CMFRI

Course Manual

Winter School on

Recent Advances in Breeding and Larviculture of Marine Finfish and Shellfish

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SHRIMP SEED PRODUCTION AND SEARANCHING

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Introduction

The pioneering efforts of Hudinaga (1942), in the successful spawning of *Penaeus japonicus* and rearing of its larvae up to the juvenile stage under controlled conditions, have paved way for the large scale hatchery seed production of penaeid prawns for aquaculture. There are two basic hatchery seed techniques for mass production of larvae of penaeid prawns are Japanese technique and Galveston Technique. These techniques have been appropriately modified to suit to the different geographical and climatic conditions as well as for different species. Sometimes combinations of these two techniques have also been used.

Japanese Technique

The well known Japanese system, also known as community culture system, fertilized system or large tank hatchery system, involves spawning, hatching, larval and post larval rearing up to fry stage in the same tank. By fertilizing the water, phyto and zooplankton organisms which form the food of the larvae are also raised along with the prawn larvae in the same tank. The hatchery tanks (cement concrete- rectangular or square) varying size from 60 – 200 t and depth of 1.5 to 2 m are kept indoor under transparent roof or out door conditions and vigorously aerated with rotating agitators. To start with, the tanks are cleaned, sun dried and filled 1/5th with fresh filtered sea water. Spawners are treated, with 3ppm potassium permanganate or any other recommended disinfectant in the required strength, and introduced in to the tank in cage nets at the rate of 1 spawner per m^3 of tank capacity. After spawning the spawners are removed with the cage nets. The eggs hatch out in to nauplii within 12 – 18 hrs depending on the water temperature. The tank is now fertilized with nitrates (KNO_3) - 2ppm and phosphate ($KHPO_4$) - 0.2 ppm which helps in the growth of naturally occurring diatoms on which the protozoeae feed. The water is fertilized daily to maintain a good growth of diatoms (20,000 cells/ml). By the time protozoea develops in to mysis stage, a good population of zooplankton also develop in the tank. Thus an environment closely similar to sea is created. Anaerobic decay of the dead organisms is prevented by vigorously aerating the entire column of water. From mysis I to postlarva IV, fresh sediment free, clean filtered sea water is pumped ever day until the water level is raised to the to the maximum tank capacity. Artemia nauplii or minced and washed clam meat or formulated feed of appropriate size are provided for post larval stages as supplementary feed. It is difficult to maintain the initial stocking density of nauplii constant throughout the run in this system. Generally the initial stocking density is around 100 nauplii/L. Rearing of nauplii to PL 25 – 30 in the same tank is achieved in a period of 35 – 40 days at a production rate of 5 – 25 nos./L. A minimum of 10,00,000 PL 25 – 30 can be obtained from a larval rearing tank of 200 t capacity in a single run. Modifications in the feeding pattern have been effected subsequently. In Taiwan, oyster larvae produced by artificial fertilization was used as feed when phytoplankton failed to bloom. 'Bread – yeast' @ 2 g/ton/day is being used along with mixed diatom to feed protozoeae and mysis stages in Philippines. Rotifer is cultured separately and fed at a concentration of 10 – 25nos. /ml for mysis and early post larval stages.

The maintenance cost and technical expertise required are low for this system of hatchery compared to the Galveston system. Using the same tank for rearing larvae through various stages up to the seed size and transferring them to the farm directly are the notable advantages of this system. The high initial cost of construction, lack of control over the density of phytoplankton and the frequent blooming of undesirable species of organisms such as dinoflagellates and Noctiluca, which leads to mass mortality of larvae are its disadvantages. As the shrimp larvae at this stage do not hunt for food but filter the food particles, a good amount of food will remain unutilized when the larval population is less.

The Galveston System

This system is more sophisticated and consists of a few independent functional processes such as mass production and storage of pure algal culture, mass production of freshly hatched Artemia nauplii and larval rearing operations which involve higher technical skill. Culture of desirable species of food organisms and proper control over the water quality, as small containers are used, make this method more dependable.

Originally a fiberglass tank of 946 L capacity was used for spawning in a 19 L polyurethane carbouy for rearing nauplii to post larvae. Phytoplankton feed in the larval tank is kept in suspension by aeration. Half the water in the carbouy is replaced every day with fresh filtered seawater, which dilutes the metabolite concentration. Using this system, a total of 133 postlarvae were obtained from 266 nauplii per litre (50%). Later, a modification to this system took place in which 1890 litre cylindrical polyethylene containers connected to a seawater re-circulation system were used with crushed oyster shell as filter bed for spawning and larval rearing . The re-circulation is stopped when pure cultures of diatoms or unicellular algae are added to the tank water for feeding protozoa in order to maintain a concentration of 10,000-15,000 cells/ml. Freshly hatched Artemia nauplii are given at the rate of 3-5/ml. of water for feeding in the larvae from mysis stage onwards.

This system has been further improved by introducing cylindro-conical fiber glass tanks of $2m^3$ capacity for rearing larvae. The shape of the tank facilitates efficient dispersal of food items and larvae in the water column. This system has been adopted and modified in Tahiti (Philippines) and U.K. Algae in higher concentrations (30,000 – 1,00,000 cells/ml) are maintained separately and used for feeding protozoa larvae. Brachionus culture maintained separately is also fed at a concentration of 5-10 rotifers/ml. of medium. From PL-1 onwards freshly hatched Artemia nauplii are fed at a density of 5 nos./ml. in the medium. Nauplii are stocked at the rate of 100-200 nos./litre and reared up to PL -5 at a survival rate of 70% and are transferred to nursery tanks.

This system has many advantages. Few spawners will suffice the larval requirement of the hatchery. It is easy to manage the water quality and the water requirement is less. Further, the water temperature can be controlled. Diseases can be checked and prevented by water management and antibiotic treatment. Food is not washed and survival rate is high.

Hatchery systems developed in India

CMFRI Technology

A low cost technology for hatchery production of prawn seed has been developed by the CMFRI and used successfully for the large-scale production of PL 15-20 of *P. indicus, P. monodon, P. semisulcatus* and *P. canaliculatus.* Cost of production of PL 15 -20 is made minimum, making use of the natural advantages of Indian cost, such as good sun light, ideal temperature, clean sea water of above 30% salinity, and native candidate species. It is established, all feeding larval stages could be reared successfully by feeding them exclusively with the diatom *Chaetoceros* spp. Which are abundant along the Indian costal waters. They are cultured in indoor fiberglass tanks in hatcheries with transparent roof facilities. Mysis III to PL 20 are fed with hen egg-prawn custard. The technology developed by CMFRI for *Penaeus* species is in fact a package of practices involving the following components: i). Broodstock management, ii). Spawning, iii). Larval rearing, iv) Livefeed culture and v). Preparation of particulate feed (egg – prawn custard) for feeding post larvae.

Broodstock management

Spawners are collected either from marine trawl catches or from broodstock maintained in the hatchery. The females are also induced to mature under controlled conditions. Induced maturation is effected by unilateral eyestalk ablation and providing congenial conditions for faster ovary development. Females of appropriate size and weight are collected from broodstock tanks, from shrimp farms or from sea. Shrimps brought are disinfected and acclimatized to the hatchery conditions for 24 – 48 hours. Unilateral eyestalk ablation is done using electrocautery apparatus or using red hot forceps. Only female shrimp is ablated. After ablation they are maintained in maturation pools, for further

gonadal development and mating, along with males in the ratio of 1:1. Generally 10 t tanks with biological filter are used as maturation pools. Depending on the species selected 20 – 30 animals can be maintained in one tank. The tanks are covered to prevent light for about 14 – 16 hours every day.

Clear sea water conforming to the following hydrological parameters are conducive for maturation"

Parameter	Permissible range
Salinity	29-34 ppt
Temperature	27-29 ^{0}C
pH	8.0-8.2
Dissolved oxygen	4.0-5.5 ml/litre
Total ammonia	0.002-0.07 ppm
Nitrite	0.003-0.02 ppm
Light intensity during day time in the shed	500-3600 lux

The pH of the sea water was maintained by addition of sodium carbonate (ca. 25 g/m^3 of water every day). The prawns are fed with fresh clam or mussel meat at the rate of 12.5-15.0% of prawn biomass per day in the evening. The unused food and faecal pellets are siphoned out in the morning. Under these conditions about 70% of the ablated females mature and spawn within 4-5 days after eyestalk ablation. The water in the maturation pool is totally replaced before introducing a fresh batch of ablated females. Spent females can also be used for re-maturation when fresh spawners are not readily available.

Spawning

Impregnated females with fully mature ovary (dark green in colour, occupies major portion on the dorsal side of the animal along its entire length) are transferred at the rate of one each to a 250 L capacity spawning tank containing 200 L of sea water of 30-34ppt salinity, after filtering through 50 micron mesh. Spawner transfer is done in the evening. Disodium salt of EDTA is added to the water at the rate of 0.1 g/100 L of water. The ideal temperature range is 27-30^{0}C and pH 8.0-8.2. A mild aeration is provided and the tank is covered with black cloth to protect the spawner from strong light and to prevent it from escape. Spawning usually takes place between 8 p.m. and 2 a.m. In the same day, female after spawning is removed from the tank at 6 am. Continuous increased aeration is maintained in the tank. Depending on the temperature, hatching takes place within 12-18 hrs and by afternoon all viable eggs hatch out to nauplii. After 5-6 hours, aeration is stopped and nauplii are allowed to congregate at the surface. Dead and unhatched eggs that sink to the bottom are siphoned out along with bottom sediments. Once again aeration is switched on and the water is mixed thoroughly and three, 100 ml samples are collected with beakers and the number of nauplii in the samples counted and the total number of nauplii estimated.

Larval rearing

2 to 5 ton capacity cylindroconical tanks are used for rearing larvae up to PL 3-5. Fiberglass or cement tanks with an inner coating of non toxic epoxy paint are used. The tanks are cleaned with bleaching powder, washed with freshwater, and sun dried for 24 hours. They are again washed with filtered sea water and then set for larval rearing. Tank is half filled with sea water, filtered through 50 micron mesh . Continuous good aeration is provided throughout the rearing period. Counted nauplii are transferred to the larval rearing tank at a stocking density of 75-100/litre. During stocking the temperature difference of water in the spawning and larval rearing tank should not be more than 1^{0}C. Desired temperature for larval growing is 28^{0}C-30^{0}C. After 36 hrs of hatching, the nauplii will be in its 5th ot 6th stage depending on the temperature of the medium. During this time 100 l of mixed algal culture dominated by *Chaetoceros* sp. or *Skeletonema* sp. is added into the larval rearing tank. Concentration of the algal cell in the medium must not be below 20,000 cells/ml. The diatoms thus added will ensure the availability of food with in the easy reach

of first protozoea. From first protozoeal stage onwards 150-200 litres of algal culture is added and the water level is made up to the maximum by adding filtered sea water. From 4th day onwards daily 1/4th to 1/3 rd water is replenished. Filter bags suitably meshed are used while siphoning out water to prevent escape of larvae. If the algal culture used is dominated with *Chaetoceros* species, no supplementary feed is required until third mysis stage. Otherwise supplementary feeding with particulate feed or hen egg-prawn custard may be given from first mysis stage onwards. Usually it is provided from third mysis stage onwards. The quantity of algal diet is reduced from first postlarval stage. Larvae are reared upto PL 3-5 in the same tanks than transferred to nursery tanks for further rearing.

Protozoea 2nd feed on a variety of diatoms such as *Skeletonema costatum*, *Chaetoceros* sp. *Thalassiosira* sp.etc. But it was found that larvae up to last mysis stage could be reared exclusively on *Chaetoceros* sp. Every day bottom sediments of the tank are removed while aeration is stopped to allow larvae to surface before siphoning. Clear seawater conforming to the following hydrological parameters are conducive for larval rearing

Salinity	-	29-34 ppt
Temperature	-	26.0-32.5^{0}C
pH	-	8.0-8.5
Dissoved oxygen	-	3.0-8.0 ml/litre
Light intensity in the hatchery during the time	-	20,000 – 1,25,000 lux
Total ammonia	-	<0.1 ppm
Nitrite	-	<0.05 ppm

Few guidelines to the management of larval rearing tanks are given in the table No.1. But it must be noted that the volume of water exchanged and the amount of feed given should be judiciously varied to meet the exigencies of the situation.

Table 1. Management of larval rearing tanks

Day	Stage	Seawater Removed (litres)	Algal culture added (litres)	Egg- prawn custard (g)	Seawater addition (litres)	Total vol. of water made upto (litres)
1	N 2	-	-	-	1000	1000
2	N 5	-	100	-	-	1100
3	PZ 1	-	150-200	-	700-750	2000
4	PZ 2	500	150-250	-	250-350	2000
5	PZ 3	500	150-250	-	250-350	2000
6	M 1	500	150-250	-	250-350	2000
7	M 2	500	150-250	-	250-350	2000
8	M 3	500	150-250	80-100*	250-350	2000
9	PL 1	750	100-150	80-100	600-650	2000
10	PL 2	750	100-150	100-125	600-650	2000
11	PL 3	750	100-150	100-125	600-650	2000
12	PL 4	750	100-150	100-125	600-650	2000
13	PL 5	750	100-150	100-125	600-650	2000

*The daily ration of egg custard may be split into 4 to 6 equal doses and fed at suitable intervals.

Diatom culture initiation and maintenance:

For initiating the algal culture, fresh unpolluted sea water (30-34 ppt salinity) is filtered through 50 micron mesh bolting cloth and kept in 1000 litre capacity, white fibre-glass tanks placed indoors under transparent roofed shed. The sea water is fertilized as below:

Sodium nitrate	..	12 ppm
Potassium orthophosphate	..	3 ppm
Sodium silicate	..	6 ppm
EDTA di-sodium salt	..	6 ppm

Sodium silicate has to be completely dissolved in freshwater. Other chemicals can be dissolved in freshwater/ seawater, and mixed thoroughly in the sea water of the algal culture tanks.

Two air-stones connected to the aeration grid are kept in each tank. The intensity of sunlight in the shed can very from 20000 to 120000 lux during day time and the temperature of the culture medium from 28 - 35^{0}C. Under these conditions the diatom cells present in the sea water multiply rapidly and give rise to a golden-brown bloom of diatoms in 24-48 hrs. Although many species of diatoms may be originally present in the sea water, under the above temperature conditions, *Chaetoceros* spp. Become the dominant diatom forming 75-90% of the cells in the culture. Other diatoms like *Thalassiosira, Skeleronema* and *Nitzschia* may also be present in lesser densities. A culture containing a concentration of 3-4 lakh cells/ml. is preferred for feeding. This culture is used for feeding the prawn larvae and also as inoculum for developing bach cultures on succeeding days. Algal culture are thus started everyday using the previous day's culture as inoculum (at the rate of 30-55 litres per m^3 of filtered seawater and fertilized as above). It attain feeding concentration within 16-20 hrs. after inoculation. On cloudy days, diatom multiplication will be delayed, hence the quantum of inoculum can be increased.

During prolonged cloudy conditions, maintenance of mixed culture becomes difficult. Therefore it is advisable to have a separate, small, diatom culture unit on the hatchery with air-conditioning and artificial lighting to ensure a study supply of diatom.

Preparation of egg-prawn custard

Yolk and albumen of hen's egg and prawn meat of small prawns are mixed well in a mixie at the ratio of 1:5 and cooked for 10 minutes in a pressure cooker and kept in refrigerator. A solid block of this custard, after thawing can be made into suitable particle by passing through proper sieves. Custard should not be stored more than 3 days.

Kerala Fisheries Technology

At Azhicode prawn hatchery of Kerala State, outdoor concrete tanks or pools of 6 – 60 ton capacity, having a height of 75 – 100 cm, are used for larval rearing. Cleaned and sun dried tanks are filled to 1/5th of its capacity with filtered seawater and aerated.. Wild spawners are introduced in to the tank at the rate of one spawner/ton of water. Spawners are removed after spawning, water level is raised to half the tank capacity and vigorous aeration continued. As the nauplii start metamorphosing to protozoeae prawn meat suspension made out of juvenile *M.dobsoni* or squilla meat is fed to the larvae. Acetes and Mysids are also used for making meat suspension. The approximate weights of meat feed for different larval stages are given below.

	M.dobsoni	Squilla
Protozoea I (1000) larvae/day)	0.5 g	1.0g
Protozoea II & III ,,	0.75g	1.5g
Mysis I ,,	1.0 g	1.75g
Mysis II ,,	1.25g	1.75g
Mysis III ,,	1.5 g	2.0 g
PL I ,,	2.0 g	3.00g

From mysis I stage onwards 1/3rd tank water is exchanged daily. In case of development of phytoplankton bloom water exchange should be suitably regulated. A stocking density of 100 – 250 nauplii/l is the norm. They are reared up to PL 10 in the same tank and an average survival rate of 75% has been reported.

Nursery rearing

Postlarvae of penaeid prawns at PL II – V are very fragile and sensitive to change in water condition and fall easy prey to predators. When stocked in growout ponds, mortality will be high initially due to handling, transportation and acclimatization stress. Stress factors in pond are unavoidable. Hence a nursery phase is advisable in shrimp farming to enable the aquaculturist to have hardy shrimp seed for stocking in farms.

In the Japanese system, since larvae are stocked in low densities in large tanks, the same can be used continuously for further rearing of postlarvae to PL 15 or above an ideal age for stocking in farms.

In small cylindro-conical tanks, larvae can be reared upto PL 3-5 when they turn benithic in habit or swim around the sides of the tank leading to over crowding, which increases mortality due to cannibalism. To avoid this PL 3 to 5 are transferred to large nursery tanks which offer enough surface area for good growth. Thus in this system nursery rearing becomes a necessity. When grown in nursery upto PL 15 or more and stocked in ponds farmer can be sure of the size of the stock and determine the right feeding regime.Nursery rearing can be done in cement tanks, earthen ponds and in net cages(hapas) in deeper ponds.

Cement tanks

Large outdoor rectangular tanks of 1 m depth provided with good aeration can be used for nursery rearing. They are cleaned, dried and filled with filtered sea water of suitable salinity. Pure culture of diatom is than added to maintain water quality and reducing water transparency. Stocking density depends on the species and efficiency of water management. Post larvae of *Penaeeus indicus* can be stocked at a density of 7500 to 10,000 nos/m^3 and *Penaeus monodon* 3500 to 5000 nos/m^3. Because of its habit of clinging to the walls and bottom the surface area of the tank may be increased using additional substrates. Nylone screen can be placed length wise in the tank. About 40-50% water should be exchanged daily. Chopped mussel meat or palletized feed are given. Early stages can be fed with adult Artemia. Raceway system and flow through systems are also used to improve the water quality and survival rate. In higher stocking rates water quality is maintained by increasing water exchange (300 % or mare) and providing strong aeration. PL 15 and above can be harvested and stocked in the grow out ponds.

Liao developed a 'ladder system hatchery' to increase the survival of larvae. This consists of 4 inter connected hatchery tanks on sloping grounds-algal culture tank (1-2 tons) larval rearing tanks (1-2 ton capacity) where larvae are reared upto third protozoea stage 10-15 ton tanks to rear Mysis to PL 5 - PL 20 and above. All tanks are built one below the other on a slopping ground to facilitate transfer of larvae directly without much handling.

Earthen ponds

500-2000 m^2 earthen ponds are used generally as a nursery to rear PL 3-5 to PL 15-25. The earthen nursery ponds have to be dried tilled, limed and fertilized. After fertilization and filling of water, enough time may be given for the development of lab-lab in the ponds. When lab-lab is developed, stock the ponds with PL 3-5 at the rate of 100 – 200 PL/m^2. Formulated palletized feed or chopped mussel meat at the rate of 20-25% of biomass is provided daily as supplementary feed. When natural productivity of the pond is less, supplementary feed is increased suitably. Depending on the pond conditions, 10-40% pond water is exchanged daily. If the water source is tidal flow, water is let in through a properly designed sluice gate, provided with nylon screen of suitable mesh size to prevent entry of predators and escape of postlarvae. Screens should be checked regularly for any damage and cleaned periodically to remove biofouling. In these nurseries, postlarvae 3-5 are reared for 20-25 days.

Nursery cages

Cages made of synthetic netting of small mesh size (0.5 mm) are used for nursery rearing of PL 3-5. The cage can be floating or fixed type. Floating cages are normally supported by farmes and floating buoys made of bamboo. The cages can be stationary and held in position by bamboo/wooden poles. They can be fixed in calm water in bays, lagoons or even inside grow out ponds. Biofouling of the cage is a problem and it must be cleaned properly. 1000-2000 PL 3-5/m^3 can be stocked in the cages. They are fed with mussel meat/prawn-egg custard or palletized feed at the rate of 15-20 % of the total biomass/day. PL 3-5 are reared in these cage for 15-20 days.

Larval diseases

Most serious diseases affecting the larval stages are caused by fungi(*Legenidium, Fusarium*) bacteriae (Vibrio, filamentous bacteriae - *Leucothrix*) and protozoans (Zoothamnium, *Vorticella*). Several antimycotic compounds and antibiotics are available for the treatment of fungal and bacterial diseases. Clotrimazole, crystal violet, malachite green, trifluralin, etc. are some of the chemotherapeutic agents employed for the control of fungi. These chemicals should be used with great care as they themselves are toxic to the larvae if used in excess. The best method of control of fungal disease is by disinfecting the spawners which are the potential source of infection, even though they do not show any visible sign of infection. Antibiotics such as streptomycin, gallimycin, penicillin, etc. are used to prevent and treat bacterial disease in larval rearing tanks.

Treating infected larvae is very difficult and often expensive. Normally when infection is detected, it is advisable to destroy the entire batch of larvae and decontaminate/disinfect the hatchery before starting a fresh batch. The best remedy by far, is to prevent diseases. To achieve this, the spawning tanks should preferably be separated from the larval rearing tanks, spawners should be disinfected, and good quality filtered or purified seawater should be provided in adequate quantity.

Sea ranching

The term sea ranching, also referred to as re-seeding or culture-based fisheries, is used to refer to the process of large scale release of hatchery produced seed / farm grown juveniles of fish or shell fish in a responsible way in selected wild areas of the sea for augmenting marine fish production. Sea ranching is distinct from conventional aquaculture/mariculture in two ways:

- First, the approach relies on extensive rather than intensive techniques, in other words, growth occurs in the open waters of the natural habitat and not in cages, pens or nets; (there is no artificial feeding of the released juveniles)
- Second, the approach is closely integrated in to the commercial fishery.

Depending on the purpose and extent of intervention sea ranching can be broadly categorized in to 1) restocking i.e. release of juveniles to severely depleted stocks (non-operational fisheries) and 2) Stock enhancement – to increase yield by overcoming recruitment limitation.

Restocking is resorted to rebuild severely depleted stocks to levels where they can once again provide substantial, regular yields when long timeframes are predicted for replenishment using other means, and when it is indicated that release of juveniles will “fast-track” the process. As the prime goal of restocking is to rebuild the spawning biomass to productive levels as soon as possible, it is necessary to protect the remaining wild stock, the released individuals, and their progeny until the stock is replenished to the desired level. This could be achieved by a moratorium on fishing for as long as is necessary.

Stock enhancement is the management tool of releasing juveniles in a way that uses the carrying capacity of the ecosystem to the extent possible to deliver substantial harvests. Stock enhancement can increase productivity of

operational fisheries by supplying additional juveniles to the natural population. Supporting measures for successful stock enhancement programmes and increased productivity are addition of habitat, monitoring of compliance with fishing regulation to ensure regular natural replenishment, and periodic independent assessment of the efficacy of the release. The carrying capacity of a habitat changes from season to season and year to year. These changes would increase variation in annual harvests. In general enhanced stocks do not require specialized management provided the animals are released at the size, in the habitat and time of the year, that optimizes their survival and cost. The cultured animals simply add to the stock available for capture and should be managed as such.

Release of cultured juveniles should be considered as a management tool for fisheries only when there is good evidence that the stock, or part of it, is either at a chronically low level, or consistently limited by recruitment. Even then, the cost – benefit of using cultured juveniles to rebuild the stock (restocking) to productive levels, or to increase productivity (stock enhancement), must be assessed and compared to other available management measures. In addition to the biological and ecological considerations, security and return on investment are two important implications to be critically evaluated for culture based fisheries.

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