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

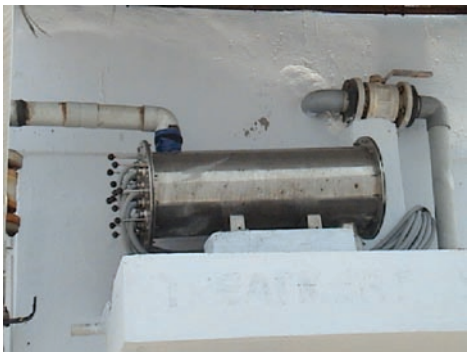
An example of an unhygienic, biologically insecure situation caused by improper management

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.



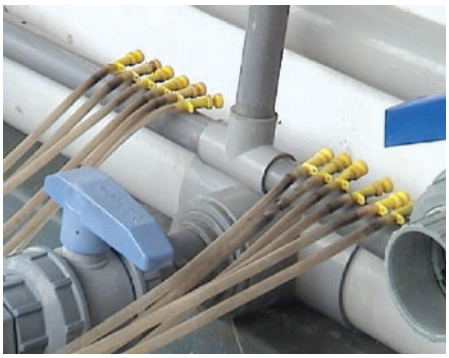
Wipers are installed to clean the UV lamp at some hatcheries

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.

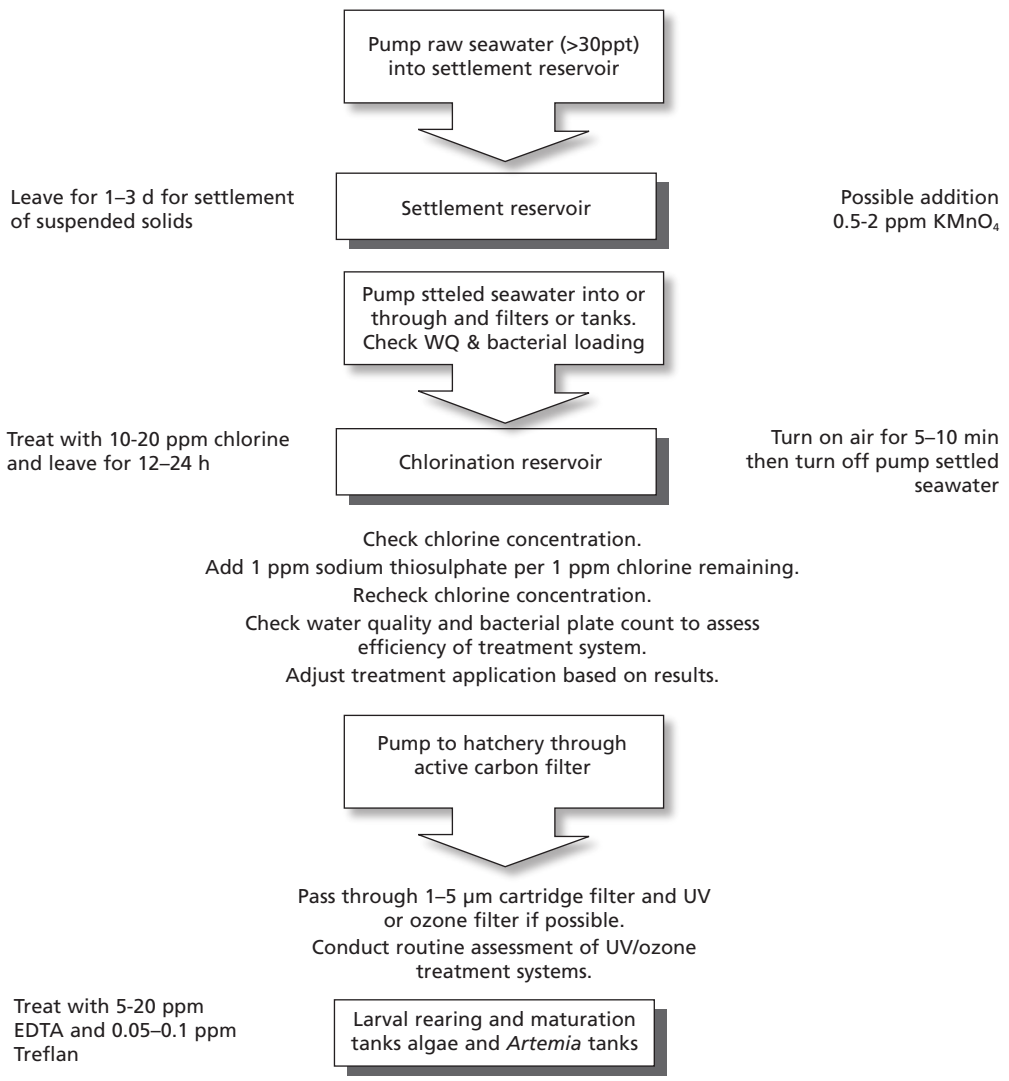
The flow and processing of inlet seawater to the hatchery facilities are shown in Figure 5.

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.

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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