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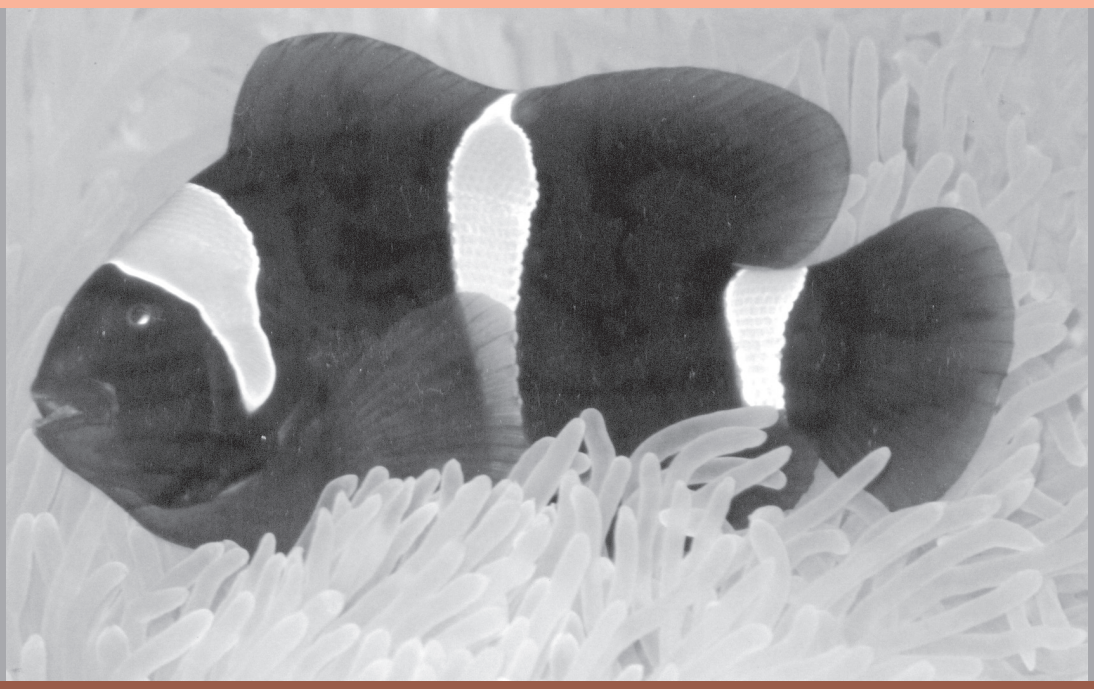

TECHNICAL AND EXTENSION SERIES

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE

COCHIN, INDIA

(INDIAN COUNCIL OF AGRICULTURAL RESEARCH)

1197 Breeding, larval rearing and seed production of maroon clown *Premnas biaculeatus* under captive conditions

The members of the family Pomacentridae commonly known as damselfishes and anemonefishes are a diverse group of marine fishes found in tropical oceans, and have very high demand in marine ornamental fish trade. The family includes 29 genera and 350 recognized species living mainly in coral reef environments. The subfamily Amphiprioninae, have two genera *Amphiprion* and *Premnas*. The maroon clown *Premnas biaculeatus* is the sole member in the genus *Premnas*.

Premnas biaculeatus

P. biaculeatus is commonly known as 'Spine-Cheek Anemone fish' because of the presence of a pair of long spines on its pre-operculum. Their most striking bright red to maroon colouration on entire body and fin, demarcated abruptly with white to golden yellow narrow bands make them as one of the most attractive species among the marine ornamental clown fishes. *P. biaculeatus* has wide distribution from Indo-Malayan Archipelago to northern Queensland. In India, this species is abundant in the coral reef ecosystem of Andaman and Nicobar Islands situated in Bay of Bengal, and the adults have 3 bright golden stripes, whereas all the juveniles possess white stripes. Generally, it is a peaceful fish for the reef aquarium and spends much time among the tentacles of bulb tipped sea anemone *Entacmaea quadricolor* and occssionally found in *Heteractis magnifica, H. crispa* and *Stichodactyla haddoni*. In the international aquarium trade, fishes having size range 3/4" to 3" fetch US $29.99 to $59.99 and an adult pair costs US $129.99. Most of the salt water ornamental fishes are collected from the wild and hence there is a serious concern in respect of their conservation. Recent studies also showed that wild collected specimens have a dismal survival history in captivity, whereas captive-bred clownfish are generally hardier, more disease free, easily adjust to life in aquaria and retain normal colouration. Due to the very high demand of the species in the aquarium fish trade, development of an appropriate technology for its captive production is felt as an alternative means of providing fish for the trade rather than wild collection which may cause depletion of the stocks. In this juncture a viable technique has been developed in the marine hatchery of CMFRI, Kochi for the captive breeding and juvenile production of *P. biaculeatus* for the first time in India.

Pair formation and broodstock development

Being a protandric hermaphrodite, fishes having size 55 to 60mm (presumptive male) and 120 to 140 mm (presumptive females) were stocked in 500-litre FRP tanks for pair formation along with host sea anemone (*H. magnifica*). All the experimental tanks were provided with biological filter and kept in the hatchery where an incident light intensity of 2500 to 3000 lux was available. The fishes were fed with wet feeds such as meat of shrimp and green mussel at the rate of 15% of their body weight twice per day. After a period of 3 to 4 months rearing, in each tank, one pair grew ahead of others and became the monogamous pair. The pairs thus formed were then transferred to 500 litre glass aquaria for broodstock development and provided with *H. magnifica*. The broodstocks were fed with 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 and also provided live feeds : adult artemia and rotifers after bioencapsulation with vitamins, minerals and fatty acids. The female and male fishes were also administered with estrogen and testosterone respectively intramuscularly at every 30 days interval to accelerate the development of gonad (ovary and testis) and sexual maturation. The temperature in all the breeding tanks were maintained between 27 to 29°C and water was recirculated to ensure water movement and water quality was maintained with the aid of filter system. Each broodstock tank was provided with tiles and earthen pots as substrate for the egg deposition.

Breeding and morphological changes of egg

Few days prior to spawning, spawning behaviour was exhibited and a nest site adjacent to sea anemone was selected by the pairs. The developed broodstock fish were successfully spawned at 1500hrs on 27. 07.06 and the spawning lasted for one to one and a half hour. A total of 115 numbers fertilized eggs were obtained and the newly spawned capsule shaped eggs were adhered to the tiles or earthen pots with stalk. The eggs are bright red in colour for the initial two days (Fig. 1) and as the embryo developed, these turned to black on third and fourth day and thereafter turned to silvery colour on fifth to sixth day of incubation. At this stage the glowing eyes of the developing larvae inside the egg capsule was clearly visible when viewed from a short distance. Incubation period lasted for 6 to 7 days at a water temperature range of 28 to 30°C. During incubation, the egg size ranged between 2.8 to 3.5 mm in length with a width

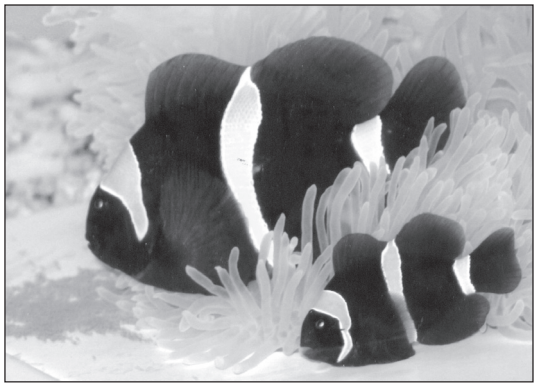

Fig. 1 A pair of *P. biaculeatus* with newly spawned eggs deposited on tiles

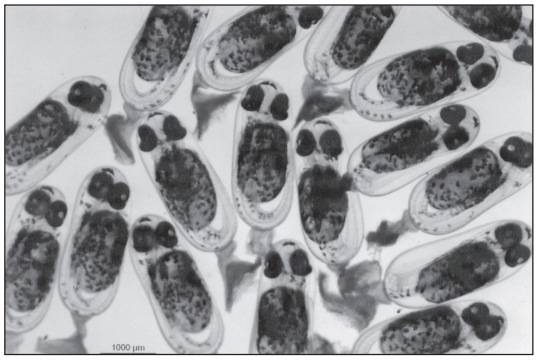

Fig. 2 Development of embryo on third day of incubation

of 1.1 to 1.7 mm (Fig. 2). On the final day of incubation the developing larvae occupied the entire space in the egg capsule.

Frequency of spawning

In the present study under laboratory condition spawning was achieved every 13 to 15 days interval giving an average of 2 spawning per month per pair and the number of eggs varied between 115 to 1000 nos./ spawning/pair. Spawning was obtained 1 to 5 days before and after the full moon and new moon.

Parental care

Both parents take care the eggs in day time during incubation period and it involved two basic activities *viz.* fanning and mouthing. Fanning was achieved by fluttering the pectoral fins and created a cooling effect which helped to reduce the damage of eggs. By mouthing, the parents removed the dead, decayed or weakened eggs and dust particles.

Egg hatching

On the expected day of hatching, two hours before sunset, the eggs along with the substratum were transferred from the parental tank to hatching tanks (100 liters). Mild aeration was provided near to the egg cluster to ensure sufficient water current for oxygenating the eggs and provided complete darkness. At a water temperature of 27 to 29° C, the larvae broke the egg capsules and the hatchlings emerged tail first and peak hatching took place shortly after sunset between 1830 to 1930 hrs.

Newly hatched larva

The newly hatched larvae measured 2.5 to 3.6 mm in total length and each had a transparent body, large eyes, visible mouth, and a small yolk sac and is free swimming. The mouth gape of the newly hatched larvae

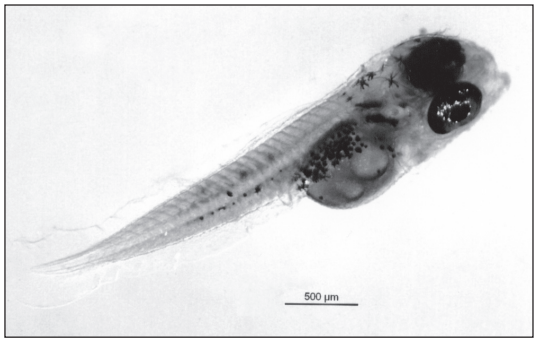

Fig. 3 Newly hatched larva

ranged from 235 to 350µm (Fig. 3).

Larval rearing and feeding schedule

The larval rearing was carried out in the hatching tank itself up to 20lh day of post hatch (DPH). Soon after completion of hatching, light was provided using (40W) bulb which was hung 30 cm above the water surface and the larvae were fed with micro algae *Chlorella marina* and *Nannochloropsis occulata* in 1:1 proportion at 1.5 x l0^6 cells/ml. First day onwards the larvae were fed with mixed culture of microalgae (1.5 x 10^6 cells/ml) and super small rotifer *Brachionus rotundiformis* (6 to 8 nos./ml) up to 8th day. From 9th day onwards, the larvae were weaned to newly hatched *Artemia* nauplii (4 to 6nos/ml) along with rotifer (6 to 8 nos./ml) and mixed culture of micro algae 1.5 xl0^6 cells/ml. On 12th day to 17th DPH, the larvae were fed with newly hatched *Artemia* nauplii (4 to 6nos/ml). A water temperature range of 27 to 29° C, salinity 33 to 36ppt, dissolved oxygen 4.6 to 6.2 ml/1 and pH 8.1 to 8.6 were maintained in all the rearing tanks.

Pigmentation

The larvae were transparent for initial three days and became black on fourth day onwards and the first sign of brownish pigmentation appeared on 7 to 10th day of post hatch. Feeble opercular white band appeared on 11 to 12th day of post hatch and feeble middle band noticed on 12 to 13th day and feeble caudal band developed on 15th to 20th DPH. At 15 to 17th day of post hatch, all the juveniles attained bright reddish colour and most of the fry metamorphosed and began to shift from partially pelagic to epibenthic and started eating minced shrimp, fish flesh, mussel meat, clam meat and formulated diets.

Juvenile growth

The juveniles attained 14 to 20 mm at 30 days of post hatch (Fig. 4). At 45 to 50 days of post hatch, most of the hatchery produced juveniles attained adult colouration and banding pattern. After 3 months of rearing, the juveniles attained a size of 30 to 40 mm (Fig. 5). As the juveniles were very aggressive, they were culled to different groups (15 to 20 Nos. /250 liter tank/ anemone). On attaining 30 to 40 mm size, 50 to 100 juveniles were stocked in 1 ton FRP tanks fitted with biological filter and provided 3 to 5 sea

Fig. 4 Thirty days old Juveniles of *P. biaculeatus*

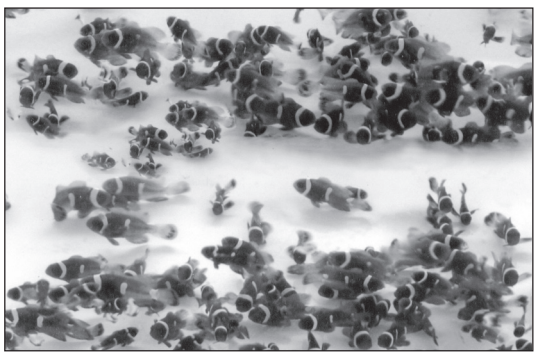

Fig. 5 Three months old Juveniles of *P. biaculeatus*

anemones per tank to ensure maximum survivability.

Water quality maintenance

Maintaining good water quality and ensuring slight water circulation is also found very essential for better survival of larvae. In the larval rearing tank 25% water was exchanged daily with sand filtered sea water. Aeration was provided at four corners of the tank through the PVC columns covered with 200 micron bolting silk cloth and aeration was adjusted to create a mild water circulation. In order to reduce "head-butting syndrome", four sides of the glass tanks were covered with black cloth to avoid reflection of light inside the tank. A low intensity light (40 w) was provided to the larvae during day and night to locate the feed and it also helped to keep the larvae swimming towards the surface at night rather than sinking to the bottom. Through different experimental trials, various hurdles associated with larval rearing have now been overcome and hatchery production of *P. biaculeatus* was achieved with 75 to 85% survival at each spawning.

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