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66. LARVAL REARING AND SPAT PRODUCTION OF THE BROWN MUSSEL *PERNA INDICA* AT VIZHINJAM

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ABSTRACT

The brown mussel *Perna indica* was spawned in the laboratory and larval rearing experiments were conducted. The larvae undergo early development and metamorphose into veliger, eyed stage, pediveliger and spat. Settlement commences between the 15th and the 20th day. Various stages from fertilized egg to spat are described. Laboratory cultured phytoflagellates like *Isochrysis galbana* and *Pavlova* sp are given as food for larvae till settlement. Details of larval rearing, spat settlement and post-set growth were studied and the results are given.

INTRODUCTION

Very little work has been done on the rearing and spat production of mussels from Indian waters. Rao et al (1976) described spawning, fertilization and larval development of green mussel *Perna viridis* from Goa coast. Attempts have been made to produce brown mussel seeds in laboratory conditions at Vizhinjam (Appukuttan et al 1984). Loosanoff and Davis (1963) have successfully reared *Mytilus edulis* larvae in the laboratory using phytoplankton as larval food. Recently the aquaculture team of Centre Oceanologique due Pacifique. French Polynesia have carried out larval rearing and spat production of the mussel *Mytilus viridis* in the tropical conditions. In the present paper details of early development, larval rearing, spat settlement and post-set growth of *Perna indica* are given.

MATERIAL AND METHODS

Experiments were done from June to September in 1983, 1984 and 1986 and larval settlement was studied in the laboratory and post-set growth in the farm at Vizhinjam Bay. One year old mature mussels of 45-60 mm were used in all the experiments. Mussels collected from natural bed, culture ropes and hatchery produced seed reared to adult stage in the farm were used as spawners. Induced spawning by thermal stimulation was tried successfully. Induced spawning method adopted was that of Nayar et. al. (1984) in edible oysters. A 4°C jump in temparature gave good results. During peak spawning period (June-August) mussels spawned in the laboratory at temperatures ranging from 26 to 29°C, without any external stimulii. One or two spawners were kept in spawning trays (Fig. 1 A) or six or ten mature mussels were placed in 50 l fibre glass tanks. Males expell milt as a jet and subsequently females eject eggs rythmically 3 to 4 times within 3h. Eggs were removed from the spawning tanks immediately to 5 l glass beakers and fresh sperm was added and stirred well. Fertilized eggs sink to the bottom of the beaker. Supernatant water containing the sperm and unfertilized eggs was removed and fresh filtered sea water was added to the beaker. The morula stage is reached in four hours after fertilization and healthy larvae aggregate at the surface. Early 'D' shaped larvae were siphoned out from the beaker and transferred to 50 x 50 x 40 cm fibreglasss rearing tanks with 50 l filtered sea water. Water was changed daily from tanks and continued till the apperance of settting stage and the tanks were changed on alternate days till the eyed stage appeared in the tanks. Aeration was given to all the rearing tanks.

The sea water for larval rearing was initially drawn from the Bay, but later water from open sea near Kovalam was brought in plastic bins and filtered through 30 μ bolting silk and then passed througn sand filters and stored in 1500 l capacity fibreglass tanks. Before transferring

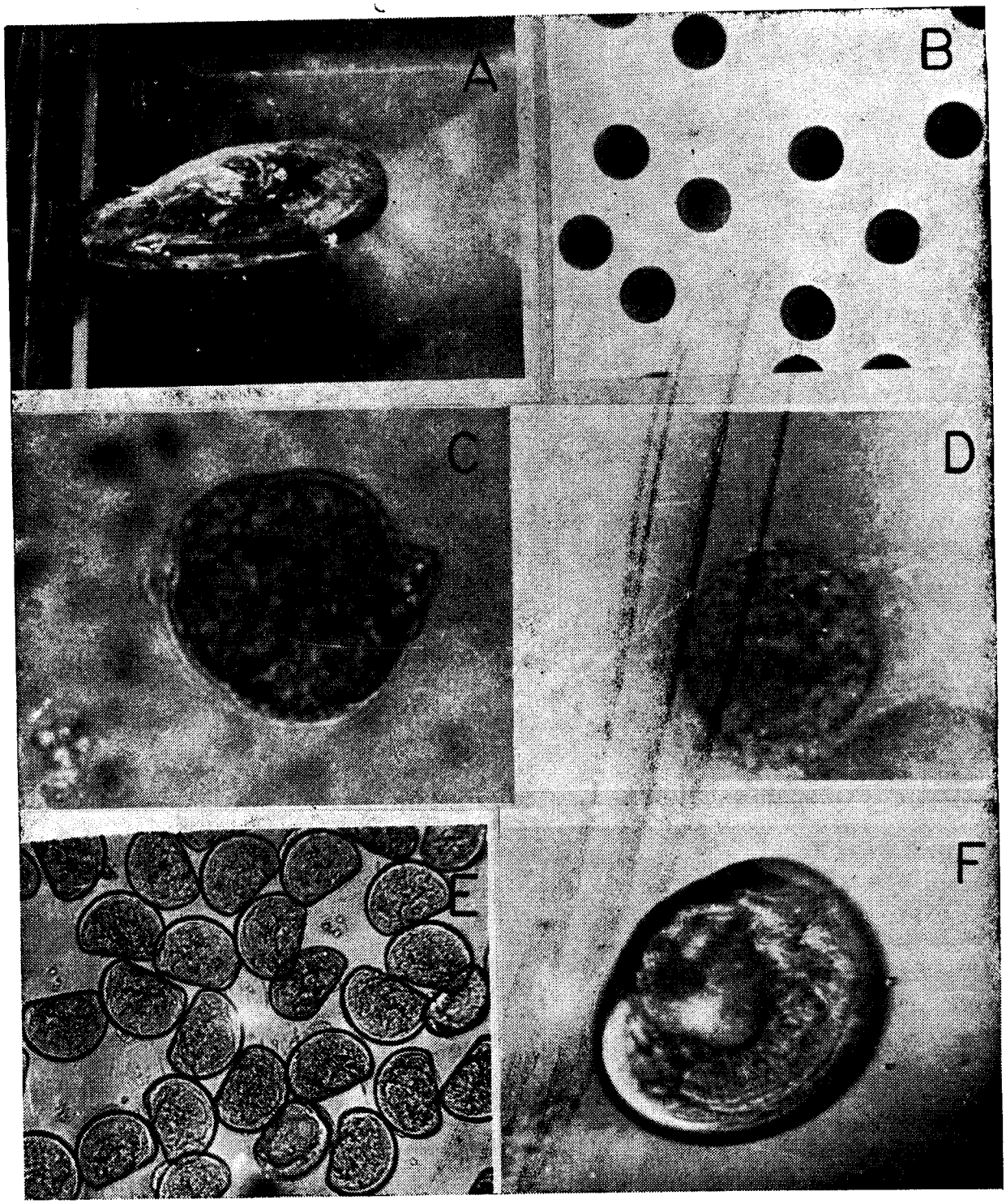

Fig. 1 A Mature male *Perna indica* kept in spawning tray ejecting sperm.
B. Spherical eggs of *P. indica* C. Fertilized eggs with polar bodies. D. Trochophore larvae.
E. D-shaped veliger F. Early umbo stage.

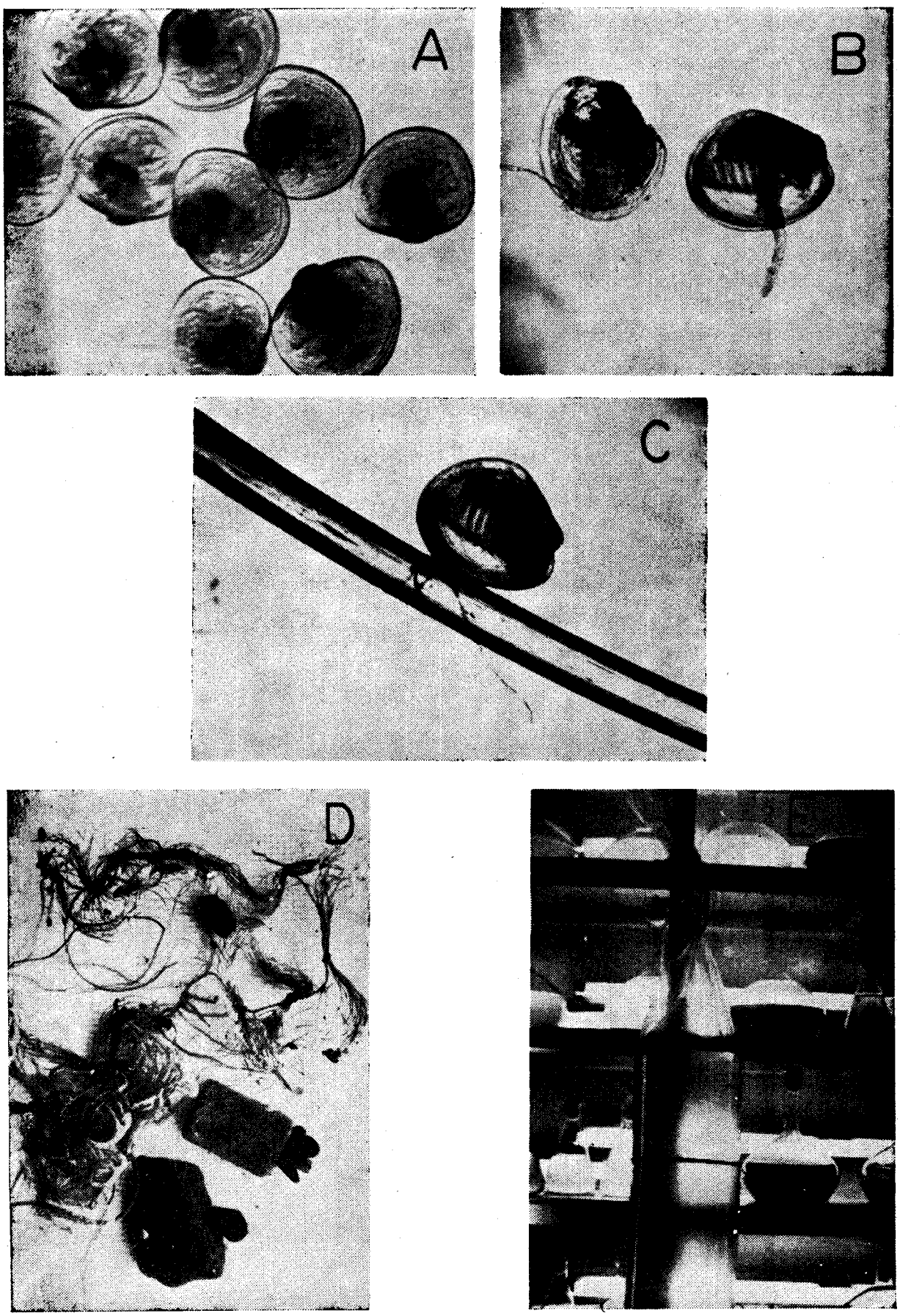

Fig. 2. A Eyed stage; B. Pediveliger stage C. Spat settled over polyethylene monofilament by weak byssus threads. D-Spat settlehd over polyethylene monofilament and granite ctones. E. Mass culture of *Isochrysis galbana* in polythene tubings.

to rearing tanks the water was filtered through surgical cotton to remove smaller particles entering into tanks.

The ambient temperature during June to August ranged from 25.5°C-28.5°C in 1983, 25.5°C-27.5°C in 1984 and 25°C-29°C in 1986. The salinity had a range of 34-34.44 °/$_{oo}$ in 1983, 30-10-33.97°/$_{oo}$ in 1984 and 33.35-34.39°/$_{oo}$ in 1986. Dissolved oxygen in the rearing tanks ranged from 4.03-4.95ml/l and pH from 7.8-8.2 during the experiments.

Yellow-brown flagellate *Isochrysis galbana* was used as larval food in most of the experiments. *Pavlova* sp in addition to *Isochrysis galbana* was given to three batches of larvae in 1984 experiments. Pure cultures of these flagellates were stocked in the laboratory throughout the year by reculturing. Mass culture of algae in 50 l perspex tanks and 30 l polythene tubings (Fig 2 E) was done to meet the larval food requirements. Filtered and heated sea water enriched with Walne's medium was used for stock culture. Flurescent lights were used to provide illumination. The peak growth phase was observed from 3rd to the 6th day. Open culture in sun light in perspex tanks of 50 l capacity was also successfully tried. Temperature below 30°C was maintained by providing shade over the culture tanks. Good bloom was obtained within 4-5 days.

The shell length and width of the larvae were measured in the antero-posterior axis and the dorso-ventral axis respectively. The mean lengths were taken for growth studies.

RESULTS

Rao et al (1976) have described the early development of *Perna viridis* from egg to spat stage in the laboratory. The present observations show that the development of *Perna indica* is very much identical. Healthy eggs are brick-red in colour and spherical (Fig 1 B). They are heavily yolked and measure between 45 and 50μ m. The first and second polar bodies are formed within 20 minutes after fertilization when segmentation commences (Fig 1 C). Oval shaped morula with minute cilia all over the body was observed in four hours after fertilization and these larvae measure 58-60μ m in the longer axis and 52-55μ m in the shorter axis. Trochophore larvae measuring 65-70μ m long and 52.5-55 μ m wide (Fig. 1 D) with an apical tuft of cilia and a long flagellum were noticed within seven hours after fertilization.

Early straight-hinged or D-shaped veliger appeared in the tanks within 17-20 h (Fig 1 E.) The veliger has two transparent valves, a well developed velum, a velar hood with small cilia all around with a strong flagellum at the centre which helps in active movements of the larvae. These larvae measured 70 - 76 μ m in the anteroposterior axis and 62-65μ m in the dorsoventral axis. Feeding of larvae with flagellates began at about this stage. The third day veliger has adductor muscles and a well developed alimentary canal. The early umbo stage (Fig. 1. F) was noticed from the 7th day. The larva is oval in shape with well developed umbo in the mid-dorsal part of the velum. It measured 120-140 μ m in the antero-posterior axis and 95-110μ m in the dorso-ventral axis. A 9 day old larva measured 200μ m in the antero-posterior axis and at this stage the rudimentary gill folds, foot and the yellow food mass in the antero-dorsal region are clearly visible.

The eyed stages (Fig 2 A) measuring 208-260μ m in the antero-posterior axis and 200-260 μ in the dorsoventral axis are seen in tanks between the 13th and 14th days. This stage is characterised by the presence of a black, pigmented rounded spot below the food mass. A much developed adductor muscle, concave valves and reduced functional velar hood are unique features of this stage.

Pediveliger stage (Fig 2 B) could be seen from the 16th day onwards. A light brownish and slight oblique shell with 280-320 μ m length in the antero-posterior axis is characterestic of this stage. Foot becomes functional, labial palps and gill filaments are well developed. Shell has weak radial striation. The larvae crawl on the bottom of the tanks with the help of foot and temperory attachment is made with the help of weak byssus secretion.

Large-scale spat settlement is noticed from the 20th day onwards. The young ones bear all unique features of adult in shape and structure. Shell is brownish in colour with radial striation and the functional foot protruded

through the gaping shells and establish as temperory settement (Fig 2 C) with the help of weak byssus thread. Spats show aggregating tendency and settle on the smooth surface of tanks or on spat settlers. The largest spat found in the tanks on the 20th day was 490 μ m in the antero-posterioraxix and 440 μ m in the dorso-ventral axis

The larval density in rearing tanks varied in each experiment. The number of larvae was estimated while transferring the D-shaped larvae to the rearing tanks. Initial stocking density in tanks ranged from 5000 to 15000/l. Estimation of larval density for advanced stages was done while changing the water in the tanks. Though initial stocking density was 5/ml to 15/ml in rearing tanks, at settling stage it came down to 2-3/ml. The tanks with initial stocking density of 5/ml showed good settlement.

Larval feeding commenced the day when D-shaped veligers were transferred to 50 l rearing tanks. *Isochrysis galbana* was used as larval food. The cell concentration of mass cultures ranged from 7 lakhs/ ml to 12.5 lakhs/ ml with a mean of 9.75 lakhs/ml. The average cell count of feed for different stages are 5850/ larvae, for D-shaped larvae, 11,700/ larva for eyed stages and 17,550/ larva for pediveliger stage. Mixed pytoplankton fortified with *Isochrysis galbana* at about 30,000 cells/ml was given to the spat in the laboratory. In tanks with high larval density the quantity of food supplied was increased by two to three times more than what was given in tanks with 5000 larvae/l.

The D- shaped veliger reached the umbo within seven days and the average growth increment in antero-posterior axis was from 73 to 135.5 μ m. This shows a growth of 62.5μm at the rate of 10.4μm/day. On the 13th day, the eyed stage had a mean length of 234.5 m with a growth rate of 16.5 μm/day. On the 16th day the mean length of pediveliger was 300μm showing a growth rate of 21.8 μ m/ day. The measurments given are based on the averages of 100 larvae per day and the days of appearence of various stages are noted by observing maximum number of that stages available in the tanks.

Spat settlement commenced on the 20th day though in a few experiments it was noticed from the 15th day onwards. Fibreglass tanks of 75 x 50 x 50 cm dimension were used for spat setting. Filtered sea water was used in these tanks under good aeration, and the water was changed on alternate days Bunches of polyethylene monofilament, pieces of granite stones, plastic sheets, tile pieces and glass plates were used as spat settlers. Among these the monofilaments (Fig 2 1) and granite stones showed good settlement. The sides and bottom of the spat rearing tanks also provided good substratum for settlement. The setting process continued for 5-9 days in these tanks. When the spat reached 2-3 mm size, they were transferred to the farm and kept in nursing cages for studying further growth.

Growth of spat was traced for 52 days in the laboratory, and for 16 months in the farm. The results are presented in Fig-3. with the bars represents the range of shell length and the dot, peak modes in each month. In the laboratory the spat grew to 690μ m in 25 days at the rate of 43.3μ m/day. On the 35th day the shell length was 1390.3μ m at 73.18 μm/day, on the 45th day 3630μm growth at 363μm/day and on the 52nd day 4190μ m at 198μm/day. In the farm, spat with peak model size of 6-10mm reached 46-50 mm within six months showing a 6.6 mm growh rate per month; after six months it was at 66-70mm, with a growth rate of 3.33 mm/months. In the next four months the peak mode observed was at 76-80 mm indicating a growth rate of 1.66 mm/month.

DISCUSSION

Among bivalves, larvae of *Mytilus edulis* from European waters have been extensively studied and frequently referred to in the literature. The work of Loosanoff and Davis (1963), Hrs-Brenko (1973) Bayne (1976) and Morse et al (1978) have described successful methods of induced spawning, studies on morphology and metamorphosis, spat settlement and mass production of spat of temperate and tropical species of mussels. Rao et al (1976) have studied induced spawning, fertilization and larval development of the green mussel *Perna viridis*.

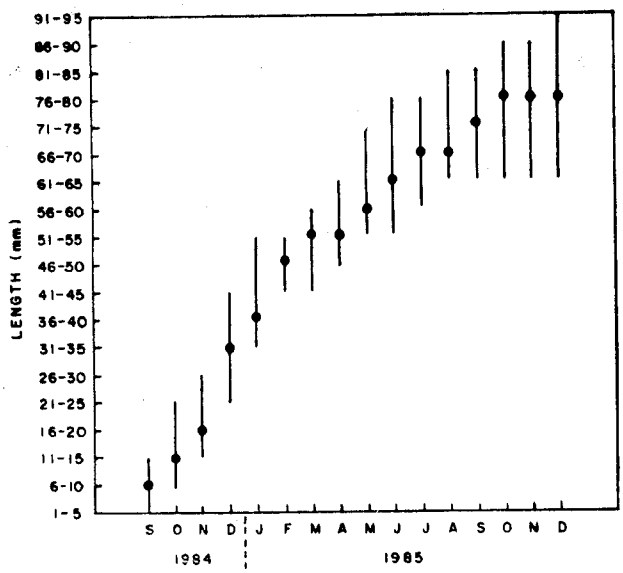

Fig. 3. Growth of *Perna indica* spat in the rearing cages inside the Bay for 16 months. Bars represent the range of shell length and dot peak modes present in each month.

Sea water used for rearing was sand filtered and further filtering through bolting silk and cotton wool has given good results. Aquacop (1983) has treated the water used for rearing *Mytilus viridis* with Treflan, chlorine and sulfadimerazine to prevent fungal attack and control bacterial action. Changing of water daily and changing of rearing tanks on alternative days till settlement gave good results.

Ukeles (1975) reviewed the nutritional requirements of bivalve larvae and suggested that the best food for rearing larvae of oysters and other bivalves are some of the yellow-brown flagellates of the family Chrysophyceae. Rao et al. (1976) used *Tetraselmis gracilis* as larval food for green mussel larvae. Alagarswami et al (1983) and Nayar et al (1984) used *Isochrysis galbana* and *Pavlova* sp. successfully. Aquacop (1983) has given *Isochrysis* sp and *Monochrysis lutheri* as food for mass production of green mussel *Mytilus viridis* in French Polynesia, and after metamorphosis *Skeletonema costatum* was added to the larval diet. *Chaetoceros gracilis* was also used as larval food in French Polynesia. At Vizhinjam *Isochrysis galbana* fed larvae showed good growth, metamorphosis and settlement. Amixture of *Isochrysis* and *Pavlova* was tried for advanced larval stages.

The smallest normal straight-hinge larva of *Mytilus edulis* measured approximately 93 x 64μ m (Loosanoff and Davis 1963). According to Rao et al (1976) the smallest D-shaped veliger of *Perna viridis* measured 73 x 50.9μ m. In this study the early D-shaped veliger of *Perna indica* measured 58 x 52μ m, thus indicating that the early veliger of this species is smaller than that of other mussels studied. D-shaped veliger appeared within 24 hours, umbo stage on the 7th day, eyed stage on the 13th day, pediveliger on the 16th day and settling stage on the 15th to 20th day in the present observation. As noted by Rao et al (1976) large variation in the growth rates was observed among the larvae of the same batch. In *Perna viridis* (Rao et al 1976) early veliger appeared within 18 h after fertilization,eyed stage on the 14th day, pediveliger on the 16th day and settling stage on the 19th day. This closely agrees with the present observations on *Perna indica*. Spat esttlement over bunches of polyethylene monofilament, granite stones and on the bottom and sides of rearing tanks was good. The growth rate studies showed that spat when transferred to farm at a size of 2-3 mm length grew faster than that kept in the laboratory.

Alagarswami (1980) indicated the need for mass production of mussel seed, since large scale removal of seed from the natural bed for farming comes in conflict with the interest of traditional mussel fishery as the mussel beds in India are limited and scattered. Thus it is imperative to develop suitable techniques for mussel seed production in India. The present work deals with the basic techniques for larval rearing and spat production of *Perna indica* at Vizhinjam and further studies are required to determine the optimum larval density in rearing tanks, critical cell concentration and spat settlement rate for initiating mass production of mussel spat.

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