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ABALONE CULTURE

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ABSTRACT

Considering the economic importance of the Indian abalone, *Haliotis varia* the Central Marine Fisheries Research Institute initiated experiments on its culture at the Research Centres, Tuticorin and Mandapam in 1996. A breakthrough was achieved in the larval rearing and juvenile production of this species in 1998 at Mandapam. The larva obtained from the spawning of *H. varia* passes through various stages of development namely the motile trochophore, veliger, gliding stage before settling as juvenile on the 26th day. The technology has been tested repeatedly in the hatchery and standardized.

In 1999, experiments were initiated to rear juvenile *H. varia* to adult under controlled condition adopting tank culture, cage culture and barrel culture methods. Among them, cage culture was found to be the most suitable method when compared with the other methods. The juveniles were fed with different types of algal food namely, *Ulva lactuca, Gracilaria edulis* and *G. crassa*. Of these, *U. lactuca* was found to be the most ideal food.

In 1999, efforts were made to produce half pearls in *H. varia* at Mandapam. Success came immediately when the first set of half pearls were produced at Mandapam in the year 2000 heralding the development of half pearl production technology in Indian indigenously.

INTRODUCTION

Abalones, commonly known as ear shell, are economically important marine gastropods belonging to the genus *Haliotis*. There are about 100 species of abalones in the world. They are found in both the hemispheres, with the larger varieties in temperate regions and the smaller ones in tropical and Arctic seas. As the commercial value of these shelled animals has been known from ancient times, much has been written about their natural history beginning with Aristotle (Crofts, 1929). Though the fishery of abalone had been conducted for the first time in China and Japan about 1500 years ago, it is only during the last 30 years the fishery for abalone spread worldwide and became economically important in those countries.

Abalone meat is highly priced and contains about 20 % protein. It is considered to be one of the best and most valuable seafoods in various parts of Asia. They have a blue grey mother of pearl that can be shaped into several types of ornaments. Abalone is a valued ingredient in the Chinese system of medicine (Guo *et al.*, 1999). The viscera can be made into good quality glue. Abalones can also be induced to produce pearls. The first recorded reference for abalone pearls occurs in one of the Japan's oldest historical writings (Shirai, 1970). The nacre of abalone shell is often multihued in tones of silver, orange, pink, green, blue and lavender. The colour play tends to be very beautiful in these pearls as the nacre produced by an abalone is thick and can reflect the full spectrum of rainbow colours. The quality of abalone pearls as reflected in their surface texture, is superior to the pearls produced in freshwater mussels and comparable to the best of pearls cultured in marine pearl oysters, *Pinctada* sp.

Cultivation of abalone has spanned over nearly a 50-year period. Pioneering efforts to cultivate North American Haliotids began in 1940 (Carlisle, 1962). The 1970s witnessed a continued interest in red abalone cultivation in California. Since then improvement of hatchery, culture technology and expansion of farm area in abalone aquaculture have rapidly developed and production has greatly increased, particularly in China, Taiwan and Japan.

Although large abalones are very popular in many Asian countries, small or cocktail size abalones of 40 - 70 mm shell length are also popular in some countries. Chen (1989) states that in Taiwan, small abalones are preferred to large species owing to their delicate flavour, appropriate size for banquets and price.

World production of abalones through aquaculture has increased from 2,179 tonnes in 1996 to 2,484 tonnes in 1997 (FAO, 1999). China and Taiwan contributed to around 89% of the total abalone production. USA contributed 265 tonnes to the world abalone aquaculture production in 1997. *Haliotis discus hannai* is the primary abalone species supporting abalone culture industry around the globe accounting for more than 70% of the world abalone aquaculture production.

The pearl culture in abalone is a recent enterprise. Unlike oysters, cultivating pearls in abalone is extremely difficult. The pearl nucleus is attached to the inside of the shell in a way in which the abalone will coat the nucleus with nacre, the pearl secretion, without being able to expel it. There is great chance for the rejection of the nucleus by the violent movements of abalone's large foot. As the production of round pearls in abalone met with only partial success owing to the nucleus dislodgement and infections precipitated by the muscular foot, most farmers are now attempting to culture both "mabe' and blister pearls in abalone.

Only 10 species are currently commercially exploited and they are mostly from temperate waters. The red abalone, *Haliotis rufescens* is the largest of the abalones in the world, often reaching length greater than 27.5 cm and weighing over 1.7 kg. Red abalone has traditionally been the most popular and commercially important species in California. The other important species in North America include H. *fulgens* (green abalone), *H.corrugata* (Yellow abalone), *H.sorensini* (white abalone), *H.cracherodii* (black abalone) and *H.kamtschatkana* (pinto abalone). Ezo awabi (*H.discus hannai*) found in Japan, is probably the most thoroughly studied abalone species in the world growing to between 18 and 20 cm in shell length. The other species found in Japan are *H. discus* (kuro awabi), *H. diversicolor supertexta* (tokobushi) and *H. gigantean* (madaka). *H.ruber* is the major Australian abalone species with *H.laevigata and* H. roei. *H.tuberculata* or *ormer* is the only commercial species in Europe. *H. midae* represents abalones in South Africa.

Unlike the temperate species mentioned above, the tropical abalone species are smaller in size and less in abundance. The major tropical abalones are *Haliotis diversicolor supertexta, H. asinina, H. ovina* and *H.varia.* Of these, H. *asinina* enjoys distribution in Japan, Thailand and Philippines. The other two species are abundantly distributed along the Andaman coast. All the tropical abalone species grow to less than 10 cm in shell length.

In the Indian Ocean, abalones are found in the Arabian Sea, the Persian Gulf, and in around Andaman and Nicobar Islands and off near Sri Lanka. In India, abalone is represented by only

one species, *Haliotis varia*. It grows to a maximum shell length of 80 mm. It is moderately distributed in Gulf of Mannar along Pamban and Tuticorin coasts of southern Tamil Nadu. Hornell (1917) states that abalones, which are highly valued in other parts of the world and occurring there in good abundance are scarce and small in size in India thereby becoming unnoticeable by the fishermen and researchers. But owing to the importance of abalones in aquaculture, it is important to push-start research on the abalones in India.

SEED PRODUCTION OF ABALONE

With the increased demand for the abalone meat, various countries have started culturing abalones on a commercial scale. Japan is the pioneer in abalone culture and the interest in cultivating this valuable shellfish has spread later to various parts of the world. Many Research and Developmental projects and hatcheries are in operation in Australia, the British Isles, Canada, Chile, France, Mexico and the United States. Japan is at present the acknowledged leader in developing techniques for the mass production of juvenile abalones.

Owing to its importance in the world aquaculture scenario, it has become imperative to initiate research on abalone culture in India with the native species. The restricted and moderate distribution of abalones necessitates production through aquaculture. The first step in abalone culture is to standardise the techniques for seed production, which include spawning, fertilisation and hatching, larval rearing, induced settlement and metamorphosis and production of juveniles with respiratory pores.

CMFRI achieved a breakthrough in the seed production of abalone *Haliotis varia* at Mandapam Regional Centre during 1998 - 1999.

During the breeding season, *Halioits varia* attains full sexual maturity on full moon and new moon days. During these periods, adult abalones were collected from the intertidal rocks of Gulf of Mannar with the aid of a chisel without causing damages to their foot and transported to the hatchery for conducting induced spawning experiments. On reaching the hatchery, they were acclimatised to laboratory conditions by placing them in 1-ton FRP tanks with running water facility.

Although natural spawning of mature animals was often observed in the hatchery, desiccation method (exposure to air for about 2 hrs) was found to be the most effective method of inducing the abalones to spawn. In *H. varia,* fully ripe male gonads were creamy white in colour and in the females they were dark blue/green in colour. In general, spawning takes place either during the late night hours or in the early morning hours. The eggs were fertilized within an hour of spawning. The fertilized eggs, were spherical in shape, measuring between 180-200 µm in diameter. The fertilized eggs were collected by siphoning at the bottom water and filtering through a 50 µm sieve. If the excess sperms attached on the eggs were not removed in time, it will be detrimental to the success of hatching. Repeated washing with clean filtered seawater removes the excess sperms. The average fertilisation rate was between 40 and 60 %. The fertilized eggs were transferred to another tank for larval rearing.

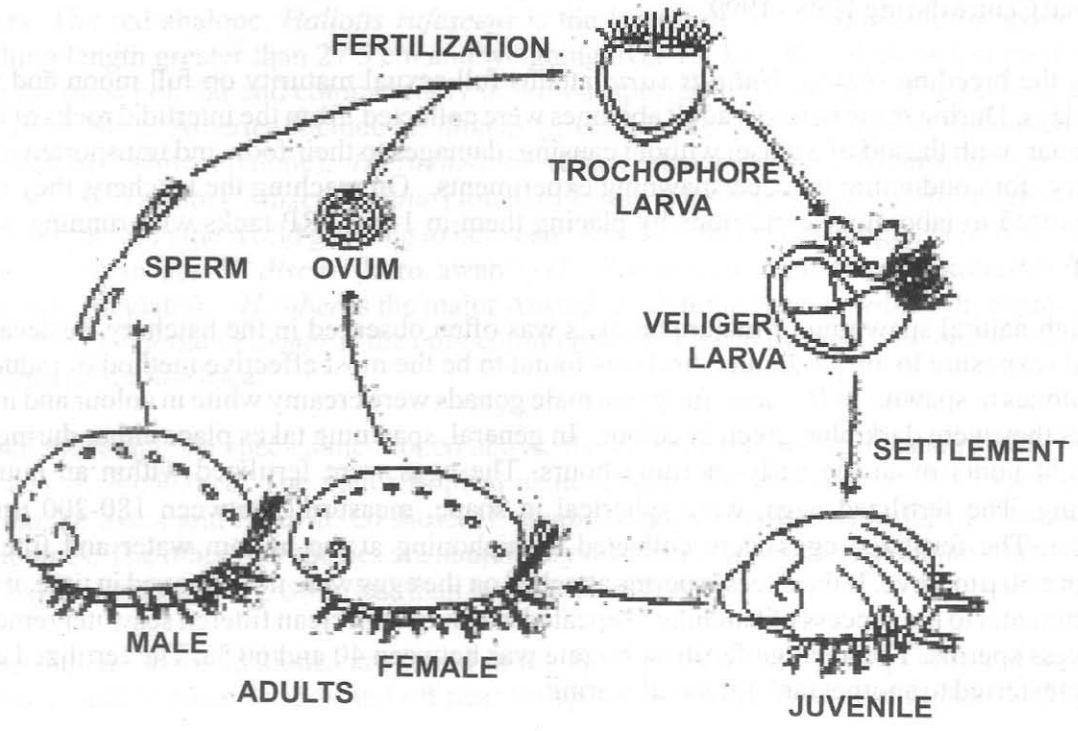
In about 12 hours after fertilisation, free-swimming trochophore larvae hatched out from the egg. The average rate of hatching was around 70%. The phototactic trochophores were siphoned out from the water and transferred to larval rearing tanks containing clean filtered seawater. The trochophore larval stage extends only to 10 - 12 hours.

The trochophore larvae transformed into the next stage, namely the veliger in about 24 hours after fertilisation. The veligers were also phototactic and exhibited the same behaviour as that of the trochophore larvae. At this stage, the larvae measured about 210 µm in length.

In the late veliger stage, the foot mass protruded to the top of the shell on the completion of the larval shell. The late veliger stage of *H. varia* extended upto 3rd day of the post fertilisation until it reached the gliding stage.

On the 4th day of post fertilisation, the floating veliger began to settle on substrates. This stage is termed as the gliding stage. At this stage, the larvae were transferred to the settling containers. The larvae actively crawl with its foot, but do not stop their swimming behaviour unless a suitable settlement substratum is found.

The gliding larvae when they reach the next stage namely the creeping stage, require suitable feed in sufficient quantities. A mat of benthic diatoms comprising mainly *Nitzchia* spp. and *Navicula spp.* was found to be the most ideal food. For *Haliotis varia,* the larval rearing period ranged from 4 to 5 days when water temperature was at 27°C. The creeping larva measured about 260 µm in length.



On the 5th day of post fertilisation, most of the larvae ceased swimming and crawled over the diatom mat provided in the settling tanks. Maximum settlement was observed along the vertical surfaces of the container. Once the larvae settled on the mat and started feeding on it, they seldom detached themselves from the diatom mat.

Peristomial growth, the first step in the metamorphosis, started on the 6th day leading to the transformation of the round tubular larval shell to one, which resembles the flat abalone shell. The animal at this stage actively feeds on the diatoms and clears the diatom mat areas by moving with their muscular foot. The settled larvae may die if sufficient quantity of the algal mat is not provided. The peristomial growth stage spat measured 288 ìm x 218 µm in diameter. On the 16th day, the shell becomes almost flat like the adult and shell colouration started as violet, but still transparent. At this stage,the animal measured 820 µm x 670 µm in diameter.

On the 26th day, the larvae transformed into juvenile with the formation of first respiratory pore at the anterior end of the shell. At this stage, it measured about 2.2mm. On the 46th day , the animal grew to a size of 2.6 mm with the formation of 3 respiratory pores.

As the abalone spat grew, there comes a time when they are too large to feed on micro algae, instead, they are ready to feed on seaweeds. The abalone spat grew faster when they are fed with the micro algal food but their growth rate slow down when they reached the juvenile stage. Hence the juveniles had to be fed with seaweeds like *Ulva lactuca and* other red algae.

FARMING OF ABALONES

Seed production is still a large scale enterprise in Japan and 15 - 20 mm seed are grown, sold to fishermen's cooperative unions and are stocked in the open sea. Few abalones are grown to marketable size in farms, but in recent years the industry has developed in several fronts and there has been grow-outs in land-based farms and from rafts in the ocean.

In Taiwan, the small abalones (*H.diversicolor*) are farmed successfully. Water temperature is relatively high in Taiwan, and hence this species is able to grow faster. During the last two decades, the production of abalones has increased steadily.

On the pacific coast of California and Mexico, considerable interest in abalone farming has been shown. The favoured species here is red abalone (*H. rufescens*). On the northern coast of the North American continent, the water gets too cold for the red abalone *H. rufescens* and hence the pinto abalone (*H. kamtschatkana*) is favoured here.

In Europe, farming of ormer (*H. tuberculata*) has gained some momentum along with pinto abalone *H. kamtschatkana.* In Australia,commercial abalone farming techniques are being developed mainly in the black lip abalone (H. *rubra*) and the green lip abalone (*H. laevigata*). In New Zealand also, there has been considerable development of farming of the common paua (*H.iris*).

Farming methods

Various methods are in vogue in abalone culture and their applicability depends on cost factors operating in the area.

Ponds or natural tide pools : The disadvantage with still water ponds is that abalones do not grow fast and hence they can be stocked only in low densities. The main reasons are that abalones are not stimulated to feed without current flow and that water quality quickly declines if the abalones are heavily stocked or fed at high rates. Water movement and improvement in water quality can be obtained by aerating the still water ponds.

Cages: Another way of farming abalones is by placing them in an enclosure in the sea. The natural movement of water removes wastes and stimulates the abalones to feed. The common cage for abalone farming is a small barrel. The open end is closed with cloth and they are hung from buoys and rafts in the open sea.

One of the advantages of cage culture in the sea is that water flow is by natural forces. This can be effective for water exchange and as a stimulant for feeding. For safety, cages are usually placed in sheltered bays where the water movement is less. Algae settle on the meshes of the cages, reaching a stage they can block the water flow.

As a replacement of the cage, submerged concrete pipes with mesh covering the open ends can be used. They are laid on the sea bottom and can be serviced by divers.

Raceways: Raceways ensure a fast movement and good exchange of water. This helps to keep the containers clean and maintain water quality. Turbulence generated by water movement helps to remove toxic gases. The water flow in a raceway stimulates abalone to feed and therefore grow faster.

The main disadvantage of raceways is that it generally requires vigorous pumping of water and hence it is expensive. Generally raceways are straight channels. Often the abalone raceways are rectangular in shape, with a width of 1.5m and a depth of 0.5 - 1.0m. The common length of the raceways could be 10 - 20 m. Another type is the circular raceways.

The common material used for the construction of raceways is concrete. But compared to concrete, fibreglass is an excellent material for constructing raceways.The cheapest material used to construct raceways is waterproof canvas supported by steel or plastic frames. The big advantage of the canvas is its low cost and it can be quickly dismantled and moved to other locations. The desirable flow rate in the raceways is about 1000 litres/hour.

Shelters: In nature abalones hide in shady areas. Even in captivity they move away from light. So in a farm it is desirable to provide hideouts, which may or may not improve the final harvest. The most common shelters are the traditional roofing tiles. These tiles are semi circular when placed with open side down on the floor of the raceway.

In recent times materials made with PVC and even 100mm PVC pipes sliced lengthwise into two halves are being used.

Feeding: In the wild, the adult abalones feed on macro-algae (sea weed). It is a common belief that abalones feed only at night. Most species of abalones prefer to seek shelter during daylight and they feed rarely. However, some abalones do feed during daylight.

Cost efficient feeding of grow out abalones is a key to economic success. Different types of feeding can be used. Farmed abalones can be fed with fresh or preserved seaweed. As an alternative to natural food, grow out abalones can be fed with formulated diets or even normal land grown vegetables.

Because of the abundance and availability, brown seaweeds are used as feed for many of the abalone farming operations. But the brown seaweeds are generally not as nutritious as red, with low levels of protein and they are often much tougher. Moreover, brown seaweeds contain phenols, which are repellent to abalones.

Green seaweeds are usually poor in nutrition and abalones do not prefer them. But *Ulva* spp. and *Caulerpa brownii* are the two exceptions to this. *Ulva* is very common seaweed and grows readily on the shoreline. *Ulva* has a thin transparent leaf and is readily acceptable to abalones.

Red seaweeds are the preferred food of abalone and they are highly nutritious. The main species of red algae used in abalone farming are *Corallina, Lithothamnium, Gracilaria , Jeanerettia and Porphyra.*

The ideal quantity to feed an abalone is to provide as much food as it can eat. Feeding rate should be of 1/5 weight of the abalone in case of fresh seaweeds. But in case of dry feeds, 1/20 weight of the abalone should be given as feed per day. The best way to determine whether the abalones are being fed enough is to offer plenty of food and then reduce the quantity until none is left over. Feeding frequency may be of one or two times a day.

Rearing experiments of *Haliotis varia* conducted by CMFRI

Juvenile rearing: In CMFRI, the juveniles were reared in FRP tanks with 100 % water exchange daily. They were fed with chopped green seaweed *Ulva lactuca*. Tanks were provided with bits of dead coral stones as hideouts for juvenile abalones. After duration of 200 days of rearing, the survival of juveniles was around 70%. During this period, the abalones have grown to a size of 8.96mm. Different kinds of feeds like red coralline algae and green filamentous algae were tried in juvenile rearing. While the juveniles fed with red coralline algae exhibited a growth of 11.32mm, the juveniles fed with green filamentous algae gave a growth of 9.70mm. The juveniles fed with the green filamentous algae showed signs of retarded growth after a certain time of rearing.

Rearing of adult abalones: Adult abalones collected from the wild were reared in captivity in order to study their adaptability in captive condition. For rearing of adult abalones different methods like miniature raceway, cage and barrel culture were tried.

FRP tank of 1-ton capacity was converted into a raceway type culture system. This tank was kept in a slightly inclined position with fresh seawater entering into the tank from one end and leaves the tank from the other end. Flow was regulated so that the feed given in the tank was not washed out from the tank. Coral stones were placed at one end of the tank as hideout. Chopped green seaweed *Ulva lactuca* was the feed given to the abalones once in three days. The survival in these tanks was about 70 % after 6 months of rearing. Growth was observed to be poor as the abalones subjected in this experiments were adults collected from the wild.

Cage culture was also attempted for the culture of abalones. Iron cages of size 40x40x10cm covered with old fishnets were used for this purpose. For providing substrate for attachment, shelter and hideouts, bits of coral stones were placed inside the cages. These cages were then hung from the pearl oyster racks erected in the Gulf of Mannar, Mandapam and also in 100-ton capacity RCC tanks. Feeding was done with chopped *Ulva lactuca*. They were fed once in two days. While the survival was good in the cages kept in the tanks, the survival was poor in the cages suspended from the racks. The poor survival was due to the settlement of suspended materials inside the cage and also because of fouling. Although cleaning of cages was done regularly, the survival was very low. It was found that abalones required clear, well-aerated water for their growth and survival. Few abalones reared in cages in tanks mature after 3 months of rearing.

In another method of culture of abalones, old barrels were cut into two halves vertically and the open end was covered with old fishnets. Holes were drilled on the body of the barrel to ensure sufficient water flow. Coral stones were placed in the barrel to act as a substratum and also as weight to keep the barrels submerged. These barrels were suspended from racks and survival was not encouraging mainly because of sedimentation.

Experiments with different kinds of feeds like *Ulva lactuca* and *Gracilaria edulis* and *Gracilaria crassa* showed that *Ulva lactuca* was suitable feed for the abalone *H. varia*.

Half pearl production in abalone *Haliotis varia*

Fixing a nucleus on the inner side of the shell of the animal was found to be very difficult due to the dislodgement of the nucleus by the powerful movement of the foot. A novel technique developed by CMFRI was found to be very effective in fixing the nucleus on to the shell, which enabled the production of half pearl /mabe pearl a reality.

Healthy adult abalones collected from the natural grounds without any injuries were selected for the implantation. Before implantation they were reared in the FRP tanks. Suitable feed was given to them for two or three days to recover from the effect of stress.

Healthy abalones were taken out from the tanks and dried for 10 minutes before fixing the nucleus. This enables the easy retrieval of the foot muscle for drilling at the appropriate site.

A hole of required size, which depends upon the size of the nucleus to be fixed, was drilled on the inner side of the shell using an electrically operated hand drill. Extreme care was taken to avoid any type of injury to the animal. Drilling was done in one swift action and the drilled abalones were returned to the tank containing well-aerated seawater in order to recover from the drilling shock and also to remove the drilling dust.

After half an hour, the drilled abalones were taken out, their mantle pushed aside with the aid of a sterile scalpel's blunt end and the inner shell was wiped with dry cotton wool. The commercial grade adhesive 'Anabond' was used as fixative. A drop of the glue was placed in the hole, immediately followed by placing a shell bead with fine tweeters and pressing the nucleus gently till the adhesive was completely dried. The animal was returned to FRP tanks with running seawater and aeration.

Active nucleated abalones were collected from the FRP tanks on the subsequent days and stocked in conventional box type cages knitted with appropriate mesh size and suspended from the racks. Feeding was done with green seaweed *Ulva lactuca.* At the end of first month, slight nacre coating was observed over the nucleus. The stocked abalones were harvested on the 4th month when the nucleus had thick uniform nacre coating. About 40 % of the abalones had good nacre coating in the experiments.

Breakthrough achieved in pearl production through tissue culture

Production of natural and cultured pearls is environment dependent. When the sea becomes polluted or its condition deteriorates the survival of pearl producing animals is not ensured. Such eco-degradation is being observed at global level. Therefore attempts are being made to produce pearls through tissue culture by avoiding the natural environment.

Due to the adverse environmental conditions that prevailed in some locations in the sea, the production of pearl was affected. Hence research on pearl oyster mantle tissue culture was initiated in Japan some 25 years ago in order to produce *in vitro* pearl. Machii (1974) has achieved deposition of organic substance in an organ culture of mantle tissue of pearl oyster, *Pinctuda fucata.* Machii and Wada (1989) demonstrated the production of organic substance, which they call as organic pearl. Samata *et al.* (1994) reported the black secretion in the mantle tissue culture of *P.fucata* that induced the crystal growth. Wada (1961), during the study on shell formation and regeneration of *P.fucata,* found the induction of nucleation from the submicroscopic nuclei for the development of crystals and reported the formation of nacreous layer.

India too felt the need of initiating tissue culture experiments in pearl oysters as the population of pearl oysters in the natural environment has become sparse. Therefore the work was initiated to culture the mantle tissues of pearl oysters and abalones and produce *in vitro* pearls at the Tissue Culture Laboratory of Tuticorin Research Centre of Central Marine Fisheries Research Institute, Tuticorin in 1997. A breakthrough was achieved in 2003 in this laboratory in the fundamental research for the *in vitro* pearl production in the abalone, *Haliotis varia* through mantle tissue culture method.

In the explant tissue culture, the cells proliferated in large numbers and produced two types of round cells, namely granular and agranular. These cells migrated away from the explants and multiplied *in vitro.* This resulted in the formation of a cell sheet. The round cells developed pseudopodia that later covered the entire surface of culture plates and formed an organic matrix and pearl sac. The granular cells consisting of granules became larger and released out the granules to form a nucleus for crystal growth.

In an organ culture, the mantle tissue of *H.varia* and a sterile shell bead nucleus were placed in the culture flask containing a nutrient rich medium. The shell bead got coated with a distinct nacreous layer with organic matrix and a pearl sac was formed within 3 months. The mantle graft produced calcite and rhombohedral crystals. Polygonal prismatic layer was formed with thick interlamellar matrix at its boundary. The nacreous layer consisted more of calcium (51% by weight). The present research in *H.varia* is highly significant in view of the fact that culturing the

mantle tissue in nutrient rich medium and achieving crystallization of nacre has not been reported hitherto. This basic technology developed through tissue culture eliminates the dependence on natural environment for pearl production. Production of large size pearls as well as more number of pearls is also possible through *in vitro* culture (CMFRI, 2003).

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