Bivalve Seed Production

Anil M.K, Gomathi P, Raju B, Krishnapriya P.M Shalini O. Sharanya M P

Vizhinjam RC of ICAR-Central Marine Fisheries Research Institute
mkanil65@gmail.com

The mussels are bivalve molluscs found attached to the hard surfaces in the littoral and sublittoral zones. They attach themselves to the substrate by secreting long threads called byssus. Though they are considered sedentary, they may move from one area to another if faced with unfavourable environmental conditions. Mussels are regarded as one of the best candidates for aquaculture since they are filter feeders feeding at the lowest level in the food chain (primary consumers); they feed on phytoplankton, detritus and associated microscopic flora and fauna. The two important species of mussels in India are the Green mussel *Perna viridis*(Peacock mussel/MayilpeeliKakka/Kallummekaya) and the Brown mussel *Perna indica* (Red chippi). The green mussel has a wider distribution along the west and east coasts of India, including the Andaman Islands. In contrast, the brown mussel is restricted to the southwest coast of India. Along the east coast, the green mussel is found in Chilka Lake, Kakinada, Madras, Pondicherry, Cuddalore and Porto Novo and along the west coast it forms on extensive beds from Muthalappuzha estuary in Trivandrum, Quilon, Alleppey, Cochin, Calicut to Kasargod, Mangalore, Karwar, Goa, Bhatia Creek, Malvan and the Gulf of Kutch (CMFRI, 2000). Now fishery for the green mussel exists in the region from Kollam to Kasargod and for brown mussel from Quilon to Kanyakumari along the Kerala Coast.

Mussel provides animal protein of high nutritional value. Fast growth rate, adaptability to varying environmental conditions such as short periods of exposure to extreme temperatures, salinities, desiccation, relatively high levels of turbidity and simple culture technique make it as a candidate species for aquaculture in coastal waters.

Difference between green (*Perna viridis)* and brown mussel (*Perna indica)*

Brown mussel is dark brown, and a small percentage has a greenish tinge (parrot mussel); its ventral margin is straight, and the dorsal profile has a distinct angle or hump. The hinge area is pointed and straight and has only a single tooth on the left valve.

The Indian peacock mussel or green mussel has a bright peacock blue-green colour during the juvenile and adult stages. It gradually turns to dark green or black dorsally, and the concavity of the ventral margin increases with age; the hinge region or beak is pointed down and has two hinge teeth on the left valve and one on the right.

Seed Production

A primary requisite in any farming operation is an abundant, reliable and inexpensive supply of juveniles (seed). At present, most bivalve culture operations in the world are moving to hatchery-produced seed rather than obtaining their seed by collecting from natural sites. The natural seed is collected by keeping substrate or spat collection ropes in breeding areas to collect metamorphosing larvae, or the juveniles are collected are transferred to growing areas for culture (grow-out) to market size. In other operations, juveniles are gathered from areas of natural abundance and are transported to growing fields that may be distant from the source of the seed. The alternative to the collection of the natural spat of bivalves is to produce seed in the hatchery. The uncertainty in the availability of natural spat in quality and quantity has led to the stagnation of mussel farming in Kerala, which showed fast development in the early years of the last decade. And this has prompted CMFRI to develop hatchery technology for bivalves. The hatchery must be located close to the sea where pollution-free seawater of desired salinity is available throughout the year. Preferably an area where adult and mature mussel of the required size is available.

Water Intake and treatment system

Water is pumped directly from the sea through in situ filters which is first filtered using slow sand filters that filter out most particulate material greater than 20-40µ. A slow sand filter consists of a tank inside of which lies a bed of sand supported by gravel, lying on a suitable under-drainage floor. Water is allowed to flow through this layer of sand with particles of varying sizes and depth. The layer is not dense but contains a number of channels and holes created between the particles that constitute filter medium. When water passes through the filter medium, particles larger than a specific size will be trapped in the medium and get filtered.

Water filtered through the slow sand filter will be collected in a water storage sump and treated with chlorine to remove the microbial load and after de-chlorination again subjected to filtration using rapid sand filter to remove minute particles and stored in overhead tank so that the effect of gravity maintains a sufficient water flow through various units of the hatchery. Before utilizing the water for various hatchery purposes final sterilization is achieved by UV irradiation. Sea water intended for stock culture of algae will be further sterilized by ozone treatment to achieve 100% disinfection, which is highly essential for maintaining the pure culture of desirable species of algae for feeding bivalve larvae.

Procurement of broodstock:

In mussels, the sexes are separate, and they attain sexual maturity within a year or less than one year. The mature broodstock having a minimum size of 6-7 cm size is collected

from the wild, quarantined and maintained primarily in the broodstock holding tank of 1-ton capacity at a density of 3-4 g/l of its live weight. The water in the tank is replaced daily to avoid build-up of bacteria and metabolic waste before feeding during the morning and provided with *Isochrysis sp*. and *Chaetoceros sp.* cells @ 5-6 million/ml. Around 60-80 l of algal culture per tank is used to feed daily. If sufficiently mature brood-mussels are available, they can be directly used for spawning or kept under low-temperature recirculation system for a long time.

Maturation

Maturation of broodstock is done in FRP tank of 1-ton capacity which has special provision for photoperiod adjustment and heat and cold water facilities. Adult male and female mussels are placed in the tank at a density of 3-4 g/l of the total live weight biomass for gonadal maturation by adjusting the photoperiod (12 hr light and 12 hr dark) and maintaining the water temperature between 20-26^{o}C.It is fed with *Isochrysis galbana* and *Chaetoceros sp* cells @ 7 million/ml. Algal culture of 80-100 l per tank is used for daily feeding.

Spawning

The mussel can be easily stimulated to spawn in a hatchery if they are fully mature with turgid gonad. In mussel, sexes are separate, and the reproductive condition of broodstock is determined by visual examination of the gonad which includes the assessment of the physical extent, fullness and colour of gonad and the degree to which it is filled with gametes. The testis is creamy white in colour while that of the ovary is orange or reddish. During spawning, mussel loses up to one-third of its body weight. Spawning of the mature brooders can be carried out in spawning tank of 200-500 l capacity or trays at a density of 3-10 numbers. A rise in 4-8^{0}C above the ambient temperature depending on the ambient temperature induces spawning of green mussel. Millions of eggs are freely released by the female into the water are fertilized simultaneously by the sperms of males, and the eggs settle down. Usually, the fecundity of adult mussel is 5-20 million, and the hatching rate from egg to larvae is 95 %.

Incubation

After spawning, the adults are removed from the spawning tank, and the fertilized eggs are collected and rinsed by pouring onto a 20µ sieve held in a basin of filtered seawater to remove the excess sperm, unfertilized eggs and metabolic waste. It is then incubated at a density up to 500-1000 no./ml in a glass tank (200 l) or FRP tank (0.5-1 t) provided with gentle aeration. The fertilized egg starts cell division in 20 min, divides repeatedly and hatches-out into morula larva. After hatching, the embryos are passed through a 100-150µ mesh screen suspended in the tank to remove larger debris. Optimum salinity is 25-35 ppt and temperature is 24-27 ^{0}C.

The morula exhibits phototropism and swims and congregates at the surface. After 5 hr of fertilization, it gets transformed to blastula by the reorientation of the cells. The cells

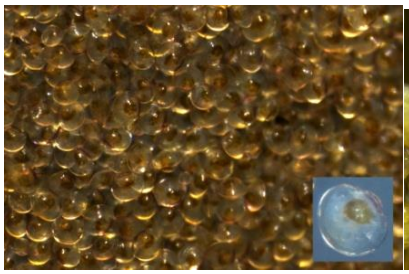
then convolute in and form dermal layers and gastrula stage formed within 6-7 hr after fertilization.

Gastrula stage transforms into trochophore within 7-8 hr by developing a long single flagellum and tuft of cilia at the apical side and the rear side and swim with the flagellum. The ectodermal cells of trochophore secrete embryonic shell material and assume a 'D' shaped veliger or straight hinge stage by 18-20 hr at 27ºC in which the flagellum and tufts of cilia disappear, and a new locomotory organ called velum develops. The early embryonic development of the larva is completed by veliger stage which measures 50-55µ dorso-ventrally.

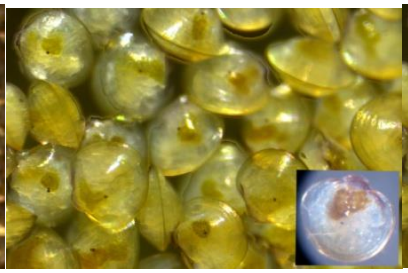
Spawning of *P. viridis*

Fertilized egg

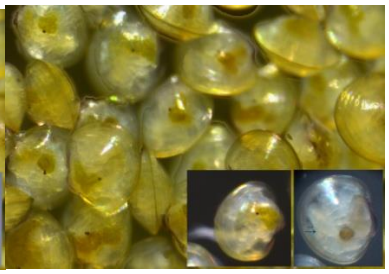
D-veliger stage



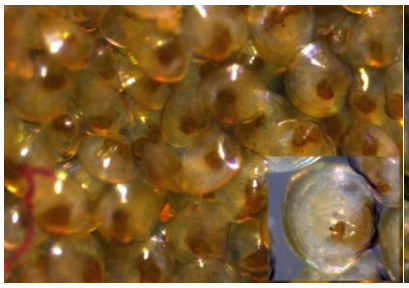
Umbo stage



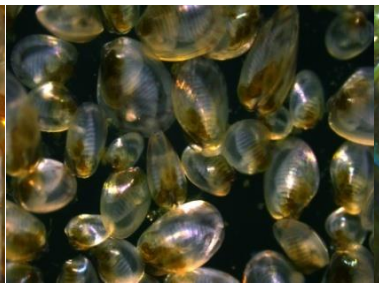
Eye-spot stage larvae



Pediveliger stage



Plantigrade stage



Spat (21st day)

42-day old *P.viridis* spat

Rearing of larvae

The D-veliger larvae are transferred into the FRP larval rearing tank (2-10 t capacity) for rearing till the settlement (or transferred at eyespot stage to a downwelling system). Washing, grading, counting and measuring the larvae is done every alternate day. In 18-20 hours, larvae attain the D-shape veliger stage, the early veliger larval stage of bivalves, also known as straight-hinge larva. D veliger larvae are filtered out from the incubation tank with 40µ mesh. The mesh should be kept immersed in a tray containing seawater so that larvae will not get exposed or dehydrated without seawater. Filtered larvae are transferred to a beaker of known volume. After thoroughly mixing sub-samples, 1ml is taken and counted under a microscope to estimate the total number. A drop of 10% NBF can be used to

immobilise the larvae. Larvae in D shape are counted and stocked in FRP Larval Rearing Tank (LRT) of 2-ton to 5-ton capacity. Larvae were stocked @ 0.05 to 0.1no/ml.

The veliger metamorphosed into umbo stage (130-260 µ) within 7-15 day in which shell valves are equal, and mantle folds develop. The umbo stage reaches the eyespot stage (260-367µ) within 14-17th day when the blackspot is seen at the base of foot bud with the development of ctenidial edges. Development of foot is observed on 17-19 days indicating the pediveliger stage with the appearance of gill filaments. The larvae are fed with mixed algal diet consisting of *Isochrysis galbana, Nanochloropsis oculata and Pavlova sp.*

Larvae need to be washed thoroughly and shifted to a new tank or restocked after thoroughly cleaning the tank. Samples are observed under a microscope to check the quality of the larvae, and measurements such as Dorso ventral measurement (DVM)/anterior-posterior measurement (APM) of 10-15 larvae are taken from each stage. Survival and mortality percentages are calculated. Large larvae are segregated and stocked in different tanks when a size difference is observed. Small size, weaker larvae are discarded. Feeding rates are based on larval count and size. When more than 50% of larvae reach the eye-spot stage, the larvae can be shifted to the down-weller system of the micro-nursery or reared in the same tank till settlement or further. Instead of settling the larvae in the tank, they must be raised in the micro nursery, which is ten times more efficient than rearing the larvae in the tank.

Nursery rearing

The nursery rearing of mussels can be done using a micro-nursery system, micron mesh cages and an integrated multitrophic aquaculture system.

Micro-nursery system

Micro-nursery system consists of down-welling and upwelling sub-systems, each with separate reservoir tanks and pumps for water circulation. In the down-welling system eyed-stage, larvae of mussels, oysters or clams can be stocked at high-density for settlement and further growth. When the settled spat reaches 2 mm size, it can be transferred to the upwelling system for further rearing.

Down-welling system is of 2000 litre capacity divided into four compartments of equal size. Each compartment has eight PVC wells of 30 cm diameter and 25 cm height, totalling 32 wells in the three compartments. Wells are provided with an airlift mechanism for pumping water to the well from the compartment. The bottom of the well is covered by a mesh cloth of 150; as growth proceeds, the spat is transferred to 250µ, 500 µ and 1mm. Through all 32 wells, water passes from the surface to the bottom through the mesh (down-welling) to the compartment.

Eyed-stage bivalve larvae can be transferred directly to down-welling wells with 150-micron mesh at the rate of 3 to 3.5 lakh larvae per well. The eyed stage will metamorphose and settle in the wells and can be grown to 2mm size by changing to 250µ, 500 microns and

1mm wells after 6 and 12 days after stocking and during the period, stocking density is gradually reduced to 1 lakh. On the 30th day, transfer the spat to the Up-welling system or other nursery system described below.

The upwelling system has a total 1500 litre volume and two raceway compartments. Each compartment with eight wells provided a bottom mesh of 1 and 2 mm in size. Here spat can be grown from 2 mm to a seed size of 10 mm in 40 days. Water flows from these compartments up through the mesh upwards (upwelling) in the wells to the middle drainage section through a half-inch pipe and from where water is drained back to the reservoir. The stocking rate in the upwelling wells is from 50000-100000, depending on size. Seawater with required feed is circulated through the systems from the reservoirs (500 litres each) by two dedicated pumps of 0.2 hp. The required quantity of the feed, proportional to the stocking density and size of the spat, is directly poured into the reservoirs of upwelling and downwelling systems or pumped to the system using an adjustable peristaltic pump with a timer. In the micro nursery, usually, the feed given is the ratio of 3:1:1:1*Chaetoceros calcitrans*: *Isochrysis galbana: Nanochloropsis salina, Tetraselmis.*

Micro-nursery system

About 0.05 million spat per well can be nursery reared to seed size 17-20 mm in 60 days. On the other hand, spat reared in hatchery tanks, even at low density, shows only limited growth. Seed grown in the nursery cages can be used for seeding ropes or on-bottom farm nurseries for further rearing. Every alternate day the wells are cleaned with seawater using a spray nozzle connected to a 0.5 hp pump. Water is fully drained from the compartments and reservoirs and refilled with fresh seawater to remove all accumulated waste materials. The nursery rearing of mussel spat can be done in micron-meshes cages or multitrophic hybrid systems.

Nursery rearing in multitrophic hybrid systems

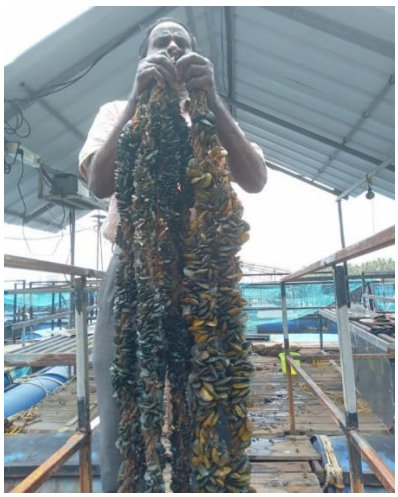
Mussels spat of 1mm size can be nursery-reared successfully in a multitrophic hybrid biofloc system that incorporates the benefits of Integrated multitrophic aquaculture (IMTA), Biofloc Technology and Recirculating Aquaculture Systems with apparent advantages such as reduced environmental impact, higher production potential from limited land and water usage and sustainability. The main objective is nursery rearing of bivalve spat along with biofloc farming of white leg shrimp (*Litopenaeus vannamei*), which is based on the principle of IMTA where we use two or more species belonging to different trophic levels where one organism utilises metabolic wastes of the other species as a source of energy.

Seed raised in silo

Seed grown in ring net

The seed produced from the system can be seeded on ropes, can be kept for a week in the system for attachment, and sold as seeded ropes. Seeded ropes are taken to the backwater farms and tested in floating rafts and have shown good growth and survival.

Seeded ropes



Growth of seeded strings in farmers' field

There is a high demand for mussel seed as the quantity of seed available from the wild is erratic, and most of the time, it reaches the farmer in low quality. For successful farming, mussel seeds of the required quality and quantity can be made available using the present technology.

Edible oyster- Seed Production Technology

Edible oysters are the most widely cultivated bivalves in the world. The edible oyster is popularly known as “Kadal Muringa” in Malayalam is a sedentary bivalve. It grows attached to the substratum by the left valve (lower valve) and the right valve (upper valve) act as a lid. Oysters occur naturally in estuarine water with salinities of about 10-25ppt, though it can tolerate higher salinity. It forms dense aggregations, often called as reefs or beds. Edible oysters are permanently attached to the hard substratum in the intertidal areas, backwaters, muddy bays, lagoons, and creeks. The coastline of Kerala is endowed with a large number of

backwaters, estuaries and brackish water lakes and edible oysters are regularly exploited from these waters.

In India there are four species of edible oysters have commercial value.

Sl. No	Scientific name	Common name	Distribution
1	*Crossostrea madrasensis*	Indian back water oyster	Orissa, Andhra Pradesh, Tamil Nadu and Kerala. It also occurs in Andaman Islands at Port Blair, Havelock Island, Maya bander and Diglipur
2	*C.gryphoides*	West coast oyster	Northern Karnataka, Goa, Maharashtra
3.	*C.rivularis*	Chinese oyster	Gujarat
4	*Saccostrea cucullata*	Indian Rock oyster	Distributed all along the coast, including Andaman, Laccadives

Crossostrea madrasensis is the most dominant species and commonly called as Indian backwater oyster. Oysters of this species have irregularly shaped shell valves. The left valve is deep while the right one is slightly concave. Abductor muscle kidney-shaped and the shell has a dark purple coloured aductor scar. The inner surface of the shell valve is glassy and white.

Edible oyster fishery forms the 2nd important component of bivalve fishery after edible clam. The flesh of oyster is highly nutritious containing 8-10% protein and 2% fat, in addition to minerals like calcium, phosphorus, zinc and iodine.

Indian edible oyster *Crossostrea madrasensis*

Oysters are filter feeders, and they feed on phytoplankton, detritus and associated microscopic flora and fauna in the natural condition, whereas in captivity oysters are provided with a mixed culture of microalgae in different cell concentrations. The spawning season of oyster in the wild varies on East and West coast of India. In East coast, it reported to be on Feb-April and in West coast of India it is in November- February.

Though the complete package of seed production and oyster farming technology has been developed by CMFRI, oyster culture is not yet developed in India due to lack of awareness regarding the nutritional quality, non-availability of seed and lack of entrepreneurship. However, it is one of the most preferred seafood items in Europe, USA and many south-east Asian countries and there is immense scope for export if produced in sizable quantities.

Hatchery techniques

Throughout the world, source of seed is changing from natural spat collection which formed the basis of most oyster culture industries in the past to hatchery-produced triploid oyster spat. Cost of hatchery produced seed is expensive than wild-collected ones. But natural spat-fall is unpredictable and low in quality and quantity. Advantages of hatchery production of the oyster seed is that desirable stage (eyespot stage/spat) and the required number and quality of larvae throughout the year could be guaranteed.

Broodstock collection and conditioning

Adults oysters collected from the wild are brought into the hatchery, and their shells are thoroughly scrubbed and rinsed to remove epifaunal (fouling) organisms and sediments. After that these brooders are rinsed with fresh water followed by 10 ppm chlorinated seawater and placed in broodstock conditioning tank.

Broodstock conditioning tank should always be kept separate to prevent the transfer of pathogens and parasites to the culture system and also without disturbance. Only 5 kg of live weight can be stocked in 120-150 LFRP tank. Effluent water discharged from wild-collected animals should be treated with more than 100 mg/ L free chlorine or Ozone for a minimum period of 24 hrs before releasing if oysters and brought from faraway places or contries.

Oysters usually mature in one year age. In oysters sexes are separate; occasional hermaphroditism is also reported. Males are smaller than females; in oysters zero year class (length up to 78 mm): 75 % presumed to be males and one year and above (length range of 80-120mm): 72% to be females. Oysters of more than 3 inch (76 mm) size are selected for breeding. (Richard *et al*,2008). Oysters of length ranging from 60-120 mm are ideal, and 30% of them should be 60-75mm to have assured availability of males.

Selected broodstocks were fed with mixed algal culture diet of *Isocrysis galbana* and *Chetoceros calcitrans and Pavlova sp*. Feeding schedule for most warm water bivalves are the same and is given in the Mussel culture chapter. Mature females will have creamy white gonad whereas males will have white gonad with oozing milt. Maturity is checked by taking smear from the gonad examining under microscope. Mature eggs are pear-shaped 48-62µ size.

Spawning

In India, induced spawning is mostly achieved by thermal stimulation. For which 20-25 numbers of oysters are selected and kept in seawater water with aeration in air-conditioned room at 23˚C for 12 hr followed by transfer them into FRP tank (1-ton capacity) at 30-32˚C. The water temperature is usually raised with the help of immersion water heater. Mild aeration is provided in the tank. Sudden rise in the water temperature induces the oysters to spawn. Chemical stimulation is another method where ammonium hydroxide, sodium hydroxide/tris-buffer is added to the broodstock kept in a tank, but here viability of

eggs be less. In another method, freshly stripped sperm is added to the broodstock tank, which in turn induces the female to release eggs. Among these, thermal stimulation offers less stress to the animal. A fully ripe animal may spawn just due to handling stress while cleaning and may not require any induction.

Generally, male oyster responds within 1-2 hr and releases sperm as a continuous stream of milky fluid whereas after15-60 min, female releases eggs into the surrounding water with periodic shell closures; where fertilization takes place externally in water. The eggs will settle down to the tank bottom. After spawning it is necessary to remove the females from the spawning tank to prevent accidental filter-feeding of eggs by themselves. Excess sperm in spawning tanks can cause abnormal fertilization of the eggs so it can be avoided by removing surface water with sperm replacing with fresh seawater. If an adult doesn't respond within the given period, it should be returned to the conditioning tank for further one week. The salinity of seawater should be in the range 32-35 ppt and pH is 8-8.4.

Oyster spawning in progress (release of milky fluid)

Initially, eggs of oyster are pear-shaped which measures 48-62µ in diameter and become spherical in shape after water hardening. Eggs that do not round-off after 15-20 min should be discarded. When the fertilized eggs settle at the bottom, aeration is suspended. It is then siphoned and filtered through 90µ mesh to remove the metabolic waste of adults from the egg. Then eggs are filtered-out with 20 µ mesh and washed with fresh seawater. Cleaned eggs are transferred to 10 l container. Eggs are gently mixed, taken 1 ml of sample is taken with a pipette and placed on Sedgwick-Rafter cell for counting the number of fertilized eggs. Usually, the fecundity of oyster is 20 million, and the survival rate from egg to larvae is 50%.

Incubation

FRP tank of 1 ton is cleaned, disinfected by chlorination, filled with filtered seawater and stocked with fertilized eggs at a stocking density of 500-1000 no./ml, and the tank is aerated gently. The first polar body is formed after 20-40 min of fertilization. Fertilized egg undergoes cleavage within 45 min and reaches morula stage after 6th division. Gastrula stage is reached between 5-6 h after fertilization

Rearing of larvae:

D shell or Straight hinge larval stage

D shell or straight hinge larval stage is reached after 20 hrs. Larvae are transparent, swim vigorously and measured 66µ (average size). Water is drained slowly from the incubation tank through 40µ size sieve (kept partially immersed in seawater trough to avoid dry filtration). The larvae retained in the sieve are transferred to a beaker of a known volume

of treated seawater (e.g., 10 L). One ml samples are taken, the larvae are counted in a Sedge wick-Rafter cell. The formula for calculating the total number of larvae is given below.

$$\text{Total number of larvae} = \frac{\text{Av. no. of larvae in subsample X total volume (ml)}}{\text{Volume of subsample(ml)}}$$

The counted larvae are stocked in the larval rearing tanks of 1-2 ton capacity (cleaned and disinfected tank filled with treated seawater) at the density of 5-10 no./ml. Mild aeration is provided. Larvae are fed with culture of *Isochrysis galbana.* Every alternate day process of filtering and cleaning of the tank is repeated till the settlement; sometimes larvae are transferred to a cleaned and dried new tank.

Umbo stage: On the third day, the larvae appear slightly oval (100 µ size) and reach the early umbo stage. Second sieving is also done using 40µ mesh. On the seventh day, umbo will have concentric rings on the shell. Between 12-15 days, the larvae will reach late umbo stage and measure 150 µ size.

Eyed stage: Eyespot develops between 13- 17 days larval rearing and larvae measure 280 µ in size. From D shape larvae to eyespot larvae 40 µ mesh is used for filtration. From eye spot onwards 150 µ mesh is used for filtration.

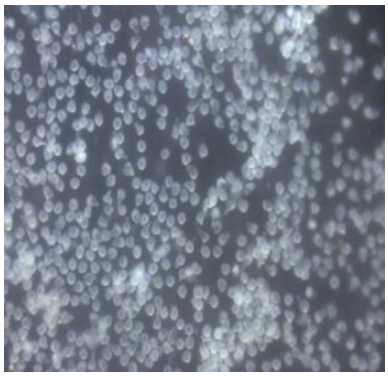
Pediveliger stage: Larvae reach pediveliger stage between 14-18th day; a functional foot develops and could be seen. Larvae measure 330-350 µ in size.

Spat: Once the pediveliger larvae lose its velum, it will start settling down, and larvae will develop adult features and metamorphose into a spat. The larvae are fed with mixed algal diet consisting of *Chaetoceros calcitrans*, *Isochrysis galbana*, *Pavlova* spp., and *Nannochloropsis* spp. The feeding schedule of oyster larvae at different stages is similar to that given in mussel chapter.

Feeding Schedule

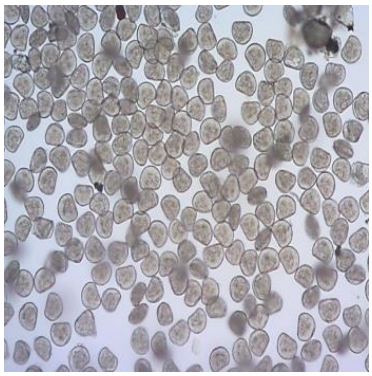
Stage	Day	No. of cells/larvae/day
Fertilized Egg	0	0
D- veliger	1-2	3,000-5,000
Umbo	3 - 14	10,000
Eyespot larvae	14 - 17	15,000
Pediveligers and Plantigrades	17 - 21	25,000
Spat	24 - 29	30,000
Spat	30 - 60	50,000
Spat	60 - 90	>100000

Larval stages of edible oyster, *C.madrasensis*

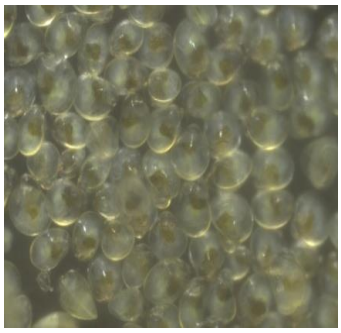


Pear shaped eggs

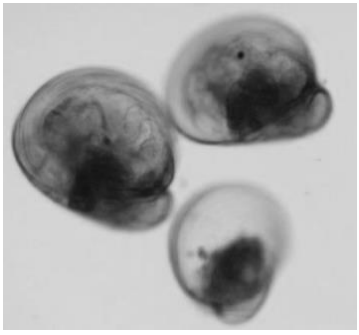
Two cell stage



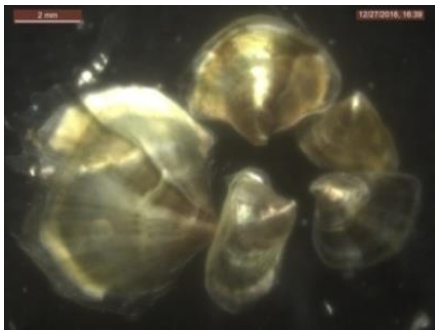
D-Veliger stage



Umbo stage



Eye spot stage



oyster spat

Settling of larvae and rearing of spat:

Once eye spot develops, the larva is ready to attach to a surface and undergo metamorphosis into spat. The eyed pediveliger larva of more than 290µ starts to settle and moves shorter distances. The process of settlement is prolonged for additional 2-6 days, and at this stage, finding a hard substratum is essential for survival. The setting of larvae can be done using different materials. The setting of larvae on 'cultch' (dead oyster shell) is the most common method. The shell is dried at least for a month to reduce the risk of pathogens, cleaned and aged in seawater for a few days for the formation of a biofilm on cultch which enhances the setting of larvae.

Oyster ren making

Oyster cultch is made either as oyster shell string or shell bag. In a 1.5 m length synthetic rope of 4 mm diameter, 8-10 shells are placed with regular interval. These strings are suspended in 1 ton tank by hanging rens from plastic pipes or wooden sticks. Or in one ton FRP tank, prepared oyster shells are spread as a layer, and the then eye spot larvae are added.

Cultch-less spat can be produced in micro-nursery using upwelling downwelling systems as described in the mussel chapter.

Setting of oyster larvae

Oyster spat grown on the shell

Prepared oyster rens kept in the tank for settlement and rearing

Setting on a whole shell or other large cultch is done by placing the cultch in large mesh bags. These bags are transferred to the tank with treated seawater. Eyed larvae are introduced @ 100 no./shell which will settle within 2-3 days and attaches permanently to the hard substratum and transform into the spat. Usually, 5-10 spats may get attached on a single oyster shell. Mild aeration should be given, and a mixed alga is given as feed. Tank containing shell bags is cleaned to remove algae. Since this method occupies more space and labour, after 1 or 2 weeks these shell bags are transferred to cultch farming sites. Once spat attains 10 mm to 12 mm size shell bags are opened, and individual oysters are spread on the bottom for further growth. At eyespot stage larvae can be transported in moist cloth and used for remote setting near the farm. The larvae are released to the tank containing cultch near the farming area and fed by pumping natural water into the tank before transferring them to the grow-out area.

Cultchless spat of *Carssotrea madrasensis* by stripping and hormone-induced settlement.

Ripe Indian backwater oyster *Crassostrea madrasensis* are used for stripping. After opening the shell, male and female gonads were scooped out and squeezed. Squeezed gonads were suspended in seawater separately. Filtrate of male and female gonads are separately

taken in beakers. The sperm suspension is activated using ammonium hydroxide and mixed thoroughly. Later 100-200 ml of sperm suspension was added to a beaker containing eggs to fertilize it. Further eggs were observed under the microscope to check the fertilization rate. Fertilized eggs were transferred to an incubation tank for hatching. The hatched-out D shaped veliger larvae were reared till they reached the eyespot stage (17-21 dph), feeding a combination of microalgae.

Single oyster spat in silo

Once the larva reached the eyespot stage, they were treated with Epinephrine. Treated Larvae were settled as single spat 20-30% success rate. After treatment, the set spats were transferred to the Micro nursery system for further rearing.

****