Recent Advancements in Edible Oyster (*Crassostrea madrasensis*) Hatchery Technology in India

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Oysters, revered as culinary treasures in the global seafood market, have a rich history steeped in luxury and tradition, particularly in the Western world. The practice of oyster cultivation dates back to ancient civilizations, with notable roots in ancient China. As far back as the 4TH century BC, the Chinese employed rudimentary techniques along coastal areas to foster oyster growth and boost production. Oysters held significant value for the ancient Romans and Greeks as both a food source and for their reputed aphrodisiac qualities, resulting in the establishment of basic oyster farming methods in the Mediterranean region.

Throughout the Middle Ages, oysters retained their esteemed status in Europe, notably in regions like France and England. The French, in particular, are credited with pioneering more advanced oyster farming techniques in the 17th century. They introduced the concept of artificial oyster beds known as "claires," which were shallow, saltwater ponds designed to fatten oysters before they were enjoyed as a delicacy among the European elite. These innovative methods played a crucial role in meeting the growing demand for oysters in Europe.

Scientific oyster farming has a relatively short history, with efforts to improve oyster cultivation techniques commencing in the 1860s. The allure of cultivating this luxurious delicacy, highly sought after by the affluent willing to pay premium prices, was undeniable. Beyond its reputation as a protein-rich culinary delight in Western countries, oysters hold tremendous ecological value. They serve as vital nursery grounds for various fish species that rely on oyster reefs as a primary food source while also contributing to coastal protection and water quality maintenance. Given their economic and ecological significance, oysters occupy a paramount position in aquaculture.

As industrialization gained momentum in the 19th and 20th centuries, the demand for oysters surged, resulting in overfishing and habitat destruction that severely depleted natural oyster populations. To address this, modern oyster farming techniques emerged, shifting the focus from wild harvesting to controlled cultivation. Hatcheries were established to produce oyster larvae, which could be strategically seeded onto artificial reefs or oyster beds, ushering in a more sustainable approach to oyster production. Since 1990, aquaculture oyster production has consistently grown, reaching 6 million tons in 2018, with a total value of US$7.5 billion. China stands as the major contributor, accounting for approximately 85% of globally farmed oysters, while other significant contributors include Korea, Japan, the USA, France, the Philippines, Indonesia, Taiwan, Thailand, and India, the latter representing less than 5000 tonnes of production.

Subtropical countries with well-established hatcheries for mass seed production dominate the oyster production landscape. They employ two primary techniques: single-set culture, which utilizes small cultch materials (such as shell pieces) to foster individual oysters for the half-shell market, grown in bags, racks, or rafts/longlines; and remote setting, which employs large tanks filled with cleaned oyster shells as cultch. This cultch can be used to create oyster reefs or strings and is farmed on racks, rafts, or on the sea floor in designated leased areas.

The development of hatchery technology for edible oysters in India commenced in the late eighties along the southeastern coast of India at the Tuticorin Research Centre of the Central Marine Fisheries Research Institute (CMFRI). While successful seed production was achieved, it was not adopted commercially. Oyster farming along the western coast of India, particularly in the states of Kerala, Karnataka, and Goa, began in the 1990s, relying on the traditional method of collecting spat from the wild using shell rens during the breeding season and farming them.

Over the last three decades, significant advancements have occurred in hatchery technology worldwide, particularly for the Pacific *oyster, Crassostrea gigas*, including methods such as larval production through stripping, micro nurseries, flupsy systems, and the production of triploids.

A consistent demand exists for shucked oysters (oyster meat) and "live shell stock," (shell-on fresh), emphasizing the need for high-quality whole single oysters with appealing shell shapes. To support a sustainable aquaculture sector, land-based hatchery technology is imperative for oyster seed production. In alignment with global developments, the Vizhinjam Regional Centre of CMFRI has developed essential techniques for oyster hatchery development, including strip-spawning, hormone-induced single oyster production, micro nurseries, and field silo systems for seed production. These advancements position India to boost oyster farming, particularly in the high-demand markets of Europe, America, China, the Middle east and Southeast Asian countries. This article presents the latest developments in edible oyster (Crassostrea madrasensis) seed production technology at the Vizhinjam Regional Centre of CMFRI.

Broodstock Development and Spawning Process

The process commences with the meticulous selection of high-quality oyster broodstock, emphasising specific traits such as size, shape, disease resistance, and growth rate. The choice of the right broodstock is pivotal for ensuring the production of robust and marketable oyster spat. To obtain brooders, one can either directly collect them from spawners in the wild during their ripe conditions or opt for hatchery-based broodstock development, which mitigates the impact of unpredictable climate-related factors on natural reproductive cycles.

Fig 1. Oyster broodstock

Fig 2. Spawning tray for thermal stimulation

In hatchery-based broodstock development, oysters are conditioned in a temperature-controlled Recirculating Aquaculture System (RAS). These oysters are accommodated in one-ton FRP tanks housed within an air-conditioned room (Fig 1). The RAS system has a comprehensive filtration system, incorporating mechanical and biological filters, a foam fractionator, and a chiller maintained at 24°C. Oysters are sourced either from the farm or the wild, then thoroughly brush-cleaned and quarantined before being introduced into the broodstock tanks. They are nourished with a mixed algal diet, and during feeding, the filtration system is temporarily halted.

Spawning

Mature oysters can be made to spawn using thermal shock or used for strip spawning. Ripe animals collected from the wild may spawn spontaneously due to the change in conditions or stress during transportation and cleaning.

Thermal shock method: Following are the steps to spawn oysters using thermal shock: It is essential to ensure they are in good condition and disease-free. It is crucial to use a clean tank or spawning tray set up large enough to accommodate the oysters without overcrowding (Fig 2). The container should be deep enough to submerge the oysters completely.

Prepare two reservoirs; one hot and one cold. The hot bath should be heated to approximately 32 - 35°C, and the cold bath should be maintained at a temperature of 20 - 24°C. Place the oysters in the cold-water bath initially. This serves to acclimate them to the lower temperature. After a few minutes (around 5 - 10 minutes), transfer the oysters to the hot water in the spawning tray. The sudden increase in temperature serves as a thermal shock, which triggers the oysters to release their eggs and sperm. Oysters typically spawn within a few minutes to an hour after being placed in the hot water.

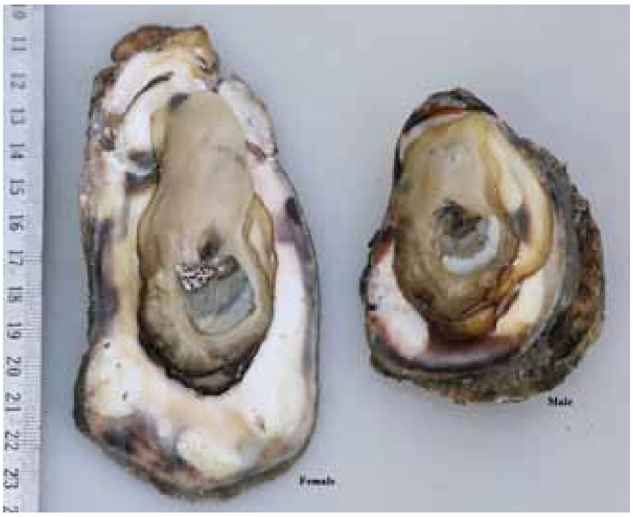

Fig.3. Female and male mature oysters

Fig.4. Sperm activation for fertilisation

Strip Spawning: Mature oysters can be strip-spawned by extracting the gonads of both males and females (Fig 3). To ascertain the sex and maturity of the oysters, gonadal tissue smears are observed under a microscope before extracting eggs and sperm. Oysters ready to spawn exhibit firm, thick, creamy white gonads occupying over 70% of the shell cavity, while immature oysters display thin, transparent gonads with the gut visible in the background. Strip spawning enables the selection of high-quality, vigorous broodstock, including large, high-quality egg-bearing females and males. This process can be carried out even when the oysters have not attained the ripe stage of maturity, ensuring continuous spat production and a year-round supply of oyster spat.

Gamete Extraction and Fertilization: Mature gametes are extracted and suspended in seawater in beakers. Stripped eggs, initially pear-shaped, become spherical upon hardening. Sperm suspension is activated using ammonium hydroxide (Fig 4). Once activated, the sperm suspension is added to the eggs to initiate fertilization. Subsequently, fertilized eggs (42-55µ) are filtered through a 20µ mesh and transferred to an incubation tank, with mild aeration provided during this period. Fertilized eggs are monitored under a microscope to track their development. The first polar body formation, typically occurring around 20 minutes after fertilization, marks the initial step in the developmental process. Free-swimming trochophore larvae emerge approximately 6 hours after fertilization at a temperature of 28±1ºC.

Larval Rearing and Spat Production

The first D-shaped veliger larvae appear approximately 16 hours after fertilisation (Fig 5). These larvae, measuring between 60 - 65µ, are then stocked at a concentration of 0.5 to 1 per millilitre (ml) in two-ton Fiberglass Reinforced Plastic (FRP) tanks for further rearing. Initially, they are fed with a culture of Isochrysis

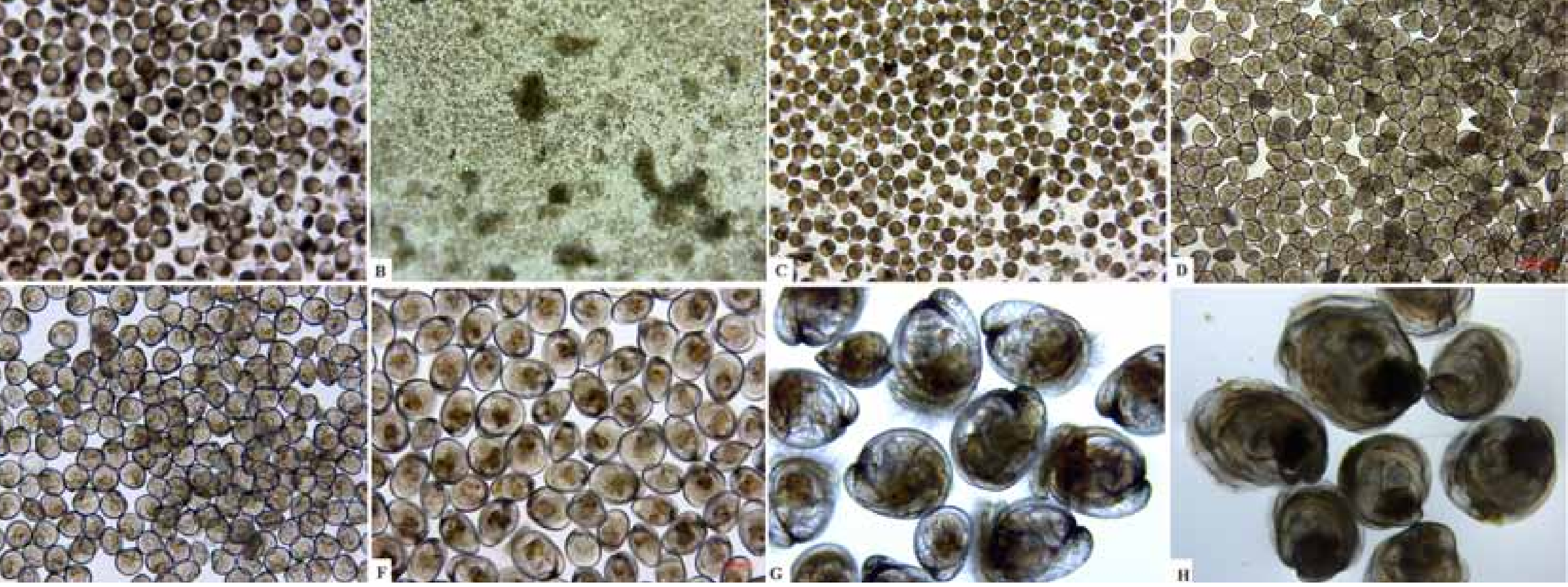

Fig.5. Unfertilised eggs, B. Sperms C. Fertilised and developing eggs, D. D-veliger E. Early umbo, F. Late umbo, G. Eye spot larvae, H. Spat

galbana, at a cell density of 10,000 cells/ml, and fed twice a day, both in the morning and evening. To maintain water quality, a 100% water exchange is given every second day, and the larvae are filtered through a 40µ mesh until they reach 7 days post-hatch (dph). After each filtration, larval samples are collected for microscopic examination, enabling the assessment of growth, survival, health, gut content, and overall development of the larvae.

Stock maintenance and mass culture of various microalgae species are integral components of any bivalve hatchery. Comprehensive details regarding their cultivation can be found in a previously published article.

In the initial stages, larvae are fed from Haffkine and round-bottom flasks, ranging in capacity from 3 to 4 litres. In later stages, the 20-litre carboy culture is used for feeding. As the larvae progress to the settling stage, they require a substantial quantity of algae and bag cultures are employed to meet their nutritional needs.

From the umbo stage onward, larvae are provided with a combination of microalgae, typically composed of four or five species, including Isochrysis galbana, Chaetoceros sp., Pavlova sp., and Dicrateria sp., mixed in a ratio of 2:1:1:1.

Various mesh screens of different sizes, including 20, 40, 100, 150, 250, and 500µ, in duplicates, play a vital role in larval filtration at different stages, ensuring the larvae are appropriately separated.

The D-shaped veliger larvae are reared until they reach the eyespot stage, typically occurring between 11-15 dph (Fig 6). During this phase, a combination of microalgae is provided in two-ton FRP tanks.

Once the larvae reach the eyespot stage with a size of around 250µ, they are subjected to treatment with Epinephrine Bitartrate, administered at a rate of 0.1 - 0.22 mg per 5 lakhs (500,000) larvae (Fig 7). Treated larvae are then settled individually as single spat, achieving a success rate ranging from 33 - 60%, depending on the quality of the broodstock and larvae.

Fig 6. Filtered eye-spot larvae

Fig 7. Hormone treatment of the Pediveliger larvae

Following treatment, the set spats are transferred to the downwelling component of Micro nursery system (Fig 8) for further rearing, with each well or silo accommodating approximately 3 to 3.5 lakh (300,000 to 350,000) larvae.

Spat typically reach a size of 1mm between 9 - 12 days after treatment. When they reach 2mm size they can be transferred to the upweller system (Fig. 9 & 10) of the micro nursery.

Spat measuring 5mm can be reared in bottle nurseries in a fluidised state (Fig.11) or in upwelling systems itself. By the time they reach a size of 15 - 20mm, baby oysters are transferred to the field nursery for further growth.

Fig 8. Micro nursery (upwelling and downwelling systems)

Fig 9. Single oyster spat different growth stages

Field Upweller Nursery System

The Field Upweller Nursery System (Fig. 12) represents an innovative solution designed for the nursery rearing of bivalves, offering a practical approach to growing hatchery-produced spat to seed stocking size, ready for direct use in grow-out operations. While achieving this growth in the hatchery using mass-cultured phytoplankton is possible, the sheer volume of phytoplankton required for commercial hatcheries, which produce hundreds of millions of seeds, makes it economically unviable.

The Field Nursery Silo System is highly adaptable and scalable to overcome this challenge. The system comprises 6 nursery silos, with the option to expand the capacity by adding more silos as required. Each silo boasts a diameter of 60 cm and a height of 90 cm.

The nursery silos are divided into two parts: the bottom unit serves as the entry point for natural water, which then passes through a mesh attached to the bottom part of the upper unit. Spats are stocked in the mesh bottom of the upper unit. The water that passes over the spat returns to the natural water body, such as an estuary or the sea, creating an ideal environment for the bivalves' growth. A pump is seamlessly integrated into the system to facilitate this continuous water flow, ensuring a steady supply of natural water rich in phytoplankton and other organic and inorganic materials.

Fig 10. Harvesting single oyster spat from micro-nursery silos

Fig 11. Bottle nursery

Fig 12. Field Upweller Nursery System

Fig 13. Upweller-grown oyster seed

Fig 14. Oyster juveniles ready to be taken to farm in net bags

By adopting this field nursery silo system, significant cost savings and faster growth are achieved compared to the alternative of producing vast quantities of phytoplankton in the hatchery. Notably, each nursery silo can comfortably accommodate up to 200000 oysters spat, streamlining the seed production process, and making it efficient and cost-effective. (Fig. 13 & 14).

Remote Spat Production

In remote spat production, mature larvae are strategically placed onto deceased shells (cultch) to facilitate their attachment to the shell. This process can be carried out within a hatchery or at a remote location proximate to the farms. These spat-attached shells are used to make strings that can be suspended from a rack, floating raft, long line, or the sea floor within designated leased areas. This cultch, bearing attached oyster spat, is extensively utilized in the United States to enhance natural oyster production through ranching and to create artificial reefs, offering various ecological benefits in addition to resource conservation. Farmers employ these attached spats to cultivate oysters for shucked meat production.

The process commences with the meticulous cleaning of the dead shells using a pressure washer or a brush. A chlorine wash is also recommended to eliminate any residual meat particles attached to the shells. Subsequently, the cleaned shells are drilled (if intended for making rens), placed in mesh bags for ease of handling, and arranged in tanks, typically exceeding one ton in capacity. These tanks are filled with filtered seawater.

Fig 15. Transfer of eyespot larvae to the cultch materials

Fig 16. Spat attached to the shells, Fig 17. Oyster seed in the cultch material

Fig 18. Preparation of oyster rens for farming

Fig 19. Oyster rens with remote set spat ready for farming.

To facilitate the attachment of larvae, a soaking period of 24 - 48 hours is recommended before introducing the larvae to the shells. This soaking aids in the formation of a microfilm, which contributes to the larvae settling more effectively on the shell surface.

Ready-to-set larvae can be sourced freshly from the larval rearing tank or larvae transported from the hatchery in oxygen-filled packaging in seawater or delivered as dry-pack larvae at 4°C in styrofoam boxes in case if it is done in farm locations. Filtered-out larvae can be transported without water in a nylon wet cloth covered with a damp paper towel within a styrofoam box, accompanied by a frozen gel pack for up to 24 hours. Upon arrival at the setting site, these larvae should be gradually acclimated to room temperature before transferring them to the setting water, which is contained in a suitable container.

The larvae are gently poured into the setting tank over the arranged shells, and proper aeration, preferably delivered through air bubbles from a PVC frame with drilled holes for uniform distribution, is essential. The larvae are introduced at a rate of 80 - 110 larvae per shell. The tank should be covered with a black cloth or an opaque sheet to facilitate the setting of the light-sensitive, eyed larvae. (Fig.15). This process typically takes a couple of days to complete.

Feeding the larvae during this stage is accomplished using a mixed algal culture or commercial algal paste. The successful attachment of larvae to the shells can be assessed both microscopically and by monitoring the larval count in the water (Fig 16 & 17). After approximately one week, these oysters can be relocated to field nursery tanks equipped with a flow-through system, allowing them to take advantage of natural food sources and promote rapid growth. Following a month in the nursery, they can be transferred to the farm for further cultivation.

This method ensures the controlled and efficient production of oyster spat, promoting a sustainable oyster industry.

During the period of 2022 - 23, Vizhinjam Regional Centre of CMFRI successfully produced and supplied various oyster seeds to different locations. In response to the request from Mangrove Cell of Maharashtra, Vizhinjam Regional Centre provided oyster seeds worth three lakh rupees in three consignments during

Fig 20. Packed remote set seed

Fig 21. Packing of single set oyster seed

Fig 22. Maharashtra Govt. officials receiving oyster spat &
Fig 23. Maharashtra farmers using oyster seed

January and March of 2023. This included remote set spat on oyster shells for making oyster rens packed in thermocol/styrofoam boxes and as single set/cultchless spat (Fig. 18 & 19). The cultchless spats were packed in oxygenated polythene bags and sent to Maharashtra by train (Fig. 20 & 21).

The officials from Mangrove Cell received the first batch of oyster shell-attached spat and cultchless spat, ensuring a smooth and successful transfer of these bivalve seeds to support aquaculture activities in Maharashtra. (Fig 22 & 23).

Conclusion

Oyster farming remains a vital industry, catering to the culinary preferences of millions while also championing responsible aquaculture practices and environmental conservation. As we forge ahead in our quest for sustainable seafood production, oyster farming serves as a remarkable example of how human innovation can coexist harmoniously with nature, ensuring a legacy for these exquisite molluscs for generations to come. Currently, the time required for freshly produced spat to mature into marketable-sized oysters stands at three to four years in the subtropical countries that dominate the industry. India, with its tropical climate, faster growth rates, abundant suitable areas for oyster farming, and cost-effective labour, has a promising opportunity to enter the oyster export market.

In recent decades, oyster farming has gained recognition for its ecological contributions. Oysters' remarkable ability to purify water and create natural habitats for various marine species has spurred oyster reef restoration projects across the globe. These initiatives aim to rejuvenate oyster populations and enhance coastal ecosystems, thereby playing a crucial role in conserving marine biodiversity and safeguarding shorelines against erosion. The continued evolution of oyster farming not only satisfies our gastronomic desires but also serves as a beacon of sustainability and environmental stewardship in the world of aquaculture.