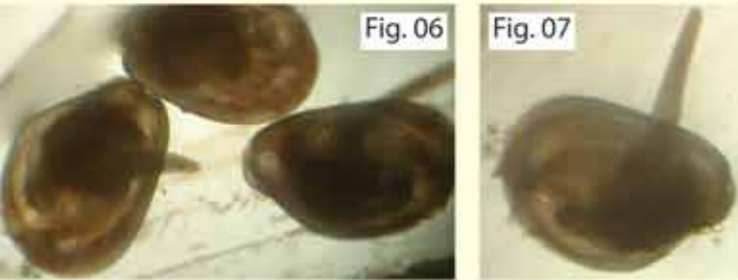


## Plantigrade stage

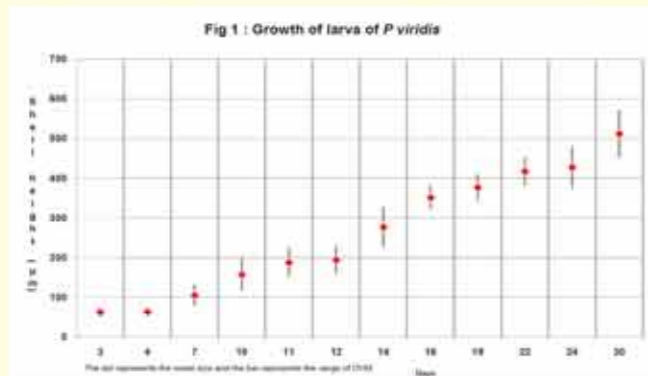
The pediveliger, at the end of the crawling stage settled on the substratum and became plantigrade and began its sessile life. Spat settlement could be observed from the 21<sup>st</sup> day onwards. The larva measured 400-490 µm in the anteroposterior axis and 380-480 µm in the dorsoventral axis. The velum almost disappeared, labial palps appeared, and additional gill filaments appeared. The concentric growth lines, foot, heart, posterior adductor muscle, mantle edge, visceral mass, intestine and chromatophore pigments were distinctly seen (Fig. 6).

## Spat

The plantigrade transformed into young spat by developing the characteristic adult shell. The shell had by now assumed the typical oblong shape like the adult mussel. The hinge line, the anterior and posterior auricles and the byssal notch typical of the adult

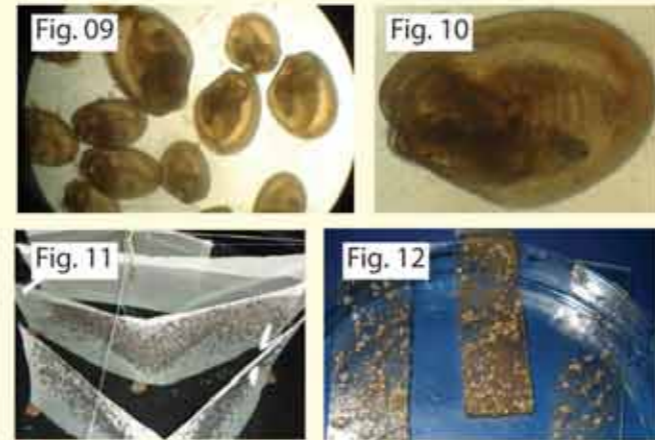


mussel were formed. The spat attached to the bottom substratum by secreting the byssus threads. Spatfall or settlement began on the 21<sup>st</sup> day and continued up to 35<sup>th</sup> day. The spat measured 510×390 µm on 21<sup>st</sup> day and 910×460 µm on 28<sup>th</sup> day (Fig.7, 8).



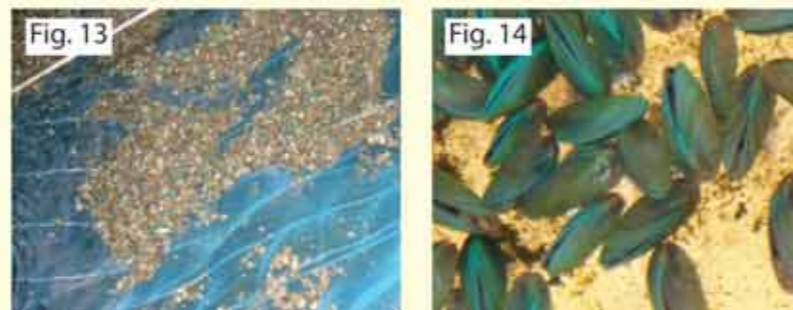
## Larval growth

The growth curve of larvae is shown in Fig. 8. The mean shell heights were plotted up to 30 days. A high degree of differential growth was observed in the different larval stages during the two attempts of larval rearing of *P. viridis*. The relationship between the shell length (APM) and shell height (DVM) of the larvae was linear. The linear relationship is given by the following equation:  $Y = -0.397 + 1.149 X$ ;  $r = 0.987$ ; where Y is the shell height (DVM) and X is the shell length (APM).



## Spat production and settlement

Spat settlement was facilitated by providing different substrata just prior to the setting stage (i.e. pediveliger stage). Black and white granite plates with a coarse surface, glass slides, nylon ropes (16 mm) and nets of different mesh sizes (10 mm, 5 mm, 3 mm, 1 mm, and 0.5 mm) were used as substrata. The settlement of spat occurred on all the substrata provided (Figs. 9, 10, 11, 12). However, the most significant finding was the highest settlement on the 0.5 mm velon screen. The larvae not only preferred the velon screen of 0.5 mm mesh, they also tended to distribute them-



selves on it for better growth. The velon screen was set on the tank bottom and this prevented the larvae from settling on the tank bottom. This gave very interesting results and a higher percentage of spat settlement. Further spat growth was also faster on the velon screen (Fig. 13, 14). Four different size groups of spat were obtained at the end of 65 days of rearing after spat settlement. The size of spat ranged from 3 mm to 14.9 mm.

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# Hatchery Technology for *Perna viridis* Spat Production

The green mussel *Perna viridis* (Linnaeus 1758) is cultured in the estuaries of Central and Northern Kerala, South west coast of India. The fishery and culture of this species are generally dependent on natural spat settlement in the granite and lateritic rocks along the North-West Coast of Kerala. Natural spat settlement is highly variable and dependent on rainfall and various other environmental factors. Mussel farming in North West and Central Kerala is entirely dependent on natural spat settlement in the beds of the North West Coast. The demand for seed has been increasing with expansion of farming in various estuaries. Hatchery production of mussel spat could, therefore, provide an alternate source of seed to the farming sector thus enabling the enhanced production of mussels through culture and reduce dependence on wild seed and reduce social conflicts.

Green mussel spawning and larval rearing trials were conducted in the marine hatchery of Visakhapatnam Regional Centre of Central Marine Fisheries Research Institute, Visakhapatnam (17.7° N–83.3° E), Andhra Pradesh, India in order to develop mass scale production of spat in the hatchery. The larval development and the production of spat of the green mussel have been standardized in the hatchery and the technology is described below.

Adult green mussels were collected, cleaned and maintained in a 300 l tank containing gently aerated, filtered seawater of 35 ppt. The brood stock ranged from 45 to 65 mm in total length. After spontaneous spawning of the mussels within 24 hrs, the adult mussels were removed from the spawning tanks. The larvae were filtered through 40 µm mesh and transferred to 1 m<sup>3</sup> larval rearing tanks.

The chrysophycean yellow-brown flagellate *Isochrysis galbana* was used as a standard food for the larvae. Filtered (1 µm) and UV treated seawater was enriched with Walne's medium and inoculated with *I. galbana* and *Chaetoceros calcitrans*. The pH of the cultures was maintained between 7 and 8. The microalgae were grown under 24 h light conditions at a temperature

of 21±1 °C and harvested daily in the exponential growth phase. Algal concentrations were counted daily using a Sedgwick counting cell.

### Larval rearing

Larvae were reared in 1 m<sup>3</sup> at a density of 5–10 larvae/ml. As larvae grew in size the stocking density was reduced and at the settlement stage the larval stocking density was 2–3 larvae/ml. They were fed with *I. galbana* at a cell concentration of 5000 cells larva<sup>-1</sup> day<sup>-1</sup> on the 2nd day onwards, when the 'D' shape was attained. Feeding was increased to 8000 cells larva<sup>-1</sup> day<sup>-1</sup> when the advanced umbo stage was attained and further increased to 10,000 cells larva<sup>-1</sup> day<sup>-1</sup> from the pediveliger stage onwards. 50% water change was done on alternate days. Mild aeration was started only after settlement of spat. Mixed algal food of *I. galbana* and *C. calcitrans* was given to the spat after settlement. The temperature ranged from 28 to 31 °C and the salinity 30–33 ppt. The larvae were measured along the two axes, the longer anteroposterior axis (APM), which is the shell length and the shorter dorsoventral axis (DVM), the shell height. Since the growth axis of the mussel is along the dorsoventral, the growth of the larvae was deduced from the DVM. The mean values of the larval stages and spat were derived from 30 to 40 measurements.

### Larval development

Eggs released by the females were brick red in color, spherical in shape and measured 45–50 µm in diameter (Fig. 1). Fertilization was 85–90%, however survival to 'D' veliger was 92–95%. Fertilization was complete within 20 min of spawning and the first and second polar bodies were observed 20 min after fertilization. The 8-celled stage was observed after 40 min; larvae with apical tuft of cilia and long flagellum were



attained 6–8 h after fertilization and the 'D'-hinge veliger by 20–22 h.

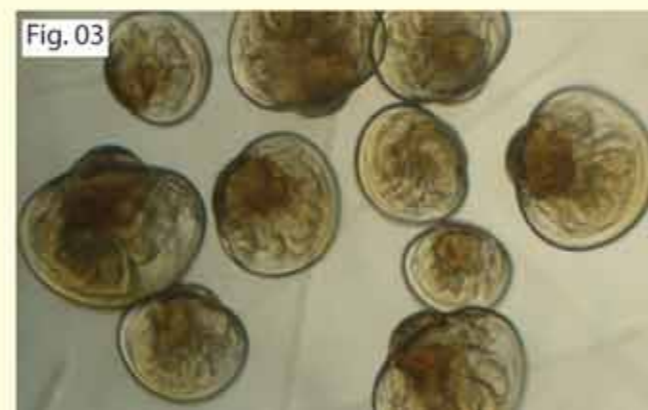
### 'D'-hinge veliger or the straight-hinge stage

The 'D'-hinge shells of the veliger (Prodissoconch I) were transparent with conspicuous granules. The velum was well developed, with a velar hood covered with small cilia which aided the fast clockwise circular movements of the larvae. The internal organs were heavily granulated. The straight hinge larvae measured 70–90 µm in the anteroposterior axis and 60–70 µm in the dorsoventral axis (Fig. 2). The larvae were very active, spinning and swimming around rapidly.



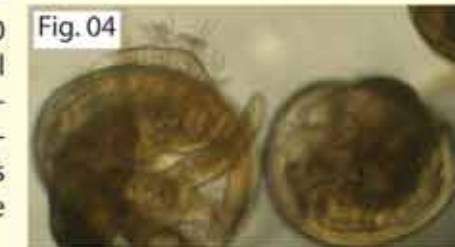
### Umbo stage

The straight hinge stage, transformed into the umbo stage on day 7. This stage was characterized by yellow digestive caecae, concentric ridges and lack of radial striae, typical of Prodissoconch II. The larvae, ranged from 90 to 260 µm in the anteroposterior axis and from 70 to 240 µm in the dorsoventral axis (Fig. 3). The larvae which were clam shaped with both valves equal became more globular and developed mantle folds.



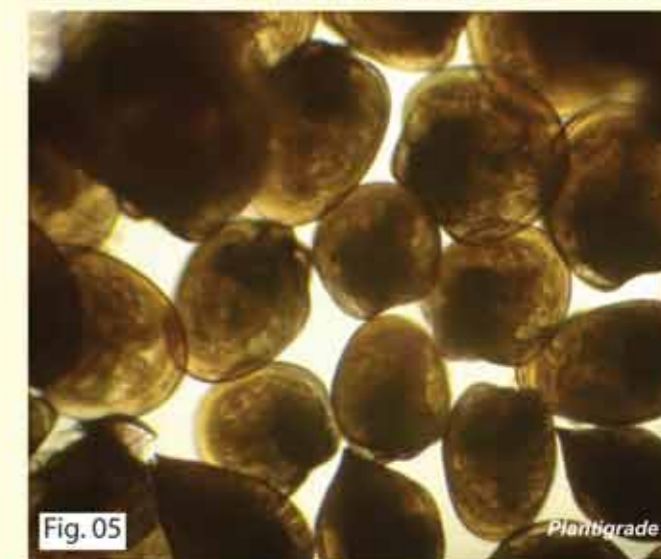
### Eye spot stage

This stage was characterized by the presence of a black rounded spot below the food mass. The eye spot and the rudimentary foot became distinctly visible by days 13–14 and the larvae measured 220–370 µm in the anteroposterior axis and 200–330 µm in the dorsoventral axis. The eye spot became deeply pigmented and ctenidial ridges also developed in the larvae (Fig. 4).



### Pediveliger stage

The development of the functional foot indicated the pediveliger stage by the 16–19<sup>th</sup> day. The larvae measured 280–400 µm in the anteroposterior axis and 360–380 µm in the dorsoventral axis. Larvae at this stage were capable of swimming with the velar cilia as well as crawling with the foot. The velar crown was reduced in size and the larvae now transformed from the free swimming pelagic larvae to the creep-



ing, crawling benthic stage ready to attach to the substratum. Gill filaments were clearly visible. The radial ridges were also distinct and green coloration was noticed along the margins of the shells (Fig. 5).