Success in Captive Breeding of Maroon Clown *Premnas biaculeatus* (Bloch, 1790)

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The clown fishes that belong to the family Pomacentridae and subfamily Amphiprionae, are classified into two genera. These are Amphiprion and Premnas. The Maroon clown, Premnas biaculeatus is the sole member in the genus Premnas. It is commonly known as "Spine-Cheek Anemone fish", because of the presence of a pair of long spines on its preoperculum. They have very striking bright red to maroon colouration on the entire body and fins, demarcated abruptly with relatively narrow white to golden yellow bands. This colouration pattern makes them as one of the most attractive species among the marine ornamental clown fishes. P.biaculeatus is known to have symbiotic relationship with bulb-tipped or purple base sea anemone Entacmaea quadricolor and both have wide distribution from Indo-West Pacific to Indo-Australian Archipelago including India, Myanmar, Thailand, Malaysia, Indonesia, Philippines, New Guinea, New Britain, Solomon Islands, Vanuatu, and Australia (northern Queensland) (Fautin and Allen 1997). Being a protandric hermaphrodite, its males are much smaller than females and are brighter red with brilliant white or golden yellow stripes. Under integrated hatchery operations, pair formation, broodstock development breeding of P.biaculeatus has been successfully achieved and juveniles of the fish were produced in the Marine Hatchery of Central Marine Fisheries Research Institute, Kochi.

This is the first report on the captive breeding of P.biaculeatus in India.

Fig 1. A pair of *P. biaculeatus* with newly spawned eggs deposited on earthen pot

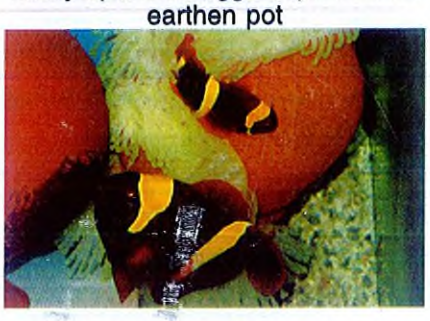

Fig 2. Three day old egg cluster showing black colouration

Fig 3. Silvery egg cluster on final day of incubation

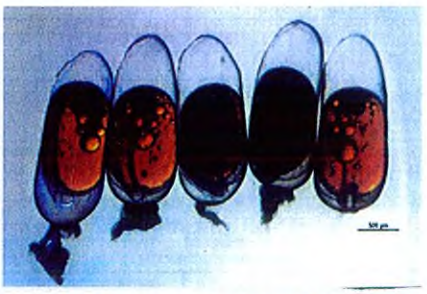

Fig 4. Microscopic view of capsule shaped eggs of *P. biaculeatus* after 24 hrs of fertilisation

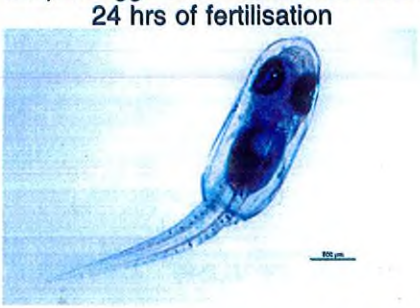

Fig 5. Larvae emerging from the egg capsule

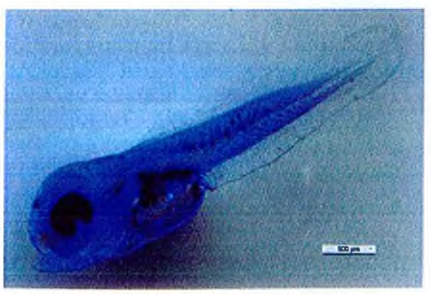

Fig 6. Newly hatched larva of *P. biaculeatus*

Fig 7. Hatchery produced juveniles showing adult colouration and banding pattern

Pair Formation and Broodstock Development

Pair formation of *P. biaculeatus* was carried out by establishing social groups of different age groups in 500 litre FRP tanks fitted with biological filter and by providing sea anemone *(Heteractis magnifica)* as the host to reduce the aggression. Pairs formed were then transferred to separate 500 litre capacity glass aquaria for broodstock development with provision of *H. magnifica* as host sea anemone. The broodstock was fed wet feeds such as mussel meat, shrimp and clam meat at the rate of 10% of their body weight in split doses, 4 times per day. They were also given live feeds i.e., artemia and rotifers, after bioencapsulation with vitamins, minerals and fatty acids.

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The standard length of the females varied between 120 to 150 mm (presumptive females) and that of male varied between 55 to 70 mm (presumptive males). The presumptive females and males were administered with estrogen and testosterone respectively intramuscularly at intervals of every 30 days to accelerate the development of gonads (ovary and testis) and sexual maturation. Each broodstock tank was provided with tiles and earthen pots for the egg deposition.

Breeding

The pairs bred successfully under captive conditions, within a period of 4 to 6 months of rearing. In one breeding event, they laid 115 numbers of capsule shaped eggs in the first spawning at 1500hrs during day time and the spawning lasted for one to one and a half hour. The newly spawned eggs were bright red/ brownish red/ maroon in colour for the initial two days (Fig 1) and as the embryo developed, these turned to black on 3rd to 4th day (Fig 2) and later turned to silvery colour. On 5th to 6th day of incubation, the larvae's large eyes and the glowing eyes of the developing larvae inside the egg capsule were clearly visible when viewed from a short distance (Fig 3). The egg size ranged between 2.78 to 3.0 mm in length with a width of 1.1 to 1.3 mm (Fig 4) and attached to the surface of the earthen pots with a stalk. In the subsequent spawning, the number of eggs produced varied between 150 to 1,000 nos/spawning pair and spawning was achieved once every 15 to 20 days interval, giving an average of 2 spawnings per month per pair. Hoff (1996) also reported 20.7 annual number of spawnings (1.7 spawnings on an average/ month) and the number of eggs per spawning by *P. biaculeatus* varied between 146 to 986 nos, giving an annual number of 1,752 to 11,832 eggs/pair/ year.

Parental Care

During incubation period, both the parents carefully looked after the eggs during day time. This care involved two basic activities *viz.*, fanning by fluttering of the pectoral fins and mouthing to remove the dead or weakened eggs and dust particles

Hatching and Larval Rearing

On the 6th day of incubation, the larvae emerged tail first from the egg capsule (Fig 5) and peak hatching took place after sunset between 1830 and 1930 hrs. at a water temperature range of 27 to 29° C, under complete darkness. The newly hatched larvae, free swimming, measured 1.5 to 3.1mm in length. They had a transparent body, large eyes, visible mouth, and a small yolk sac. (Fig 6). The mouth gape of the newly hatched larvae ranged from 350 to 410 μ. The larvae fed on a mixed culture of micro algae *Chlorella marina* and *Nannochloropsis oculata* (1.5×10^6 cells/ml) and super small rotifer *Brachionus plicatilis* (6 to 8 nos./ml) up to 8th day. From 9th day onwards the larvae were weaned on to newly hatched *Artemia* nauplii (4 to 6nos/ml) along with rotifers (6 to 8 nos./ml) and mixed culture of micro algae 1.5×10^6 cells/ml. From 12th day to 17th day of post hatch, the larvae were fed with newly hatched artemia nauplii (4 to 6nos/ml). At 15 to 17th day of post hatch, the size of the juveniles ranged between 12.0 to 16mm. All these attained bright reddish colour and most of the fry resembled juvenile / adult fish and began to shift from partially pelagic to epibenthic and started eating minced shrimp, fish flesh, mussel meat, clam meat and formulated diets. Daily 25% of water was replaced with filtered sea water. Most of the hatchery produced juveniles attained adult colouration and banding pattern at 45 to 50 days of post hatch (Fig 7). Under hatchery conditions, with management of water quality and feeding, 75 to 85% of the larval and juvenile survivability was obtained.

Water Quality Management

Maintaining good water quality and ensuring mild water circulation are also found to be very essential for better survivability of larvae (Job *et al* 1997, 1998). For this, aeration was provided at all four corners of the tank through PVC columns covered with 200 micron bolting silk cloth to avoid possible rubbing effects on delicate larvae. A water temperature range of 26 to 29° C, salinity 33 to 36 ppt, dissolved oxygen 4.6 to 6.2 ml/l and pH 8.1 to 8.6 were maintained in all the rearing tanks. One of the critical problems encountered during the larval rearing was the "head-butting syndrome". In order to reduce this, measures were taken to avoid reflection of light inside the tank. Low intensity light (40 w) was provided to enable the larvae to locate feed. This had also helped to keep the larvae swimming towards the surface at night rather than sinking to the bottom. It was found that larval period from 3rd and 9th day of post hatch stage was critical. Mortality occurred during this period due to change in feeding behaviour. At this stage provision of feed that is nutritionally adequate and of suitably sized feed in optimal quantity is of vital importance to overcome the critical stage. For this, the rotifers and *artemia* were bio-encapsulated with vitamin A,D,E,K and fatty acids (DHA and EPA) before being fed to the larvae. Through different experimental trials, various hurdles associated with larval rearing have now been overcome and a production protocol has been developed for the hatchery production of *P. biaculeatus*.

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