

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 dormant 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/cm² 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 (NH₃) (< 0.1 ppm), nitrite (NO₂) (< 0.1 ppm) and nitrate (NO₃) (< 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 ₂ S)	<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- μ m 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 μ m 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.

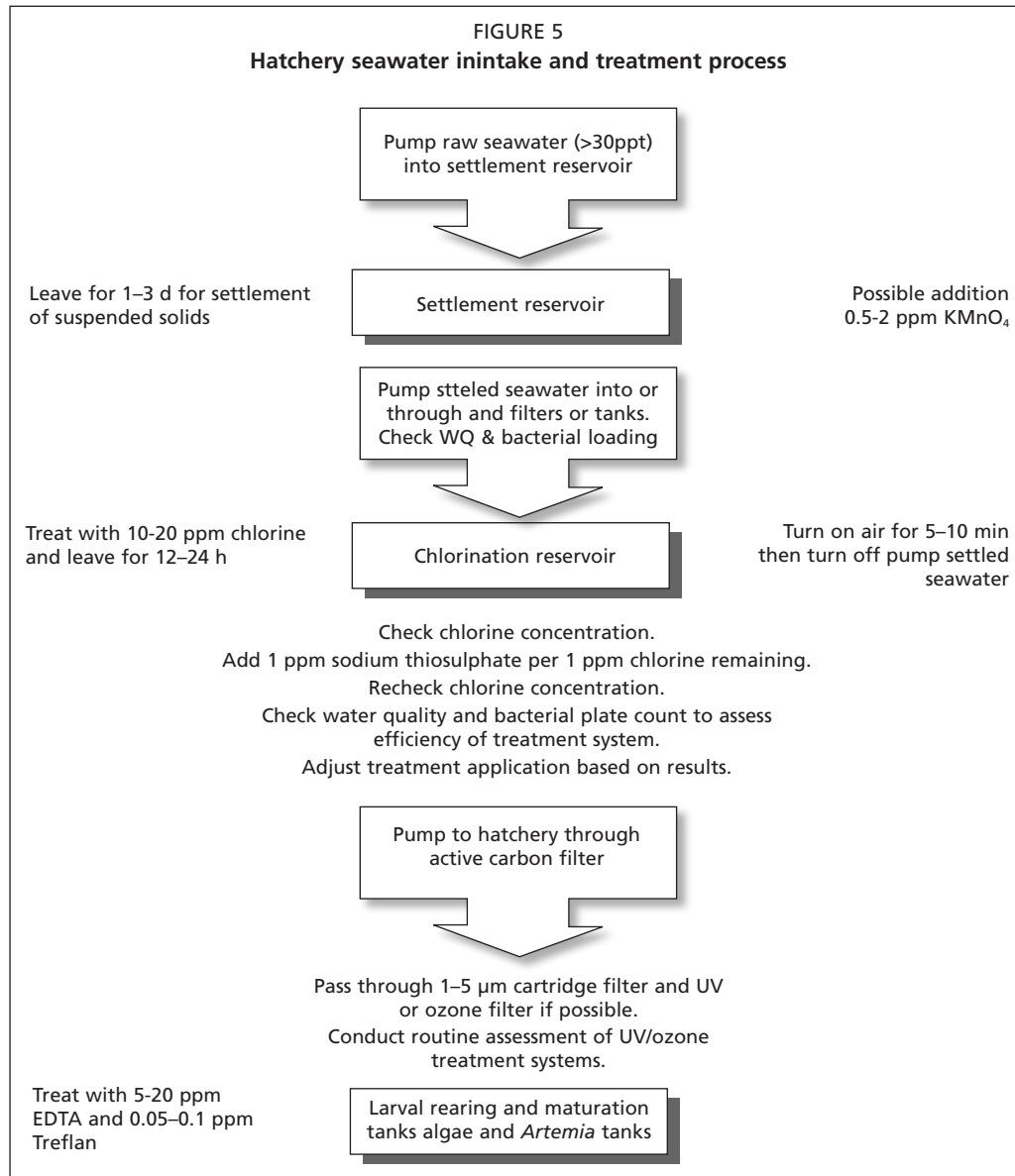
The flow and processing of inlet seawater to the hatchery facilities are shown in Figure 5.



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.



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 Diagnostic 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 immunodiagnosics (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.

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
Level 2	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
Level 2	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 immunodiagnosics or PCR
Level 3	Screening of nauplii and PL by dot blot or PCR



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