CMFRI

Course Manual

Winter School on Recent Advances in Breeding and Larviculture of Marine Finfish and Shellfish

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PEARL OYSTERS AND PEARL PRODUCTION TECHNIQUES



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Introduction

Pearl oysters are soft bodied marine pearl producing bivalve mollusk with hard protective shell. These animals produce pearls. About 29 species of pearl oysters are available in the world and distributed in tropical and subtropical regions.

Taxonomy

Phylum	:	Mollusca
Class	:	Bivalvia
Subclass	:	Pteriomorphia (Suzuki, 1985)
Order	:	Pterioidea (Suzuki, 1985); Mytiloidea (Richard, 1985)
Sub-order	:	Pteriaceae
Family	:	Pteriidae
Genus	:	Pinctada
Species	:	fucata (Gould, 1850)

The term "cultured pearl" was used for the first time in 1920 for the pearls produced "akoya gai " in Japanese pearl oyster and marketed in Europe. Mikimoto is the first man when cultured pearls and believed to deserve the credit for the original development of the technique. His technique involved taking a piece of mantle tissue from one oyster and implanting it in another. The term `artificial pearl` dose not denote a cultured pearl, but would refer to cheap imitation made of plastics, glass, etc. by using the extract" guanine" from fish scales for artificial shine. The core material called "shell bead nucleus" is produced from the shells of freshwater mussel in America which is imported to Japan, China, Thailand and Australia where they produce the spherical beads of 2-22mm size according the size of oysters to be used for pearl production. The surgical tools have designed and developed by CMFRI. Implanting the images of required object in between the mantle and shell cavity without affecting the mantle produces the "Mabe" image pearls. This technique has been developed by CMFRI, during 2002. The tissue culture pearl production is under perfection.

Morphology

The shells of pearl oyster *Pinctada fucata* are reddish brown but may exhibit different colour patterns. Externally 6-8 radial reddish brown bands emerge from umbo towards the free margin of the shell. The hinge line is fairly long. The hinge teeth are well defined, one on either end of the ligament. The anterior and posterior ears are well developed. The anterior ear is ventrally bound by byssal notch. In fresh specimens, prominent scaly growth processes can be seen at the distal border. The left valve is deeper. The adductor muscle scar is large and sub central, small scars are also present for the attachment of pallial muscle. The shell of pearl oyster is composed of three layers; the organic conchiolin layer (or Periostracum), the middle prismatic layer and the innermost nacreous (mother-of-pearl) layer.

Species of pearl oysters available in India Family - Pteriidae (Pearl oysters



Anatomy

The soft body of the pearl oyster consists of a pair of mantle lobes, visceral mass, gills, foot, posterior adductor muscle and other musculature. The soft creamy yellow coloured mantle that follows the contour of the valves (shells) envelops all the other soft parts of the body.

Mantle

Mantle is a fold of skin covering the soft body below the shell. It is formed of two lobes on either side ((Please see graft tissue preparation) .The mantle has three zones viz; The marginal , Pallial and Central zones. The two lobes are fused together dorsally" The mantle isthmus "below the hinge line, from which point they hang down on both sides of its body. The most important function of the mantle's external surface is the secretion of shell's organic matrix and deposition of calcite crystals. The edge of the mantle has three folds viz. the ciliated inner fold, the middle fold and the outer fold. The outer fold is shorter and flattened against the valve's interior face. It is separated from middle fold by the periostracal groove, from the bottom of which arises a translucent organic membrane, the periostracum. They ensure the longitudinal growth of the valves, which is sporadic, due to periods when growth stops, when temperature and nutritional conditions are unfavorable. The pallial mantle secrete calcite and aragonite as polygonal /hexagonal crystals, which add to the thickness of the shell by strong binding. It is in this part that the graft tissues are cut. If graft tissues are taken from the mantle edge, the pearl sac will secrete prismatic layers and the pearl will not be a nacreous one.

Gills

The gill consists of a longitudinal axis from which hang down two lamellae of ciliated filaments. Each blade is folded in a V-shape, which increases the length and number of filaments. The inhaled water current enters the shell anteriorly and reaches the gills. The ciliary movements redistribute the water by channeling it along the filaments. Two veins follow the bronchial axis; the afferent vein brings blood coming from the kidneys and the efferent vein takes it away towards the auricle. A network of capillaries, which follow the filaments where blood is oxygenated, links the two veins. The blood is colourless.

Heart

The heart consisting of a ventricle and two auricles enclosed by the pericardium it is located ventral to the intestine. The auricles are covered with pericardial glands, which function as excretory organs. From a single ventricle, arise the short posterior aorta, which supplies blood to the rectum and the adductor muscle, and the anterior aorta, which continues as arteries and arterioles. These minor arteries open into lacunae of connecting tissue, where blood circulates freely before reaching some large spaces called sinuses. The deoxygenated blood is collected in veins and carry into the nephredia where it is purified before it reaches the gills.

Filtration

Sea water contains suspended particles of varying sizes, shape and nature. The gills must expel them immediately upon contact or suffocate. They sort out the organic and inorganic materials and pass them towards the digestive tube. Larger particles are discharged at the rear end of the shell through the exhalent current. The ciliary movements of the filaments bring small plankton and other nutrients to the mouth. Bacteria and mud are aggregated by mucus secreted by the gills before they are sent to the labial palps.

Labial palps

The mouth is framed by labial palps which are two superposed curved leaves whose opposing faces are stripped with grooves. The small plankton brought in by branchial currents undergoes another selection. Some organisms are undesirable, because of their spiny ornamentation or chemical character. They are directed along the furrows to the end of the palps, where they fall into the pallial cavity and are periodically ejected as pseudo-faces into the seawater.

Sensory organs

The animal does not have a brain and only cerebral gangleae is seen. The nervous system consists of 3 pairs of ganglia viz, cerebropleural, pedal and visceral from which the nerves arise and connected by connectives and commissures. A complex network innervates the mantles, the pallial plexus which make it react at the least tactile or chemical stimulus. The gill responds to stimuli. A branchial eye is present on the first left hand branchial filament. It can detect variation in light intensity. In adult condition the eye disappears and form as chromatophores in the base of the foot.

Reproduction

In pearl oyster, the sexes are separate, but the males and females cannot be distinguished from the characters of the shell. The reproductive system consists of a pair of gonads, which spreads superficially over the hepatopancreas and intestine in mature state. It is pale yellow in colour in male and is of a deeper shade in the female. The eggs or sperms are spawned through the paired gonoducts ending in the genital openings located at the base of the anterior ends of the gills. *P.fucata* attains first sexual maturity when it is about 15.5mm, i.e when about 3-4months old, Pearl oysters spawn twice in a year. In different populations 5-10 % matured animals are observed in good productive months. In the Gulf of Mannar, the spawning season is June-August and November- January coinciding with the southwest and northeast monsoons respectively.



1. Hinge line 2. Posterior aorta 3. Anterior aorta 4. Liver diverticula 5. Stomach 6. Ligament 7. Oesophagus 8. Mouth 9. Anterior ear 10. Labial palps 11. foot 12. byssal threads 13. Gonad 14. Intestinal loop 15. Muscular ctenidial axis 16. Outer fold of left mantle 17. Middle fold of left mantle 18. Inner fold of left mantle 19. Growth process 20. Left outer ctenidial 21. Left inner ctenidial 22. Pallial fold 23. Anal process 24. Rectum 25. Adductor muscle 26. Auricle 27. Pericardial cavity 28. Ventricle 29. Posterior ear



Life cycle of pearl oyster

Age and growth

Observations made on cultured pearl oysters collected near Krusadai Island and at Tuticorin show that the oysters can grow to a height of about 35-45, 50-55,55-60, 60-65 and 65-70mm and weight of 10,30,45,60 and 70g at the end of the first, second, third, fourth and fifth years respectively.

Tracing the growth hatchery produced *P.fucata* in the farm at Tuticorin Harbour revealed that specimens attained a size of 47.0, 64.5 and 75.0mm and weight of 8.3, 31.6 and 45.4 g at the end of the 1st, 2nd and 3rd year respectively. The estimated longevity of pearl oysters is about 5.0-5.5 years in natural beds whereas it can live up to seven years when reared in the farm.



Distribution

In India *P.fucata* were found along the east and west coast especially in the pearl banks of Gulf of Mannar ("paars") and in Gulf of Kutch (Khaddas) respectively. The Black-lip pearl oyster *P. margaritifera* famous for black pearls is distributed only in Andaman & Nicobar Islands while occasionally 1: lakha/ per *P.fucata*. Other species found along Indian coast are *P.chemnitzii, P.sugillata, P.anomioides* and *P.atropurpurea*. They are also distributed in the Red Sea, Persian Gulf, China, Korea, Japan, Indonesia, Australia, Margarita, Africa and Venezuelan Islands. This cosmopolitan species are found in the Persian gulf, Red Sea, Seychells, Philippines, Thailand, Malaysia, Indonesia, Micronesia, South Sea Islands and Cook islands . French Polynesia, Papua New Guinea, Gulf of California, Gulf of Mexico, Panama Bay and Peru.

Pearl formation

In salt-water lakes and rivers, mussels produce pearls. Natural saltwater (also called oriental) pearls are formed when sand or hard particle entered inside into an oyster. The irritant settles in the soft body of the mollusc between the shell and mantle, finally that particle being entrapped into the epithelial layer. The oyster secretes a (Extra pallial fluid containing calcite and aragonate chrystals called nacre), which solidifies to avoid the irritation and smoothen the foreign body. Nacre secretion surrounds the particle ("nucleus or core material") and it gives the iridescent color to the precious pearl.

Pearl sac theory

Most accepted theory to be known as the pearl-sac theory explains how a pearl is formed when the pearlsecreting cells of the mantle migrate into the body of the oyster under the stimulus of a foreign body (undischarged eggs of the oyster; sand grains got into the shells and formed pearls; and that parasites or other eggs or other organic matter formed the core of the pearls); and form a pearl-sac that secretes nacre which gets deposited on the foreign body and in course of time a pearl is produced according to the shape of the foreign body. The pearls produced by the oysters through the implantation of a nucleus along with a mantle piece. They have become highly desired because of their uniformity in size and shape. Most of the world's cultured pearls are produced in Japan, Australia and the South Seas.



Implantation and rearing

A skilled technician opens the live oyster, makes an incision in the gonad (A, B, C & D positions) and inserts a nucleus. The nucleus usually made from fresh water mussels 'pig-toe "shells. Following the insertion, the oyster is placed in a tray of water to rest. In Indian waters, the deposition of nacre on nuclei is much faster than in sub-tropical and temperate waters. The duration of post-operative culture varies from four to eighteen months depending on the size of nucleus and the maturity of pearl. When a 3 mm nucleus is introduced in an oyster, it takes a minimum of 4 months for the pearl to attain maturity and it is 5-7 months for a 4mm dia nucleus. Floating rafts are used for farming oysters in the open sea. In shallow sheltered bays, racks are employed. In rack system which is a fixed structure, teak wood poles are driven vertically into the sea bottom and a rack is constructed by lashing horizontal and cross poles on them with coir ropes at a convenient height of 0.5 m above the water level so that the rack thus erected remains always above the water. The oyster cages are suspended from the wooden frame.

Implantation technique

The healthy adult pearl oysters are anesthetized using mentholated seawater in closed containers or pegging in between shells with wooden pieces. A passage is made from the base of the foot towards the gonad of the host oyster without damaging any of the vital organs of the oyster, for which it is mounted on an oyster stand. After that skillful surgery, the mantle piece (graft tissue) and the shell bead (nucleus) are implanted into the gonad (through the passage already made) to lie in contact and proper orientation. The oysters are maintained in the laboratory for two to three days for convalescence with sufficient fresh seawater and aeration for healing of the wound. The care is given to form the pearl-sac over the nucleus by implanted grafted mantle piece to get a good quality pearl from each oyster.

¹⁸⁶ CMFRI - Winter School Course Manual on "Recent Advances in Breeding and Larviculture of Marine Finfish and Shellfish". 30.12.2008 - 19.1.2009





Cutting of mantle for graft preparation













Useful tips in pearl culture: Site selection, mother oyster culture, implantation, post -operative care and pearl production process

Selection of suitable sites	Areas with Salinity above 30ppt, Good food (Phytoplakton) availability, Mild/ moderate current, Low siltation, Depth 2-3 m and above
Selection of oysters for operation	Age of oyster - 1.5-2.0 years, Weight - >25g (40mm), Stage of maturity - Spent resting stage, Overall health - Good, free from polychaets/ sponge, trematode infection.
Conditioning.	Arranging oysters in a container with their hinge pointing downwards. Narcotization of selected oysters by sprinkling menthol in the seawater Insertion of a small wooden peg between the two valves to facilitate nucleus implantation.
Preparation of Graft Tissue	Select healthy non narcotized oyster cut mantle into thin strips of 5 cm length and 0.5 cm width Remove mucous and muscle from the mantle cut the mantle strip into 20 to 25 pieces of 2-3 mm squares keep cells live by adding Azumin/Eosin solution in sterilized SW and use within 15 minutes.
Implantation	Mounting oyster in the stand with the valves facing upwardsMaking incision at the right place and placing the graft mantle piece. 1.Single implantation - in the gonad near the intestinal loop, 2. Double implantation - additionally, close to hepatopancreas, 3. Multiple implantation – more than double implantation. Placing the sterilized nucleus on the graft mantle piece.
Convalescence	Placing the implanted oysters in fresh seawater with mild circulation for two to three days. Maintaining water quality by water exchangeRemoving dead oysters and shifting the healthy implanted oysters into the natural environment.

Harvesting

The pearls are harvested by cutting and separating the two valves and squeezing out the pearl from the gonad of the oyster. The harvested pearls are washed in distilled water, polished with concentrated salt solution and again washed in distilled water wiped with soft cloth and dried and stored. The percentage of pearl production varies with efficiency of the operation, environmental and health conditions of the animal.

Pearls are measured in millimeters across the diameter of the pearl.

Once removed from the oyster, pearls are first sorted by size and then by colour and shape. Because they are created by nature, pearls will have their own birthmarks-blemishes or irregular surfaces. The pearls are graded as "A" with spherical and good luster, "B"some times a pimple like spot with good shining and all the characters of "A" and "C" with more teeth.

Pearls with only a single blemish will be used for ear rings. If a pearl has blemishes on opposing sides, a hole will be drilled through the middle and used in necklaces.

Characterizing pearl quality

Seven key factors define the quality of pearl. Here's how each one affects the value and beauty of pearl jewellary.

1. Lustre

Lustre is the direct result of the amount of nacre used in the formation of the pearl. It's what gives a pearl its unique glow and beauty.

2. Size (diameter)

As with most gems, the larger the size of a pearl, the greater its value. Pearls are generally between 3mm and 10mm in diameter.

3. Colour

The color is largely determined by the species of oyster that produced the pearl. Different colors are more highly desired in different cultures around the world. This creates a market where all colours, such as cream, off white /ivory, rose, gold, gray and golden yellow, pea-cock hue are equally in demand. The value of the pearl is then determined by the demand in particular parts of the world. Pearls can be dyed or bleached with medicinal dyes to enhance their look; black, gray and blue pearls from the South Seas come by their color naturally, which makes them extremely valuable.

4. Nacre thickness

The thickness of nacre affects the durability and sometimes the beauty of a pearl. A thick nacre will sustain more handling and normal wear. A thin coating can be more easily worn away, thereby exposing the nucleus of the pearl.

5. Blemishes/ Spotting

Perfect pearls are extremely rare. The value of a pearl is determined by a lack of blemishes and other imperfections. Blemishes affect the value of pearl.

6. Shape

The most desirable pearls are perfectly round or spherical in shape.

7.Consistency

Consistency in size, colour, shape, blemishes and transparency help to define the value of a pearl/ pearl strand.

8.Drilling

The quality of the drilling in the pearl/ pearl strand is also important. Pearls that are drilled off center lowers its value.

Options in pearls

Marine pearls includes

South Sea pearls /Black pearls : South Sea pearls are both very rare and valuable. They are quite large, ranging from 10-22 mm in diameter, compared with a typical cultured pearl of 2-10 mm. Black pearls are cultured in the South Pacific, primarily Tahiti.

Seed / Blister pearls : Created by farm-raised oysters when sand particles enters the shell during feeding and is surrounded by nacre.

Mother of pearl : A secretion from the oyster that is thinner than nacre and protects the mollusc from its own shell. Its iridescent layers are used in decorative purposes.

Mabe pearls : Image pearls. Placing an image, flat side againt the shell of the oyster, produces Mabe. Mabe are used for making high fashion jewellary

Fresh water pearls includes

Rice pearls	:	Produced in China, these naturally occurring pearls have a long, irregular, dented shape resembling Rice crispiest.
Biwa pearls	:	Originating in Japan's Lake Biwa, these naturally occurring pearls are larger and flatter than the Chinese pearls, with a fine colour and luster.
Cultured fresh water pearls	:	These fresh water pearls cultivated to produce a smooth, more uniform pearl. Fresh water mussels produce them.



Marketing.

In the international market pearls of larger size are highly valued. India is importing pearls worth Rs.29 crores every year. The major countries involved are Bahrain, Hongkong, Japan, the UAE and the U.K. In the present condition in India some private companies have produced pearls and sold internally and exported very little. China produces 118 types of value added products from pearl oyster and shells.

Import of Raw Pearls by India

Role of CMFRI

Based on the packages developed, CMFRI has been offering regular training to officials from State Government, Universities, Research Institutes, Krishi Vigyan Kendras, industry and progressive

farmers on pearl oyster hatchery, pearl culture and SCUBA diving for studying the under water ecology of pearl oyster beds and resources. India is offering pearl culture training to candidates from other nations. Recently CMFRI has conducted First Indian pearl congress and exposition during 5-8th February, 2003 inviting all the pearl workers in the country to discuss and sort out the problems encountered in the pearl research and production of pearls in India.

Transfer of technology and Economics of pearl production

Pearl culture training given in National and International level to 11 South East Asian countries, Egypt and Bahrain.

Advances in Pearl culture in India

Many attempts were made over the years to entice oysters to produce a greater number of high quality pearls. But success didn't come until the 20th century. In early 1900 Japanese scientists discovered a method of inducing pearl growth that gave rise to the modern cultured pearl industry. Now India, the proud owner of a technology developed for tissue culture pearl production.

In-vitro pearl production: First time in the world, India has developed an indigenous technology for in-



Mabe Pearl production

vitro pearl production in Indian pearl oyster Pinctada fucata and the abalone Haliotis varia.

Coloured pearl production (Make-up pearl production)

CMFRI is also in a proud position in the production of make-up pearls. Iron and manganese are the two salts using for the production of these coloured pearls. Iron added oysters will produce violet and mercury coloured pearls while manganese enriched will make the pearls with light orange to grayish orange colour.



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