

CMFRI

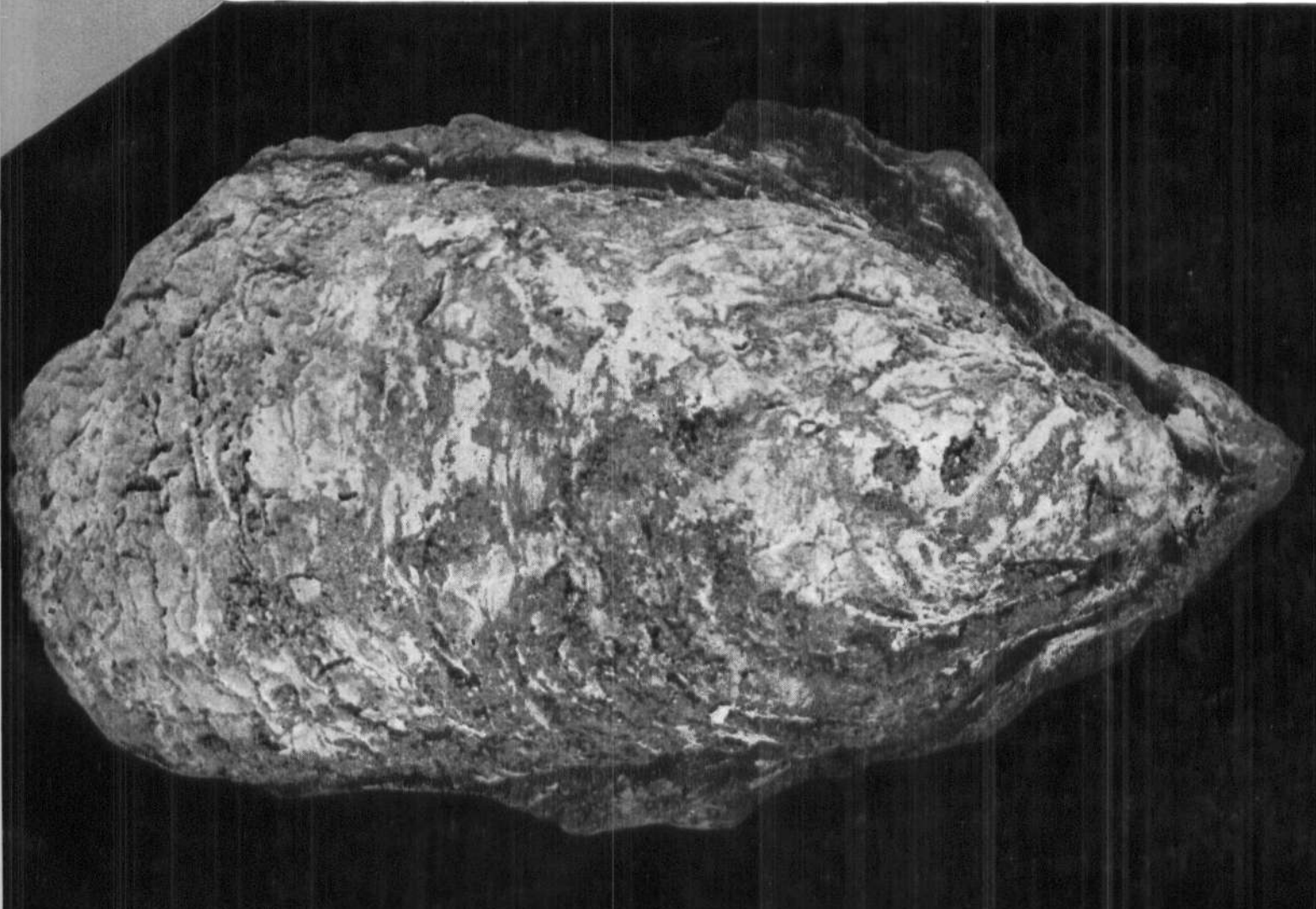
bulletin 38

JANUARY 1987



OYSTER CULTURE—STATUS AND PROSPECTS

Edited by : K. NAGAPPAN NAYAR AND S. MAHADEVAN



CENTRAL MARINE FISHERIES RESEARCH INSTITUTE

(Indian Council of Agricultural Research)

P.B. No. 2704, Cochin 682 031, India

PRODUCTION OF OYSTER SEED IN A HATCHERY SYSTEM

K. NAGAPPAN NAYAR¹, K. SATYANARAYANA RAO¹, M. E. RAJAPANDIAN¹, C. P. GOPINATHAN¹
AND A. D. GANDHI¹

INTRODUCTION

Although oyster culture dates back to First century B.C., the development of hatchery techniques for the production of oyster seed on a year round basis is a recent innovation. Since early 1950s attempts for large scale production of oyster seed have been initiated. Loosanoff and Davis (1952), Dupuy *et al.* (1977) and AQUACOP (1977) have successfully produced seed of the oysters *Ostrea edulis*, *Crassostrea virginica* and *Crassostrea gigas*. Nayar *et al.* (1984) have successfully accomplished the production of seed of *C. madrasensis* on a large scale at the molluscan hatchery laboratory of Central Marine Fisheries Research Institute at Tuticorin. At this hatchery cultched as well as free or cultchless spat are produced. The production of oyster seed by hatchery techniques is accomplished in six phases of operations viz., (1) Conditioning adult oysters for maturation of gonads (2) Induced spawning (3) Larval rearing (4) Culture of algal food (5) Preparation of spat collectors and (6) Setting of spat. These six functions although interrelated are independent phases of operation and easy to follow and implement.

CONDITIONING OF ADULT OYSTERS FOR MATURATION OF GONADS

The aim of conditioning oysters is to induce maturation of gonads rapidly by feeding the oysters with a rich supply of food which provides adequate nutrition required for gamatogenesis and build up of the reproductive organs.

Selection of broodstock

The oysters for broodstock are selected keeping in view the area, growth and condition factor, size and age of the standing oyster population. They are

collected from population in areas where they are known to occur in healthy condition. The oysters chosen for conditioning are collected from areas where the salinity regime is comparable to that in hatchery. Otherwise the oysters have to be acclimatised before they are conditioned. The prevailing temperature of the collection area has to be recorded first, since on this basis manipulation of temperature regime is effected for conditioning of the oysters for maturation and induced spawning.

Oysters of the size (length) range 60 mm to 90 mm are ideal and 30% of this should be of 'O' year class or just one year old (60-75 mm) in order to be assured of the availability of males in the broodstock. Before stocking the oysters a sample of 10 numbers are opened to ascertain whether they exhibit uniform maturity stage, preferably immature or spent. If there are more than one stage the conditioning cannot be done effectively. A minimum of 500 oysters are kept as broodstock in the hatchery.

The selected oysters, 25 in each batch are cleaned thoroughly and placed on a synthetic twine knit P.V.C. frame in a 100 litre fibreglass tank (75 × 50 × 25 cm) and raw seawater is filled in the tank and well aerated. The water level inside the tank is maintained at half the height of the tank and 15 litres of mixed phytoplankton cultured in outdoor tanks using fertilised medium (Gopinathan, 1982) are added twice during a day between 09.00 and 17.00 hours at 4 hours intervals. The phytoplankton diet is composed of diatoms such as *Chaetoceros affinis*, *Skeletonema costatum*, *Thalassiosira subtilis* and *Nitzschia closterium*, the phytoflagellates, *Isochrysis galbana* and *Pavlova* sp. and the microgreen alga *Chlorella salina*. On an average the cell concentration of the algae should be about 1.0 million cells/ml. The oysters are conditioned at

¹ Present address : CMFRI, Research Centre, Tuticorin-628 001.

about 5°C below ambient temperature for 10 to 20 days. The tanks in which the oysters are reared are cleaned every day to remove dirt and faeces and filled with fresh raw seawater before commencing feeding.

On the tenth day a sample of three oysters are opened to observe the condition of the gonads. The right valve of the oyster is removed with the help of a shucking knife. By using a Pasteur pipette a few shallow cuts are made on the mantle surface, a small quantity of gonadal contents are drawn with the pipette, a drop of this is placed on a slide with a cover slip and examined under microscope to determine if the gonads of the oyster contain ripe sperms or ova. Rapid directional movement of the particles indicates sperms. The ripe ova of a mature ovary of *Crassostrea madrasensis* are pear shaped and compressed. The presence of spherical and subspherical ova in the sample indicates the ripeness or the readiness of the oyster to spawn. Such ova measure 50 to 60 μ in diameter. If the oysters are not mature they are conditioned for another five to ten days. The oysters conditioned to mature stage by this process are transferred to spawning tanks.

INDUCED SPAWNING

The conditioned oysters spawned on giving thermal stimulation by transferring them to water of higher temperature. Firstly the conditioned oysters are thoroughly washed and transferred to a 100 lit. Perspex spawning tank containing 50 l. of filtered seawater with temperature 2 to 4°C above the ambient temperature level. This is achieved by operating a heating element with porcelain coating which is controlled by a thermostat (Pl. I A). Proper aeration of seawater is provided in the tank. During the first hour the oysters stimulated thus commence spawning. If spawning cannot be achieved by this method, fresh sperms stripped from a sexually ripe male are introduced in the tank containing the broodstock.

The sperms from a stripped male (0.25 ml.) are diluted with 10 ml of filtered seawater. This suspension is drawn in a long pipette (10 ml.) which is immersed and placed above the oyster along the incurrent side and sperms are slowly released from the pipette. This stimulation should be given to all the oysters individually in the tank. At intervals of 10 to 15 minutes more sperm suspension are released till the oysters start spawning.

Once an oyster starts spawning, it is transferred to a three litre glass tray containing filtered seawater at ambient temperature, 15 to 20 numbers of such

trays are kept ready for operation. Only one oyster is placed in each tray. Each individual oyster is allowed to complete its spawning in the tray. If the female is a heavy spawner and the water becomes highly milky it is transferred to another tray to complete spawning. The males spawn by continuous ejection of white stream of seminal fluid containing spermatozoa (Pl. I B) and the females by rhythmic ejection of ova at intervals. The spawning oysters in the glass trays are carefully watched and each removed as spawning is completed. This is done to prevent the oysters from filtering the gametes. The filtered seawater containing eggs (Pl. I C) from each spawning tray is poured through a 100 μ stainless seive or nylobolt seive into a 10 litre glass beaker. At this stage mild aeration is given to ensure sufficient supply of oxygen from air bubbles produced by an aquarium air stone.

FERTILIZATION OF EGGS AND ESTIMATION OF FERTILIZED EGGS

The ova in each beaker are fertilized within 45 minutes after being spawned, from a pooled sperm suspension in the individual trays in which the males have spawned (Pl. I D).

50 ml. of a pooled sperm suspension obtained from as many males as possible is added to each beaker containing egg suspension. After 20 to 30 minutes a 2 ml sample of the fertilized eggs is taken and examined under microscope to ascertain the extent of fertilization. This can be determined by noting the formation of polar body. If 10% or more of these eggs do not exhibit this feature, then more sperm suspension (15-20 ml.) is added.

When fertilization is complete, the 10 l. beaker containing the fertilized eggs is filled with filtered seawater. After proper mixing of the eggs in the container a 1 ml. sample is drawn quickly with a blowout pipette. The sample is drawn quickly as the eggs have a tendency to settle out of suspension. If the concentration of the eggs is high the sample is made up to 10 ml and one ml of subsample is taken. The sample is then transferred to a counting chamber and the number of eggs counted with a compound microscope. After counting and recording the number of fertilized eggs/ml. will be multiplied by the dilution factor and the number of fertilized eggs in 10 l. is estimated.

At the end of one hour, aeration is suspended. The fertilized eggs settle at the bottom. After 15 minutes, the supernatant water, containing sperms, unfertilized eggs and debris is removed. Fresh seawater is added

and decanting is carried out 3 times. Finally 40 to 50 l. seawater is added to the tank and mild aeration is provided. At the end of 4 hours following cell divisions, the eggs attain the morula stage and begin to swim in the column layers (Pl. I E and F and Pl. II A and B). The larvae are allowed to develop for the next twenty hours.

LARVAL REARING

The sequence of developmental stages and growth and rearing of larvae from the straight hinge to the eyed or pediveliger stage are described below.

Straight-hinge stage

The straight hinge or 'D' shell larval stage is attained at the end of 20 hours (Pl. II C). The larvae are semi-transparent with the velum protruding out and creating a strong ciliary current which directs minute particles of food into the stomodaeum. The larvae swim vigorously and some of the larvae show a slow circular movement under the microscope. The actively swimming larvae are separated by siphoning them from the tank, leaving the sluggish ones. This process of culling is continued during the first two days or till the entire stock of the larvae are of uniform size and movement. On an average, the larvae measured 66μ in length on the first day. At this stage the larvae are reared in one ton fibreglass tank. The total number of the larvae is estimated and stocked at the density of 4 larvae per ml in the rearing tank. The larvae are fed with phytoplankton at the end of 24 hr.

Umbo stage

On the third day, the larvae become slightly oval in shape and measure 100μ . This stage is considered as early umbo stage. At this stage the larvae are filtered through 80μ filter in order to segregate the smaller ones or the non-growing larvae.

On the 7th day, the umbo is seen distinctly and pronounced concentric rings are found on the shells as the larvae grow (Pl. II D). The larvae measure 150μ at this stage.

In 12 to 15 days the 'late umbo' larvae measure between 260 and 270μ . During the rearing period, on every third day the total number of larvae in each tank is estimated to determine the mortality rate. On an average, per day a larval mortality rate of 2% to 3% is normal. If the mortality rate is very high it means the conditions are unfavourable. In such a case the larvae should be filtered every day to remove the

dead and non-growing larvae. Further it is advised to treat the larvae with specific doses of water soluble antibiotics such as Streptomycin sulphate or Chloramphenicol.

The larvae are filtered out from the tank and immersed for 15 minutes in seawater containing the antibiotic Streptomycin sulphate at a strength of 50 PPM. This process of treatment controls to a large extent fungal diseases. The dipping of larvae in antibiotic medium may be given once or twice during the larval period if necessary.

Eyed stage

An irregular eye spot is observed between 13 and 17 days when the larvae grow to a size of 280μ (Pl. II E). It becomes distinct at the size of 290μ . The eye spot is present in the lower quadrant of the ventral region close to the right angle of the dorso-ventral and antero-posterior planes.

Pediveliger stage

Between the 14th and 18th day, the functional foot emerges. At this stage the larvae measure $330-350 \mu$ (Pl. II F). At this stage they descend to the bottom and start crawling. This is known as 'swimming creeping' stage which has been designated as pediveliger by Carriker (1961). The spat start setting within 24 hours or sometimes setting is prolonged to 2 to 6 days depending on the availability of favourable substratum.

Spat

The pediveliger larvae settle down, losing the velum totally. The shell edges grow hexagonally and the larvae develop the characteristic adult features and metamorphose into the spat (Pl. III A). The young spat measures 450μ . The eye spot is traceable at the stage and it disappears 24 hours after attachment.

Larval density

As the larvae grow their number per tank is reduced. It is absolutely necessary to reduce the number of larvae since the growing oyster larvae require greater space for optimal feeding and growth.

The larvae are transferred to 10l beakers using 100μ mesh sieves. In the beaker they are mixed well and one ml is drawn and transferred to cell counter. The number of the larvae is counted under microscope and the total number is estimated. This study is carried out in the case of all individual containers. Measured quantities of the larval medium is added to the rearing tanks so

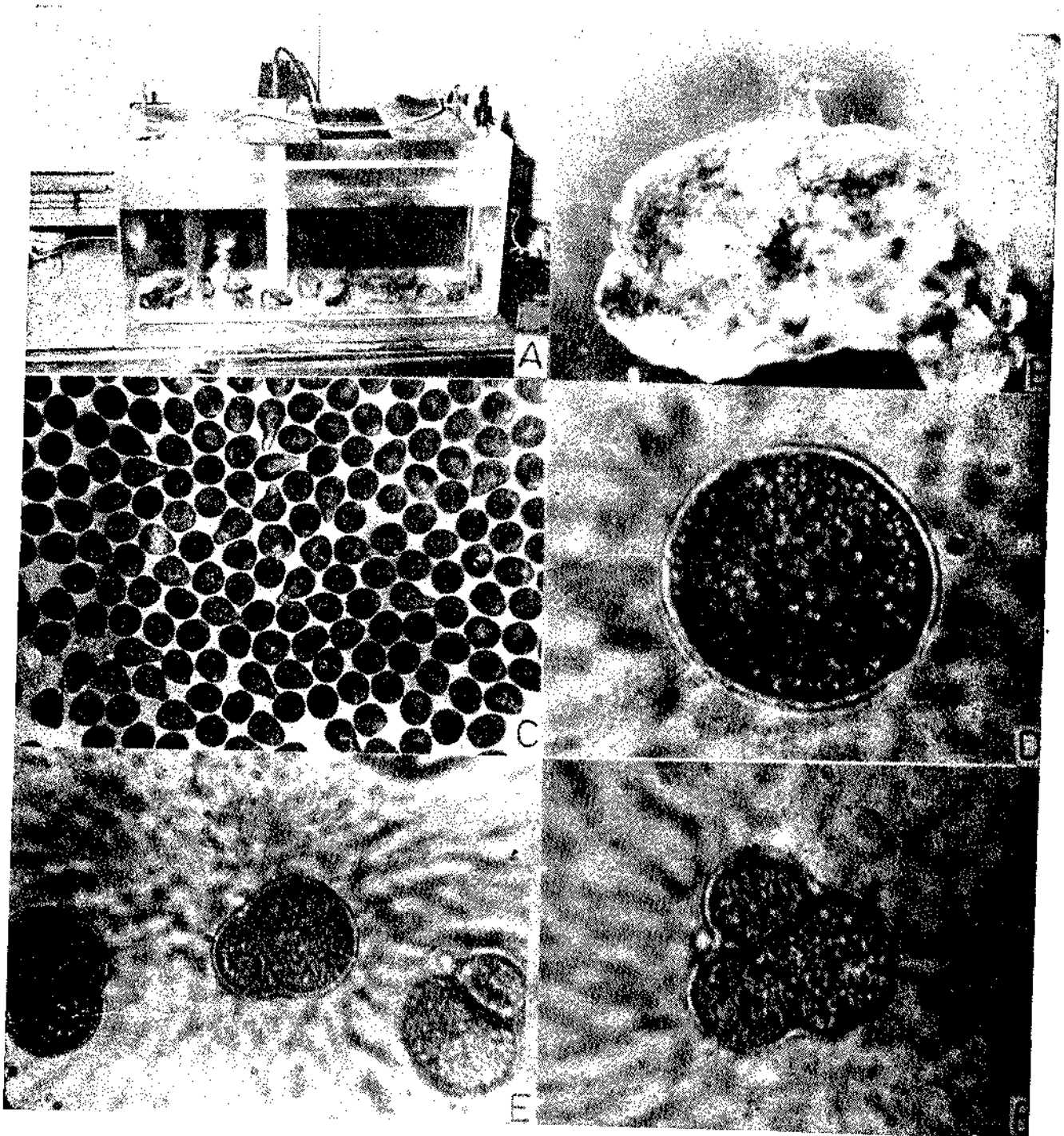


PLATE I. A. Thermal stimulation of broodstock to induce spawning in *Crassostrea madrasensis*. B. Spawning of male oyster in hatchery. C. Unfertilized eggs of oysters spawned in hatchery. D. Fertilized egg. E. Two celled stage of fertilized egg. F. Four celled stage of fertilized egg.

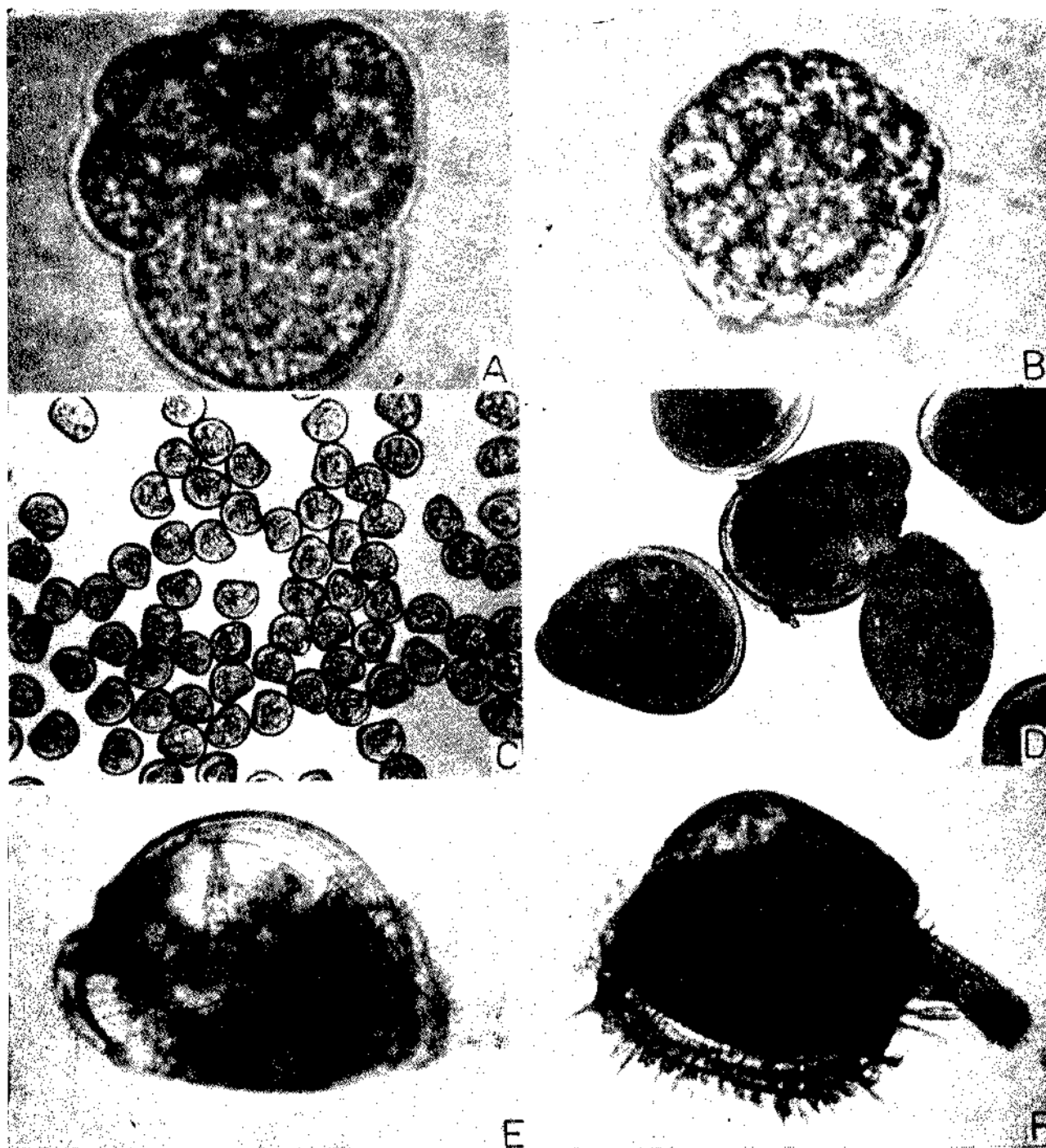


PLATE III. A. Eight celled stage of fertilized egg. B. Morula stage. C. Straight hinge stage. D. Umbo stage. E. Eyed stage. F. Pediveliger stage.



PLATE III. A. freshly set oyster spat. B. Oyster spat which had set on oyster shells. C. A view of the Oyster hatchery of CMFRI Institute at Tuticorin. D. Isolation of pure phytoflagellate cultures in conical flasks in controlled conditions. E. Stock cultures of phytoflagellates, F. Mass cultures of phytoflagellates.

as to maintain the required larval density in the rearing tanks. The following densities of the larvae are advised at different stages of development during the larval period.

1. Fertilized eggs to 'D' shaped larvae : 25/ml
2. 'D' shaped stage to umbo stage : 5/ml
3. Umbo to eyed stage : 2/ml

CULTURE OF ALGAL FOOD

The success of the hatchery operation depends mainly on the availability of adequate quantity of the larval food, the microalgae. In the natural environment, the larvae feed on nanoplankters which are readily available to them. In a hatchery the forms which will be acceptable to the larvae for their growth and development have to be identified and isolated. The production and constant supply of the algal food, especially selected species to the larval organisms is a pre-requisite in the hatchery systems throughout the world.

Realising the importance of the nanoflagellates, measuring less than 10 microns as the essential food of the larvae of edible oyster, the isolation, identification, maintenance of stock culture, laboratory mass culture and large scale open tank culture of these flagellates are being carried out at the molluscan hatchery of Central Marine Fisheries Research Institute at Tuticorin.

Algal species

Since the oyster larvae could feed only on nanoplankters less than 10 microns in size upto the stage of spat, suitable phytoflagellates have to be isolated from the seawater. It has been observed that the ideal phytoflagellate for feeding larvae of *Crassostrea madrasensis* is *Isochrysis galbana*, a member of the Class Haptophyceae. Apart from this, species of *Pavlova*, *Dicrateria* and *Chromulina* have also been tried as food and satisfactory results obtained. All these flagellates measure 7-8 μ and have 26-38% of protein by body weight. Once the larvae set and become spat, they are fed with mixture culture of microalgae comprising mostly diatoms and other phytoplankters.

Culture media

For the successful culturing of the microalgae various chemical culture media have been recognised depending on the organisms, Class and genera. Although most algae are photoautotrophic and can grow in

purely inorganic media, many require organic compounds, the requirements of which may be either absolute or stimulatory. Usually for culturing the flagellates, Conway or Walne's medium is used in the laboratories for the maintenance of the stock culture as well as mass culture (Walne, 1970). Since this culture medium contains the chemicals, trace metals and vitamins (B_{12} , B_{12}) required for microalgae, the flagellates such as *Isochrysis*, *Pavlova*, *Dicrateria* and *Chromulina* are being cultured by using this media alone.

Isolation

For the isolation of the required species of phytoflagellates (Pl. III D) 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}) or 4 steps (0.001, 0.01, 0.1 and 1 ml) are required for the isolation of the phytoflagellates. After filtering the seawater through 10 microns sieve, the filtrate is inoculated to 5 series of culture tubes in various concentrations and kept under sufficient light (1 k. lux with uniform temperature (25°C) conditions. After 15 days, we can see some colouration of the culture tubes. On examination, the growth of a unialgal species could be noted in these tubes. Further purification of these organisms can be done by sub-culturing the same in 500 ml. or 1 litre conical flasks. Finally, if the culture is fully purified it is transferred into a 3 litre or 4 litre Hauffkin culture flasks as stock culture.

Stock culture

Stock cultures of all the flagellates are maintained in a special room, adjacent to the mass culture laboratory. The stock cultures are kept in 3 or 4 litre Hauffkin culture flasks (Pl. III E). The autoclaved or heated seawater after cooling is poured to the culture flasks and required nutrients are added. Walne's medium enriched with vitamins is the ideal one suitable to maintain the stock of all the nanoflagellates. About 10 ml of the inoculum in the growing phase is transferred to the culture flask and the latter placed in front of two tube lights (800 lux). When the maximum exponential phase has reached, only one tube light (400-500 lux) is used for further growth. Normally the flagellates will enter the stationary phase of growth after 10 days. In the stationary phase, the culture can keep for a period of 2 months in the stock culture room, under controlled conditions of light and temperature with or without aeration. At the time of maximum exponential phase of growth, the colour of the culture will turn dark brown and the cells are found in suspension without movement. It is believed that during

the stationary phase, the cells form cyst or matrix individually for thriving in the unfavourable conditions.

Mass culture

Utilizing the inoculum from the stock culture, the flagellates are grown on largescale. The containers for the mass culture of flagellates are 10 litre polythene bags, 20 litre glass carbuoys and 100 litre Perspex tanks (Pl. III F). These containers are kept in specially light provided wooden racks with aeration facilities. Fully grown culture from the stock culture room is used as inoculum for the mass culture in these containers. About 100 ml of the culture is used for mass culture in the polythene bags, 500 ml in the glass carbuoys and about 2 litres of fully grown culture is used in the 100 litre Perspex tanks providing adequate light and aeration facilities. These containers will have the maximum concentration of cells in the growing phase on 5-6th day for harvest. After noting the cell concentration using a haemocytometer, the culture is siphoned to plastic buckets or bins and supplied to the hatchery for the rearing operations. Leaving one litre of the same culture in a 20 litre glass carbuoy, fresh sterilized seawater can be added for further mass culture in the same container.

Illumination

One of the most important factors determining the successful culture of the microalgae is the type and quantum of illumination. Most of the flagellates require less light during the stationary and declining phases. Too much of light will cause the culture to decline early. For the growing phase of mass culture 1,000—1,500 lux is optimum upto 5-6 days and for maintaining the stock culture, 400-500 lux is enough for keeping the microalgae in live condition. Twelve hours of light and 12 hours darkness is ideal for maintaining the stock as well as the mass culture, which can be controlled by control switch clocks.

Temperature control

Normal temperature (28-30°C) is not ideal for the maintenance and culture of flagellates. Hence air-conditioned rooms are used for keeping the stock as well as mass cultures. Both the rooms have 23-25°C during daytime when all the tube lights are burning.

Aeration system

Similar to light and temperature control aeration of the culture tanks provides healthy culture as well as to enhance the exponential phase for a few days more. It was noticed that if aeration is given to the mass culture,

the culture will remain in the growing phase for 2-3 days more than the tanks where there is no aeration. Aeration will help the nutrient salts to distribute uniformly in the medium and also for supplying carbon-di-oxide required for photosynthesis. Lastly, aeration will prevent settling out of the cells at the bottom of the culture tanks and causing eventual death due to the lack of supply of carbon-di-oxide.

Anticontamination procedure

In working with the various species of microalgae, the most important is the cleanliness of all surfaces, containers and especially of the personnel's hands. When handling the equipments, glassware and cultures, one should wash hands after working with one species and before starting to work with another species of algae. Transfer of tube cultures is done where there is a minimal movement of air to reduce chances of contamination. Further, all the stock cultures should be checked for contamination periodically using sterilized pipettes.

Harvest of the culture

The fully grown culture is harvested during the growing phase of the phytoflagellates, after determining the cell concentration. If the culture has entered the declining or stationary phase, the metabolites will be very high and the cells are not in healthy condition. The larval organisms reared will not have the expected growth if fed with such dietary organisms.

FEEDING PROTOCOL

Since the nutritional requirement increases with the growth of larvae, a schedule of feeding has been developed with different cell concentrations depending on the age and size of larvae. The cell concentration of the larval food *Isochrysis* sp. in respect of different stages of development is as indicated below.

Stages	Cell concentration in ml/larva
'D' shape	3,000—4,000
Umbo	4,000—5,000
Late umbo	5,000—8,000
Eyed stage	8,000—10,000
Pediveliger	10,000—12,000

GROWTH OF LARVAE

From 'D' shell stage (day 1) to umbō stage the larvae grow from a mean size of 61μ to 156μ in seven days with an average growth rate of 13.6μ per day. On the 17th day the mean size of the eyed larvae is 289.2μ with an average growth of 13.3μ per day. On the 19th day the pediveliger is 348.2μ showing an average growth of 29.0μ per day.

ENVIRONMENTAL CONDITIONS

The water temperature in the oyster hatchery (Pl. III C) varies from 23.5 to 27.6°C on the minimal range and between 28.2 and 32.6°C at the maximum. Although the temperature varied periodically during the course of year, each experiment has been concluded within the temperature regime of the period. There was no marked fluctuation in the salinity except during November and December. The annual average salinity varied between 34.11 and 36.32‰ . The pH range of the seawater measured from 7.76 to 8.20 .

SEAWATER SOURCE AND MANAGEMENT

The seawater required for the hatchery is drawn from the Tuticorin Bay and filtered through a bed of sand filters. Before pumping the seawater to the rearing tanks it is further filtered through sterilized cotton. In this manner the seawater is filtered effectively up to a rating of 5μ . The salinity, oxygen content and pH of the filtered seawater are monitored periodically (twice in a week). Water temperature is recorded daily.

EQUIPMENT AND ITS CARE

It is very important that the pipes which carry filtered seawater from the filtration facility (filter bed and storage sump) to the larval culture and setting rooms should be kept very clean and free from contamination. These pipelines should be flushed with freshwater at least once a week. The flushing of freshwater will kill the protozoans and other organisms that might get past the last filtration stage.

The filters of nylobolt and stainless steel meshed sieves, after use are dipped in warm fresh water and dried.

Equipment required for spawning and rearing operations are Perspex tanks, fibreglass tanks, glass beakers and fibreglass setting tanks. Once in two days the rearing tanks are emptied and scrubbed and washed with freshwater and filtered seawater.

Aeration tubes should be periodically changed and aquarium air stones should be washed in boiling freshwater once in a week.

PREPARATION OF SPAT COLLECTORS

The type of spat collectors used for setting of oysters should fulfil several requirements. They should be non-toxic and be favourable for oyster spat to set. They must be compact enough to allow sufficient water circulation for the growth of spat. The spat collectors should be robust enough to withstand handling and it should be possible to separate the spat individually.

The materials used for setting of spat in oyster hatchery, are oyster shells, polythene sheets, shell grit and lime-coated tiles. Oyster larvae prefer shells which have retained the natural tanned protein. Extracts of oyster tissue enhance settlement of larvae.

The spat collectors are sterilized with chlorinated water and pretreated by soaking and repeated washing in seawater for a week so as to remove any toxic substances present. The oyster shells are brushed well and washed in seawater.

SETTING OF SPAT

The eyed larvae are released into the setting tanks of size $2 \text{ m} \times 1 \text{ m} \times 0.5 \text{ m}$ lined by polythene sheet inside and containing filtered seawater which is aerated. The larvae are released into the tank at the rate of 2 larvae/ml. Water change is done once in two days just before feeding. The larvae are fed with *Isochrysis* at the rate of $10,000$ — $12,000$ cells/larva. The process of setting extends for 5 - 6 days. The spat are reared in the tank for a period of three weeks, feeding them with mixed phytoplankters such as *Chaetoceros* sp., *Skeletonema costatum*, *Thalassiosira subtilis*, *Nitzschia* spp. etc. Average setting on polythene sheet is 4 spat/sq.cm.

Oyster shell rens with 6 to 8 shells strung on 3 mm thick synthetic rope of 1 m length numbering about 25 - 30 are suspended in the setting tanks which are covered inside by polythene sheet. The released larvae settle in large numbers on the shells and grow into spat (Pl. III B). When the spat grow to a size of 5 - 10 mm the shell rens are transferred to the oyster farm for further rearing of the seed.

CULTCHLESS SPAT

Oyster shell grit and polyethylene sheets have been used for the production of cultchless spat. Oyster shell grit 500μ in size are washed thoroughly, sterilized in seawater with 10 ppm chlorine, washed once more in running filtered seawater and dried. The shell grit are uniformly spread at the bottom of one ton capacity setting tank and oyster larvae about to set are released into it. For the setting of spat, polyethylene sheet is spread at the bottom and along the sides of the setting

tank. Three weeks after setting, the spat are separated from the polyethylene sheet, put in cages lined by velon screen and reared on racks in the farm.

BASIC FACILITIES REQUIRED IN A HATCHERY FOR THE PRODUCTION OF OYSTER SEED

The selection of site for the hatchery is an important aspect which will determine the technological success of the system. The site should have the following environmental features. The sea water along the coast should be free from pollutants, both domestic and industrial. Waters with a wide range of salinity will not be suitable. Seawater with salinity range of 25‰-30‰ has been found to be suitable for oysters of our coasts. Places with seawater in which the level of suspended organic and inorganic matter is too much should be avoided as the cost of operation of filtration system will be high.

A set of sedimentation tanks and sand filter bed is necessary to filter a large quantity of seawater daily for the hatchery. In this system the sea water is filtered up to 10-20 μ . The filtered sea water is taken to a storage sump and is further purified by passing through cotton filters before pumping to larval and spat rearing tanks. It would be expensive to filter large quantities of seawater using microfilters. Ultraviolet light water sterilizer in which seawater is made to flow through a series of U shaped quartz tubes kept exposed to ultraviolet lamps will be required to sterilize the seawater when bacterial and fungal load is noticed in seawater.

Pump sets are required to pump seawater from the sea to sedimentation tanks and filter bed and from the latter to the storage sump and hatchery. In pipe lines PVC gate valves and pumps are used to avoid corrosion.

A 7.5 H.P. air compressor fitted with auto start switch and oil filter and a PVC pipeline system connected with it is required to supply oil free air for aerating the tanks in which the broodstock as well as oyster larvae and spat are reared.

Fibreglass tanks of various capacities 100 lit., 300 lit. and 1 ton are needed for rearing broodstock, mixed phytoplankton cultures, oyster larvae and spat.

In the room where oysters are conditioned for maturation the temperature is controlled with air conditioners.

Mass phytoflagellate culture facility in an airconditioned room with controlled temperature of 22-25°C and aseptic conditions is essential for isolation of phytoflagellates and maintaining stock cultures and production of mass cultures of the flagellates for feeding oyster larvae.

Stainless steel test sieves of mesh size 40 μ , 50 μ , 75 μ , 125 μ and nylobolt filters with mesh sizes of 30 μ and 40 μ are needed for segregation and culling of eggs and larvae.

A hot air oven is required to dry glassware used in culture of phytoflagellates. Chemicals for preparing culture medium for the culture of phytoflagellates have to be procured.

The development of hatchery techniques for the mass production of oyster seed is a major breakthrough which is of great significance as it will be possible to produce and supply adequate quantities of oyster seed at any time during the year for conducting oyster culture. By adopting hatchery techniques it is possible to develop disease resistant strains of oysters with regular shape, fast growth and high meat content.

REFERENCES

- AQUACOP. 1977. Elevage larvaire et production de naissainode *Crassostrea gigas* en milieu tropical. Proc. 3rd Meeting. ICES Working Group on Mariculture. Actes de Colloques du CNEYO, 4 : 331-346.
- CARRIKER, M. R. 1961. Interrelation of functional morphology, behaviour and autoecology in early stages of the bivalve *Mercaenaria mercenaria*. Jour. Elisha Mitchell Scient. Soc., 77 (2) : 168-241.
- DUFUY, J. L., N. J. WINDSOR AND C. E. SUTTON. 1977. Manual for design and operation of an oyster seed hatchery for the American oyster *Crassostrea virginica*. VIMS, Gloucester Point, Spl. Rep., 142 : 1-104.
- GOPINATHAN, C. P. 1982. Methods of culturing phytoplankton. In : *Manual of Research Methods for Fish and Shellfish Nutrition*. CMFRI. Spl. Publ. 2 : 113-118.
- LOOSANOFF, V. L. AND H. C. DAVIS. 1952. Temperature requirements for maturation of gonads of Northern oysters. *Biol. Bull.*, 103 : 80-96.
- NAYAR, K. NAGAPPAN, M. E. RAJAPANDIAN, A. D. GANDHI AND C. P. GOPINATHAN. 1984. Larval rearing and production of spat of the oyster *Crassostrea madrasensis* (Preston) in an experimental hatchery. *Indian J. Fish.*, 31 : 233-243.
- WALNE, P. R. 1974. *Culture of bivalve molluscs*. Fishing News (Books) Ltd., Surrey, 173 pp.