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CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
(Indian Council of Agricultural Research)
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HATCHERY TECHNOLOGY FOR PEARL OYSTER PRODUCTION

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INTRODUCTION

The successful development of hatchery technology for production of pearl oyster *Pinctada fucata* spat in 1981 at the Central Marine Fisheries Research Institute (Alagarswami *et al.*, 1983 a, b, c) is an important milestone in the progress of research and development of molluscan shelfish culture in India. This is evident from the fact that subsequently, similar developments have been achieved in the production of spat of the oyster *Crassostrea madrasensis* in 1982 (Nayar *et al.*, 1984), green mussel *Perna viridis* (Rangarajan, 1983) and brown mussel *P. indica* in 1983 (Appukuttan *et al.*, 1984). These developments have resulted in the establishment of a moderate shellfish hatchery at Tuticorin which has become capable of mass production of spat of any of these marine bivalves with a certain degree of predictabilitly. Although the larval rearing technology for the bivalves of temperate and sub-tropical regions had been known for over two decades prior to the above developments through the works of Loosanoff *et al.* (1963), Walne (1964), Imai (1977) and others, the achievements in India have established the validity and relevance of hatchery technology for the tropical species and situations. The present paper considers the subject in three parts ; first, it outlines the hatchery system ; secondly, it deals with the experimental results obtained in pearl oyster spat production ; and last, it critically evaluates the technology with reference to its expected role in shellfish research and development.

HATCHERY SYSTEM

Site selection

Many factors are to be taken into account in the selection of a hatchery site. The primary requirement of an operational hatchery is the uninterrupted supply of good quality seawater free from pollutants. The seawater used in the hatchcery should be free from suspended particles and silt and hence the site of drawal of water should be rocky, coralline or sand mixed. It should be away from industrial and domestic sewages. It should not be close to river mouths, the flooding of which will dilute the seawater, resulting in problems during the times of monsoon. Places experiencing dust and hot summer winds are not ideal. Other aspects to be considered are proximity to the natural resource and farm sites and logistics of transplantation.

Hatchery building

The hatchery building should be designed and constructed in such a way as to get maximum light and air inside the hatchery. The roof should partly be provided with translucent fibreglass sheets. This will provide light for the algal culture inside. In order to minimise heat radiation, the height of roofing should be sufficiently high. Glass panelled, large windows with ventilation for free passage of light and air should be provided. Air vents and exhaust fans are required in sufficient numbers. Entry of insects, flies and birds should be avoided by fixing fine wire mesh panels to the air vents. Concrete flooring with sufficient gradient and gutters for easy drainage of water should be provided. Provisions must be given for fresh water supply and power supply inside the hatchery. In case of extremely high ambient temperature airconditioning would help in successful larval rearing.

Water management

Seawater drawn from beyond the low water mark is usually devoid of silt and suspended particles. This water is collected in a well, by placing PVC pipes of

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15 cm diameter, by gravitational force. The well acts as a sump-cum-sedimentation tank. The water is then pumped to a biological filter where the water passes through coarse river sand, pebbles and charcoal. The filtered water is stored in a sump with two compartments, facilitating easy cleaning and maintenance. The water stored in the sump is lifted to overhead tank. This water is then drawn to the hatchery through PVC pipelines. The water is allowed to pass through an ultraviolet sterilising chamber to destroy the bacteria and distributed to culture tanks.

The seawater requirement of a hatchery is about 10,000 litres a day when static or recirculation systems are under operation. In the flow-through system, the requirement will be very high. Metals which get rusted while in use should be avoided in the water circulation system. Parts made out of materials like PVC and stainless steel are preferable in the hatchery operation.

The seawater filter system should be maintained properly. Removal of sediment, replacement of river sand and charcoal and washing and drying of pebbles should be done periodically. The frequency for this maintenance operation can be decided by monitoring the bacterial load of the filtered water. Having an additional filter bed as a standby will help in uninterrupted supply of filtered seawater.

Aeration

Air compressors can be either of piston or rotary vane type. Rotary vane models give a high output at low pressure and are less prone to mechanical failure. Air is compressed into a storage tank. The automatic cut off allows the compressor to rest for a while, when the storage tank is full. The air is passed through a series of filters to remove oil and moisture and supplied to the hatchery through PVC pipes of diameter 2.5 cm. Air can be drawn at the required places from these pipes running the entire length of the hatchery at a height of 3 m through the nozzles. The air is supplied to the culture tanks through diffuser stones.

By using electrical air blower, oil free air is supplied to the tanks. The disadvantages of air blower are (i) the air blower should run non-stop since there is no storage tank ; (ii) there is no provision to regulate the air flow at the source and (iii) power failure will affect the air supply to the tanks.

Larval food production

Flagellates measuring less than 10 μm form the main food of pearl oyster larvae. The important phytoflagellate used as larval food is *Isochrysis galbana* (class : Haptophyceae). It measures 7 μm. Besides, species of *Pavlova, Chromulina* and *Dicrateria* have also been found to be satisfactory food for the larvae. Various culture media have been used depending on the organism, class and genera. Usually for culturing flagellates, Conway or Walne's medium is used in the laboratories for the maintenance of the stock culture as well as for mass culture. The composition of Walne's enrichment medium is as follows :

Solution—A

Ferric chloride ($FeCl_3$. $6H_20$)	..	2.60 g
Manganese chloride ($MnCl_2.4H_20$)		0.72 g
Orthoboric acid (H_3BO_3)	..	67.20 g
Sodium EDTA	..	90.00 g
Dibasic sodium acid phosphate (NaH_2PO_4. $2H_20$)	..	40.00 g
Potassium nitrate (KNO_3)	..	200.00 g
Distilled water	..	2 l

Solution—B

Zinc chloride	..	2.10 g
Cobalt chloride	..	2.00 g
Ammonium paramolybdate	..	0.90 g
Copper sulphate ($CuSO_4$. $5H_2O$)	..	2.00 g
Distilled water	..	1 l
Acidify with HCl to obtain a clear liquid.		

Solution—C

Vitamin B_{12}	..	10.00 mg
Thiamine	..	200.00 mg
Distilled water	..	2 l
(To be stored in a refrigerator)		

Culture medium

Solution—A	..	10 ml
Solution—B	..	1 ml
Solution—C	..	1 ml
Seawater	..	10 l

Isolation : For the isolation of the required species of phytoflagellates, the serial dilution culture technique is employed. In this method mainly 5 dilution steps (the inocula corresponding to 1, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} ml) are employed. After filtering the seawater through 10 micron sieve, the filtrate has to be inoculated to 5 series of culture tubes in various concentrations. These are kept under sufficient light with uniform temperature (25°C) conditions. After 15-20 days, discolouration of the tubes can be observed. On examination, the growth of unialgal species can be observed. Purification of these organisms can be

done by sub-culturing the same in 250 ml, 500 ml, 1 l and finally 3 or 4 l Haufkin culture flasks as stock culture.

Stock culture : Required quantities of nutrients are added to autoclaved or boiled and cooled seawater in Haufkin flasks. Walne's medium is found to be the ideal and suitable one to maintain the stock of all the Haptophycean flagellates. About 10 ml of the inoculum in the growing phase is transferred to the culture flask and placed under tube lights (800 lux). When the maximum exponential phase is reached, light intensity is reduced to 400 lux to enable further growth. Normally the flagellates will enter the stationary phase of growth after 15 days. In this phase the culture can be kept for a period of 2 months without aeration.

Mass culture of algae : Utilising the inoculum from the stock culture room, the flagellates are grown in large scale in 20-l glass carbuoys or in 100-l perspex tanks. Fully grown stock culture is used as inoculum for the mass culture in these containers. About 250 ml of the inoculum is used for the glass carbuoy and 2 l for the perspex tank. These containers will have the maximum concentration of cells in the growing phase within 5 to 6 days. After observing the thick brownish yellow colour and noting the cell concentration using a haemocytometer, the culture is drawn for use as food for the pearl oyster larvae. The composition of the medium used for mass culture as well as mixed algal culture is as follows :

Potassium nitrate	..	0.4 g
Potassium dihydrogen orthophosphate	..	0.2 g
Sodium silicate	..	0.2 g
Sodium EDTA	..	0.2 g
Filtered seawater	..	30 l

Culture conditions : The right amount of illumination is an important factor for algal culture. Most of the flagellates require less light during the stationary phase. Too much of light causes early declining of culture. For the growth in mass culture 1,000-1,500 lux is optimum upto 5-6 days, and for maintaining the stock culture 400-500 lux is sufficient. A photoperiod of 12 hr light and 12 hr darkness is ideal for maintaining the stock as well as mass cultures. The algal cultures are maintained best at 23-25°C. It has been noticed that under aeration, the cultures remain in the growing phase 2-3 days more. Aeration helps the nutrients to be distributed uniformly in the medium.

Broodstock maintenance

In order to get spawners throughout the year, the brood oysters are kept in seawater, the temperature of which is maintained at 25-28°C. They are fed with mixed culture of algae at the ration of 4 l per oyster per day, the quantity being supplied twice a day at equal intervals. The algal food is supplemented by raw corn flour at 30 mg per oyster per day. Pearl oysters with maturing gonad fed with the above food for 45 days would spawn with 30% response. The mature oysters can be kept for prolonged period under 25-28°C and the spawning of these oysters can be induced with a slight elevation of temperature.

Larval rearing system

The eggs are fertilised as soon as they come in contact with sperms. The fertilised eggs settle at the bottom of the vessel along with unfertilised and undeveloped eggs, broken tissues and mucus. The column water contains the excess sperm. The developing embryos are sieved carefully through 30 μm mesh and are transferred to fresh seawater for further development. At 3-4 hrs after fertilisation the morula stage is reached. The embryos which are phototrophic rise from the bottom of the vessel and congregate near the surface leaving behind the unfertilised eggs, under developed embryos and other debris at the bottom. The morulae are collected by siphoning them out gently to another fresh vessel. Most of the embryos reach straight-hinge stage within 20 hrs. The size of larva is 57.5 μm. Congregation behaviour of the larva still persists. One more sieving with 40 μm mesh at this stage yields pure larval culture. Estimation of larval concentration and stocking the larvae is done at this stage. The larvae are fed from this stage onwards. Further growth depends mostly on the supply of right type of food. Otherwise the larvae would remain in this stage upto 30 to 40 days without any further development. The straight-hinge stage is considered to be a critical one.

In the larval rearing vessels, water is changed once in two days. The larvae are sieved through 40 μm mesh and introduced to clean vessels containing seawater. A differential growth rate is noted in larvae beyond straight-hinge stage and on any given day during larval rearing, a wide range of sizes is seen. To obtain better results the method of culling is resorted to at different stages of development and the slow growing ones are discarded.

Aeration seems to affect the growth of pearl oyster larvae and leads to greater mortality. The effect of

aeration is more pronounced in smaller vessels than in larger ones. Agitation caused by aeration weakens the larvae. The larvae prefer difiused light or darkness. Fibreglass tanks are found to be ideal for larval and spat rearing. Mass production of spat is done in tanks of one tonne capacity. The larvae show better growth and settlement in tanks painted with dark colour.

In the water circulation system, the tanks should have provision for inlet and outlet of water. The outlet should be of an appropriate size fitted with a sieve of nylobolt which prevents the passage of larvae out.

Disease control of larvae

Natural mortality of larvae/spat may occur even under the best conditions. The weak and dying larvae are found infected heavily by bacteria and protozoans. In some cases the mortality is due to epizootic organism. Within a day or two after infection, heavy mortality is seen among the larvae/spat. Infection by fungus is perahps through untreated seawater. The mortality of larvae/spat can be minimised to a large extent by following certain precautionary measures such as (i) general cleanliness of all utensils used in larval rearing; (ii) sterilisation with germicidal ultraviolet irradiation of the culture water and (iii) use of appropriate antibiotics to control bacteria. Streptomycin, Kanamycin, Aureomycin and Combistrep in low concentration have been suggested for use in larval rearing systems. Use of antibiotics in higher doses beyond certain limits is likely to retard the growth or kill the larvae. The commercial preparation of Terramycin, Sulfathiazone and Sulfanilamide is considered to be somewhat toxic even in low concentrations.

Spat production

To enhance the survival rate of spat during the nursery rearing phase, the fully developed larvae are allowed to set on suitable collectors in the spat setting tanks (Pl. III A). The inexpensive, long lasting, light materials such as fibreglass plates, bunches of monofilament, old fish net, seasoned bamboo splits and coconut shells are selected as spat collectors. In general, these materials should not leach out any chemical on reacting with seawater which may affect settlement and growth of spat. The spat collection units should be compact and should facilitate easy *in situ* transplantation to the farm.

The spat on collectors are transplanted to the farm. These are kept in cages which are again covered by old fish net on the outer side. This provides protection to the spat from predators. The spat settled on the culture tanks (Pl. III B) are removed carefully by means of soft foam rubber and reared separately in spat rearing net-cages. When the spat on the spat collectors attain a size of 10-15 mm, they are removed from the spat collectors and reared in net-cages (Pl. III C, D, E). Periodical maintenance of the spat and cages and transferring spat to fresh cages will minimise mortality during the phase of juvenile rearing (Pl. III F).

Flow chart

The different functions involved in hatchery operation are shown in the form of a flow-chart in Fig. 1. The chart depicts the basic steps in each of the subsystems, namely seawater treatment, algal food production and pearl oyster breeding.

Results of Experimental Production of Pearl Oyster Spat

Spawning and fertilisation

In pearl oysters with mature gonads, a simple change of seawater often leads to spawning. The shock of shell cleaning is enough for some oysters to spawn when they are returned to seawater. During some months the pearl oysters from the natural beds spawn when they are brought to the surface and supplied with seawater drawn from the surface area. In all the above cases the males invariably initiate the act of spawning, followed by females.

In hatchery operation, occassional natural spawning in the laboratory alone cannot be depended upon. A series of experiments have been conducted on induced spawning of pearl oyster (Alagarswami *et al.*, 1983 a) and the results are as follows (Table 1):

Table 1. *Induced spawning in pearl oyster* Pinctada fucata (After Alagarswami *et al.*, 1983a)

Treatment	No. of oysters			
	Tested	Spawned		Percentage of spawning
		Male	Female	
Tris, pH 9.0 ..	28	4	18	78.6
Tris, pH 9.5 ..	28	8	2	39.3
Tris, pH 9.1 + H_2O_2 (3.064 mM) ..	16	3	7	62.5
NaOH, pH 9.0 ..	21	5	5	47.6
NaOH, pH 9.5 ..	19	6	7	68.4

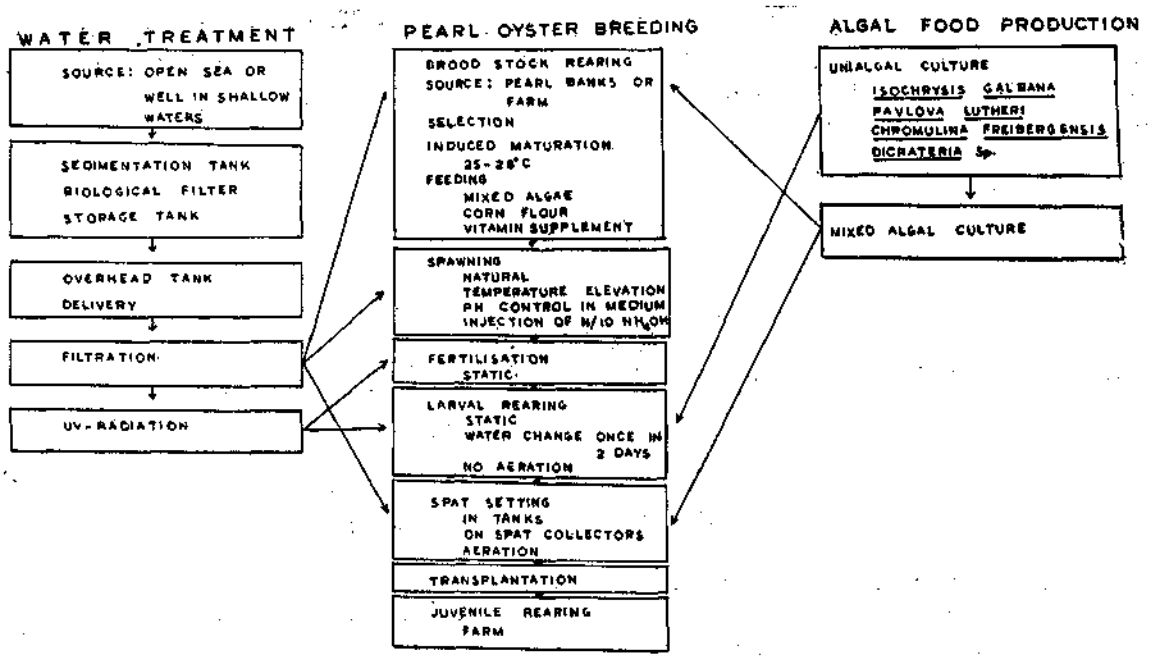

FIG. 1. Flowchart showing the hatchery operation.

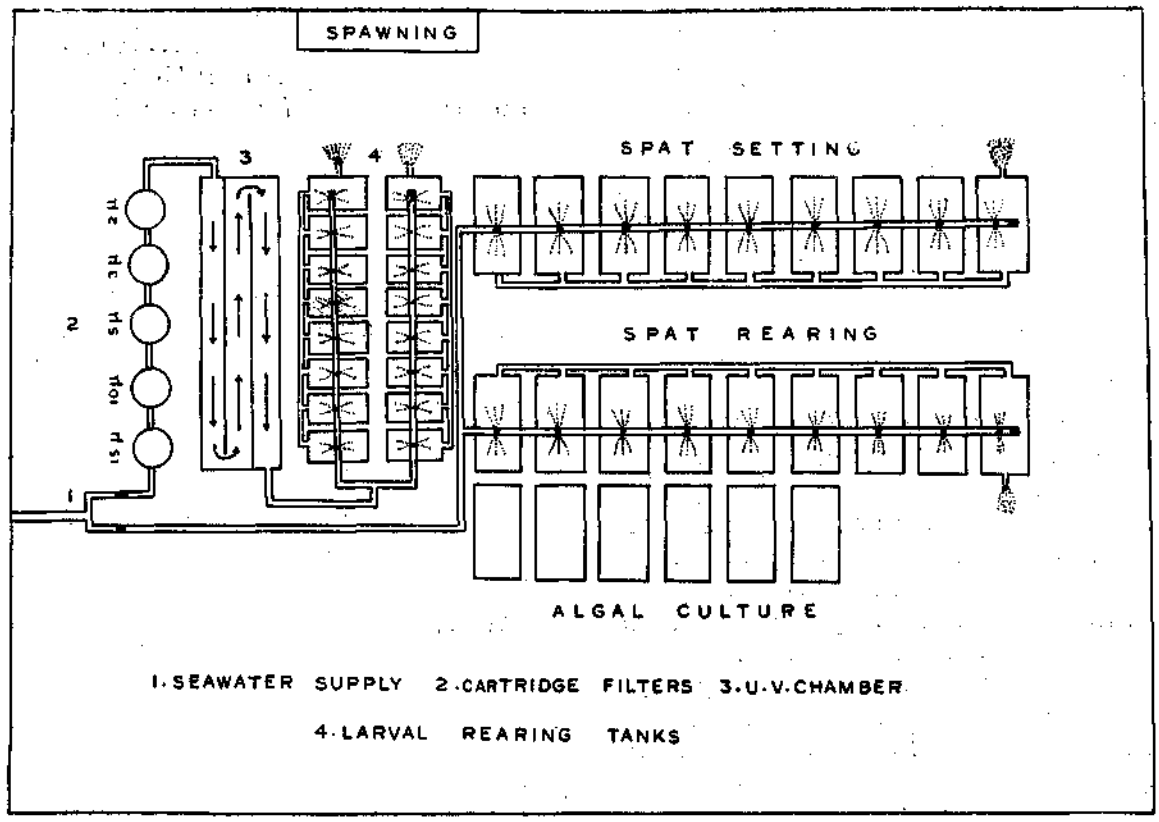

FIG. 2. Layout of hatchery.

Effect of hydrogen peroxide (H_2O_2): Different concentrations of H_2O_2 at 1.532, 3.064 and 6.128 millimolars (m^M) were prepared by adding 6.25 ml, 12.5 ml and 25.0 ml of 6% H_2O_2 solution to 6 l of fresh seawater. The oysters were acclimatised for 12 h before experimenting. The oysters are kept in the experimental medium for 2 h at the end of which the solution was siphoned out and fresh identical seawater was added. Spawning (18.12%) occurred after the change to fresh seawater in the concentrations of 3.064 m^M and 6.128 m^M H_2O_2.

Effect of Tris buffer: The buffers with different pH such as 8.5, 9.0, 9.5 and 10.0 were prepared by slowly adding Tris buffer to fresh seawater. Normal seawater with a pH of 8.1-8.2 was used as control. The duration of the experiment was 3-4 h. A maximum of 78.6% of oysters spawned in the pH 9.0, 37.3% in the pH 9.5 and 20% in the pH 10.0. Spawning response started between 1-2 h in the Tris medium itself. There was no spawning in the control.

Effect of alkali (NaOH): Solutions having a pH of 8.5, 9.0, 9.5 and 10.0 were prepared by dissolving pure pellets of sodium hydroxide in seawater. Normal seawater having a pH of 8.0 and 8.1 was kept as control. The duration of the experiment varied between 3-4 h. 68.4% of oysters spawned in pH 9.5 and 47.6% in pH 9.0. Spawning did not occur in other pH. In most cases, spawning occurred in NaOH medium itself.

Effect of H_2O_2 *in alkaline medium* (H_2O_2 + *Tris*): The alkaline medium by Tris buffer acts to increase the porportion of animals that will spawn in response to H_2O_2. Oysters in Tris buffer solution at pH 9.1 formed the controls. The concentrations of H_2O_2 used here were the same as in the earlier experiment. 62.5% of oysters spawned in 3.064 m concentration of H_2O_2 buffer after the oysters were changed to fresh seawater at the end of 4 h of treatment.

Effect of H_2O_2 *in alkali medium* H_2O_2 + NaoH): The pH was adjusted to 9.0 by adding NaOH pellets. The H_2O_2 concentrations were the same as above. The duration of the experiment ranged between 2-5 h. Two oysters spawned mildly out of 21 tested at 6.128 m^M concentration after changing to seawater. Spawning was observed in the control also.

Effect of injection of N/10 ammonium hydroxide: A dilute solution of N/10 NH_4OH was prepared from a stock solution. 0.1 ,0.2 and 0.3 ml of the solution was injected in the adductor muscle. 48% of oysters spawned at 0.2 ml injection but none at 0.1 and 0.3 ml. In one experiment when the injection was given at the base of the foot, all the oysters spawned profusely. Oysters without injection were kept as control and no spawning occurred in them.

Thermal stimulation: Experiments on thermal stimulation also resulted in spawning when the temperature was increased gradually from 28.5°C to 35.0°C.

Larval rearing

Spawning: Invariabily the males initiate spawning in the normal condition (P. I A). The presence of sperm in the water stimulates the females which follow act within 30 minutes. Majority of the eggs released first are pyriform, and measure 73.9 μm along the long axis and 45.2 μm in breadth on an average. A large clear germinal vesicle (nucleus) measuring 24.7 μm in diameter, is distinctly seen. The yolk cytoplasm is heavily granulated and is opaque (Pl. I B). The egg is enclosed in a vitelline membrane which remains upto trochophore stage.

Fertilisation: Soon after discharge, the eggs are fertilised. They assume a spherical shape and the germinal vesicle breaks down. The fertilised egg measures 47.5 μm in diameter (Pl. I C). During the process of fertilisation the first and second polar bodies are released. The polar bodies persist on the embryo even in the blastula stage.

Cleavage: The first cleavage begins 45 min after fertilisation resulting in a micromere and a macromere. Now the polar body lies at the cleavage furrow. During the second cleavage the micromere divides into two and the macromere divides unequally into a micromere and a macromere. The stage with three micromeres and a macromere is called Trefoil stage. (Pl. I D). Macromere does not take part in further divisions. Micromeres become smaller and smaller in size after passing through eight cell, sixteen cell and so on and reach morula stage (Pl. I E). Then each micromere develops a small cilium which makes rotation movement of the embryo.

Blastula: The stage is reached 5 h after fertilisation. Reorientation of cells result in the formation of blastopore and blastocoel.

Gastrula: Gastrulation takes place by epiboly. The cells convolute arid differentiate into different layers. The archenteron formed at this stage communicates to the exterior through the blastopore. The embryo exhibits phototrophism. It takes 7 h to reach the stage.

Trochophore larva : The early trochophore larva develops preoral and postoral tufts of cilia thus marking antero-posterior region of the embryo (Pl. I F). A single apical flagellum is developed in the typical trochophore stage. The minute cilia noticed in the blastula stage disappear. A shell gland of the dorsal ectoderm secretes the prodissoconch I. The stage is attained in 10 h.

Veliger : The veliger stage is reached by the formation of straight hinge line, mantle, rearrangement of preoral cilia into a velum and disappearance of the apical flagellum, preoral and postoral ciliary bands. The straight hinge larva measures on an average 67.5 μm along the antero-posterior and 52.5 μm along the dorso-ventral axis (Pl. II A). The stage is reached in 20 h.

Umbo stage : The development of straight-hinge larva to umbo stage is gradual. Typical clam shaped umbo stage is reached between 10-12 days measuring 135 × 130 μm (Pl. II B). The shell valves are equal and develops mantle folds.

Eye spot stage : Eye spot is developed on 15th day when the larva measures 190 × 180 μm. The eye spot is situated at the base of the foot primordium. Eye spot is visible in a spat of 3.9 mm. The larva develops ctenidial ridges (Pl. II C).

Pediveliger stage : The foot is developed on 18th day at the size of 200 × 190 μm. The transitional stage from swimming to crawling phase has both velum and foot (Pl. II D). Later the foot becomes functional with the disappearance of velum. 2-4 gill filaments are seen at this stage.

Plantigrade : The stage is seen on 20th day and it measures 220 × 200 μm. Labial palps and additional gill filaments develop. The shell growth is by the formation of a very thin, transparent, uniform conchiolin film around 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 (P I.II E).

Spat : The plantigrade transforms to a young spat. The hinge line, anterior and posterior auricles and the byssal notch assume specific shape. The left valve is slightly more concave than the right one (Pl IIF). The spat attaches itself to the substratum with byssal threads. The typical spat is recognised at this size 300 μm on 24th day.

The sizes and days given for the different larval and post-larval stages vary from experiment to experiment according to the environmental conditions prevailing at that time.

Larval growth under different conditions

Larval density : Under identical conditions the larvae showed differential growth rate at different larval densities and was low in higher densities. In the experiment 1 of the Table 2 it can be seen that the growth increment from the day of stocking to first settlement was 137.9 μm, 134.7 μm, 131.2 μm, 132.8μm, 118.9 μm and 115.8 μm in the densities of 1, 2, 3, 4, 5 and 10 larvae/ml. The highest percentage (99.9%) of settlement occurred in 2 larvae/ml concentration and the lowest (18.9%) in 10 larvae/ml. A similar trend of settlement was also seen in the experiment 2 (Table 2)

TABLE 2. *Larval density, growth and settlement of larvae of* P. fucata

Expt. No.	Larval density (larva/ml)	Volume of water (l)	Average size of larvae (in μm)				Number of spat settled	Percentage of spatfall
			2nd day	8th day	15th day	28th day		
1	1	4	64.0	85.4	141.5	201.9	3,588	91.5
	2	4	64.0	93.4	142.6	198.7	7,991	99.9
	3	4	64.0	91.8	144.8	195.2	11,054	92.1
	4	4	64.0	95.6	142.8	196.8	15,540	97.1
	5	4	64.0	95.8	138.8	182.9	15,892	79.5
	10	4	64.0	95.2	133.7	179.8	7,562	18.9
2	2	500	67.5	88.0	174.8	302.2	3,16,334	31.6
	3	500	67.5	67.1	125.9	214.8	2,86,833	19.1
	4	500	67.5	83.8	164.8	281.5	2,81,666	14.0
	5	500	67.5	77.5	144.1	323.1	3,39,333	13.6

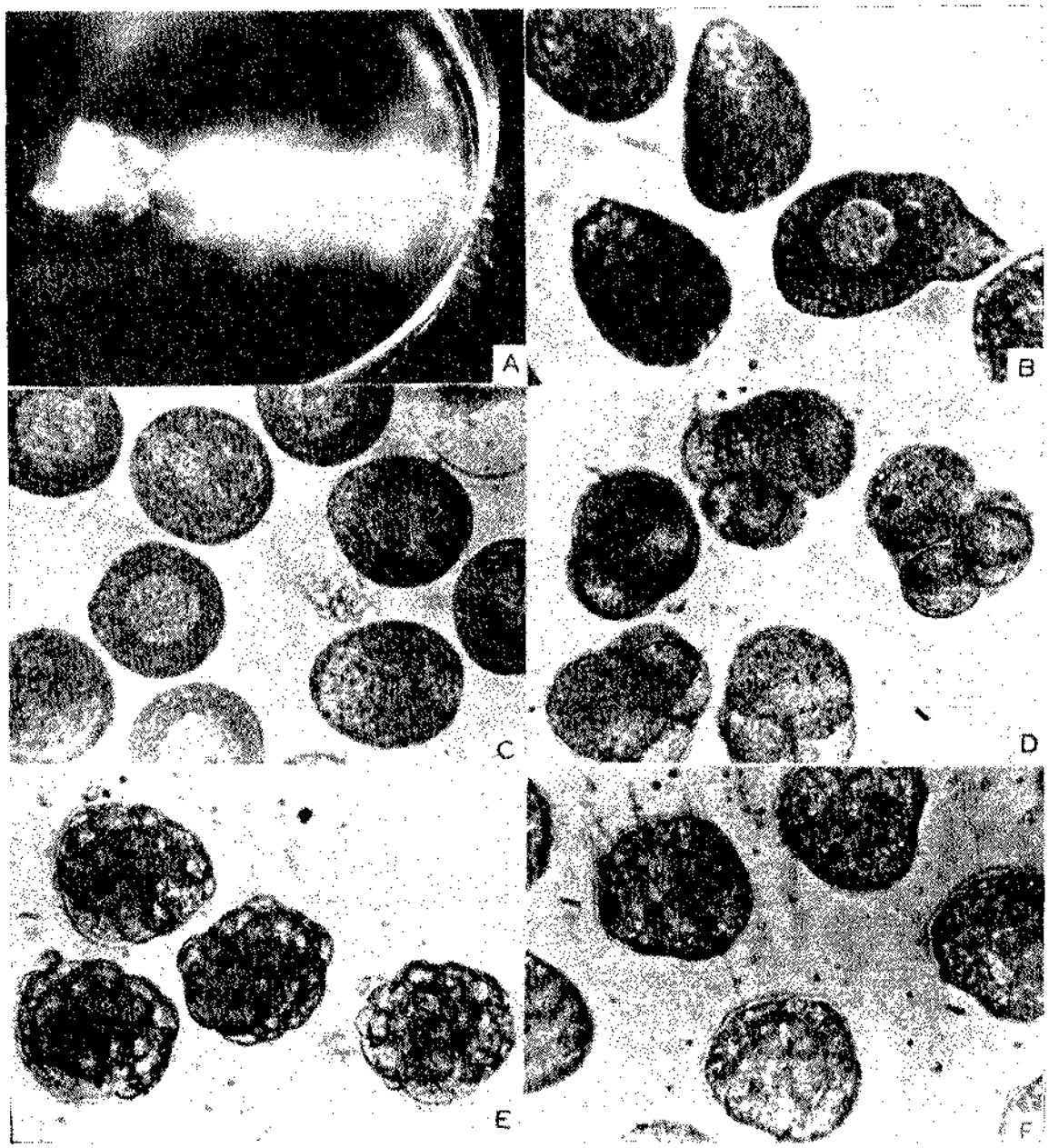

PLATE I. A. A spawning male ; B. Released eggs ; C. Fertilised eggs assuming spherical shape ; D. Cleavage, 2-celled and 4-celled stages ; E. Advance stages of cleavage ; F. Trochophore larvae.

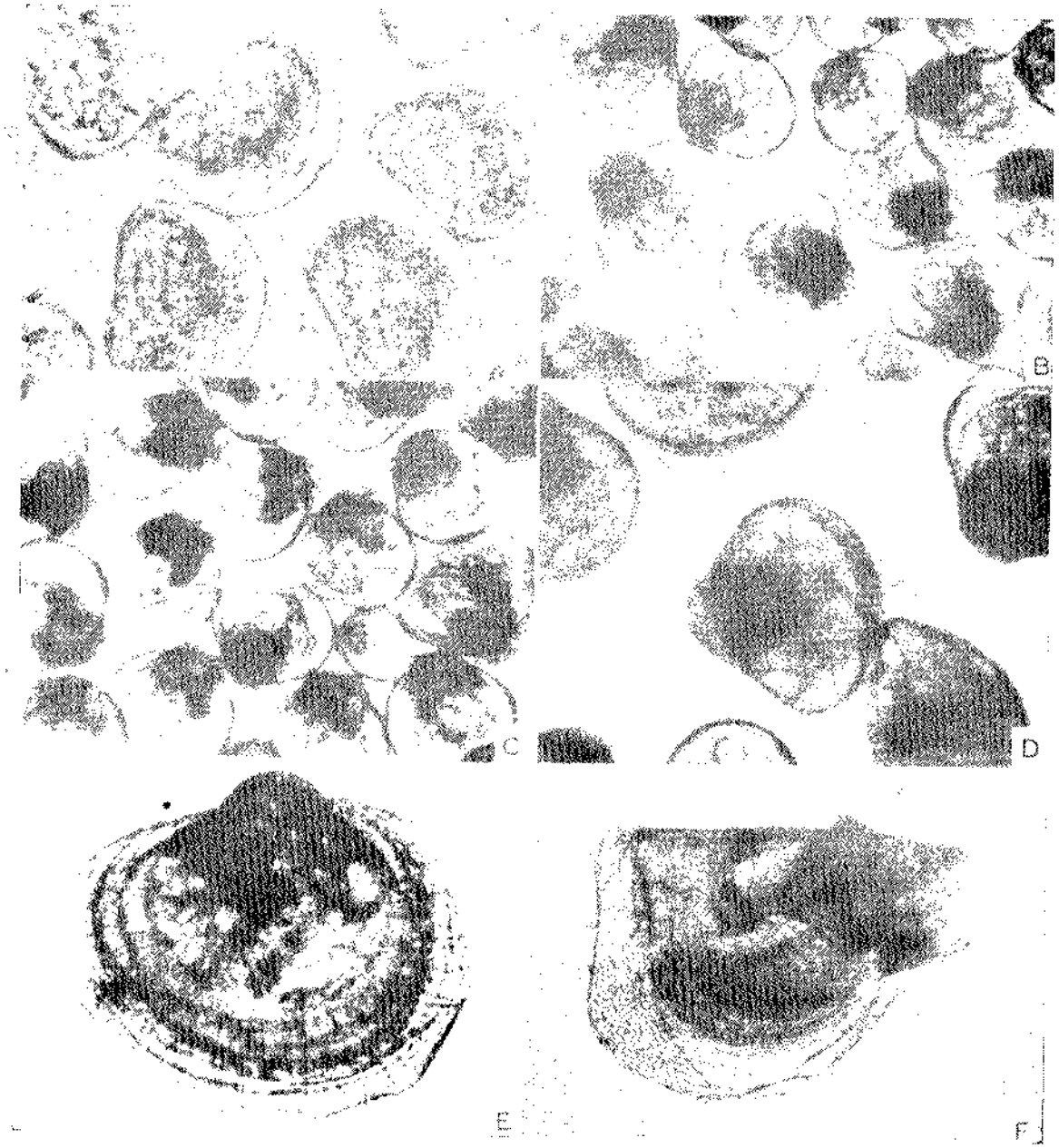

Plate II. A Straight-hinge stage; B. Umbo stage; C. Eyed stage; D. Pediveliger stage; E. Plantigrade stage; F. Young spat.

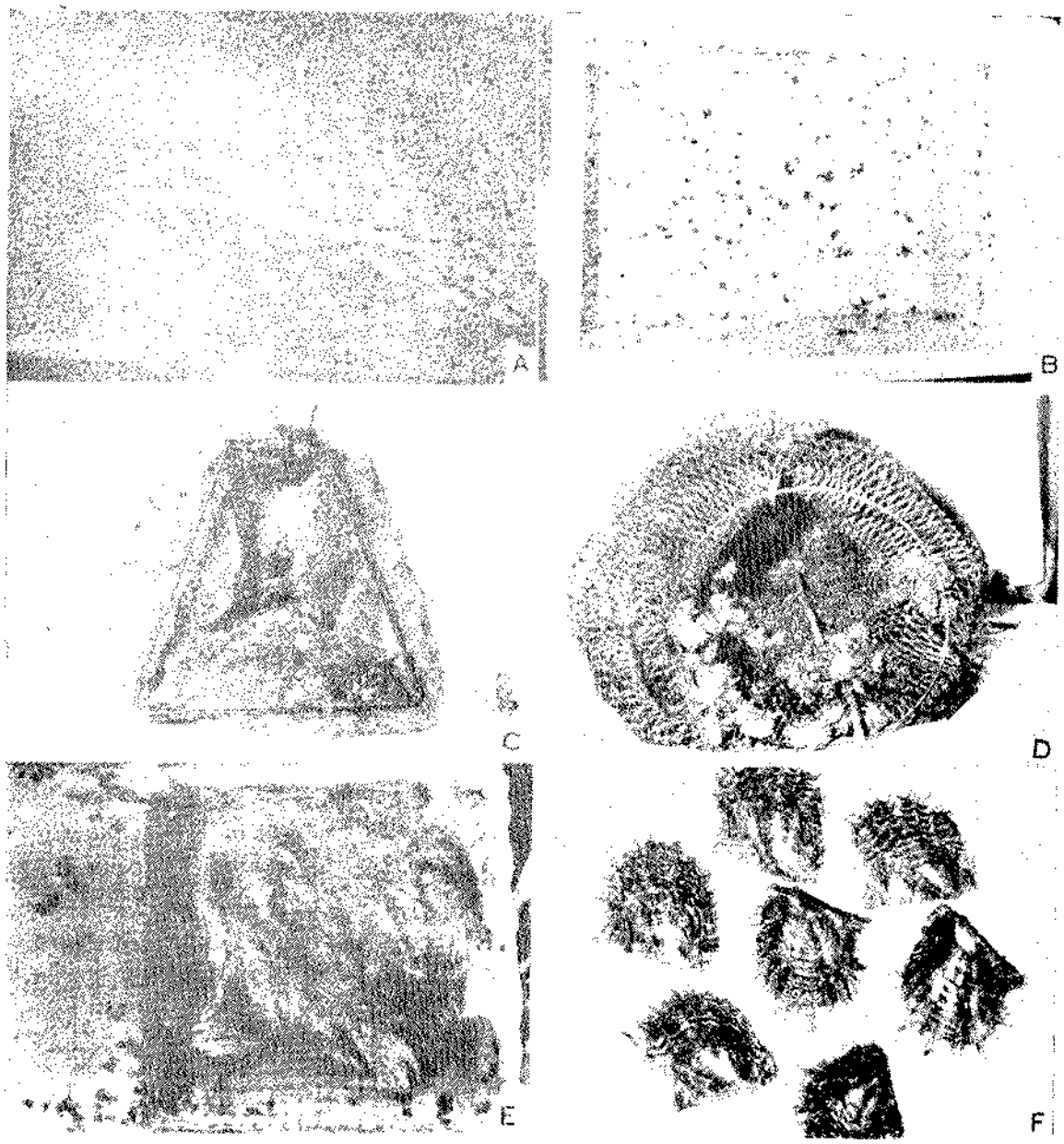

PLATE III. A. Spat settlement on a glass plate (spat collector); B. Spatfall in the FRP tank; C. Spat rearing in the farm (velon screen net-cage); D. Large spat reared in a fish net-cage; E. Spat on a bunch of monofilament in box-cage; F. Hatchery produced young oysters.

where the percentage of spatfall was 31.6, 19.1, 14.0 and 13.6 in the densities of 2, 3, 4 and 5 larvae/ml. In this particular experiment where the volume of culture water was 500 l, the increase in growth was 252.7 μm, 147.3 μm, 214.0 μm and 255.6 μm for the larval densities 2, 3, 4 and 5 larvae/ml. From the experiment it was seen that 2 larvae/ml was found to be the optimum both for larval growth and spatfall.

Colour of larval rearing vessels : Apart from the larval densities and feeding levels, the colour of the larval rearing vessels was also found to influence the spatfall. Vessels of black colour received more settlement followed by white and light blue ones (Table 3). In the three experiments conducted 1.4, 2.4 and 7.3 per cent larvae settled respectively. It was also seen that the total spatfall in the black colour vessels was 64.5% in the first experiment, 73.7% in the second and 47.7% tn the third. Among the white and blue colour vessels the spatfall was almost similar.

TABLE 3. *Effect of colour of larval rearing tanks (FRP) on spatfall*

Expt. No.	Colour of tanks	Total No. of larvae stocked (million)	Volume of water (l)	No. of spat settled (%)	Percentage in total settlement
1	Black	.. 4.0	60	70,262 (1.8)	64.5
	White	.. 4.0	60	38.762 (0.97)	35.5
2	Black	.. 3.0	60	71.679 (2.4)	73.7
	White	.. 3.0	60	25,560 (0.85)	26.3
3	Black	.. 11.85	500	86,555 (7.3)	47.7
	White	.. 11.85	500	53,000 (4.5)	29.2
	Blue	.. 11.85	500	42,055 (3.6)	23.2

Culling : The effect of culling was not felt much in the growth of larvae (Table 4). The survival rate of spat obtained from the experiment was worked out. Under identical treatment for 44 days in the hatchery the survival was 91.3% in the non-culled case, 71.2% in one culling, 62.4% in two cullings and 68.2% in three cullings. On transplantation of the spat to the farm condition, the spat of non-culled experiment 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.

Aeration : Agitation of the culture water through aeration was found to have direct impact on the growth and settlement of larvae. The result of the three exerpiments conducted on this aspect is given in the Table 5. The effect of agitation was more pronounced in smaller vessels of 5 l capacity than in larger vessels. The growth as well as the settlement of the larvae was affected by aeration. The percentage of spat settlement in aerated condition was 0.6%, 9.0% and 8.7% in the three sets of experiments whereas it was 15.9%, 40.6% and 14.3% for the non-aerated experiments.

Spat production and rearing

Larval rearing was conducted almost throughout the year in the hatchery laboratory of Central Marine Fisheries Research Institute at Tuticorin and spatfall was achieved in all the cases (Table 6). However, during the months May-August, the spatfall was at the minimum level. This was because of the high salinity of the rearing water. This was particularly true in the year 1983 (Table 6). In addition to the rise in salinity, heavy dust fall and warmer landward wind were experienced during May-August at Tuticorin. Occasional spurt in the growth of ciliates resulted in heavy mortality in larvae. This was more common only during May-August. It may be seen from Table 6 that higher rates of mortality in the larval rearing were observed in the initial period. Later, steps were taken to overcome some of the problems of larval rearing which resulted in higher survival rates.

TABLE 4. *Effect of culling on the growth and settlement of larvae and survival rate of spat*

Treatment	Av. growth of larvae (in μm)					No. fo spat settled (%)	Percentage of survival in lab (44 days)
	2nd day	6th day	12th day	19th day	22nd day		
No Culling ..	67.5	68.9	90.9	183.6	293.2	24,776 (24.8)	91.3
One Culling (at umbo) ..	—	—	99.7	192.8	291.9	26.597 (66.5)	71.2
Two Cullings (at umbo & eyed) ..	—	—	107.0	192.4	315.2	15,130 (33.6)	62.4
Three cullings (at umbo, eyed & Pediveliger) ..	—	—	105.6	187.7	288.7	16,296 (47.9)	68.2

TABLE 5. *Effect of aeration on the grwoth and settlement of larvae*

Expt. No.	Vessel	Treatment	Larval density (ml	Vol. of water (l)	Average growth of larvae in μm				No. of spat settled & %	Percentage in total spatfall
					3rd day	10th day	17th day	24th day		
1	Glass beaker	Non-aerated ..	2	5	60.7	95.4	152.5	242.4	1591 (15.9)	96.36
	Glass beaker	Aerated ..	2	5	60.7	97.6	103.0	286.7	58 (0.6)	3.64
2	FRP tank (Blue)	Non-aerated ..	2	50	62.1	100.8	173.2	351.0	40,567 (40.6)	81.87
	FRP tank (Blue)	Aerated ..	2	50	62.1	80.5	99.3	314.8	8,980 (9.0)	18.13
3	FRP tank (Black)	Non-aerated ..	2.5	500	—	—	—	—	1,69,943 (14.3)	62.31
	FRP tank (Black)	Aerated ..	2.5	500	—	—	—	—	1,02,777 (8.7)	37.69

Transplantation

After spat setting in the hatchery they are further grown in nursery tanks. Feeding with mixed algae, especially with *Chaetoceros* enhances the juvenile growth. Spat of 3 mm and above withstand transplantation to the new farm environment. High mortality is noted in transporting spat of size smaller than 3 mm. Silt deposition and settlement of other organisms on the spat and culture containers greatly affect the minute spat. The spat collectors with the spat *in situ* are transported in wet condition and reared. The spat settled on the tank are removed with sponge and transported in water and reared further in net-cage.

TABLE 6. *Pearl oyster spat production in the experimental hatchery at Tuticorin during* 1981—86

Expt. No.	Date of spawning	No. of spat set
1	20-8-81	70,496
2	16-9-81	14,450
3	2-11-81	1,374
4	4-12-81	42,151
5	17-8-82	—
6	1-12-82	5,17,521
7	23-2-83	1,05,856
8	23-4-84	1,818
9	1-6-83	71
10	10-8-83	3,314
11	16-9-83	6,627
12	23-11-83	1,30,292
13	7-1-84	99,308
14	22-3-84	20,559
15	2-5-84	5,36,015
16	16-8-84	22,260
17	1-1-85	6,00,000
18	17-2-85	6,700
19	19-3-85	10,32,000
20	24-4-85	7,60,000
21	23-6-85	10,500
22	28-8-85	9,93,000
23	26-11-85	70,000
24	10-12-85	6,18,000
25	15-2-86	5,60,534
26	19-5-86	2,14,131
27	26-6-86	13,06,965

CRITICAL EVALUATION OF HATCHERY TECHNOLOGY

The development of hatchery technology for production of pearl oyster seed has been timely (Alagarswami *et al.*, 1983) both in respect of this specific resource and for marine bivalves in general. Seed availability has been felt a major constraint in the development of shellfish culture in India. The pearl oyster resource in the natural beds of Gulf of Mannar and Gulf of Kutch have fluctuated unpredictably in the past and no one would venture into pearl culture with total dependence on supply of oysters from the natural source. Also, experimental spat collection in the open-sea has not been successful and that in the coastal waters has yielded mixed species populations at Tuticorin and Vizhinjam, with a very low and uneconomical component of *Pinctada fucata*. Under these circumstances, the hatchery technology has proved very useful and given a positive direction to the effort on pearl culture in solving the resource problem.

High level of production achieved in certain batches of rearing, upto a maximum of 1.3 million spat, under minimal conditions would show the viability of the technology. Such mass production has enabled development in another direction to attempt to repopulate the natural beds themselves by sea-ranching. This has a great potential to control or minimise the vagaries of natural production and re-open pearl fisheries for natural pearls if successful. Pearl culture

also can benefit considerably if full grown oysters can be taken from the beds after a period for nucleus implantation and subsequent culture.

The technology developed and practised is simple for adoption and is of low cost in terms of equipment and operational expenditure. The technology has been tested under severe conditions of fluctuations of environmental parameters and, under more ideal situations, the results can even be excelled.

Besides establishing a source of supply of pearl oysters to pearl culture operations, the hatchery technology has opened up new possibilities of improving the quality of pearl oyster and thereby the pearls through genetic selection and breeding. The Japanese pearl culture industry is paying utmost attention to the genetic improvement of the stocks, without which the production and value of cultured pearls is likely to suffer. The areas for genetic studies are to increase the shell growth, shell cavity size, and strains with potentiality for secreting nacre of desirable quality to improve lustre and colour. The breeding programme takes considerable time to establish strains of desirable characteristics, but this approach has become possible only through the hatchery technology.

The spin-off from this development has been the possibility of research in the microalgae which form the food of the larvae. The techniques of isolation of local species of microalgae and their mass culture in the hatchery facility have enabled several lines of investigations on these organisms.

Hatchery technology is multidisciplinary in nature and there are several aspects which need further directed research. These relate to broodstock management for continuous supply of quality mature oysters for the breeding programme ; genetic improvement as already stated ; water quality management ; nutritional needs of larvae at different stages ; larval physiology ; identification of diseases and control measures ; improvement in larval survival ; synchronisation of growth and metamorphosis ; spat setting requirements ; spat nutrition and juvenile rearing.

Economics of hatchcery production of seed have to be duly considered in terms of fixed assets and working capital requirements. Given the fixed assets, the break-even point of production can be minimised and profitability enlarged only through achieving scheduled production rates, high survival, fast growth, early spat setting and high amount of success in juvenile rearing. These aspects need careful consideration and would decide the future of shellfish hatchery programme in India.

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