

CHAPTER 18

INDUCED BREEDING AND SEED PRODUCTION OF MOLLUSCS

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INTRODUCTION

Study on the hatchery breeding of oysters dates back to early 19th century. In 1880 W.K.Brookes of John Hopkins University had studied the development of eggs and early larval stages of the American oyster *Crassostrea virginica*. J.A.Ryder in 1883 and F.Winslow in 1884 made unsuccessful attempts to bring oyster larvae to metamorphosis. In 1920 W.F.Wells of the New York Conservation Commission succeeded in rearing the oyster larvae to setting which opened door for further development of hatcheries. Later, Wells also succeeded in rearing the larvae of the mussel *Mytilus edulis*, the clams *Mercenaria mercenaria* and *Mya arenaria* and the scallop *Pecten irradians*.

In the mid-1940s Dr.V.L.Loosanoff, H.C.Davis and other colleagues in the U.S Bureau of Commercial Fisheries Laboratory at Milford, Connecticut, U.S.A. developed the techniques for induced spawning and rearing of larvae of commercially important molluscs and production of micro algal feed for the larvae. These developments have led to the establishments of commercial hatcheries along the Pacific and Atlantic coasts of U.S.A and the Maritime Provinces of Canada, the U.K., France and Japan. The technology was useful for the temperate and sub-tropical species. The technology for tropical species succeeded in French Polynesia for oyster and mussel (AQUACOP, 1979) and the Central Marine Fisheries Research Institute in India for the pearl oyster (Alagarwami *et al.*, 1983). The decline stock of pearl oysters and uncertainty of the resources in the traditional pearl banks , the consequent reduction in the availability of seeds for farming led to the formation of a research project at the Central Marine Fisheries Research Institute on the development of hatchery technology for the production of seed of pearl oyster in 1978 which gave results in 1981. Subsequently a hatchery system has been developed for the seed production of molluscs such as the edible oyster in 1982, clams in 1-1988, mussels in 1988 and abalones in 2000.

MATURATION AND SPAWNING

Higher temperature promotes the development of gonad, maturation and spawning. The pearl oysters in the Gulf of Mannar have two spawning seasons coinciding with north-east and south-west monsoons. Maintenance of brood stock with active reproductive phase is possible to produce seeds round-the-year. Therefore, the hatcheries develop the brood stock by temperature manipulation for gonad maturation and then for spawning. The edible oysters in shellfish hatcheries in the U.S.A., Canada and Europe are reared in water temperature from 10°C to 24°C in steps for 3 months. They are induced to spawn in 28°C.

In tropical country like India, the annual temperature of sea water ranges from 25°C to 32°C. Availability of rich food provides the energy for reproductive growth and breeding can be induced with some manipulation of temperature between 25°C and 32°C. To obtain larvae both natural and induced spawnings are used. Fully mature oysters spawn spontaneously on many occasions when they are brought from the natural beds or from the farm and placed in sea water. At all times the males initiate spawning first. The chemical substance present in the sperms stimulates other oysters to release their gametes. In case the gravid oysters are not responding to spawning, they are induced to spawn by thermal, chemical and physical means.

INDUCED SPAWNING

Various stimuli such as thermal shock, pH changes, injections and exposure to hydrogen peroxide (H_2O_2), ammonium hydroxide (NH_4OH), potassium chloride (KCl), addition of gonadal products are effective in induced spawning and obtaining viable gametes.

By thermal stimulation: The inducement of spawning by heat stimulation is a practical method which is successfully used in aquaculture programmes in obtaining viable gametes. Mature pearl oysters are conditioned in a air-conditioned room at 22°C for 12 h. When these oysters are brought to hatchery and immersed in sea water of 28°C, the increase in temperature induces the oysters to spawn. If the oysters are not responded at 28°C, the water temperature is gradually increased to 34°C. The increase in temperature causes spawning. As soon as spawning is noticed, the oysters are transferred from the medium to normal sea water to save the sperms and eggs from heat stress.

The edible oysters (*Crassostrea madrasensis*) with full ripe gonad are conditioned in the conditioning room at 22°C as brood stock. These oysters are from placed in 50 l filtered sea water in a Perspex tank with temperature 2° to 4°C above the ambient temperature. This is achieved by operating a silica cased immersion heater which is controlled by a thermostat. Proper aeration is provided in the tank. The rise in water temperature from ambient level stimulates the ripe oysters to spawn. If the oysters are not spawned by this method, sperm suspension is given to all the oysters individually through their inhalant Water current. The chemical substance present in the sperm

stimulates the oysters to spawn. Nayar *et al.*, 1988 experimented spawning in *C.madrasensis* conditioned by feeding with mixed phytoplankton, micro green alga *Chlorella salina* and corn flour. The results are given in the Table 1.

Table 1. Induced spawning in *Crassostrea madrasensis* at different feeds.

Name of feed	Period of conditioning (Days)	No. of oysters tested	No.of oysters spawned	
			Male	Female
Mixed algae	14.5	25	6.9	32
Chlorella salina	13.3	25	2.6	0.8
Com flour	14.6	25	2.8	1.6

The venerid clams (*Meretrix meretrix*) popularly known as the great clam are kept in the conditioning room in water in FRP tank. They are fed intensively with *Isochrysis galbana* once a day. The water temperature ranges from 24°C to 26°C. After 10-15 days they are given thermal shock in the hatchery by raising the water temperature 4-5°C above the ambient level and no spawning occurred. When they are shifted back to conditioning room spawning took place. Under similar conditions the blood clam *Anadara granosa* was also made to spawn. Kanno (1965) was able to induce spawning in *Anadara* sp. by repeated heat stimulation. The clams were conditioned at 14° to 20°C for 7 days in water circulating tanks. They are stimulated with heat at 21° C within 180 minutes.

The windowpane oysters (*Placuna placenta*) collected from the Tuticorin Bay are kept in a FRP tank containing 500 I filtered sea water with 28°C for 24 h. They were given thermal shock by immersing them in the preheated sea water. The temperature of the water was 37°C. Males initiated spawning first and then followed by females after 45 min. Thermal stimulation was employed on mussels. Spawning could be induced in *Mytilus edulis* by a sudden rise of temperature from 7°C to 15°C. Rao *et al.*, (1976) obtained spawning in *Perna viridis* by raising the temperature from 26.5°C-28.0°C to 32°C-35°C. Hrs-Brenko (1973) was successful in conditioning mussel for spawning by raising the temperature from 1°C to 18°C within 13 days. The tropical scallop *Pecten ziczac* was induced by rapidly raising the water temperature from 20°C to 29°C. The animals have been previously kept at 20°C for 12 h. (Velez *et al.*, 1990).

By Chemical Stimulation

1. **Effect of hydrogen peroxide (H₂O₂):** The Indian pearl oyster *Pinctada fucata* was induced to spawn by using different concentrations of H₂O₂ viz., 1.532, 3.064 and 6.128 mM. The oysters were acclimatized for 12 h. before testing. The oysters were kept in the experimental medium for 2 h. and then transferred to isothermal fresh sea water. 18.2 % spawning occurred after change over in the concentrations of 3.064 mM and 6.128 mM H₂O₂. (Table 2, 3)

Table 2. H₂O₂ induction of spawning in the pearl oyster *Pinctada fucata*

Treatment	No. of oysters		
	Tested	Spawned	
		Male	Female
H ₂ O ₂ , (pH 8.1 - 8.2 control)	37	0	0
H ₂ O ₂ , 1.532 mM	37	0	0
H ₂ O ₂ , 3.064 mM	37	3	1
H ₂ O ₂ , 6.128 mM	37	2	2

(Source: Alagarwami *et al.*, (1983))

Morse *et al.*, (1976) reported that the H₂O₂ is an inexpensive chemical used for the control and synchronous induction of spawning and reproduction in molluscan species.

Table 3. H₂O₂ induction of spawning in abalone.

Treatment	No. of oysters			
	Tested		Spawned	
			Male	Female
H ₂ O ₂	14	17	14	17
H ₂ O	15	13	0	0

(Source: Morse *et al.*, (1976))

Effect of H₂O₂ in TRIS buffer medium: The alkaline medium of TRIS buffer is used to increase the action of H₂O₂. Out of the above three concentrations of H₂O₂, the concentration 3.064 mM resulted in 62.5 % spawning after the oysters were changed to fresh sea water at the end of 4 h treatment.

Morse *et al.*, (1978) reported that addition of TRIS buffer, though not essential for induction of spawning, acts to increase the proportion of animals that will spawn in response to a given concentration of peroxide. They found that TRIS at pH 9.1 was effective in the case of abalones.

H₂O₂ can be used conveniently to induce broadcast spawning in all the following species of abalones, mussels, oysters and scallops of current importance to global mariculture. (Table 4 & 5)

Molluscs induced to spawn with H₂O₂

1. Red abalone	-	<i>Haliotis rufescens</i>
2. Green abalone	-	<i>H. fulgens</i>
3. Pink abalone	-	<i>H. corrugata</i>
4. Bay mussel	-	<i>Mytilus edulis</i>
5. Sea mussel	-	<i>M. californianus</i>
6. Mangrove oyster	-	<i>C. rhizophora</i>
7. Purple hinged rock scallop	-	<i>Mini gigantesus</i>

Table 4. Induction of spawning in pearl oyster *Pinctada fucata* by H₂O₂ in alkaline medium of TRIS

Treatment	No. of oysters		
	Tested	Spawned	
		Male	Female
TRIS, pH 9 .1 (control)	16	0	1
TRIS, pH 9.1 + H ₂ O ₂ , 1,532 mM	16	0	0
TRIS, pH 9.1 + H ₂ O ₂ , 3.064 mM	16	3	7
TRIS, pH9.1 + H ₂ O ₂ , 6.128 mM	16	0	0

(Source: Alagarswami, *et al.*, 1983)

Table 5. Induction of spawning in abalone by H₂O₂ in alkaline medium of TRIS

Treatment	Female abalone	
	Tested	Spawned
H ₂ O (pH-7.8) 2	0	
H ₂ O + H ₂ O ₂ 0.2mM	2	0
H ₂ O + H ₂ O ₂ 1.0mM	2	0
H ₂ O + H ₂ O ₂ 4.2mM	2	1
TRIS (6mM, pH 9.1)	2	0
TRIS + H ₂ O ₂ 0.2 mM	2	0
TRIS + H ₂ O ₂ 1.0 mM	2	1
TRIS + H ₂ O ₂ 4.2 mM	2	2

(Source: Morse *et al.*, 1976)

Effect of H₂O₂ in Alkaline Medium of NaOH

Sodium hydroxide is as effective as TRIS buffer in facilitating induction of spawning in pearl oyster. At pH 9.0 and at 6.128 mM of H₂O₂ combination only 9.5 % of oysters spawned whereas at control pH 9.0 47.6 % oysters spawned. (Table 6)

Table 6. Induction of spawning in pearl oyster *Pinctada fucata* by H₂O₂ in alkaline medium of NaOH

Treatment	No. of pearl oysters		
	Tested	Spawned	
		Male	Female
NaOH, pH 9.0 (control)	21	5	5
NaOH, pH 9.0+H ₂ O ₂ - 1.532 mM	21	0	0
NaOH, pH 9.0+H ₂ O ₂ - 3.064 mM	21	0	0
NaOH, pH 9.0+H ₂ O ₂ - 6.128 mM	21	2	0

(Source: Alagarwami, *et al.*, 1983)

Effect of TRIS Buffer: Sea water with pH values of 8.5, 9.0, 9.5 and 10.0 was prepared using TRIS buffer. Normal sea water with a pH of 8.1 - 8.2 was kept as control. A total of 94 oysters were tested in TRIS solution and 27 oysters in normal sea water as controls. The duration of immersion in TRIS medium was 3-4 h. A maximum of 78.6 % oysters spawned in pH 9.0 and 39.3 % in pH 9.5. No spawning occurred in the control (pH 8.1-8.25) and in pH 8.5. (Table 7)

Table 7. TRIS induction of spawning in pearl oyster *Pinctadafucata*

Treatment	No. of oysters		
	Tested	Spawned	
		Male	Female
H ₂ O, pH 8.10-8.25 (control)	27	0	0
TRIS, pH 8.5	28	0	0
TRIS, pH 9.0	28	4	18
TRIS, pH 9.5	28	9	2
TRIS, pH 10.0	10	2	0

(Source: Alagarwami, *et al.*, 1983)

Effect of Sodium Hydroxide (NaOH)

Sea water with a pH of 8.5, 9.0, 9.5 and 10.0 was prepared using NaOH and a total of 67 oysters was tested in these concentrations. The normal sea water with a pH of 8.0 and 8.1 was kept as control. 68.4 % oysters spawned in pH 9.5. (Table 8)

Table 8. NaOH induction of spawning in pearl oyster *Pinctadafucata*

Treatment	No. of oysters		
	Tested	Spawned	
		Male	Female
H ₂ O, pH 8.0-8.1 (control)	20	0	0
NaOH, pH 8.5	19	0	0
NaOH, pH 9.0	19	0	0
NaOH, pH 9.5	19	6	7
NaOH, pH 10.0	10	0	0

(Source: Alagarwami, *et al.*, 1983)

Injection of Ammonium Hydroxide

A dilute solution of 0.1 N NH₄OH was prepared. A total of 47 pearl oysters were treated with injections of 0.1, 0.2 and 0.3 ml of the NH₄OH solution at the adductor muscle. Controls were kept without injection. 48.1 % oysters spawned in 0.2 ml injection. (Table 9)

Table 9. Induction of spawning by the injection of 0.1 N NH₄OH solution

Treatment	No. of oysters	
	Injected	Spawned
Injection of 0.1 N NH ₄ OH - 0.1 ml	10	0
“ - 0.2 ml	27	13
“ - 0.3 ml	10	0

(Source: Alagarwami, *et al.*, 1983)

Sagara (1958) has induced spawning in the clam *Meretrix* by injection of NH₄OH 1/20 N in the gonad. Iwata (1948 a,b) has succeeded in spawning several species of clams by injection of 2 ml neutral potassium salt solutions into the visceral cavity. Velez (1990) reported spawning induction of the tropical scallop *Pecten ziczac* by injection of 0.4 ml (2.0 mM) of serotonin in the gonad and the adductor muscle.

Table 10. Injection of serotonin in the tropical scallop *Pecten ziczac*

Expt. No.	Treatment	No. of animals		
		Tested	Spawned (%)	
			Male	Female
1	Temperature	20	5	5
	Serotonin	20	55	0
	Temperature + Serotonin	–	–	–
	Control	20	0	0
2	Temperature	15	25	33
	Serotonin.	15	100	0
	Temperature + Serotonin	15	100	0
	Control	15	0	0

(Source: velez,et al., 1990)

Chemical stimulation was carried out by a single intragonadal injection of 0.5ml of 2mM serotonin solution to ripe windowpane oyster *Placuna placenta* broodstock. 15 females and 15 males were individually injected with 0.5ml serotonin solution and another 30 were injected with 0.5ml of filtered seawater only as controls at the gonad. The animals were individually kept in 500ml of filtered seawater of 34ppt at 30°C. Ripe oysters spawn 15 - 30 min. after serotonin injection. Ladjja(1997) experimented with 16 animals in 30 l. rectangular plexiglass tank at 1 : 3 male: female ratio. U.V.treated filtered seawater was added up to the 30 l. mark. 16 animals were also kept in a similar container with filtered seawater only. A maximum of 10h. exposure time was allowed. No aeration has given. The ripe oyster started spawning in 30 - 60 min. after exposure to U.V. light - irradiated seawater (Table 10).

Development of Larvae and Production of Seeds

In India, the development of hatchery system for the breeding and rearing of molluscan larvae was achieved in 1981. The shellfish hatchery laboratory established at the Tuticorin Research Centre of the Central Marine Fisheries Research Institute, Tuticorin is the first of its kind in the country (p1. 1, Fig. 1) and has done a pioneer work in the breeding and rearing of bivalve larvae. Following the success achieved in the seed production of commercially important Indian pearl oyster *Pinctada fucata* in 1981, breeding and rearing of other bivalves like the edible oysters, mussels, clams, windowpane oysters and the gastropods like the abalones, chanks etc., and the sea-cucumber were continued.

The Indian Pearl Oyster *Pinctada Fucata*

Development of larvae: In natural spawning the males initiate spawning followed by the females after 30-45 min. The ripe eggs are pyriform in shape measuring 73.9 μm along the long axis and 45.2 μm in breadth. After fertilization the pyriform egg becomes spherical in shape and measures 47.5 μm in dia. During the process of fertilization first and second polar bodies are released. First cleavage starts 45 min. after fertilization resulting in a micromere and a macromere. During the second cleavage the micromere divides into two and the macromere into a micromere and macromere. The stage with three micromeres and a macromere is called 'Trefoil stage'. The micromeres divide repeatedly and spread over the macromere. The macromere does not take part in further cell divisions. After completion of the cleavage each micromere develops a cilium in about 4 h. by which the embryo rotates itself and moves to water column. The stage is called morula which transforms to blastula with a cavity-the blastocoel and a blastopore. By transformation of cells the blastula becomes gastrula. The gastrula has three dermal layers and an archenteron. Gastrula transforms to trochophore stage in 10 h. The ectodermal cells secrete the first embryonic shell material and form a veliger larva with straight hinge line. It is reached in 18-20 h.

Veliger: The veliger is fed with *Isochrysis galbana* on day 2 onwards. The size of the veliger is 67.5 \times 52.5 μm . The shell valves are equal and transparent with conspicuous granules. (Plate I, Fig. 2)

Umbo stage: The straight hinge veliger larva reaches umbo stage at 135 \times 130 μm in 10-12 days. The straight hinge line disappears and umbo region is formed. The mantle folds develop on the inner side of the shell. The larva swims with velum. (Plate I, Fig. 3).

Eye-spot stage: The eye-spot stage is reached at 190 \times 180 μm on 15th day. The eye-spot is situated at the base of the foot primordium. During the transitional stage from eye-spot stage to pediveliger, the larva has both a velum and a functional foot. (Plate I, Fig. 4).

Pediveliger: A functional foot is developed at 200 \times 190 μm on 18th day. When the foot becomes functional, the velum disappears. (Plate I, Fig. 5).

Plantigrade: The larva attaches itself to the substratum initially by the foot. Now the byssal gland is activated and byssus threads are formed for attachment. Later the foot is drawn inside. Additional shell growth is by the formation of very thin, transparent, uniform conchiolin film all along the globular shell margin except in the vertex of the umbo region. This is the beginning of the formation of the adult shell or the dissoconch (Plate I, Fig. 6).

The stage is reached at 220 \times 200 μm on 20th day.

Spat: By the addition of shell growth the plantigrade transforms to a young spat. The hinge line, anterior and posterior auricles and the byssal notch are formed. A miniature shape of the adult oyster is reached at 300 μm on 24th day. (Plate I, Fig. 7).

Feeding schedule: The feeding schedule of in the Indian pearl oyster *Pinctada fucata* with *Isochrysis galbana* at different stages of development is given below (Table 11).

Table 11. Feeding schedule of in the Indian pearl oyster *Pinctada fucata* with *Isochrysis galbana* at different stages of development.

Stage	Day of development	No. of cells fed per larva per day
Veliger	2 nd day	5,000
Umbo	12 th day	10,000
Eye spot stage	15 th day	15,000
Pediveliger	18 th day	20,000
Plantigrade	20 th day	25,000
Spat	24 th day	30,000

Seed production of *Pinctada fucata*: Seed production was carried out throughout the year and as high as 1.3 million pearl oyster seeds were produced in a single experiment in the hatchery at Tuticorin. But, however during the months May- August, the spat fall was at the minimum level. This was due to the rise in salinity, heavy dust fall and warmer land wind during south - west monsoon. It resulted in the spurt of ciliates in the water medium. Yearwise production of seeds of the pearl oyster *Pinctada fucata* is given below (Table 12).

Table 12. Yearwise production of seeds of the pearl oyster *Pinctada fucata*

Year	No. of seeds produced
1981	1,28,470
1982	5,17,520
1983	2,48,000
1984	6,78,140
1985	40,90,200
1986	30,03,630
1987	5,48,600
1988	7,25,650
1989	3,52,460
1990	3,13,800
1991	2,07,690
1992	2,12,050
1993	5,84,710
1994	3,42,680
1995	3,25,550
1996	4,46,500
1997	2,95,090
1998	2,62,460
1999	6,99,300
2000	3,07,160

Mortality rate tends to be high due to delicate nature and sensitivity of the larvae to changes in environmental parameters. But a survival rate of up to 50% can be achieved under ideal conditions.

Spat /juvenile rearing of pearl oyster *Pinctada fucata*: After settlement the spat are reared in the hatchery for two months and by that time they reach 3-5 mm size. The spat are transferred to farm and reared in a net cage enclosed with a velon screen netting at a density of 10,000 spat each cage. In a period of one month the spat reach 10 mm with 50% survival of the initial stocking density. In the subsequent rearing, mortality is less and the juveniles are reared in net cage of 40 × 40 × 10 cm (Plate II, Fig. 1) At the end of 15 months 30-35% survived up to adult stage. (Plate II, Fig. 2). (Table 13)

Table 13. Details of spat /juvenile rearing in the farm.

Spat size (mm) net	No. of spat per rearing	Months of (%)	Cumulative mortality	No. of survivals	Net size (mesh in mm)
3-5	10,000	1	50	5,00,000	0.5
5-10	5,000	2	20	4,00,000	1.0
10-20	2,000	3	10	3,60,000	1.5
20-30	750	6	5	3,42,000	10

Development of larvae and production of seeds of the black-lip pearl oyster *Pinctada margaritifera* : The black-lip pearl oyster *P. margaritifera* is a precious species for the production of black pearls. (Plate II, Fig. 3) The oysters are available in the oceanic islands like Andaman & Nicobar Islands. Stray specimens of the species occur in the main land in the Gulf of Mannar.

It was bred in the shellfish hatchery at Tuticorin. Profuse spawning occurred. (Plate II, Fig. 4). The spawned eggs were spherical and measured 45 µm. (Plate II, Fig. 5). The early D shaped veliger appeared in 20 h. has a size 75 × 60 µm (Plate II, Fig. 6) The umbo stage is reached at 140 × 130 µm on day 12; eye-spot stage at 210 × 200 µm on day 16; the pediveliger at 220 × 210 µm on day 20; the plantigrade at 260 × 240 µm on day 23 and the spat at 350 × 300 µm on day 28. The laboratory-reared juveniles of 10 mm shell height showed no growth processes (Plate II, Fig. 7). The farm-reared juveniles have prominent growth processes (Plate II, Fig. 8).

Seed production rate: In 1986 the initial larval population stocked in all the culture tanks was 7.75×10^5 in a total volume of 775 l of sea water. Total number of larvae which metamorphosed as spat was 48,800 giving a survival rate of 6.3% and the production rate of 63 numbers per litre. In subsequent years a total of 98,020 seeds were also produced.

Development of larvae and production of seeds of the windowpane oyster: The windowpane oyster *Placuna placenta*, another pearl producing bivalve mollusc, collected from the Tuticorin Bay were bred in the shellfish hatchery in 2000 and seeds were produced for the first time in India.

The eggs of the windowpane oyster were yellow in colour and measure 50 μm (Plate III, Fig. 1). Cleavage starts in 30 min. after fertilization. Morula stage is reached in 4 h. 45 min. The veliger larva is reached in 18 h 45 min. and measured 79.9 and 65.2 μm . in average (Plate III, Fig. 2). The shell valves of the veliger are equal and transparent with conspicuous granules. The veliger larvae are fed with *Isochrysis galbana* at 5000 cells/larva /day. The typical umbo stage is at 140 \times 130 μm on day 4. (Plate III, Fig. 3) and the rate of feeding is increased to 10,000 cells /larva/day. The eye-spot stage is obtained at 210 \times 200 μm on day 5; The pediveliger is reached at the size of 215 \times 205 μm on day 7 and is fed with 15,000 cells/larva /day. The plantigrade stage is resulted at 235 \times 210 μm on day 8 . On day 10 the plantigrade transformed to spat stage at 340 \times 300 μm (Plate III, Fig. 4). As spat grows faster the feeding rate is increased to 25,000 cells/ larval day. The feed is switched over to mixed algae nom day 36 onwards. The hatchery-reared spat are highly transparent (Plate III, Fig. 5) and the farm-reared spat are opaque (Plate III, Fig. 6). In the first experiment a total of 2700 seeds were produced and sea-nanche. Large scale production of seeds is yet to be continued.

Development of larvae and production of seeds of the edible oyster: The spawned egg of the edible oyster *Crassostrea madrasensis* measures 55.7 μm and the fertilized egg is about 60.7 μm . (Plate IV, Fig. 1) The early embryonic development of the larva is similar to *P. fucata* attaining the veliger stage (66 μm) in about 20 h. (Plate IV, Fig. 2). The veliger larvae are stocked in 1 ton tank at 4 larvae/ml. The larvae are fed with *I. galbana* on day 2. On day 7 the larvae reach umbo stage measuring 150 μm . The typical umbo is reached at 260 μm on day 12 (Plate IV, Fig. 3) An eye-spot is developed at 280 μm on day 13 (Plate IV, Fig. 4). A functional foot is emerged at 330 μm on day 18. At this stage the larva can swim or crawl exploring a suitable place to set as spat. The spat setting takes place on day 20. The spat measures 450 μm .

The feeding schedule of *Isochrysis galbana* in *Crassostrea madrasensis* is given below. (Table 14)

Average setting on a given polythene sheet as a substratum for attachment was at the rate of 4 spat per sq. cm. 25-30 oyster shell rens each with 6 to 8 shells are suspended in a rearing tank as spat collectors. When the settled spat grow to 5-10 mm the shell rens are transferred to the oyster farm for further rearing.

Table 14. The cell concentration of larval food at each stage of development

Stages	Day of development	Cell concentration per larva per day
Veliger	1 st day	3000-4000
Umbo	7 th day	4000-5000
Late umbo	15 th day	5000-8000
Eye spot	17 th day	8000-10000
Pediveliger	18 th day	10000-12000
Spat	20 th day	12000-15000

Seed production of *Crassostrea madrasensis*

Rack & string method: The oyster spat attached to shell string are enclosed in velon screen bags and suspended from racks in nursery areas. A string can hold six shell valves containing around 80 to 100 spats. After 40 to 50 days the bags are removed and the strings are transferred to farm areas.

In each rack 90 strings are suspended which occupy 80 sq. m area. In one hectare 125 racks can be constructed. After 12 month each string weighs around 7 to 7.5 kg. The production rate per hectare is estimated as 80 tonnes.

Rack & Tray method: The cultch free spats are reared in rectangular trays (90 x 60 x 15 cm) in a rack. Each rack occupies 25 sq .m and in one hectare area 400 trays can be constructed After 12 months 120 t is produced per hectare.

Stake culture: The collectors with spat *in-situ* are fixed on to a stake by a nail. The production rate is 20 t. per hectare per year.

Development of larvae of mussels: The hatchery technology has been developed at CMFRI for the production of seeds of the mussels *Perna viridis* and *P. indica*. Large scale production of seeds is yet to achieve at hatchery level. The eggs are brick red in colour and measure 45-50 µm. After completion of cleavage the embryo reaches morula stage in about 4 h. and it measures 58-60 µm. The morula starts moving to the water column. The trochophore stage is reached 7 h. after fertilization which measures 65-70µm. The trochophore transfers to D shaped veliger larvae in 20 h. The size of the veliger larva is 70-75 µm.

The veliger larvae of *Perna viridis* are collected, estimated and stocked at a density of 2 larvae per ml. They are fed with *Isochrysis galbana* at 2000 cells/larva /day. The other microalgae such as *Pavlova lutheri*, *Chromulina freibergensis* and *Dicretaria sp.* are also acceptable to the mussel larvae. The veliger transforms to umbo stage at

146 × 120 µm on day 7. Eye-spot stage obtained at 260 × 208 µm on day 13-14 day and the pediveliger at 320 × 280 µm on day 16. The settlement of larvae takes place on day 20. The rate of feeding is increased at every stage of development. The spat is fed at 30,000 cells/ spat day at the time of settlement. Since day 30 the spats are fed with mixed algae consisting of *Chaetoceros*, *Skeletonema*, *Nitzschia* etc.

Development of larvae and production of seeds of the venerid clam *Meretrix meretrix*: The venerid clam *Meretrix meretrix* was bred in the shellfish hatchery at Tuticorin in 1988 (Narasimham *et al.*, 1998) (Plate IV, Fig. 5). The fertilized egg is spherical measuring 75.9 µm in average. The development of larvae is similar to other bivalves up to veliger stage. The veliger measures 116 × 91.3 µm (Plate V, Fig. 1). When it is fed with *I. galbana*, it grows to umbo stage on day 4 (Plate V, Fig. 2). and measures 147.2 × 126 µm. The eye-spot stage is not recorded. The pediveliger is reached on day 6. On day 8 the larva measures 183.7 × 161.9 µm. This can be taken as the average size before the commencement of metamorphosis. On day 13 the size of the post-set clams is 224.2 × 196.8 µm and on day 18 it was 328.4 × 303.8 µm. (Table 16)

The nutritional requirement increases with the growth of larvae. Hence a schedule of feeding has been developed with different cell concentrations depending on the age and size of larvae. The cell concentration of larval food of *I. galbana* has been standardized at each stage of development (Table 15).

Table 15. The cell concentration of larval food at each stage of development.

Stages	Day of development	Cell concentration in ml/larva/day
Veliger	1 st day	5,000
Umbo	4 th day	8,000
Eye-spot	Not recorded	—
Pediveliger	6 th day	10,000
Plantigrade	8 th day	10,000
Spat	10 th day	12,000
Spat	27 th day	15,000

Table 16. Seed production of *Meretrix meretrix*

Expt. No.	No. of larvae stocked	No. of spat produced	Percentage of survival (%)
1.	36,000	5630	15.64
2.	3,72,380	27,500	7.38

Development of larvae and production of seeds of the blood clam: The blood clam *Anadara granosa* was bred in the shellfish hatchery at Tuticorin and the seeds were produced in 1992. (Muthiah *et al.*, 1992)

The eggs are spherical, light pink red in colour and measured 50- 60 μm with an average of 51.9 μm . After fertilization cleavage starts within 10 min. The morula stage is reached 3-4 h., the trochophore in 5 h. and the veliger in 20-26 h. measuring 83 \times 65.5 μm . They are fed with *galbene* at 5000 cells/larva/day. Early umbo measuring 131.6 \times 106.3 μm on day 7 is fed at 7000 cells/larva/day. Feeding is increased to 10,000 cells/larva/day from pediveliger and 15,000 cells/day from spat stage onwards.

Spat rearing of *A. granosa*: The hatchery produced seeds are reared in the fann in cages since 242 mm onwards from day 60. The seeds reach 17. 60 mm in 5 months indicating a survival rate of 93 %. (Table 17)

Table 17. Seed production of *Anadara granosa*

Expt. No.	No. of larvae stocked	No. of spat produced	Percentage of survival (%)
1.	1,56,000	7576	4.86
2.	6,000	514	8.57

Development of Larvae and Production of Seeds of the Abalone

Brood stock of the abalone *Haliotis varia* (Plate V, Fig. 3) was collected from the wild and acclimatized in the hatchery for spawning. After fertilization the eggs settle at the bottom. The abalone eggs are 100-200 μm in dia. and released in 100-200 l cap. tank. A gentle water flow-through is maintained and the outflow of water is screened to prevent embryo loss. The embryo grows to trochophore stage in about 10 h. and the larva measures 180 μm . Trochophore transforms to veliger stage in 12 h. As the larvae of the abalone are lecithotrophic, feeding is not required. The veliger larvae start setting down on substrates. The larva has fully developed cephalic tentacles and eye-spots. The veligers are transferred to tanks with a mat of benthic diatoms like *Nitzschia sp.* and *Navicula sp.* On day 6 the embryonic tubular shell is formed and the larva a flat abalone. On further development, the juvenile stage is reached with a respiratory pore on day 26.

Seed production in abalone: Large scale production of seeds of abalone is yet to be achieved.

TECHNIQUES IN LARVAL REARING

Screening of embryos: In pearl oysters mass spawning is followed to increase the probability of spawning and to ensure obtaining genetically viable larvae from mixed

population. During spawning the water medium becomes milky by the heavy discharge of sperms. The medium contains not only the sperms but also the excreta, shell bits, byssus threads, mucus from mother oysters, unfertilized eggs and other debris. Fertilization is immediate and the fertilized eggs settle at the bottom of the tank. If the eggs are continued to develop, as they are in the medium, contamination is likely to occur after few hours. The life span of sperm is about 2 h. and starts putrifying after 24 h. For proper development of embryos screening of eggs should be done at this stage. During the first phase of screening the sperm laden supernatant water is siphoned out gently without disturbing the bottom samples. During the second phase the bottom samples containing fertilized eggs and other wastes are collected carefully in a sieve of 30 Jim mesh and flushed out repeatedly with filtered sea water to remove the remaining sperms and smaller particles. The filtrate is again passed through 80µm sieve to remove larger dusts. The eggs less than 80µm pass through the sieve. They are collected and released in 500 l. sea water in FRP tank. After 24 h. the embryos develop into a viable veliger larvae which congregate at the surface. The larvae are collected in glass beakers and estimated. The required number of larvae are stocked for production of seeds. The rest of the larvae are either sea-ranched or discarded. A similar procedure is followed in *Meretrix meretrix* and *Anadara granosa*.

In *C. madrasensis*, after initiation of spawning, each oyster is placed separately in glass tray to complete the spawning. Once the spawning is over, the parent oysters are removed to prevent filtering the gametes. The eggs are collected to a 10 l. glass beaker and 50 ml of sperm suspension is added to it. The process of fertilization is observed under microscope. The excess sperm is removed and replaced with fresh filtered sea water. The water containing embryos is released to 40 to 50 l water in a tank for further development.

Water change: The water in the pearl oyster larval rearing tank is totally changed at intervals of 2 days. The tank is first washed in fresh water to kill the marine bacteria and rinsed with sea water (Dharmaraj and Alagarwami, 1997). The larvae collected in a sieve are released back to the tank. The tank is covered with thick black cloth to prevent fall in a dust and sunlight. The schedule of water change promotes larval growth and spat setting to 25 % of the total larval stocked (Alagarwami, 1991).

Water tanks are changed often to prevent any adverse effect from diatoms and bacteria which might adhere to the tank walls. To avoid the growth of such adhering organisms, the brightness inside the tank is maintained at a lower level. Kanno (1965) reported that the survival and growth rate of larvae were better when a part of the water was changed during the course of rearing *Macra sp.* The growth of larvae of European oysters could be improved by merely changing the water tanks. By this the role played by the metabolic products of the adhering organisms on the walls of the rearing tanks is curtailed.

The growth and survival rates are monitored at every water change once in two. 50 numbers of random sample of larvae are measured along the dorsoventral axis and anteroposterior axis. The growth of the larvae is determined by the mean values. In *Perna indica* larvae changing of water daily and changing of rearing tanks in alternative days till settlement gave good results (Appukuttan, 1988).

Effect of larval density: Extensive studies were made on the effect of density in pearl oyster larval growth and metamorphosis (Dharmaraj and Alagaraswami, 1997). Larval growth and percentage of spatfall in different densities in 4 l., 50 l. and 500 l. of sea water are given in the Table 18.

Table 18. Larval growth and percentage of spatfall in different densities in 4 l., 50 l. and 500 l. of sea water

Volume of water(lit.)	Larval density/ml	Initial mean size of larvae (mm)	Mean growth on the day of first spatfall (μm)	Rate of growth per day (mm)	Day of first spatfall	Percentage of spatfall(%)
4	1	64.0	184.6	6.35	19 th	89.7
	2	64.0	190.0	6.63	19 th	99.9
	3	64.0	191.6	6.72	19 th	92.1
	4	64.0	180.9	6.15	21 th	97.1
	5	64.0	163.3	5.23	21 th	71.8
	10	64.0	158.6	4.98	21 th	18.9
50	1	64.8	126.8	3.88	20m	17.6
	2	64.8	141.5	4.79	20th	67.0
	4	64.8	117.8	3.31	23th	14.3
	6	64.8	89.0	1.51	25m	0.2
	8	64.8	81.0	1.01	25m	0.04
500	2	67.5	174.8	7.15	15th	31.6
	3	67.5	125.9	3.89	15th	19.1
	4	67.5	164.8	6.49	15m	14.1
	5	67.5	144.1	5.11	15th	13.6

The study indicated that larvae density of 2-3 larvae /ml is optimum for better growth and good spatfall in *P fucata*. Anuradhakrishnan and Alagaraswami (1987) reported that in *P fucata* the percentage settlement of spat was 8.8, 6.0 and 1.4 in the densities 5, 15 and 20 larvae /ml respectively. The larvae of *Perna indica* at an initial stocking density of 5 ml showed good settlement (Appukuttan *et al.*,1988). Walne (1956) experimented the larvae of *Ostrea edulis* in different densities viz., 0.3 ,0.9 and 1.3 per ml which gave the spatfall of 67.5 ,54.9 and 27.0 % in the respective densities. Dharmaraj and Alagaraswami (1997) reported that the densities 5, 10 and15 per ml resulted in the spatfall of 11.7, 6.1 and 5.4 % respectively. Davis (1953) used 0.6, 2.8, 18.5 and 32.9 concentration of larvae of *Crassostrea virginica* per ml of water and reported that there was an inverse relationship between the concentrations and the rates of growth of larvae. Rao *et al.*, (1993) recommended the optimum density at different stages of larval development of *Crassostrea madrasensis* and given below. (Table 19).

Table 19. Optimum density at different stages of larval development of *Crassostrea madrasensis*

Stage of larvae	Density of larvae per ml
Veliger	5
Umbo	3
Advanced umbo to eyed stage	2
Pediveliger	2

Feeding protocol: Determination of right feed dosage is an important factor in larval rearing. The optimum feed dosage increases the larval growth. The dosage differs as per the rearing water temperature, population density, type of feed and mode of feeding. When the population density is high , the concentration of feed is also high. In such conditions the larvae tend to waste the algal food as pseudofaeces and sending as waste.

Loosanoff and Davis(1963) suggested the micro algal feeds such as *Isochrysis galbana*, *Pavlova lutheri*, *Chromulina pleiades*, *Dicrateria inomata* are the suitable feeds for the larvae of American oyster. Davis and Guillard (1958) proved that the single microalgal feed *I.galbana* and *P. lutheri* are the best feed but also suggested that better results were obtained in a mixture of *Isochrysis*, *Pavlova*, *Dunaliella* and *Platymonas* than a diet of *Isochrysis* and *Pavlova*. They further reported that the larvae of the American oyster at a density of 10 to 15 larvae per mI requires 4,00,000 cells of *Isochrysis* and 2,50,000 cells of *Pavlova* per mI. When the concentration of feed was increased to 5,00,000 cells per ml the growth of larvae was restricted. Loosanoff *et al.*, (1953) reported that the growth of larvae of European oyster was best when the concentration of *Monochrysis* and *Chaetoceros calcitrans* was 30,000 cells per ml.

Dharmaraj and Shanmugasundaram(1999) found that the veligers of *P. fucata* were stocked at a density of 2 larvae per ml and fed with *I.galbana* at different concentrations viz., 1000,2000,4000,5000,6000 and 8000 cells / larval day, the concentrations 4000-5000 cells larval day gave better growth and high spat settlement.

Performance of single food indicated that *Pavlova* fed larvae showed slightly higher growth than *I.ga/bana*. When the larvae fed with *Isochrysis* and. *Pavlova* at 1:1 combination better growth was obtained than in *Isochrysis: Chromulina* and *Isochrysis: Dicrateria* combination. The growth in two species combination was higher than in single food. In three species combination viz., *Isochrysis: Pavlova: Chromulina* and *Isochrysis: Pavlova: Dicrateria* the growth and spat settlement were good in the former. The growth in three feeding was less than in single food.

Davis (1953) reported that *P. lutheri* gave slightly better growth than *I.galbana* in the larvae of *Ostrea edulis*. The food value of *Dicrateria inomata* on the growth of larvae of *O.edulis* was poor than in *Igalbana* and *Chromulina pleiades* (Walne,1956). Dharmaraj and Shanmugasundaram (1999) demonstrated that the percentage of spat settlement was high when fed with *Dicrateria* singly or in combination with other algae.

Dunaliella euchlora and *Dunaliella sp.* found to induce better growth in the oyster larvae after 6 days of development (Loosanoff and Davis,1963). *Dunaliella sp.* gave good growth in the larvae of *Ostrea edulis* (Bruce et al.,1939). The larvae of *P.fucata* neither metamorphosed nor survived beyond 13 days when fed with *Dunaliella sp.* (Dharmaraj and Shanmugasundaram,1999). *I.galhana* results in best growth of the larvae of *C.madrasensis* (Loosanoff and Davis, 1963; Dupuy et al., 1977 and Bruce et al., 1939).

The D shaped larvae of *Perna indica* are fed with *Isochrysis* at 5000 cells/ larva day; eyed stage 11700 cells larval day; pediveliger 17550 cells/larva /day and spat at 30,000 cells of *Isochrysis* and mixed algae combindly. AQUACOP (1983) had given *Isochrysis sp.* and *Monochrysis lutheri* as food for mass production of green mussel *Mytilus viridis* in French Polynesia.

Use of U.V water and antibiotics in larval rearing: In Aquaculture systems the use of U.V.irradiated sea water is advocated to prevent pathogenic bacteria. Waugh (1958) used U.V. irradiated sea water for rearing larvae of the European oyster *Ostrea edulis* with considerable success. Loosanoff and Davis (1963) reported that fungal diseases of young clam *Mercenaria mercenaria* were prevented with the use of U.V.light. Since bivalve larvae are mostly reared in standing water where the water is changed daily, it is extremely important to estimate pathogenic bacteria present in the sea water. Brown and Russo (1979) indicated that U.V. irradiation filtered sea water can be an effective disinfectent if used the proper dose along other sanitary procedures. Walter et al., (1979) showed that the ozone-U. V.quarantine system designed by them provides a reliable and thorough method for complete disinfection of shellfish systems.

Dharmaraj and Shanmugasundaram (1999) studied that the effect of U.V. irradiated sea water on larval growth and spat settlement in the pearl oyster *Pinctadafucata*. It showed that the U.V. treated sea water resulted in relatively lesser growth and spat settlement in *P. fucata* than in non-U.V. irradiated sea water. The U.V. treatment of sea water affects organic material and destroy thiamine which useful to bivalves (Armstrong *et al.*, 1966; Button, 1968).

Walne (1958) reared the larvae of European oyster in water to which antibiotics were added to prevent the bacterial growth. 50,000 U of penicillin and 50 mg streptomycin to one liter of water would accelerate growth of larvae. Larval growth is also favourable with antibiotics such as Chloromycetin, streptomycin and oleomycin etc. But the large scale use of antibiotics is not economical in larval rearing. Loosanoff and Davis (1963) recorded rapid growth of clam larvae in cultures containing about 100 ppm of streptomycin(or combistrep),. about 33 ppm of sulfamerazine(sulmet) and 3 ppm of aureomycin. Hidu and Tubiash (1963) reported that the formation of combistrep consisting dihydrostreptomycin sulfate, streptomycin sulfate, phenol, sodium citrate, sodium disulfate., water etc. consistently resulted in 25-100 % increase in larval growth. Dharmaraj and Shanmugasundaram(1999) found that the growth of larvae of the pearl oyster *P fucata* and spatfall were more in kanamycin at 50 and 100 ppm concentrations. Though the use of streptomycin and crys 4 resulted in good growth and spatfall, there was mortality.

Effect of aeration: Apart from the chemical factors of sea water, mechanical disurbance in larval rearing medium plays a key role in larval development. The necessity for agitation in larval rearing medium for the larval of a given species larvae was determined based on their ecological adaptations. Edible oysters, mussels and clams inhabit shallow waters and therefore the larvae of these organisms were able to adjust to rough conditions. This was practically demonstrated in *C .madrasensis* (Nayar,*et.al.*,1984); mussels *P. indica* (Appukuttan,1988) and great clam *meretrix* (Narasimham1988) where agitation through aeration in the larval rearing medium was essential for better growth,survival and spatfall. The pearl oysters live in deeper waters (15-20 m depth) on sea bed where the disturbance is, minimal when compared to shallow coastal waters.Hence. providing aeration during the larval phase of *P fucata* adversely affected the growth and spatfall.

Effect of colour of FRP tanks on larval growth and spatfall: The colour of the larval rearing tanks was also found to influence the growth and spatfall. The tanks with black colour resulted in more settlement of spat followed by blue and white colour tanks. (Table 20).

Effect of culling on the survival of spat: The effect of culling was not seen on the growth of larvae. In the hatchery the survival rate of spat was 91.3% in non-culled ones;71.2 % in one culling;62.4%in two cullings and 68.2% in three cultings was obtained. On transplantation of the spat to the farm, the spat of non- culled ones suffered mortality but the spat got through culling withstood the farm condition well and more than 50% of survival was obtained here than in the non-culled spat.

Table 20. Effect of colour of larval rearing tanks on growth and spatfall in *P. fucata*

Expt. No.	Colour of tank	Total larvae stocked (in million)	Volume of water (L)	No. of spat settled	Percentage of spatfall (%)
1	Black	4.0	60	70,262	1.8
	White	4.0	60	38,762	0.97
2	Black	3.0	60	71,679	2.4
	White	3.0	60	25,560	0.85
3	Black	11.85	500	86,555	7.3
	Blue	11.85	500	53,000	4.5
	White	11.85	500	42,055	3.6

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PLATE-I



Fig.1. Pearl oyster hatchery at Tuticorin- An inner view.

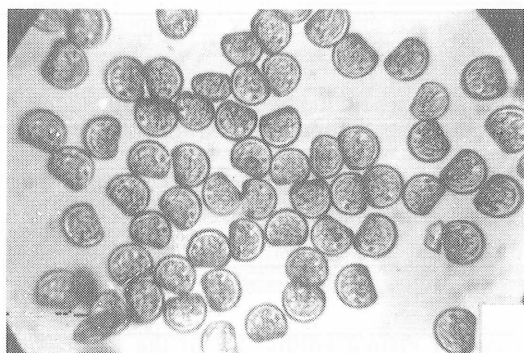


Fig. 2. Straight-hinge larvae of *Pinctada fucata*, size $72.5 \times 57.5 \mu\text{m}$

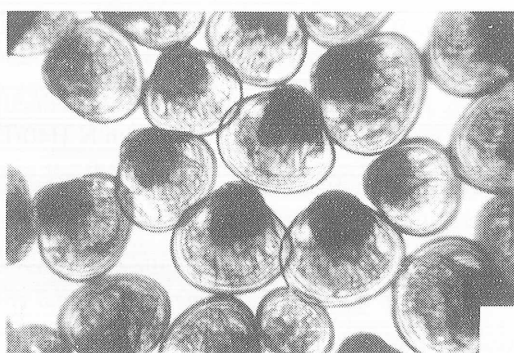


Fig. 3. Umbo stage larvae, size $135 \times 130 \mu\text{m}$.

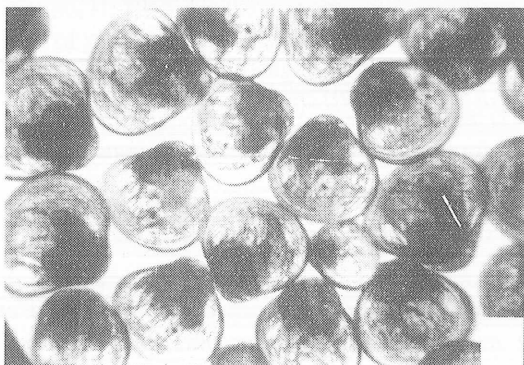


Fig. 4. Eye-spot stage, size $190 \times 180 \mu\text{m}$.

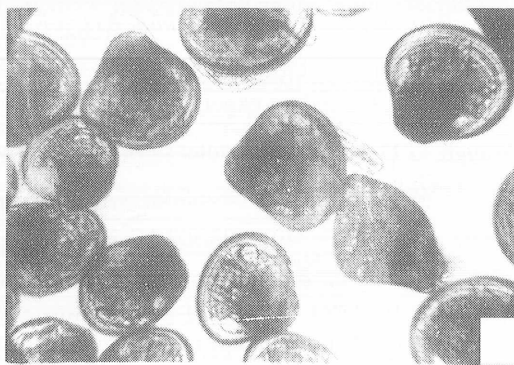


Fig. 5. Pediveliger stage, size $200 \times 190 \mu\text{m}$.

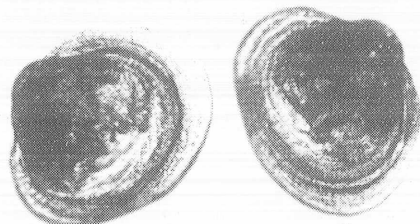


Fig. 6. Plantigrade stage, size $220 \times 200 \mu\text{m}$.

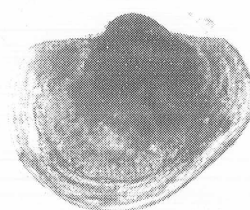


Fig. 7. Spat of *Pinctada fucata*, size $300 \mu\text{m}$.

PLATE-II

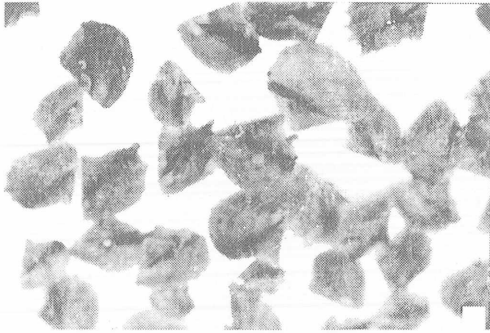


Fig.1. Farm reared juveniles of *Pinctada fucata*.

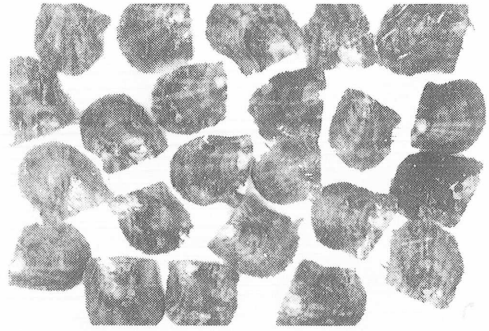


Fig.2. Adult pearl oyster *Pinctada fucata*.



Fig. 3. Brood stock of the black-lip pearl oyster *Pinctada margaritifera*.

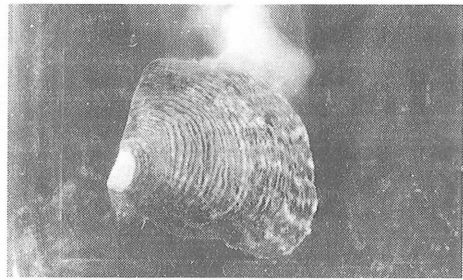


Fig. 4. A spawning male of *Pinctada margaritifera*.

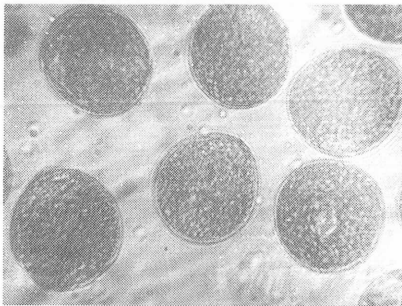
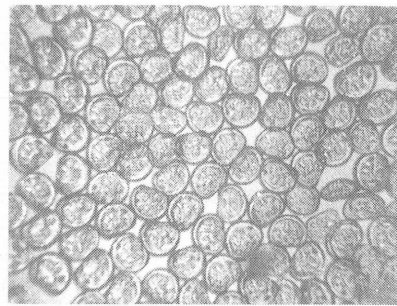


Fig. 5. Fertilized eggs (45/112 dia.) of



Pinctada margaritifera.

Fig. 6. D shaped veliger larva, size 75x60 μ m.

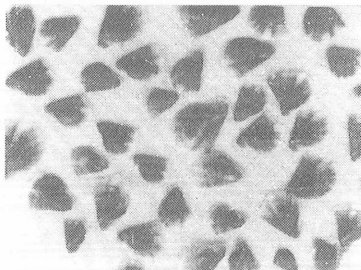


Fig. 7. Laboratory-reared juveniles showing no growth process, size 10 rom in shell height.

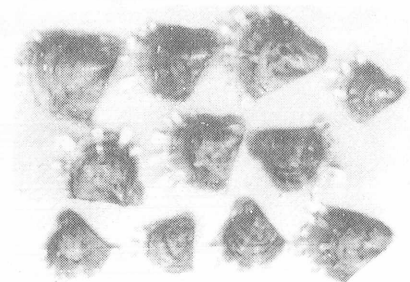


Fig. 8. Farm-reared juveniles with typical shell characters, size 21.1 rom in shell height.

PLATE-III

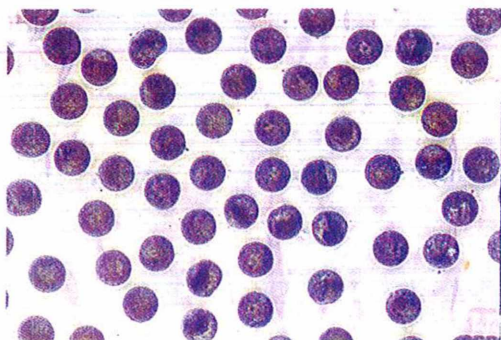


Fig. 1. Spawned eggs of *Placuna placenta*, size 50 μ m

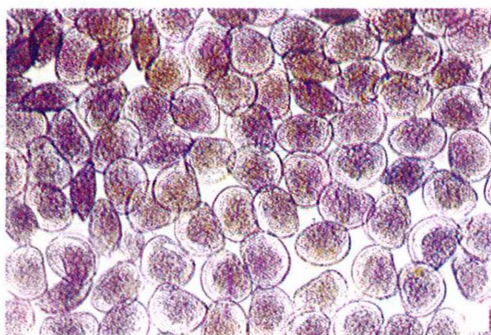


Fig. 2. Veliger larvae of *Placuna placenta*, size 79.9 \times 65.2 μ m.

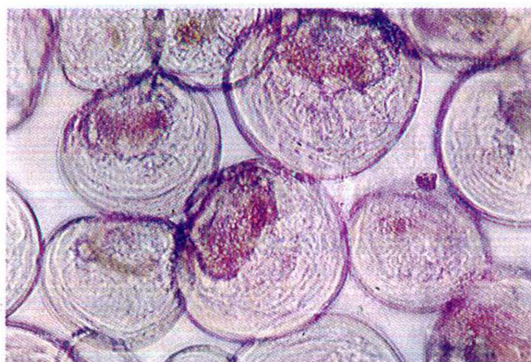


Fig. 3. Umbo stage of *Placuna placenta*, size 140 \times 130 μ m.

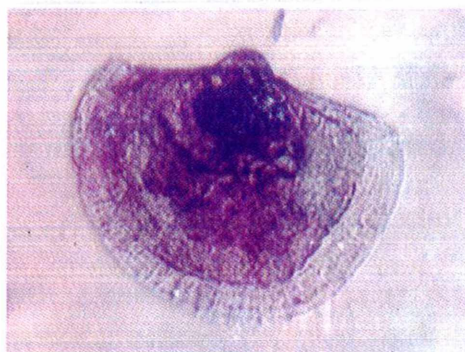


Fig. 4. Spat of *Placuna placenta*, size 340 \times 300 μ m



Fig. 5. Hatchery-reared spat of *Placuna placenta* with transparent shell.

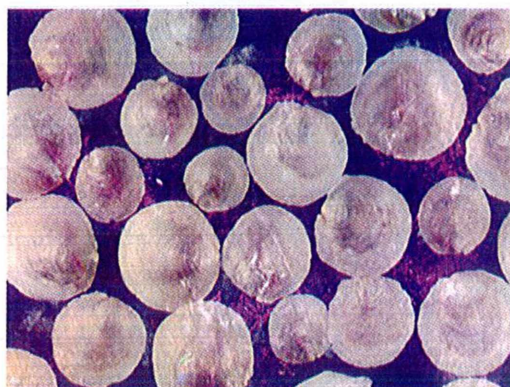


Fig. 6. Farm-reared spat of *Placuna placenta* with opaque shell.

PLATE-IV

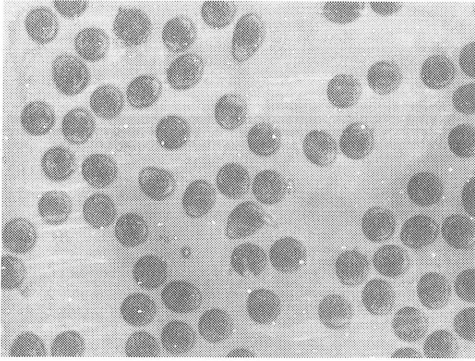


Fig. 1. Spawned eggs of *Crassostrea madrasensis*, size 55.7 μm .

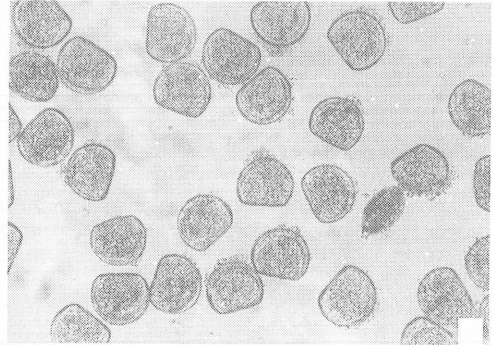


Fig. 2. Veliger larvae of *Crassostrea madrasensis*, size 66 μm .

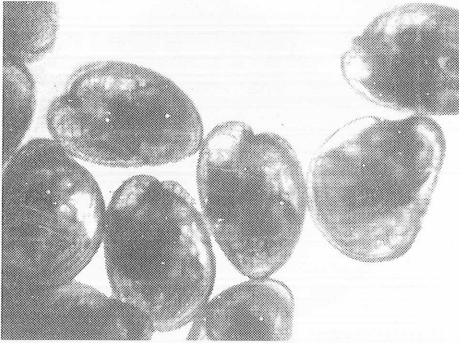


Fig. 3. Umbo stage of *Crassostrea madrasensis*, size 260 μm .



Fig. 4. Eye-spot stage of *Crassostrea madrasensis*, size 280 μm .

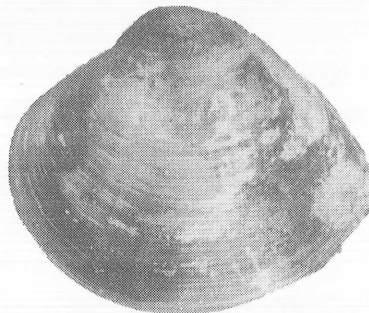
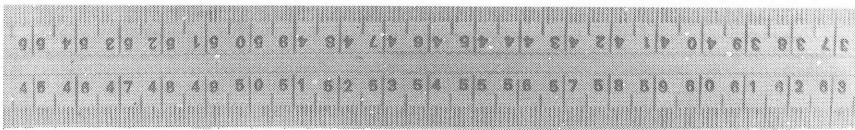


Fig. 5. The venerid clam *Meretrix meretrix*.

PLATE-V

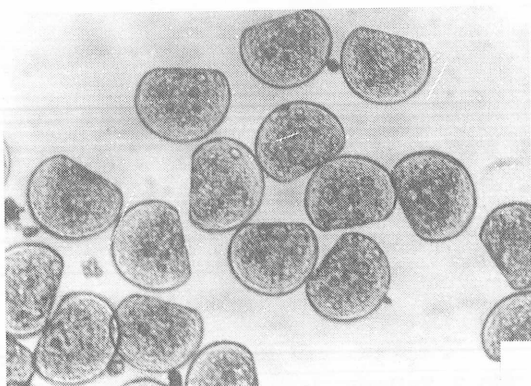


Fig. 1. Veliger larvae, size 116 x 91.3 μm

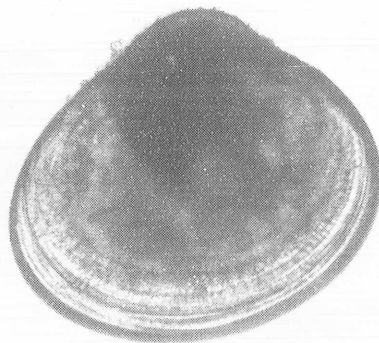


Fig. 2. Umbo stage, size 147.2 x 126 μm .

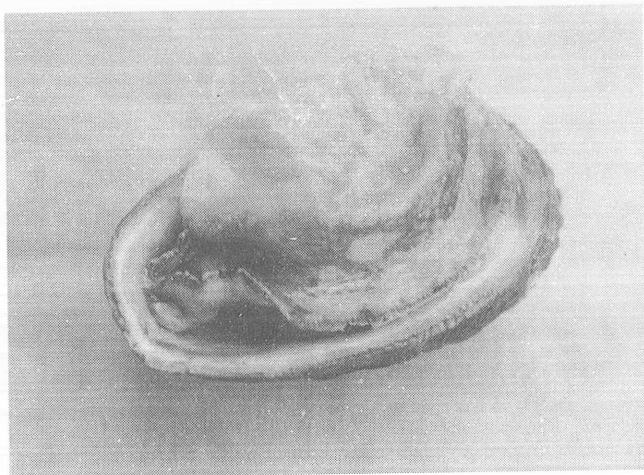


Fig. 3. The abalone *Haliotis varia*.