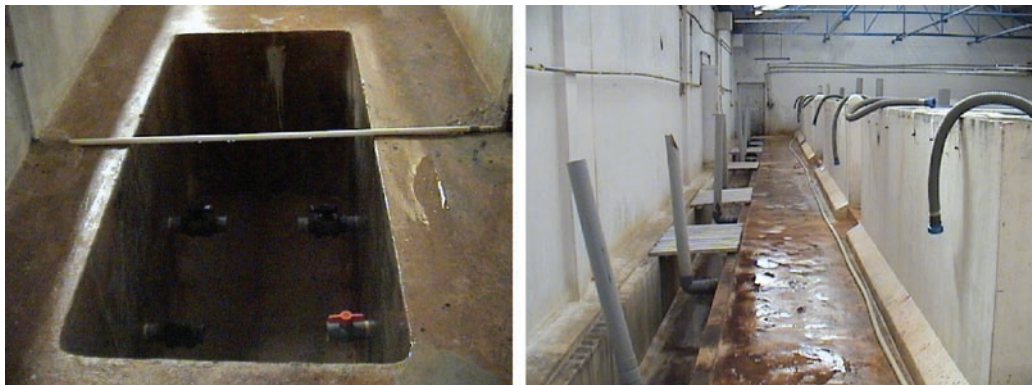




Some hatcheries have good laboratory facilities for polymerase chain reaction (PCR) diagnostics, water quality and microbiology, although day-to-day management system may not reflect the existence of such facilities

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:

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.



Recirculating sand filters (RSF) are properly maintained 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



Wipers are installed to clean the UV lamp at some hatcheries



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.

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



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



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 settleable 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 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