# MARINE PEARLOYSTER HATCHERY

S. DHARMARAJ[1], A.C.C. VICTOR[2] AND A CHELLAM[1]
*Central Marine Fisheries Research Institute, Cochin - 682 014.*

## INTRODUCTION

In india, the Gulf of Mannar is well known for the resource of pearl oysters belonging to the species *Pinctada fucata* which has been exploited for natural pearls and mother-pearl shells since several decades. In a period of 298 Years from 1663 to 1961, a total of 40 pearl fisheries exploitation had taken place along the Tamil Nadu coast from which pearls worth several million rupees had been obtained. Since 1961, these resources had totally disappeared from the pearl oyster beds and as a result the prospects for a pearl fishery in the near future looks very bleak. This sort of uncertain condition is neither conducive for obtaining natural pearls nor for a sustained supply of mother oysters for culture purposes. Therefore it has become imperative to look for the alternative - development of a system through which a continuous supply of pearl oysters is ensured throughout the year. The Central Marine Fisheries Research Institute (CMFRI) took up this challenge and the breakthrough came in 1981 when the first batch of pearl oyster spat was produced in the laboratory. This technology has been tested over the last 4 years and scaled up for large-scale production. The dwindling of pearl oyster resources in the pearl banks is not a constraint any more for the pearl culture industry. The hatchery technology besides being a source of sustained supply of pearl oysters to pearl culture operations has opened up new possibilities of improving the quality of pearl oyster and thereby the pearls through genetic selection and breeding. The hatchery methods developed are simple to adopt and cost effective.

## HATCHERY ESTABLISHMENT

In hatchery establishement, selection of site is an important aspect. The hatchery should be in an area where ideal environmental conditions prevail during most part of the year. Areas with wide fluctuations in hydrological conditions, high levels of suspended matter or blooms of harmful phytoplankters are unsuitable. The hatchery establishment consists of facilities for supply of good seawater, aeration, live feed production, broodstock maintenance and spawning of oysters, larval rearing and spat rearing. These aspects are briefly described in this paper.

### Seawater supply

The success of hatchery production of pearl oyster seed largely depends on the supply of good quality seawater. The water should be free from pollution as the larvae are very sensitive. The seawater is usually drawn from the sea beyond the low water mark into a well through PVC pipes and it is pumped to sedimentation tanks where

Present Address: 1. Tuticorin Research Centre of CMFRI, 90, North Beach Road, Tuticorin 628 001.
2. Regional centre of CMFRI, Marine Fisheries P.O.623 520, Ramanathapuram District, Tamil Nadu.

large particles in the water settle at the bottom. Then the supernatant water passes through a biological filter which contains coarse river sand at the top, pebbles below and charcoal at the bottom. The seawater thus filtered is collected in a sump and lifted to an overhead tank for supply to the hatchery. Periodic cleaning of the filter bed keeps the seawater uncontaminated. The seawater sterilized by ultraviolet irradiation is used only in specific cases.

### Aeration

The air supply system consists of air compressors, filters, PVC air grid, polythene aeration tubes, diffuser stones and air regulators. Air compressors with storage tank are used to aerate seawater in the rearing tanks. The compressed air is regulated and passed through a series of filters to remove the oil and moisture mixed in the air. The clean air is supplied to the various culture containers through PVC pipes.

### Live feed production

Flagellates measuring less than 10μ form the main food for pearl oyster larvae. *Isochrysis galbana* is an important algal food for the larvae. Other *microalgal* cells such as *Pavlova*, *Chromulina* and *Dicrateria* are also suitable. The growth and spat fall timing varies with the different algal food. *Chromulina* promotes faster growth while *Dicrateria* gives better spat fall.

The flagellates are grown in 4 l Haufkin flasks as stock culture and in 20 l glass carbuoys and 100 l perspex tanks in mass culture. Conway or Walne's medium is used for the culture. These flagellates reach stationary phase of growth in about 15 days in Haufkin flasks and are maintained for two months without aeration. In mass culture, the maximum cell concentration is reached within 5-6 days. The composition of the medium used for mass culture as well as mixed algal culture is as follows:

| | |
|---|---|
| Potassium nitrate | : 0.4g |
| Sodium Silicate | : 0.2g |
| Potassium dihydrogen orthophosphate | : 0.2g |
| Sodium EDTA | : 0.2g |
| Filtered seawater | : 30.0 l |

## BROODSTOCK MAINTENANCE AND SPAWNING

In a hatchery system, broodstock has to be maintained in active reproductive phase continuously to enable year-round operations for seed production. Sexually ripe oysters collected either from the natural beds or from the farm are maintained in an airconditioned room at a constant temperature of 25 °C. The oysters are fed with mixed algal food dominated by *Chaetoceros* sp. at a ration of 4 l per oyster/day. Under such condition the oysters retain their gonadial maturity for a longer duration. In a static water system, a constant aeration is required for the oysters.



### Natural spawning

In the Gulf of Mannar, the pearl oysters naturally spawn twice a year, in June-August and Novembe-December coinciding with the south west and north east monsoons respectively. Fully mature oysters brought from the natural beds or from the farm spawn instantaneously when they are placed in seawater drawn from the surface area or due to mechanical shock caused while cleaning the shell surface or with change in water pressure. In all cases it is found that males spawn first which triggers the females to spawn within 45 minutes.

### Induced spawning

When the mature oysters fail to spawn naturally, they are induced to spawn by other means. The most common one is by thermal stimulation in which mature oysters are placed in a 100 l perspex tank filled with seawater. The water temperature is either increased gradually from the ambient of about 28.5 °C or the oysters are suddently transferred to seawater medium having high water temperature. In both these cases, the oysters spawn instantaneously.

Spawning of pearl oysters can also be effected by chemical stimulation. Oysters kept in Tris buffer solution at pH 9.0 for 1-2 hours and later transferred to normal seawater spawn with a success rate of about 78%. Similarly, seawater medium at pH 9.5 using sodium hydroxide had effected spawing in 68% oysters. Different concentrations (0.5,3.0 and 6.1 millimolars) of hydrogen peroxide in combination either with normal seawater or with alkaline seawater (pH 9.1) are also used in induced spawning. Injection of 0.2ml of N/10 ammonium hydroxide solution into the adductor muscle or foot of the pearl oyster results in 48% spawining. In induced spawning, as soon as spawning is noticed, the oysters are transferred from the medium to normal seawater so that the sperms and eggs are not subjected to any physiological stress and remain viable for fertilization.

### Fertilization

Once the spawning is initiated, the act will continue till all the ripe gametes are extruded. When the eggs are released in the medium, they are pyriform in shape measuring 73.9μ. along the axis and 45μ. in width. The yolk cytoplasm is heavily granulated and opaque. The egg is enclosed in a vitelline membrane and a large germinal vesicle is seen at the centre. The eggs get fertilized in the water medium as soon as they come in contact with the sperm. Following fertilization, the fertilized eggs extrude a polar body. The fertilized eggs assume a spherical shape with the break down of the germinal vesicle. It measures 47.5μ. in diameter. The fertilized eggs settle at the bottom of the vessel along with the unfertilized eggs, broken tissues, dead sperms and faecal matter. The supernatant water loaded with sperms is siphoned out. The water with the fertilized eggs filtered gently through a sieve of 30μ and the eggs thus collected in the sieve are once again filtered through a sieve of 60μ. All unwanted debris retained in the sieve are discarded. The fertilized eggs that pass through the sieve are transferred to FRP tank holding 50 l filtered/sterilized seawater.

## EARLY DEVELOPMENT AND LARVAL REARING

The first cell division takes place 45 minutes after fertilization resulting in the formation of a micromere and macromere. The polar body is placed at the cleavage furrow. During the second cleavage the micromere divides into two and the macromere divides unequally into a micromere and macromere. The stage with three micromere and a macromere is called Trefoil stage. The macromere does not take part in further divisions.

Micromeres divide repeatedly and the morula stage is atained in about 3-4 hours. The morula looks like a ball of minute trasparent cells enclosing a hollow space, the blastocoel. Each cell has a cilium which is used for movement. The morula at this stage lifts itself in the water column and congregates at the surface, exhibiting phototrophism. The morula stage passes on to blastula stage. A blastula stage is reached with the formation of blastocoel inside the embryo. The blastocoel communicates to the exterior through a minute hole called balstopore. The blastula stage is reached 5 hours after fertilization.

In the next stage of gastrulation, the process of orientation of cells into dermal layers and the formation of archenteron is completed in about 7 hours. The embryo develops a single flagellum surrounded by a pre-oral tuft of cilia at the apical end and a post oral tuft of cilia at the rear end. This larva is called as Trochophore larva. The trochophore larva exhibits fast movement.

The ectodermal cells start secreting the embryonic shell known as prodissoconch, I. The shell valve assumes a definite 'd` shape. A straight hinge line is formed. This stage is called the veliger or straight hinge stage. The single flagellum and the pre and post oral tufts of cilia transform into a locomotory organ called the velum. The veliger larva has two equal shell valves. The size of the veliger is about 67.5µ in anteroposterior axis and 52.5µ in dorsoventral axis. This stage is reached in about 18-20 hours.

The veliger larvae are counted, estimated and stocked in the larval rearing tanks. A culture density of two larvae per ml. has been found to be optimum concentration for stocking. Stocking in higher densities results in poor growth. From the second day onwards, the larva is fed with the unicellular microalga *Isochrysis galbana*. The optimum ration for a larva is 5000 cells/day.

### Umbo stage

Further development of the veliger to the umbo stage is gradual with the development of the shell, prodissoconch II. The straight hinge line disappears. The typical clam shaped umbo stage is reached between 10-12 days. The shell valves are equal with mantle folds on the inner side. The larva swims with velum. It measure 130µ.



### Eyed stage

After attaining the full umbo stage, the larvae develop an eye spot at the base of the foot. A well developed velum effects the movement of the larvae. The ctenidial ridges develop at this stage. At this stage, the larva measures 180 x 170µ. The eyed stage is reached usually in 15 days.

### Pediveliger stage

The pediveliger stage is reached on the 18th day when the larva measures 200x190µ. The foot is well developed. The transitional stage from the eye to pediveliger has both velum and foot. Later, the foot becomes functional while the velum disappears. Gill filaments are now visible.

### Planntigrade stage

When the pediveliger larva selects a substratum for settlement, additional shell growth is seen all along the periphery. In the meantime, the byssal gland becomes functional which secretes byssal threads for attachment. Labial palps and gill filaments develop. The stage is reached on 20th day and it measures 220 x 200µ.

### Spat

By the repeated addition of dissoconch, the plantigrade metamorphoses into a spat on 24th day. To accomodate byssus threads, a byssal notch is developed at the anterior end. Gill rakers are formed. The shell valves are transparent. The spat attaches itself to the substratum with the aid of the byssal threads. The size of spat is 300 µ at the time of settlement.

## LARVAL REARING CONDITIONS

Even under indentical conditions, the larvae of the same brood show differential growth rates and time in settlements. This may vary from batch to batch. Mortality of the larvae is more till they reach the umbo stage and becomes negligible beyond this, unless there is any change in the water quality or food. Factors like high larval concentration, colour of the inner surface of the culture tanks, aeration and overfeeding may affect the larval growthand survival. Larval density plays a significant role in the growth. At higher densities, the growth and spat fall are poor. A culture density of 2 larvae per ml produces optimum growth and spat fall rates. The colour of the culture tanks also influences the settlement of larvae. Spat fall is much higher in FRP black tanks than in white and blue tanks. Aeration during larval rearing affects growth and spat fall. The effect of aeration is more pronounced in smaller volume of water. However, aeration is required after the settling of the pearl oyster larvae.

In static water system, change of water is done on alternate days. Water is gently siphoned out through appropriate sieves. A 40µ sieve is used upto umbo stage, 80µ

seive upto eye spot stage and 140 μ seive afterwards until settlement. At every water change, the larvae thus collected on the sieve are washed gently and released in fibreglass tanks filled with fresh filtered seawater. The tanks are covered with black cloth to prevent algal growth.

The unicellular microalga *Isochrysis galbana* is provided as food to the larva from the veliger stage onwards. The optimum ration for a larva is 5,000 cells per day upto umbo stage. The dose is doubled from the umbo to the pediveliger stage and tripled afterwards upto settlement. Apart from *I.galbana* other unialgal food such as *Pavlova lutheri, Chromulina freibergensis* and *Diratera* sp. are also given as food. Feeding is given once a day. Feeding in low or high doses affects the larval growth.

Normally spat fall occurs between 18th and 20th day. In exceptional cases the spat fall is either advanced to 14th day or delayed corresponding to conditions prevailing at the time. In the hatchery, a spat production rate of upto 30% (compared to the initial stock at veliger stage) can be achieved under ideal conditions in one ton capacity tanks and 40-50% production rate in tanks of smaller capacity. Mortality of spat is negligible under normal conditions.

## TRANSPLANTATION AND SPAT REARING IN THE FARM

The spat are reared in the hatchery for about 3 months. By then, they grow to 3mm or more. They are transferred to the farm in velon screen net cages with a mesh size of 400μ. Mortality may occur if spat measuring less than 3mm are trasplanted. Spat growth is monitored carefully and the net cages are changed whenever necessary. Normally, after a month of rearing the spat are transferred to another net cage having 2mm mesh size. On attaining a size of 10-15m, they are reared in a box type cage having 3mm mesh. The stocking density varies with the size of the spat and net cage.

## JUVENILE REARING

On attaining a size of 20-30mm, the spat are transferred to a cage having 10mm mesh size. The stocking density of juveniles ranges between 750 and 1,000 according to the size of the cages. The spat attain a size of 40-45mm in a period of 12-15 months.

## SURVIVAL

A survival rate of 30% is achieved in 500 I capacity tanks and 40 to 50% in 50 l capacity tanks, The survival rate for transplanted spat in the farm is about 30%.

## ECONOMIC EVALUATION OF PEARL OYSTER SEED PRODUCTION

In the shellfish hatchery laboratory of CMFRI at Tutitcorin, the economics of production has been worked out. The actual production cost of a single 3 mm spat amounts to 9 paise. This was arived at by taking into account the capital and recurring expenditure.



## PRODUCTION CAPACITY OF THE HATCHERY

The pearl oyster hatchery has the production capacity of about 5 million spat annually. The details of spat production are given below.

## HATCHERY SPAT PRODUCTION

| | | |
|---|---|---|
| Total No. of tanks used | : | 10 (1 tonne capacity) |
| Stocking rate of veliger larvae per tank | : | One million |
| Total veliger larvae stocked in one run | : | Ten million |
| Expected production of spat (20%) | : | Two million |
| Percentage of spat survival at the end of two months in the hatchery | : | 50% |
| Net spat production per run | : | One million |
| No. of runs in a year | : | 5 |

## CAPITAL EXPENDITURE

| A. Buildings, constructions etc. | Rs. |
|---|---|
| Hatchery building with translucent roofing 150 sq.m. @ RS.1500/- $m^2$. | 2,25,000 |
| Generator/compressor room 27 sq.m. @ Rs.1000/- per $m^2$. | 27,000 |
| Water sump 14 sq.m. @ Rs.1500/-per $m^2$. | 21,000 |
| Retention tank 8.4 sq.m. @ Rs.1500/-per $m^2$. | 12,600 |
| Filter bed 4.5 sq.m. @ Rs.1500/-per $m^2$. | 6,750 |
| Pump house 14.6 sq.m. @ Rs.1500/- per $m^2$. | 21,900 |
| Overhead tank - 10,000 l capacity | 1;00,000 |
| Total | 4,14,250 |

| B. Fiberglass/perspex tanks etc. | RS |
|---|---|
| 6 Nos. 100 l capacity broodstock FRP tank @ Rs.1600/- | 9,600 |
| 5 Nos. 100 l capacity spawning/algal culture perspex tank @ Rs.3000/- | 15,000 |

| | |
|---|---|
| 24 Nos. 1 tonne capacity rectangular larval/spat rearing/mixed algal culture FRP tanks @ Rs.8000/- | 1,92,000 |
| 6 Nos. 3 l capacity Haufkin flask for stock culture @ Rs.1200/- | 7,200 |
| Total | 2,23,800 |

| **C. Major equipments and instruments** | **Rs.** |
|---|---|
| 10 KVA Generator - 1 NO. | 1,00,000 |
| Air Compressor - 1 No. | 50,000 |
| 7.5 HP Electric pump and motor - 1 No. | 30,000 |
| 1.0 HP Electric pump and motor - 2 Nos. | 20,000 |
| Microscope, pH meter, salinometer | 45,000 |
| Chemical balance | 10,000 |
| UV Chamber, Hot air oven | 15,000 |
| Furniture | 50,000 |
| ECE Controller, Silica cased immersion heater, Jumo thermometer | 10,000 |
| Air conditioner - 2 Nos. | 1,00,000 |
| Total | 4,30,000 |
| Total capital cost (A+B+C) | 10,68,050 |

| **RECURRING EXPENDITURE** | |
|---|---|
| **A. Interest** @ 15% on Rs 10,68,050 | (approx.) 1,60,200 |
| **B. Depreciation** @ 5% on building and fibreglas tanks | (approx.) 31,900 |
| @ 10% on equipment | 43,000 |
| **C. Salaries** to the Manager, Technicians and skilled workers | 1,27,500 |
| **D. Contingencies** Plasticware, flexible PVC hoses, glassware, bolting silk etc. | 10,000 |
| Energy cost (Electricity and Diesel) | 30,000 |
| Chemicals | 10,000 |
| Other contingencies | 5,000 |
| **E. Maintenance** | 20,000 |



| | Rs. |
|---|---|
| **F. Annual lease for land** | 3,000 |
| Total recurring cost (A to F) | 4,40,600 |

**ECONOMICS OF HATCHERY PRODUCED SPAT**

| | |
|---|---|
| Total spat production | 5 million |
| Cost of production per spat(3mm) | 9 paise |

**PROFIT**

(N.B: If the selling price of a spat is 15 paise)

| | | |
|---|---|---|
| Annual revenue for 5 million spat | | 7,50,000 |
| Annual expenditure | | 4,40,600 |
| Annual net profit | | 3,09,400 |
| Annual repayment of loan | | 1,25,100 |
| Net profit after repayment of loan | | 1,84,300 |
| Cost of production of 3mm spat/thousand (inclusive of interest and depreciation) | | 90 |
| Profit cost ratio (Rs.3,09,400 : 4,40,600) | 0.414 | |
| Profit investment ratio (3,09,400 : 10,68,050) | 0.218 | |

[redacted] rate of loan rapayment i.e. Rs.2,00,000 per year (Rs.74,900 depreciation + 25,100 as loan repayment) the entire loan will be repaid in about 6 years and a [redacted]lative net profit of Rs.11,05,800 will be received in 6 years.

[redacted]nomics given here are based on the facilities available at the Molluscan Shell-[redacted]tchery of CMFRI, Tuticorin.