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ICAR-CMFRI DEVELOPS AND TRANSFERS MUSSEL HATCHERY TECHNOLOGY

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ICAR-CMFRI is set to establish the first mussel hatchery for *Perna viridis* (Indian Peacock mussel) in the world for the State Government of Kerala. It will be the third mussel hatchery after New Zealand's (The SpatNZ hatchery Cawthorn, New Zealand for *Perna canaliculus*) and Chilean hatchery at Chincui Bay-Puerto Montt (for the Chilean mussel (*Mytilus chilensis*)). Mussel is a highly sought-after seafood delicacy worldwide, especially in Europe, Australia, America, Southeast Asia, and China. In India, it is a preferred seafood item along the southwest coast of India, especially in Kerala, Karnataka, and Goa. A significant advantage of mussel farming is that they come under non-fed aquaculture as they do not require feed or fertiliser and feed directly on primary producers (Phytoplankton). They also utilise dissolved and suspended organic matter because of their filter-feeding mode of nutrition. Being in the lowest rung in the food chain makes them one of the most efficient producers of the much-needed animal protein and they are also a good source of essential fatty acids and minerals. They can be farmed using several methods, including the on-bottom method or other three-dimensional farming methods like rack, raft and pole culture, utilising the whole water column.

Global aquaculture production reached a record of 122.6 million tonnes in 2020, of which aquatic animals contributed 87.5 million (USD 264.8 billion) and 35.1 million tonnes of algae (USD 16.5 billion). Of this, 68.1

million were from marine and coastal aquaculture, and the remaining were from inland waters. Of the 17.54 million tonnes contributed by marine and coastal molluscs, most of which come from the non-fed aquaculture of marine bivalves (16.2 million tonnes) (FAO 2022). Mussels are farmed by China, Spain, France, Netherlands, Canada, the UK, Ireland, Germany, Norway, Italy, Greece, Bulgaria, Chile, New Zealand, the USA, Dominican Republic, Thailand, Philippines and India, producing about 2.1 million tonnes (2018) worth USD 4.5 billion.

Mussel is farmed in India mainly in three states; Kerala, Karnataka and Goa, with commercial-scale farming mostly restricted to Kerala. Indian mussel farming relies exclusively on wild seed collected from the intertidal area during the spat fall season. This decade has witnessed a considerable increase in demand for mussels pushing the price from less than a rupee to Rs. 10 - 15 per piece. Farmed mussel production peaked in 2009 (18000 tons), and then declined due to diseases and lack of seed availability, and the current output is less than five thousand tons, mostly from northern Kerala. There is an ever-increasing demand for mussel seed for farming. Hatchery production of mussel seed in a technologically sound & cost-effective manner is obligatory for increasing mussel production. Settlement and metamorphosis of free-swimming mussel larvae and their rearing to seed size are critical for the success of bivalve seed production.

To date, mussel culture has relied entirely on wild seed from natural mussel beds in inter-tidal and subtidal regions of the marine ecosystem and estuarine areas. A significant problem faced by mussel farmers is the unpredictability of seed availability; the amounts of wild seed available are variable and erratic; further, climate change has worsened the situation. Large-scale collection of seed from the natural mussel beds for farming has led to conflicts between mussel pickers/ fishermen and farmers in the past with the increased adoption of green mussel farming in northern Kerala. Hatchery-produced seeds can supplement wild seed supply as and when they are required by farmers rather than restricting the seed availability to natural spat fall time. Furthermore, spent mussels are low in meat content, less preferred and fetch a low price. Hatcheries can also produce triploid non-maturing mussels and thereby avoid the seasonality of meat content. This article describes the mussel hatchery technology standardised by ICAR-CMFRI at its Vizhinjam Regional Centre.

Species of mussels: Two species of mussels occur along India's coast: the brown mussel *Perna indica* and the Indian Peacock mussel (Mayilpeelikakka- in Malayalam) or green mussel *P. viridis*. The former has a restricted distribution from Varkala on the southwest coast of Kerala to Thiruchendur on the east coast. In contrast, the latter occurs both on the east coast and west coast of India, including the estuarine areas like the Gulf of Kutch, Malvan, Goa, Karwar, Malpe, Kasargod and Kozhikode. Due to the seawall construction, now it is available at Cochin, Alappuzha and Quilon and Perumathuara in the Trivandrum

Fig. 1. Brown mussel (Perna indica)

Fig. 2. Indian peacock mussel (Perna viridis)

district. They are also present along the east coast in Chilka lake, Kakinada, Madras, Pondicherry, Cuddalore and Porto-Novo.

Brown mussel is dark brown, and a small percentage has a greenish tinge (parrot mussel) (Fig. 1); 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 (Fig. 2), 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.

Mussel hatchery components

Essential components of a hatchery include **1.** Seawater intake, filtration and storage. **2.** Microalgal culture systems, **3.** Broodstock development and Spawning, **4.** Larval rearing, **5.** Nursery rearing

1. Seawater intake, filtration and storage

Proximity to unpolluted seawater sources is crucial for the success of a bivalve hatchery. The area must be free of domestic and industrial pollution, and a stable salinity will ensure the year-round functioning of the hatchery. Seawater for hatchery can be pumped from surf water or a bore dug near shore. Water from the bore is preferred as it would avoid many organisms entering the seawater system, as the sand over the suction provides a primary filtration at the intake point. The seawater collected from surface can be pumped through a pressure sand filter to remove suspended particles. Pumped water is settled in a 50 T concrete tank and chlorinated before use (60 g TCC chlorine/30 tons of seawater) and kept overnight with aeration. Dechlorinated water was then pumped to the storage (overhead tank of 50-ton capacity) tank made of either concrete or fibreglass. For each million spat production, the hatchery requires approximately 30 tons of seawater every alternate day. Seawater from the overhead tank is passed through a cartridge filter and UV before transferring it into the LRT tanks. Cartridge filters and filter bags used in the hatcheries must be washed with chlorine water after everyday use, and water needs to be checked for chlorine levels to ensure that it is totally free from residual chlorine.

2. Microalgal culture systems

To a great extent, successful larval rearing and seed production depends on the quantity and quality of the microalgal output. Microalgae constitute the sole source of food for larvae and juveniles of bivalves, and their production cost accounts for about 40% of the expenses of bivalve seed production in a hatchery.

Fig. 3. Algal stock culture

Fig. 4. Intermediate-level culture

Fig. 5. Photobioreactor

Fig. 6. Raceway system

Production of an adequate quantity of microalgae of different species is crucial and challenging. A bivalve hatchery should have dedicated stock culture, starter culture, and intermediate and mass culture facilities for a continuous microalgae supply. Walne's medium is used for microalgal culture in the hatchery, where treated natural seawater is enriched with additional nutrients, which include nitrates, phosphates, trace elements, vitamins and carbon dioxide as the carbon source. The most commonly used microalgae species in bivalve hatcheries based on cell size, nutrient composition and ease of culture are *Isochrysis, Chaetoceros, Dicrateria, Pavlova, Skeletonema, Thalassiosira, Tetraselmis, Nannochloropsis*, and *Chlorella*.

Microalgae-Mass production systems: The Vizhinjam Regional centre has developed a Photobioreactor (Fig. 5) with daily output of more than 1000 litres of high-density algae of different species simultaneously and a raceway system (Fig. 6) for mass microalgal production with a production capacity of 1500L/day.

3. Broodstock development and spawning

Broodstock is usually collected during the spawning season. The spawning season can be extended by keeping the mature spawners in recirculatory aquaculture systems (RAS) at low temperatures feeding

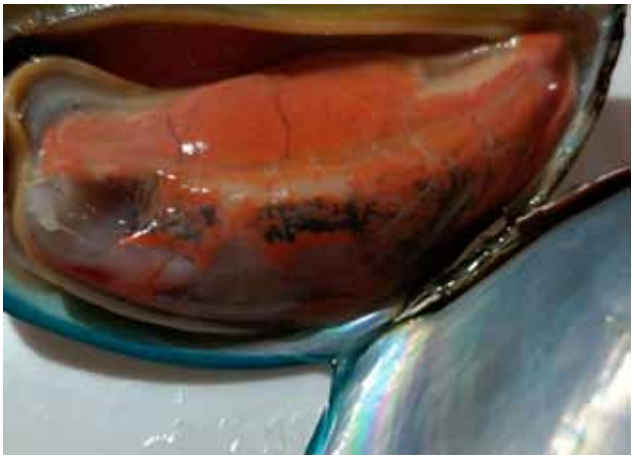

Fig. 7. Mature female

Fig. 8. Mature male

with mixed algal culture. Wild-collected mussels must be cleaned thoroughly by scrubbing off any attached fouler and other invasive species and disinfected with a mild chlorine solution (5 - 10 ppm) before assessing the gonad. The reproductive condition of the gonad can be evaluated by visual examination of the gonad: the colour of the gonad, fullness and extent of its fullness. Female green mussel with ripe gonad has an orange-red colour (Fig. 7), and their entire mantle cavity is filled with gonadal tissue, whereas the male has cream colour gonad with oozing milt (Fig. 8).

Spawning: Two methods of spawning are commonly used in mussel hatchery: natural spawning and induced spawning.

Natural spawning: Ripe mussels may spawn instantly when placed in fresh seawater at ambient water temperature because of transportation stress and desiccation. This spontaneous spawning occurs only if the animals are fully ripe or gravid and usually results in good-quality gametes and better fertilisation. Male responds first and release sperms like smoky fluid, followed by females, which release orange-red colour eggs.

Induced spawning: This is achieved by keeping the mature spawners in seawater at a low temperature (22 - 24º C) overnight and transferring them to a spawning set up with a heater where the water temperature is maintained 8 - 10ºC above the temperature (Fig. 9) of spawners. Spawning may commence within 15 min to 1 hr.

Fig. 9. Spawning by thermal stimulation

Fig. 10. P. viridis female spawning

Fig. 11. Male spawning - smoky white milt

The release of the egg (Fig. 10) and sperm (Fig. 11) can be observed. Excess males can be removed from the mass spawning tray to avoid too much sperm concentration.

4. Larval rearing

The eggs (Fig. 12) get fertilised immediately after spawning and after 2 hrs, they are filtered using a 20-micron filter cloth by siphoning the water from the spawning tray along with the eggs. It is important to keep the eggs immersed in water while siphoning by keeping the filtering silo immersed in water. Wash it with clean filtered seawater and stock them in the incubation tank after passing through a 100-micron filter to remove any debris or faecal matter, at a density of 50000 eggs per litre with mild aeration for development, in tanks of capacity 200 - 2000 lit. Fertilised eggs start dividing in 20 minutes and attain the trochophore stage in 6 to 8 hours.

In 18 - 20 hours, larvae attain the D-shape veliger stage (Fig. 13), 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 do not get

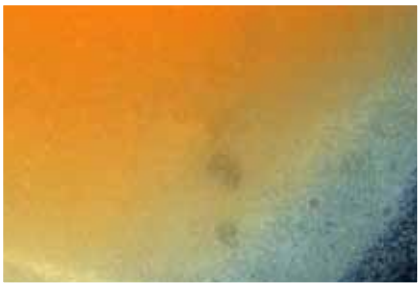

Fig. 12: Fertilized egg of P.viridis

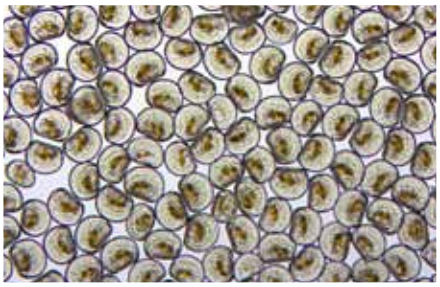

Fig.13: D veliger stage

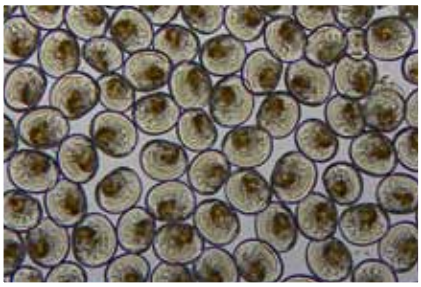

Fig. 14: Early Umbo stage

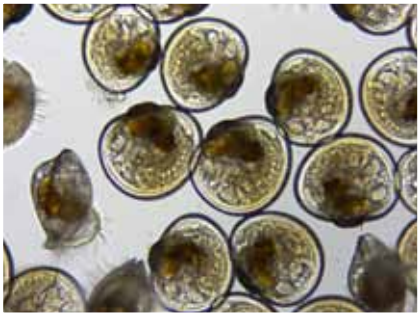

Fig. 15: Late Umbo stage

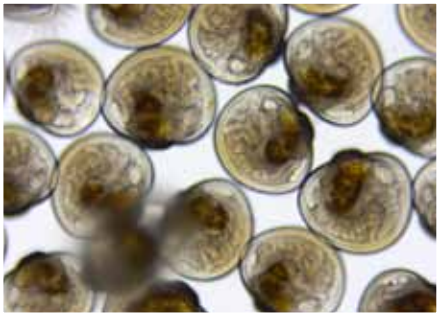

Fig. 16: Eye-spot stage larvae

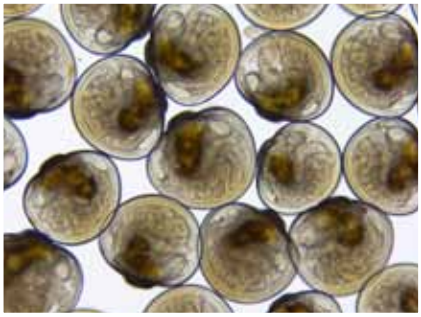

Fig. 17:Pediveliger stage

Fig. 18: Spat stage

Fig. 19: Juvenile 42 Day

Fig.20 Larval rearing tanks (LRT) of mussels in the Molluscan hatchery at Vizhinjam

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 at a density of 0.5 to 1 larva/ml.

On day 7, the D-veliger transforms to the umbo stage with a beak-like structure at the hinge of the shells (Fig.14 &15). Larvae attain the eye-spot stage: (a black spot-like structure that develops at the centre of larvae) (Fig. 16) on days 12 - 13 with the development of ctenidia. Development of the foot is observed on days 15 - 17,, indicating the pediveliger stage (Fig. 17) with the formation of gill filaments. During the pediveliger stage, larvae are competent for swimming with velar cilia and crawling with the functional foot. Along with the formation of the foot, the ciliated velum disappears, and the larvae start settling to the bottom with the appearance of the gill. Settlement of spat (Fig. 18) starts from the 19th day ahead and continues till the 28th day.

Larval rearing is usually done in FRP tanks of 2 - 5 T with a side drain valve (Fig. 20) which facilitates filtering the larvae every 3rd day, and the tank water is refilled with fresh seawater. The larval density has to be maintained at 0.5 - 1 larvae/ml for D-veliger which has to be reduced to 0.25 larvae/ml when the larvae reach the late umbo-stage. Mussel's early larval stages (D shape to umbo stage) are fed with the culture of Isochrysis species for up to 4 - 5 days. In later stages (late umbo to the eye-spot stage), larvae were fed with multiple microalgae species; comprising *Isochrysis galbana, Tetraselmis, Pavlova lutheri,* and *Chaetoceros calcitrans* in the proportion 2:1:1:1 at the rate of 5000 - 50000 cells/larvae according to the size of larvae. Mussel larvae in the settling stage were fed with *Chaetoceros* sp. in addition to other algal species. Complete water change has to be given every third day after filtering and separating the larvae using mesh cloth of required size.

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 sized, 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 even 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.

5. Nursery rearing.

The nursery rearing of mussels can be done in three methods: micro-nursery system, micron mesh cages and 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 (Fig. 21). 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 (Fig. 21 - 22). 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 150µ; 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 of the compartment through the mesh (down-welling).

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, 500µ and 1mm (Fig. 23) wells after 6 - 12 days of stocking and during the period, stocking density is gradually reduced

Fig.21 Micro-nursery system

Fig. 22 Down-welling compartments

Fig. 23 Well with settled spat

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 (Fig. 24). Each compartment has eight wells provided with a bottom mesh of 2 mm in size.

Here spat can be grown from 2 mm to a seed size of 10 mm in 40 days (Fig. 25). 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 there 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 in 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.

Fig. 24. Upwelling system

Fig. 25. Mussel seed in the upweller silo

Water is fully drained from the compartments and reservoirs and refilled with fresh seawater to remove all accumulated waste materials.

Nursery rearing in micron-meshed cages

The spat of 2 mm size can be nursery reared in micron-mesh cages by stocking 0.1 million spat in a meshed cage of 100 cm length and 10 cm diameter (Fig. 26). The cage is made of 1mm mesh and has a zip lock to close the mesh bag. Nursery cages can be hung from a floating raft in the bay water. The spat attains 10 mm in 45 days (Fig. 27), whereas it grows only to 5 mm in FRP tanks. The cages must be cleaned every fifth day to avoid fouling and subsequent clogging of the meshes. Lack of maintenance of the cage will result in poor growth and survival.

Nursery rearing in multitrophic hybrid systems

Mussels spat of 1 mm size can be nursery-reared successfully in a multitrophic hybrid biofloc system (Fig. 28) 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 (Fig. 29 & 30) can be seeded on ropes, can be kept for a week in the system for attachment, and sold as seeded ropes (Fig. 31). Seeded ropes are taken to the backwater farms and tested (Fig. 32) in floating rafts and have shown good growth and survival.

Fig. 26 Spat stocking in the micro-meshed cage

Fig. 27 Seed grown in a micro-meshed cage

Fig. 28 Multitrophic hybrid system for bivalve seed nursery rearing

Fig. 29 Seed raised in silo

Fig. 30 Seed grown in ring net

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. ICAR-CMFR has signed MOUs with the Governments of Maharashtra and Kerala for transferring bivalve hatchery technology. The Kerala govt has already begun the bivalve hatchery work at Puthiyangadi in Kannur District. The design proposed by ICAR-CMFRI was drawn by Kerala State Coastal Area Development Corporation (KSCADC) in consultation with ICAR-CMFRI and is given below in Fig. 33.

Fig. 31 Seeded ropes

Fig. 32 Growth of seeded strings in farmers' field

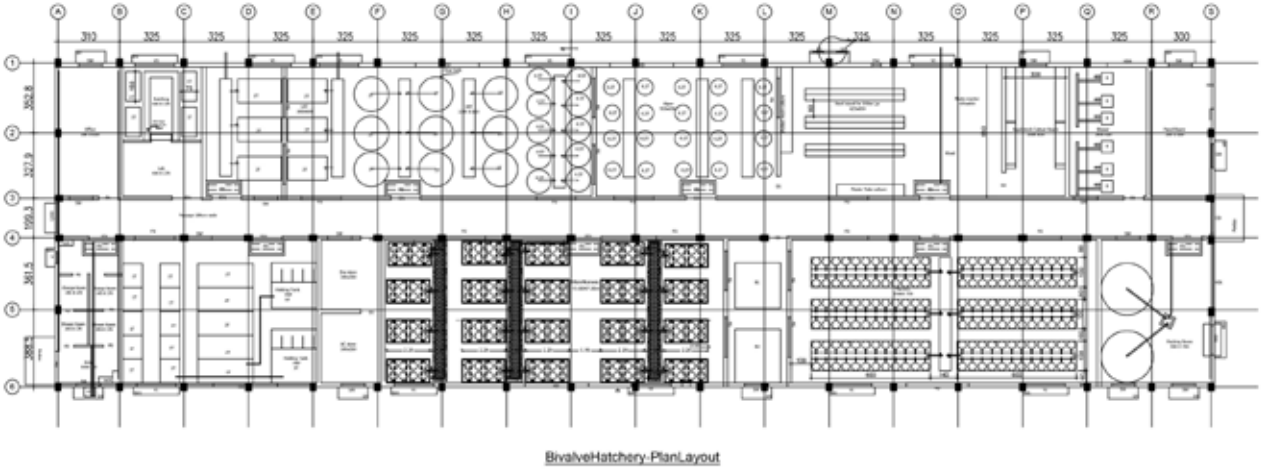

Fig. 33 Bivalve hatchery design for KeralaState Fisheries Department

It is anticipated that establishing bivalve hatcheries in these states would help develop a sustainable bivalve farming industry. This industry will potentially export mussels, oysters, and clams; it would allow the farmers and fisherfolk to earn a substantial income and increase the availability of protein-rich food. Farming based on the hatchery-raised seed will help conserve the natural population and avoid the conflict between farmers and the fishermen whose livelihood depends on the harvest of mussels from the wild.

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EVENTS

UPCOMING AQUACULTURE EVENTS

INTERNATIONAL CONFERENCE ON RECENT ADVANCES IN ANIMAL SCIENCE -ICRAAS'23 (Hybrid Mode)
Date: 01 Feb 2023
Location: B1 Seminar Hall, The American College, Madurai

AQUACULTURE AMERICA 2023
Date: 23 - 26 Feb 2023
Location: Marriott New Orleans, Louisiana, USA

11TH INTERNATIONAL ABALONE SYMPOSIUM
Date: 27 Feb – 6 Mar 2023
Location: Auckland, New Zealand

LACQUA 2023
Date: 18 - 21April 2023
Location: Panama City, Panama

WORLD AQUACULTURE 2023
Date: 29 May - 1 June 2023
Location: Darwin, Northern Territories, Australia

"BALANCED DIVERSITY IN AQUACULTURE DEVELOPMENT"
Date: 18 - 21 September 2023
Location: Vienna, Austria.

AQUACULTURE AFRICA 2023
Date: 13 – 16 November 2023
Location: Lusaka, Zambia.