be divided into the same three basic categories. The details of the different levels of assessment techniques are given in FAO/NACA (2000, 2001a, 2001b). They provide a simple and convenient separation based on the complexity of the techniques used (Table 6).

TABLE 6
Descriptions of diagnostic levels as adapted for use in shrimp hatchery systems

Level 1	Observation of animal and environment. Examination based on gross features
Level 2	More detailed examination using light microscopy and squash mounts, with and without staining, and basic bacteriology
Level 3	Use of more complex methods such as molecular techniques and immunodiagnostics (e.g. PCR, dot blots etc.)

2.9.1 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 operators and technicians 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 also 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 phototactic and weak swimming behaviour, it will be rejected without further examination.

2.9.2 Level 2 health assessment techniques

Level 2 techniques are also frequently used in the decision-making process in shrimp hatchery management. Most if not all hatcheries will have a microscope that is used to make more detailed examinations of the condition of the shrimp larvae and to observe directly various health-related features (cleanliness, feeding behaviour, digestion, etc.).

Many hatcheries also routinely employ basic bacteriology to gain an understanding of the bacterial flora of the tanks and to identify possible pathogens when the larvae become weak or sick. This information may then be used to make a decision on whether the tank should be discarded or treated.

The Andhra Pradesh State Institute of Fisheries Technology (SIFT) is now equipped with modern technology to provide better service for quality seed production

2.9.3 Level 3 health assessment techniques

Level 3 techniques are becoming more commonly employed in shrimp hatcheries. Polymerase chain reaction (PCR) methods are used for the screening of PL and broodstock for viral diseases, as are dot blot and other immunodiagnostic tests. The various applications of the different diagnostic techniques in a shrimp hatchery are given in Table 7. The use and application of these techniques are described in later sections.

TABLE 7
Use of level 1, 2 and 3 diagnostics in shrimp hatcheries

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

3. Pre-spawning procedures

For ease of reference, technical guidance on how to manage health and maintain biosecurity in shrimp hatcheries is arranged according to the basic hatchery production process, starting from broodstock options through to transportation of PL out of the facility. This has been divided into two broad categories: the pre-spawning process and the post-spawning process. The pre-spawning process includes procedures for broodstock collection/production, landing and holding, selection, transport, utilization, quarantine, health screening, maturation and nutrition. Also covered are spawning, egg/nauplius hatching, selection, disinfection and washing, holding and disease testing of nauplii and their transportation. As these procedures require different facilities, the facility maintenance guidelines are described under the different specific facilities used in the hatchery production process.

Indian shrimp hatcheries are totally dependent upon wild broodstock, with the bulk of the production coming from gravid females. Although there appears to be sufficient supply of these broodstock in Indian waters to satisfy the current demand, future problems are expected. These include probable broodstock shortages from the wild, as the Indian shrimp aquaculture programme expands to meet the Indian Government's plan to double shrimp production by 2010 and a high infection rate of broodstock with pathogenic viruses and bacteria during peak demand periods, leading to poor quality broodstock, diseases and losses in the hatcheries and farms. Data exist to show that unhealthy and infected PL lead to frequent crop failures with estimated losses of US$ 110–220 million per annum (1US$=44.9 INR, 1 crore = 10 million). To date there is no existing broodstock programme to support production of high quality seed.

3.1 WILD BROODSTOCK

3.1.1 The broodstock capture fishery

Information on broodstock availability in India is difficult to find. As part of the FAO study that lead to this document, discussions were held with shrimp trawlers' associations, trawler crews and hatchery owners on different occasions to collect primary information. However, middlemen and deep-sea trawler operators could not be contacted. More information is needed to assess the current status of the sector before presenting suggestions for its improvement.

Presently broodstock is obtained as by-catch from shrimp trawling and by the use of specialized traps, except in seasons of peak demand and value, when exclusive fishing for gravid female broodstock is done by a small percentage of trawler operators for short duration. The broodstock capture fishery has been dominated by near-shore operators; the extent of involvement by offshore deep-sea operators was impossible to review as information was limited. Near-shore trawlers supplied about 90 percent of the broodstock requirement while the deep-sea trawlers may have fulfilled the rest.

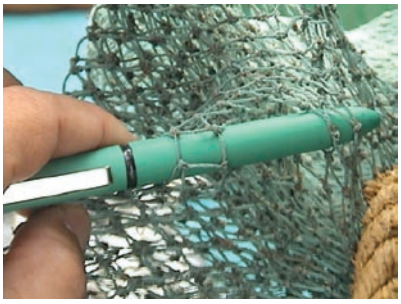
Sona baby trawlers at Vizag Fish Landing Complex

There are about 1 540 mechanized fishing vessels in Andhra Pradesh, of which 900 to 1 000 are 12–13 m "Sona baby trawlers," which mainly trawl for fish and shrimp. A survey of 26 Sona trawlers at Vishakapatnam (10), Kakinada (10) and Machilipatnam (6) indicated the availability of broodstock. Vishakapatnam has 500 trawlers which catch 21–28 percent shrimp, 3–5.8 percent of which is *P. monodon*, with an average

TABLE 8
Information on tiger shrimp broodstock as by-catch by mechanized trawlers in three districts of Andhra Pradesh

District	Fishing depth (m)	Vessel length (m)	Time (days/ fishing trip)	No. trawls/ day	Mean No. BS caught day/ boat	Transport time (h) (point of catch to jetty)	Total Mechanized Vessels (CMFRI/ DOF)	No. trawlers operating daily for BS
Vishakapatnam	30–50	12–13	1	3–5	2.5	8–14	500/600	375
Kakinada	20–36	12–13	3–7	5–6	1.6	4–7	600/500	200
Machilipatnam	18–28	12–13	5	4–7	2.6	9	200/238	170
	30–45	14.5	5	4–7	2-4	8		

Source: Broodstock fishery questionnaires, 2004



Small-mesh nets are used by most Sona trawlers to catch broodstock

daily harvest of 1–6 broodstock/boat; Kakinada has 600 trawlers catching 18.6–31.4 percent shrimp of which 1–2.3 percent is *P. monodon*, with a capture of 1–3 broodstock/d/boat; and Machilipatnam has 200 trawlers, catching an average of 4 broodstock/d/boat. If an estimated 25 percent of the Sona boats in Andhra Pradesh collected broodstock as by-catch, then about 500–700 could be made available to hatcheries every day.

A summary of information obtained from broodstock fishery personnel on broodstock fishing in Andhra Pradesh is shown in Table 8.

There are specific broodstock grounds, and trawlers usually do not cross to other waters of different districts for catching brooders. Most trawlers fished near shore at a depth of between 20 and 50 m. The impact of pollution below 50 m depth may be less, and a study is necessary to explore the availability and cost-efficiency of catching quality broodstock from the 50–100 m depth range.

Off the east coast of Andhra Pradesh fishing for broodstock is conducted 5 to 20 km from the shore where there is soft loam or sandy clay or clay-loam substrates with seaweed. Broodstock caught from the sandy coast of the Andaman Islands was reportedly of better quality than that from silty bottom areas.

Although trawling usually lasts from three to four hours, to reduce stress, broodstock-specific trawling lasted only 1 to 1.5 hours. The total catch per haul is spread on board, and any gravid female brooders are quickly collected and put into 50–100 litre containers. Battery-operated portable aerators are used to aerate the tanks.

As shrimp broodstock is largely by-catch, the fishermen need to modify present practice in order to reduce stress, improve general quality and minimize the time from capture to delivery of broodstock to the auction centres. There is a need for targeted short-duration trawling with nets having mesh size larger than the 1 cm mesh currently used (this should be discussed with trawlers and possibly incentives offered). Additionally the fishermen require training in selecting the right quality broodstock and in handling, storage and transportation techniques. The containers and aeration systems present on the Sona trawlers are often substandard and unreliable. After collection, the greatest risk to broodstock is thought to be due to bacterial-related mortality during transportation.

Broodstock-holding container and aerator on a Sona Trawler

Ideally individual animals should be transported in transparent plastic bags

filled with oxygen, sealed and placed on ice within insulated foam boxes to maintain a temperature of <29 °C. The use of bioreactor technology and/or anaesthetics to reduce metabolic activity during the holding and transportation of broodstock should be investigated. The literature indicates two possible anaesthetic compounds that could be used for the purpose: MS-222 (tricaine methane sulphanate at 150 ppm) and Aqui-(2-methoxy-4-propenolphenol – a major constituent of clove oil - at 20 ppm). MS-222 is the only anaesthetic agent licensed for use on fish intended for human consumption. A withdrawal period of 21 days is suggested following anesthetization of animals with MS-222 destined for human consumption; however, this does not apply to spawners destined for hatchery use only. Aqui-S™ is considered to be the safest anaesthetic since all ingredients are food grade and thus no withdrawal time is required. The use of these chemicals is not widespread and more research is required into their utility.

Shrimp fishing is a seasonal activity throughout India. The main season for fishing in Andhra Pradesh is June to February with the low (banned) season from March/April to May. In Vishakapatnam shrimp are landed throughout the year, but the main season is from July to December. The peak fishing seasons for Kakinada and Nellore are from September to December and from November to March, respectively.

Some trawler operators claim to have knowledge on locations where high quality broodstock can be caught. Through trial and error, some hatchery operators from Nellore also have a good knowledge of the seasonal and locational changes that affect broodstock quality; however, they tend to keep this information for their own use. In general large hatcheries with strong and diversified businesses tend to plan ahead to get good broodstock despite seasonal and locational changes in broodstock quality by closely coordinating with fishery operators and by paying at least 30 percent extra for high quality broodstock.

Fishing trip duration is about two to three days for the small trawlers; however when demand and price are high, a trawler will return to shore within a day with the broodstock gathered by all the trawlers to provide better quality.

Deep-sea trawlers tend to fish in depths of about 60 m where higher quality and larger broodstock is found. These trawlers usually spend around two to three weeks at sea and thus send their broodstock to port or landing centres via utility boats. According to some hatcheries, nauplii of better quality and quantity can be obtained from deep-sea gravid females but they are unable to use them for eyestalk ablation. However, some hatchery operators who also own fishing vessels have formed groups to get breeders from their deep-sea trawlers.

Due to the rapid expansion of the Sona trawler fleet in Andhra Pradesh since the early 1990s, there are concerns that over-fishing has occurred, and at least the artisanal fishery was clearly affected. For Vishakapatnam, Andhra Pradesh, the landings of *Penaeus monodon* have declined gradually from 5.8 percent in 1993–1994 to 3.0 percent in 1996–1997. For catch per hour, the decline was from 0.129 kg in 1994–1995 to 0.088 kg in 1996–1997. This indicates the importance of planning and management efforts aimed at improving the availability of tiger shrimp broodstock.

In terms of catch per hour by Sona boats of Vishakapatnam for 12 month periods, penaeid shrimp landings increased from 1.70 kg in 1993–1994 to 2.96 in 1996–1997. Overfishing tendencies were reported for *P. monodon* and *Metapenaeus affinis*, while stocks of other penaeid species appeared healthy.

In the Kakinada region from 1995 to 2002–2003, while there was an increase in total landings for all six varieties of shrimp, the catch composition percentage varied for different species. Discussions with trawler operators indicated that catches of tiger shrimp and Indian white shrimp (*Fenneropenaeus indicus*) have declined drastically, the catch per boat decreasing significantly because of the increase in the number of fishing vessels over the period. Currently (before the tsunami) there are 600 mechanized boats involved in fishing activity in Kakinada region. The lowest percentage composition in

the catch is for *P. monodon* (1.0–2.6 percent), followed by *F. indicus* (3.3–9.5 percent), and the highest is for *Metapenaeus dobsoni* (16.1–37.9 percent).

In other discussions, catches of *P. monodon* broodstock were reported to be consistent but comprising only a small percentage of the total landings. More information is required to predict future availability of the broodstock, which may be a crucial factor in the sustainability of the hatchery sector.

Tables 9, 10 and 11 give some historical data on the catches of shrimp from around India.

TABLE 9
Penaeid shrimp landings in Vishakapatnam by Sona boats from 1993–1994 to 1996–1997

Species	1993–1994	1994–1995	1995–1996	1996–1997	Mean
Shrimp (tonnes)	1 224	1 165	980	1 220	1 147
Metapenaeus monoceros (%)	27.0	25.3	30.3	23.6	26.6
M. dobsoni (%)	12.2	34.5	20.4	33.0	25.0
M. affinis (%)	12.4	7.9	3.0	3.0	6.6
Fenneropenaeus indicus (%)	14.5	8.2	14.1	11.7	12.1
Penaeus monodon (%)	5.8	5.7	5.1	3.0	4.9
P. monodon (tonnes)	70.6	67.0	49.7	36.1	55.9
P. semisulcatus (%)	1.0	1.0	0.7	0.7	0.9
Other penaeids (%)	27.0	17.3	26.3	25.0	23.9

TABLE 10
Marine shrimp landings by all mechanized boats in Kakinada between 1995 and 2002–2003

Species	1995	1996	1997	1998	1999	2000–2001	2001–2002	2002–2003
Marine shrimp (tonnes) [all boats total]	1 537	1 433	1 723	1 790	2 490	5 647	10 111	10 631
Sona boats (tonnes)	-	-	-	-	-	1 828	3 842	5 226
Sorrah boats (tonnes)	-	-	-	-	-	2 720	4 448	4 048
Penaeus monodon (%)	2.3	2.3	1.1	1.0	2.0	1.1	2.6	2.5
P. monodon (tonnes)	35.1	33.5	19.4	17.7	49.7	61.7	267.5	267.5
Fenneropenaeus indicus (%)	5.2	5.3	5.2	4.3	3.3	3.5	9.5	8.7
Parapenaeopsis stylifera (%)	12.6	15.3	12.0	9.6	10.6	15.1	17.1	20.3
Metapenaeus monoceros (%)	16.7	15.0	17.0	16.1	9.3	6.2	10.4	9.1
M. dobsoni (%)	31.6	30.1	37.9	32.0	25.5	16.1	18.4	19.5
M. brevicornis (%)	3.9	4.4	7.3	9.9	5.0	10.4	10.0	7.3
Others (%)	27.7	27.6	19.5	27.1	44.3	47.6	31.9	32.6

Source: Department of Fisheries, Kakinada, 2004

TABLE 11
Landings (tonnes) of penaeid shrimp on Indian coasts over the ten-year period from 1991 to 2000

Coastal States	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000
West Bengal	1 233	2 677	2 754	1 247	3 352	3 799	3 030	3 123	2 704	4 272
Orissa	1 972	2 738	2 986	2 520	5 350	3 557	2 966	2 276	4 323	6 911
Andhra Pradesh	10 759	10 797	16 200	15 513	13 863	15 138	14 193	19 011	24 967	22 573
Tamil Nadu	18 523	20 286	19 833	30 176	28 038	27 528	27 284	28 348	23 443	21 868
Pondicherry	654	400	146	785	458	361	104	702	368	437
Kerala	60 318	51 068	47 988	71 871	43 224	46 143	56 131	58 523	42 133	56 462
Goa	3 231	2 997	2 202	2 617	1 853	3 178	2 914	1 726	986	1 668
Maharashtra	57 976	58 055	56 416	52 413	40 450	52 984	49 819	45 832	31 840	47 611
Gujarat	26 376	29 980	20 151	39 061	34 533	27 935	42 621	48 630	34 414	38 354
Total	190 202	189 819	173 204	224 621	178 874	187 791	208 532	214 679	174 071	206 729

3.1.2 Broodstock quality

When considering the availability *of Penaeus monodon* broodstock, it is important to think in terms of quality as well as quantity. Sufficient numbers of gravid female broodstock appear to be consistently available to meet the current demand. However, measurements of broodstock quality remain somewhat elusive because of the lack of standardization and monitoring. Currently there is no precise information available on the percentage of broodstock infected with pathogens in the different areas (hatcheries, landing centres or catching sites). Additionally there are no quality criteria available, only morphological parameters being used currently.

Although there are 30 shrimp hatcheries in Andhra Pradesh equipped with PCR laboratories, only a few screen the broodstock for white spot syndrome virus (WSSV) infection. Random checking is performed by selecting one or two individuals from a batch of 50–60 broodstock. Moreover, quality appears to vary with season, and there are some indications that average size has become smaller and quality poorer in recent years.

In Andhra Pradesh at least, although the number of trawlers has increased and the catch per unit effort has declined, the total landings for at least one district remain unchanged. During the last three years the fishing crews have reported that they often caught broodstock with black spots on their heads and pleopods, necrosis and reddish colouration. These substandard broodstock are rejected by hatchery technicians except for the gravid females that are at stage IV.

As wild-caught broodstock from different trawlers are held in one container at the fish landing, they are marked by tieing a knot to their antenna to indicate their source. Such practices should be discouraged by proper training

The availability and supply of high quality broodstock is vital in successful shrimp hatchery operations and the production of high quality PL that can support the grow-out farming sector. With the emergence of several serious pathogens of cultured shrimp in recent years, disease-free wild broodstock have become rare around Indian coasts, as in all other Asian countries. Therefore extreme care must be given to the collection, transportation, handling and maintenance of broodstock free from such pathogens and to the biosecurity of hatcheries producing larvae from them. New areas for the capture of broodstock are required.

Most hatcheries in Vizag and some from other districts such as Kakinada have reported that broodstock quality has declined since 1995 and that the females are unable to undergo the maturation process. Mortality occurring during maturation, before or after performing eyestalk ablation, has caused the hatcheries to suspend their maturation operations and obtain nauplii from the two functioning maturation systems in Vizag or to obtain gravid females to satisfy their demands for PL production.

Highly stressed broodstock like this one could still be sold to hatcheries when there is a supply shortage. This situation poses a danger to the production of healthy shrimp seed

During the visits to hatcheries in Nellore some problems with a reduction in egg production from the female spawners were reported (this was disputed by others), but most of the hatcheries there have maturation programmes and have become centres for the production of nauplii, supplying them to hatcheries in other districts. Analysis of field information from Andhra Pradesh suggests that there has been a decline in broodstock quality since the year 2000 (see Table 12). Average data for both eyestalk-ablated and gravid females show a trend towards decreasing quality. However, this has yet to be scientifically documented and confusion remains.

TABLE 12
Performance of gravid and non-gravid female broodstock used at hatcheries in Andhra Pradesh

	Before 2000			After 2000		
	100–150 g	150–200 g	Mean	100–150 g	150–200 g	Mean
Eyestalk ablated (EA)						
Mean fecundity (millions)	0.50	0.59	0.55	0.43	0.49	0.46
Mean hatching rate (%)	80	78	79	76	78	77
Mean survival to PL20 (%)	43	41	42	42	43	43
No. spawners required to produce 1 million PL20	5.8	5.3	5.5	7.3	6.1	6.6
Gravid female (GF)						
Mean fecundity (millions)	0.42	0.65	0.54	0.40	0.57	0.49
Mean hatching rate (%)	80	81	81	77	83	80
Mean survival to PL20 (%)	40	34	37	35	39	37
No. spawners required to produce 1 million PL20	7.4	5.6	6.2	9.3	5.4	6.9

Field survey data, 2004

In order to solve their broodstock problems, some hatcheries have looked into the possibility of importing broodstock from sources outside mainland India such as the Andaman Islands, where more and better *P. monodon* broodstock have recently been reported to be available.

It is also possible that it is not broodstock quality that is causing this problem but rather poor water quality. Poor water quality may cause deficiencies in reproduction, maturation, spawning, fertilization, egg quality, hatching and survival of larvae and PL. This idea is lent support by the fact that the hatcheries in Nellore (where there is little other industry) are currently fulfilling the majority of the seed demand to the Indian industry, while the water supplying hatcheries in the Vizag and Kakinada areas is known to be contaminated with heavy metals. This could explain the difficulties that hatcheries in these areas are having with maturation, but further investigation is required, since some hatcheries are still able to conduct maturation, often with foreign technicians.

A further problem with broodstock quality is that there has been a high seasonal and spatial prevalence of viral and bacterial pathogens in wild shrimp broodstock. During 2001–2004 the broodstock tested by state and private laboratories confirmed varying degree of WSSV infections, although the exact source of these infections still requires further investigation.

Currently there is a certain degree of hatchery accreditation available in the form of a code of practice (COP) by MPEDA, while guidelines are in preparation by the Coastal Aquaculture Authority of India (CAA). Close coordination among stakeholders to establish an accreditation scheme for broodstock quality as an alternative may improve the broodstock supply business networks. Applying such accreditation schemes to seed production centres would indirectly improve the supply of quality broodstock, thereby reducing the risk of vertical transmission of pathogens.

Industrial pollution may be a major cause of poor water quality in broodstock fishing grounds close to Kakinada, as an industrial complex is located on the coast

3.1.3 Pollution

Increasing industrial pollution (and a lack of information about its extent) is a potential threat to the marine environment, including the availability and quality of shrimp broodstock and the hatchery and farm-culture operations. The expansion of chemical and oil industries in Andhra Pradesh State,

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:

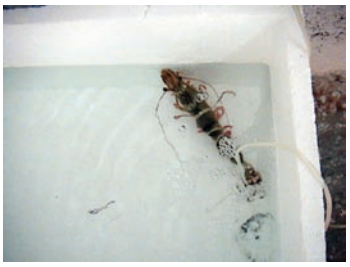
- It should be adequately isolated from all rearing and production areas to avoid any possible cross-contamination.
- It should be in an enclosed and covered building with no direct access to the outside.
- There should be means provided for disinfection of feet (footbaths containing hypochlorite solution 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

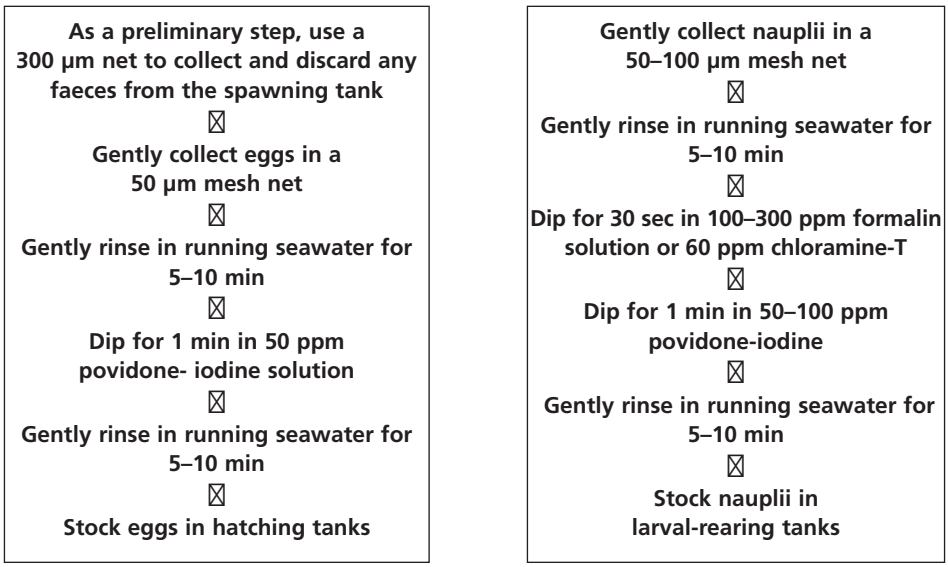
The nauplii should appear approximately 8 h after stocking the eggs. After this point (typically after 12–15 h, at about midday of the day following spawning), the aeration is stopped in order to harvest the nauplii (typically at stage 3–4).

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



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.

4. Post-spawning procedures

The post-spawning process includes the following components:

- larval-rearing unit preparation;
- larval-rearing management;
- health management;
- larval nutrition and feed management;
- important larval diseases;
- general assessment of larval condition;
- quality testing/selection of PL for stocking;
- PL harvest and transportation;
- nursery rearing;
- timing of PL stocking;
- use of multiple species in shrimp hatcheries;
- documentation and record keeping; and
- research and development and extension requirements.

4.1 LARVAL-REARING UNIT PREPARATION

In many cases, where hatcheries and farms form distinct economic units, larval quality is often sacrificed for economy or profit. However, in reality the most economic strategy is to produce PL that will grow quickly, are free from disease and that will give a high survival and production rate in the grow-out facilities. In order to achieve this, all areas involved in larval rearing must be designed for optimal efficiency, cleanliness and production of the best quality, high-health PL possible.

Entrance to the larval-rearing area(s) should be restricted to the personnel that work in these areas. Sanitary mats or footbaths containing a disinfectant solution (e.g. calcium or sodium hypochlorite solution, >50 ppm active ingredient) must be placed at the entrance of each room of the hatchery. The disinfectant solution must be replaced as necessary. At each entrance to the larval-rearing room(s), container(s) with povidone iodine (20 ppm) and/or 70 percent alcohol should be available, and all personnel must wash their hands in the disinfection solution(s) on entry to and exit from the rooms.

Each room should have a full complement of materials for routine operation (filters, meshes, buckets etc.). A tank of approximately 500–600 litres containing disinfectant (hypochlorite solution, 20 ppm active ingredient) should be provided to disinfect hoses, buckets etc. Common-use equipment can be placed in this disinfecting tank at the end of every day and rinsed before re-use the following day. The disinfectant in this tank should be replaced daily or as required.

Additionally, beakers, nets etc. used for each tank should be maintained in a bucket filled with sodium hypochlorite solution (20 ppm active ingredient) and dedicated to that one tank to prevent cross-contamination between tanks within the same unit.

The infrastructure for larval culture consists principally of one or more units of larval and PL rearing tanks that are either used from nauplius to PL harvest or sometimes in two phases. The design of the larval-rearing tanks should support effective cleaning of waste and appropriate water exchange. The first phase comprises tanks for larvae from nauplius up to PL4-5, which should preferably have conical, “U” or “V” shaped bottoms with a perforated air pipe or airstones situated at the base of the tank. The second phase (if utilized) comprises larger, flat-bottomed tanks or raceways for PL or nursery culture and stocked at lower density with PL4-5 until harvest (typically at PL15). Supporting infrastructure (discussed in more detail in Section 2) includes a

water storage, treatment, heating and distribution system; an aeration system; live feed production facilities for algae and *Artemia* (and others); laboratories for health checks, bacteriology and feed preparation; offices and an area for packing and shipping PL.

Protocols for tank disinfection and preparation for stocking are given in Section 2.2.3. These procedures should be followed to minimize the risks of transferring disease from one cycle to the next or from one tank (or unit) to the others.

4.2 LARVAL REARING/HEALTH MANAGEMENT

There are many factors involved in managing larval rearing and health in the hatchery. Tight control must be maintained on all of these factors throughout the larval-rearing cycle if good numbers of high quality PL are to be produced. Some of the more common factors affecting larval health during the larval culture cycle (assuming that high quality nauplii have been stocked according to the methods outlined in Sections 1.12, 1.13 and 1.14) are shown in Table 15.

4.2.1 Stocking rate

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

Before stocking the nauplii into the larval-rearing tanks, the high quality, disinfected and washed nauplii should be counted by taking at least three small samples from the nauplius holding tanks and calculating the average. They should then be acclimated to the larval-rearing tank water conditions by flow-through of water from the larval-rearing tank until temperature and salinity levels are equal. The nauplii should then be stocked into the larval-rearing tanks. Stocking density should be between 75 and 120 nauplii per litre (75 000–120 000/tonne), assuming a full larval–rearing tank (even though it will only be 50 percent full at stocking).

Ideally nauplii from individual spawners should be reared separately in individual larval-rearing tanks to avoid cross-contamination and maintain traceability. At a

TABLE 15

Some factors affecting shrimp larval health and possible control measures

Factor	Effects	Control measures	Standard
Excessive stocking density	• Stress • Cannibalism • Poor water quality	Reduce stocking density	750–150 nauplii/litre
Poor water quality: (A) sea water (B) tank water	• Mortalities • Late moulting • Deformities	(A) Improve water quality by proper filtration, chlorination &/or sterilization (B) Increase water exchange	• Filter < 5 µm • Activated carbon Chlorination (>10 ppm) followed by neutralization • UV or ozone • 20–100% water exchange per day
High bacterial loading	• Mortalities • Fouling • Deformities	• Improve tank & water disinfection protocols • Disinfect nauplii & live feeds used • Use probiotics	Zero green Vibrio colonies and low levels of yellow vibrios on TCBS agar plates
Long stocking period	Increased infection rates of later stocked larvae	Limit number of days to stock hatchery	3–4 d/ unit/hatchery
Poor feed (quality &/or frequency)	• Cannibalism • Malnutrition • Epibiont fouling • Poor water quality	• Appropriate feeding programme • Frequent checks on feed consumption & water quality	Feed every 2–4 h to satiation with high quality feeds
Poor quality and/or quantity of algae	• Mortality in zoeal stages • Fouling of larvae	Routine counts & quality checks	Chaetoceros or Thalassiosira at 80 000– 130 000 cells/ml
Infected Artemia nauplii	Source of bacteria leading to mortality	• Decapsulation of cysts • Disinfection of Artemia nauplii	Hydrogen peroxide or hypochlorite at 20 ppm active ingredient

production rate of 400 000–500 000 nauplii/female and a stocking density of 75–120 nauplii/litre, this would entail conducting larval rearing in individual larval-rearing tanks with a capacity of four to six tonnes.

Unfortunately the current setup in most Indian hatcheries is not suitable for rearing larvae spawned from single spawners, as the tanks are too large (generally >10 tonnes each). If individual spawning and rearing is not possible, mixing of two or more batches of only WSSV-negative (tested by PCR) nauplii from negative spawners is recommended.

4.2.2 Water exchange protocols

Indian hatcheries commonly have issues with water quality due to inadequate checking, control and treatment of inlet water and inappropriate management practices such as inadequate water exchange. Daily water exchange during larval rearing varies depending on the stocking density, the larval stage, the feeding regime and the water quality conditions in the larval-rearing tanks. In general the quantity of water exchanged should be sufficient to maintain the critical water quality parameters. The optimal levels for these parameters are shown in Table 16. The most important parameters that must be controlled are ammonia, nitrite, bacterial loading and quantity of suspended or settled wastes.

The quality of the new water (treated as detailed in Section 2.3) used for exchange should always be better than the quality of the water in the tanks in terms of bacterial loading and toxic metabolite (ammonia and nitrite) concentrations. However, it should be similar in terms of temperature, salinity and pH to avoid stressing the larvae with an abrupt change in these parameters. Whenever new water is added, it should first be treated with 5–10 ppm EDTA for chelation of heavy metals and bacterial inhibition, 0.05 ppm treflan to kill fungi, and (if desired) probiotics at 1–5 ppm to populate the rearing water with beneficial bacteria. A generalized scheme for water exchange (in a 10-tonne tank) is shown in Table 16.

Normally larval-rearing tanks should be filled to only 50 percent of their full capacity with clean, disinfected, filtered seawater at 30–35 ppt and 28–30 °C prior to stocking with nauplii. Then, during zoea stage, about 10 percent (of a full tank) of new water (including the volume of live algae used) should be gradually added per day until the tanks are full by the time mysis stage is reached. Water is not exchanged during the delicate 3–5 d zoeal stages (length of zoeal stage depends on temperature) since they are very delicate and the water quality should be good because the shrimp biomass is low and feeding comprises mostly live algae, which does not foul the water.

During the mysis stages 10–30 percent of the water is exchanged per day. This is accomplished by first draining water out of the tanks and then refilling the tanks gradually. When exchanging water, it can be either drained from the bottom drain of

TABLE 16
Water volume/exchange rate and mesh strainer size during different stages of larval rearing (for a 10-tonne larval-rearing tank)

Larval stage	Water volume (tonnes)	Drain water (tonnes)	Water exchange rate (%/d)	Flow-through	Mesh size	
					(µm)	(mesh)
Nauplius	5	-	0	-	-	-
Zoea	5–10	-	add 1 tonne	-	-	-
Mysis	10	7–9	10–30	-	300	85
PL1-4	10	6–9	30–40	-	400	65
PL5-8	10	5–6	40–50	-	500	50
PL9-12	10	2–5	50–80	If required	700	35
PL13-16	10	1–4	60–90	If required	1 000	25

the tank or siphoned out from the bottom. In either case mesh-net strainers will be required to prevent the removal of the larvae along with the water and wastes. The size of the mesh required will vary depending on the larval stage and should be changed according to the data given in Table 16.

Due to the possibility of stress on the delicate zoeal and mysis stages, some operators prefer not to change any water until the PL-1 stage is reached. In this case water can be added daily as for zoea and only exchanged after metamorphosis to PL.

During the early PL stages, water is exchanged in the same way but at greater exchange rates, aiming for 30–40 percent/d for PL1-4 and 40–50 percent/d for PL5-8. Later PL stages have greater requirements for water exchange, and rates should be increased to 50–80 percent/d for PL9-12 and 60–90 percent/d for PL13-16. If high levels of waste, ammonia, nitrite and/or bacteria are encountered, the water level in the tanks can be lowered and then new water allowed to flow through the tanks for some time, before refilling the tanks.

Typically in Indian hatcheries, less water than the recommended rates (as shown in Table 16) is used. Under these circumstances the major water quality problems encountered are the accumulation of unionized (NH_3) ammonia and nitrite (NO_2) above the 0.1 ppm critical concentrations, resulting in sublethal toxicity and allowing the proliferation of *Vibrio* sp. bacteria. To counteract this it is recommended that more water is exchanged (if available) and biological filters and/or ammonia and nitrite-consuming probiotic bacteria are used to control the levels of these toxic metabolites and repress the growth of pathogenic vibrios.

4.2.3 Siphoning of wastes

In addition to daily water exchanges, the bottom of the tanks will need to be siphoned from zoea 2-3 throughout larval rearing. Uneaten food and faeces may need to be siphoned from the bottom of the tanks periodically (although the 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. If excess sediment/wastes can be seen (using a light) on the tank floor, siphon them into a net and then transfer the contents of the net into a bucket. Any larvae siphoned from the tank can then be returned to the tank.

4.2.4 Aeration

Uniform aeration in all parts of the tanks should be provided through use of a perforated air pipe or air diffuser stones (1 stone/ft^2) situated at the base of the tank to help promote thorough oxygenation and turn-over of the water in the tank and to keep the larvae and the feed uniformly distributed in the tank.

4.2.5 Water quality monitoring

Water quality parameters of temperature, salinity, pH (optimum 7.8–8.2), ammonia (optimum <0.1 ppm NH_3), nitrite (optimum <0.1 ppm NO_2) and bacterial concentrations should be monitored daily (or every two days) in each tank and recorded on data sheets to ensure optimal conditions are maintained throughout the larval-rearing period. The results should be analysed and together with the results of the analysis of larval quality, used as a basis for determining the water exchange and disease treatment requirements for each tank. Water quality and health monitoring records must be kept at each larval-rearing tank, as well as in a central recording at the managers' office.

If any disease or water quality problems are noted, the water exchange rates mentioned above should be increased. Water temperature should be maintained at 28–30 °C day and night throughout the larval-rearing period (or increased to 32 °C for zoea), while salinity should be maintained at 30–35 ppt until acclimation for pond conditions from PL10-12 onwards occurs, if required.

Currently most Indian hatcheries have to shut down and dry out their operations periodically due to supposed chronic *Vibrio* infections, which cause massive mortality after a few continuous larval-rearing cycles. It is theorized that these vibrios are colonizing and forming resistant (even to scrubbing with detergents and formalin) microbial biofilms on the inner walls of the water pipes and larval-rearing tanks that support colonization with vibrios and their liberation into the water and subsequent infection of the larvae, often by the luminous *Vibrio harveyi*.

Recommendations for resolving this problem are as follows:

- routine monitoring (using bacteriology with TCBS agar) of *Vibrio* levels in the entire hatchery system to identify their source and hence facilitate eradication or suppression;
- appropriate clean-up, disinfection and dry-out procedures for facility, pipes and tanks;
- application of a steam gun between the culture cycles on concrete tanks to kill bacteria;
- proper protocols for filtration and disinfection of incoming seawater (see Section 2.3);
- effective disinfection of broodstock, eggs and nauplii prior to stocking (see Sections 3.1.3 and 3.6);
- rapid stocking of the entire hatchery (or at least each unit) within three to four days to prevent contamination of young larvae;
- use of separate equipment for each tank and routine disinfection of equipment, hands and feet when in production (see Sections 2.2 and 2.4);
- use of disposable plastic covers for larval-rearing tanks, at least until the PL stages;
- use of clean stocks of live algae;
- decapsulation and disinfection of *Artemia* nauplii prior to feeding;
- maintenance of optimal water quality through larval rearing by water exchange, siphoning, feed control and testing;
- reduced reliance on antibiotics whose overuse leads to drug-resistant strains of pathogenic bacteria; and
- application of effective probiotic bacteria throughout the larval-rearing cycle to out-compete and suppress pathogenic vibrios.

Following these protocols will not only help minimize disease problems due to *Vibrio* spp., but all other disease problems also.

4.2.6 Chemical/antibiotic use

Chemicals should be used only where absolutely necessary and then minimally and responsibly applied. A list of chemicals recommended as safe and suitable for use in shrimp hatcheries for specific reasons is shown in Annex 2.

In December 2005 the Government of India notified the Coastal Aquaculture Authority Rules, 2005, which lists the antibiotics and pharmacologically active substances banned for use in Indian aquaculture. This list is included as Annex III of this report.

MPEDA cites serious problems with the use of antibiotics including increased drug resistance of pathogenic bacteria, limits of the export potential for Indian shrimp and negative health consequences for consumers of contaminated shrimp. They therefore recommend that farmers attempt to replace the use of antibiotics with probiotics, comply with regulations governing the use of antibiotics, be conscious of and cautious with the health of consumers and gain awareness of the maximum permissible limits and withdrawal periods for antibiotics whose use is permitted in aquaculture.

The leaflet provided by MPEDA recommends that farmers and hatchery operators:

- check that the products they purchase are approved for use in aquaculture, clearly labeled and free from antibiotics;
- store chemicals correctly;
- only use chemicals approved for specific purposes;
- maintain detailed records of chemical use;
- verify that seed and feeds are tested to ensure freedom from antibiotics (and if not free, immediately inform MPEDA);
- be aware of recent developments and educated on the problems with antibiotic use;
- try to control the environment of the shrimp to prevent disease rather than treat their gross clinical signs;
- do not import or use chemicals that are not approved;
- try to use probiotics as replacements for antibiotics;
- maintain a withdrawal period before harvest if antibiotics are used; and
- obtain advice from MPEDA and other approved sources rather than from chemical salesmen.

In addition to this advice from MPEDA, it is recommended that a full compendium providing a list of all the banned drugs, a list of all permitted drugs, suitable withdrawal periods after use of antibiotics and guidelines and licensing requirement etc. be prepared through an expert consultation. Draft guidelines should be subjected to general discussion before submission to the Ministry of Agriculture, who should be charged with enacting and enforcing any laws generated.

Proper use of chemicals within the industry will also permit traceability, which will inevitably become a growing issue with exportation of cultured shrimp.

4.2.7 Use of probiotics to replace antibiotics

Hatchery managers should attempt not to use antibiotics during their operations. Antibiotics are problematic for many reasons including:

- they are often ineffective for major problems;
- they are expensive;
- some are dangerous to workers and consumers and/or illegal;
- they can create strains of bacteria resistant to antibiotics (even for humans);
- they can reduce the effectiveness of antibiotics to treat human sickness;
- they cause slow growth and low immunity in the larvae; and
- it is illegal to export shrimp containing certain antibiotic residues.

Currently many Indian hatcheries use a wide range of antibiotics but are gradually changing towards the use of probiotics. However, some problems are encountered with lack of knowledge on selecting good probiotics, inappropriate dose rates and lack of knowledge on proper probiotic procedures. Effective probiotics should be used prophylactically throughout the larval-rearing period. Probiotics confer a number of advantages over antibiotics for shrimp undergoing larval culture. These include:

- the use of probiotics is usually more effective and cheaper than using antibiotics;
- probiotics actually treat the cause of the problem rather than the symptoms as antibiotics do;
- there is no acquired immunity to probiotic bacteria, so they will continue working for a long time;
- probiotics are able to reduce levels of pathogenic bacteria through out-competing them for food and producing natural inhibitory compounds (i.e. bacteriocins) of their own;
- some probiotics utilize toxic metabolites (ammonia and nitrite) directly from the tank, thereby enhancing water quality;
- some probiotic formulae contain bacterial enzymes that are able to reduce levels of organic material including faeces, uneaten food and other particulate organics, leaving cleaner water and tank floors;

- some probiotics (i.e. *Lactobacillus* sp.) enter the guts of the shrimp where they assist digestion and serve as a source of nutrition and hence improve food assimilation and growth of the larvae and eliminate toxic metabolites at source; and
- some probiotics can compete for gut adhesion sites, limiting colonization with pathogens and perhaps activating the immune system .

Protocols for use of hatchery probiotics are as follows:

- Select a probiotic that has a good reputation and a high concentration (minimum 10^9 CFU/g) of a large number of bacterial species, together with yeasts and enzymes for their effects in reducing organic matter accumulation in larval-rearing tanks.
- The probiotic is first weighed depending on tank size and dose rate, and then put into a bucket with seawater and aerated for 4–24 h. It is then filtered through a 100 µm (250 mesh) net. The solid material (bran carrier) should be discarded and the liquid filtrate added to the larval-rearing tanks daily. Typical dose rates are 1–3 ppm/d for zoea, 2–5 ppm/d for mysis and are 3–10 ppm/d for PL, according to the manufacturers' recommendations.
- Some probiotics can also be added to the larval feed or "bioencapsulated" in algae and/or *Artemia*/rotifers to help administer them into the larval shrimp body. This is an area of promising research and may have a good future in shrimp larval rearing.
- Do not use probiotics and antibiotics together since the antibiotics will also kill the beneficial bacteria in the probiotics.

4.2.8 Responsible use of antibiotics

Where use of antibiotics is unavoidable, the following guidelines should be considered:

- Only use antibiotics that are specifically recommended and approved for use in Indian aquaculture operations.
- Where the product will be consumed, strictly follow guidelines on withdrawal periods after use of antibiotics (generally for farmers).
- Do not use antibiotics prophylactically, but rather only where absolutely required to treat specific bacterial problems.
- Conduct laboratory-based (histopathological and bacteriological) analyses to ensure that the problem is caused by bacteria, since antibiotics are only able to treat diseases of bacterial origin.
- Before using antibiotics attempt to locate and remediate the root cause of the problem, since antibiotic use will only treat the symptoms of the problem and not the underlying cause, which is invariably associated with poor environmental control. If this is accomplished first, antibiotic use or (at least) repeat doses may not be required.
- Conduct sensitivity testing of the recommended antibiotics on the particular bacteria causing the problems. Antibiotic sensitivity discs are available cheaply from local companies and can be used on bacterial culture plates inoculated with the problematic bacteria to determine which antibiotics are capable of killing them. Only after this analysis is done should the selection of antibiotic be done. (This work may need to be done under trained supervision in a dedicated disease laboratory).
- Follow recommendations on dose rates and time of treatment for the particular antibiotic selected. Use of suboptimal doses for short periods may create problems with bacterial resistance, while overdoses result in bacterial contamination of the environment on discharge and may cause reduced growth rate, feed assimilation and/or survival in the shrimp themselves.

4.3 LARVAL NUTRITION AND FEED MANAGEMENT

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 helps maintain good water quality while promoting fast growth and high survival of the larvae and hence optimal production from the hatchery. Some of the major biosecurity issues and disease problems facing Indian shrimp hatcheries result from a lack of control over the nutrition and feeding regime used during larval rearing. Particular areas of concern include a lack of, or poor algal-culture facilities and protocols, poor feeding management, unchecked health and quality status of the feeds and poor or non-existent disinfection protocols for feeds.

All steps of feed preparation, especially of live feeds (algae, *Artemia* and others) are a critical control point (CCP), because feed can be contaminated through inappropriate management. All sources of live, fresh or frozen food should be considered from the point of view of pathogen risk. The source, treatment, storage and use of feed items should be reviewed and steps taken to ensure that they are safe and properly managed.

The feeding regime used in shrimp hatcheries should be based on the use of live/preserved algae for zoea and mysis. Dead *Artemia* nauplii (killed by freezing) may be used only for mysis stages (since mysis have difficulty catching live *Artemia* nauplii). However, some Indian hatcheries prefer not to feed the larvae with *Artemia* at all during the mysis stages. In this case, feeding directly with live newly hatched *Artemia* nauplii is started at the PL-1 stage (see subsequent sections for live feed procedures and dose rates).

However, these live diets require supplementation with artificial dry or liquid diets to achieve optimal production of larvae. Dose rates for artificial diets should be based on observations of the larval feeding habits and water quality, manufacturers' recommendations and experience. Care must be taken not to overfeed with artificial diets, as this may lead to water quality problems and fouling of the larvae. Algae dose rates are shown in the next section. *Artemia* dose rates are calculated based on the use of about 6–7 kg/million PL produced, while trying to maintain 3–5 *Artemia* nauplii/ml in the larval-rearing tanks.

It is difficult to detail exact feeding protocols for larval rearing. The feeding regimes should be based on the specific requirements of the various larval stages, backed by frequent and detailed examination of the feeding activity of the larvae in each tank. Indications are given in this section of significant points to bear in mind.

TABLE 17
Guide to feeding regime for zoea and mysis stage larvae [1]

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	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

[1] Note: A = algae (live or preserved *Chaetoceros*, *Thalassiosira* or *Skeletonema* spp.); F1 = artificial feed (Fippak/Lansy/liquid feed/*Spirulina* etc) 10–80 µm; F2 = artificial feed (Fippak/Lansy/liquid feed/*Spirulina* etc) 50-150 µm; D = dead (well-frozen) *Artemia* nauplii (if required).

For zoea, microparticulate diets (i.e. microencapsulated Frippak CAR and Lansy Z-M and dried powdered *Spirulina*) or liquid emulsion diets (i.e. Epicore LHF or Cargill Liqualife) of 10–80 µm particle size are required. These should be fed alternately to the addition of live algae, up to six times per day (each for algae and formulated diets) over a 24-h period, to satiation. If the zoea maintain long trails of faeces and all stages of larvae are seen to have full guts at all times, 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 amount of faeces and uneaten food on the tank bottom.

For mysis, similar dry or liquid diets of 50–150 µm-sized particles should be fed in the same manner, as shown in Table 17.

For PL a variety of similar dry, liquid and crumbled flake diets of 200–300 µm-sized particles for PL1-8 and 300–500 µm from PL9-15 should be fed. This feeding is in the same manner, little and often, once every 4 h (six times/d) over each 24-h day period, alternating with six feedings per day of newly-hatched, live *Artemia* nauplii, as in Table 17.

For all of these artificial diets, sufficient aeration should be provided to maintain the dry or liquid feed particles in suspension at all times. This is so that they are always available to be eaten by the larvae and that they do not settle out onto the tank bottom where they are unavailable and may lead to deterioration in the tank water quality.

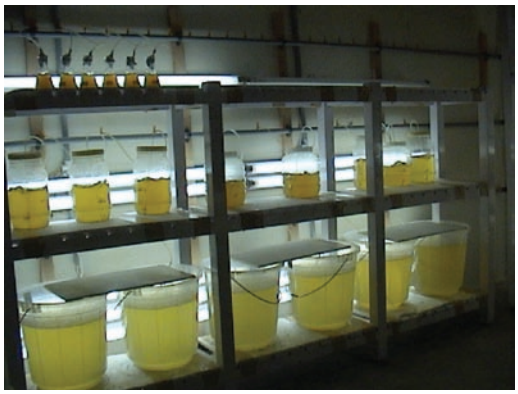
4.3.1 Use of live algae

Live (or preserved, if live are unavailable) diatom microalgae are the best food for early larval stage (zoea and mysis) shrimp. Not only are they self-suspending in the water column, they offer the perfect nutrition, contribute to enhancement of water quality (by absorbing ammonia, nitrite and carbon dioxide and producing oxygen), maintain shade in the water, produce natural and helpful bacteriocides and act as enrichment, increasing the nutritional value of the *Artemia* fed. Live algae should therefore always be offered to the early-stage larvae, as long as the algal culture can be kept clean.

However, one of the most common sources of pathogenic *Vibrio* spp., fungi and protozoa in larval rearing is the live algal cultures. Thus when culturing live algae, it is vital to start from a clean stock culture, maintain systems of hygiene and ensure that the algal-culture tanks, the equipment and the water used in them is clean and free from pathogens. General water disinfection procedures are provided in Section 2.3, but for algal culture, additional filtration of water and air (to 0.5–1 µm), and UV treatment of the water is required for all stages of production. Ozonation has also been used but any residual ozone can cause problems for algal growth and it is best avoided. Additionally all water, equipment and pure laboratory-grade fertilizing chemicals used in the algal-culture laboratory should be sterilized by autoclaving prior to use.

All algal-culture tanks must be washed and disinfected after each harvesting. Following disinfection of the tanks with calcium (sodium) hypochlorite solution (10 ppm active ingredient), they should be rinsed with clean, treated water and washed with 10 percent muriatic acid before being left to dry. The tanks should be plastic, fibreglass or epoxy-painted or plastic-lined concrete to aid cleaning operations and maintain clean algal cultures. If possible the tanks should be transparent or white in colour and the water depth should not exceed 1 m to increase light availability and algal growth.

Pure cultures of algae must be obtained and maintained using appropriate sanitary and microbiological procedures. The algal-culture laboratory should be well-lit with fluorescent tubes and air-conditioned to maintain temperatures between 18–24 °C. The algae are cultured here using extreme hygiene and sanitation to at least until 20-litre bag or carbuoy size. The algae are then passed outside the laboratory to cylinders or small tanks that are either well-lit artificially and/or in direct sunlight.



Indoor algal culture with different phases of multiplication at a hatchery

It is important to continuously monitor *Vibrio* concentrations (particularly the pathogenic green colonies) in the algal-culture system prior to feeding to maintain control over possible pathogens. Use of probiotic bacteria in the mass algal production systems has shown promise in maintaining clean algal cultures. It also doses the larval culture tanks with beneficial bacteria at the same time as adding the algae.

Due to the high risk of contamination from/to these areas, entry to the algal-culture and *Artemia*-culture rooms must be restricted to authorized personnel only, and staff from these areas should not be able to enter other production areas. A footbath containing a disinfecting solution (calcium (sodium) hypochlorite, >50 ppm active ingredient) should be placed at the entrance of each room. This solution must be replaced as often as necessary. As in other areas, container(s) of disinfectant solution (20 ppm of povidone iodine and/or 70 percent alcohol) should be placed at the doors and all staff should wash their hands on entering and leaving the room.

Single-celled algae such as *Chaetoceros*, *Thalassiosira*, *Tetraselmis*, *Isochrysis* and *Skeletonema* spp. are most commonly used. Pure cultures of all the algal species used should be maintained and cultured and subcultured on site, at all stages (from agar plates and tubes/bottles in the laboratory to massive on-growing outside). Alternatively pure starter cultures can be purchased from reputable algal-culture laboratories and be on-grown in the hatchery's massive tanks. Appropriate sanitary and microbiological procedures should be used to ensure the quality of the culture. The procedure of buying one lot of pure algal culture and continuously subculturing it throughout each larval culture cycle (commonly done with *Skeletonema* spp.), is not recommended, as it can easily lead to contamination of the algae and eventually, of the larvae themselves.

Once bloomed, the algae are examined using a microscope to determine cell concentration, growth phase, condition and presence of bacteria and protozoa. If free from contamination, at high density (>1 million cells/ml) and determined still to be in the highly nutritious log-growth phase, the algae is then pumped directly from the massive algal-culture tanks (through clean pipes) into the larval-rearing tank. Alternatively, with some species it can be filtered through a fine-mesh bag and the concentrate added to the larval-rearing tanks. Feeding with live (or preserved) microalgae is usually done two to six times per day, depending on the levels of algae remaining in the tanks after algal cell counts have been made.

The microalgae are usually offered to the larvae from the last naupliar stages (at 50 000–80 000 cells/ml), so that upon metamorphosis to the first feeding stage (zoea 1), the larvae will be able to begin feeding immediately. Concentrations are usually maintained at 80 000–130 000 cells/ml throughout the zoeal and mysis stages (peaking at Z3), and then decline to 50 000–60 000 cells/ml through the PL stages as the larvae become more carnivorous (as shown in Table 18). During PL or nursery culture, benthic algae are often used, as the PL will begin grazing algae from the walls of the tanks.

If concentrated preserved algal products are to be used, they should be purchased fresh and maintained in a refrigerator until required. They are then added up to six times per day in small quantities as per the manufacturer's recommendations, to maintain the desired algal cell densities as shown in table 18.

At least twice daily (and preferably six times per day), the number of algal cells in each tank should be counted (using a haemocytometer and a microscope) and

TABLE 18
Algae maintenance regime for zoea, mysis and early PL stage [1]

Day	Stage	Time					
		0:00	4:00	8:00	12:00	16:00	20:00
1	N/Z1			50–80	50–80	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

[1] Numbers are algal cells/ml to be maintained in larval-rearing tank.

compared to the desired range as shown in Table 18. Any deficiency in algal cell numbers should then be made up by adding appropriate volumes of algal cultures for which the cell density has already been established. Excess algal density should not be used since it may lead to fouling of the larvae. Regular monitoring of algal density in the larval-rearing tanks is crucial. This is especially the case with tanks open to the sunshine, where natural blooms will occur in the larval-rearing tanks with little need to add more algae beyond the first application, except where high water exchanges are conducted.

4.3.2 *Artemia* use

Artemia nauplii are the most important food item available to the shrimp hatchery. They are the main food fed to larval shrimp from early mysis all the way through to harvest of PL10-15 or more. Typically 6–7 kg of *Artemia* cysts will be fed for each million PL produced in *Penaeus monodon* hatcheries. Feeding with *Artemia* is conducted four to six times per day, always attempting to maintain 3–5 *Artemia* nauplii/ml in the larval-rearing tank so that they are always available to be fed on.

Because *Artemia* cysts are normally expensive and a potentially serious source of contamination, a number of procedures (involving decapsulation, hatching and disinfection) must be followed to optimize cost-efficiency of use while minimizing the risks of contamination of the larvae. Although the cost of *Artemia* cysts varies depending largely upon hatch rate, the best quality (highest hatch rate) *Artemia* available should be purchased since it is of superior nutritional quality and leads to optimized resource use in the hatchery. Whichever cysts are purchased, measures should be taken to ensure that the *Artemia* do not pose a risk of disease introduction. Certification may be requested for freedom from TSV, WSSV and YHV viruses by PCR analysis for all batches of *Artemia* cysts purchased.

Artemia *decapsulation*

Although *Artemia* cysts may not carry major viral pathogens, they are certainly a significant source of bacterial, fungal and protozoan diseases. Therefore decapsulation (removal) of the cysts is recommended to increase the hatching rate and avoid contamination of the *Artemia* culture water and the larval-rearing tanks with viruses, bacteria, fungi, microsporidians, other parasites and organic matter that can lead to disease and mortality.

Placing Artemia *hatching tanks in a separate room increases biosecurity*

First open the *Artemia* cans and pour the cysts into a large plastic bucket. Add freshwater and hydrate with continuous aeration for 1 h. Then put the hydrated cysts into a 80–100 µm (250–320 mesh) net and wash under running clean freshwater (preferably) or seawater.

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

Method 1: Place the hydrated cysts back in the bucket and add 40 g of sodium hydroxide (NaOH) crystals and 4 litres of sodium hypochlorite liquid (chilled to 4 °C) and 4 litres of seawater (also chilled to 4 °C)/kg of cysts. Stir continuously for 5–8 min until the eggs begin to turn orange. Maintain the temperature at 18–25 °C through the addition of ice.

Method 2: Place the hydrated cysts back into the bucket and add about 7 litres of seawater and ice at a temperature of 20 °C. Add calcium oxide (CaO) at the rate of 125 g/kg of cysts. Stir until mixed. Then add calcium hypochlorite at the rate of 275 g/kg of cysts. Stir until mixed. Stir for 5–8 min, maintaining the temperature at <40 °C through the addition of ice.

Then add some more ice to decrease the temperature to 30 °C, add calcium oxide and calcium hypochlorite at the same dosages as before and stir for another 5–8 min until the eggs begin to change from white to orange in colour. The whole procedure will now have taken 10–16 min.

Stopping the reaction and washing the eggs

Immediately after the eggs have turned orange, transfer them into a 80–100 µm (250–320 mesh) net, wash with running freshwater (preferably) or seawater and then place into another bucket containing a solution of sodium thiosulphate crystals at 100 g/kg of cysts in a little water and stir until mixed. This will neutralize the remaining chlorine and stop the reaction. The decapsulated eggs will then sink and any floating debris can be discarded. Then collect the decapsulated eggs, wash once again and they are then ready for hatching or preservation in salt.

Preservation

There are two methods for storage of the decapsulated eggs if they are not required immediately. They can either be preserved in a solution of super-saturated brine (highly concentrated seawater at about 300 ppt) or in dry refined salt at about 300 g NaCl/kg of eggs. The first technique keeps the eggs in good condition for about one week, during which time they should be used. The second technique can preserve the eggs for up to seven 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 or in a fridge.

Artemia *hatching*

Specific *Artemia* hatching techniques should be used to obtain the highest possible number of clean 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 (>10 ppm chlorine for >12 h, then dechlorinated with sodium

thiosulphate at 1 ppm for every 1 ppm of chlorine remaining). To further improve the hatching rate, instead of full-strength seawater, 18–25 ppt seawater (made from seawater diluted with clean freshwater) should be used.

Probiotics antagonistic to vibrios may be used in the *Artemia* hatching tanks to ensure the *Artemia* nauplii are free from pathogens when fed to the larvae. Alternatively 30–60 ppm chloramine-T may be added to the hatching tank together with the cysts and the cysts hatched in this solution. The use of this product can result in *Artemia* nauplii virtually free from contamination after this procedure.

Add the decapsulated cysts to the conical *Artemia* hatching tank at <1 kg cysts/1 000 litres of water (1 can of 425–450 g/500 litre tank). Place one or more airstones in the tank and provide full and constant aeration. Switch on a light placed 30 cm above the centre of each tank and leave it on constantly (day and night). After about 20–24 h (when most of the cysts have hatched), or 12–18 h for umbrella stage, switch off the air, allow the nauplii and debris to settle and harvest the live nauplii from the tank into a 100 µm (250 mesh) net. Wash thoroughly under clean running freshwater (preferably) or seawater then disinfect.

Artemia *nauplius disinfection*

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

Harvest the hatched *Artemia* nauplii from the hatching tank into a 100 µm (250 mesh) net. Wash the nauplii thoroughly in running clean freshwater (preferably) or seawater. Place the washed nauplii into a 15–20 litre bucket. Add 10 litres of clean seawater. Add 125 ml of 50 percent hydrogen peroxide liquid and stir. Leave for 5 min and all the unhatched cysts and debris will form bubbles and float to the top of the bucket and the live nauplii will go to bottom. Use a 100 µm mesh net to scoop out all floating debris and throw it away. Pour the live nauplii into a 100 µm mesh net and wash thoroughly in running clean freshwater (preferably) or seawater.

Alternatively *Artemia* nauplii can be disinfected with a 20 ppm sodium hypochlorite solution, or better, chloramine-T at 60–100 ppm for 3 min, and washed with freshwater. However, these latter procedures will only disinfect the *Artemia* nauplii and not remove any unhatched eggs or cysts that may lead to contamination of the larval-rearing tanks. The *Artemia* nauplii are then ready to be fed live or frozen, or kept in a fridge at 4 °C at up to 5 million nauplii/litre for up to two days. They can then be fed when needed or placed into separate tanks for enrichment (for 3–12 h), or for on-growing for feeding to PL stages.

Frozen *Artemia* nauplii or adults should be stored in a separate, exclusive freezer. Basic hygienic protocols (SOPs) must be implemented at all times. For mysis (frozen) and early PL stages (live), only the nutritionally superior "umbrella" (just hatching) or instar I nauplii should be used as larval feed for the small-sized larval shrimp. However, for older PL, the *Artemia* metanauplii can be enriched with vitamins and minerals, probiotics and HUFA lipids to increase their nutritional value. Alternatively (and more economically), frozen, enriched adult *Artemia* biomass can be produced in-house or bought and fed (up to 12 times/d) from PL 5 until harvest.

After harvest the tanks used to hatch *Artemia* must be washed with detergent and water, and then disinfected using a sponge dipped in sodium hypochlorite solution (20 ppm active ingredient), rinsed with abundant treated (filtered and sterilized) water and washed again with a 10 percent solution of muriatic acid before sun-drying.

4.3.3 Artificial feeds

Many kinds of artificial or formulated feeds are available for use during larval rearing. These types of feeds generally do not pose the same health risks as live feeds because they can be maintained free from contamination. Generally they should not present any health-related difficulties as long as high quality feeds are selected, and they are stored correctly in cool, dry conditions, used promptly once the container is opened and not used excessively, as this can lead to water quality issues.

Artificial feeds include dried algae, liquid feeds, microencapsulated feeds, flakes and crumbled pellets, and mineral and vitamin supplements and enrichments. These are used in various sizes according to the stage of larval development and in various combinations depending upon hatchery preferences, water quality and nutritional requirements. However, they are usually used primarily as supplements to live feeds. Feeding regimes for artificial feeds will depend upon the quantity and frequency of live feed (algae and *Artemia*) offered, and a general guideline is shown in Tables 17 and 18.

4.4 IMPORTANT LARVAL DISEASES

4.4.1 Monodon baculovirus (MBV)

MBV is a major pathogen of shrimp that is enzootic to India and present in a large proportion of wild broodstock. Even though harmless during the initial stages, the virus may cause mortality in PL. However, mortality due to this virus is seldom reported in hatcheries or in farms, where its effects are seen more as slow growth if environmental conditions are sub-optimal.

MBV forms large, dark tetrahedral occlusion bodies (OBs) that occur mostly in the nuclei of the deep R and S cells of the hepatopancreas. They are eosinophilic (pink-staining) in the early stages and basophilic (dark-purple staining) in advanced stages, and occur in large numbers in a single nucleus. Early infection may be detected by the presence of hypertrophied nuclei with marginated chromatin and displaced nucleolus. The average size of the MBV nucleocapsid is 246 x 42 nm.

Severely infected PL may also exhibit reduced feeding and growth rates, increased levels of surface and gill fouling, and sometimes a white midgut line through the abdomen. However, definitive diagnosis is based on the histological demonstration of eosinophilic occlusion bodies in the nuclei of hepatopancreocytes. OBs may also be detected in fresh squash preparations of the hepatopancreas or in faeces stained with 0.05 percent aqueous malachite green. DNA probes for MBV and the PCR method of detection are also available.

The virus is transmitted by the ingestion of free virus and by cannibalism. It is also believed to be transmitted vertically (probable false vertically, attached to the outside of the eggs) from broodstock to offspring. Since the virus is easily found in the faeces of infected broodstock, it is probably transmitted to the eggs through faecal contamination during spawning. The virus may remain viable in the external environment for some time due to the protective nature of the polyhedral OB.

MBV can be controlled in hatcheries by avoiding contamination and by adopting strict disinfection regimes. All shrimp coming into the system should be quarantined and any infected animals should be removed and destroyed immediately. Water entering the facility must be thoroughly disinfected. All equipment and tanks should be thoroughly disinfected between cycles and equipment used in the spawning area should be segregated from the rest of the hatchery. Eggs should be separated from the spawner faeces and disinfected as detailed in Section 3.13. Exposure to 1 000 ppm calcium hypochlorite (400 ppm chlorine) for 24-h is necessary to inactivate MBV.

Often MBV prevalence in PL is related to stressful environmental conditions, and when a batch of PL tests positive for MBV, if water quality conditions can be improved, the batch can test negative on rechecking after a couple of days. Such procedures can save a lot of money if the hatcheries are educated as to their efficacy.

Finally, if SPF broodstock were to be made available and could be maintained in biosecure facilities, the current problems with MBV would be eliminated.

4.4.2 White Spot Syndrome Virus (WSSV)

Although WSSV does not have any direct adverse effects on the hatchery production of PL, many farmers will reject a batch of WSSV-positive PL. It has been conclusively shown that stocking PL with high levels of WSSV (as demonstrated by nested PCR) is more likely to result in a failed harvest then stocking uninfected PL. Production of WSSV-positive PL is thus a source of lost revenue for hatchery operators and is a high risk factor for the farmers.

WSSV enters the hatchery system either through water exchange, contaminated feeds or most commonly, through infected broodstock, of which greater than 60 percent are believed to be carriers in India. It is believed that in hatcheries the virus is vertically (probably false vertically on the outside of the eggs) transmitted from positive broodstock. Additionally WSSV in the hatchery can be transmitted via cannibalism of moribund shrimp larvae.

Detection is by histopathology by looking for hypertrophy of the cell nuclei of infected tissues, by DNA probes and through nested PCR analysis.

Prevention is the only cure, entailing testing and disposal of each infected broodstock; disinfection of water, tanks and equipment; and washing and disinfection of eggs/nauplii to prevent transmission of the virus from infected broodstock. Maintaining each batch of eggs, nauplii and larvae separate throughout the larval-rearing process can also reduce transmission of the virus within the hatchery.

Unfortunately apparently WSSV-free broodstock may still harbour the virus undetected until stressed, i.e. through spawning. Thus broodstock shrimp need to be tested following spawning (or some other stress) to ensure that they are really disease free, before continuing to culture larvae from the suspect shrimp. Of course development of SPF broodstock *P. monodon* would help eliminate this virus from the hatchery, but so far such animals are not commercially available.

4.4.3 Baculoviral midgut gland necrosis virus (BMNV)

Clinical signs reported from various Indian and Vietnamese hatcheries indicate the presence of BMNV in *P. monodon* hatcheries. BMNV is a rod-shaped, enveloped virus with a tail-like appendage and is 36 x 250 nm in size. It may be in the same family of viruses as WSSV and two insect viruses.

This virus is probably transmitted vertically or false vertically from the broodstock during spawning. It can appear in zoea or mysis, but it is most frequent in later PL stages. The first gross sign of infection is the pale, turbid appearance of the hepatopancreas ("white gut"). The epithelial cells of the hepatopancreatic tubules become necrotic with hypertrophied nuclei that contain large inclusion bodies and marginated chromatin. Severely affected PL may float inactively on the surface of water and display a white midgut line and white spots on the cephalothorax. Heavy mortality can result very rapidly.

Similarly for the other viruses affecting the hatchery, the only defense is prevention, including screening and discarding positive broodstock and proper disinfection of water and eggs/nauplii to prevent transmission from infected broodstock.

4.4.4 Vibriosis

Vibriosis has a high prevalence in all hatcheries in India and is caused by several species of bacteria belonging to the genus *Vibrio*. The disease appears with a variety of clinical signs such as necrosis of appendages; exuvial entrapment; reddening of the pleopods, pereiopods and gills; cessation of feeding; white intestine; excessive fouling; luminescence in the water and larval bodies and so on.

One of the most important species leading to vibriosis in India (and elsewhere) is *Vibrio harveyi*. On many occasions this species is present in such high numbers that high mortality results even after heavy doses of antibiotics. Due to the increasing resistance of many strains of *Vibrio* to a large number of antibiotics, a more comprehensive approach to their management in hatchery systems has to be adopted. This entails observing the strict biosecurity protocols detailed throughout this report, aimed at preventing the ingress and proliferation of pathogenic bacteria in the hatchery and reducing levels of stress. Additionally the use of probiotic bacteria that can out compete or inhibit the pathogenic bacteria can reduce incidences and/or the severity of vibriosis.

4.4.5 Larval mycosis

Larval mycosis, most often caused by *Lagenidium* and *Sirolpidium* spp., may be introduced into hatcheries through broodstock, live feeds and/or carrier hosts present in the seawater supply. Fungal spores can also arrive in the air and are ubiquitous in the hatchery. The fungal spores can also survive in seawater for long periods of time and readily attach to and encyst on the cuticle of the egg or the larvae or onto *Artemia* cysts. Prevention is thus almost impossible, although the regular filtration and disinfection procedures outlined in Sections 2.2 and 2.3 will help limit the problem. Once the spores start to grow, the most common method of treatment is to destroy the spores in the water by using either treflan (at 0.05–0.1 ppm up to daily) or benzalkonium chloride (BKC) (at 0.01–0.05 ppm daily). Treatment with UV light can also yield good results.

Microsporidians (as well as BMNV) have been implicated in the white-body and or white-gut disease found in Vietnamese hatcheries and may also be involved in diseases with similar gross clinical signs in Indian hatcheries.

4.4.6 Ciliate infestation

Stalked ciliate protozoans such as *Zoothamnium* and *Vorticella* spp. often grow in large numbers attached to the gills or appendages of the larvae, causing suffocation, cessation of feeding, moulting inhibition and death. Such infestations usually result from poor treatment of water, use of infected live feeds, inadequate water exchange and/or overfeeding. At the onset of infestation, treatment of larvae with 0.01–0.05 ppm of BKC may yield results. Later on during the PL stages problems generally become more severe due to the high organic loading of the larval tanks. In this case treatment with formalin (at 30 ppm for 1 h, with high aeration, followed by high water exchange) can usually eradicate the problem, provided the larvae are sufficiently healthy to withstand the treatment and to resume feeding and moulting.

4.4.7 Swollen hind gut (SHG)

Swollen hind gut syndrome has been a common problem in many Indian shrimp hatcheries since about 2002. Gross clinical signs are a bloated or swollen hind-gut area, with the posterior digestive tract appearing to convolute or meander through this distal portion of the tail. Signs only become apparent in the later PL stages from PL10 onwards. Although this problem rarely results in mortality, it has negative effects on digestion and excretion, and significantly reduces the value of the PL produced. This is because farmers suspect (although it is yet to be conclusively proven) that it results in digestive problems and differential growth rates during pond on-growing.

The cause(s) of this syndrome are not known, but may possibly include poor water quality (i.e. heavy metal toxicity), low quality/diseased nauplii, bacterial infection (Professor Donald Lightner's laboratory in the United States of America found only some Gram-positive bacteria in positive samples) and/or inferior quality dry feeds (use of liquid feeds appears to help avoid this problem). More research is urgently required to discover the causative agent(s) (if any) and remedial measures for this syndrome.

4.4.8 Diseases of unknown aetiology

Apart from the above mentioned specific diseases, there are reports from various hatcheries about varied health problems having characteristic signs, but for which no causative agent has yet been identified. These include: abrupt cessation of feeding and moulting, reduced growth rate, breakage of the body in the middle of the abdomen, appearance of an extra segment in the larval tail, differential expansion of the chromatophores on the underside of the tail leading to opaque tail muscle from PL9 onwards, and settlement of moribund larvae to the tank bottom without apparent infection. A comprehensive, multicentric research programme (with support from the industry) aimed at investigating the diseases of shrimp larvae in hatcheries is urgently required.

In case of high mortality due to disease, the infected larval-rearing tanks should be chlorinated as soon as possible (>50 ppm chlorine for 60 min) before being drained with care (into the waste-water treatment tanks) The larval-rearing tanks should then be suitably disinfected and thoroughly dried (preferably with direct exposure to sunlight) before they can be restocked with nauplii.

4.5 GENERAL ASSESSMENT OF LARVAL CONDITION

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

A lack of knowledge regarding the importance of routine health monitoring, a lack of protocols for routine health monitoring, a lack of emphasis on disease prevention and the lack of a diagnostic and problem-solving approach are currently major impediments in Indian hatcheries. In Indian hatcheries, health monitoring and general checks on larval development, survival estimation, moulting and feeding regimes are conducted during the larval-rearing cycle. However, there is generally a weak monitoring system for water quality, larval health and survival rate, and routine bacteriology. Similarly organized recording systems are virtually absent, and PCR checking of broodstock and larvae is also very infrequent.

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. All observations are recorded so that details are not forgotten and later problems can be referenced to historical records in each tank.

Samples of larvae and PL for routine checking should be taken in disposable plastic containers (paper cups or 300 ml plastic beakers) that are disposed of once used or in glass containers that can be disinfected. After the daily check is complete, the larvae or PL sampled should be discarded into a plastic container with sodium hypochlorite (20 ppm active ingredient) or another suitable disinfectant. Larvae and PL used in the daily checks must never be returned to the larval-rearing rooms or larval tanks.

The larvae in each tank should be inspected at least twice daily, preferably more frequently. Observations are made on the larval stage, health, activity, behaviour and abundance of feed and faeces in the water and the shrimp body. Records may also be taken of water quality parameters and the amount of food in the tank.

The same or a separate sample of larvae should also be taken to the laboratory for a more detailed microscopic examination. This will provide information on the stage, condition, feeding and digestion and the presence of any disease or physical deformity. Routine monitoring of the bacteriology of the larval-rearing tanks, at least at five stages (nauplius, zoea 2, mysis 2, PL1 and PL5) should also be conducted along with the routine larval health monitoring.

Samples should also be sent to a PCR laboratory once (two to three days before harvest) or twice (at nauplius and PL5) during the cycle for screening for viral pathogens (particularly WSSV and MBV).

PL samples from hatcheries are being checked at the SIFT laboratory for diseases before purchasing. This practice reduces the risk of pathogens entering into ponds

TABLE 19
Diagnostic level descriptions adapted for use in shrimp hatchery systems

Level 1	Observation of animal and environment. Examination based on gross features.
Level 2	More detailed examination using light microscopy and squash mounts, with and without staining, and basic bacteriology.
Level 3	Use of more complex methods such as molecular techniques and immunodiagnostics (e.g. PCR).

The type of observations that are made can be categorized into three levels, based on the health assessment levels described in Table 19. They provide a simple and convenient separation based on the complexity of the techniques used.

4.5.1 Level 1 Health assessment observations

Level 1 observations are commonly employed in most hatcheries. Detailed examination of large numbers of larvae is not practical, and hatchery operators and technicians frequently use Level 1 techniques to get a preliminary feel of the health status of larvae and to prioritize more detailed examination. Level 1 observations are also 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 without the need for further examination. 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 tanks of larvae in the facility.

Level 1 observations are based on simple visual features of the larvae and water condition that can be easily seen with the naked eye in a glass beaker of animals taken from the tank. Special attention is paid to the activity of the larvae, their swimming behaviour (according to the larval stage), water quality, presence of feed and faeces and later on, white-body disease, size disparity and homogeneity. These observation and the scoring system used are summarized in Table 20.

Swimming activity

The swimming activity of the larvae changes dramatically but characteristically through the larval cycle. Zoeal stages will swim rapidly and consistently forwards, usually in circles, filter feeding on phytoplankton. Mysis, by comparison, swim backwards with intermittent flicks of their tails, maintaining themselves in the water column and feeding visually on phyto- and zooplankton. PL again start to swim rapidly and consistently forward, searching for food while being maintained in the water column by strong aeration. Within these distinct modes of swimming, if >95 percent of the larvae are observed to be swimming actively, they are given a score of 10; if 70–95 percent are active, they are given a score of 5 and if < 70 percent are active, they are given a score of 0.

TABLE 20
Summary of Level 1 assessments of larval health

Criteria	Score	Stage	Observation
Swimming activity - Active (> 95%) - Intermediate (70–95%) - Weak (on bottom) (< 70%)	 10 5 0	All stages	Daily (2–4x) observations
Phototaxism - Positive (>95%) - Intermediate (70–95%) - Negative (< 70%)	 10 5 0	Zoea	Daily (2–4x) observations
Faecal string (cord) - Present (90–100%) - Intermediate (70–90%) - Absent (<70%)	 10 5 0	Zoea	Daily (2–4x) observations
Luminescence - Absent - Present (<10%) - Abundant (>10%)	 10 5 0	Mysis	Night observation of the tank
White-body disease - Absent - Present (<10%) - Abundant (>10%)	 10 5 0	PL	Daily (2–4x) observation
Homogenous stage - High (80–100%) - Intermediate (70–80%) - Low (< 70%)	 10 5 0	All Stages	Daily (2–4x) observation
Intestinal contents - Full (100%) - Half full (50%) - Empty (<20%)	 10 5 0	Mysis	Daily (2–4x) observation

Phototaxis

Zoeal-stage larvae should retain a strong positive phototaxis and move towards light. To test this, a sample of larvae is placed in a translucent container (glass or beaker) next to a light source and the displacement of the animals is observed. The zoea should move strongly towards the light. Mysis and PL do not show such attraction to light. If 95 percent or more of the larvae move strongly towards the light, the larvae are good and given a 10; if 70–95 percent respond, they are acceptable and given a 5; and if less than 70 percent move towards the light, they are considered weak and given a score of 0.

Faecal string

During the zoea l 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 90–100 percent of the larvae have these long, continuous strings all along the digestive tube, through their bodies and continuing outside, they are considered well fed and given a score of 10. When 70–90 percent have these strings or they are short or discontinuous, they are given a score of 5; and when <70 percent of the larvae have these strings, the larvae are not eating and are given a score of 0.

Luminescence

This factor is observed directly in the larval-rearing tank in total darkness. Larval luminescence is generally due to the presence of luminescent bacteria such as *Vibrio harveyi*. There should be no luminescence in the tanks. If there is, 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 the case is severe and treatment fails, the tank should be dumped quickly to prevent transfer of the infection to other tanks, as this problem is easily transferred and can result in mass mortalities. If no luminescence is observed, a score of 10 is given: if the observed

luminescence appears low (up to 10 percent of the population), the score is 5; and if above 10 percent of the population are luminescent, the score is zero.

White-body disease

There appear to be two forms of white-body disease (which may have separate causes and therefore be different diseases). The first form displays necrosis and whitening of the hepatopancreas and midgut, 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 lighter mortality. The water also appears to go reddish in colour, with white strands in it. If no white-body disease is observed, a score of 10 is given; if the observed disease appears low (up to 10 percent of the population), the score is 5; and if above 10 percent of the population have white bodies, the score is zero.

The exact causes of this disease(s) are unknown, but may be related to the presence of microsporidian parasites and/or viral disease such as baculoviral midgut gland necrosis virus (BMNV). Very occasionally, antibiotics are reported to work if treatment is applied quickly, but they are nearly always ineffective and the tank must be destroyed rapidly before disease can be transmitted to neighbouring 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.

Stage homogeneity (Size uniformity/size variation)

This indicates the uniformity of larval stages in a tank. Most of the larvae should be within one moult stage of each other. Where there are many stages in a single tank, this indicates a problem (such as disease or poor water quality) needing attention. It should be noted that when larval shrimp moult, it is normal to see a decrease in the stage homogeneity, so the time at which the stage homogeneity is determined has to be taken into consideration. This is also true for PL when they are moulting. If 80 percent or more of the population is in the same stage, a score of 10 is given; if between 70–80 percent are at the same stage, the score is 5; and if less than 70 percent are in the same stage, the score is zero.

Intestinal contents

The intestinal contents (gut contents) can be observed in older larval stages. The intestine is visible as a dark line extending posteriorly from the hepatopancreas in the larva's head region that is easily observed in larvae held in a clear container such as a glass beaker. This is useful as a guide to larval feeding and feed availability. Most larvae should appear full and dark and if they do not, they are probably being underfed or are diseased and remedial action is indicated. If most of the larvae observed are full, a score of 10 is given; if half of the larvae have food in the intestine, a score of 5 is given; and if <20 percent of the larvae have food in the intestine, the score is zero.

4.5.2 Level 2 Health assessment observations

Level 2 observations are also frequently used in the decision-making process in shrimp hatchery management. All hatcheries should have a microscope that is used to make more detailed examinations of the condition of the shrimp larvae and to observe directly various health-related features. Level 2 observations 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, swollen hind gut (SHG) and intestinal contents, necrosis and deformity of limbs, fouling organisms and the presence of baculovirus in the faeces

TABLE 21
Summary of Level 2 assessments of larval health

Criteria	Score	Stage	Observation
Hepatopancreas (lipid vacuoles)			
- High (>90%)	10	All stages	Daily (2–4x) observations
- Moderate (70–90%)	5		
- Low (< 70%)	0		
Intestinal content/Swollen hind gut			
- Full (>95%), (0% SHG)	10	All stages	Daily (2–4x) observations
- Moderate (70–95%), (1-10% SHG)	5		
- Empty (< 70%), (>10% SHG)	0		
Necrosis			
- Absent (0%)	10	All stages	Daily (2–4x) observations
- Moderate (<15%)	5		
- Severe (>15%)	0		
Deformities			
- Absent (0%)	10	All stages	Daily (2–4x) observations
- Moderate (<10%)	5		
- Severe (>10%)	0		
Epibionts			
- Absent (0%)	10	All stages	Daily (2–4x) observations
- Moderate (<15%)	5		
- Severe (>15%)	0		
"Bolitas" [1]			
- None	10	All stages	Daily (2–4x) observations
- 1 to 3	5		
- >3	0		
Baculovirus			
- Absent (0%)	10	Mysis	Daily (2–4x) observations
- Moderate (<10%)	5		
- Severe (>10%)	0		

[1] Sloughed cells of hepatopancreas and intestine, expressed as number of "bolitas" in the digestive tract

or hepatopancreas of older larvae. These observations and the scoring system used are summarized in Table 21.

Condition of the hepatopancreas and gut

The condition of the hepatopancreas and gut gives an indication of larval feeding and digestion. It is observed using a wet-mount of a sample of larvae on a microscope slide at a magnification of 40X. In healthy larvae showing active feeding and digestion, the hepatopancreas and midgut will be full of small, easily observed bubbles (digestive or "lipid" vacuoles) and strong peristalsis will be seen in the intestine. PL should not have a swollen hind gut (SHG) or a coiled posterior digestive tract. If the hepatopancreas appears empty or pale, without lipid vacuoles, then the larvae are either underfed and/or diseased and require treatment. If 90 percent or more of the animals sampled show abundant lipid vacuoles and/or a full gut without SHG, a score of 10 is given; if the sample shows 70–90 percent of individuals with lipid vacuoles and/or a moderately full gut or traces of SHG, a score of 5 is given; and if the gut is less than 70 percent full and/or the intestine is empty or there is >10 percent SHG, the score is zero.

Necrosis

Necrosis of the larval body and limbs, which is an indication of cannibalism or possible bacterial infection, can be observed by light microscopy under low power. 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. If necrosis is absent, a score of 10 is given; where <15 percent of the animals show some necrosis, a score of 5 is given; and where >15 percent show necrosis, indicating a severe infection, a score of 0 is given.

Deformities

Deformities may indicate poor-quality nauplii, if in the early stages, and bacterial infections or mishandling and stress later on. Typically the fine setae on the limbs of the larvae and/or their rostra may appear bent, broken or missing; the tail may appear bent; or the gut may terminate before the anus. 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 as soon as possible to prevent infection of other tanks. Deformities should be monitored and if they are encountered frequently on many larvae, the water quality and disease status of the tank should be checked and rectified. Where deformities are absent, a score of 10 is given; if <10 percent have deformities, a score of 5 is given; and if >10 percent present deformities, a score of 0 is given.

Epibiont fouling

The larvae may become host to a range of fouling organisms ranging from bacteria and fungi through to protozoans of many species. These will typically attach to the exoskeleton on the head and body, and particularly around the gills of the larvae. Where the infections are slight, the next moult may remove the fouling without further problems, but in severe cases the fouling will persist or reoccur in the next stage, indicating poor water quality and necessitating action such as the application of 20–30 ppm formalin (with high aeration) for 1 h, followed by a large water exchange. Where fouling is absent, a score of 10 is given; if <15 percent have temporary or permanent fouling, a score of 5 is given; and if >15 percent are fouled continuously, a score of 0 is given.

"Bolitas"

"Bolitas" is the Spanish name given to 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. If no bolitas are found a score of 10 is given; where 1–3 bolitas are present, a score of 5 is given; and when more than 3 are found a score of 0 is given.

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 high-powered light microscope to spot the characteristic viral occlusion bodies (which, in the case of MBV, are dark coloured and tetrahedral). Apart from MBV, HPV and MGNV can and should also be assessed through stained hepatopancreas squashes. The expression of baculoviruses is often mediated by stress, and if seen, reductions in levels of stress (i.e. improving water quality) can often reduce prevalence and the associated problem of growth depression. Emphasis should be placed on prevention, which entails the selection of uninfected broodstock and disinfection of eggs and nauplii, together with proper disinfection and treatment of incoming seawater. Where baculoviruses are absent, a score of 10 is given; if <10 percent have baculovirus, a score of 5 is given; and if >10 percent are infected, a score of 0 is given.

All hatcheries should also routinely (preferably daily, or at least five times during each cycle) employ basic bacteriology to gain an understanding of the bacterial flora of the tanks and to identify possible pathogens when the larvae become weak or sick. If they do not have these facilities, samples should be sent to a competent laboratory for analysis, particularly if problems become evident. This information may then be used to make a decision on whether the tank should be discarded or treated (and how).

The value of Level 1 and 2 scoring

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

4.5.3 Level 3 Health assessment techniques

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

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

TABLE 22
Summary of PL quality assessment using Level 3 procedures

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

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

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

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

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

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

4.6 QUALITY TESTING/SELECTION OF PL FOR STOCKING

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

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

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

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

Gross examination (level 1)

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

Microscopic examination (level 2)

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

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

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

Salinity stress test (level 1)

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

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

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

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

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

Vibrio *examination (level 2)*

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

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

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

PCR testing (level 3)

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

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

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

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

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

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

4.7 PL HARVEST AND TRANSPORTATION

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

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

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

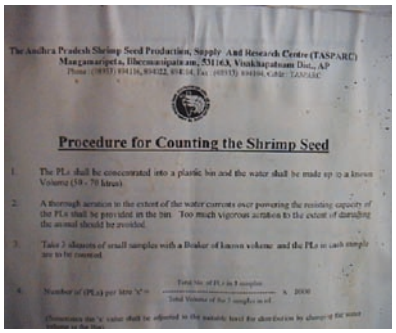
If possible the PL should be acclimated in the hatchery to the expected salinity in the on-growing farms. This is to reduce the stress on stocking the ponds, a critical point in the process that can lead to high mortalities if not done smoothly. Procedures for acclimation are currently not standardized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

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

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

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

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


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

Procedure for Counting the Shrimp Seed

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

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

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

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

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

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

4.8 NURSERY REARING

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

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

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

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

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

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

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

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

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

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

4.9 TIMING OF PL STOCKING

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

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

4.10 USE OF MULTIPLE SPECIES IN SHRIMP HATCHERIES

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

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

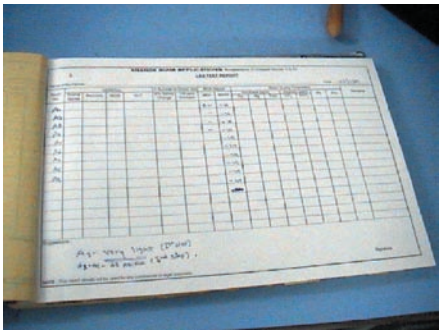
Where essential, such multi-species hatcheries should be registered as two different units within the same hatchery premises. However, the practice of culturing more than one species is not recommended, and should be phased out if possible. Meanwhile close monitoring and assessment of multi-species hatcheries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

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

4.11 DOCUMENTATION AND RECORD KEEPING

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

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



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

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ANNEX 2. Chemicals and treatments used in shrimp aquaculture in India

Part A. Summary of chemicals and their uses mentioned in this document

Use in hatchery	Chemical	Recommended concentration (parts active ingredient)
Disinfection of inflow seawater	Sodium hypochlorite [1]	20 ppm for not less than 30 min (or 10 ppm for not less than 30 min)
Chelation of heavy metals in inflow seawater	EDTA, versene	5–30 ppm, depending on concentration of heavy metals in water
Disinfection of discharge water	Sodium hypochlorite	>20 ppm for not less than 60 min
Determination of presence of chlorine in water	Ortho-toluidine	5 drops in 5 ml water sample [2]
Neutralization of chlorine in treated water	Sodium thiosulfate	1 ppm for every 1ppm residual chlorine
Chelation of heavy metals in broodstock tank water & hatching tank water	EDTA	Must be determined based on heavy metal loading at location up to 20 ppm or both at 20–40 ppm
Disinfection of broodstock upon entry to quarantine	Povidone iodine Formalin	20 ppm 50–100 ppm
Disinfection of broodstock following spawning	Povidone iodine	20 ppm for 15 sec (dip)
Washing and disinfecting eggs	Povidone iodine or Formalin, Chloramine-T & Treflan	50–100 ppm for 1–3 min, (or for 10–60 s) 100 ppm for 30 sec 60 ppm for 1 min (for nauplii) 0.05–0.1 ppm (to reduce fungal infections)
Disposal of discarded larvae	Sodium hypochlorite	20 ppm
Removal of epibiont fouling from PL	Formalin	up to 20–30 ppm for 1 h with full aeration
Stress testing of PL	Formalin [3]	30 min
Decapsulation of *Artemia* cysts	Caustic soda (NAOH) & chlorine liquid [4]	40 g in 4 litre (8–10% active ingredient)
Disinfection of *Artemia* nauplii	Sodium hypochlorite solution or Chloramine-T or both	20 ppm Wash with 60–100 ppm for 3 min, or use 30–60 ppm during hatch-out)
Treatment of water in spawning & hatching tanks	Treflan	0.05–0.1 ppm
Footbath	Sodium (calcium) hypochlorite solution	>50 ppm (or >100 ppm)
Disinfection of equipment (containers, hoses, nets etc.)	Sodium hypochlorite or Muriatic acid	20 ppm (or 30 ppm) 10% solution
Disinfection of hands	Povidone iodine or Alcohol	20 ppm 70% solution
Cleaning & disinfection of tanks used for broodstock spawning, egg hatching holding for nauplii & PL, hatching of *Artemia*	Sodium hypochlorite and/or Muriatic acid [5]	30 ppm (or 20–30 ppm) 10% solution (pH 2–3)
Disinfection of previously cleaned & disinfected tanks prior to starting a new cycle	Muriatic acid	10% solution
Disinfection of algal culture tanks	Sodium hypochlorite followed by Muriatic acid	10 ppm 10% solution
Disinfection of sand filters	Sodium hypochlorite or Muriatic acid	20 ppm 10% solution (pH 2–3)
Disinfection of cartridge filters	Sodium hypochlorite or Muriatic acid	10 ppm 10% solution (pH 2–3) for 1 h
Washing of feed preparation equipment (knives, tables, mixers, pelletisers etc.)	Povidone iodine	20 ppm

[1] or calcium hypochlorite
[2] Presence of chlorine is indicated by a yellow color
[3] Salinity change can also be used.
[4] See Section 4.3.2 for details.
[5] In the past, muriatic acid referred to 3:1 HCl and HNO_3, but currently it refers to 34–37% HCl.

Part B. Characteristics of some chemicals and treatments commonly used in shrimp hatcheries

Chlorine

Chlorine is available as hypochlorites (sodium hypochlorite, NaClO and calcium hypochorite $Ca(OCl)_2$). A variety of micro-organisms such as bacteria, fungi, protozoans and viruses are killed by chlorine at various concentrations. Hypochlorites act by releasing hypochlorous acid, which is the primary active ingredient, a potential germicide. They are particularly effective in acidic conditions. The bactericidal effect of hypochlorite is 10 times greater at pH 6 than at pH 9. Shelf life is very short. Sodium hypochlorite will remain stable only at 4 °C, and the entire contents of the package should be used quickly once the container has been opened. Calcium hypochlorite must be maintained in properly sealed polythene bags protected from sunlight in a cool dry place. The effectiveness of chlorine is also affected by the amount of organic matter, reduced compounds and turbidity present in the water to be treated. If chlorine is used in water with high organic matter content, the rate of application should be higher. The dosage depends on the active ingredient of the residual chlorine.

Hypochlorites are not consumed by the animals nor do they penetrate their tissues. It is an active oxidizing agent and its activity is limited to the animal's surface. WSSV is inactivated by contact for 10 min at room temperature with a final concentration of 100 ppm sodium hypochlorite.

Chloramine–T

Chloramine–T is one of the most useful chemicals available for use in aquaculture. It is used as a disinfectant and also as an antimicrobial and anti-protozoan. In comparison with formalin, chloramine–T has greater efficacy against bacteria but much lower efficacy against protozoans. Chloramine–T is considered to be a safer disinfectant than chlorine because chlorine combines with organic matter to form carcinogenic trichloromethane.

Formalin

Formalin is a generic term that describes a solution of 37 percent formaldehyde gas dissolved in water. At 1 percent it is effective in killing spores of protozoans. Formalin effectively kills external parasites. It is not the preferred treatment for external bacterial or fungal infections. Formalin is not effective against internal infections of any type. It acts as a disinfectant, antiseptic and astringent. For application, dilution is necessary in order to insure that therapeutic dosages may be safely discharged to receiving waters. In most current applications, this dilution will occur before discharge. Treatment concentrations are typically 100 ppm for 30 seconds for control of MBV in eggs and nauplii and 10–30 ppm for 1 h for treating external parasites of larvae and PL. Prolonged bath treatment is not safe at any larval stage. Water quality parameters such as DO, CO_2, pH, total ammonia and nitrite are influenced when used at recommended rates, as it is toxic to many aquatic plants, especially phytoplankton and algae. Formalin is toxic to aquatic life at low concentrations with 96-h LD_{50} values ranging from 1 to 1 000 ppm depending on species. Formalin toxicity is increased at high water temperatures. If water temperatures exceed 21 °C, the concentration used should be reduced. Formalin is a potential carcinogen and should be handled very carefully to avoid skin contact, eye irritation and inhalation.

EDTA

EDTA (di-sodium ethylene diamine tetraacetic acid [$(C_{10}H_{14}N_2O_8Na_2.2H_2O)$] is used to treat ectocommensal fouling by stimulating juvenile molting. Added to larval-rearing water in shrimp hatcheries it chelates divalent and trivalent metal cations,

reducing the bioavailability of heavy metals by complexation. It is used to improve water quality by reducing heavy metal concentrations. EDTA may also help reduce bacterial contamination of the eggs, allowing better oxygen transfer into the egg and enhancing hatching rate. EDTA can inhibit the capacity for agglutination and adhesion in some bacteria and the production of extracellular proteases by some pathogenic vibrios, including *Vibrio harveyi*.

In shrimp larval rearing, it is applied at 5–30 ppm for spawning and hatching and at 5–10 ppm prior to stocking of nauplii. It may also be applied at 1–5 ppm to remove organic substances in the water.

Iodine

Iodine (I_2) is available as polyvinyl pyrrolidone iodine (PVPI)/iodophor compounds (povidone iodine). These preparations are complexes of iodine with a solvent or carrier that liberates free iodine into solution slowly. They can prevent and control diseases caused by *Aeromonas*, *Pseudomonas*, *Vibrio*, fungi and several viruses. These compounds are particularly used to treat eggs and larvae and to disinfect equipment. They are lethal to viruses, which are killed within 15 min in a 50 ppm solution. Iodine is an oxidizing agent that can oxidize and inactivate proteins with sulfhydril groups (-SH group). The action involves halogenation of tyrosine units of enzymes and other cellular proteins requiring tyrosine for activation. The preparation is diluted in water and added as required. The iodine compounds should be maintained in airtight dark bottles in a cool dry place. In such situations they can remain stable for long periods. Shrimp eggs and nauplii are dipped in iodine solution for 30 seconds at concentrations of 50 ppm and 100 ppm, respectively, to avoid the transmission of MBV and other viruses from broodstock. The shrimp body does not consume iodine and no residual effects are reported.

Treflan

Treflan is a,a,a-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine having the empirical formula $C_{13}H_{16}F_3N_3O_4$. Trade names include Flurene SE, Treflan, Tri-4, Trust, M.T.F., Trifluralina 600, Elancolan, Su Seguro Carpidor, Trefanocide, Treficon, Trim, L-36352, Crisalin, TR-10, Triflurex and Ipersan. Treflan is commonly used as a prophylactic chemical against fungal infections such as larval mycosis in shrimp caused by *Lagenidium callinectes*, *Haliphthoros* sp. etc. It inhibits cell wall synthesis in fungi. The chemical has to be diluted in water and applied to the rearing water at appropriate concentrations. When refrigerated it remains stable for more than two years. Dose rates of 0.2–1.0 ppm have no inhibitory effect on hatching rate of eggs of *Penaeus monodon*. However, survival rate of hatched nauplii subsequent to treatment will be significantly reduced in most cases. Treflan at 0.05–0.1 ppm is recommended to treat *Lagenidium* sp. infections in nauplii or larvae. Treflan is not known to be absorbed by animal bodies, and residual effect in host species is not recorded. Exposure of non-target species such as *Teraselmis cheuii*, a phytoplankton used as food for penaeid larvae, to 0.1ppm trifluralin delays its growth and reduces protein content. Treflan is rapidly degraded in soil-water systems.

Ascorbic acid (Vitamin C)

Known as ascorbic acid, the L-isomer is the physiologically active form of vitamin C. The R-isomer, which is called erythorbic acid (or occasionally iso-ascorbic acid), has no vitamin value, although it does function as an *in vitro* antioxidant. The ingredients used for the manufacture of compounded feeds are unlikely to contain any measurable amounts. Vitamin C is usually measured in weight units of pure crystalline L-ascorbic acid. Diets supplemented with vitamin C have been reported to give protection against bacterial pathogens.

Ascorbic acid is transported to all living cells for use in important oxidation/reduction reactions in cell metabolism. It is essential for the formation and maintenance of function of the intercellular substances of skeletal tissues, particularly collagen. It also exerts a stimulant action on defensive mechanisms. According to recent research, it plays an essential role in transporting iron from plasma to storage sites. It is an important intercellular antioxidant and is involved in quenching highly reactive free radicals. In addition to reacting directly with aqueous free radicals, ascorbic acid indirectly affects the balance between oxidative products and antioxidant defense mechanisms. Accordingly ascorbic acid can donate an electron to the tocopheryl free radical, regenerating the active tocopherol. The concentration of ascorbic acid in phagocytes in blood is many times higher than in the erythrocytes and is approximately 150 times the concentration in plasma. These phagocytes use free radicals and other highly reactive oxygen-containing molecules to help kill invading pathogens. The antioxidant action of ascorbic acid helps protect these cells from oxidative damage.

Crystalline ascorbic acid is relatively stable in air if moisture is completely absent. In the presence of even small amounts of moisture there is rapid oxidation, first to dehydroascorbic acid, and then to other, non-vitamin-active products. To optimize stability of vitamin C during feed production and storage as well as bioavailability in fish/shrimp, the use of vitamin C in its phosphorylated form is recommended. Active uptake of vitamin C seems to be very important at low doses while at high doses, uptake by passive diffusion also occurs.

Benzalkonium chloride (BKC)

Benzalkonium chloride is a quaternary ammonium compound containing four carbon-containing groups and a negatively charged ion such as bromine or chlorine. A large number of different quaternary ammonium compounds have been synthesized and evaluated for their antimicrobial action. Bactericidal concentration ranges from 1 part in a few thousand to 1 part in a million under dilution. The combined properties of germicidal and detergent action, low toxicity and high solubility in water, stability in solution and noncorrosiveness are its important characteristics.

The mode of action includes denaturation of proteins and interference with glycolysis and membrane damage. The most likely site of damage to the cell is the cytoplasmic membrane where the compound alters the vital permeability feature of the cell structure.

Benzalkonium chloride is targeted at a variety of microorganisms including *Lagenidium* and *Haliphthoros* species. Shrimp farmers use it to reduce the concentration of plankton and dinoflagellates in closed pond systems. In shrimp hatcheries and grow-out systems it is used in low concentrations of 0.1–0.5 ppm. At 10 ppm no inhibitory effect on the hatching rate of eggs of *Penaeus monodon* has been noted, but survival rate of hatched nauplii was significantly reduced. At 0.01 ppm, BKC has immunostimulatory properties and at 1.0 ppm it can induce moulting. If applied in large amounts, the resulting decomposition of organic matter will have an effect on animal health.

β-1-3 glucan

β-glucans are the cell wall component of mushroom and yeasts, which appear to be the most promising of all immunostimulants so far examined in fish and shrimp. β-glucans are poly-glucose molecules linked through β-1,3 bonds in a long chain with β-1,6 branches consisting of single or multiple glucose molecules. Such glucans can exist in various structural forms and may be in the form of water-soluble oligomers, water-soluble or insoluble macromolecules or particulates. Glucans extracted from *Saccharomyces cerevisiae* (baker's yeast) is one such type and is an important structural element of the yeast cell wall. Yeast glucans are polysaccharides composed of smaller

units linked together by β-1,3 bonds. These bonds hold the glucan molecule together, hence the name, β-1,3 glucan.

The mode of action of β-1,3 glucan is that there is a specific receptor for β-1,3 glucan on the surface of macrophages that when activated, stimulates a cascade of events turning the body into "an arsenal of defense". There is now evidence to show that glucan is, from an evolutionary point of view, the most widely and commonly observed macrophage activator in nature and is proven to overcome the negative effects of immunosuppression.

The phenol oxidase system is an important element of the disease resistance of crustaceans. Crustaceans use lipopolysaccharide and the β-1,3 glucan structure as specific signals to activate the prophenol oxidase system. The crustacean blood contains proteins that specifically bind β-1,3 glucans. Activation of this protein on the hemocytes by β-1,3 glucan induces degranulation and release of the prophenol oxidase, which can be converted from its proform into an active enzyme by serine proteases. Phenol oxidase then oxidizes the phenolic group containing amino acids (thyrosine) into semiquinones, which have microbicidal action, and these semiquinones are polymerized into melanin.

Commercially available forms of β-glucan are Macrogard, Betafectin, Lentinan, Schizophyllan and Scleroglucan. They are normally added to the feed and fed directly to the shrimp at concentrations recommended by the manufacturers.

Ozone

Ozone is a powerful oxidizing agent with numerous beneficial uses in aquaculture. The quality of aquaculture production water can be improved by ozone treatment, including improvement in solids settling and reductions in nitrite-nitrogen (NO_2-N), colour, fine particulate matter and microbial activity. Ozone shows excellent potential for many aquaculture systems because of its rapid reaction rate, few harmful reaction by-products and the oxygen produced as a reaction end product.

Ozone use for aquaculture began in the mid-1970s and was initially focused on disinfection and colour reduction of aquarium water with low fish densities and low feed loading. Since then ozone has been used to improve water quality in various types of aquaculture systems, ranging from flow-through raceway systems to indoor recirculating systems. Although ozone has proven effective in the reduction and control of certain water quality characteristics, it is not a one-step water treatment technology. Because less ozone is needed when supporting water treatment technologies are also used, the use of ozone as part of a larger water treatment system maximizes its efficacy and cost-effectiveness. The effective concentration for ozone to reduce WSSV infectivity to zero is 0.5 µg/ml as a total residual oxidant for 10 minutes at room temperature.

Ultra-violet (UV) radiation

UV rays are non-ionizing radiations that can cause permanent damage to DNA and consequent death of cells, if doses are high enough. It has been calculated that WSSV becomes inactivated by 60 min UV irradiation at 900–000 mws/cm^2. However, most commercial UV systems are only rated at 15 000–30 000 mws/cm^2, which is still sufficient to kill most bacteria, fungi and protozoans, but will have little effect on most viruses. Therefore appropriate standardization and calibration has to be made for effective treatment.

ANNEX 3 - List of antibiotics and pharmacologically active substances banned for use in aquaculture in India[1]

1. Chloramphenicol
2. Nitrofurans including Furazolidone, Nitrofurazone, Furaltadone, Nitrofurantoin, Furylfuramide, Nituratel, Nifursoxime, Nifurprazine and all their derivatives
3. Neomycin
4. Nalidixic acid
5. Sulphamethoxazole
6. *Aristolochia* spp. and preparations thereof
7. Chloroform
8. Chlorpromazine
9. Colchicine
10. Dapsone
11. Dimetridazole
12. Metronidazole
13. Ronidazole
14. Ipronidazole
15. Other Nitroimidazoles
16. Clenbuterol
17. Diethylstilbestrol (DES)
18. Sulfonamide (except approved sulfadimethoxine, sulfabromomethazine and sulfaethoxyrpyidazine)
19. Floroquinolones
20. Glycopeptides

[1] Source: Coastal Aquaculture Authority (2006).

ANNEX 4 - 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 (°C)	Salinity (ppt)	Notes

ANNEX 5 - Spawning/hatching tank daily data sheet

Tank No:

Date	Volume	No. females	Ovary stage	No. Source tank (ST or tonnes)	No. Spawned	No. Eggs	No. Nauplii	Temperature (°C)	Salinity (ppt)	Transferred to LRT No.	Notes

ANNEX 6 - 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 (Tonnes)	Water exchange (%)	Temp (°C)	Algae			*Artemia*		Artificial feed		Notes
								Species	Cells/ml in tank	Cells/ml fed	No./ml in tank	No./ml Fed	Type	g. fed	
1		N6/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		PL15													

ANNEX 7 - Level 1 larval health data sheet

Tank No: **Date:** **Average Score: (>60 good, 40–60 medium, <40 poor):**

Larval sample	Swimming activity	Phototaxism	Faecal string	Luminescence	White-body disease	Homogenous stage	Intestinal contents	Notes
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
Total								
Notes: Average								

Scores: 10 Good, highest, most; 5 Medium; 0 Poor, lowest, least.

ANNEX 8 - Level 2 larval health data sheet

Tank No: **Date:** **Average Score: (>60 good, 40–60 medium, <40 poor):**

Larval sample	Hepatopancreas (Lipid Vacuoles)	Intestinal Contents	Necrosis	Deformities	Epibiont fouling	Bolitas	Baculovirus	Notes
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
Total								
Notes: Average								

Scores: 10 Good, highest, most; 5 Medium; 0 Poor, lowest, least.

ANNEX 9 - 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 12 mm)			
Swimming activity			
Feeding			
Colour			
2. Microscopic examination			
Hepatopancreas	Full, dark (10)	Medium (5)	Empty, pale (0)
Gut condition	Full, vacuoles (10)	Medium (5)	Empty, SHG (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. V*ibrio* testing			
Green	<60/plate (pass)	>60/plate (fail)	
Yellow	<80/plate (pass)	>80/plate (fail)	
Luminescence	Absent (pass)	Present (fail)	
5. PCR testing			
WSSV	Negative (pass)	Positive (fail)	

ANNEX 10 - Research and development and extension requirements

Broodstock:

- Reasons and solutions for the seasonal variations in quality/quantity of broodstock caught at each landing centre
- Development and design of transportation containers for broodstock from the wild
- Comparison and evaluation of performance of induced matured spawners versus wild gravids
- Anaesthetics and transportation of broodstock
- Nutritional requirements and the role of live and artificial diets in promoting maturation and good quality larvae
- Genetic selection of families/domestication and captive production of SPF/SPR lines of *Penaeus monodon*
- Vertical disease transmission issues
- Cryopreservation of eggs and sperm of *P. monodon*
- Reasons for failure/success of induced maturation programmes in hatcheries
- Validation of re-circulation system with bioreactor technology for maturation and larval rearing systems

Larval culture:

- Nutritional requirements and the development of appropriate artificial diets for each larval stage
- Probiotics and their role in nutrition and health management
- Larval diseases and methods for their prevention
- Research on replacement of live feeds to minimize use of *Artemia* and reduce feed cost
- Development of reliable algal culture laboratories to supply algal stock to hatcheries
- Analysis of the options for nursery rearing of PL

Others:

- Bioremediation of wastewater

Extension requirements:

- Harmonization and intercalibration of PCR methods used in diagnostic centres and hatcheries
- Suggested annual closure of all hatcheries on the east coast during November, December
- Training on broodstock capture/handling and transport on-board vessels, at landing centres/nauplii centres etc.
- Awareness programmes for broodstock collectors, handlers and auctioneers
- Hands-on training for hatchery technicians for better management practices
- Awareness programmes on better management practices for commercial nursery owners
- Awareness programmes to eliminate chemicals, antibiotics and other drugs by adopting alternate approved methods such as probiotics
- Information dissemination to farmers using the cluster approach on seed selection, pond preparation, stocking and pond management
- Training of nontechnical and managerial staff
- Upgrading skill of technical staff
- Development of quarantine at landing centres

- Definition and utilization of biosecure procedures in hatcheries
- Government-run training of hatchery technicians for maturation, larval and PL rearing about phytosanitary measures and critical control points

Shrimp aquaculture is an important and valuable production sector that has been growing rapidly over the past two decades. Success is largely based on the quality of postlarvae, particularly their health condition, thus making hatchery production of quality postlarvae crucial to the sector's sustainability. This document reviews the current status of the *Penaeus monodon* shrimp hatchery industry in India and provides detailed guidance and protocols for improving hatchery productivity, health management, biosecurity and sustainability of the sector. The information provided is equally useful in all regions where *P. monodon* aquaculture is practised.

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