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Larval and Juvenile Rearing of Black-Lip Pearl Oyster, *Pinctada margaritifera* (Linnaeus)

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ABSTRACT

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The black-lip pearl oyster, *Pinctada margaritifera* (Linnaeus), has been cultured in the experimental shellfish hatchery at Tuticorin, India. The flagellates *Isochrysis galbana* and *Pavlova lutheri* were used independently as larval food at a concentration of 5 cells/μl up to day 5 and the ration was doubled thereafter until spat setting. The initial larval density was 1/ml. Straight-hinge veliger stage (75×60 μm) was reached in 20 h, umbo stage (140×130 μm) on day 12, pediveliger (220×210 μm) on day 20 and plantigrade (260×240 μm) on day 23, and spat of 350×300 μm appeared on day 28. *I. galbana* promoted faster growth and early spat setting as compared to *P. lutheri*. The modal component of the larval population showed an average growth of 10.98 μm/day. A total of 6.3% of the initial larval population metamorphosed as spat. Juveniles cultured in the laboratory showed a growth rate of 0.09 mm/day. On transplantation to the culture raft in the farm, growth rate increased to 0.4 mm/day. The juveniles suffered heavy mortality after 4 months. It remains to be tested whether *P. margaritifera* juveniles would have a greater chance of survival in oceanic island conditions, as the natural distribution of the species in India is confined to the Andaman and Nicobar Islands.

INTRODUCTION

The black-lip pearl oyster, *Pinctada margaritifera* (Linnaeus), is one of the three valuable species of pearl oyster for the cultured pearl industry of the world, the other two being *P. maxima* (Jameson) and *P. fucata* (Gould). In India, *P. margaritifera* is second in importance, after *P. fucata*. Its distribution is limited to the Andaman and Nicobar Islands (Alagarswami, 1983); however, stray oysters may occasionally be collected from the Gulf of Mannar. The resource is not substantial in the islands; it is not exploited for the shell or the pearl, but collected by the Nicobarese for food. As a species native to the is-

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lands, and with a favourable ecosystem for its cultivation, the potential for black pearl production has been indicated (Alagarswami, 1983). This would depend on raising of stocks through husbandry practices and development of pearl production techniques specific to the species. *P. margaritifera* produces one of the finest and most expensive pearls in French Polynesia (Ward, 1985), where it is the second largest export product (AQUACOP, 1982).

P. fucata was bred in India earlier (Alagarswami et al., 1983) and an experimental hatchery has been established for mass production of spat (Alagarswami et al., 1987). Experimental success has been achieved in artificial breeding of silver-lip pearl oyster, *P. maxima* in Australia (Tanaka and Kumeta, 1981), but it has yet to be commercialized. In spite of repeated trials in French Polynesia, culture of *P. margaritifera* larvae has been a failure (Coeroli et al., 1984). The success achieved in artificial breeding of *P. margaritifera* at Tuticorin (lat. 8°47′N; long. 78°08′E), India is reported in this paper.

MATERIAL AND METHODS

Four *P. margaritifera*, collected from the pearl oyster beds of the Gulf of Mannar during 1984–85, were maintained in the pearl oyster farm at Krusadai Island and brought to the shellfish hatchery at Tuticorin by road in May 1986. These oysters were 77–92 mm in shell height (dorsoventral measure) and 70–97 g in weight. They were placed in a meshwork basket and suspended at 5 m depth from a raft in the experimental pearl oyster farm in the harbour basin at Tuticorin. Two of the oysters subsequently died. The surviving pair, one male and one female (Fig. 1), spawned successfully in December 1986 and died later.

The oysters spawned spontaneously during their transportation from Krusadai to Tuticorin. The spawn was discarded. On placing them in a tank in the hatchery, they spawned again on 15 May 1986, producing viable larvae. The same animals, when subjected to thermal stimulation (temperature elevated from 24.7 to 30.5°C) on 30 May 1986, spawned mildly, but the larvae were not viable. After maintaining them for 7 months in the farm, the surviving pair was brought to the hatchery on 31 December 1986 and they spawned spontaneously and profusely. The male released sperm at 11.10 h and the female spawned at 13.20 h. Fertilization was normal and the larvae were viable. The results presented in the paper relate to the culture of larvae of this batch.

The larval culture procedures were generally the same as described for *P. fucata* by Alagarswami et al. (1983). Fibreglass tanks (75×50×25 cm) holding 50 l of seawater, previously filtered through a sterile cotton plug, were used for culturing the larvae. In the static system, seawater was changed every second day and no aeration was given. During larval culture, water temperature in the tanks was 26–30°C, salinity 34.2–35‰ and pH 7.87–8.07. The initial larval density was 1/ml. Pure cultures of two flagellates, *Isochrysis galbana* and

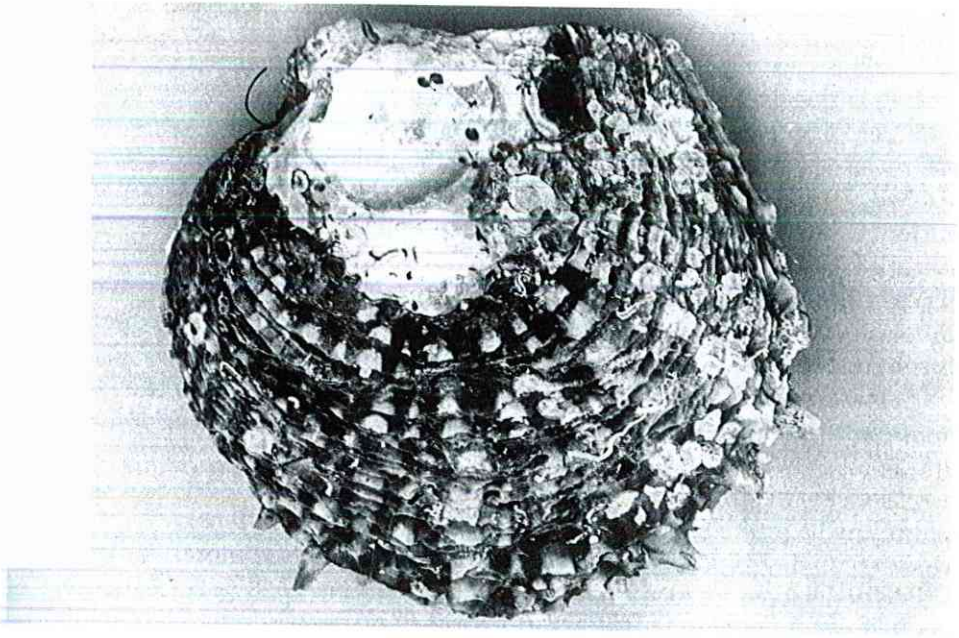

Fig. 1. *Pinctada margaritifera* (Linnaeus), adult female of shell height 85.6 mm and weight 88.0 g, which spawned in the laboratory.

Pavlova lutheri, were used independently as larval food at a concentration of 5 cells/μl from day 2 and 10 cells/μl from day 5 until metamorphosis. Food was supplied once a day. Thirty larvae randomly collected were measured under a microscope on the days of observation to study growth. Unless stated otherwise, the data presented in the paper relate to larval culture with *I. galbana* as diet.

Spat were allowed to set on the sides and bottom of the larval culture tanks. Subsequently they were carefully detached with a piece of foam rubber, washed and placed in fresh tanks. The spat were cultured in static seawater which was changed daily and given aeration. They were fed a mixed algal diet of *I. galbana*, *Skeletonema* sp. and *Nitzschia* sp. The ration was 25 cells/μl until spat size of 1 mm and was doubled thereafter. Feeding was done once a day.

At transplantation to the farm, the spat were placed in a pyramidal lantern-net having a triangular base of 35-cm side, covered with velon fabric of appropriate mesh, and suspended from the raft at a depth of 5 m. The average density was 600 spat/net. The annual range of surface temperature was from 25.2°C in December to 31.6°C in May and that of salinity was from 30.5 ppt in December to 35.75 ppt in September.

RESULTS

Most of the spawned eggs were spherical and measured an average 45 μm in diameter (Fig. 2). The first cleavage was seen 35 min after the first polar body was released. The 4-cell, 8-cell, 16-cell and morula stages of the embryo were reached, respectively, in 1 h 5 min, 1 h 38 min, 2 h 20 min and 4 h from fertilization. The early D-shaped veliger, which appeared in 20 h, had an anteroposterior measure (APM or shell length) of 75 μm and dorsoventral measure (DVM or shell height) of 60 μm (Fig. 3). Velar abnormality was seen in a few larvae with the velum protruding from a single point in the shape of a tongue or a stalked hood.

Umbo formation commenced on day 9 at 110×100 μm, with the larva becoming globular (Fig. 4). The typical umbo larva on day 12 measured 140×130 μm. Shell growth from early umbo stage was characterized by addition of distinct concentric growth lines on the margin. The ventral margin of the shell maintained a pinkish tinge from umbo stage till metamorphosis. The eye spot was seen on day 16 in larvae measuring 210×200 μm and more.

The pediveliger (220×210 μm) appeared on day 20. The dorsal aspect of the foot was pigmented black with irregular opaque white patches, while the creeping sole was fully opaque white. The plantigrade was seen on day 23 at

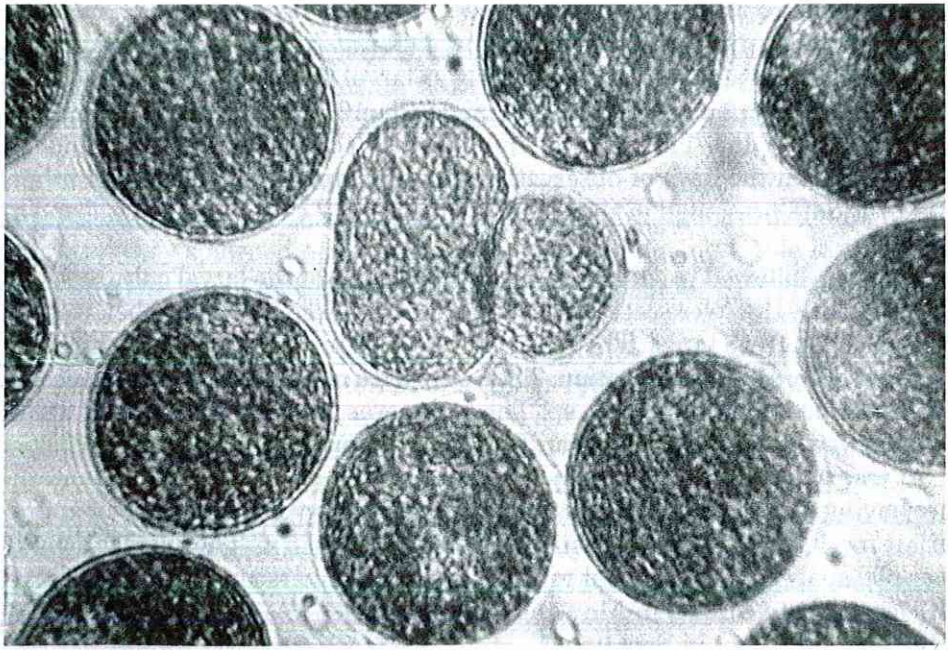

Fig. 2. Fertilized eggs (average 45 μm diameter) of *P. margaritifera*. The first unequal cleavage is seen in the egg at the centre.

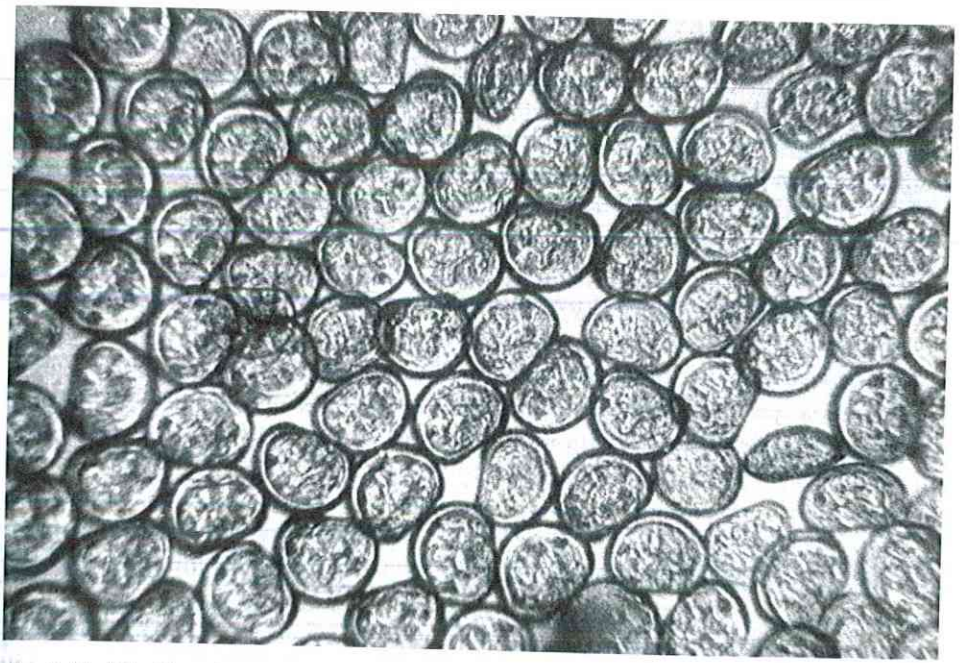

Fig. 3. Straight-hinge larvae, average size 75 × 60 μm.

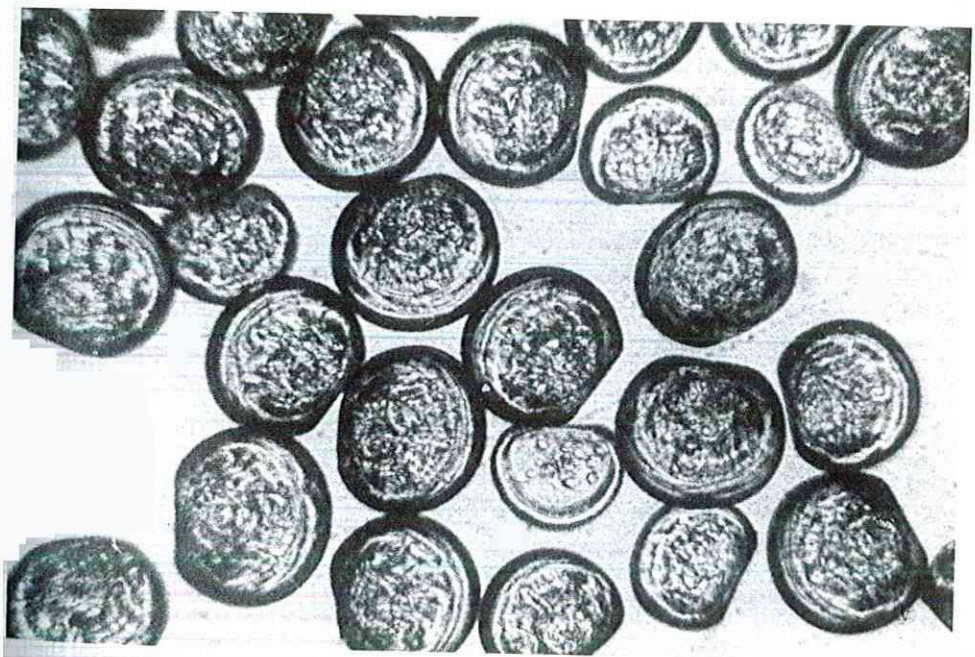

Fig. 4. Veligers becoming more globular and about to reach umbo stage.

the size of 260×240 μm; it metamorphosed to a typical spat. The smallest spat observed in the tanks measured 350×300 μm on day 28. The *P. margaritifera* spat of 1.9 mm shell length in Fig. 5 shows the umbo-shaped larval shell and the pattern of subsequent shell growth, assuming the typical pearl oyster form. The shell had a dark colour which later turned to light bronze.

Up to day 9, all larvae were in the straight-hinge stage. Thereafter differences in growth rate and stages were observed. The umbo stage was dominant on day 12 (73.3%) and day 16 (90%), and the pediveliger on day 23. On day 28, the umbo stage accounted for 26.7%, pediveligers for 26.7%, plantigrades for 16.7% and spat for 30%. Spat constituted 73.3% on day 31 and 100% on day 35. Spatfall in the tanks had spread over a period of 8 days.

In view of the above heterogeneity, the modal size of the dominant larval stage was considered in working out the growth rate. The modes were at 82.5 μm on day 6, 97.5 μm on day 9, 112.5 μm on day 12, 182.5 μm on day 16 and 252.5 μm on day 23. The average growth rate of the dominant segment of the larval population from day 0 to day 23 was 10.98 μm/day. On day 23, the minimum larval size was 180 μm, showing a growth rate of 7.83 μm/day, and the maximum was 410 μm, recording a growth rate of 17.83 μm/day.

Testing the two algal diets *I. galbana* and *P. lutheri* under parallel larval culture, it was seen that *I. galbana*-fed larvae showed a higher growth rate. On

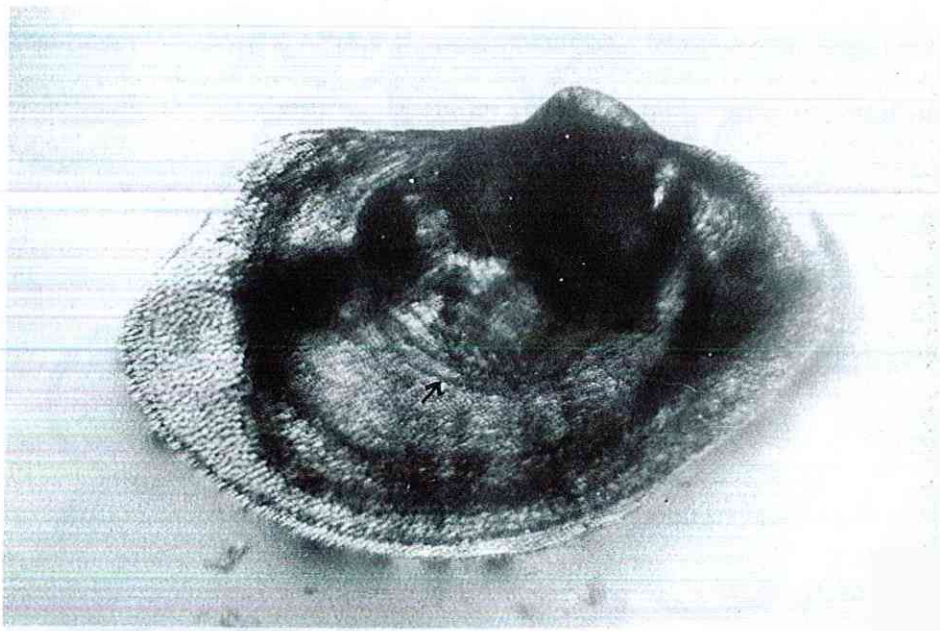

Fig. 5. Spat of *P. margaritifera*, shell length 1.9 mm. Arrow shows the margin of the larval shell.

day 23, *I. galbana*-fed larvae had reached a mean shell height of 244.8 μm, but *P. lütheri*-fed larvae only 202.8 μm.

The initial larval population in all the culture tanks was 7.75×10^5 in a total volume of 775 l of seawater. Total number of larvae which metamorphosed as spat was 48 800, giving a survival rate of 6.3% and production rate of 63 spat/l.

Shell growth of early juvenile of *P. margaritifera* is by deposition of shell material in a transparent honey-comb-like lamina (Fig. 5) which becomes opaque with further deposition of calcium carbonate crystals all along the margin. Patches of light yellow pigment appear on the shell when the spat is about 0.75 mm. Simultaneously the mantle deposits dark pigments on the inner side of the shell. The shell, which appears dark at this stage, turns light bronze with further growth. The first growth process appears at the broad end of the oblique dorso-ventral axis, arising from the pigmented spot on the transparent shell margin. Additional growth processes appear later along the same growth ring on either side of the first one. Growth rings are many and imbricate in nature, and the process arises from below the growth ring. It is flat, tender, flexible and opaque white in colour. In a spat of 17.8 mm shell height, the largest growth process measured 4.8 mm in length and 3.3 mm in width at the free end, narrowing to 2.5 mm at the base. In the laboratory-cultured juveniles, no growth process developed even at 10 mm shell height (Fig. 6) whereas in the farm-cultured specimens it was distinct at 4.5 mm.

The juvenile shell has a light bronze background colour. Along the growth rings, a continuous, diffusely bordered, smooth or wavy brownish band is present. Radially, discrete opaque white bands denoting the axes of growth processes are seen (Fig. 7). The anterior ear is well developed with a conspicuous byssal notch on the right valve. The posterior ear is not developed. On the inner aspect (Fig. 8), the anterior nacreous border does not project beyond the hinge margin and slopes backwards. The posterior nacreous border slopes forwards. The nacre is white and iridescent with no particular change along the margin. The non-nacreous portion is light bronze in colour with opaque white bands along the radial lines of the growth processes. Brownish pigmentation marks the zone of separation of nacreous and non-nacreous areas.

In juveniles up to 21.1 mm hinge length (HL), the DVM and APM are subequal. The ratio of DVM to HL is 0.7 at 3.8 mm HL and increases to 0.9 at 21.1 mm HL. The APM appears more variable as compared to DVM, and the ratio of APM to HL is within the range of 0.7–0.9.

In juveniles of 2.8–19.5 mm DVM, the APM and HL are linearly related to the DVM, and the equations describing the relationships are:

$$Y_1 = 0.96783 + 0.94183\,X$$

$$Y_2 = 1.49061 + 1.01276\,X$$

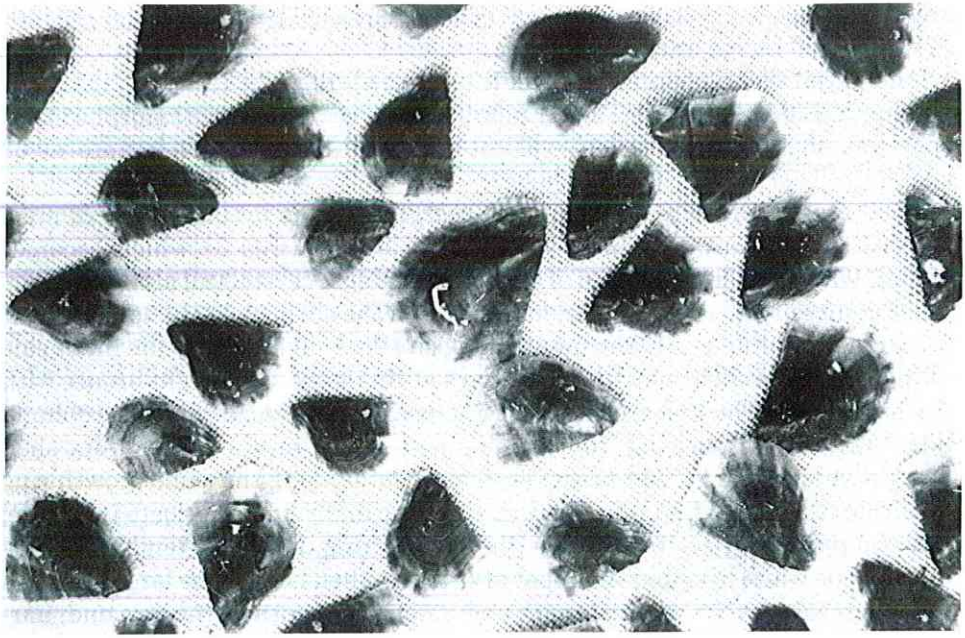

Fig. 6. Laboratory-reared juveniles, which did not develop growth processes. The largest at the centre measured 10.6 mm.

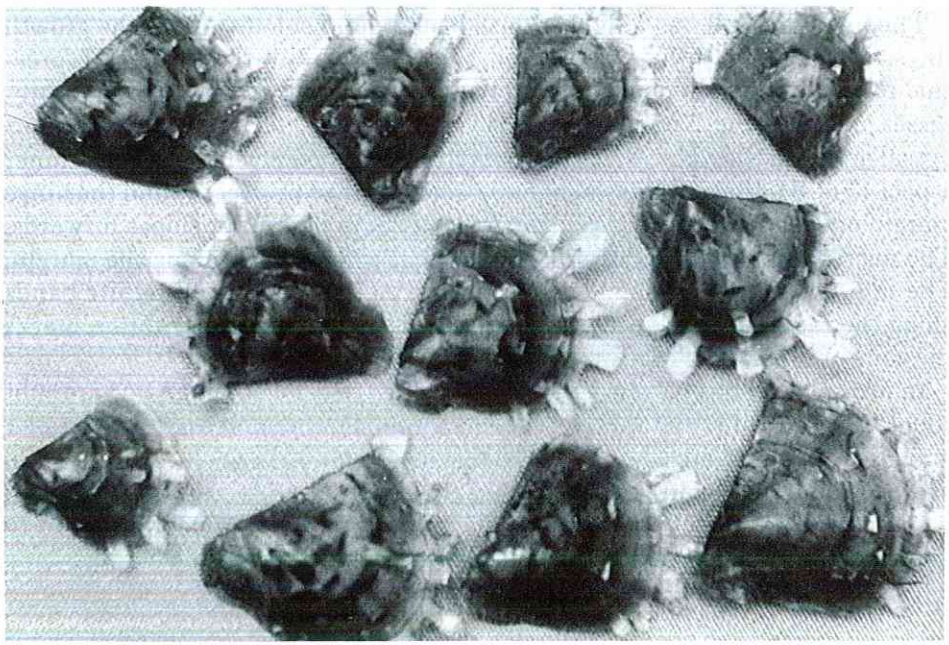

Fig. 7. Juveniles of *P. margaritifera* reared in the farm, showing typical shell characters and prominent growth processes. The largest measured 21.1 mm in shell height.

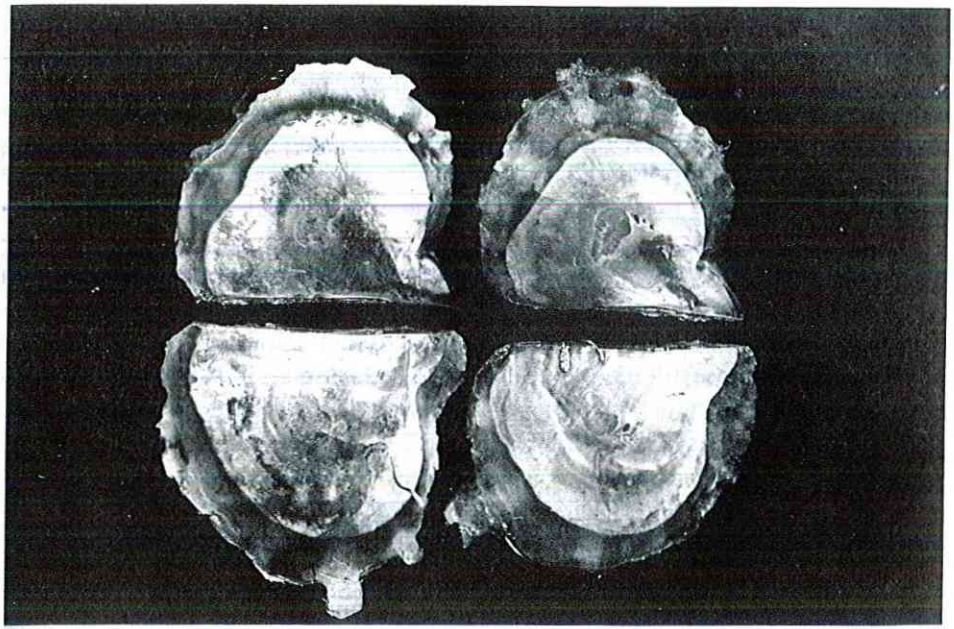

Fig. 8. Two pairs of shells of juvenile *P. margaritifera* showing the characters of nacreous and non-nacreous regions. The larger of the pair measured 18.0 mm in shell height.

where Y_1 is APM, Y_2 is HL and X is DVM, with correlation (r) values of 0.982 and 0.996 respectively.

In the laboratory the growth of juveniles was very slow. Tracing those which showed the maximum growth, it was seen that the growth rate was 0.09 mm/day from date of setting for a 3-month period. When the juveniles were transplanted to the farm on 18 March, the growth rate improved to 0.4 mm/day as compared to 0.15 mm/day for spat which were retained in the laboratory over the next one-and-one-half months. The following equation was fitted to the juvenile growth data: $y = ae^{bt}$ where y = DVM in mm and t = time in days. The fitted equation for laboratory-reared spat was $y = 0.4313\, e^{0.03345t}$ with an r value of 0.997, and that for the farm-transplanted spat was $y = 0.3985\, e^{0.040833t}$ with $r = 0.998$. It is obvious from the above equations that the farm-transplanted spat had a higher instantaneous growth rate (b). The growth curves with observed and calculated values are presented in Fig. 9.

As compared to the spat of *Pinctada fucata* which were concurrently reared in the farm, *P. margaritifera* spat suffered heavy mortality. The survival rate ranged from 15.16% to 17.40% in different lantern-nets on 2 May 1987. Mortality rate increased suddenly and, by 7 May, it was total except for a single spat. The 11-month-old survivor measured 38.8 mm in shell height, 33.2 mm in hinge length and 6 g in weight on 29 December 1987.

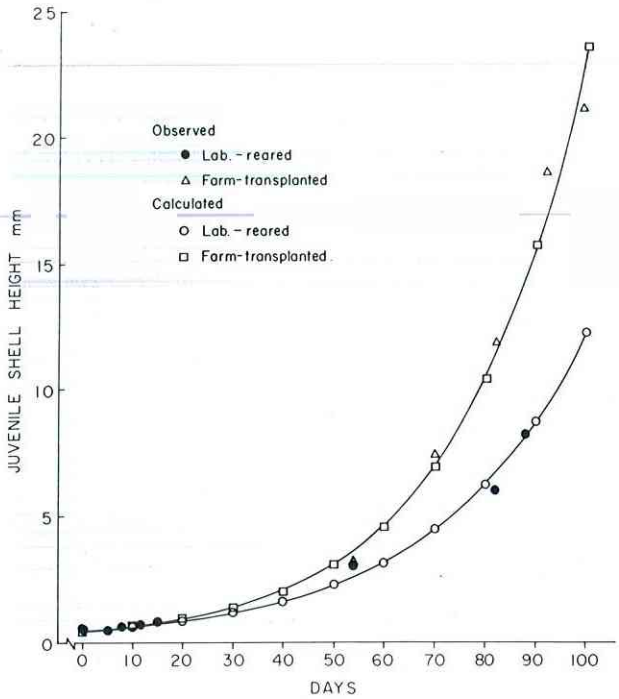

Fig. 9. Growth curves of *Pinctada margaritifera* representing the maximum growth of laboratory-reared and farm-transplanted juveniles from date of setting to day 99.

DISCUSSION

Crossland (posthumous 1957) successfully developed methods for collection of spat and cultivation of *Pinctada margaritifera* in Dongonab Bay, Red Sea. The methods were further improved by Reed and a cultivation-based shell industry was established (FAO, 1962; Rahma and Newkirk, 1987). Similar commercial cultivation of natural stocks of the species from spatfall is carried out in French Polynesia (AQUACOP, 1982) and some success has also been achieved in Papua New Guinea (Lock, 1982). In the tended stock of Dongonab Bay, massive mortality was observed in 1969, 1973 and 1978 (Nasr, 1982; Gideiri, 1983). In *P. maxima* heavy natural mortality has been reported from Australia (Pass et al., 1987). Environmental degradation due to repeated use of the farming sites, pollution and diseases lead to large-scale mortality of pearl oysters. There is, however, an increasing demand for fresh stocks for pearl cultivation. Mizumoto (1979) stated that development of a method for artificial seed production would facilitate more stable production of pearls from *P. maxima* and *P. margaritifera*. In such a context, development of hatchery tech-

niques for controlled production of *P. margaritifera* reported here assumes importance.

Basic experiments on propagation of *P. margaritifera* were carried out in Japan by Setoguchi (1964, 1966; Tanaka et al., 1970). Several attempts at seed production of black-lip pearl oyster were made in French Polynesia, but larval culture was unsuccessful in promoting growth beyond the tenth day (Millous, 1980; Coeroli et al., 1984). Following larval culture and spat production of *P. fucata* in India (Alagarswami et al., 1983), success was achieved in breeding of *P. margaritifera* in the present study.

The progression of larval growth of *P. margaritifera* does not differ much from that of *P. maxima* (Tanaka and Kumeta, 1981) and *P. fucata* (Alagarswami et al., 1983). The presence of spawned eggs in different stages of development in the stomach and digestive diverticula of *P. margaritifera* (these had been previously ingested by the animal) enabled Tranter (1958) to describe the embryonic development of the species up to the gastrula. Larval growth data for *P. fucata* and *P. maxima* as observed by earlier workers (Ota, 1957; Minaur, 1969; Tanaka and Kumeta, 1981; Alagarswami et al., 1983) and those of *P. margaritifera* from the present study are compared in Table 1. It is seen that, despite species and regional differences and variations in rearing conditions, the time–size series data for the different larval stages show similarity. Metamorphosis of larvae of the three species takes place around day 20–23 at size of about 230–266 μm. The minor differences noted are to be expected even in the larval culture of the same species at the same time. The differences in larval characters noticed are the pinkish tinge in the shell margin of the larvae of *P. margaritifera* which is absent in *P. fucata*; dark pigmentation in the foot of the former which is absent in the latter; and delayed column formation of larvae in the former (seen only from day 12) as compared to the latter (from early veliger stage). Minaur (1969) observed this in *P. maxima* from the early veliger stage.

Hynd (1955), in his revision of Australian pearl shells, described *P. margaritifera* of size more than 2 cm DVM. Alagarswami (1983) dealt with *P. margaritifera* specimens of 34.0 mm DVM and above from the Andaman and Nicobar Islands. Since no description of juveniles less than 2 cm is available in the literature, details of shell growth and coloration have been briefly described in the present study.

Coeroli et al. (1984) reported that in the Takapoto Lagoon, a closed atoll of the Tuamotu Archipelago in French Polynesia, the juveniles of *P. margaritifera* raised by spat collection from the natural grounds reached 0.2–0.3 mm, 2–3 mm, 8–10 mm and 40–50 mm in 1, 2, 3 and 6 months respectively, and 70–80 mm, 100–120 mm, 120–150 mm, 140–150 mm and 140–150 mm at the end of 1, 2, 3, 4 and 5 years respectively. In the present study, the farm-transplanted spat reached the maximum size of 21.1 mm (minimum 8.2 mm; average 14.2 mm) in 99 days counted from the date of spat setting. As compared to 8–10

TABLE 1

Time series growth data for three species of pearl oyster. The larval measurements are in μm. Wherever two measurements are given with an × sign, the first is APM and the second is DVM. Time from fertilization is given in hours (h) and minutes (m) or in days (d).

Stage	*P. fucata*				*P. maxima*				*P. margaritifera*	
	Alagarswami et al. (1983)		Ota (1957) and others		Minaur (1969)		Tanaka and Kumeta (1981)		Present study	
	Size	Time	Size	Time	Size	Time	Size	Time	Size	Time
Egg, spherical	47.5	-	-	-	59- 60	-	-	-	45	-
D-shape	67.5× 52.5	20 h 40 m	72× 60	20 h	-	-	75- 77	20 h	75× 60	24 h
Early umbo	100 × 95	-	96× 87	d 8	96× 87	d 5	110-125	d 10	110× 90	d 9
Umbo	135 ×130	d 10-12	-	-	125×112	d 12	-	-	140×130	d 12
Eye-spot	210 ×190	d 15	170 - 200	-	-	-	-	-	210×200	d 16
Pediveliger	230 ×200	d 20	209×195	d 25	180×160	d 14-20	234	d 19	220×210	d 20
Plantigrade	250 ×240	d 22	200-230	-	-	-	266	d 20	260×240	d 23

mm attained in 3 months in the Takapoto Lagoon, the growth attained by *P. margaritifera* juveniles (8.2–21.1 mm) for about the same period in Tuticorin is higher. In the Takapoto Lagoon the range of seawater temperature is from 26–27°C (August–September) to 29–30°C (March–April) and that of salinity is from 37 ppt (January–August) to 39 ppt (November–December) (Coeroli et al., 1984). The salinity values are much higher in Takapoto than in Tuticorin. It is not known whether this factor be responsible for the differences in juvenile growth rate observed in the two regions.

Both in the laboratory and in the farm the mortality of spat was high. Spat production itself was low at 6.3% of the initial larval population. There was heavy juvenile mortality after about 4 months. Prior to the rearing reported here, 50 spat of *P. margaritifera* had resulted from spawning on 15 May 1986 and these too did not survive. The species does not occur in the coastal waters of the mainland of India, whereas it has a natural distribution at several centres in the Andaman and Nicobar Islands. It remains to be tested whether hatchery-produced juveniles would have a greater chance of survival in oceanic island conditions.

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