Larvae Rearing

The techniques described in the following sections have been adapted from practices generally used in a brine-based recirculation hatchery established in southeastern Bangladesh in 1991. Recirculation systems for shrimp and prawn hatcheries have wide applicability wherever brine is available. Artificial sea salts could also be used in a recirculation system; **this enables prawn hatcheries to be set up almost anywhere.**

Water supply and treatment

Generally, some kind of treatment is required to render the water suitable for larvae rearing. The nature and degree of treatment depends upon the quality at the source, It bears repeating that **no amount of treatment will bring badly polluted and turbid water up to standard.**

Brine collection

Salt farms are divided into reservoirs, condensers and crystallizers. Brine can be taken from the condensers when the salinity has reached at least 180 ppt, but 220-240 ppt is preferable. Supernatant water from the crystallizers is toxic and should never be used. Brine is pumped from the condensers into plastic barrels, which are capped and transported by truck to the hatchery.

Water treatment

Underground water is ideal for hatchery operation if it fulfils the criteria mentioned in Table 2.

Table 2 : Hatchery water quality criteria

Parameter	Level
Salinity	12 - 15 ppt
Temperature	28- 31°C
pH	7.0-8.5
Nitrite nitrogen	0.1 ppm
Nitrate nitrogen	20 ppm
Chlorine	0,0
Hydrogen sulphide	0.0
Hardness	00 ppm
Iron	2 ppm

Tapwater may also be used, but it should be aerated for 24-48 hours, or passed through activated carbon, to remove chlorine. Groundwater has no oxygen and must be well aerated before use.

Dissolved iron is precipitated by aeration in the mixing tank and subsequent removal by sand filtration. Very high levels require special treatment. This can include filtration through a soda lime bed, followed by settling and sand filtration. If dissolved iron is **greater than 2ppm,** alternative sources of water or sites should be considered.

Pond or riverwater requires more elaborate treatment, but may be employed if no other source is available. Such water often contains very fine particulate organic matter and silt, making filtration difficult. Flocculation with alum at 150 ppm may be needed to ensure efficient filtration. Usually a rapid sand filter (see Figure 7) is used for this purpose.

Municipal water can be dechlorinated by activated carbon filtration. Sodium thiosulphate may be used if the water is stored in an overhead tank.

In the mixing tank, 12 ppt saline water is prepared by mixing the freshwater and brine. Table 3 shows treatment procedures for the mixed water. Bleaching powder is added to the 12 ppt brackishwater which is then aerated for 24 hrs. Excess chlorine is removed by treating the water with sodium thiosulphate. Treated water is allowed to stand several hours after thorough aeration, followed by sand filtration.

Table 3 : Culture water treatment regimen

Chlorination	*5 t water*	*10t water*	*Duration*	*Detoxification*
a. 70 % calcium hypochlorite	50 g	100 g	12 hrs static. Aerate initially for 1 hour to mix thoroughly	Mix 12 ppm sodium thiosulphate and aerate for 12-24 hrs
b. 5 to 6% sodium hypo- chlorite* (chlorox)	*½1*	*11*	*12 hrs static*	*Mix 12 ppm sodium thiosulphate and aerate* for 12-24 hrs

* Can be doubled to 10 ppm if the water is suspected to carry a heavy load of organic debris. This should be done prior to filtration, as chlorine tends to precipitate iron and flocculate organic matter.

Note If water treated with chlorine is high in organics, it is suggested that it be passed through activated carbon to detoxify chloramines after chlorination and sand filtration.

Broodstock and spawning tank management

To get enough quality eggs, careful management of the broodstock is required.

The depth of the water in holding tanks should be 0.9-1.0 m.

Daily, in the morning and evening,. 50 per cent of the water should be changed.

Enough shelter should be provided at the bottom of the tank to reduce stress. Tank covers, or a shed, should be installed for shade.

Rainwater stimulates sexual activity of the prawn, so better results can be expected if rainwater is allowed to fall into the holding broodstock tank.

Feed equivalent to 5 per cent of the bodyweight of the stocked prawns should be supplied twice every day, in the morning and evening. It is preferable to supply raw food in the night and prepared food during daytime. Chopped fish and mussel, small shrimp, chopped fish, and adult Artemia are good for broodstock. Dry pellets with good food value can be provided, if available.

Before the food is given everyday, the bottom of the tank should be cleaned by siphoning. This removes uneaten food, leftover moults and faecal waste. The tank bottom and wall should be brushed and cleaned every two days.

The tank and broodstock can be disinfected by introducing 20 ppm formalin solution in the tank, followed by a 100 per cent change of water after 24 hrs. This must be done very carefully.

From time to time, the water level should be decreased and injured prawns removed,

Berried females should be transferred to the spawning tank as they become available.

Fig. 15 Plastic tube used for long distance transport of berried females.

Broodstock collection and maintenance

The female prawn carries the fertilized eggs under her abdomen in a brood chamber. Prawn that are carrying eggs are referred to as 'berried'. They are generally found in rivers and their tributaries, canals, ponds and deep depressions. Berried prawns can also be produced in the holding tanks in a hatchery. During cool periods, broodstock can be produced in a hatchery under controlled temperature conditions.

Berried female prawns are available throughout the year in the lowlands of Malaysia, Thailand, Indonesia, southern Vietnam and similar locations where water temperatures remain high through the year. In Bangladesh and West Bengal, natural reproduction occurs only during the warm months – from late March through September.

Transport of berried prawns is similar to that of adult prawn, but because of the eggs in the abdomen, they need to be handled with more care than immature prawns. Berried female prawns should be held in individual perforated plastic tubes if they are to be transported long distances (Figure *15).* The ends of the tube are closed with gauze held in place by rubber

bands. The tubes are transported in plastic bags containing oxygenated water. After broodstock are collected, they should be disinfected with formalin. Weak, wounded and diseased animals should be discarded and only healthy and disease-free berried prawns stocked or transferred to the hatching tank.

In the hatching tank, it is advisable to stock four prawns/sq.m in water of depth 30-40 cm. The tank bottom should be cleaned every morning and evening and 50 per cent of the water exchanged. The salinity should gradually be increased to 12 ppt.

The feed ratio should be 5 per cent of the total bodyweight of broodstock. Bivalve meat, snails and worms can be fed.

Aeration should be continuous and the temperature maintained at 28-29°C. If temperature is not controlled, the growth of embryo will be delayed and the newly-hatched larvae will be weak and undersized. The main purpose of this tank is to provide proper conditions for the embryo to grow.

Full development of the eggs in the abdomen of the female prawn takes about 19 days. The female prawn remains busy during these 19 days by brooding the eggs. As the eggs develop, their bright orange colour changes to a greyish colour.

Ag. 16a Newly **spawned** eggs.

Fig. 16b **Berried female** ready **for transfer to hatching tank**

Selection and disinfection

Egg development should be observed every alternate day: a 'conical scoopnet is used to take out each prawn and its yellowish ventral egg sac examined. When the colour of the eggs in the sac becomes dark grey, the prawn should be transferred to hatching tanks after disinfection (see Figure 16). Disinfection is done by keeping them in aerated water with 25 ppm formalin for 1½hour. When the elliptical eggs are ready to hatch, fully developed larvae may be seen inside the egg with the aid of a microscope.

Berried females should be selected carefully, applying the following criteria. They should be:

- Healthy and disease free;
- Strong and active;
- Bright-coloured;
- Laden with a large number of eggs;
- As large as possible.

The chances of larvae surviving from prawns having these qualities is good. Their growth will also be fast.

Hatching tank management

Hatching can be done in the larvae rearing tanks. However, the use of a separate hatching tank helps to prevent the spread of disease to larvae rearing tanks. Larvae rearing density is also more easily controlled. In case separate tanks are used, for every 100 g prawn weight a tank or aquarium of at least 500 l capacity is required.

Before stocking prawns in a hatching tank, it should be filled to a depth of 30-40 cm with treated 12 ppt brackishwater. The sodium salt of ethylene diamine tetracetic acid (EDTA) appears to have very beneficial effects on the hatching rate and larvae survival, so 5-10 ppm EDTA should be added to the water in the hatching tank. Aeration should be continuous and temperature maintained at 30°C. After hatching of larvae, spent broodstock should be returned to the holding tank or broodstock pond.

No **feed** need be supplied to berried prawn in the hatching tank. During the time they spend in this tank, they are busy taking care of their eggs and **do not feed. Larvae generally hatch during** the first half of the night, although sometimes hatching may take place during late evening. In some cases, partial hatching also occurs.

Movement of larvae starts five minutes after hatching. At this time, larvae swim in a head-down position.

Larvae are made to concentrate in one corner of the hatching tank by covering all but a small portion of the surface with an opaque material. Larvae are attracted to the light and will gather in the exposed corner, from which they can be easily removed with a siphon or small bucket.

Larvae rearing tank preparation

The larvae rearing tank should be filled with treated 12 ppt water. The water is conditioned by circulating it through a biofilter for several days prior to the tank being stocked with newly-hatched larvae (Refer to page 19 for water treatment procedures). Add 10 ppm of EDTA before stocking with larvae. If the biofilter is new, a few handfuls of urea, or another ammonia salt, can be thrown into the tank to enhance the growth of denitrifying bacteria in the filter during the conditioning period.

Stocking larvae rearing tank

Stage I larvae are stocked at 100/1 in the larvae rearing tank. The remaining larvae should be transferred elsewhere. After ten days, the density of larvae can be adjusted to 60-80l.

By keeping all these parameters favourable and by controlling management accordingly, the end result can give up to 30-40 PL/l. In some cases, 60-100 PL/l have been produced. Fifteen to 20 PL/l seems to be about average.

Tank management

Maintaining stable water conditions in the tank is what ensures successful larvae rearing. This is much more critical in a recirculation system than in an open system hatchery. Salinity, temperature, ammonia and pH must all be kept within the limits necessary for the good health of the larvae.

Salinity control

The salinity of water in the larvae rearing tank should be maintained at 12 ppt up to the PL stage. However, ±2ppt does not affect growth of the larvae. Sudden fluctuations during water changes are to be avoided. Salinity can be checked and controlled by a hand refractometer.

In a recirculation system, salinity may increase a little due to evaporation. Freshwater of the same temperature as the tank water should be added as needed to maintain the salinity at 12 ppt. Salinity should be checked every four or five days and freshwater added as required. If turbidity increases dramatically, an immediate water change is required. For this reason, **treated 12 ppt water should always be available in the mixing tank.**

Temperature regulation

The ideal temperature for rearing is 28-30°C. Temperature can be controlled by using immersion heaters. It has to be borne in mind that temperature below 24°C and above 33°C are lethal to larvae. Fluctuations of temperature by more than 1°C are stressful and cause mortality.

To heat the water in the tank, different types of heaters are available on the world market. About 400 watts per 1000 l of water is required. Water temperature should be checked five or six times daily if a thermostat is not being used. Covering the tank at night will help to reduce diurnal fluctuations.

Larvae at all stages are attracted by light, but **direct sunlight is harmful.** It has been shown that growth and survival of larvae are improved in lighted tanks compared to dark tanks. Low, even illumination is preferable. Uneven illumination will cause 'clumping' of larvae in the brightest areas of the tank. Strong aeration counteracts the clumping tendency. Covering individual tanks is recommended to maintain temperature and to inhibit the spread of disease between tanks. Fluorescent tubes can be used to evenly disperse light if tanks are inside a closed building.

Ammonia, Nitrite and pH control

Chemical changes take place in the water of the larvae rearing tank. Such changes take place because of the waste products of larvae and *Artemia,* dissolved fractions of feed supplied to the larvae, unused feed and spoilage of dead larvae. Some of these changes are very harmful.

Un-ionized ammonia is the result of one such change. High pH increases the amount of un-ionized ammonia. Both nitrates and nitrites are harmful. Excessive nitrates increase mortality and retard growth, while 1.8 ppm of nitrite is lethal. The presence of un-ionized ammonia in very small quantities also induces mortality.

The concentration of nitrites and nitrates in the water of the larvae tank should not exceed 1 ppm and 20 ppm respectively. The concentration of ammonia nitrite and nitrate is reduced and controlled by recirculating the water through the biofilter. Oyster or clamshells are good media for the biofilter, as the calcium carbonate content acts as a buffer against sudden changes in pH. The best pH range is 8.0 - 8.2.

Maintaining water quality

Cleanliness must be strictly maintained to ensure best results. Constant vigilance is required to prevent or control outbreaks of disease. Instruments and glassware should be kept separately for each tank, to prevent the transfer of disease between tanks. All tools and glassware should be disinfected by soaking them in a solution of potassium permanganate or formalin. After every larvae cycle, the tank should be washed and disinfected to prevent the growth of *Zoothumnium, Epistylis,* hydroids and other disease organisms.

The following precautions should be taken:

- Larvae should not be given feed in excess of their requirements.
- The walls of the tank should be cleaned with a soft brush every third day.
- After the first feed every day, aeration should be stopped and solid waste and dead larvae allowed to settle at the bottom. These should then be removed by siphoning and the aeration turned on immediately thereafter.
- If the hatchery is located on a sea beach, only 50 per cent of the water needs to be exchanged daily. In a recirculation system, an even smaller proportion of tank volume needs be changed. A 20 per cent replacement on Day 10 and on Day 20 is beneficial.
- If for any reason the condition of the water deteriorates, or the movements of the larvae become weak, then 100 per cent of the rearing water should be changed.
- EDTA (usually 5-10 ppm) added to both hatching and larvae rearing tanks improves production.
- After completing a larvae rearing cycle, the side walls of the tank should be brushed well and kept moist for 24 hours with a strong solution of commercial bleaching powder. Formalin at 250 ppm may be used in place of bleaching powder. The disinfectant rinse should be followed by washing with clean water and drying for at least one day. Before starting work again, the tank should be rinsed with tapwater.

Counting larvae

Larvae are strongly phototactic (light sensitive) and tend to group in even a well-aerated tank. It is almost impossible to get an accurate count under these conditions. Taking several samples with a 250 ml beaker and counting the larvae caught in it will give a rough estimate. It is worthwhile estimating the Stage I larvae so that the stocking rate could be kept within reasonable limits.

Counting dead larvae during daily tank cleanings will give a clear indication if something is amiss and enable remedial measures to be taken. If there is a large increase in dead larvae, behavioural **and colour changes will usually be seen in the live** larvae in the tank.