HATCHERY AND FARMING TECHNOLOGIES FOR MOLLUSCS

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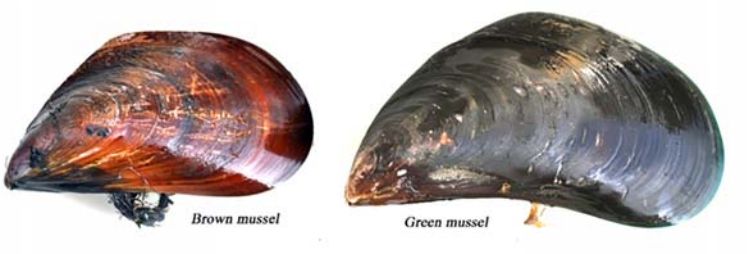
Introduction

In the ever-evolving landscape of aquaculture, the cultivation of molluscs has emerged as a pivotal domain, marked by advancements in hatchery and farming technologies. Molluscs, encompassing a diverse array of species such as oysters, mussels, and clams, play a crucial role in both ecological systems and global seafood markets. As the demand for sustainable and nutritious seafood grows, the development of innovative techniques in mollusc hatchery and farming becomes increasingly significant. This exploration into the hatchery and farming technologies not only addresses the challenges of meeting rising consumption demands but also underscores the importance of conservation practices to ensure the long-term health and viability of mollusc populations. In this context, the integration of cutting-edge technologies and environmentally conscious approaches becomes imperative to strike a balance between economic viability and ecological responsibility in the realm of mollusc aquaculture.

Mussels

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*).

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 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 seeds 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. 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 are first filtered using slow sand filters that filter out most particulate material greater than 20-40ì. 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 several channels and holes created between the particles that constitute the 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 the rapid sand filter to remove minute particles and stored in an 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 the 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 the 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 a low-temperature recirculation system for a long time.

Maturation

Maturation of broodstock is done in FRP tank of 1-ton capacity which has special provisions for photoperiod

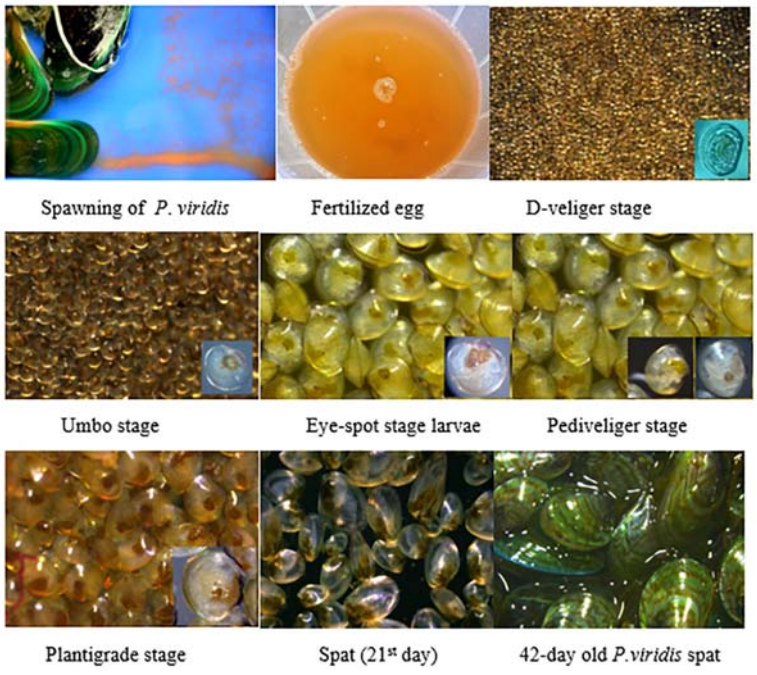
adjustment and heat and cold water facilities. Adult male and female mussels are placed in the tank at a density of 3-4 g/l of the total live weight biomass for gonadal maturation by adjusting the photoperiod (12 hr light and 12 hr dark) and maintaining the water temperature between 20-26°C. It is fed with *Isochrysis galbana* and *Chaetoceros sp* cells @ 7 million/ml. An 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 a 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 the 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 tanks of 200-500 l capacity or trays at a density of 3-10 numbers. A rise of 4-8 °C above the ambient temperature depending on the ambient temperature induces spawning of green mussels. Millions of eggs that are freely released by the female into the water are fertilized simultaneously by the sperm of males, and the eggs settle down. Usually, the fecundity of adult mussels 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 a morula larva. After hatching, the embryos are passed through a 100-150µ mesh screen suspended in the tank to remove larger debris. The optimum salinity is 25-35 ppt and the temperature is 24-27 °C. The morula exhibits phototropism and swims and congregates at the surface. After 5 hr of fertilization, it gets transformed into a blastula by the reorientation of the cells. The cells then convolute in and form dermal layers and the gastrula stage formed within 6-7 hr after fertilization. The gastrula stage transforms into a trochophore within 7-8 hr by developing a long single flagellum and tuft of cilia at the apical side and the rear side and swimming 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 the veliger stage which measures 50-55µ dorso-ventrally.



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 the 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 the umbo stage (130-260 µ) within 7-15 days 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 the 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 a 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

The 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. The 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,

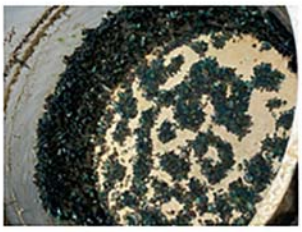
transfer the spat to the Up-welling system or other nursery system described below. The upwelling system has a total 1500 litre volume and two raceway compartments. Each compartment with eight wells provided a bottom mesh of 1 and 2 mm in size. Here spat can be grown from 2 mm to a seed size of 10 mm 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*.

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.

Micro-nursery system

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. 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



Seed raised in silo

Seed grown in ring net

Seeded ropes

Growth of seeded strings in farmers' field

and have shown good growth and survival. 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.

Mussel farming on racks in backwaters

Site selection

Coastal waters free from navigation are suitable for mussel farming. Fluctuation in salinity during monsoon season is one of the main constrain in estuarine mussel farming. Usually, the culture period is from November to May along the Kerala coast.

Water quality parameters

Optimum water quality parameters are given below:

Water current:17-35 cm/s

Temperature:25-33^{o}C

Salinity:22-33 ppt for green/peacock mussel

Farming structure

Rack culture of green mussel *P. viridis*

Rack culture is ideal for estuarine conditions where the water depth is between 1.5-3 m. The ideal size of fixed rack culture is 25 m^2 (5x5 m) which is fabricated by placing bamboo /casuarina poles and tying with nylon ropes. Nine poles having length more than the water depth during maximum high tide is driven into the bottom and spaced at a distance of 2.5 m apart, and it is connected in both directions by horizontally placed six poles of more than 5 m lengths. The horizontal poles should be above the water level at high tide, and the seeded ropes should be suspended from it. In shallow areas of below 1.5 m depth, both ends of the seeded ropes are horizontally tied onto poles.

Rack for Mussel farming in Kali estuary (Karwar)

Harvested mussel rope

On-bottom culture of green mussel *P. viridis*

In on-bottom culture, mussel seeds are relayed on the bottom of a water body leaving them to grow until the harvest, and this is generally practised in open waters or pens which can also be practised in shrimp or fish ponds at a low stocking density. In this case, the mussel seeds will form clumps within a week and grow. This method is widely practised in Ashtamudi and Kasaragod.


On-bottom culture in open water


Pen for on-bottom culture


Clump formation of seed

Raft culture of green mussel *P. viridis*

Raft culture is ideal if the water depth is more than 3 m, where the ropes are suspended from a floating raft 25 m^2 (5x5 m) at the surface of the water. The raft is made of bamboo poles placed parallel and across and tied with synthetic rope, and it is held afloat by tying with four airtight barrels of 200 l capacity at the corners and moored with concrete block. A protected bay and harbour are ideal for this.

Seeding of green mussel

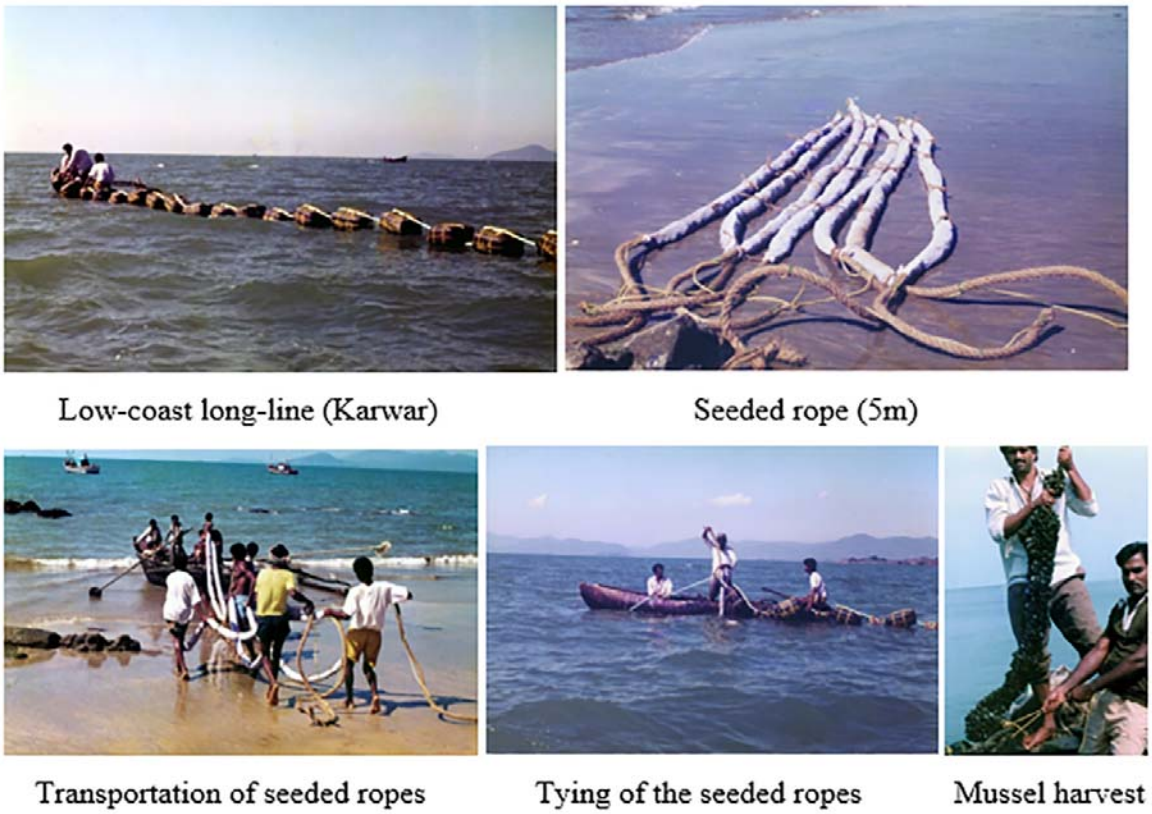

Seeding the rope

Farming of mussel is mostly dependent on wild-collected spat which is collected manually during low tide from the natural bed available in the intertidal and sub-tidal waters. At first, the collected spat is thoroughly cleaned to remove epifauna and other organisms. The length of the seeded rope ranges between 1-2 m depending on the water depth. At first, a mosquito net of 20-25 cm in width and the required size is cut and spread on a smooth and flat surface in a shady place. In the middle of these pre-arranged netting, a rope of 18-22 mm diameter is placed length-wise. The spat of 15-25 mm @ 600-1000 g/m is spread uniformly in the netting and over the rope and thereafter wrapped the netting by keeping the rope at the centre and stitched tightly to get the spat cover around the rope. To avoid slippage of mussels, knots are made or 10-15 cm length bamboo peg is inserted horizontally in between the twists of the seeded rope at a regular interval of 25 cm.

The seeded rope is suspended immediately after seeding from the farming structures. Generally, 60-120 no. of seeded strings with a length of 1-2 m are suspended 0.5-1 m apart. Within 2-3 days, the cloth starts to disintegrate, and the seed gets attached to the culture rope using byssus thread.

Long line culture of green mussels in the sea (Farming in the sea)

The long line culture is ideal for marine conditions at a depth of 5-20 m. The mussel can be farmed in the sea using rafts or long lines; protected areas like bays are preferred compared to open waters. Seeding and other management procedures are the same except that it has to be appropriately moored using heavy anchors or gabion boxes loaded with rocks. The long line is made of a 50-150 m long and 16-22 mm diameter synthetic rope which is held afloat with barrels or large floats and moored with anchors. The seeded ropes are suspended from the mainline. Sea farming of mussels is vulnerable to poaching, unpredictable climatic conditions and predation.



Longline farming of green mussels

Care and monitoring

The growth of the mussel depends on tidal flow and primary production. When the mussel is continuously submerged in water having good phytoplankton productivity and adequate particulate organic matter comprising of detritus, it grows rapidly. The seeded rope should be regularly examined and cleaned gently with a brush made of natural fibre to remove mud, silt and any fouling organisms. The major predators of mussels are crab, lobster and starfish.

Harvesting

Typically, harvesting of the mussel is done from April to June along the west coast of India and farmers are forced to sell their crop before the onset of monsoon to avoid mass mortality due to freshwater influx depending on the distance from bar mouth. Under culture conditions, green mussels and brown mussels attain a size of 80-88 mm (36-40 g) and 60-65 mm (25-40 g) respectively and yield production of @ 5-10 kg/m over 6-7 months. The farmed mussels give a better meat yield compared to mussels from the natural bed.

Harvested mussels using hatchery produced green mussel seed

As a filter feeder, it harbours microorganisms and contaminants present in the growing waters. Hence, a cleaning process called depuration is necessary to render the animal free of bacterial load and contaminants. When blooms of dinoflagellates occur, the harvest of mussels should be suspended as consumption of mussels from the affected area may cause gastrointestinal disorders to the consumer.

Edible oyster seed production technology

Edible oysters are the most widely cultivated bivalves in the world. The edible oyster 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) acts as a lid. Oysters occur naturally in estuarine water with salinities of about 10-25ppt, though they can tolerate higher salinity. It forms dense aggregations, often called 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, four species of edible oysters have commercial value.

Sl. No	Scientific name	Common name	Distribution
1	*Crossostrea madrasensis*	Indian backwater oyster	Odisha, Andhra Pradesh, Tamil Nadu and Kerala. It also occurs in the 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 is commonly called an Indian backwater oyster. Oysters of this species have irregularly shaped shell valves. The left valve is deep while the right one is slightly concave. The abductor muscle is kidney-shaped and the shell has a dark purple-coloured abductor 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. 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 oysters in the wild varies on the East and West coasts of India. On the East Coast, it is reported to be from Feb-April and on the West Coast of India it is from 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 a 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, the USA and many Southeast Asian countries and there is immense scope for export if produced in sizable quantities.

Indian edible oyster *(Crossostrea madrasensis)*

Hatchery techniques

Throughout the world, the 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. The cost of hatchery-produced seed is more expensive than wild-collected ones. But natural spat-fall is unpredictable and low in quality and quantity. The advantages of hatchery production of the oyster seed are that the desirable stage (eyespot stage/spat) and the required number and quality of larvae throughout the year could be guaranteed.

Broodstock collection and conditioning

Adult 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 the 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 far away places or countries. Oysters usually mature at one year of 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 inches (76 mm) in 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 a mixed algal culture diet of *Isocrysis galbana, Chetoceros calcitrans* and *Pavlova sp*. The feeding schedule for most warm-water bivalves is 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 a smear from the gonad and examining it under a microscope. Mature eggs are pear-shaped 48-62µ in size.

Spawning

Oyster spawning in progress (release of milky fluid)

In India, induced spawning is mostly achieved by thermal stimulation. For 20-25 numbers of oysters are selected and kept in seawater water with aeration in an air-conditioned room at 23ÚC for 12 hr followed by transfer into FRP tank (1-ton capacity) at 30-32ÚC. The water temperature is usually raised with the help of an immersion water heater. Mild aeration is provided in the tank. A sudden rise in the water temperature induces the oysters to spawn. Chemical stimulation is another method where ammonium hydroxide, sodium hydroxide/tris-buffer are added to the broodstock kept in a tank, but here viability of eggs is 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 and replacing it with fresh seawater. If an adult doesn't respond within a period, it should be returned to the conditioning tank for a further one week. The salinity of seawater should be in the range 32-35 ppt and pH should be 8-8.4. Initially, the eggs of oysters 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 a 10 l container. Eggs are gently mixed, taken 1 ml of sample is taken with a pipette and placed on the Sedgwick-Rafter cell to count the number of fertilized eggs. Usually, the fecundity of oysters 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. The fertilized egg undergoes cleavage within 45 min and reaches the morula stage after 6th division. The Gastrula stage is reached between 5-6 hour after fertilization

Rearing of larvae

D shell or Straight hinge larval stage

The D shell or straight hinge larval stage is reached after 20 hrs. Larvae are transparent, swim vigorously and measured 66 µ(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, and the larvae are counted in a Sedge wick-Rafter cell. The formula for calculating the total number of larvae is given below.

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

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 the culture of *Isochrysis galbana*. Every alternate day process of filtering and cleaning of the tank is repeated till the settlement; sometimes larvales 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, the umbo will have concentric rings on the shell. Between 12-15 days, the larvae will reach the late umbo stage and measure 150 µ size.

Eyed stage

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

Pediveliger stage

Larvae reach the pediveliger stage between 14-18th days; a functional foot develops and can be seen. Larvae measure 330-350 µ in size.

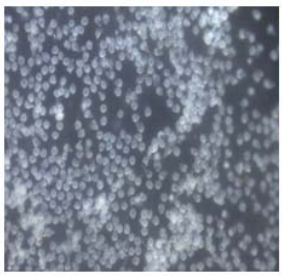
Spat

Once the pediveliger larvae lose their velum, it will start settling down, and larvae will develop adult features and metamorphose into a spat. The larvae are fed with a 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 the mussel chapter.

Feeding schedule

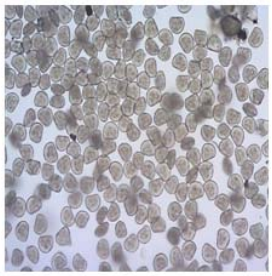
Stage	Day	No. of cells/larvae/day
Fertilized Egg	0	0
D- veliger	1-2	3,000-5,000
Umbo	3 - 14	10,000
Eyespot larvae	14 - 17	15,000
Pediveligers and Plantigrades	17 - 21	25,000
Spat	24 - 29	30,000
Spat	30 - 60	50,000
Spat	60 - 90	>100000

Larval stages of edible oyster, *C.madrasensis*



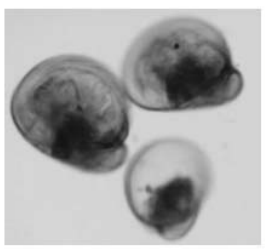
Pear-shaped eggs

Two cell stage

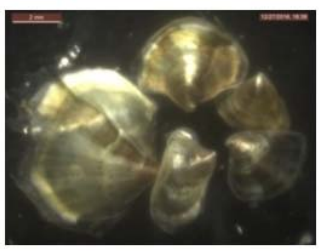


D-Veliger stage

Umbo stage



Eye spot stage



oyster spat

Settling of larvae and rearing of spat

Once the eye spot develops, the larva is ready to attach to a surface and undergo metamorphosis into a spat. The eyed pediveliger larva of more than 290µ starts to settle and moves shorter distances. The process of settlement is prolonged for an 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 the 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 at regular intervals. These strings are suspended in 1-ton tank by hanging rens from plastic pipes or wooden sticks. Or, in one one-ton FRP tank, prepared oyster shells are spread as a layer, and then eye spot larvae are added. The cultch-less spatula can be produced in micro-nursery using upwelling and 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 attach permanently to the hard substratum and transform into the spat. Usually, 5-10 spats may get attached to a single oyster shell. Mild aeration should be given, and a mixed alga should be given as feed. A 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 cultche 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 the eyespot, stage larvae can be transported in a moist cloth and used for remote settings 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

Single oyster spat in silo

hatched-out D shaped veliger larvae were reared till they reached the eyespot stage (17-21 dph), feeding a combination of microalgae.

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.

Oyster farming

Rack and ren method

Oyster- rack culture (7x7 m) tying of the ren to the rack

Ren with harvestable sized Oyster

The rack and ren method is ideal for estuarine conditions where the water depth is between 1.2-3 m. The ideal size of a fixed rack is 5X5 or 7 X 7 m in dimension (49 m^2) which is fabricated using bamboo/casuarina poles and tying them together with nylon ropes. Sixteen poles having a length more than the water depth (at maximum high tide) are driven into the bottom and spaced at a distance of 2.3 m apart and it is connected in both directions by horizontally placed 8 poles of 4-6 m length which are above the water level during high tide, and the 'rens' (oyster shell strings) are suspended from these racks.

On bottom culture

The on-bottom culture of oysters is ideal where the water depth is below 1.2 m where the oyster seeds attached to the collectors are directly planted on the bottom and allowed to grow.

The tray unit having the size 60 x 45 x 30 cm with 3 horizontal shelves of 10 cm height, suspended in the water column using float and anchor is also used for oyster farming.

Seeding

The oyster spat is attached to the 'cultch' at the hatchery itself @ 5 no./cultch which is then transported to the culture site. In the rack and ren method, such 8-10 spat attached cultch is connected through a string of 1-1.5 m long to form a 'ren' and usually 80-100 such 'ren' are suspended from a rack of 7x7 m size. Here the survival rate is about 55-60%. In the tray method, the nursery-reared single spat (cultch-free) measuring about 25 mm is kept in a tray unit of size 60 x 45 x 30 at an oyster-ling density of 200-250 no./unit.

Care and monitoring

The ren is periodically checked for replacement of broken structure and fastening of loosened ren or if it touches the bottom. If the ren falls on the ground, survival will be low. In the case of farming in the tray, once the oyster reaches 50 mm in length, it is segregated, and slow-growing ones will be culled and fast-growing ones will be placed back on the tray. The barnacle that settles on the wooden structure, tray and oyster may add more weight to the ren or tray and compete for food. Crabs and starfish, polychaetes and gastropods are the primary predators. The average growth rate of the oyster is 7 mm/month, and at the end of 12 months, the oysters attain an average length of 85 mm. Compared to the ren method, the tray method gives more production, but the production cost is higher. Other aspects are the same as that practised for mussel farming.

Harvesting

The ideal period for harvest is in May or September in Vembanad Lake and Ashtamudi Lake when the gonad is ripe before spawning. It attains a size of 50 g (shell on) over 6-7 months and yields production of 10 kg/m rope. The yield of the meat usually ranges between 7-20%. The farming of edible oysters with triploidy (all-season oysters) or tetraploidy can give better meat yield, but they are not available in India. As oysters are filter-feeding, after harvesting, depuration is necessary to clean the animal free of bacterial load, faeces, sand particles, silt and other contaminants from its gut. Initially, it is placed for 12 hr in a tank under a flow of filtered seawater. After draining the tank, it is then cleaned by a strong jet of water. The tank is again filled with filtered seawater, and the oysters are placed for another 12 hr. Then again the tank is drained and flushed with a jet of filtered seawater. The oysters are held for about 1 hr in 3 ppm chlorinated seawater and then washed once again in filtered seawater before marketing. Oysters can be kept alive for up to three days under moist and cold conditions. The removal of the meat from the oyster is called shucking, which is a hard process. The oyster is subjected to processes such as immersion in hot water, freezing, vacuum, and steaming for 5-8 min to make the oyster open their valves and facilitate the shucking process. A stainless steel knife is usually used to shuck the meat from the live shell. The edible oyster is even eaten in the fresh condition in the half shell.

Farming of cultchless oyster

Cultivating oysters without the traditional reliance on cultch materials, a practice commonly referred to as cultchless oyster farming, has emerged as an innovative and sustainable approach within the aquaculture industry. Unlike traditional methods where oyster larvae attach to cultch material like shells or limestone to form the oyster bed, cultchless oyster farming utilizes specialized bags as substrates for oyster settlement and growth.

In this method, oyster juveniles are introduced into the bags typically made of mesh or other permeable materials, allowing for water circulation and the exchange of nutrients essential for oyster development. This technique not only eliminates the need for natural cultch, streamlining the farming process but also offers several advantages.

Farming of cultchless oysters in HDPE Net bags

Cultchless oyster farming in bags allows for increased scalability and flexibility in production. Farmers can efficiently manage oyster density, monitor growth parameters, and mitigate the risk of predation. Moreover, the method proves particularly advantageous in areas with limited access to traditional cultch materials or where environmental considerations prioritize resource conservation.

The adoption of this innovative approach underscores a commitment to sustainable aquaculture practices, as it minimizes the impact on natural oyster beds and promotes efficient resource utilization. As the aquaculture industry continues to seek environmentally responsible solutions, cultchless oyster farming in bags stands out as a promising avenue for meeting the rising demand for oysters while preserving the delicate balance of marine ecosystems.