ISSN 0254-380 X

No. 201
July-September 2009

Marine Fisheries Information Service

Technical and Extension Series

भाकृ अनप
ICAR

Central Marine Fisheries Research Institute
(Indian Council of Agricultural Research)
Post Box No. 1603, Cochin - 682 018
www.cmfri.org.in

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Broodstock development, breeding and seed production of selected marine food fishes and ornamental fishes

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In recent years the contribution of marine finfish in the global aquaculture production has been steadily increasing. Marine food fishes like groupers, snappers, siganids, pompano, cobia and ornamental fishes have great potential for domestic and export trade. Availability of fingerlings has been one of the most critical factors for the commercial success in marine fish farming. Marine finfish farming is yet to be commercialized in India and the major constraint is the lack of viable seed production techniques for the candidate species. Even though technological advancements in broodstock development by manipulating temperature and photoperiod and induced spawning techniques have been developed for many marine finfishes internationally, a lot of research is needed in this area in India for developing viable technologies for selected species. The major constraints for successful larviculture are identified as (i) the small mouth gape of the larvae and hence their requirement for small prey at first feed and (ii) the occurrence of high mortality at various stages of larval rearing. Hence, rearing techniques, including the advantages of 'green water', appropriate live feeds, nutritional enrichment and larval rearing protocols have to be standardized for selected species having the potential for culture. In recent years, a lucrative global marine ornamental fish trade has been developed and hatchery production methods have to be evolved for the development of a long term sustainable trade in the country. The Central Marine Fisheries Research Institute has been focusing on brood stock development and standardisation of hatchery production methods for important species of marine food fish as well as ornamental fish, a brief account of which is presented in this paper.

Food fishes

The honeycomb grouper, *Epinephelus merra*

Broodstock development, breeding and seed production trial of *E. merra* was attempted at Mandapam Regional Centre of CMFRI. One broodstock tank of 5 t capacity was set up with undergravel filter. Six pre-adult fishes were stocked in the tank and fed *ad libitum* with fresh sardines. The fishes ranged in length from 20-36 cm and in weight from 100 g to 650 g.

The fishes above 30 cm formed pair and natural spawning was obtained. During August-September 2005, seven spawnings were obtained. The periodicity of spawning ranged from 3 days to 12 days, but the interval in majority of spawnings ranged between 3 to 4 days. The approximate number of eggs in the different spawnings ranged from 11,220 to 63020. The eggs hatched on the same day of spawning. The average length of newly hatched larvae was 1.5 mm.

Successful trial of seed production of *E. merra* was also carried out. Larval rearing was conducted in 5 t capacity FRP tank. Before the introduction of larvae, the tank was filled with filtered seawater and micro-algal culture was added to make the water green ('green water technique'). Calanoid copepods were introduced into the tank at an average concentration of 500 numbers per litre. The copepods were maintained in the tank in the multiplicative phase as was noted by the availability of egg bearing copepods, nauplii and copepodites.

About 2,000 newly hatched larvae were introduced. Eighty percentage mortality occurred during 3rd and 4th day. Thereafter the availability of sufficient copepod nauplii in the rearing tank was the key factor noted for the survival of the larvae upto two weeks. Whenever there was a decline in the availability of nauplii, mortality of larvae was noted. Additional copepods were collected from the wild and added to the rearing tank to maintain the density of nauplii. The addition of rotifer to the rearing tank resulted in the blooming of rotifers with a consequent depletion of copepods in the rearing tank. This was found to increase the mortality of the larvae. Hence the maintenance of copepods in sufficient densities in the rearing tank was found to be the critical factor for the survival of the larvae. After two weeks, freshly hatched *Artemia* nauplii were also added to the rearing tank. From the 25th day onwards, in addition to *Artemia* nauplii, adult *Artemia* was also supplied as feed. The larvae started metamorphosing from the 40th day onwards and all the larvae metamorphosed by the 60th day. A total of 33 numbers of young ones were produced in this experiment. The young ones ranged in total length from 20-64 mm and the majority was in the length range 30-49 mm (Fig. 1).

Fig. 1. Juveniles of hatchery produced honeycomb grouper

Epinephelus malabaricus

At Vizhinjam, pre-adults of *E. malabaricus* weighing >1 kg and upto 2 kg were collected from April 2006 onwards and reared for developing into broodstock. They were fed enriched diet and the required hormones for sex reversal also were administered to them. Male hormone was administered by incorporating through feed, twice a week from the first week of August. The dosage of hormones was @ of 3 mg per kg body weight of the fish. Eleven numbers of broodstock of *E. malabaricus* weighing from 2.85 to 5.45 kg were developed at the mariculture laboratory at Vizhinjam (Fig. 2). Biopsy examination of the brooder was carried out in January as well as during March 2007. Two of the females were found to have the ova in the tertiary stage of vitellogenisis, measuring in size from 360 to 400 µ. Though there was no free flow of milt, sex inversion had taken place by the hormone application in the male brooders.

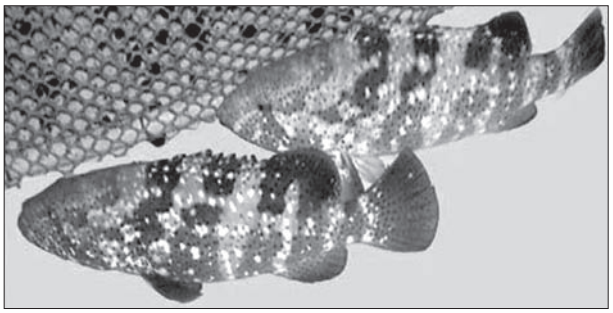

Fig. 2. Broodstock of *Epinephelus malabaricus*

Epinephelus polyphekadion

Broodstock development of *E. polyphekadion* was initiated at Mandapam. The fishes in the broodstock tank ranged in total length from 46 to 53.5 cm. Even though one spawning was obtained in February, the eggs were unfertilized. It indicated that sex reversal for male formation had not taken place.

Siganus canaliculatus

At Cochin hatchery, four pairs of broodstock of the rabbitfish *Siganus canaliculatus* were maintained in 5 t FRP tanks having *in situ* biological filters (Fig. 3 and 4). Feeding of fishes was done with chopped fish meat, prawn meat, mussels, clam meat and fish eggs. Occasionally intertidal green seaweeds like *Ulva* were also given as feed. Water exchange in this tank was done 2 -3 days before and after full/new moon days.

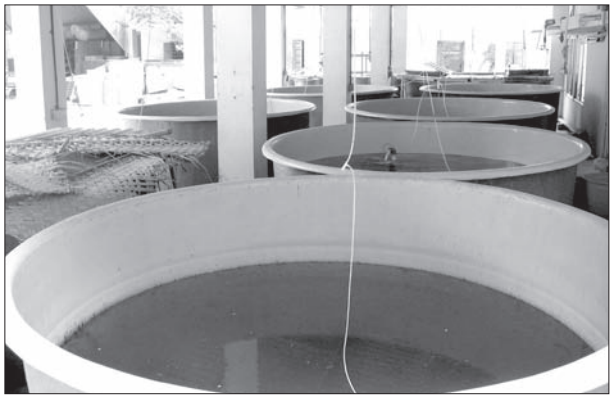

Fig. 3. Broodstock facility for marine foodfishes at Marine Research Hatchery, Kochi

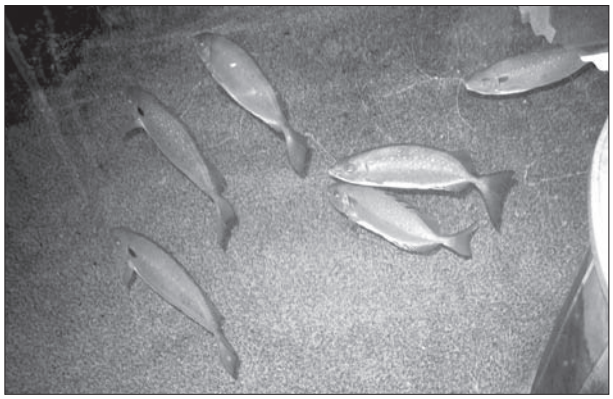

Fig. 4. Broodstock of *S. canaliculatus*

Spawning of the fishes was observed during November. Two spawnings were obtained during this month (01/11/2006 and 18/11/2006). The interval between two spawnings was 18 days. Since the fertilized eggs were demersal and adhesive in nature, collection of eggs from the tanks was not possible. So the hatched out larvae (Fig. 5) were collected next day morning and transferred to larval rearing tanks.

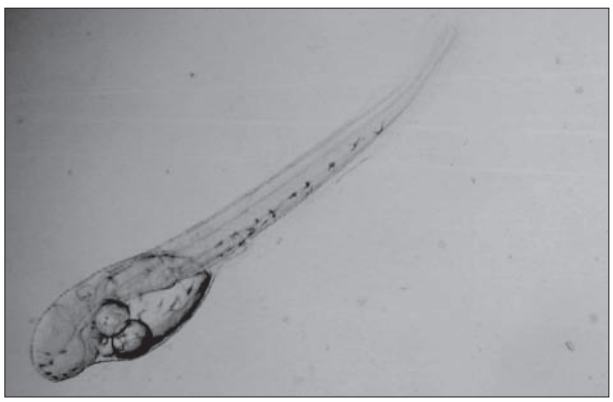

Fig. 5. Newly hatched larva of *S. canaliculatus*

The larvae collected from the tanks were stocked in 1 t FRP tank kept under roofing for further rearing. The larvae were stocked at a density of 5/l. The size of the larvae on day 2 was 2.87 mm. The mouth size of the larvae at this time was 100-125 µ (Fig. 6). Rotifers at a density of 5-10/ml were maintained as first feed. Everyday morning the bottom of the tank was siphoned out and 5-10% water was replaced with fresh seawater. The rotifer density was adjusted by adding fresh rotifers every day. To provide green water to the larvae and also as feed to rotifers, *Nanochloropsis* was added to the tanks everyday morning and evening.

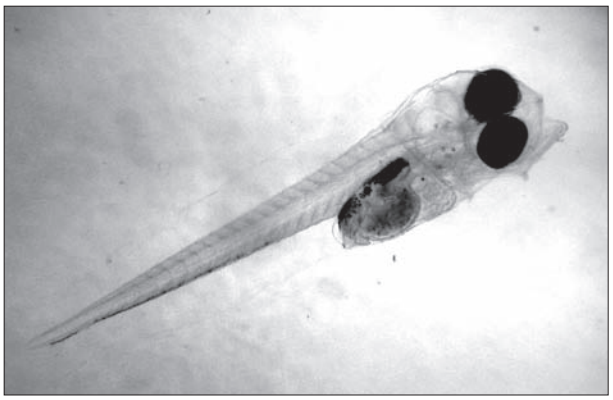

Fig. 6. Three day old larva of *S. canaliculatus*

On 4th day a slight reduction in the number of larvae was observed. On 6th day the larvae had grown to a size of 3.25 mm and mouth size increased to 150-175 µ. The gut content analysis of larvae revealed the presence of rotifers along with algae in the gut of the larvae. There was a gradual reduction in the number of larvae during succeeding days. Large reduction in the number of larvae was observed

Fig. 7. Hatchery produced juveniles of *S. canaliculatus*

between 12th – 15th day. At this time larvae reached size of 5-6 mm and were actively feeding on rotifers. On day 16, freshly hatched *Artemia* were added to the tanks as feed to the larvae. Between day 20 and 25 the larvae metamorphosed to juvenile fishes (Fig. 7). A total of 35 juveniles were produced from this tank.

Siganus javus

A 5 t capacity broodstock tank was set up for *S. javus*. A total of eight numbers of pre-adult fish were stocked in the tank. The length ranged from 20.5 to 31 cm. Two fishes died during the period. The length range (by the end of March 2006) was 29 to 35 cm. The broodstock development of *S. javus* was unsuccessful due to the incidence of severe mouth infection and consequent mortality of the fish in January 07. The biological details of the broodstock reared were as follows:

Length (cm)	Weight (g)	Sex	Maturity stage
42	850	Male	V
37	650	Male	II
36	750	Male	III
36	648	Male	III
390	760	Female	IV

Trachinotus blochii

A 5 t capacity broodstock tank was set up for *T. blochii.* A total of 11 pre-adults were stocked. At the time of stocking (May 05) the fishes ranged in length from 11 to 15 cm. The fishes were fed *ad libitum* with trash fish and squid meat. Three fishes died. Very good growth was noted during the period. However, In March 2006 the length of the broodstock ranged from 29 to 43 cm. Canulation was done during March 2006 and revealed that the fishes were maturing. The maturity condition of dead fishes from the broodstock development tank showed that fishes above 35 cm had developed gonads (stages II to III).

Marine ornamental fishes

Damsel fishes

The methods of seed production for the three species *viz., Dascyllus trimaculatus, Dascyllus aruanus* and *Pomacentrus caeruleus* were standardized during 2006 -2007 by repeated seed production trials and the protocols were standardized.

***Dascyllus trimaculatus* (Three spot damsel)**

Four successful experiments on hatchery production of three spot damsel *Dascyllus trimaculatus* was conducted during this period and the methods were standardized.

The three spot damselfish *D. trimaculatus* constitutes one among the topmost ten marine ornamental fishes in the international trade. The broodstock of the species was developed in captivity and successful breeding and larval rearing was achieved. *D. trimaculatus* is dioecious and the pair was developed in one tonne glass aquarium tanks. The mature fish ranged in total length from 9-10 cm. Breeding was observed during early morning hours. Approximately 12,000 to 15,000 eggs were present in a single spawning. The eggs were attached either to the sides of the tanks or on the substrata provided inside the broodstock tanks. The average periodicity of spawning was 2 weeks. Parental care by the male was noted. Hatching occurred on the evening of the fourth day of incubation. Larvae were altricial type with no mouth opening at the time of hatching. The average length of newly hatched larvae was 2.5 mm. The larvae were transferred to 5 t capacity circular FRP tanks in which harpacticoid copepod cultures were maintained in green water. Mouth opening appeared on the second day and the gape measured around 150 µm. The larvae started feeding on copepod nauplii from the third day of hatching. After two weeks when the average size of the larvae had reached 4 mm with average mouth gape of 450 µm, freshly hatched *Artemia* nauplii were fed *ad libitum*. The larvae started metamorphosing from 35th day of hatching and all the larvae metamorphosed by 40th day. The just metamorphosed young one measured from 12 to 13 mm in length. The average survival rate in the four rearing experiments ranged between 10-15%.

***Dascyllus aruanus* (Humbug damsel)**

Hatchery production of *Dascyllus aruanus* was also standardized. Five successful experiments were conducted during the period.

The striped damselfish *D. aruanus* constitutes one among the most sought after ornamental fishes in the international trade. The broodstock of the species was developed in captivity and successful breeding and larval rearing was achieved. *D. aruanus*

is dioecious and the pair was developed in 250 l FRP tank. Boiled and finely chopped clam meat was provided during morning and adult *Artemia* were fed during evening. Excess feed and faecal matter was removed daily and 25% water exchange was also done. The mature fish ranged in total length from 7 to 8 cm. Breeding was observed during early morning hours. Approximately 8,000-10,000 eggs were present in a single spawning. The eggs were attached either to sides of the tanks or on the substrata provided inside the broodstock tanks. The average periodicity of spawning was 2 weeks. Parental care by the male was noted. Hatching occurred on the evening of the fourth day of incubation. Larvae were altricial type with no mouth opening at the time of hatching. The average length of newly hatched larvae was 2.4 mm. The larvae were transferred to 2 t capacity rectangular FRP tanks in which calanoid/harpacticoid copepod cultures were maintained in green water. Mouth opening appeared on the second day and the gape measured around 160 µm. The larvae started feeding on copepod nauplii from the third day of hatching. After two weeks when the average size of the larvae had reached 4 mm with average mouth gape of 450 µm, freshly hatched *Artemia* nauplii were fed *ad libitum*. The larvae started metamorphosing from 25th day of hatching and all the larvae metamorphosed by the 31st day (Fig. 8).

Fig. 8. Hatchery produced *D. aruanus*

***Pomacentrus caeruleus* (Caerulean damsel)**

The methodology for the hatchery production of blue damsel was standardised. A total of five batches of about 100 numbers each was hatchery produced.

P. caeruleus is protogynous and polygamous. The broodstock was developed in 2 t capacity FRP tanks. The mature fish ranged in total length from 70-90 mm. Spawning was noted during early morning hours. Approximately 5,000-6,000 eggs were present in a single spawning. The eggs were attached on the substrata provided inside the broodstock tanks. The average periodicity of spawning ranged between 3 and 12 days. Parental care by the male was noted. The eggs were oval with an average length of 850 µ. The newly hatched larvae measured about 1.2 mm with an average mouth gape of 200 µ. The larvae were transferred to 5 t capacity FRP tanks in which green water was developed and a culture of calanoid/ harpacticoid copepods was maintained. After twelve days, freshly hatched *Artemia* nauplii were also supplemented. The larvae started metamorphosing from the 17th day and by 21st day all of them metamorphosed. The average length of just metamorphosed juvenile was 21 mm (Fig. 9).

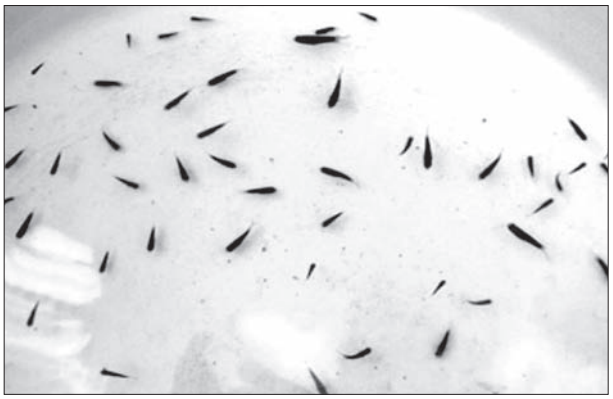

Fig. 9. Hatchery produced *P. caeruleus*

***Chromis viridis* (Blue green damsel)**

Experimental success was obtained in the broodstock development and seed production methods for the blue green damsel *Chromis viridis*.

The broodstock development of *C. viridis was* carried out in 2 t FRP tanks. Boiled and finely chopped clam meat, squid meat, earth worm and adult *Artemia* were fed to the fish. The excess feed and faecal matter was removed and about 25% water exchange was done daily.

Spawning was obtained from June 2006 onwards. The average frequency of spawning was five per month with an interval of about five days.

The egg was oval shaped with an average length of 502 µ. Hatching occurred in the evening of the fourth day of incubation. Larvae were altricial type with no mouth opening at the time of hatching. The average length of newly hatched larva was 2.25 mm. The larvae were transferred to 5 t capacity round FRP tanks in which cultures of calanoid copepod *Pseudodiaptomus serricaudatus* and the harpacticoid copepod, *Euterpina acutifrons* were maintained in green water produced by adding *Nannochloropsis* culture. Mouth opening was formed on the second day of hatching and the gape measured around 190 µ. The larvae started feeding on copepod nauplii from the third day of hatching. The average densities of egg bearing copepods, nauplii and copepodites maintained per ml in the larval rearing tank ranged between 1-13, 7-78 and 1-31 respectively for the first 20 days of larval rearing. From the 32nd day of larval rearing, freshly hatched *Artemia* nauplii was also supplemented. Metamorphosis started from 30th day and was completed by 49th day (Fig. 10). Two experiments on larval rearing were conducted and the average survival rate was about 5%.

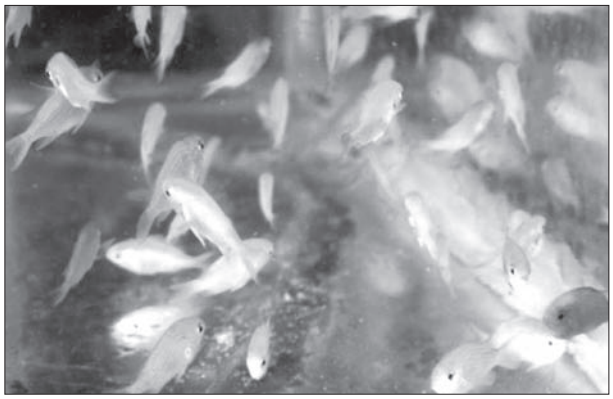

Fig. 10. Hatchery produced *C. viridis*

***Neopomacentrus nemurus* (Yellowtail damsel)**

The broodstock of the yellowtail damsel was developed in 2 t capacity FRP tanks. Spawning was obtained from April 2006 onwards. The average interval of spawning ranged from 4-5 days. The length of freshly laid egg was 870 µ. The eggs hatched on the evening of the fourth day of incubation. The freshly hatched larva measured 1.8 mm with a mouth gape of about 100 µ. The larvae were transferred to 5 t capacity FRP tanks in which mixed culture of copepods were maintained in green water produced by adding cultures of *Nannochloropsis*. The larvae started feeding on nauplii of copepods from the third day of hatching. From the 12th day onwards the larvae were also fed *ad libitum* with freshly hatched artemia nauplii. From the 16th to 21st day of hatching the larvae metamorphosed into juveniles. The length of the just metamorphosed juvenile ranged from 10 -13 mm (Fig. 11).

Fig. 11. Hatchery produced *N. nemurus*

Dascyllus carneus

Dascyllus carneus were collected from wild and brought to laboratory for further rearing and developing broodstock. These fishes were kept in 1t FRP tanks with biological filters and management protocols were same as in the case of other fishes. After a period of 2 months in the tanks, the fishes started laying eggs on to the substratum provided in the tanks. The eggs were 625 – 650 µ in length and 450 µ in width (Fig. 12). The number of eggs at single spawning were more than 5000.

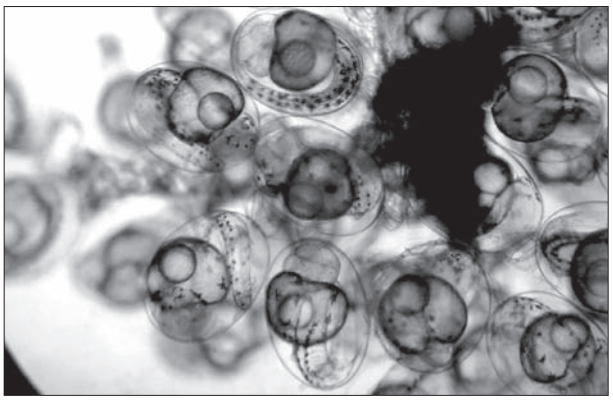

Fig. 12. Eggs of *D. carneus*

Standardistion of breeding and seed production of *Amphiprion percula* (orange clown)

Spawning of the orange clown *Amphiprion percula* was obtained and methods of hatchery production were standardised. Spawning occurred during day time (0600 -1530 hrs) and the spawning interval ranged from 14 to 18 days. The clutch size per spawning ranged from 112 to 557 eggs. Hatching was on the 8th day of incubation in the evening and the length of the newly hatched larvae ranged from 1.91 to 2.02 mm. Larviculture protocols were developed and during 19th -20th day of hatching, the larvae metamorphosed into juveniles (Fig. 13).

Fig. 13. Hatchery produced *A. percula*

***Broodstock development of Amphiprion ocellaris* (false clown) and *Premnas biaculeatus* (maroon clown)**

Pre-adults of the false clown *Amphiprion ocellaris* having total length 30 to 60 mm and spine cheek anemone fish (maroon clown) *Premnas biaculeatus* (40 to 60 mm) were collected and examination of their gonad showed that all the specimens were males. For broodstock development, six numbers of pre-adults of each species were reared in 500 l FRP tank fitted with biological filter and provided with suitable host sea anemone. Fishes were daily fed four times with meat of clam, prawn and fish egg mass at the rate of 15% of their body weight. After a period of 3 to 4 months rearing in each tank, one pair grew ahead of others and became the functional male and female. The standard length of the female varied between 89 and 100 mm and that of male varied from 40 to 60 mm in *A. ocellaris* and that of *P. biaculeatus* attained a standard length of 120 to 140 mm and 55 to 60 mm for female and male respectively. The broodstocks thus developed were then transferred to separate glass aquaria for breeding.

Breeding and seed production of *Amphiprion ocellaris*

The first pair spawned in November 2005. Thereafter spawnings were obtained at an interval of 12 to 15 days giving an average of two spawnings per month per pair. Spawning was noticed during day time between 0500 and 1530 hrs and lasted for one to one and half hour. Depending upon their size, the females spawned 300 to 1000 eggs per spawning and they deposited capsule shaped eggs in nearly round patches on the surface of earthen pots and each egg adhered to the substratum through a stalk. The newly spawned eggs were white in colour on the first day which later changed to light grey on 2nd day and as the embryo developed, these turned to black on 3rd to 6th day which later turned to silvery on 7th day of incubation (Fig. 14).

Fig. 14. Silvery eggs of *A. ocellaris* on 7th day of incubation

During the incubation period, both the parents carefully looked after the eggs (Fig. 15). The hatchling emerged on 7th day of incubation and the peak hatching took place soon after sunset at water temperature range of 27 to 29 °C. The newly hatched larvae measured 3.2 to 4.0 mm in length and each had a transparent body, large eyes, visible mouth and a small yolk sac. The larvae were initially maintained in greenwater with small rotifer *Brachionus rotundiformis* and later on with newly hatched *Artemia* nauplii. At 9-10th day of post-hatch, the larvae showed first sign of pigmentation and by 15-17th day 90 to 95% metamorphosed into juveniles and shifted from pelagic to epibenthic stage (Fig. 16 and 17).

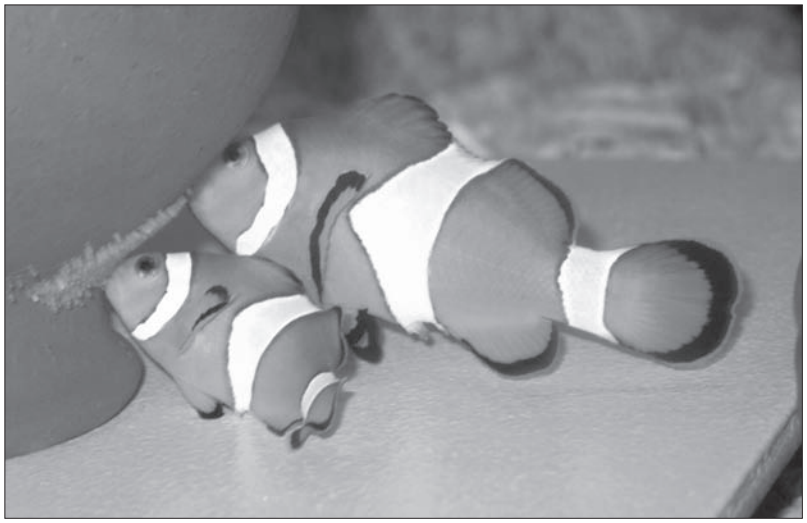

Fig. 15. A pair of *A. ocellaris* with eggs deposited on earthen pot

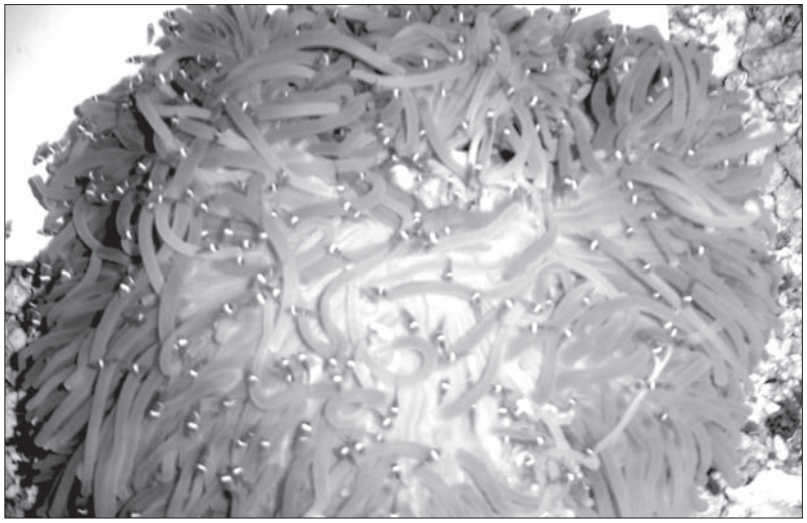

Fig. 16. Fifteen days old juveniles of *A. ocellaris* settling in sea anemone *Heteractis magnifica*

Breeding and seed production of *Premnas biaculeatus*

Under captive conditions, the pairs successfully bred for the first time in India on 27.07.06 and laid 115 eggs. The newly spawned eggs (Fig. 18) were bright red/brownish red/maroon in colour for the initial two days and as the embryo developed, these turned to black on 3rd to 4th day and later turned to silvery on 5th to 6th day of incubation (Fig. 19) and the glowing eyes of the developing larvae inside the egg capsule was clearly visible when viewed from a short distance. Subsequently spawning was achieved every 15 to 20 days interval giving an average of two spawnings per month per pair and laid 115 to 1000 eggs/ spawning. The fertilized eggs were elliptical shaped with size ranging from 2.8 to 3.5 mm long and 1.1 to 1.7 mm wide (Fig. 20). Early embryonic development was completed within 6 days of incubation (Fig. 21) at water temperature of 27 to 29 °C. Peak hatching took place immediately after sunset under complete darkness and the newly hatched larvae measured 2.5 to 3.6 mm in total length. Green water technique was employed for larval rearing and feeding protocols with enriched rotifers and newly hatched *Artemia* nauplii were developed. On 15th to 17th day of post-hatch, the size of the juveniles ranged from 12 to 16 mm.

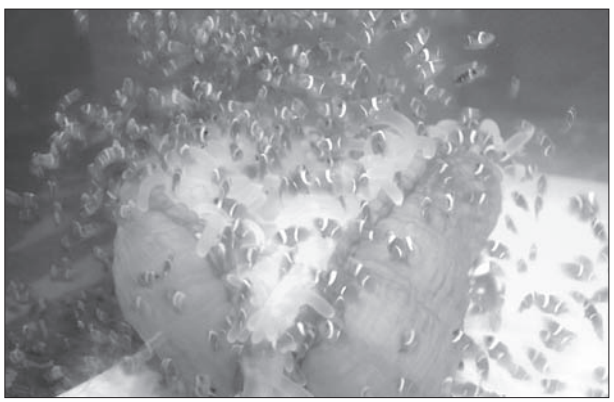

Fig. 17. One clutch of laboratory produced juveniles of *A. ocellaris*

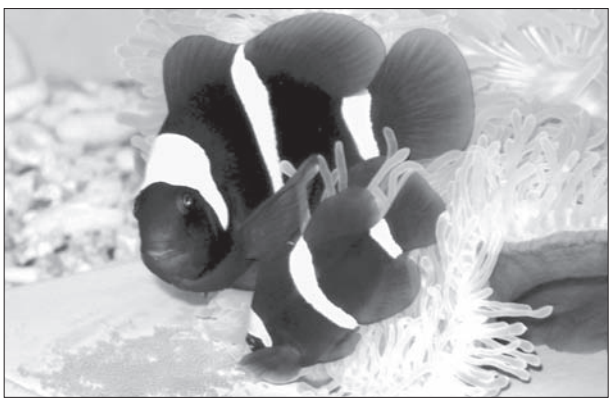

Fig. 18. A pair of *P. biaculeatus* with newly spawned eggs deposited on tiles

Fig. 19. Silvery eggs of *P. biaculeatus* on final day of incubation

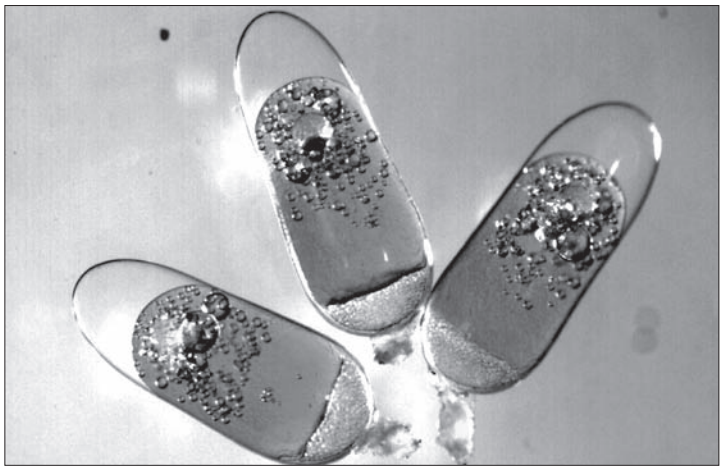

Fig. 20. Photomicrograph of capsule shaped eggs of *P. biaculeatus* after 24 h of fertilization

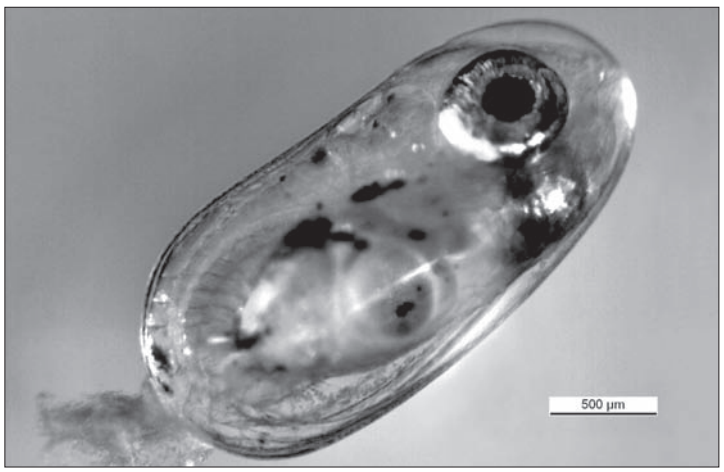

Fig. 21. Embryo of *P. biaculeatus* occupying the entire space in egg capsule on final day of incubation

Conclusion

In the context of declining returns for shrimp farming in recent years, it appears that diversification of farming practices is the only alternative to sustain mariculture production. Marine finfish farming is one of the viable options and international attention is being focused on research and development in this emerging sector. The lack of commercial scale availability of hatchery produced seed is the major bottleneck for any large scale venture of marine finfish farming in India. The availability of seeds from wild is often unpredictable and hence farming based on wild collection of seeds may not be a sustainable venture. Hence the development and standardization of seed production techniques for a few species belonging to groupers, siganids, pompano, snappers, breams and cobia should receive research priority. It is felt that the development of commercial hatcheries for ready supply of seeds is the primary step for the development and expansion of marine finfish farming in India.

The global marine ornamental species trade has grown into a multi-stakeholder industry operated almost throughout the tropics and dependent almost entirely on wild collection from coral reef habitats. However, it is well accepted that the environmentally sound way to increase the supply of marine ornamentals in order to reduce the pressure on wild population is the development of hatchery production techniques for the species which are on demand. During the past few years, the Central Marine Fisheries Research Institute has intensified research activities on breeding and culture of marine ornamental fishes which has resulted in the development of hatchery production technologies of ten species of Pomacentridae that are in good demand in the international trade. Research and development in the breeding and culture of marine ornamentals is a priority area which has to be intensified in the coming years which can result in the development of a hatchery produced marine ornamental fish trade in India.