Larval rearing and spat production of the windowpane shell *Placuna placenta*

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The windowpane shell, *Placuna placenta* is one among the pearl producing bivalves. It was identified as the second-priority mollusc species for research during the Second Conference on Aquaculture Development in Southeast Asia held in the Philippines in July 1994 and is the basis for a 'kapis' (windowpane shell) fishery in the Philippines. Windowpane shell provides fishermen an additional income-generating source through the sale of its pearls and shells. The empty shells are used as raw material in making shell craft products and are exported.

In India the distribution of the species is confined to the Kakinada Bay in Andhra Pradesh[1,2,3,4]; to the Okhamandal Coast in the Gulf of Kutch[5,6]; to the Nauxim Bay of Goa[7] and to Tuticorin Bay[8] and Vellapatti near Tuticorin[9]. The oysters were fished from these areas in considerable quantities every year for pearls and shells causing concern about over exploitation of wild stocks. The development of techniques on breeding, larval rearing and spat production of the species might eventually help to sustain the fishery. Hence efforts are made in different parts of the world to breed the windowpane shell in captivity and produce the seeds for replenishment of wild stocks. Previous research has documented the spawning and larval development of *P. placenta*[10]; documented the techniques in induced spawning and early embryonic and larval development[11]; studied the effect of salinity on the embryonic development, larval growth and survival at metamorphosis[12]; evaluated the effect of microalgal diets and rearing condition on gonad maturity, fecundity and embryonic development[13]; and reported on hatchery management techniques[14].

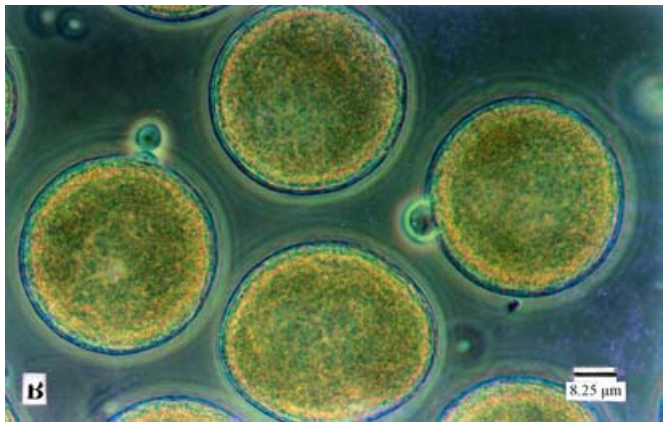
In Tuticorin Bay, the windowpane shell occurs in a 0.46 ha bed and its exploitation during the fishery is almost total leading to the depletion of stock in the bay. The natural population has to be augmented in order to meet the needs of mariculture. We therefore undertook to develop the technology for the production of seed of the species in the hatchery and to sea ranch them for the replenishment of wild stock. The report is the first to be published on larval rearing and seed production of *P. placenta* from India.

Techniques

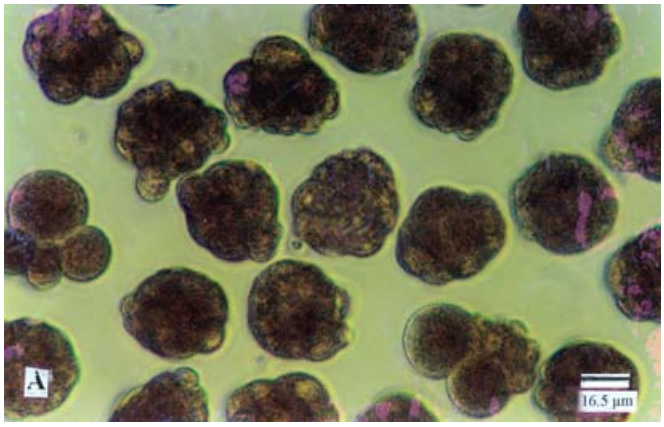
The windowpane shells, collected from the Tuticorin Bay in the Gulf of Mannar, were brought to the Shellfish Hatchery of the Tuticorin Research Centre of the Central Marine Fisheries Research Institute. Prior to the experiment, these broodstock animals were kept for 24 hours in aerated seawater held in a rectangular tank. The oysters were treated for spawning on the following day by thermal stimulation at 37ºC at 1445 hours. Males started to spawn at 1530 hours followed by females. When the spawning was over the brood stock animals were removed and the eggs allowed to fertilize. The eggs were yellow in colour.

We collected the fertilized eggs by gently siphoning through a 30µm sieve. The material collected in the sieve contained fertilized eggs, faecal matters, broken tissues, shell fragments and other waste materials. The contents of the sieve were passed through an 80µm sieve and the unwanted materials discarded. The fertilized eggs that passed through 80µm sieve were allowed to develop in the tank and the larval development was studied. No aeration was provided and antibiotics were not used during larval development. We observed the development under an inverted microscope using 10x10 magnification. After 24 hours straight-hinged larvae were collected in 40µm mesh sieve. The larvae were reared in two 75 liter rectangular fiberglass tanks. Feeding was initiated at veliger stage on day two. The unicellular microalga *Isochrysis galbana* was given as food to the larvae once in a day at a concentration of 5,000 cells larva^{-1} day^{-1} (10 cells µl^{-1}) from day two; 10,000 cells day^{-1} (20 cells µl^{-1}) from day five; 15,000 cells day^{-1} (30 cells µl^{-1}) from day seven; and 20,000 cells day^{-1} (40 cells µl^{-1} from day ten. The microalga was cultured in Conway medium[15] and harvested during its exponential phase. Algal density was assessed using haemocytometer and proportionate feed was given. Water change was done once in two days by siphoning the larvae through a sieve with the mesh size smaller than the larval size. Random sample of 50 larvae was measured along the dorsoventral axis (DVM) and anteroposterior axis (APM) as a parameter to assess the growth. The spat were allowed to settle free in the tank itself, as they did not have byssus thread or cement gland for attachment. No cultch material was provided for settlement of spat. Aeration was given only after spat setting. Unicellular microalga *I. galbana* was continued to be supplied as food for the spat up to 1.0mm and was gradually replaced afterwards by mixed algal food. The mixed algae, chiefly containing *Chaetoceros* sp.and other diatoms, were developed in outdoor tanks. During larval rearing the water temperature ranged from 28ºC to 30ºC; salinity 34.6 – 35.2 ppt. and pH 7.91-8.09.

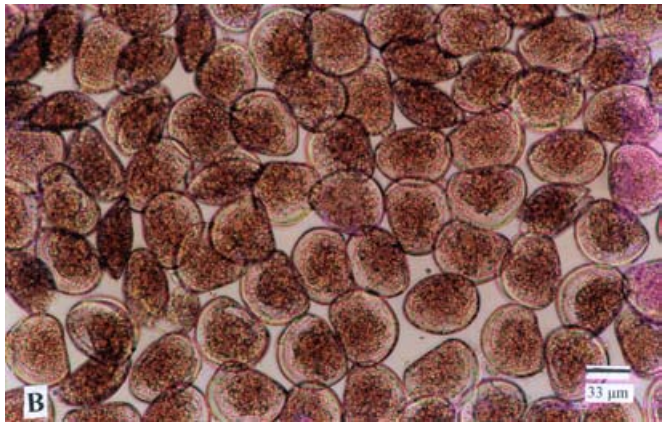
Window-pane shell Placuna placenta.



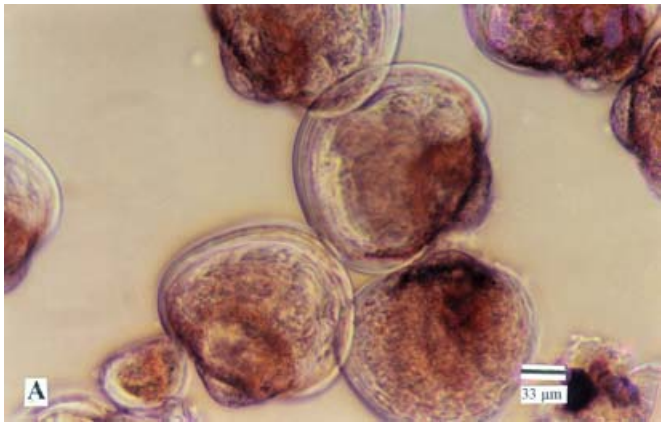
Fertilized eggs with polar body, size 50µm.



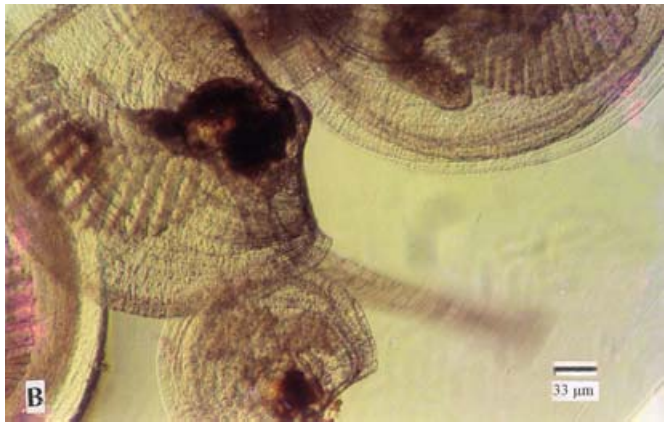
4-celled, 8-celled and 32-celled stages.



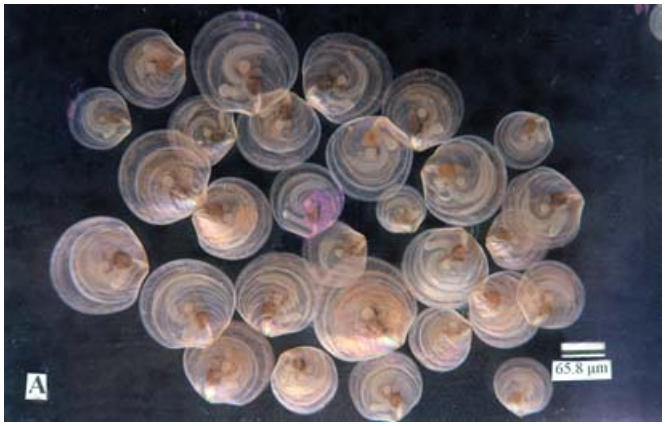
Straight-hinge larvae, average size 79.9 x 65.2µm.



Typical umbo stage, size 140 x 130 µm.



Spat with its transparent shell.



Hatchery reared juveniles of P. placenta*, average size 26.4 x 10.6 mm on day 135.*

Larval development

The spawned eggs are spherical in shape measuring 50µm. Fertilization was immediate and the first polar body was released 15 minutes after fertilization. First cleavage started after another 15minutes leading to the two-celled stage. The two-celled stage had a smaller micromere and a larger macromere along with a polar body at the furrow of cleavage. The trefoil stage, containing three micromeres and one macromere was obtained four minutes. After the two-celled stage. The cell wall of the 3 micromeres slowly disappeared and resulted in the four-celled stage 65 minutes after fertilization. The macromere did not take part in further cell divisions. The 8-celled, 16-celled and 32-celled stages are obtained in 80 minutes, 100 minutes and 155 minutes respectively after fertilization. Morula stage was reached in

four hours and 45 minutes. The embryo began to move at the morula stage. As the larvae move in water column, the morula is transformed to a blastula stage by the development of a blastocoel, which opens to the exterior through blastopore. Gastrulation commenced at this stage by convolution of cells to interior through blastopore. After completion of gastrulation, the dermal layers namely ectoderm, mesoderm and endoderm are formed along with an archenteron. By the formation of single flagellum at the apical end, the embryo reached trochophore stage in 5 hours and 41 minutes. The ectodermal cells secrete embryonic shell material (prodissoconch 1) and formed a D-shaped veliger 18 hours and 45 minutes post fertilization and measured an average of 65.2µm in DVM or shell height and 79.9µm in APM or shell length. The shell valves of the veliger are equivalve and transparent with conspicuous granules. The time sequence of early embryonic development of larvae of *P. placenta* is given in Table 1 and the time series growth data of the species from veliger to spat is presented in Table 2.

Larval growth

The relationship between the larval shell length (APM) and shell height (DVM) is linear and is described by the equation:

$Y=17.982+0.9595X$ with $r=0.9981$

Where Y represents APM and X represents DVM in µm.

On day three all larvae were at veliger stage and subsequently the D-shaped veliger became globular at 110µm APM x 100µm DVM resulting in the disappearance of straight hinge line. On day four the typical umbo stage constituted 90% of the population measuring 140 x 130µm. The late umbo stage is reached at 210 x 200µm on day five, pediveliger at 215 x 205µm on day seven, and plantigrade at 235 x 210µm on day eight. On day nine the umbo larvae constituted 14%, eyespot 28%, pediveliger 34% and plantigrade 24% of the population. The spat had grown to 340 x 300µm on day ten. On day thirteen the pediveliger formed 14%, plantigrade 36% and spat 50% of the population. In view of such heterogeneity in growth, the average size of larvae at different stages was taken into account in working out the growth rate. The average shell height on day one is 65.2µm; 81.6 µm on day three; 121.6 µm on day six; 205.8 µm on day nine and 300 µm on day thirteen. The average daily rate of growth of the larvae is 23.0 µm from day 0 to 13.

Spat setting and spat production

The larvae set as spat on day 7-8. The spat has neither byssus nor cement gland for attachment and hence they were allowed to settle on tank surface. The spat has an exceptionally long foot which would be of much use in burrowing. The shell is highly transparent with concentric growth line. The initial larval population in all the culture tanks was 1.5×10^5 in a total volume of 150 litres of seawater. The total number of larvae that metamorphosed as spat was 12,500, giving a survival rate of 8.3 % and production rate of 83.3 spat/liter.

Growth of spat

The average growth in shell height of spat was 0.300mm on day thirteen; 0.806mm on day 22; 3.09mm on day 36 and 12.44mm on day 80. The equation

Table 1. Time sequence of early embryonic development of larvae of *Placuna placenta*

Stage	Time after fertilization	
	Madrones-Ladja (1997)	Present study
Egg	0	0
First polar body	15 min	15 min
Second polar body	-	20 min
2-celled stage	30-40 min	30 min
Trefoil stage	40-50 min	34 min
4-celled stage	50-60 min	65 min
8-celled stage	115-120 min	80 min
16-celled stage	-	100 min
32-celled stage	120 min	155 min
Morula stage	-	285 min
Blastula stage	-	295 min
Gastrula stage	235 min	305 min
Trochophone stage	325 min	341 min
Water temperature Salinity	27 ºC 33 ppt	28 ºC 34.6-35.2 ppt

Table 2. Time series growth data of *Placuna placenta*. The larval measurements are in µm. Whenever two measurements are given with an X 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	Madrones-Ladja (1997)		Present study	
	size	Time	Size	Time
Egg- spherical	0		50	
D-shape	89 x 75	18 h 20 m	79.9 x 65.2	18 h.45m
Early umbo	98 x 82	d 5	110 x 100	d 3
Umbo	138 X 105	d 7	140 x 130	d 4
Pediveliger	192 x 190	d 9	215 x 205	d 7
Plantigrade	232 x 225	d14	235 x 210	d 8
Spat	-	-	340 x 300	d 10
Water temperature Salinity	24.0-27 ºC 32.0-35.0 ppt		28.0-30 ºC 34.6-35.2 ppt	

for the growth of the spat from day 13 to 80 is described as:

$Y = 0.1634 + 0.9754 X$ with r-value of 0.9998.

The spat was transferred to farm on day 80 and the growth rate of spat after 54 days in the farm was 0.59mm/day. During the same period the spat reared in the hatchery showed a growth rate of 0.08mm/day (average size 26.4 x 10.6 mm on day 135). The following equation was fitted to the spat growth data: $y = ae^{bt}$ w here y = DVM in mm and t = time in days. The fitted equation for hatchery reared spat was: $Y = 0.6973 + 0.9365 X$ with r-value of 0.9998 and that for farm reared spat was $Y = 0.3410 + 0.9955 X$ with r-value of 0.9984. It is evident from the equation that the farm reared spat had a higher instantaneous growth rate (b). The farm-reared spat attained juvenile stage with an average size of 44.4mm on day 135. The spat produced in the hatchery was ranched in the bay.

Applications

Regular fishing of windowpane shell is conducted in the Kakinada Bay in Andhra Pradesh and in Okhamandal Coast in the Gulf of Kutch. Huge quantities of these animals are exploited every year causing depletion of stock. In a notification dated July 21, 2001 the Ministry of Environment and Forests, Government of India, has included the windowpane shell in Schedule 1 of the Wildlife (Protection) Act, 1972. As a result the natural populations of *P. placenta* are protected against exploitation. While breeding of several species of bivalves has been achieved[16], this is the first report on breeding, larval rearing and spat production of windowpane shell from India.

Initial attempts to induce spawning windowpane shell were made using water manipulation techniques[10]. Others resorted to chemical and photochemical stimulations[11]. In the present study thermal stimulation was successful in the induction of spawning in *P. placenta* when water temperature was increased to 37ºC. The temperature at which *P. placenta* responded to spawning seemed to be high when compared to other bivalves studied from India such as the blood clam *Anadara granosa* which spawned at 32ºC after conditioning at 24.0-26.0ºC for 15 days[17]; the great clam *Meretrix meretrix* at 4-5ºC above the ambient level of 24.0-26.0ºC[18].

The easy response of induction of spawning by thermal stimulation and faster growth of larvae/spat has facilitated the scaling up of production of the seeds of *P. placenta* in India. The Shellfish Hatchery at Tuticorin had already demonstrated the production of pearl oyster seed to a maximum of 1.3 million per run[19]. An average survival rate of 5% of pearl oyster seeds was achieved. In the present study the rate of production of windowpane shell seed is 8.3%. The culture conditions (water temperature 28-30ºC and salinity 34.6-35.2 ppt) prevailing during this study seemed to be favorable for the production of seeds. Large-scale production of seeds of *P. placenta* to replenish natural stocks seems quite feasible.

Madrones-Ladja[12] reported the settlement after fourteen days in the salinities ranging from 22-34 ppt. The present investigation not only observed earlier settlement (between the day seven and eight) but also indicated faster growth of larvae/spat at a water temperature of 28-30ºC and salinity 34.6-35.2 ppt. The growth of larvae/spat up to day thirteen was 23.0µm day $^{-1}$ whereas the same, as reported by others[14], was 11.0 µm day $^{-1}$ up to the day fourteen. The faster growth rate in India may perhaps be related to higher water temperature. Madrones-Ladja[11] provided petri dish as cultch at the time of settlement and reported poor survival, which may be attributed to the lack of deficiency of essential nutrients in the microalgae fed to the larvae and the non-availability of suitable substrate. Similar results have been reported for tridacnid clams when suitable substrate is not available[20].

P. placenta has neither cement gland nor byssus thread for attachment. Hence at the time of settlement if a suitable substrate is provided, as in the natural habitat, high survival may be achieved. The foot of the windowpane shell is exceptionally long when compared to other bivalves, and facilitates in burrowing. In the natural habitat the lengthy foot may be beneficial in positioning the spat at the time of settlement in the clayey bottom. *P. placenta* is naturally found burrowing in muddy or sandy-mud substratum[21]. Provision of cultch material like glass or plastic items might not be useful for settling in *P. placenta*. Hence, in the present study no cultch materials were provided and therefore the larvae are allowed to set at the bottom of the fiberglass tank.

The rate, at which Madrones-Ladja[11] fed the larvae is higher than in the present study and still the growth and settlements are faster in India. The nutritional value of *Isochrysis galbana* may likely vary at different locations and this may reflect in the vigor, viability and growth of the embryo, larva and spat. Higher larval growth of *Ostrea edulis* has been reported when fed a microalgal diet of *I. galbana* and *Chaetoceros calcitrans*[22] Madrones-Ladja[12] reported the food value of *I. galbana* with 41 % crude protein and 23 % crude fat. In the present work the food value of *I. galbana* is 59.6 % crude protein (dry weight) and 14.4 % crude fat (dry weight). *I. galbana* is one of the most commonly used marine unicellular algae in mariculture and is rich in fatty acid C22:6[23]. However a detailed study is needed to determine the optimum algal cell ration to the larvae and its food value on the growth of larvae and spat.

Acknowledgements

The authors are grateful to Prof. (Dr.) Mohan Joseph Modayil, Director and Dr. K.K. Appukuttan, Head, Molluscan Fisheries Division, Central Marine Fisheries Research Institute, Cochin for their interest and encouragement. Our sincere thanks are due to Miss. Anu alias Meena, Senior Research Fellow, Tissue Culture Project, Tuticorin for the help in computerization of data.

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are continuously put in place and effectively implemented. Otherwise, the risks of major disease incursions and newly emerging diseases will continue to threaten the sector. Sometimes I wonder whether doing something or doing nothing at all can make a difference. Glenn Hurry, of AFFA (Australia) when I reported to him about these seemingly incessant disease incursions, commented 'not bad, especially when governments are told what to and what not to do!'. There are many lessons from the past and hopefully our memories will not be too short to forget the events caused by various trans-boundary aquatic animal disease epizootics (e.g. epizootic ulcerative syndrome of fresh and brackishwater fishes, viral nervous necrosis of marine fish, viral diseases of shrimps, haplosporidiosis in oysters, akoya pearl oyster mortalities, etc.). These lessons can assist us towards preparing better and improving responses to similar events when they occur in the future.

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