LARVAL REARING AND PRODUCTION OF SPAT OF THE OYSTER *CRASSOSTREA MADRASENSIS* (PRESTON) IN AN EXPERIMENTAL HATCHERY

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ABSTRACT

The Indian backwater oyster, *Crassostrea madrasensis*, was spawned in the laboratory at Tuticorin. The larvae were reared and the spat settled in 15-20 days. Two species of phytoflagellates, *Isochrysis galbana* and *Pavlova* sp. were isolated from the natural environment, cultured in mass scale and given as food throughout the larval period. Early stages of the development of fertilized egg, successive stages of developing larvae, and metamorphosis to the spat have been described. The methodology of the production of cultchless spat and rearing the post-set oysters are discussed.

INTRODUCTION

The technology of culturing the oyster *Crassostrea madrasensis* by rack-and-tray method was developed by the Central Marine Fisheries Research Institute at Tuticorin (Nayar and Mahadevan 1980). Following this attempts have been made to evolve suitable hatchery techniques for the production of oyster seed on an year-round basis.

Cole (1937), Loosanoff and Davis (1952) had successfully reared the larvae of *Ostrea edulis* and *Crassostrea virginica* on cultured phytoplankton. Dupuy et al (1977) reared the larvae of *Crassostrea virginica* on commercial propositions. In tropical conditions, larval rearing and production of spat of *Crassostrea gigas* were carried out (Aquacop 1977). Alagarswami et al (1980, 1983) successfully reared the Indian pearl oyster *Pinctada fucata* and produced the spat on a large scale. Desai et al (1980) attempted rearing of the larvae of oysters of the Gulf of Kutch, and succeeded in rearing the larvae up to straight-hinge stage. Similarly, Samuel (1980) reared the larvae of *Crassostrea madrasensis* up to straight-hinge stage. Rao (1980) succeeded in his attempts on induced spawning of *Crassostrea madrasensis* and rearing the larvae in laboratory conditions. In his experiment a few pediveligers settled on the 19th day. The present paper deals with the results of induced

spawning, larval rearing and production of spat of *Crassostrea madrasensis* at the Institute's experimental hatchery at Tuticorin. A preliminary account of the same has already been published (Nayar et al 1982).

Material and Methods

Water source and management

The water required for the hatchery was drawn from the Tuticorin bay and filtered through a set of sedimentation tanks and a filter bed. The filter bed, composed of fine river sand, gravel and activated charcoal, filtered the particles very effectively up to a rating of 10 to 20 μm. The water from filter bed was collected in a storage tank and pumped into the laboratory, where the delivery end was plugged by surgical cotton to prevent still smaller particles from entering into the rearing tanks (Alagarswami et al 1983).

The equipment required for spawning and rearing operations were perspex and fibreglass tanks (100 l), glass beakers (10 l) for holding the spawn suspension, and fibreglass setting tanks (1000 l). Twenty-litre glass carbuoys and 100-l perspex tanks were used for the culture of algal food. Once in two days, the rearing tanks were emptied, scrubbed and washed with fresh water and filtered sea water. The glass carbuoys and perspex tanks used for the mass culture of algal food were also cleaned similarly after every operation. Stainless steal test sieves of mesh 40 μm, 50 μm, 75 μm, and 125 μm and Nylobolt of 40 μm and 10 μm were used for filtering and segregation of the larvae.

Maturation and spawning

Several sets of experiments were conducted from July 1982 to June 1983 and seven batches of larvae were successfully reared in the laboratory. One-year-old oysters, with uniform gonadal condition, preferably immature or spent, were used for development of broodstock. The selected oysters, 25 in each batch, were fed in the laboratory with a mixed diet of diatoms such as *Chaetoceros affinis, Skeletonema costatum, Thalassiosira subtilis* and *Nitzschia closterium* and phytoflagellates, *Isochrysis galbana* and *Pavlova* sp., and micro-green alga, *Chlorella salina,* at the prevailing ambient temperature for 15 to 20 days. One 100-l fibreglass tank was used to hold 25 oysters.

The oysters were cleaned thoroughly and placed in the tank containing 75-l filtered sea water. Ten litres of algal culture were added to it and the tank was aerated. On an average, the cell concentration of the algae was 0.8-1.0 million cells|ml, and food was provided twice during the day between 0900 hrs and 1700 hrs at 4-hourly intervals. The water in the tank was changed in the morning before feeding. At the end of the fortnight, the condition of the gonad was examined from a few oysters and found to be fully mature.

The oysters were transferred to a spawning tank (perspex, 100-l) containing 50-l of filtered set water with temprature 2-4°C above the ambient level.

This was achieved by operating a heating element with porcelin coating which was controlled by a thermostat. Proper aeration was provided to the tank (Plate IA). After 20 to 60 min. the males commenced spawning, which in turn induced the female oysters to spawn. In the cases where the temperature stimulus failed to work fresh gonad extraction was introduced in the tank to induce spawning.

The male spawned by continuous ejection of a white stream of sperm (Plate IB), and the female by rhythmic ejections of eggs at intervals. On the commencement of spawning, aeration was suspended. Oysters which did not spawn and those which completed spawning were removed from the tank. This was done to prevent the oysters from filtering the gametes. Aeration in the tank was revived to facilitate mixing up of sperms and eggs. Within 60 min. of spawning it was observed that most of the eggs were fertilized (Plate ID). At the end of one hour aeration was again suspended, and fertilized eggs settled at the bottom. After 15 min. the supernatant water, containing sperms, unfertilized eggs (Plate 1C) and debris, was removed. Fresh seawater was added and decanting was carried out 3 to 4 times. Finally, 40 to 50 l seawater was added to the tank and mild aeration was provided. At the end of 4 h, the eggs attained the morula stage and began to swim in the water column.

Environmental parameters

The temperature varied from 23.5° to 27.6°C, on the minimal range, and between 28.2° to 32.6°C, on the maximum. Although the temperature varied periodically during the year, each experiment was conducted within the temperature regime of the period. There was no marked fluctuations in the salinity except during November-December. The annual average salinity varied between 34.11-36.31 ‰. During the experiments the pH range of the sea water was narrow, from 7.76 to 8.20.

Algal culture

The isolation of the required species of micro-algae was done by filtering sea water collected from Tuticorin bay, through a 10 μm nylon bolting sieve. A fraction each of the filtered water was poured into a number of 25 ml culture tubes which were filled with Miquel's medium about two thirds of its volume. After 8 to 10 days the tubes showed changing colouration, from yellow to brown, green, blue-green and even to red. Both phytoflagellates and nannoplankters were found to develop in these tubes. On repeated subculturing and reculturing, pure cultures of the flagellates were obtained (Plate IIID), which were gradually raised to 250 ml, 500 ml and one litre in conical flasks using Conway or Walne's medium. Stock cultures and batch cultures were obtained by transferring the axenic cultures to 3- or 4-litre Hauffkin's culture flasks and 5-litre conical flasks (Plate IIIE), and maintained at the optimum temperature of 25°C in the

culture room. The mass culture of the flagellates was done in 20-l glass carbuoys as well as in 100-l perspex tanks (Plate IIIF) under constant aeration and light (20-30 k lux) in Conway medium.

Both *Isochrysis galbana* and *Pavlova* sp. were isolated and cultured. They were 7 to 8 μm in size and appeared golden-yellow in colour. Seen under optical microscope on a magnification of 900 X, *Isochrysis* had two identical cilia, and its movement was wavy and rotatory. In the case of *Pavlova*, a rudimentary flagellum called haptonema was also present between the two cilia and the movement was slightly wavy and straight.

Normally when the algae were grown in test tubes and conical flasks, the maximum growth of cells per unit volume was obtained between 10-12 days. When one litre of stock culture having 4-5 million cells|ml has been inoculated to 20-l glass carbuoy, optimum growth was observed within 5 days. Hence the period required to produce the maximum number of cells in a 20-l glass carbuoy or 100-l perspex tank could be shortened by increasing the amount of inoculum.

For the mass culture of these two flagellates, filtered sea water was heated up to 90°C in 100-l plastic bins using an immersion heater. After cooling, it was poured into the algal culturing vessels. Required quantities of Conway medium was poured and inoculum was added. On an average, the cell concentration of the stock culture was observed as 4-5 million cells|ml. One litre of the inoculum was added to 20-l glass carbuoys and 4-l to the 100-l perspex tanks. Under favourable conditions *Isochrysis* and *Pavlova* have their peak growth phase in 1 to 6 days (Fig. 1). It has been observed that in mass culture, the experimental phase extends up to 10 days without aeration; with aeration the growth phase remains up to 15th day. The algal food supplied to the oyster larvae was always harvested during the exponential phase of growth.

The salinity of the sea water was maintained between 32-35‰, pH at 8-8.4, dissolved-oxygen content at 4-5 ml|l and the temperature at 25°C.

Results

Eearly development

After fertilization, the first polar body was observed within 20 to 40 min. and, subsequently, the second polar body appeared. The first cleavage occurred immediately after the formation of the two polar bodies and the cell was divided into two unequal halves, (Plate I, E). The second division made the blastomere into four-celled stage (Plate I, F) and at third division the cells in the animal pole divided, resulting in the formation of 8-celled stage (Plate II, A). One of the cells (macromere) formed in the first cleavage undewent division during the second cleavage, retaining the identity and remaining at the

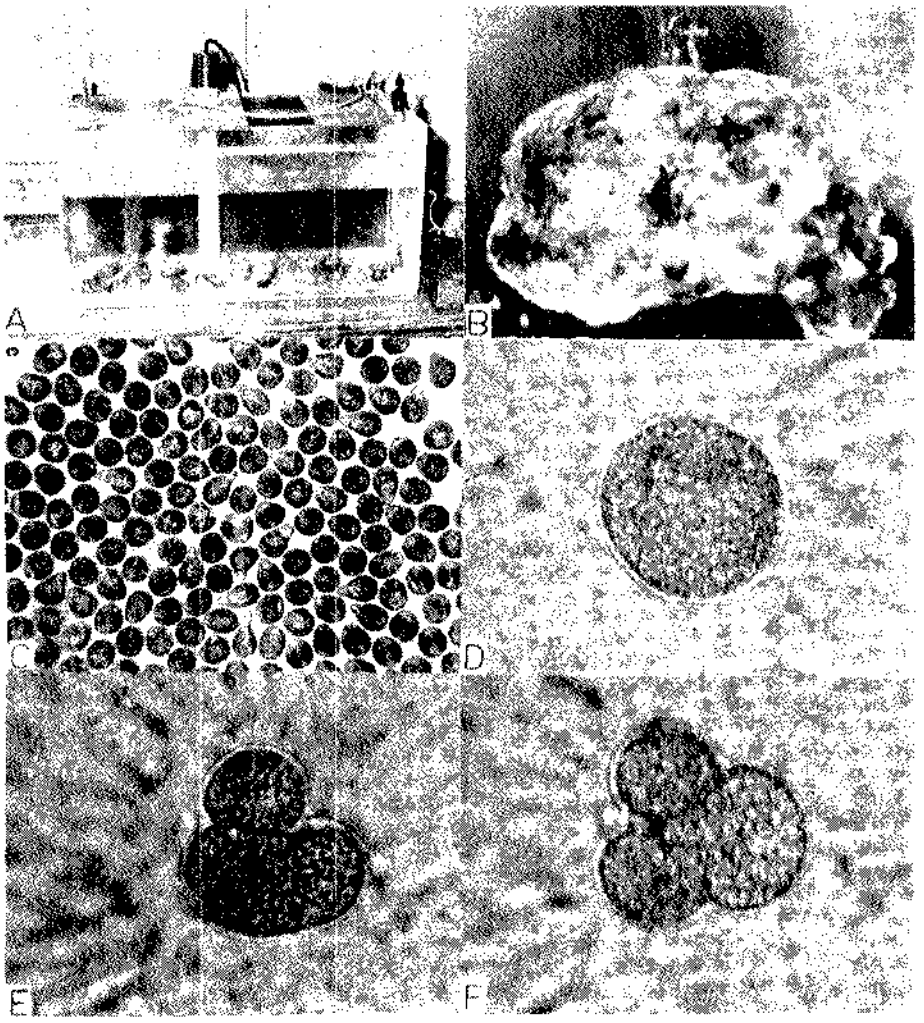

PLATE 1: A. Spawning by thermal stimulation; B. Spawning of a male oyster; C. Unfertilized eggs; D. Fertilized egg; E. Two-celled stage; F. Four-celled stage.

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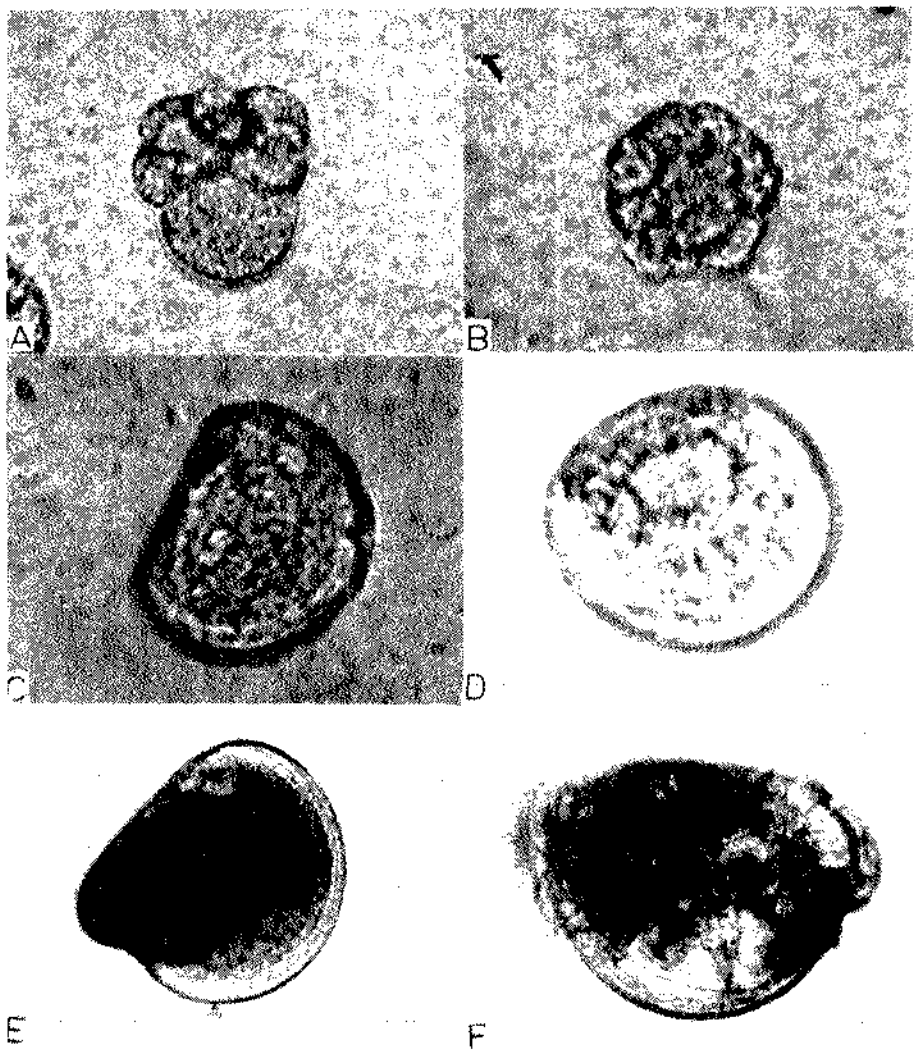

PLATE II: A. Eight-celled stage; B. 'Morula' stage; C. Straight-hinge stage; D. Early umbo stage; E. Advanced umbo stage; F. Eyed larva

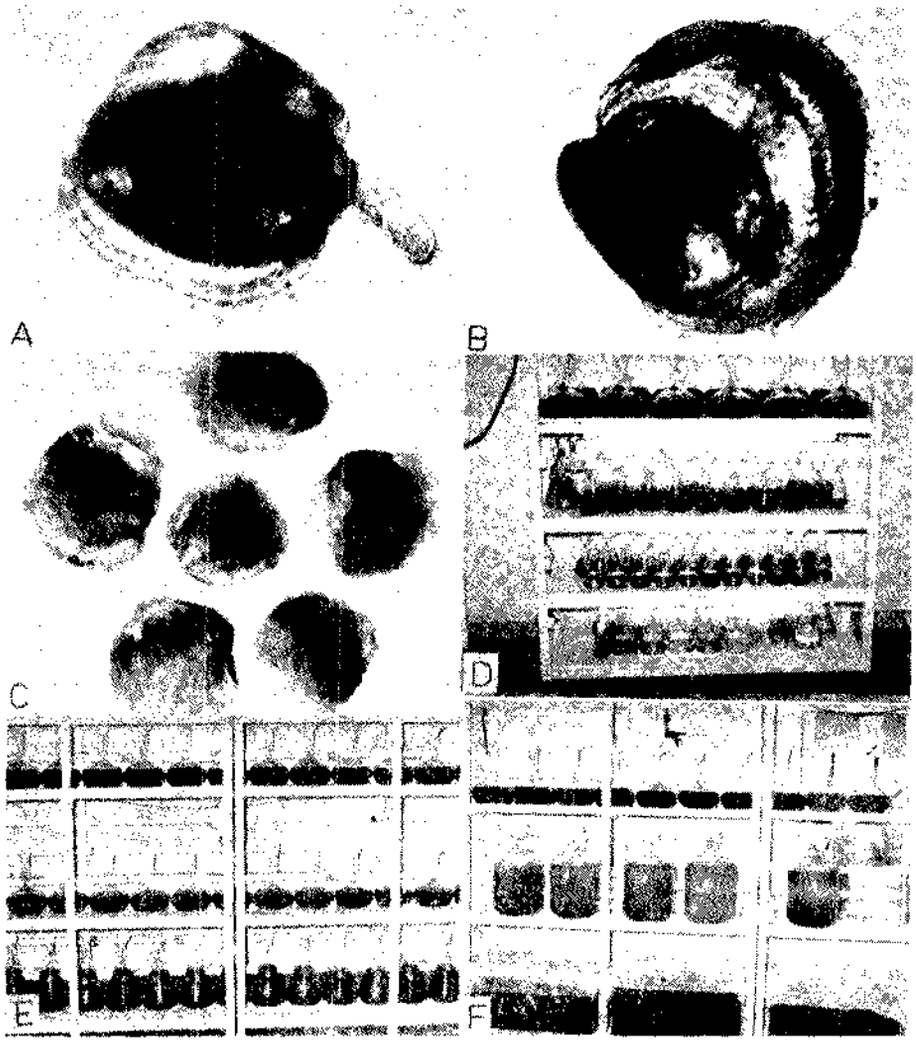

PLATE III: A. Pediveliger; B. Spat; C. Spat, three months old; D. Isolation of the required species of micro-algae; E. Stock culture maintenance; F. Mass culture of phyto-flagellates

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vegital pole as the macromere. Subsequently after the 6th division, an irregular morula was formed (Plate II, B). The gastrula stage was reached between 5-6 h after fertilization. At this stage the larvae started swimming upwards and they could be easily separated from unfertilized eggs and debris, which lay on the bottom.

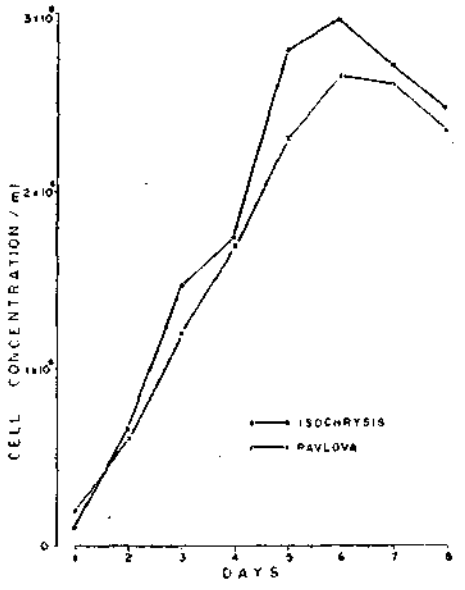

FIG. 1. Growth characteristics of *Isochrysis galbana* and *Pavlova* sp.

Straight-hinge stage

The straight-hinge or 'D' shell larval stage was reached at the end of 20 h (Plate II C). The larvae were semitransparent, with the velum protruding out and creating a strong ciliary current which directed minute particles of food into the stomodaeum. The larvae swam vigorously; and some of the larvae showed a slow circular movement under microscope. Dupuy et al (1977) have considered that such movements of the larvae were due to the abnormal development of velum. In the present study, in a few experiments, 5 to 10% of the larvae showed such movements and majority of them were found dead during the first two days. The swimming, viable larvae were separated by siphoning them from the tank, leaving the sluggish larvae. This process of culling was continued during the first two days or till the entire stock of the larvae were of uniform size and movement. On an average, the larvae measured 66 µm in length (APM) on the first day. At this stage the larvae were reared in rectangular fibreglass tanks (75 x 50 x 40 cm) containing 70-l of filtered sea water. The larvae were fed with phytoflagellates at the end of 24 h.

Umbo stage

On the third day, the larvae appeared slightly oval in shape and measured 100 μm. This stage was considered as 'early umbo' stage (Plate II, D). On the 7th day, the umbo appeared distinctly at the hinge side and pronounced concentric rings were found on the shells as the shell was growing. The larvae measured 150 μm at this stage. In 12 to 15 days, the 'late umbo' larvae measured between 260 and 270 μm (Plate II, E).

Eyed stage

An irregular eye spot was observed in 13 to 17 days when the larvae reached 280 μm (Plate II, F). Dupuy et al (1977) had observed in *Crassostrea virginica* that the appearance of eye had occurred when the larvae had assumed a size of 280 μm and the eye spot had become distinct at 290 μm. The eye spot was found at the lower quadrant of the ventral region close to the right angle of the dorso-ventral and antero-posterior planes in *C. madrasensis*.

Pediveliger stage

Between the 14th and 18th day, the functional foot emerged. At this stage the larvae measured 330-350 μm and they sank to the bottom and started crawling (Plate III, A). This is known as 'Swimming-creeping' stage which has been designated as 'Pediveliger' by Carriker (1961). The larvae started setting subsequently, within 24 h.

Spat

The 'Pediveliger' larvae settled down, losing the velum totally. The shell edges grew hexagonally and the larvae developed the characteristic adult features and metamorphosed into spat. The young spat measured 450 μm (Plate III, B). The eye spot was traceable at this stage, but it disappeared after 24 h of attached life.

Larval density

The straight-hinge larvae were transferred into 10-l beakers and uniformly suspended. After 10-15 seconds of mixing, one ml sample was drawn and transferred on to Sedwick's rafter cell counter and counted under the microscope. The study was carried out in individual 10-l containers, in different batches. The density varied in different experiments. The initial concentration of the straight-hinge larvae was estimated from 10-l beakers. The rearing concentration was estimated after filtering and transferring the straight-hinge larvae in one or more rearing tanks (100-l) to make up the concentration between 2 to 8 larvae|ml as shown below. The larvae were reared in these tanks till they reached eyed stage.

	Average No. of larvae per ml	
	Initial concentration (in 10-l beakers)	Rearing concentration (in rearing tanks)
Expt. 1	120	2
Expt. 2	406	8
Expt. 3	197	4
Expt. 4	256	5
Expt. 5	150	3
Expt. 6	113	2
Expt. 7	250	5

Feeding

Since the nutritional requirements increase with the growth of larvae a schedule of feeding was developed with different cell concentrations depending on the age and size of larvae. The cell concentration of the food, namely, *Isochrysis* and *Pavlova*, in respect to different stages of the larval development is shown below:

	Cell concentration in ml/larvae	
	Isochrysis galbana	*Pavlova* sp.
'D' shape	3000-4000	3000-4000
Early umbo	4000-5000	4000-5000
Umbo	5000-8000	5000-8000
Late umbo	8000-10000	8000-10000
Eyed stage	10000-12000	10000-12000
Pediveliger	"	"
Spat	"	"

Larval growth

From 'D' shell (day 1) to umbo (day 7) the larvae grew from a mean size of 63.9 to 133.3 μm with an average growth rate of 11.5 μm per day. On the 17th day (Eyed stage) the mean size of the larvae was 289.2 μm with an average growth of 14.9 μm per day. On the 19th day, the mean size of the pediveliger was 348.2 μm registering an average growth of 23.6 μm per day (Figure 2).

Spat setting

Once the eyed stage was reached, they were ready to be transferred to the spat-setting tanks. The spat-setting tanks were rectangular in size with dimensions 2 m x 1 m x 0.5 m made of fibreglass. The tank was covered inside with a polyethylene sheet which was pretreated by soaking and repeatedly washing with sea water for one week, to ensure that none of the finishing chemicals were

present. Fltered seawater was filled in the setting tanks and well aerated. The eyed larvae were transferred to these tanks and fed. The sea water was changed once a day just before feeding. Each time the setting tanks were drained, the water was passed through a sieve to avoid loss of larvae. Immediately after draining, the sides and bottom of the tank were thoroughly flushed to remove detritus or any other algal matter found. Mixed phytopankton at a cell concentration of 0.8-1.0 million cells/ml was added to the tank. The setting process was usually completed within 5-6 days. The spat, at the end of 20-30 days (10-12 mm), were removed from the polyethylene sheet and reared separately.

Growth of spat

The growth of spat was followed for a period of six months, both in the laboratory and in the oyster farm (Plate III, C). At the end of first month, about 2000 spat were retained in the laboratory and the rest were transferred to the special type of cages and suspended from the racks in the bay. The growth of labortary-reared oysters in the natural waters is presented in Fig. 3.

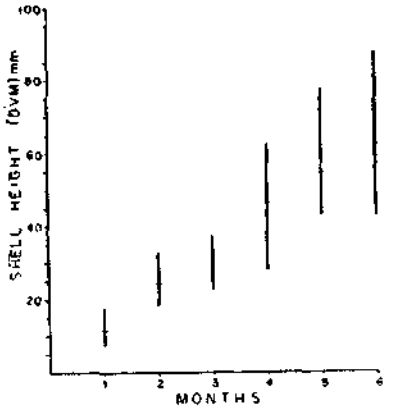

FIG. 2. Growth of *Crassostrea madrasensis* larvae up to 19 days after fertilization.

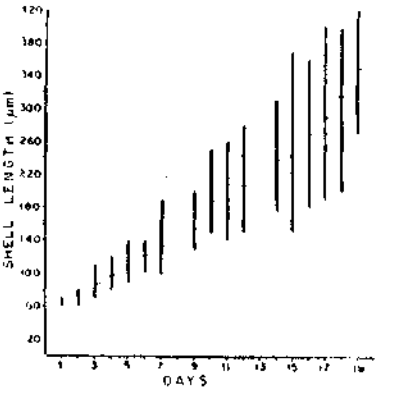

FIG. 3. Growth of the spat of *Crassostrea madrasensis* reared in the natural waters for a period of six months.

In the laboratory the spat were fed with phytoplankters such as *Chaetoceros* spp., *Skeletonema costatum*, *Thalassiosira subtilis*, *Nitzschia* spp. and other phytoflagellates. These phytoplankters were raised from natural environment using fertilizing medium. It was found that the culturing of the post-set spat in the natural waters showed rapid growth. The spat showed a growth of 62.5 mm in 6 months, registering a growth rate of 10.2 mm per month.

Discussion

Although larvae of many bivalve molluscs of temperate and sub-tropical waters have been reared in the laboratories or hatcheries, ony a few workers

have studied on the spawning and early developmental stages of Indian oysters. Hughes (1940), Imai et al (1961), Loosanoff (1945), Loosanoff and Davis (1952), Don Maurer (1968) and Dupuy et al (1977) have demonstrated that a successful hatchery mainly depends on the ready availability of a stock of ripe oysters for spawning any time in the year. Dupuy et al (1977) accomplished a round-the-year spawning of *Crassostrea virginica* by manipulating the temperature regime. In the present study significant development in stages of gonads was achieved in 15 days of conditioning at temperature 2°-4°C lower than ambient level with adequate supply of food.

Loosanoff and Davis (1963), Walne (1974) and Dupuy et al (1977) reared the larvae in running, filtered sea water, free from particles up to 1 μm, treated with antibiotics or ultraviolet light. However, the present study was carried out in the same way as was done by Alagarswami et al (1983), who had successfully reared the larvae of *Pinctada fucata* (Gould) without adopting any elaborate system of filteration or sterilization.

Cole (1937) demonstrated the suitability of pure culture of phytoflagellates as food for the larvae of *Ostrea edulis*. Following this, Bruce et al (1940), Davis (1953), Walne (1956), Davis and Guillard (1958) and Ukeles (1969) found that *Isochrysis galbana* and *Monochrysis lutheri* were the best food for the rapid growth of the larvae of *O. edulis* and *C. virginica*. Loosanoff and Davis (1963) succeeded in setting the larvae of *C. virginica* in 10 days at 30°C and in 18 days at 23°C. Dupuy et al (1977), using a combination of three algal species, namely, *Pyramimonas virginica, Pseudoisochrysis paradoxa* and *Chlorella* sp., reared the larvae of *C. virginica* and *C. gigas* to setting stage in 9 to 11 days at 27°C. Further, they found that the optimum development of the larvae occurred at a density of 5|ml and at salinities of 17.5‰ to 20.0‰. In the present experiments, the larvae of *C. madrasensis* were reared feeding with *Isochrysis galbana* and *Pavlova* sp. In two experiments (Nos. 6 and 7), the eyed stage was observed from 13th to 15th day at salinities of 30.5 ‰ to 31.5 ‰ and in temperatures of 25.4° to 27.0°C. In the rest of the experiments the eyed stage was noticed between the 16th and 19th days when the salinity varied between 33.5 ‰ and 36.3 ‰ and the temperature from 29° to 30.0°C. The larval rearing was carried out at different densities from 2 to 8|ml. From the above results, it may be reasonable to believe that a slightly lower salinity and temperature ranges may promote rapid growth of larvae. However, the optimum salinity and temperature ranges are yet to be worked out.

The recent development in the production of 'cultchless' spat is an improvement in the methodology of setting the larvae in a relatively simple manner. Loosanoff (1958) has used polyethylene film as collector and separated the grown up seeds easily after sometime. Dupuy et al (1977) have demonstrated the production of cultchless seed on a large scale by using mylar sheet. In the majority of the experiments, the setting over polyethylene sheet was obtained at a density ranging from 0.5 to 2.5 spat $|cm^2$.

Walne (1974) and Dupuy et al (1977) had demonstrated certain techques for growing the spat in laboratories and hatcheries. Walne (1974) found that 4 spat|cm^2 or 10 spat|l of water was the optimum density for rearing the spat of *O. edulis* and that their survival and growth greatly depended upon the availability of food rather than any other factors. Further, he had observed relatively high growth rate when they were cultured in unfiltered (strained through 70 µm fine mesh) water on a food of *Isochrysis* and *Tetraselmis*. Dupuy et al (1977) had reared the spat for 6 days in tanks by feeding with cultured *Pyramimonas virginica* and, afterwards, transferred to tanks receiving raw ambient water. He found that, with a rate of 1125 litre per hour, one lakh oyster spat gained optimum growth. In the present investigations, the spat were initially reared in the laboratory in filtered sea water, feeding them with phytoflagellates and diatoms. After 20 to 30 days, they were transferred to nursery cages and were suspended from the racks in the bay. The young oysters reared in the bay registered a growth rate of 10.2 mm per month during the first six months.

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PLATE I. A: Spawning by thermal stimulation; B: Spawning of a male oyster; C: Unfertilized eggs; D: Fertilized egg; E: Two celled stage; F: Four celled stage.

PLATE II. A: Eight celled stage; B: 'Morula' stage; C: Straight-hinge stage; D: Early umbo stage; E: Advanced umbo stage; F: Eyed larva.

PLATE III. A: Pediveliger; B: Spat; C: Spat three months old; D: Isolation of the required species of micro-algae; E: Stock culture maintenance; F: Mass culture of phytoflagellates.