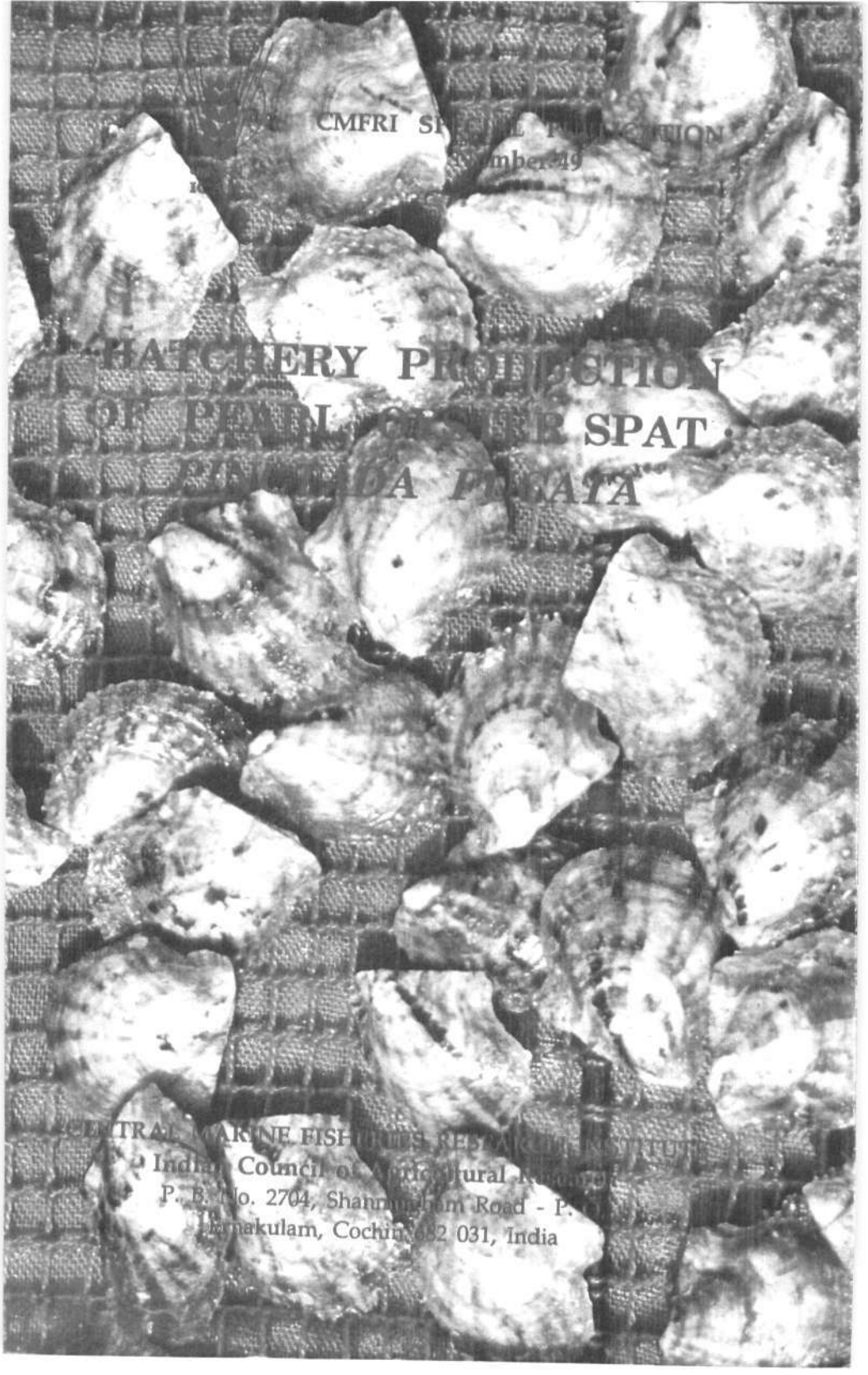

CMFRI SPECIAL PUBLICATION

Number 49

HATCHERY PRODUCTION OF PEARL OYSTER SPAT: *PINCTADA FUCATA*

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
Indian Council of Agricultural Research
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HATCHERY PRODUCTION OF PEARL OYSTER SPAT : *PINCTADA FUCATA*

TRANSFER OF TECHNOLOGY

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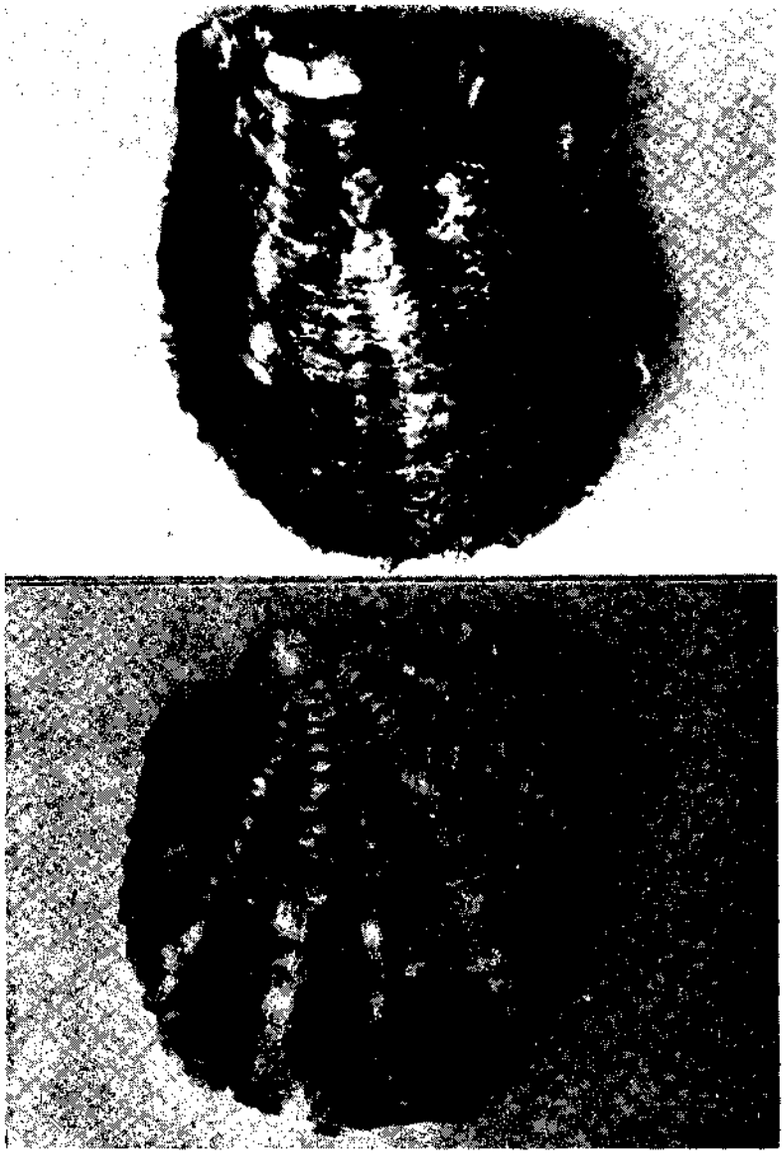

(Top) Pinctada fucata (Gould) and (bottom) Pinctada margaritifera (Linnaeus)

PREFACE

The Central Marine Fisheries Research Institute (CMFRI) on successful development of an indigenous technology for the production of cultured pearls in India in 1973, felt the urgent need for the production of pearl oyster spat in the hatchery to meet the requirement of large scale production of mother oysters for surgery. The pearl fisheries history of India shows that the population of pearl oysters in the beds of the Gulf of Mannar and Gulf of Kutch had been unpredictable and unproductive during most part. For the pearl culture to blossom into an industry, an uninterrupted supply of oysters is a prerequisite. Pressed with this need, a team of scientists of the Molluscan Shellfish Hatchery at Tuticorin Research Centre of Central Marine Fisheries Research Institute made a breakthrough in 1981 by producing the first spat of the Indian pearl oyster *Pinctada fucata* in the hatchery.

Pearl oysters, in India, are found in the waters around Andaman & Nicobar Islands and Lakshadweep besides the Gulf of Mannar and Gulf of Kutch. The Islands are considered to be more potential for pearl culture activities in future, because of the suitable environmental conditions. The shellfish hatchery at Tuticorin, with all its infrastructure facilities, can be the main production Centre of the spat of the Indian pearl oyster *P. fucata*. With the experience gained recently in the transportation of spat to long distances, it is quite possible for the Centre to send the spat to any part of the country with minimum possible mortality to the spat.

This publication is a practical guide, written in lucid language, with illustrations and photographs. I hope this publication will be of immence help to those who are intending to

handle hatchery operations for bivalve molluscs in general and pearl oysters in particular.

The efforts of the team of Scientists in the production of the spat of pearl oysters in the hatchery and also in the preparation of this publication are highly appreciated and I congratulate them on this achievement. I thank Dr. K. Rengarajan for editing and getting the publication printed expeditiously.

P.S.B.R. JAMES
Director
Central Marine Fisheries
Research Institute.

Cochin-682 031,
February 28, 1991.

1

INTRODUCTION

In India the pearl oysters occur in the natural beds in the Gulf of Mannar and Gulf of Kutch. The resources show wide fluctuations which cause great concern to the development of pearl culture industry in the country. Collection of young pearl oysters from the open sea and enclosed bays by means of spat collectors yielded no success. The hatchery technology developed at the Molluscan Shellfish Hatchery at the Tuticorin Research Centre of Central Marine Fisheries Research Institute solved not only the problem of seed scarcity, but also paved the way for commercialisation of pearl culture in India. The present publication of hatchery systems developed at the Institute would be useful to the entrepreneures. The technology is simple for adoption and low-cost one in terms of equipment and expenditure. The technology presented here is the result of continued research on various aspects of hatchery system. The infrastructure facilities mentioned are easily available. Besides, basic informations on the biology and distribution of the Indian pearl oyster and the production of feed for larvae are given.

2

DISTRIBUTION AND BIOLOGY

2.1 *Distribution*

The pearl oysters belong to the genus *Pinctada* come under the family Pteriidae. They enloy a world wide distribution occurring in almost all the seas of the tropical belt. They are distributed in the Persian Gulf (Bahrain, Kuwait, Dubai, Muscat and Bushire), Red Sea (Farasan Islands, South of Sabia and Jidda, west of Mecca and Sudan), Philippines, Japan, Australia, the Gulf of California, Mexico, Panama and Venezuela. In Indian waters, they occur in 6 regions namely the Gulf of Mannar, Palk Bay, Trivandrum, the Andaman and Lakshadweep Islands and the Gulf of Kutch. Of these, the Gulf of Mannar and Gulf of Kutch are the most productive regions where pearl oysters occur in tremendous numbers. Six species of pearl oysters occur in Indian waters are *Pinctada fucata* (Gould), *P. margaritifera* (Linnaeus), *P. chemnitzii* (Philippi), *P. sugillata* (Reeve), *P. anomioides* (Reeve) and *P. atropurpurea* (Dunker). Of these, *P. fucata* which occurs in extensive beds in the Gulf of Mannar and to a much less extent in the Gulf of Kutch alone had contributed to the pearl fisheries in the past. The black-lip pearl oyster *P. margaritifera* confined mostly to Andaman and Nicobar waters are fished more for the shells than for their pearls. In the Palk Bay, the *P. chemnitzii* are found on coarse sandy bottom. From Lakshadweep, settlement of spat of *P. anomioides* have been recorded on the ridges of the rocks and corals.

2.2 *Lifecycle*

Pearl oysters are sedentary animals found attached on corals, dead coral outcrops and sand grits by means of byssus threads. They inhabit at depths from 10 to 20 m.

Like other bivalves, pearl oysters are also filter feeders feed mainly on phytoplankters. The analyses of stomach content of pearl oysters showed the presence of diatoms, flagellates, larvae of lamellibranchs, gastropods, heteropods, crustacean nauplii, appendages and frustules of copepods, spicules of sponges and unidentified spores, algal filaments, detritus and sand particles.

In pearl oysters, the sexes are separate and they do not exhibit sexual dimorphism. The gametogenic activity commences when they are 7-8 months old and about 17-25 mm size. The sex of an oyster can be correctly determined only by histological and smear examination of gonad.

In Indian waters, two seasons of spawning, one in June - September and the other in November - December coinsiding with the southwest and northeast monsoons respectively were observed in an year.

The matured oysters release their eggs and sperms in the water and the eggs get fertilized as soon as they come in contact with the sperms. In most cases, the males spawn first which trigger the females to release their eggs. The fertilized eggs pass through different developmental and larval stages such as blastula, gastrula, trochophore, veliger, umbo, eye spot, pediveliger and plantigrade before they finally settle down as spat. In the hatchery it normally takes 3 weeks for the larvae to metamorphose into a spat. The spat measures about 0.3 mm.

Pearl oyster grows fast during the first year and reaches a size of about 45 mm at the end of the first year. During the subsequent years, the growth is slow. They attain a size of about 65 mm and 75 mm at the end of the second and third year of life respectively. The longivity of a pearl oyster is about 5 to 6 years and the maximum attainable size is 100 mm.

3

HATCHERY SITE

Selection of suitable site is a prerequisite for establishing a hatchery. The viability and success of hatchery operations depend on several factors concerned with the site.

The following are the primary factors/requirements to be considered, while selecting a site for the hatchery.

1. The hatchery must be located near seafront with an uninterrupted supply of good quality seawater free from pollutants, suspended particles, silt, etc.
2. The salinity of the seawater must be between 30-40 ppt. throughout the year.
3. The bottom of the sea adjacent to hatchery must be rocky or coralline so as to get clean seawater throughout.
4. It should be away from industrial and domestic sewages and from river mouths where dilution of seawater is possible during monsoon.
5. Freshwater supply must be ensured at the site.
6. Good approachable road must be there for easy transport.
7. The area should not be affected by cyclones and other natural calamities.

8. The site with sea erosion or soil erosion should be avoided.
9. The seashore with pure sand, would ensure supply of clear seawater through borewell.
10. Electricity supply must be available to the hatchery site.
11. The proximity of the site to farm would be advantageous for transplantation of live specimens.

4

HATCHERY FACILITIES

The infrastructure facilities required for the production of five million pearl oyster spat in a hatchery per year is described below.

4.1 *Building*

The main hatchery complex is a 15 x 10 m shed (Pl. I), half of which is roofed with translucent FRP sheets and the other half with asbestos roofing. Concrete flooring with proper slope should be given and a pair of closed gutters which run along the entire length of the hatchery collect the spilled water and keep the floor dry and clean. Sufficient height of the building, suitable air vent at the top and proper ventilation of the sides keep the hatchery building cool (Fig. 1).

The asbestos roofed section consists of (i) algal culture room and (ii) conditioning room (each 4.0 x 2.5 m) on one side and (iii) duty room and (iv) analytical room (4.0 x 2.5 m each) on the other side (Fig. 1). The translucent portion of the hatchery is used for larval/spat rearing and mixed algal food production. The filtered sea water drawn into the hatchery is further filtered and sterilized at this portion.

4.2 *Seawater supply*

Seawater supply system consists of an intake point, a draw well, sedimentation tanks, filter bed, water sump, overhead tank

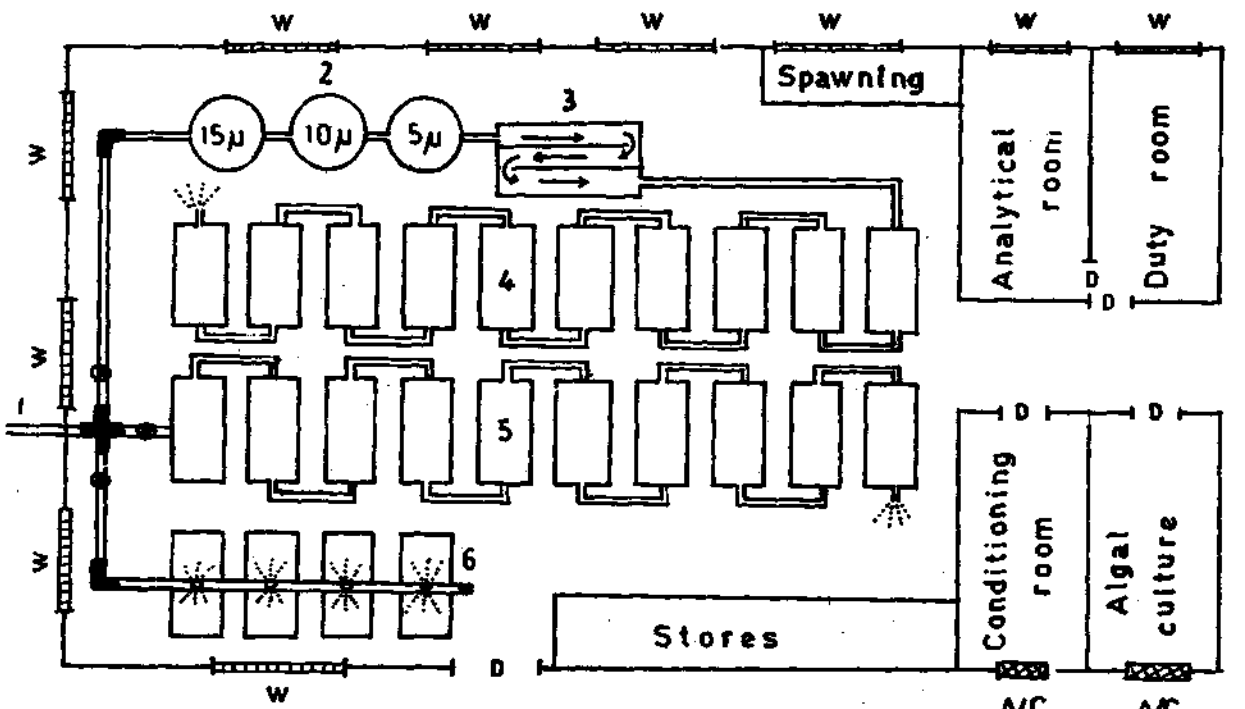

FIG. 1. Layout of hatchery (1. Seawater supply, 2. Cartridge filters, 3. UV Chamber, 4. Larval rearing tanks, 5. Spat rearing tanks, 6. Algal culture tanks, W - window, D - Door and A/C — Airconditioner).

and PVC delivery lines upto the hatchery. Seawater is drawn into the well through a 15 cm dia PVC pipe by gravitation and is pumped to sedimentation tanks where large particles in the water settle at the bottom. The supernatant water is passed through filter bed. The filter bed consists of river sand at the top, charcoal, pebbles and granite stones at the bottom. The seawater thus filtered is collected in a sump and is pumped to a overhead tank. 1 HP electric pump sets with gun metal impeller and stainless steel shaft are used to pump water from the well to the sedimentation tanks and 7.5 HP pump from the sump to the overhead tank. Standby pump sets are required in case of any emergency. From the overhead tank, water is drawn to the hatchery through PVC pipes of 12 mm diameter. The sea water used for larval rearing is further filtered at the hatchery by passing through 15 µm, 10 µm and 5 µm cartridge filters and sterilised in UV chamber. The daily requirement of seawater for the hatchery will be 10,000 l. The capacity of the storage sump is 20,000 l and that of the overhead tank is 10,000 l.

4.3 *Air supply system*

This system consists of air compressors, filters, PVC air grid, polythene aeration tubes, diffuser stones and air regulators. Air compressors of rotary vane model run with a 1 HP electric motor is used. This gives a high output at low pressure. It has got a storage tank and is automatically cut off when the tank is full, giving the compressor sufficient rest. The air flow is regulated and is allowed to pass through a series of filters. Oil and moisture in the air is removed thus. The air is supplied to the hatchery through PVC pipes of 25 mm diameter. The air pipes run, in pairs along the entire length of the hatchery. Air is drawn at the required places from the pipe lines through nozzles fixed to the pipes. Air is supplied to the tanks through polythene tubes and diffuser stones. Air supply to the tanks can be adjusted with the help of a gate valve connected to the polythene tubes. By providing a standby compressor, the air supply system can be effectively managed.

4.4 *Generator*

A 10 KVA 3 phase generator operated by a 16 HP diesel motor as a standby, is required to be used in case there is break in electricity supply.

4.5 *Conditioning room*

The size of the conditioning room is 4.0 x 2.5 m with thermocol ceiling. An air-conditioner is provided in the room. The temperature is to be controlled to a constant level. The matured oysters can retain sexually ripe condition of gonads at the temperature. These oysters are given adequate mixed algal food at the rate of 4 l per oyster per day in two instalments.

4.6 *Maturation tank*

The size of FRP tank used for maturation/conditioning of pearl oysters is 75 x 50 x 25 cm (Fig. 2). The tanks with light blue inner finish are used. The capacity of the tank is 75 l. Six such tanks are kept to accommodate different broods of pearl oysters.

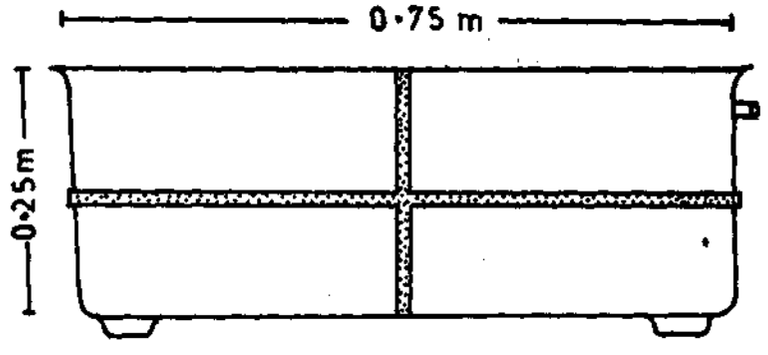

FIG. 2. Maturation tank.

4.7 *Spawning tank*

A 100 l capacity rectangular perspex tank is used in spawning of pearl oysters (Fig. 3). Jumo thermometer, silica cased immersion heater and an aerator are fitted to the tank during thermal stimulation. These gadgets are connected to an automatic controller.

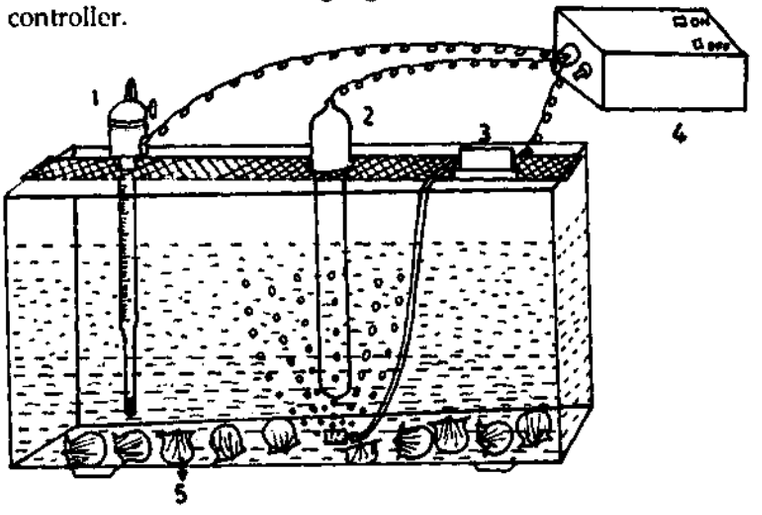

FIG. 3. Spawning tank (perspex) (1. Jumo thermometer, 2. Silica cased immersion heater, 3. Aerator, 4. Automatic controller and 5. Pearl oysters.

4.8 *Larval rearing tanks*

Rectangular (200 x 100 x 50 cm) Fibreglass tanks are used for rearing the larvae (Fig. 4). The capacity of the tank is 1000 l. Ten tanks are required for the hatchery. The tanks should have a dark smooth inner surface, which is scratch resistant for larval rearing.

4.9 *Spat rearing tanks*

The settled spat are reared in rectangular fibreglass tanks similar to larval rearing tanks (Fig. 4). Ten such spat rearing tanks are required for the hatchery. Tanks with a dark and smooth inner finish enhance spat settlement and white surface will have only less percentage of settlement.

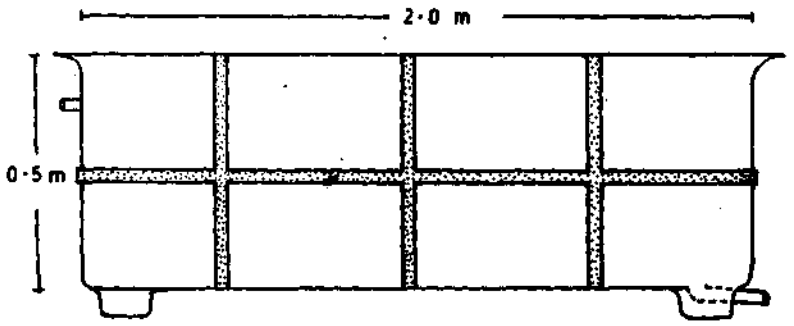

FIG. 4. Larval/spat rearing/mixed algal culture tank .

4.10 *Algal culture tanks*

These tanks are also similar to the earlier two types, but with white inner surface required for mixed algal culture inside the hatchery. Four such tanks are required for this. The seawater in the tanks is agitated with a pair of diffuser stones. The mixed algal food cultured in these tanks are suitable for feeding the spat. The larval food such as *Isochrysis galbana, Pavlova lutheri, Chromulina freibergensis* and *Dicrateria* sp. are cultured under controlled temperature conditions.

4.11 *Other equipments*

Seives of different mesh sizes (30 µm, 40 µm, 80 µm, 140 µm), a good microscope to check the condition and to measure the larvae, a haemocytometer to count algal cell concentration, a plankton counting chamber to count the larvae are the other requirements for a hatchery. pH meter, thermometer, salinometer, oxygen analyser are the qeuipments needed to monitor the water quality. Perspex tanks, trays, beakers, conical flasks, embryo cups, petridishes, pipettes, microslides, cover slips are the glassware requirements. The plasticware required include buckets, basins and mugs. The requirements are listed and shown in Annexure I.

5

HATCHERY OPERATIONS

5.1 *Broodstock maintenance and conditioning*

To obtain oysters for spawning at the required time, a brookstock of mature oysters has to be maintained. This is possible only by keeping the sexually ripe oysters under controlled conditions. A constant temperature of around 25°C and supply of mixed algal food dominated by *Chaetoceros* sp. (4 l per oyster per day) are found to make the oysters to retain their ripeness for considerable time. Supplementary food such as raw corn flour at 30 mg per oyster per day is also effective for this purpose. Constant aeration is to be given. Spawning is effected by transferring these oysters to water with slightly increased temperature.

5.2 *Spawning*

5.2.1 *Natural spawning*

Ripe oysters spawn when there is a slight increase in water temperature (Pl. II A). Ripe pearl oysters collected from the natural beds are found to spawn when they are supplied with seawater drawn from the surface area. Even the shock of cleaning shell surface had led to spawn in some cases.

5.2.2 *Induced spawning*

The pearl oysters can be induced to spawn by other means also. During thermal stimulation the oysters are kept in seawater

PLATE I. A. Hatchery Building - Front view and B. Inner view.

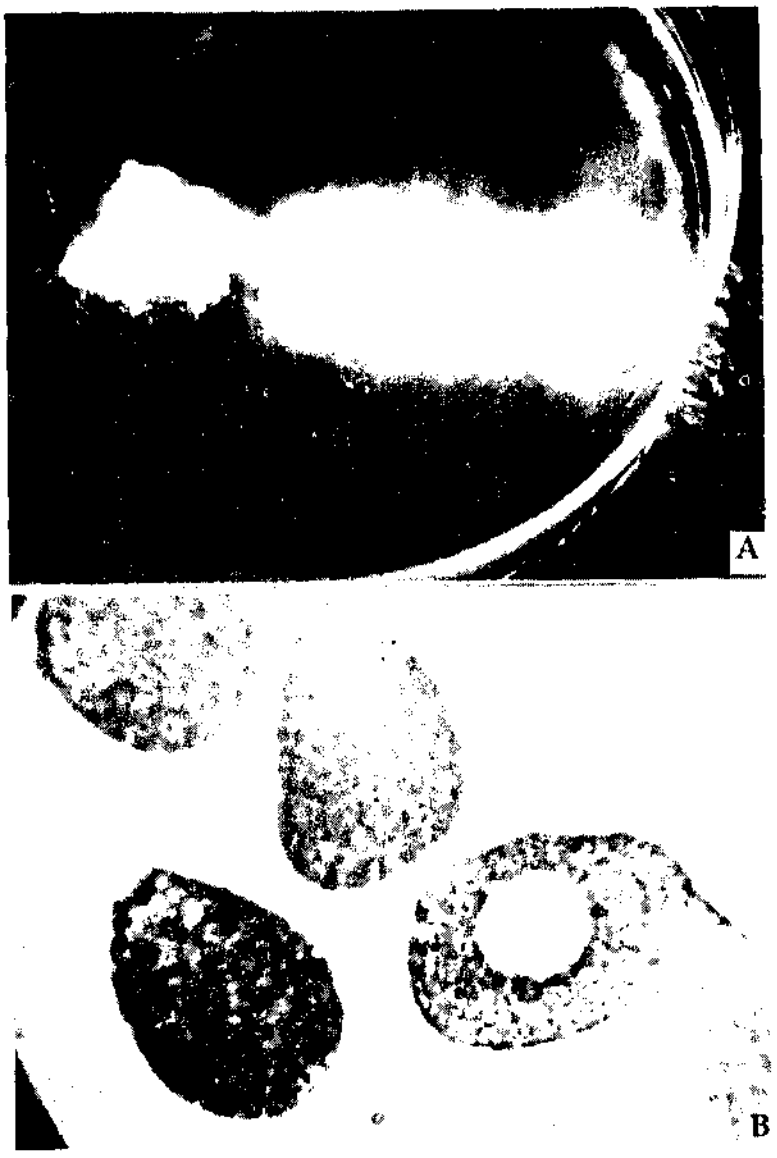

PLATE II. A. A spawning male and B. Released eggs.

in perspex tank. The water temperature is increased gradually from the ambient temperature of 28.5°C to 35.0°C. This would effect spawning in pearl oysters.

The following Chemical stimulation will also induce the oysters to spawn.

i. 6% hydrogen peroxide (H_2O_2) in 3.064 m^M concentration (12.5 ml in 6 l seawater) in an alkaline medium (pH 9.1) using TRIS buffer had induced upto 62.5% spawning in pearl oysters when they are changed to fresh seawater at the end of 4 hrs of treatment.

ii. 6% hydrogen peroxide in 6.128 m^M concentration (25 ml in 6 l seawater) in Sodium hydroxide (NaOH) buffer medium had resulted in spawning of 9.5% oysters.

iii. Seawater with pH 9.0 prepared using TRIS buffer had effected spawning in 78.6% oysters and pH 9.5 using NaOH in 68.4% oysters.

iv. Injection of 0.2 ml of N/10 ammonium hydroxide (NH_4OH) solution into adductor muscle or foot of pearl oyster had induced spawning in 48% oysters.

If the oysters are triggered to spawn they are to be transferred to tanks with clear normal seawater. Here the process of spawning continues.

5.3 *Fertilization*

Invariably males spawn first which triggers the females to spawn in about 45 minutes time (Pl. II B). Soon after the eggs are released, they are fertilized by the sperms. The fertilized eggs measure 47.5 µm in diameter (Pl. III A) and they settle at the bottom of the vessel. Unfertilized eggs, broken tissues, faecal matter and mucus also settle if kept undisturbed. The water with the fertilized eggs are filtered gently through a seive of 30 µm and the eggs thus collected are transferred to FRP tank holding 50 l filtered/sterilised seawater.

5.4 *Early development*

The first cell division takes place 45 minutes after fertilization (Pl. III B). This process goes on (Pl. IV, Pl. V A) and morula stage is attained in 3-4 hrs. The morula looks like a ball of transparent cells enclosing a hollow space, the blastocoel (Pl. V B). Each cell has a cilium which is used for movement. Hence the morula rises to the surface and congregates exhibiting phototrophism. Now they are siphoned out gently to another tank. The waste materials in the bottom are discarded. This helps to select the viable morula alone.

In gastrulation, the process of orientation of cells into dermal layers and formation of archenteron is completed in about 7 hrs. Then the trochophore larva is formed (Pl. VI A). It is pearshaped with a long single flagellum and a tuft of cilia at the apical end.

The secretion of embryonic shell material, the prodissoconch I by the ectodermal cells results in the formation of Veliger larva (Pl. VI B). It is two valved and it swims in the water column with the help of a locomotary organ, the velum. The size of the veliger is about 67.5 µm in anteroposterior axis and 52.5 µm in dorsoventral axis. This stage is reached in about 18-20 hrs. Further development of the veliger depends on the supply of suitable algal food.

5.5 *Larval estimation and stocking*

The veliger larvae are counted and estimated using plankton counting chamber and expressed in numbers per ml. The required number of larvae are taken based on the calculated volume of water and stocked in rearing tanks to get known larval density. The density of two larvae per ml has been found to be optimum concentration for stocking. Stocking in higher densities results in poor growth.

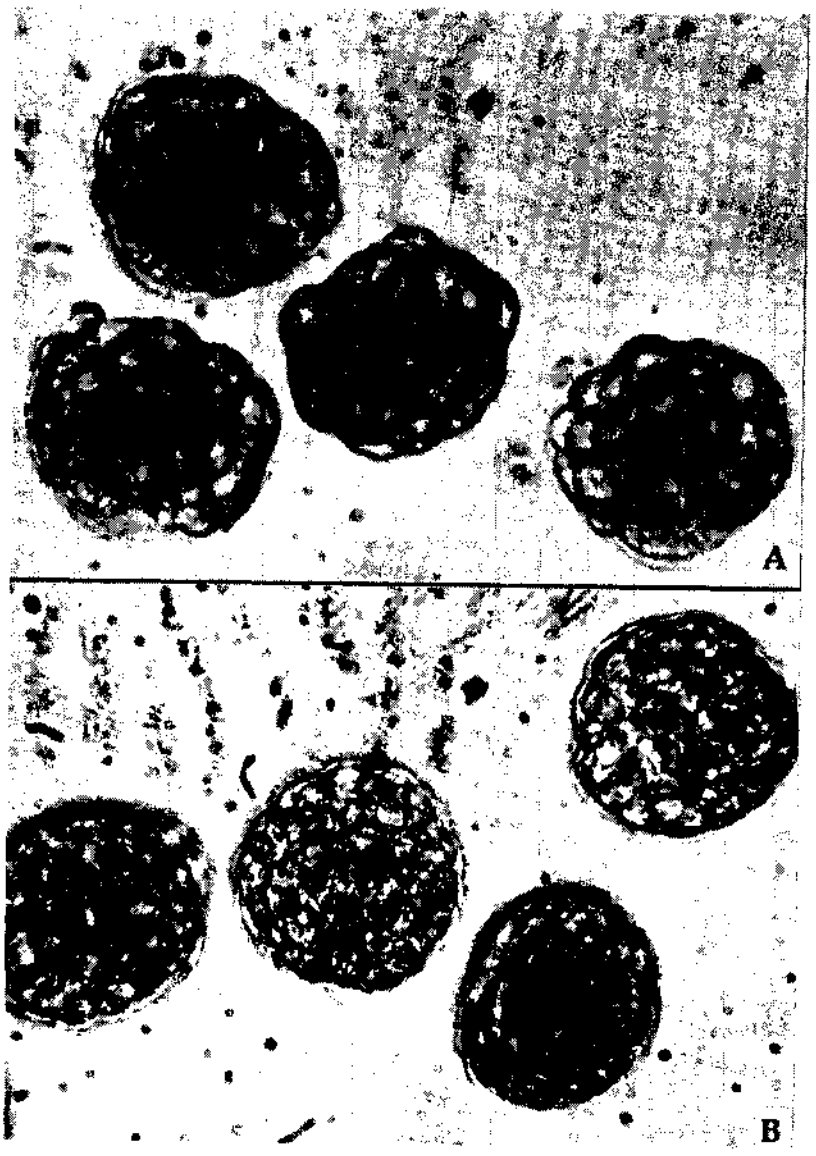

PLATE V. A. 16 - celled stage and B. Morula.

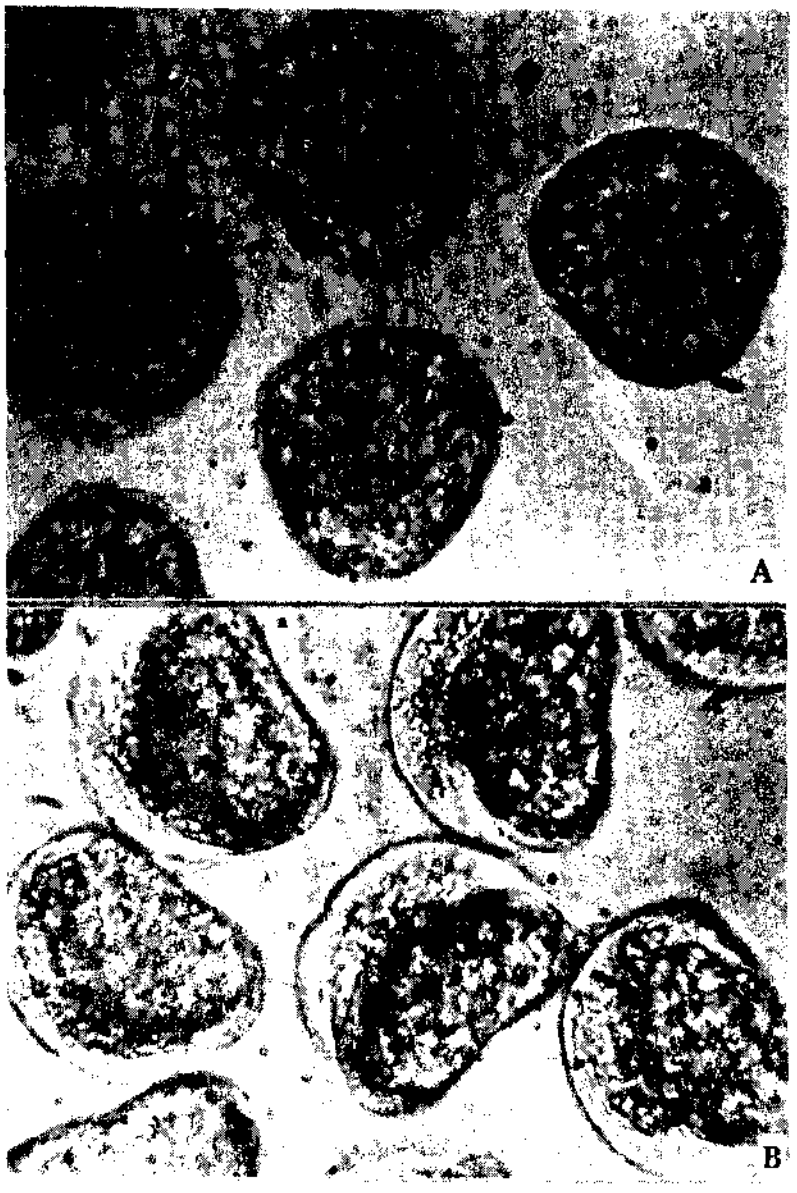

PLATE VI. A. Trochophore larvae and B. Straight-hinge stage.

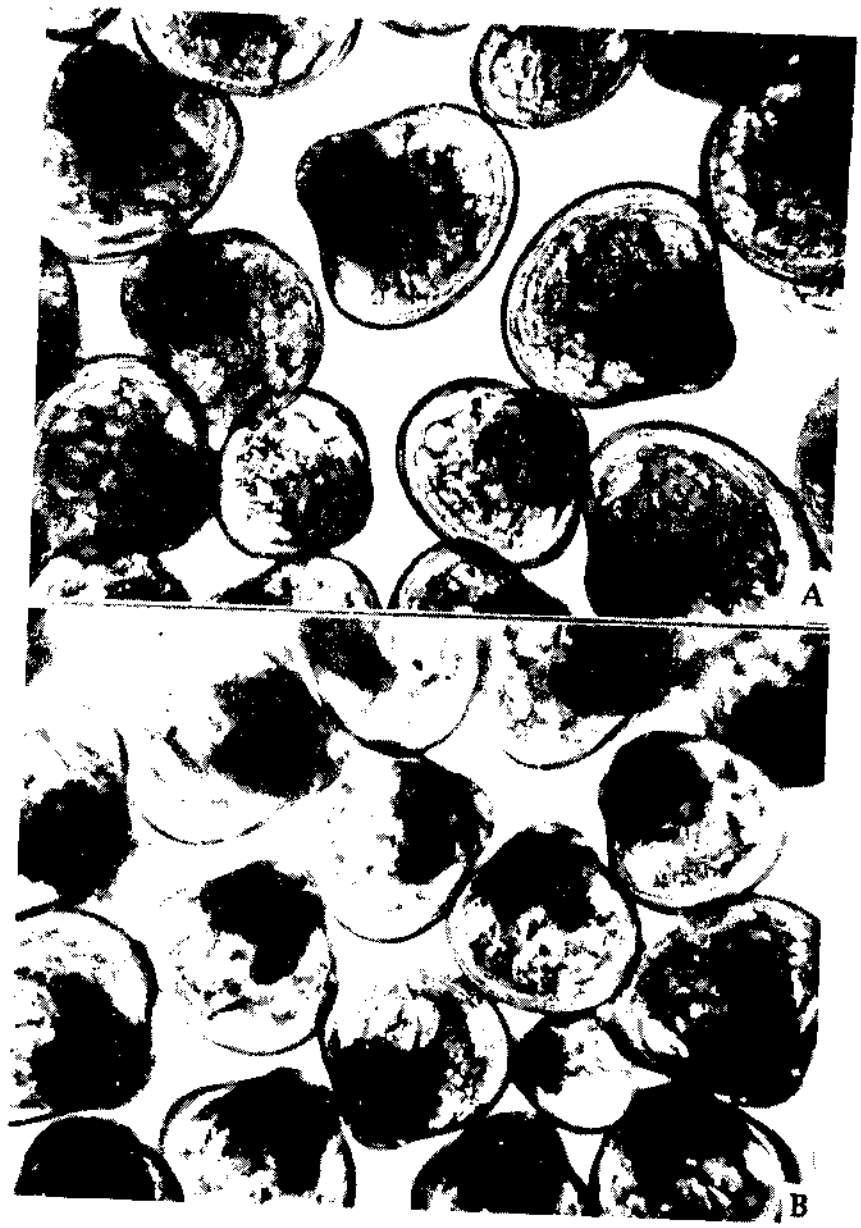

PLATE VII. A. Umbo stage and B. Eyed stage.

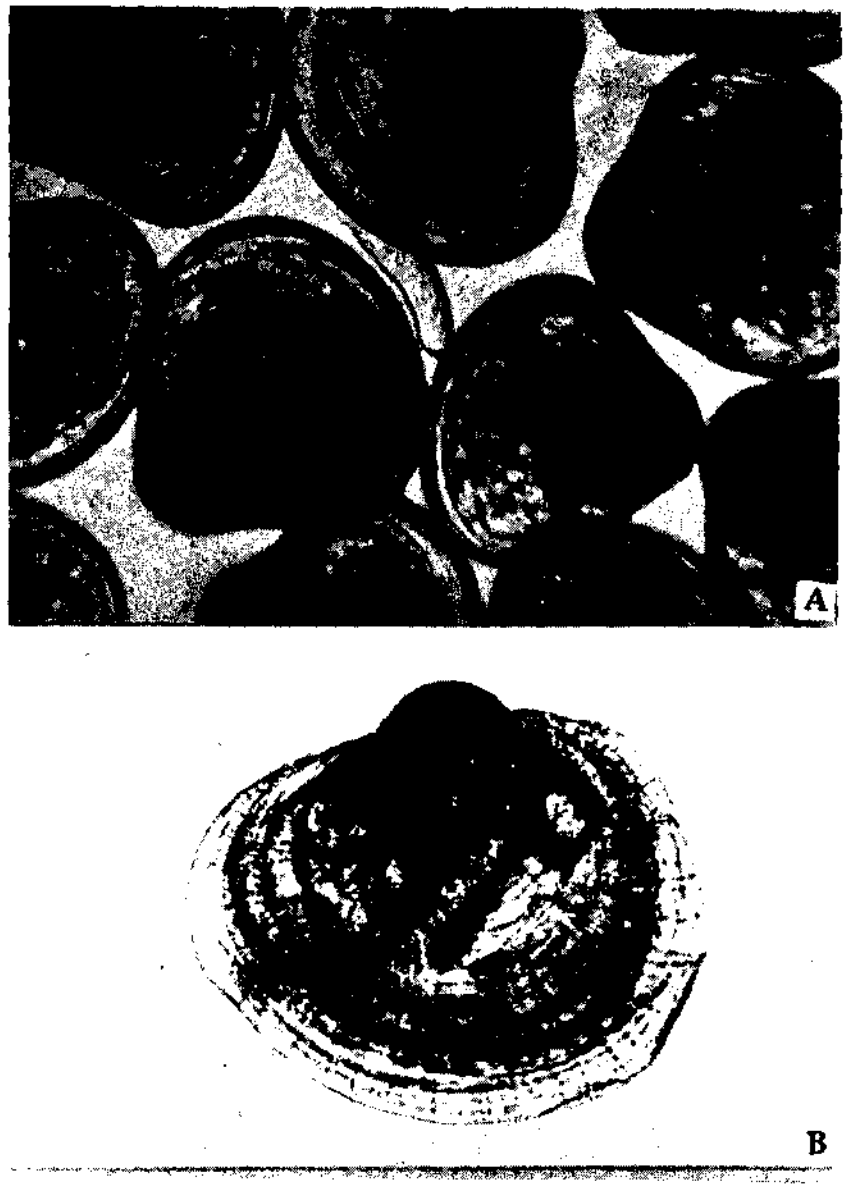

PLATE VIII. A. Pediveliger stage and B. Plantigrade stage.

PLATE IX A. Young spat and B. Juveniles.

5.6 *Larval growth*

5.6.1 *Umbo stage*

On 10-12th day the veliger reaches umbo stage by thee addition of prodissoconch II and measures 135 x 130 µm (Pl. VII A). The straight hinge line is disappeared and umbo region is formed. The shell valves are equal with mantle folds on the inner side. The larva swims with velum.

5.6.2 *Eyed stage*

On 15th day umbo stage transforms into eyed stage (Pl. VII B). The eye-spot is found at the base of the foot primordium. At this stage the larva measures 180 x 170 µm. During the transitional stage from eyed to pediveliger, the larva has both velum and foot functional.

5.6.3 *Pediveliger*

On 18th day pediveliger stage is reached (Pl. VIII A). Pediveliger has a functional foot which helps in movement and in adhering to the substratum temporarily at the time of settlement. The velum disappears. The size of the pediveliger is 200 x 190 µm.

5.6.4 *Plantigrade stage*

The plantigrade stage is reached on 20th day by the growth of additional shell meterial all along the periphery (Pl. VIII B). Byssal threads are produced for attachment. It measures 220 x 200 µm.

5.6.5 *Spat*

The plantigrade stage metamorphoses into a spat by the formation of straight hinge line and anterior and posterior ears. Byssal notch is well developed. The size of spat is 300 µm at the time of settlement (Pl. IX A).

6

LARVAL REARING SYSTEMS

6.1 *Water quality management and other conditions*

Even under identical conditions, the larvae of the same brood show differential growth rates and time in settlement. This may vary from batch to batch. Mortality of the larvae is more till they reach the umbo stage. It is negligible beyond this, unless there is any defect in the water medium or food. Factors like high larval concentration, colour of the inner surface of the culture tanks, aeration and overfeeding may affect the larval growth and survival. Rearing of the larvae at a concentration of two per ml gave better growth and higher percentage of spat settlement. The larvae prefer dark surfaces. Aeration is harmful to the larvae. Selection of the fast growing larvae by culling and discarding the slow growing ones is found to yield better survival rate in the spat after transplantation to farm.

6.2 *Water change*

In static water system, change of water is done once in two days. Water is gently siphoned out through appropriate seives. 40 µm seive is used upto umbo stage, 80 µm seive upto eye-spot stage and 140 µm seive afterwards until settlement. At every water change, the larvae are received in the seive and given gentle washing. Before releasing, the tanks are cleaned well and filled with fresh seawater. Tanks are covered with black cloth to prevent too much of light which may promote algal

growth inside the tanks. Antibiotics are not used in normal cases. A strict vigil is maintained at every water change.

6.3 *Feeding schedule*

From the second day onwards the unicellular microalga *Isochrysis galbana* is fed to the larvae. Before feeding the cell concentration is estimated using haemocytometer. Ten cells per ml is the optimum dose for the veliger larvae upto umbo stage. Feeding is doubled from umbo stage and tripled from pediveliger upto settlement. Apart from *Isochrysis*, the other unialgal food such as *Pavlova lutheri, Chromulina freibergensis* and *Dicrateria* sp. are also acceptable. Feeding is given once a day. Feeding in low or higher doses seems to affect the larval growth.

6.4 *Spatfall*

Normally spatfall occurs between 18th and 20th days. In exceptional cases the spatfall is either advanced to 14th day or delayed corresponding to conditions prevailing at the time. High percentage of spatfall is obtained in 2 larvae per ml concentration. Dark coloured surfaces enhance the spatfall whereas aeration during larval phase affects spatfall.

6.5 *Survival*

20-30% of spat production compared to the initial stock at veliger stage is obtained in mass production in one tonne capacity tanks and 40-50% production in tanks of smaller capacity (75 l). Negligible mortality of spat is experienced under normal conditions.

7

SPAT REARING

The spat rearing in the hatchery forms the first phase of nursery rearing. Supply of *Isochrysis galbana* as food is continued for one month after setting of the spat and gradually changed over to mixed algae. The spat reaches the size of 3 mm in a period of 60 days. Now the spat are ready for transplantation. Aeration is needed for the spat after settlement. As the spat are sedentary, recirculation of seawater is provided. This will help in the removal of faecal matter and other algal wastes which settle at the bottom of tanks. The spat mortality is minimum in the hatchery phase of rearing as long as the environmental and hydrological conditions are favourable.

8

PRODUCTION OF FOOD FOR LARVAE

The unicellular microalgae such as the *Isochrysis, Pavlova, Chromulina* and *Dicrateria* form the larval food. After settlement, feeding the spat with a mixture of microalgae dominated by the diatom *Chaetoceros* is found to be very effective in obtaining faster growth rates.

8.1 *Isolation*

The phytoflagellatess of the required species can be isolated from the sea water by employing serial dilution culture technique. The sea water collected from the natural pearl oyster bed area should be passed through seives of 200 µm, 50 µm and 10 µm. In fine dilution steps, the inocula are added to the sea water containing the nutrients in the concentration of 1, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} in 50 ml culture tubes. After this, the tubes are kept under light (perferably 500 lux) at a temperature of 23-25°C. After 15-20 days, decolouration of the water can be seen. On examination under microscope, the growth of unialgal species can be observed. Purification of the algae can be done by subculturing and reculturing in 50 ml culture tubes, 250 ml, 500 ml and 1000 ml culture flasks and finally in 3 or 4 l. Haufkin flasks are maintained as stock culture.

8.2 *Culture media*

For culturing phytoflagellates, Conway or Walne's medium is used. For maintenance of stock culture and also for mass

culture the same medium can be successfully used. The composition of Conway medium is as follows :

Solution A - *Chemicals:*

Potassium nitrate	-	100 gm
Sodium orthophosphate	-	20 "
Sodium EDTA	-	45 "
Boric acid	-	33.4 "
Ferric Chloride	-	1.3 "
Manganese chloride	-	0.36 "
Distilled water	-	1000 ml

Solution B - *Trace metals:*

Zinc chloride	-	4.2 gm
Cobalt chloride	-	4.0 "
Copper sulphate	-	4.0 "
Ammonium molybdate	-	1.8 "
Distilled water	-	1000 ml

Acidify with HCl to obtain a clear liquid.

Solution C - *Vitamins:*

Vitamin B (Thiamin)	-	200 mg
Vitamin B_{12} (Cynocobalamin)	-	10 "

Each dissolved separately in 100 ml distilled water and stored in a refrigerator.

Solution A, B and C in different reagent bottles are prepared. 1 ml of 'A', 0.5 ml of 'B' and 0.1 ml of 'C' each are added to 1000 ml of filtered and sterilized sea water.

8.3 *Growth phases*

A small quantity of the stock culture is inoculated into a limited volume of the medium containing the nutrients. It is then exposed to normal conditions of light and temperature under aeration. In four hours, no cell division will take place. This

phase is called the *log phase*. Then the cells will multiply and grow rapidly and in about 10-15 days, it will reach the maximum concentration. This phase is called the *exponential phase*. Once this stage is reached, the growth and multiplication of the cells are arrested and they will show a declining trend. This is the *declining phase*. After the arrested growth, the cells will remain without further division for a few days. This is the *stationary phase*. In flagellates, the stationary phase is prolonged. If the cells in the stationary phase is introduced/inoculated into a new environment, they may start further growth and reproduction. If the cells remain in the stationary phase for a long time, they will loose their vitality and will die. These cells cannot be used for reculturing and for feeding the larval stages of pearl oysters.

8.4 *Stock culture maintenance*

Conway medium is found to be the ideal one to maintain the stock culture. Required quantities of the nutrients are added to the sterilized sea water in a Haufkin culture flask. About 10 ml of the inoculum in the *growing phase* is poured into it and is kept under two tube flurescent light (1000 lux) at a controlled temperature (25°C) condition. When the exponential phase is reached in 8-10 days, light intensity is reduced to half. The flagellates will enter into the stationary phase after 15 days and this can be maintained for about 2 months with or without aeration. Before it enters into the *death phase*, the culture can be used as inoculum for mass culture.

8.5 *Mass culture*

Glass carbuoys of 20 l and perspex tanks of 100 l capacity are used for mass culture. The inoculum used in 250 ml for 20 l and 2 l for 100 l (2 million cells/ml). The maximum bloom is reached within 5 or 6 days if four tube flurescent lights are used at 25°C. Aeration is provided.

Open culture of the algae is done in 1 tonne fibreglass tanks. Algae cultured in mass culture can be used as inoculum. Sufficient sun light must be provided. The components of the

culture medium used for open culture of algae, diatoms and other nanoplankters are as follows :

Potassium nitrate	-	13.2 gm
EDTA	-	6.6 "
Sodium orthophosphate	-	6.6 "
Sodium silicate	-	6.6 "
Unfiltered sea water (from open sea)	-	1000 ml

8.6 *Favourable culture condition*

The right amount of illumination is an important factor for algal culture. Most of the flagellates require good light during exponential phase and less light during stationary phase. Too much of light causes early declining of culture. A photoperiod of 10 hrs light and 12 hrs darkness is ideal for maintaining the stock as well as the mass cultures of the algae. They are maintained best at 23-25°C. Under aeration, the cultures remain in the growing phase 2-3 days more.

9

PROBLEMS AND REMEDIES

9.1 *Care of water supply system and culture tanks*

Non-corrosive pumps are to be used wherever needed. Sedimentation tank, filter bed, sump and overhead tank should be cleaned periodically with bleaching powder. Organic and inorganic chemicals should be avoided. Fibreglass tanks, plastic buckets, aeration tubes and the UV chamber should be washed with soap solution. Before drawing water to the tanks, some water is allowed to flow waste to remove the overnight stagnant water in the tubes. Regular change of cotton wool should be done. The seives used in filtering the larvae should be cleaned well by dipping them in warm soap solution for few hours.

The larval rearing tanks should be cleaned with soap solution and washed well with fresh water and dried. The tanks should be rinsed in seawater before use. Concentrated sulphuric or hydrochloric acid can be used to clean the algal culture flasks and carbuoys and soap solution to clean the perspex tanks.

9.2 *Infestation and diseases*

Oysters infected with fungi and trematode parasites are to be avoided as brood stock. The weak and dying larvae are to be separated and eliminated. This helps to avoid the ciliate development in the larval rearing tanks. If larvae are found to be infected with ciliates, it is advised to discard the whole lot of the larvae in the tank.

9.3 *Fouling*

Algae, sponges, polychaetes, hydrozoans and ascidians may grow in the spat rearing tanks. These can be removed by pouring seawater with some force and changing the sea water. Pentachlorophenol at 1 ppm, formalin at 40 ppm and dichlorophene at 10 ppm have also been suggested to eradicate certain bryozoans, ciliates and ascidians which may sometimes infect the trays and other culture containers when the molluscan juveniles are reared in the hatchery.

10

PERSONNEL REQUIREMENT

One Manager with a overall knowledge on hatchery management, one technician with experience in larval estimation, stocking, measurement and algal cell counting; two skilled assistants to change water for the larvae/spat and feed them daily and two helpers for washing rearing tanks, glassware, plasticware and for doing farm work will be enough to produce five million spat in the hatchery per year and to rear them in the farm. Establishment of running seawater system both for the larvae and spat would minimise labour in the hatchery and the man power may be utilised for efficient management of juveniles/oysters in the farm.

11

ECONOMICS

Production capacity of the hatchery

The pearl oyster hatchery has the production capacity of about 1.2 million pearl oyster spat annually. The details of spat production are given below.

Hatchery spat production

Total No. of tanks used	: 10 (1 tonne capacity)
Stocking rate of veliger larvae per tank	: One million
Total veliger larvae stocked in one run	: Ten million
Expected percentage production of spat (20%)	: Two million
Percentage of spat survival at the end of two months in the hatchery	: 50%
Net spat production per run	: One million

CAPITAL EXPENDITURE

A. *Buildings*

	Rs.
Hatchery building with translucent roofing 150 sq.m @ Rs.750/- per sq.m.	1,12,500

		Rs.
	Generator/Compressor room 27 sq.m @ Rs.500/- per sq. m.	13,500
	Seawater sump 14 sq.m. @ Rs.750/- per sq.m.	10,500
	Sedimentation tank 8.4 sq.m @ Rs.750/- per sq.m.	6,300
	Filter bed 4.5 sq.m. @ Rs.750/- per sq.m.	3,375
	Pump house 14.6 sq.m @ 750/- per sq.m.	10,950
	Overhead tank - 10,000 1 capacity	50,000
	Total	2,07,125
B.	*Fibreglass/perspex tanks*	
	6 Nos. 100 1 capacity broodstock FRP tank @ Rs.600/-	3,600
	5 Nos. 100 1 capacity spawning/algal culture perspex tank @ Rs.1000/-	5,000
	24 Nos. 1 tonne capacity rectangular larval/spat rearing/mixed algal culture FRP tanks @ Rs.3000/-	72,000
	6 Nos. 3 1 capacity Haufkin flask for stock culture @ Rs.400/-	2,400
	Total	83,000
C.	*Major equipments*	
	10 KVA Generator - 1 No	50,000
	Air Compressor - 1 No	10,000
	7.5 HP Electric pump - 1 No	15,000
	1.0 HP Electric pump - 2 Nos	7,000
	Microscope, pH meter, salinometer	15,000
	Chemical balance	5,000

		Rs.
	UV Chamber, Hot air oven	7,000
	Furniture	25,000
	ECE Controller, Silica cased immersion heater, Jumo thermometer	4,000
	Air conditioner - 2 Nos	40,000
		1,78,000
	Total capital cost (A + B + C)	4,68,125

RECURRING EXPENDITURE

A.	*Interest*	
	@ 15% on 4,68,125	70,200 (approx.)
B.	*Depreciation*	
	@ 5% on building and fibreglass tanks	14,500 (approx.)
	@ 10% on equipment	17,800
C.	*Salaries*	
	One Manager @ Rs.3000/- p.m One Technician @ Rs.2000/- p.m Two skilled workers @ Rs.1000/- p.m. Two helpers @ Rs.750/- p.m.	1,02,000
D.	*Contigencies*	
	Plasticware, flexible PVC hoses, glassware, bolting silk, etc.	5,000
	Energy cost (Electricity and Diesel)	15,000
	Chemicals	5,000

		Rs.
	Other contingencies	5,000
E.	*Maintenance*	10,000
F.	*Annual lease for land*	3,000
	Total recurring cost (A to F)	2,47,500

Economics of Hatchery Produced Spat

Total spat production	5 million
Cost of production per spat (3 mm)	5 paise

Profit

(N.B: If the selling price of a spat at 7 paise)

Annual revenue for 5 million spat		3,50,000
Annual expenditure		2,47,500
Annual net profit		1,02,500
Annual repayment of loan		67,800
Net profit after repayment of loan		34,700
Cost of production of 3 mm spat/thousand (inclusive of interest and depreciation)		50
Profit cost ratio (Rs.1,02,500/2,47,500)	0.414	
Profit investment ratio (1,02,500/4,68,125)	0.218	

At this rate of loan repayment *i.e.* Rs.1,00,000 per year (Rs.32,200 depreciation + Rs. 67,800 as loan repayment) the entire loan will be repaid in about 5 years and a cumulative net profit of Rs. 3,23,500 will be received in 5 years.

The economics given here are based on the facilities available at the Molluscan Shellfish Hatchery of CMFRI, Tuticorin.

12

NURSERY REARING

The spat, on attaining 3 mm size after the first phase of nursery rearing in the hatchery are shifted to farm. In the farm, mortalities of varying degrees set in at various stages of nursery/juvenile rearing.

12.1 *Spat transport*

The spat are gently removed from the tank with the help of soft foam rubber. For short distances the spat are transported in water during cool weather conditions. If the duration is more, they are transported in water under oxygen. In 39 hr of transport, survival rates of 99.2%, 98.2% and 80.7% were obtained when packed in polythene bags having pearl oyster spat numbering 500, 1000 and 1500 of size 10-15 mm in 7 l of seawater with oxygen.

12.2 *Spat rearing in the farm*

Spat with average size of 3 mm are reared in box-type net cages of the size 40 x 40 x 10 cm with iron frame and encased in a retrievable synthetic velon screen bag of 0.5 mm mesh. The velon bag with the above iron frame is inserted into another bag made out of old fish net with 10 mm mesh. This serves as a protection to the velon screen bag and to the spat from the predators like crabs and fishes. The bags are easily removable.

They can be sun dried and reused. The stocking rate of 3 mm size spat is 10,000/net cage. After a month of rearing the spat are transferred to another net cage, the bag of which is 1.0 mm in mesh size. Now the density of the spat per net cage is reduced to 5000. On attaining thumb-nail size (10-15 mm) they are reared in the box cage with a covering of velon screen of 1.5 mm mesh. At this stage the stocking rate is 2000 per net cage.

12.3 *Juvenile rearing*

The juveniles of the size 20-30 mm (Pl. IX B) are reared in 40 x 40 x 10 cm cages netted with 1.5 mm nylon thread having 10 mm mesh size. Velon screen and fish net coverings are not provided. The rearing density of the juveniles will range between 750-1000 per cage. Bits of fish nets and synthetic filaments placed inside the cage provide additional space for detachment of juveniles. Quick loss of juveniles/oysters due to net damage by fish bits can be avoided thus. Further thinning is done when the juveniles reach the size suitable for implantation (40-45 mm) in a period of 12-15 months.

12.4 *Spat survival in the farm*

One million spat of 3 mm size when transplanted to farm, mortality of about 50% is expected in the first month. Then the mortality is less to 20% and 10% in the second and third months respectively. By then the spat grows to 10-15 mm. Beyond this size, the mortality is negligible amounting to 5% in 15 months. The juveniles reach 45-50 mm in 12-15 months of farm rearing and the overall survival can be expected to about 30% of the initial stock.

The details on the spat size, number of spat percentage, number of net/cages/rafts and the percentage of mortality generally expected during different periods of rearing are given in Table 1.

Table 1. *Details of spat/juvenile rearing in the farm (Total spat : One million and period of rearing : 15 months)*

Spat size (mm)	No. of spat per net	Total nets/raft	Months of rearing	Cumulative Mortality (%)	No. of survivals	Net size (mesh in mm)
3	10,000	100/1	1	50	5,00,000	0.5
10	5,000	100/1	2	20	4,00,000	1.0
15	2,000	200/2	3	10	3,60,000	1.5
20-30	750	400/4	6	5	3,42,000	10
31-40	500	600/6	12	5	3,25,000	10
45-55	250	"	15			10

12.5 *Economics of spat/juvenile rearing in the farm*

CAPITAL EXPENDITURE

		Rs.
A.	*Launch*	
	30 footer 4 cylinder, 50 HP	3,00,000
B.	*Rafts*	
	6 rafts with teak wood poles, MS barrels, 2 anchors, chain with 100 netted cages each	1,20,000
	Total capital cost (A + B)	4,20,000

RECURRING EXPENDITURE

A.	*Interest*	
	@ 15% on Rs. 4,20,000	63,000
B.	*Depreciation*	
	@ 20% on launch	60,000
	@ $33^{1}/_{3}$% on Rafts and cages	40,000 (approx.)

C.	*Salaries*	
	One Launch driver @ Rs.2000/- p.m and One Mechanic @ Rs. 1500/- p.m	42,000
	Casual labours @ two for 15 months; two for 12 months; four for 9 months and four for 6 months @ Rs. 20/- per head on 4 days per week.	37,120
D.	*Contigencies*	
	Insurance on launch @ 2 paise per thousand rupees	600
	Running cost on launch	20,000
E.	*Maintenance*	5,000
	Total recurring cost (A to E)	2,67,720
	No. of oysters at the end of 15 months	3,25,000
	Cost per oyster of 45-50 mm	Rs.0.824

(N.B : If the spat is purchased at the rate of 7 paise each the cost of the spat will be Rs.70,000 for one million spat of 3 mm size. In this case, the cost of oysters of the size 45-50 mm will be Rs.1.04 per number).

ANNEXURE I

List of Equipment Requirements

Equipment/facility		Quantity required
Generator (10 KVA)	:	1
Air compressor ELGI 200/220V 1420 RPM 50 HZ S_1 Rating output 0.75KW AMP 7.3	:	1
Fibreglass rectangular maturation tanks	:	6
Fibreglass rectangular larval/spat rearing/algal culture tanks (1000 1 capacity)	:	24
Perspex tanks rectangular (1000 1 capacity) spawning tank	:	1
Perspex tank rectangular (1000 1 capacity) algal culture tanks	:	4
Binocular microscope	:	1
pH meter	:	1
Salinometer	:	1
Chemical balance	:	1
Thermometer (0-50°C)	:	3
Hot air oven	:	1
Air conditioner 2 tonne capacity (algal culture room and conditioning room)	:	2
UV Chamber (Locally designed)	:	1
Jumo thermometer with 50°C (Thermal stimulation)	:	1

Equipment/facility		Quantity required
Silica cased immersion heater	:	1
Laboratory glassware		
Beaker 5000 ml	:	6
Beaker 1000 ml	:	6
Beaker 500 ml	:	6
Beaker 250 ml	:	6
Beaker 100 ml	:	6
Conical flasks 250 ml	:	6
Oxygen bottles 125 ml	:	12
Volumetric pipettes (assorted sizes)	:	12
Burettes (10 ml)	:	2
Burettes (50 ml)	:	1
Petridishes (150 mm dia)	:	2
Finger bowls (50 ml capacity)	:	2
Embryo cups (50 x 50 mm)	:	6
Micro slides with cavity	:	6
Micro slides (in box)	:	1
Cover slips (in box)	:	2
Haemocytometer	:	2
Plankton counting chamber (1 ml capacity)	:	2
Measuring flasks - 1000 ml, 250 ml, 50 ml, 25 ml	:	1 each
Glass troughs (5 1) 25 cm dia.	:	2
Plasticware		
Plastic buckets (15 l)	:	4
" (5 l)	:	2
" (3 l)	:	2

Equipment/facility		Quantity required
Basins (20 l) capacity	:	6
Polyethelene flexible hoses (20 mm dia)	:	6 m
PVC pipes - 150 mm for seives	:	50 cm
80 mm for seives	:	50 cm
Polythene sheets (mixed algal culture)	:	10 m
Bolting silk cloth	10 microns	25 cm
	20	25 cm
	30	50 cm
	40	50 cm
	80	50 cm
	140	50 cm
	180	50 cm
	200	50 cm
Velon screen	0.5 mm mesh	1 m
	1 mm mesh	1 m
	1.5 mm mesh	1 m
Tank cover cloth (Black)	:	50 m
Seawater drawing distribution grid made of 50 mm and 25 mm rigid PVC pipelines and valves.	:	as required
Aeration grid made of 25 mm rigid PVC pipelines with copper nozzles, 5 mm polythene tubes, plastic 'T' joints and regulators and diffuser stones.	:	as required
Seawater intake pumping and storage system (see text)		
Freshwater storage and pumping system (see text)		
Green house facilities (outside the hatchery)	:	as required

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