

**CMFRI**

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# ***Course Manual***

*Winter School on  
Recent Advances in Breeding and Larviculture  
of Marine Finfish and Shellfish*

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### Introduction

Indian pearl oyster belonging to the Genus *Pinctada* occurs in the two Gulf zones of India i.e., Gulf of Kutch in Gujarat and Gulf of Mannar in Tamil Nadu. Commercial fishery existed in GoM till 1961 and then has gone into a dormant period due to many reasons including insignificant natural settlement of spat in the pearl beds ('paars') of Gulf of Mannar. To sustain a pearl culture industry, a steady supply of pearl oyster seed and farming it to mother oysters is a pre requisite. Large scale collection of pearl oyster spats either by using spat collectors or from their natural beds was not always found dependable owing to the irregular spat fall. Hence, hatchery production of pearl oyster seed and mother oyster culture has become an essential aspect as far as pearl culture is concerned. The following account details the basic hatchery requirement, broodstock maintenance and spawning and hatchery production of spats of *Pinctada fucata* and the methods of farming the spats for mother oyster development.

#### a) Seed production in *P. fucata*

##### Site selection

The prime requirement for site selection to construct bivalve hatchery is good quality seawater free from pollutants, suspended particles and silt. The site of drawal of water may be from rocky, coralline or sand mixed area. The intake point should preferably be away from any kind of industrial/domestic sewage. River mouths should be avoided, as dilution would become a problem during monsoon. Proximity to the natural resources and farm site and logistics are also to be considered.

##### Water intake and distribution

Water intake point should be beyond low water mark. Water is collected in a well through PVC pipeline (15 cm dia.) by gravitational force/pumping. The well acts as a sump cum sedimentation tank. Sedimentation tanks can be rectangle in shape. The water that is drained into the sedimentation tank is allowed to stand for some time for settling the heavy particles. Then the supernatant water is pumped into the biological filter. The biological filter is made of coarse river sand, pebbles and charcoal and the water allowed to pass through it is then collected in a sump and then pumped into overhead tank. From overhead tank, the water is drawn through PVC pipelines to the hatchery and subsequently passed through ultraviolet sterilizing chamber or cartridge filters (mesh size i.e., 10, 5, & 1 µm) to destroy bacterial load before being taken to the larval rearing tanks.

##### Air supply system

Air compressors or blowers are used to supply the required aeration to the tanks. Air compressors can be of Piston or Rotary vane type. Rotary van model is considered suitable because they give a high output at low pressure and are also less prone to mechanical failures. Air is compressed into a storage tank. The automatic cut off valves allows the compressor to rest for a while when the tank is full. The air is then passed through a series of filters to remove oil and moisture and supplied to the hatchery through PVC pipelines. Electrical air blowers can be twin lobe oil free type for the use in hatchery. However, the disadvantage of air blower is that it should run continuously as it doesn't have storage tanks and regulate airflow at the source.

### Larval rearing tanks and sieves

Larval rearing is done in rectangular FRP tanks of assorted size. A hatchery should have various sized FRP tank i.e., 100, 500 and 1000 L for different uses. The FRP tanks could preferably be black in colour. Sieves made of Nylobolt cloths of different mesh size ranging from 20 to 250µm are essential for hatchery operations.

### Collection and broodstock maintenance

Collection of brood pearl oysters is mostly done from the natural bed. A diver can collect oysters either by skin diving or using SCUBA (Self contained underwater breathing apparatus). SCUBA equipment enhances the diver's underwater stay for a longer period for collection. Collected oysters are beached, cleaned and stocked in rearing cages and maintained in the farm. In order to get spawners throughout the year, the brood oysters are kept in conditioning room at temperature less than 25°C and fed with mixed culture of algae containing mostly *Chaetoceros* spp. at a rate of 4.0 L/oyster/day in two rations. The mature oysters can be kept for prolonged period under 25-27 °C and can be used for induced spawning purposes.

### Natural spawning

Most of the oysters collected during the spawning season (June to August & November to January) would be sexually ripe and may spawn naturally in the hatchery.

### Induced spawning

In the event of negative response from the oysters, induction of various kinds can be resorted to make the oysters spawn.

### Chemical induction

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), Tris buffer (Hydroxymethyl amino methane), Sodium hydroxide (Na OH) and Ammonium hydroxide (NH<sub>4</sub> OH) are found to have some effect on the oysters and can induce them to spawn with some degree of success.

### Thermal induction

The most preferred and suitable technique for inducing pearl oysters to spawn is thermal induction. As the term implies, the oysters are kept in room temperature of less than 25 °C in a conditioning room for some time and quickly changed to water having 5 to 6 °C higher temperature. The change would stimulate the oysters to spawn.

### Spawning

In all the cases, the male oyster responds to the induction and initiates spawning. The presence of sperms in the water column stimulates the females to respond within 30 minutes.

### Developmental stages of *P. fucata*

**Fertilization:** Majority of the eggs released is pyriform in shape. A large clear germinal vesicle (nucleus) is distinctly seen. The yolk cytoplasm is heavily granulated and is opaque. Immediately after discharge, the eggs are fertilized and assume spherical shape and attain a size of about 50µ in diameter. During the process of fertilization, the first and second polar bodies are released.

**Cleavage:** 45 minutes after fertilization, the first cleavage begins and a micromere and macromere are formed. During the second cleavage the micromere divides into two and the macromere divides unequally into a micromere and a macromere. This stage with three micromere and a macromere is called Trefoil stage. Macromere become smaller and smaller in size after passing through eight, sixteen cell stage and so on to reach the morula stage. Cilium is formed in each of the micromeres and the rotary movement of the embryo develops.



**Blastula:** Blastula is reached 5 hrs after fertilization. In this stage, reorientation of cells results in formation of blastopore and blastocoel.

**Gastrula:** Gastrulation takes place by epiboly. The cells convolute and differentiate into different layers. The archenteron is formed and communicates to the exterior through blastopore. The embryo exhibits phototropism and it takes 7 hrs to reach the stage.

**Trochophore:** The early trochophore larva develops pre oral and post oral tufts of cilia thus marking antero posterior region of the embryo. A single apical flagellum is developed in the typical trochophore stage. The minute cilia noticed in the blastula stage disappear. A shell gland of the dorsal ectoderm secretes the prodissoconch I. Trochophore is attained in 10 hrs.

**Veliger:** Veliger stage is reached at 20 hrs by the formation of straight hinge line, mantle, rearrangement of pre oral cilia into a velum and disappearance of the apical flagellum, pre oral and post oral ciliary bands. The larvae measure an average of 65µm antero posteriorly (APM) and 52.0µm dorso ventrally (DVM).

**Umbo:** The development of umbo stage is gradual. Typical clam shaped umbo is reached between 10-12 days measuring about 135 x 130µm. The shell valves are equal and mantle fold is developed.

**Eyespot :** Eyespot is developed on 15<sup>th</sup> day when the larvae are about 190 x 180µm and at the base of the foot primordium. Ctinidial ridges are also formed.

**Pediveliger stage:** The foot is developed on 18<sup>th</sup> day at a size of 200 x 190µm. The transitional stage from swimming to crawling phase. The larva has both velum and foot. The foot becomes functional later with the disappearance of velum. 2-4 gill filaments are seen in this stage.

**Plantigrade:** Plantigrade is reached on 20<sup>th</sup> day when the larvae measure 220 x 200µm. Labial palps and additional gill filaments develop. Shell growth is by the formation of a very thin, transparent, uniform conchiolin film around the globular shell margin except in the vertex of the umbo region. This is the beginning of the formation of the adult shell or the dissoconch.

**Spat:** The plantigrade transforms to a young spat. The hinge line, anterior and posterior auricles and the byssal notch assume specific shape. The left valve is slightly more concave than the right one. The spat attaches with the substratum with byssal thread. This stage is attained on 24<sup>th</sup> day and the size is 300µm.

### Algal culture

Flagellates measuring less than 10µm are the ideal food for pearl oyster larvae. The haptophycean flagellate *Isochrysis galbana* is the most suited food and measures 7µm. Apart from this, *Pavlova*, *Chromulina* and *Dicrateria spp* have also been found to be good larval feed.

### Feed volume estimate

Depending on the stocking density and stage of the larvae stocked in the larval rearing tanks the feed volume is worked out following the formulae.

$$\frac{\text{No. of larvae} \times \text{feed rate (cells/larvae)}}{\text{Cell concentration of algal culture}} = \text{ml}$$

### Larval rearing

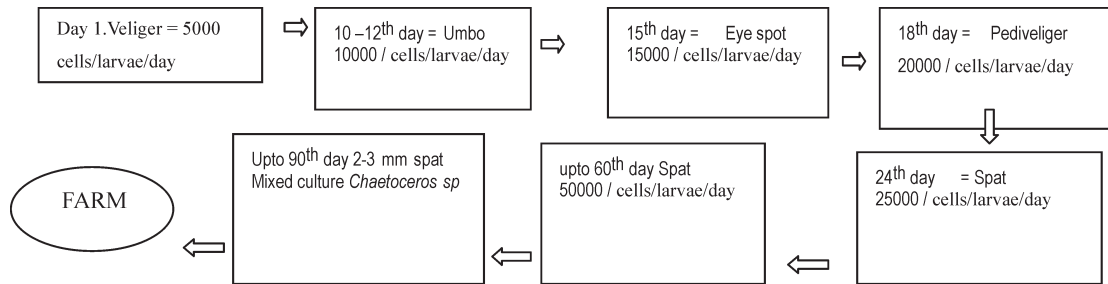
**Water exchange:** During the course of larval rearing complete (100%) water exchange has to be done on alternate days. The larva in the tanks is drained through appropriate sieves made of nylobolt cloth of various mesh

sizes to a container with filtered seawater. Later the tanks are cleaned and fresh filtered seawater is filled and the larvae transferred.

### Larval density estimate

No. of larvae in sub sample X Volume of the sample = Larval density

### Feeding protocol



Microbial contamination can be controlled by adding Chloromphenical at 200mg/ton of water. A well managed hatchery can yield up to 10% settlement of initial stocking in the rearing tanks.

### b) Pearl oyster farming

Farming of pearl oyster is essentially for meeting two demands i.e., 1) to grow the wild collected/hatchery produced spats to implantable sized oyster, 2) to grow the nucleated oysters for pearl production.

#### Oyster sources

The basic stock for the farming operations is either collected from the natural beds or from the hatchery. There are about 65 pearl beds have been identified in the Gulf of Mannar area off Tuticorin at a distance of 12-20 km at depths ranging from 15-20 m. The availability of the oysters in these beds is erratic due to the irregular spat settlement. Pearl oyster spats can also be collected to smaller extent by suspending various kinds of spat collectors for farming purposes. However, such natural collections may be of different species composition, which some time may not be useful in production of pearls. Hence, an established hatchery could be a sustained source for spats for culture operations on continuous basis.

#### Site selection criteria

Selection of farm site is of a paramount importance. The selection would be based on the biology of the organism farmed and the ambience of the prevailing environmental parameters of the site. Congenial conditions such as protection, water current, clarity, optimum temperature and salinity regimens are to be considered while selecting the farm site, apart from the site being free from any kind of pollution. A deep sheltered bay/protected water bodies with sea conditions not going too rough offers an excellent site for pearl oyster farming.

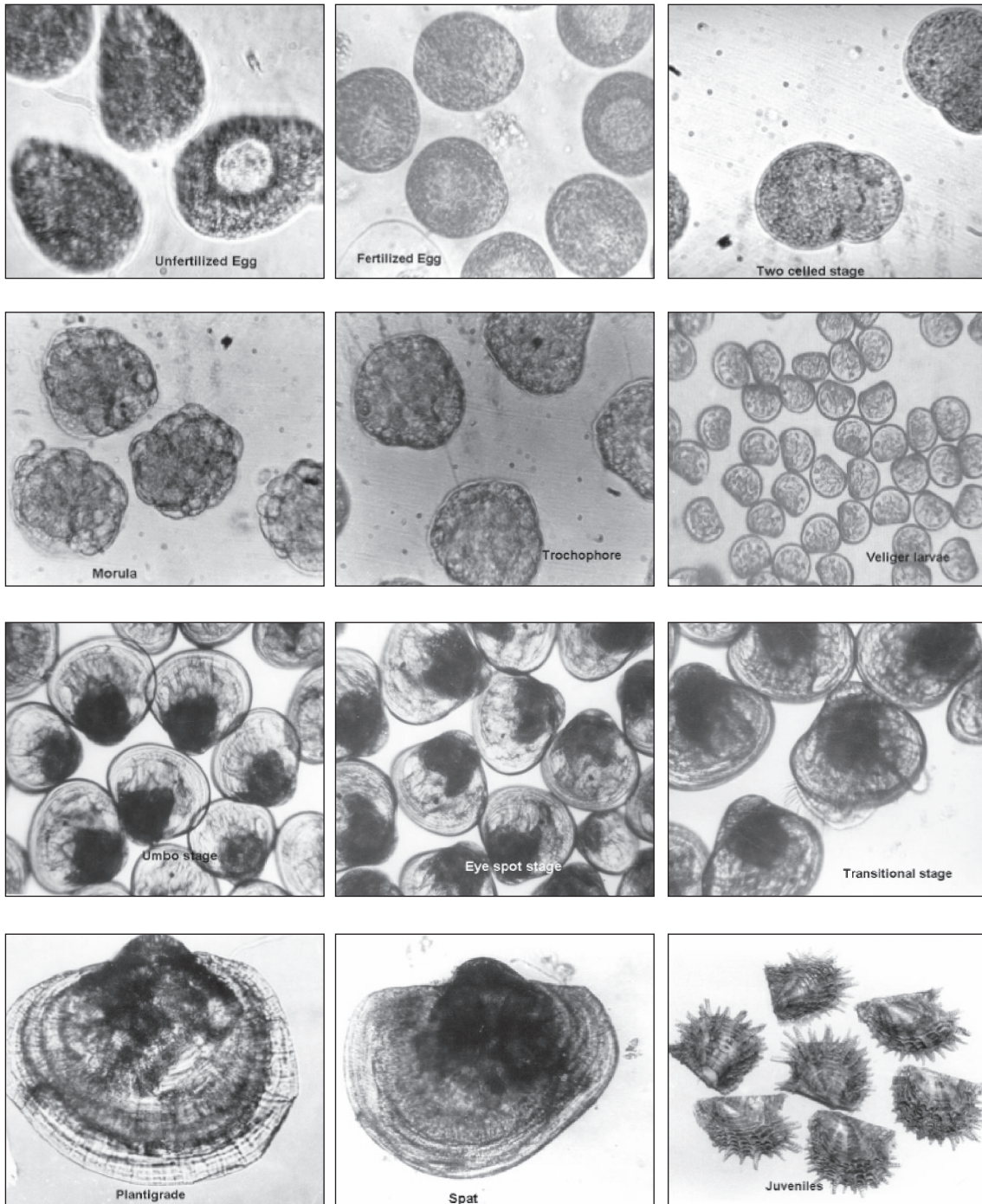
#### Farming Systems

In India, the farming practices of pearl oyster is very much limited to the two Gulf zones i.e., Gulf of Kutch in Gujarat and Gulf of Mannar in Tamil Nadu. The method of farming varies to the prevailing farm site conditions. Accordingly the following methods are developed and practiced.

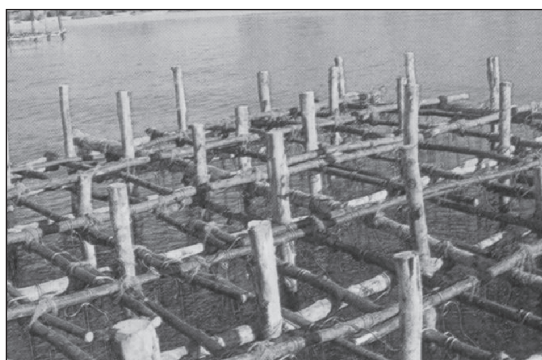
#### Rack culture system

Rack culture system is very much suitable for areas of shallow depths ranging from 2-5 m. In such shallow areas either casuarinas or teak poles of 4-5" thickness are driven vertically on the sea bottom. These vertical poles

Developmental stages of pearl oyster Pinctada fucata



**Wooden Racks with oyster cages in shallow water**



**Raft culture system**



are interconnected with similar poles horizontally and lashed with coir ropes. The horizontal poles are so arranged in a way that they are just above the highest high tide level. Driving GI pipes of 4" dia on the periphery of the rack can provide reinforcement to the racks. Generally the racks are rectangular in shape. A working platform at few places of the rack can also be provided by 6-8" wooden planks. A rack of 30 sqm can hold about 100 cages.

#### **Raft culture system**

This is a most suitable method to farm pearl oysters in the sheltered bays with considerable depth. Rafts are constructed with teak wood poles of 4-5" thickness or sliced timber of similar thickness. The teak wood poles are lashed with coir ropes and the sliced timber poles can be fixed with steel bolt and nut. Rafts are generally almost square in shape. Such constructed rafts are floated with the help of 4 buoys and moored by 2 anchors of 30-40kg weight. Appropriate anchor chains are provided after considering the depth of the area. Empty oil drums coated with fiberglass, mild steel barrels and FRP/Styrofoam floatation buoys are few alternates to serve as floats for a raft.

#### **Long line culture system**

Long line culture system is practiced in the open sea where the depth is more. This is more suitable to withstand the high wind and wave action. Long line is primarily constructed with a long synthetic rope (15-20 mm) with two main floats on both the ends. Depending on the length of the long line smaller floats is also attached to the mainline at 5 m interval. Vertical lines are arranged in 1- 2 m apart from the horizontal main line. Such line is moored in appropriate site with the help of 2 anchors. A series of such long lines are arranged horizontally.

#### **On shore culture system**

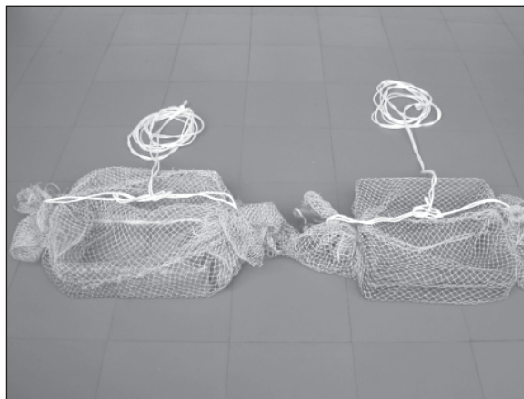
This system is of recent origin. Seeds of pearl oysters are reared in cement tanks of size ranging from 250-500 sqm. A water level of 1 m is maintained. Oysters are held above the bottom, through a grid system constructed using PVC pipes. A stocking density of 125nos/sqm is maintained. Raw seawater is pumped into the settlement tank and then to the rearing tanks. Stocked oysters are supplemented with mixed culture of *Chaetoceros spp.* Cell concentration in the rearing medium is maintained at 75,000 cells/l in the tank. Daily 25% water exchange is required. By this method an average growth of 50 mm in 6-7 months from a stocking size of 5.0 mm is achieved.

#### **Culture containers**

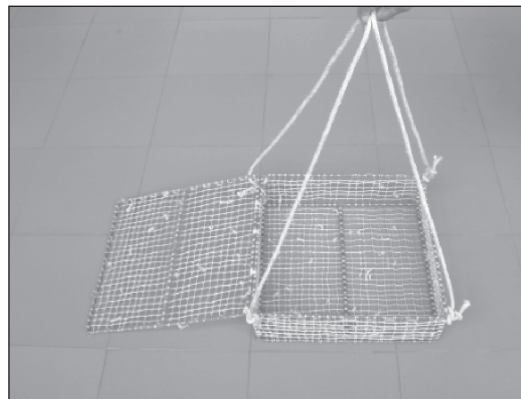
##### **Spat transplantation cages**

This type of cages is fabricated using the box cage iron frames. Suitable sized velon screen (0.5-1mm mesh) bags are prepared and the cage frame is inserted inside and both the ends are tied. Old fish net (10mm mesh) is stitched as bag and rolled over the velon bag. This provides the extra strength to the spat cages. A central rope is provided and folded in the center so that the cages are hung horizontally in the culture structures. A stocking density

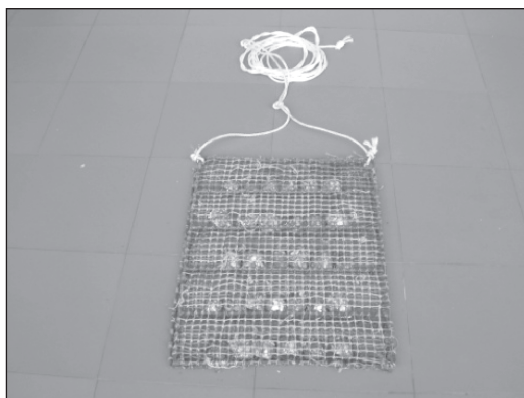
**Spat transplantation cages**



**Box cages**



**Book type cages**



**Lantern cages**



of about 1000 nos/cage (3.0mm spats) can be maintained for short period. The small meshed velon screen bag has to be replaced with large meshed velon screen bags every 15 days as the spat grows. Clogging takes place very quickly in these cages and hence periodical cleaning is very essential to avoid mortality. Culling has to be done as the spat grows.

### **Box cages**

Oysters of above 30 mm are reared in Box cages. Box cages are made of 6mm steel rods. Square in shape and measures 40x40x10cm size with lid. Frames are covered with suitable sized old fishnets and stitched. Suspense ropes are provided for tying to the culture structures. 150-200 nos of oysters of 30mm can be reared in such cages.

### **Book type cages**

Book type cages are made of two rectangular frames 75x50cm stitched with required size netting. Many sub sections are provided to hold oysters in series. Meshed and hinged at one end, open as a book. The arranged oysters are held in the compartments when the cage is closed. The space between the frames is 10mm and sufficient for the oyster to open the shells while feeding. These cages are designed for holding oysters for experimentation. About 75 oysters can be held in each cage.

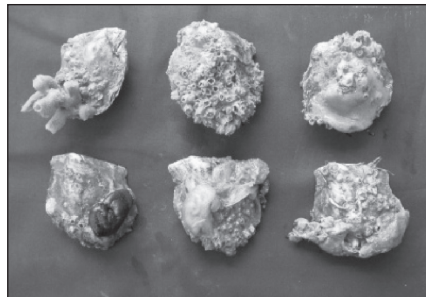


### Lantern cages

Lantern cages are fabricated using circular iron frames of 4 - 6 mm rod arranged in 2-3 layers. Plastic sheath can be provided over the iron rod for longer life of the cages. Appropriate sized mesh is stitched over the frame. An opening is given at each layer to handle the stocked oysters. The cages are vertically hung in the culture structures.

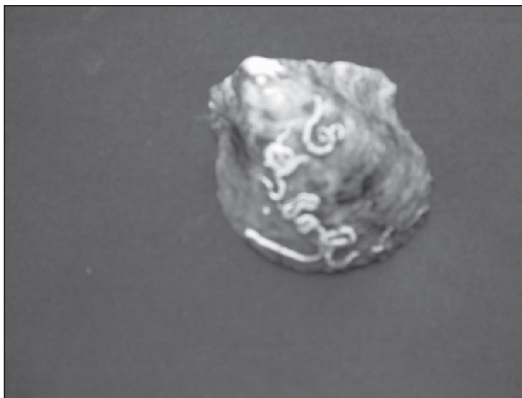
### Farm management

Major problems in the maintenance of pearl oyster stocks in the farm are the biofouling organisms. These organisms settle and grow on the shells, the boring organisms riddle through the shells and render them weak and

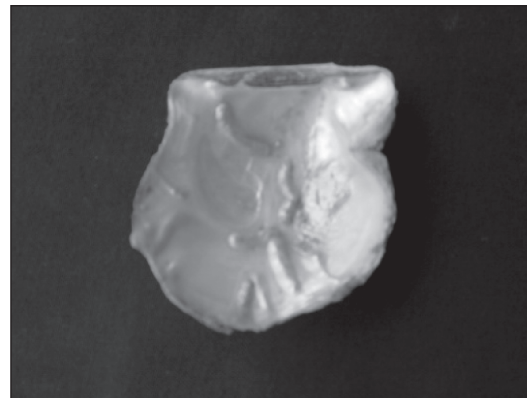


Fouled pearl oysters by Sponges, barnacles, ascidians single and compound

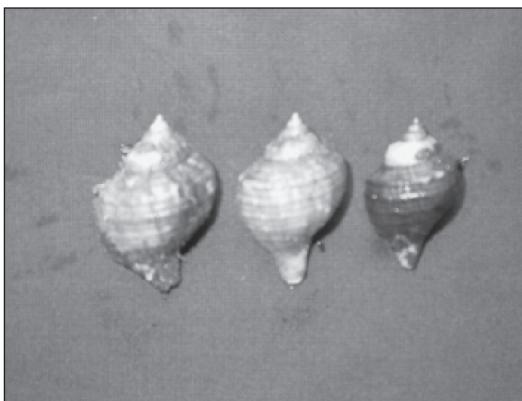
Boring Polychaete worm



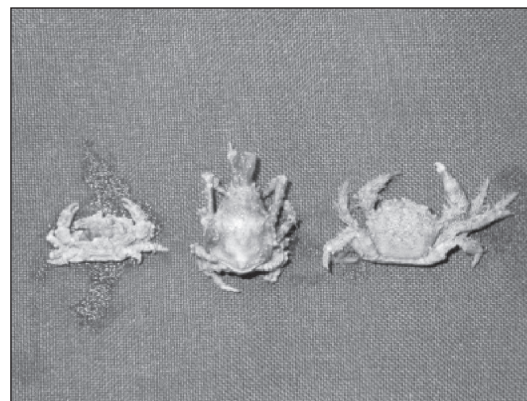
Extensive blisters - nacreous layer on pearl oyster



Cymatium spp. predators



Few important Crabs



fragile to predators. These factors either singly or combinedly can affect the farmed stocks or result in growth retardation and mortality.

### Fouling

The most dominant fouling organisms in pearl oyster farming is barnacles (*Balanus amphitrite*), bryozoans (*Membranifora* sp, *Thalamoporella* sp and *Lagenipora* sp.). Simple ascidians *Ascidia depressiuscula* and *Dicarpa* sp and compound ascidians *Diplosoma* sp and species of *Botrilloides* were found to settle and encrust upon the oysters. The profuse growth of sponges *Callyspongia fibrosa* and *Haliclona exigua* results in complete covering of an oyster or a cluster of oysters. However, the frequency of occurrence of this kind of foulers is very less.

### Boring

The dominant groups of boring organisms are the sponges and polychaetes. *Polydora ciliata*, *Polydora flava* and *Cirratulus cirratus* are the major borers and causes mortality. Cirratulid worms inhabited the pearl oyster shell in between the layers of periostracum. As a result the furrow becomes deeper by the accumulation of mud and thus causing peeling of periostracum making the shell weak. Members of the family Syllidae, Nereidae and Terebellidae burrow through the shells and causes extensive blisters in the nacreous layers. *Cliona vastifica* and *C. celata* form honey comb like ramifications in the shell with numerous openings on the nacreous surface. Such affected oysters suffer great physiological strain in repairing the shell and latter leading to mortality. Boring mollusks such as *Lithophaga* sp, and *Martesia* sp which make holes on the shells are also occasionally found on the oysters.

### Predators

The gastropods *Murex virgineus* and *Cymatium singulatum* are the most important predators on the farmed oysters. They can consume at a rate of one oyster/day/animal. Crab species like *Charybdis lucifera* and *Atergatis integerrimus* also prey upon the oysters in the farm. Sometime *Xancus* sp are also found to prey on oysters in the cages. The boring and fouling organisms can be killed by applying a number of chemicals in appropriate dosages on the individual oysters. However, in the farming conditions the best method of controlling the boring and fouling organisms on the farmed oyster is thorough periodic cleaning by scrapping off the borers and foulers. Predatory molluscs in the cages can be picked and killed. By following the judicious choice of depths for growing oysters can also avoid such problem, as the fouling in deeper waters is relatively less.

### Conclusion

Thus farming of pearl oyster is a laborious job. Appropriate selection of site, periodical attention on the farmed oysters would yield better growth and production of mother oyster. Similar attention on the nucleated oysters would enhance the rate of pearl production and to some extent pearl quality. In an ideal farm, the oysters show an average growth rate of 5-6 mm in the spat stage till it attains the juvenile stage. Further rearing would result in a growth rate of about 4 mm /month. An average of 30% survival is obtained from the spat stage to mother oyster. In an unattended farm, during the spat stage complete mortality of the transplanted spats could happen. Repeated farming in the same area is not advisable for pearl production, as it would deteriorate the culture site.

