Seed production and Hatchery management of Pearl oyster

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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.

- Pearls are highly esteemed as gems for their beauty and splendor.
- These structures are secreted by the mantle (i.e., the skin) of pearl oysters in response to irritations caused by external or internal stimuli such as sand grains, molluscs eggs, parasites, detritus, and other foreign particles.
- India has one of the highest demand for pearls for setting in jewelry, and is particularly famous for its pearl oyster resources which yield superb pearls.
- The pearl oyster fisheries are located in two main areas: 1) in the Gulf of Mannar off Tuticorin coast and 2) in the Gulf of Kutch on the northwest coast of the country.

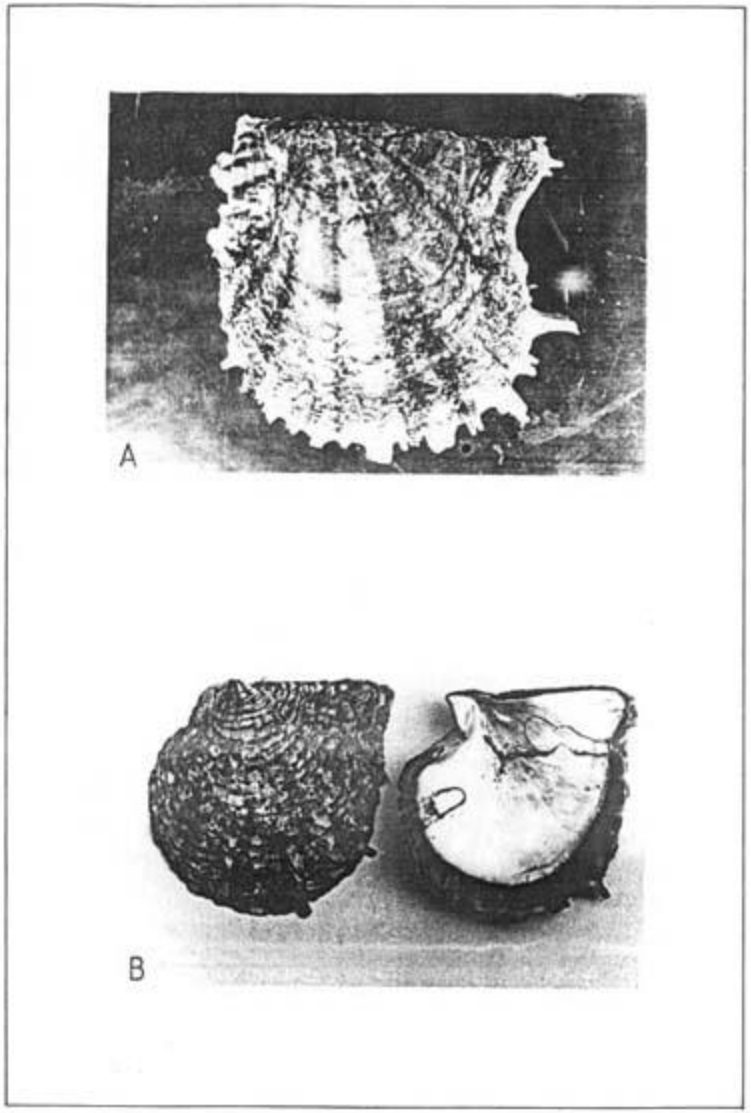
- The CMFRI succeeded in artificially spawning Pinctada fucata, rearing of larvae, and producing seed in the laboratory by hatchery techniques.
- This breakthrough is very important in light of the difficulty in obtaining sustained supplies of oysters from natural banks for culture purposes.
- CMFRI also produced seed of the black-lip pearl oyster, Pinctada margaritifera which produces the highly valuable black pearl.

Taxonomy

* Phylum : Mollusca
* Class : Bivalvia
* Order : Pterioidea (Suzuki, 1985); Mytiloidea (Richard, 1985)
* Sub-order : Pteriaceae
* Family : Pteriidae
* Genus : *Pinctada sp*

- Six species of pearl oysters, Pinctada fucata (Gould), P. margaritifera (Linnaeus), P. chemnitzii (Philippi), P. sugillata (Reeve), P. anomioides (Reeve) and P. atropurpurea (Dunker) occur along the Indian coasts.
- ***Pinctada fucata***
- The hinge is fairly long.
- Hinge teeth are present in both valves.

- **<u>Pinctada margaritifera</u> (Linnaeus)**
- The hinge is shorter than the width of the shell and has no teeth.



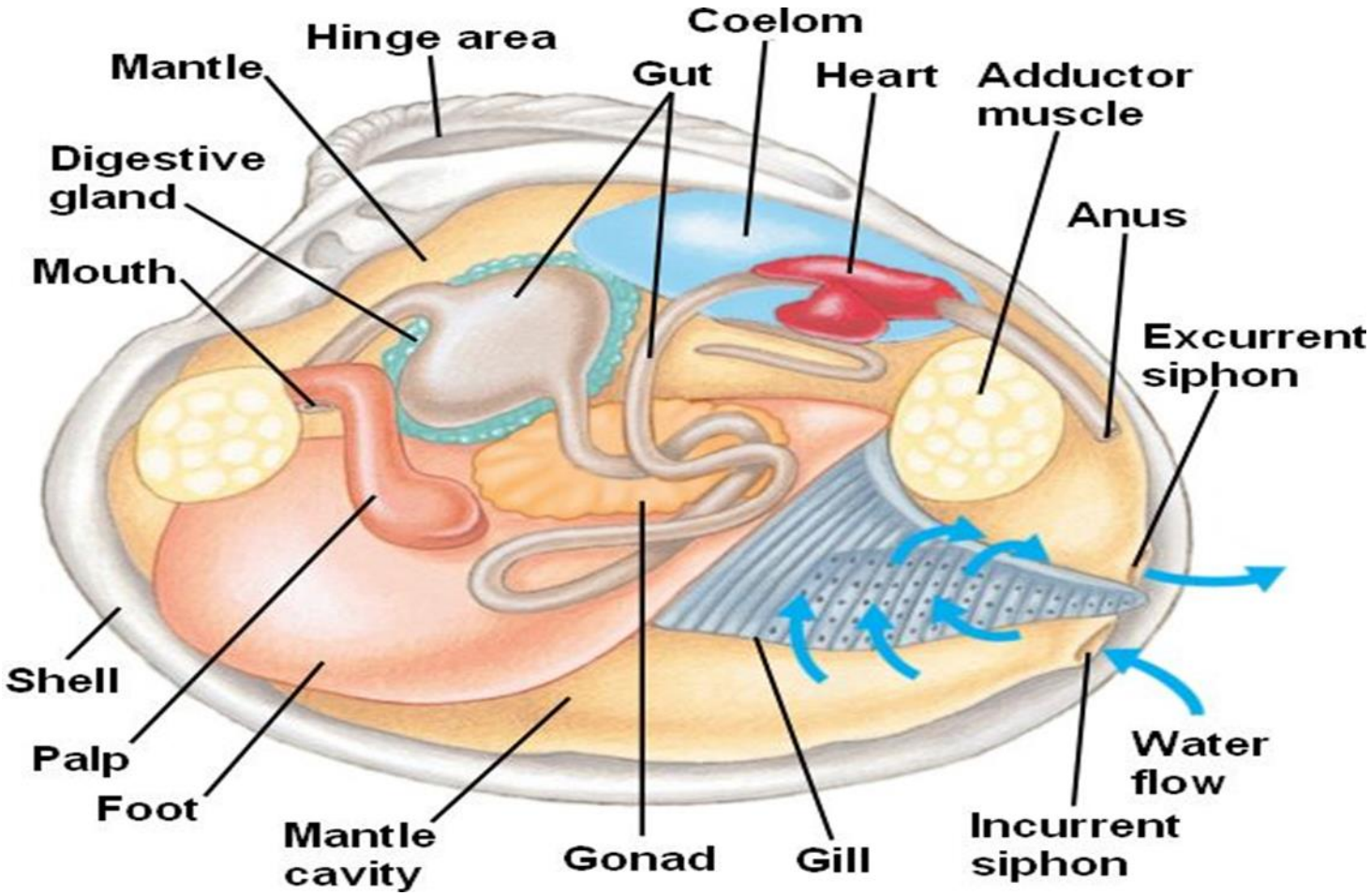
(A) <u>Pinctada fucata</u> and (B) <u>Pinctada margaritifera</u>.

Distribution

- In the Indian waters six species of pearl oysters occur but only P. fucata has contributed to the pearl fisheries in the Gulf of Mannar and Gulf of Kutch.
- In the Gulf of Mannar, the pearl oysters occur in large numbers on the submerged rocky or hard substrata known as paars. The paars lie at depths of 12 to 25 m off the Tuticorin coast along a stretch of 70 km.
- In the Palk Bay, P. fucata occurs sporadically on loose sandy substratum attached to submerged objects in littoral waters.

- In the Gulf of Kutch, the pearl oysters are found as stray individuals on the intertidal reefs known as khaddas.
- In the southwest coast of India at Vizhinjam, Kerala coast, large numbers of spat of P. fucata have been collected from mussel culture ropes.
- The blacklip pearl oyster, P. margaritifera is confined mostly to the Andaman Islands where it is common in some places.

Anatomy


Hinge area
Coelom
Mantle
Gut
Heart
Adductor muscle
Digestive gland
Anus
Mouth
Excurrent siphon
Shell
Palp
Foot
Water flow
Mantle cavity
Gonad
Gill
Incurrent siphon

Reproduction

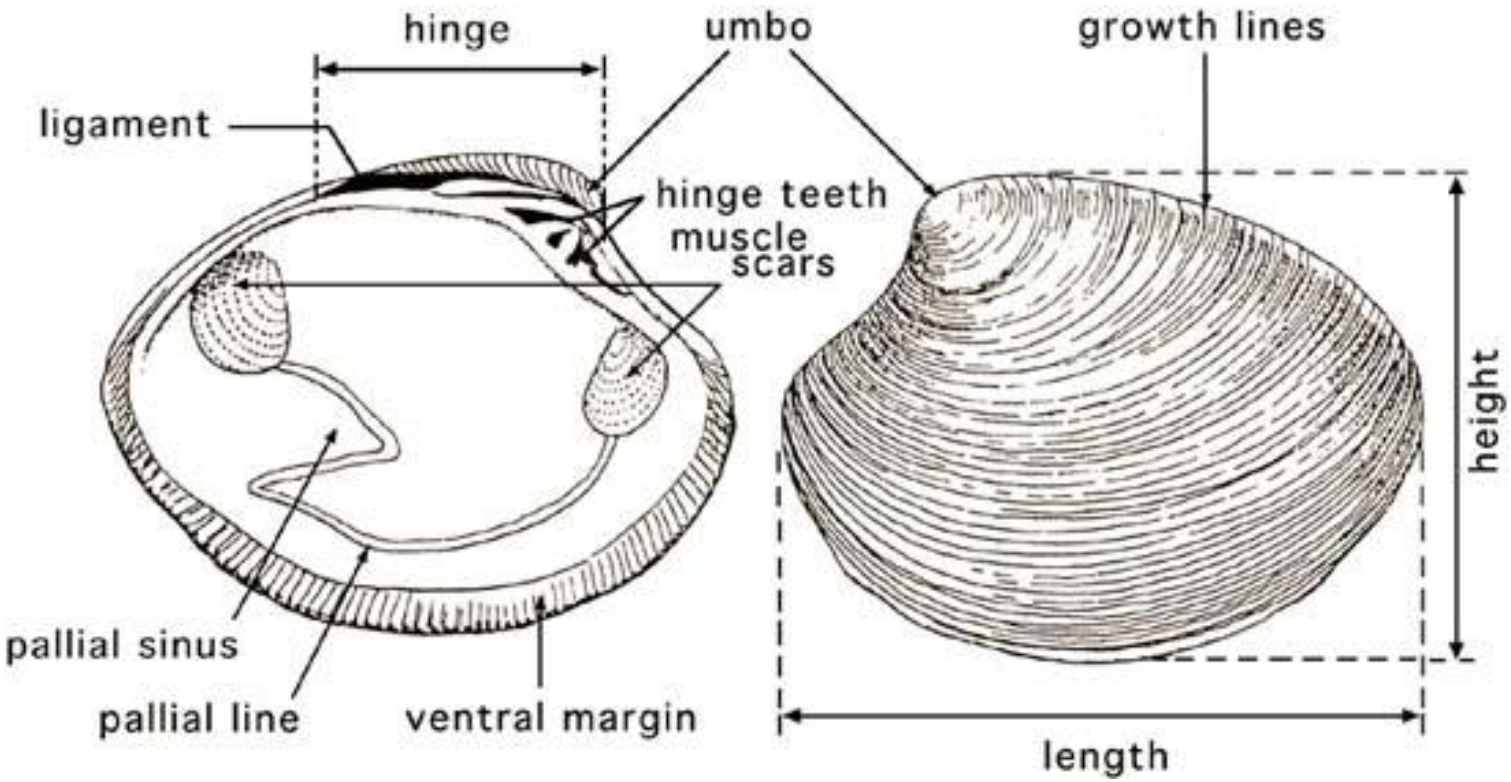
* In pearl oyster, the sexes are separate, but the males and females cannot be distinguished from the characters of the shell.
* Pair of gonads (testis in male n ovary in female) placed over the intestine and hepatopancrease.
* The reproductive system consists of a pair of gonads, which spreads superficially over the hepatopancreas and intestine in mature state.
* In the Gulf of Mannar, the spawning season is June-August and November- January coinciding with the southwest and northeast monsoons respectively.

* The male and female gonads are indistinguishable from external appearance in the initial stages.
* Both are creamy yellow in color. In the mature stage, the male gonad is pale creamy and the female gonad yellowish creamy.
* It is pale yellow in color in male and is of a deeper shade in the female.

- The gonads of the two sexes consist of branched tubules made up of large number of compartments called, the alveoli. The spermatozoa and ova develop in these.
- The accumulated ripe gametes fill these alveoli and tubules and later pass into three trunks which converge into one which leads to the external genital aperture.
- Pearl oysters spawn twice in a year.

Maturity stages

- Matures at around 40 mm dorso-ventral midline length after 1 yr.



- Pinctada fucata from the Gulf of Mannar has two peak spawning seasons in a year: June-September and November-December, coinciding with the southwest and northeast monsoons, respectively.
- A slight rise in water temperature may be considered as the stimulating factor for the onset of the gametogenic cycle and a slight reduction in water temperature stimulates the oysters to spawn.

- **Stage 1: Inactive/spent/resting**
- The gonad is completely shrunken and translucent. In some cases it is pale orange in colour. Large vacuolated yellow (fat) cells are seen in the interfollicular spaces. The sex at this stage can hardly be distinguished.
- **Stage 2: Developing/maturing**
- The transparent nature of the resting gonad is lost and it becomes distinguished from other visceral masses. Gametogenic materials begin to appear in the gonad. As the stage advances, the gonad begins to branch.

- The gametes begin to proliferate along the follicle wall.
- In advanced stages, the inter-follicular spaces become reduced and the lumen of the follicle may contain some free oocytes.
- The majority of the oocytes are irregular in shape and the germinal vesicle (nucleus) is not distinctly seen.
- The average size of the oocytes is 60.0 × 47.5 µm and the germinal vesicle, if present, is 20.0 µm.

- **Stage 3: Mature**
- The gonad spreads on to most of the visceral tissues. It is mostly yellowish cream. The lumen of the follicle is filled with free oocytes.
- The average size of the oocyte is 68.0 x 50 µm with a well defined germinal vesicle. The mean diameter of the nucleus is 25 µm.

- **Stage 4: Partially spawned**
- The gonads become loose in consistency and the visceral epithelium becomes dull. The follicles shrink with the reduction of gametes in the lumen.
- The oocytes are free and found along the follicular wall. Most of the oocytes are spherical and nucleated. The average size of the oocyte is 51.7 µm.

- **Stage 5: Spent**
- The gonads shrink further with a few left over gametes in the lumen of the follicles.
- Ruptured follicles are seen in some cases and the lumen sometimes contains ruptured cells. Oocytes, if present are few and spherical. The average size of the oocytes is 54.4 µm.

Life Cycle Of Pearl Oyster

* Each female can released million of eggs into the water.
* Fertilization is external.
* Eggs hatch and the oysters larval pass through various stages during which they remain swimming freely into the water.
* The length of the larval life varies with the temperature, food and other environmental factors.
* Just before settling, larval developed into a pair of eyespot & a foot.

Life Cycle Of Pearl Oyster

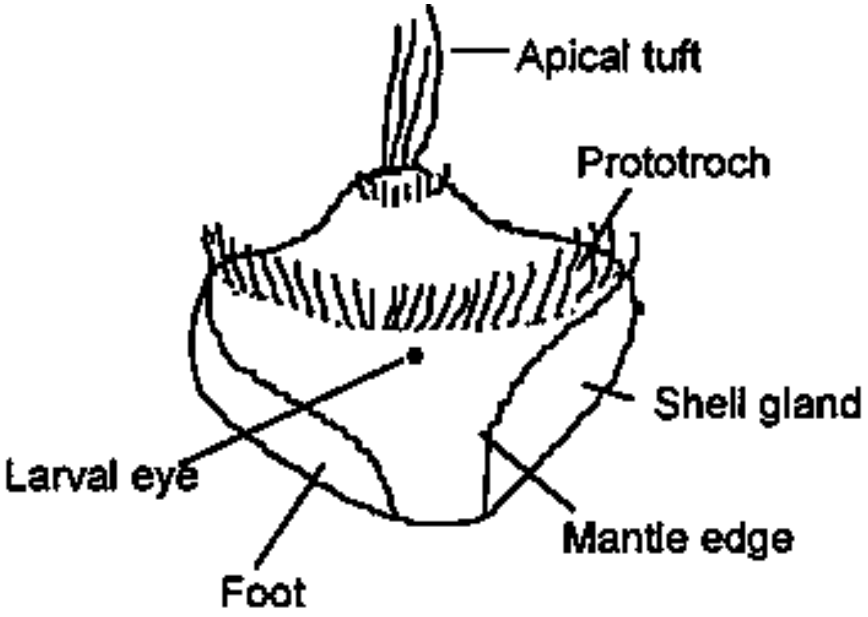
* This period is probably the most critical period of a oyster life unless a suitable substratum is available for attachment.

* After 25-35 days of ages, larval stages spending more time crawling on the bottom & finally metamorphose into juvenile pearl oyster.

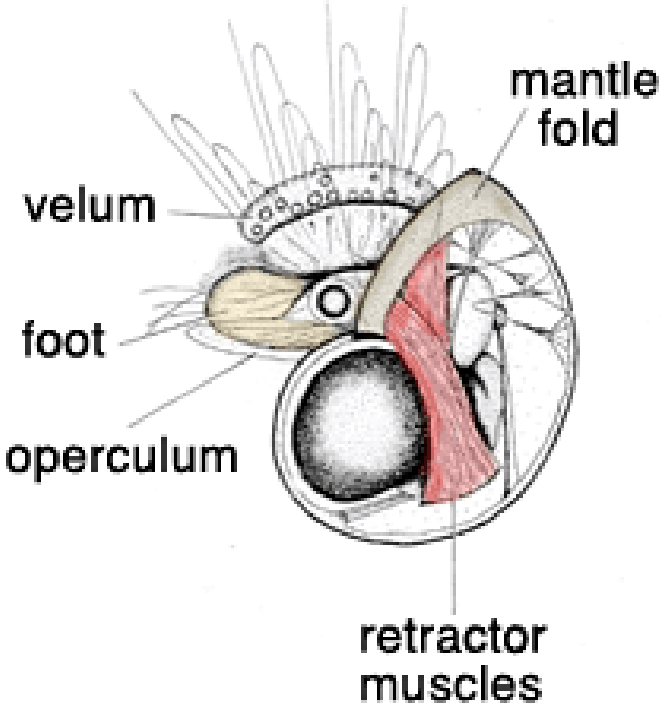
- **Cleavage**
- The first cell division is seen 45 minutes after fertilization resulting in the formation of a micromere and a macromere.
- During the second cleavage the micromere divides into two and the macromere divides unequally into a micromere and macromere. The stage with three micromeres and a macromere is called **Trefoil stage.**
- The macromere does not take part in further divisions. Micromeres divide repeatedly thus becoming smaller and smaller and passing through 8-cell, 16-cell, and so on until the **morula stage**. Each micromere develops a small cilium which helps in the movement of the embryo.

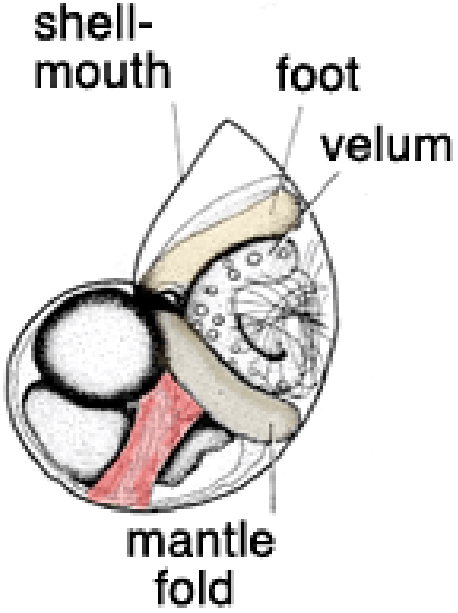
- **Blastula**
- The embryo is ball-like with transparent cells and a blastocoel. The embryos lift themselves in the water column and congregate at the surface.
- The blastula stage is reached 5 hours after fertilization.

- **Gastrula**
- **Different dermal layers and archenteron are formed.**
- The gastrula exhibits negative photrophism. This stage is reached in 7 hours.
- **Trochophore larva**
- The minute cilia present in the gastrula stage disappear and the **pre-oral and post-oral tufts of cilia** develop, thus marking antero-posterior differentiation of the embryo.
- A single **apical flagellum** is developed at the anterior side. The anterior portion of the larva is broader while the posterior end is tapering like an inverted triangle.
- The movement of the larva is affected by the propulsive movement of the flagellum. The dorsal ectodermal cells secrete the embryonic shell, known as the **prodissoconch I.**



Trochophore

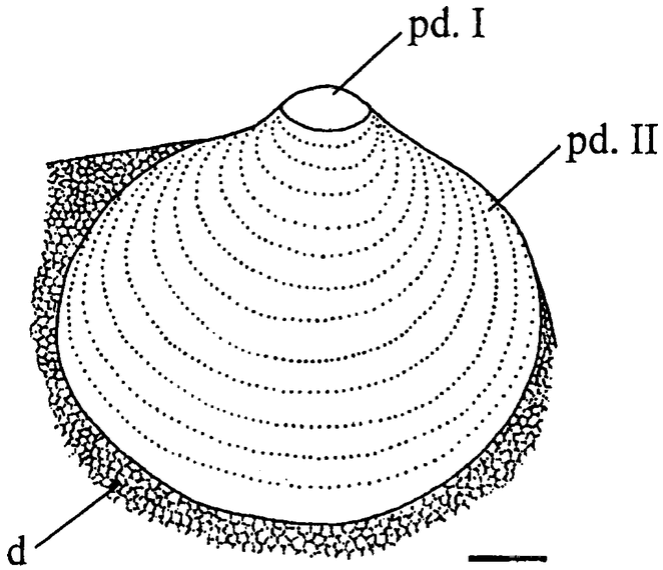




Veliger

- **Veliger**
- A definite ‘D’ shape is obtained by the secretion of the prodissoconch I having a hinge line, mantle and rearrangement of the pre-oral tuft of cilia into a velum.
- The single flagellum, pre-oral and post-oral tufts of cilia disappear. The veliger larva measures 67.5 µm along the antero-posterior axis and 52.5 µm along the dorso-ventral axis.
- This stage is reached in 20 hours.

- **Umbostage**
- Secretion of additional shell material called prodissoconch II.
- Shell valves are equal and mantle fold also develops.



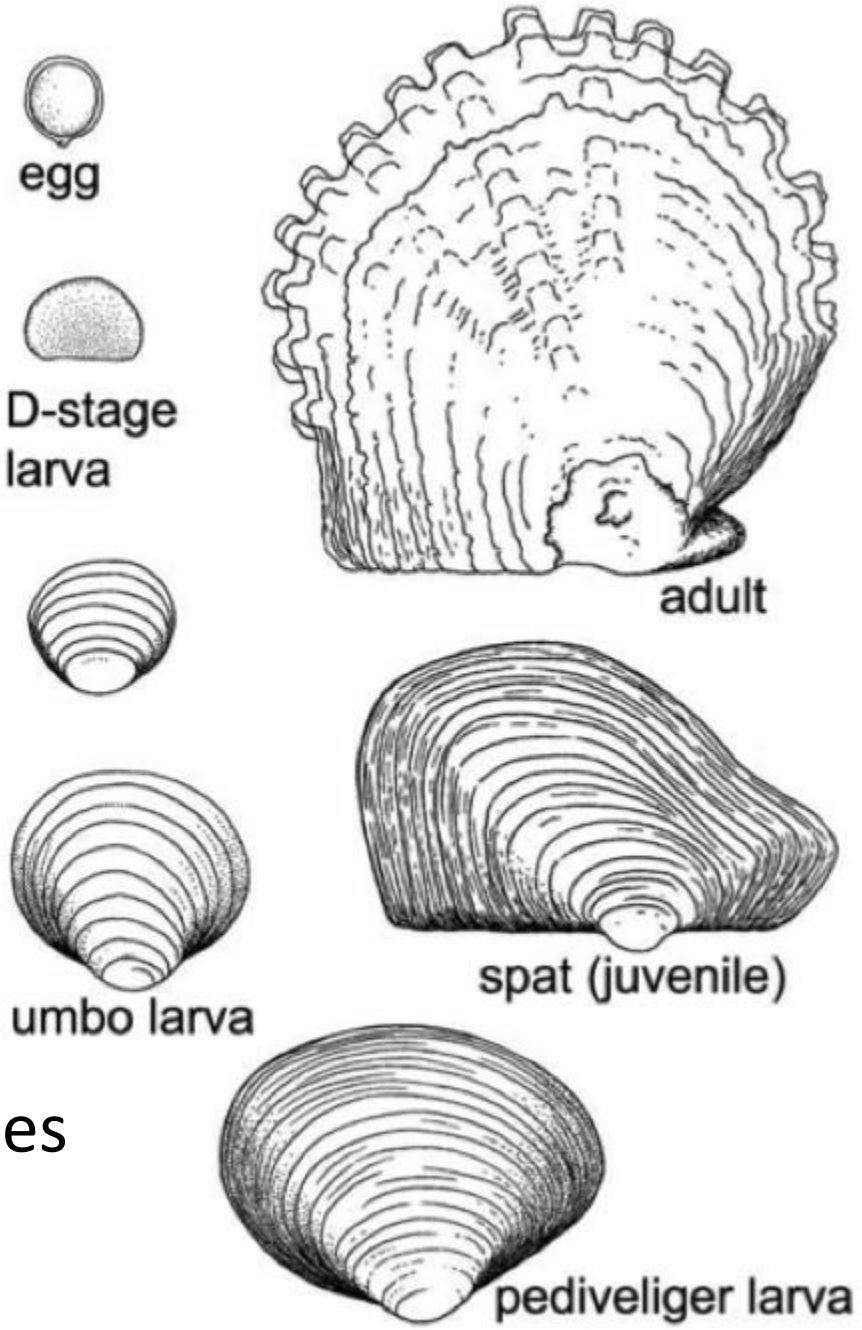


Fig showing different stages of shell secretion

P. *Margaritifera* life stages

- **Eye spot stage**
- After attaining the full umbo stage, the larvae develop an eye spot at the base of the foot primordium. A well developed velum effects the movement of the larvae.
- The minimum size at which the larva develops the eye spot is 180 x 170 µm usually in 15 days.

- **Pediveliger stage**
- The foot is developed on the 18th day when the larvae measures 200 × 190 µm. The transitional stage from the swimming to the crawling phase has both velum and foot.
- Later the foot becomes functional while the velum disappears. Gill filaments are now visible

- **Plantigrade**
- When the pediveliger larva selects a substratum for settlement, additional shell growth is seen along the globular shell margin except at the vertex of the umbo region, in the form of a very thin, transparent, uniform conchiolin film.
- In the meantime the byssal gland secretes byssal threads for attachment.
- Labial palps and gill filaments develop.
- The stage is reached in 20 days at the size of 220 × 200 µm

- **Spat**
- By the repeated addition of dissoconch, the plantigrade transforms into a spat. It resembles an adult.
- The left valve is slightly more convex than the right one. The spat attaches itself to the substratum with the aid of the byssal threads. A healthy spat measures 300 µm in 24 days.
- For collecting spats different substrates can be kept in peak seasons.

Materials used for spat collection

- **Lime-coated tiles**
- **Oyster shell strings**
- **Coconut shell string**
- **Strips of corrugated roofing asbestos**
- **Asbestos sheet**
- **Rope collectors**
- **Synthetic filamentous spindle**

Hatchery technique

❖ **Brood-stock holding**

- FRP tanks of 50 L capacity is used for holding the ripe oysters in aeration and temp 22 -25 ℃.
- They are fed with a mixed algal containing 80% *Chaetoceros* diet at a ration of 4 L culture/ oyster/day.
- The algal food is supplemented by raw corn flour at 30 mg per oyster/day.
- Pearl oysters with maturing gonad fed with the above food for 45 days will spawn with a 30 % response.

❖**Induced spawning unit**

- Fully ripe oysters spawn by thermal induction or chemical induction.
- **Thermal stimulation**
- Spawning tank of size 76 * 46* 46 cm is filled with freshly filtered sea water.
- It is provided with an immersion heater which is thermostatically controlled with thermometer.
- About 50 – 100 oysters from conditioning tank is released into the tank.
- Water temperature is gradually increased to 35℃.This induces spawning. Usually male releases sperms first then female.

- **Chemical stimulation**
- Spawning tank is provided with sea water and pH is raised to 9 – 9.5 using Tris buffer or NaOH. They usually spawns in 1-2 hrs.
- After spawning parents are removed.
- Fertilisation takes place in water and fertilized eggs settle in the bottom.
- Fertilized eggs are collected using 30 µm mesh and released into FRP tanks of 1 ton capacity filled with freshly filtered sea water.

❖ **Larval rearing units**

- About 20 hrs after fertilization, trochophore larvae that hatches out becomes free swimming veliger.
- Veliger larvae is stocked in separate FRP tanks 2 larvae per ml.
- Tank should be covered by thick black cloth to avoid light and dust.
- First day no feeding is required.
- From day 2, microalgae *Isochrysis galbana* @ 5000 cells/larva/day is fed.

- Water exchange once in two days with 45 µm mesh.
- This is continued till 10th day.
- Between 10th and 12th day veliger reaches umbo stage.
- At this time feeding is doubled and 80 µm is used for water exchange.
- On 15^{th} day umbo stages reaches eyespot stage.

- Water exchange can be done with 100 µm mesh.
- On day 18, when pediveliger stage is reached feeding 15000 cells/larva/day.
- On day 20 plantigrade, water exchange 140 µm mesh.
- On day 24 spat appears; isochrysis 20000 cells/larva/day up to day 30.
- Water exchange using 180 µm sieve.
- Spat will settle to the sides and bottom of the tank. Feeding 30000/cell/spat/day and aeration.

- On day 45th spat size increases to 1 mm and feeding dose 50000 cells/spat/day. Up to day 60.
- At this time Isochrysis is mixed with Chaetoceros in ratio 50:50.
- At size 2-3 mm only mixed algae is given.
- By 90th day it reaches to 3-5 mm and is transformed to outdoor farms.

*Rearing methods:

*Raft culture

- Raft culture is considered to be one of the most suitable farming methods in sheltered bays.
- A raft of 6×5 m in size can be easily constructed and floated with 4 buoys.
- Unit raft system is found to be convenient and well suited to the Indian sea conditions.
- Rafts are moored with anchors at opposite sides with tested quality chains.

Raft culture method

*Rack culture system:

- Rack culture system is very much suitable for areas of shallow depths ranging from 2-5 m.
- Generally the racks are rectangular in shape. A working plat form at few places of the rack can also be provided by 6-8" wooden planks.
- A rack of 30 sqm can hold about 100 cages.

Long line culture system:

- Long line culture system is practiced in the open sea where the depth is more.
- Long line is primarily constructed with a long synthetic rope (15-20 mm) with two main floats on both the ends.
- Depending on the length of the long line smaller floats is also attached to the mainline at 5 m interval.
- Vertical lines are arranged in 1- 2 m apart from the horizontal main line.

- **Onshore tank culture**
- Large concrete tanks of 75 to 150 tons capacity and of 3 m depth.
- Culture cages are made up of synthetic fabric of dimension 40 cm * 40 cm * 10 cm.
- Frame is made up of mild steel rod. One cage can hold 40 -50 oysters.
- Growth

- 1st yr – 41 mm (8.3 gm)
- 2nd yr – 64 mm (31.6 gm)
- 3rd Yr – 75 mm (45 gm)

Thank you