

Successful captive breeding and juvenile production of the tomato anemonefish, *Amphiprion frenatus*

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The tomato anemonefish *Amphiprion frenatus* belonging to the family Pomacentridae and subfamily Amphiprioninae has very high demand in the marine aquarium industry due to their attractive bright red colouration. The adult *A. frenatus* is bright orange-red in colour, with one white vertical stripe just behind the eyes, joined over the head, whereas the juveniles are dark red, with three vertical white bands and black pectoral fins. They are very hardy and easy to feed as they accept a variety of food, making them a great choice for aquarists. Usually they grow upto 14 cm in length. However, the females grow significantly larger than the males. In nature, they live in association with sea anemone species viz., *Heteractis magnifica*, *Entacmaea quadriolour*, *Heteractis aurora* and *Macrodactyla dorensis*. They have wide distribution in waters of the Western Pacific from the Ryukyu Islands, Japan to Malaysia and Indonesia, Indo-Pacific to Oceania. Being a protandric hermaphrodite, males are much smaller than females. Considering the importance of this species in marine aquarium trade, captive breeding of the species was attempted. Under captivity, pair formation, broodstock development and breeding of *A. frenatus* have been successfully obtained and its juveniles were produced in the Marine Hatchery of the Central Marine Fisheries Research Institute, Kochi. This is the first report on the captive breeding and spawning of *A. frenatus* in India.

Pairing

The breeding pairs were developed through pair formation experiments. For this, 5-6 numbers of juveniles of different age groups brought from the Andaman & Nicobar Islands were reared in 500 l FRP tanks along with one host sea anemone *H. magnifica*. The fishes and anemones were fed twice daily with wet feeds such as meat of shrimps, mussels and clams at the rate of 10% of their body weight and live feeds like *Brachionus plicatilis* and

newly hatched *Artemia* nauplii. In all the tanks, the range of environmental parameters were maintained at optimum level i.e., temperature: 27 to 29 °C, salinity: 32 to 34 ppt, dissolved oxygen: 4.6 to 6.2 ml/l and pH: 8.1 to 8.8. After 3-5 months of rearing, one pair grew ahead of others (Fig. 1) and the pair thus formed were then transferred to broodstock tanks.

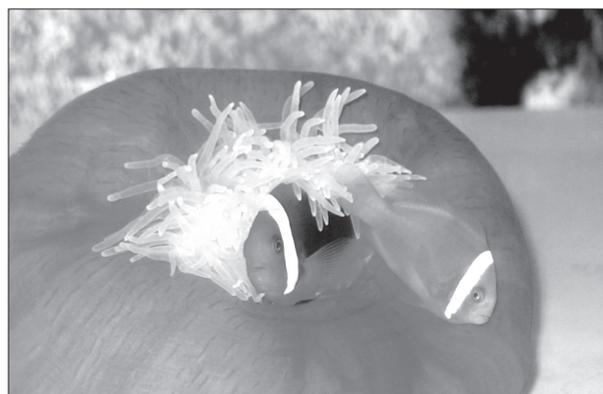


Fig. 1. Adult pair of *Amphiprion frenatus* developed through pair formation

Broodstock development

In the broodstock rearing tanks, the fishes were fed with wet feeds such as cooked mussel meat, shrimps, clam meat, fish egg mass and also provided formulated feeds enriched with vitamins, minerals and algal powder, at the rate of 10% of their body weight supplied at an interval of 3 h during day time whereas at night, they were fed with live feeds such as rotifers and *Artemia* nauplii at the rate of 3 to 5 no./ml after enriching the same with a mixed culture of micro-algae *Nannochloropsis oculata*, *Chlorella marina* and *Isochrysis galbana*. The environmental parameters were maintained as in the case of pair formation tanks. The water was recirculated to ensure water movement and provided good water quality with the aid of a specially devised filter system during

the period of rearing and once in a week, 25% of the water was exchanged. Broodstock tanks were provided with tiles or earthen pots for egg deposition which also enabled the transfer of egg clutches to hatching tank without mechanical injury.

Breeding behaviour and spawning

Few days prior to spawning, the male selected a suitable site near to the sea anemone for laying the eggs and cleared off the algae and debris with its mouth. On the day of spawning, both the parents spent considerable time for the cleaning of site which indicated that spawning may occur within few hours. The spawning was noticed between 0600 hrs and 1530 hrs during day time and the spawning lasted for one hour to one and a half hour. In each spawning, approximately 200 to 600 capsule shaped eggs were laid at an interval of 15 to 30 days, depending on the size of fish. The egg size ranged between 1.2 to 3.0 mm in length with a width of 0.8 to 1 mm. The eggs adhered to the sides of the earthen pot with stalk.

Parental care

As parental care is inevitable for hatching of the larvae, the parents were allowed to remain in the parental tank itself until hatching. During incubation period, both the parents carefully looked after the eggs during day time which involved two basic activities viz., fanning by fluttering the pectoral fins and mouthing to remove the dead or weakened eggs and dirt. No nocturnal care was noticed (Fig. 2).

The newly spawned eggs were red or reddish brown in colour for the initial two days and as the embryo developed, it turned to black from 3rd to



Fig. 2. Male and female *A. frenatus* guarding the eggs

5th day and later turned to silvery on 6th and 7th day of incubation (Fig. 3).



Fig. 3. Embryo inside the egg capsule on 5th day of incubation

At this stage, the glowing eyes of the developing larvae inside the egg capsule were clearly visible when viewed from a short distance. Male assumed nearly all responsibility of caring for the eggs and spent a higher percentage of time at the nest than the females, which increased gradually upto 70% of time as the day of hatching approached. When incubated at a water temperature range of 27 - 29 °C, the hatchlings emerged on completion of 7th day of incubation. Hatching was initiated shortly after sunset.

Hatching and larval rearing

The peak hatching took place after sunset between 1830 and 1930 hrs at a water temperature range of 27 - 29 °C. The newly hatched larvae measured 1.5 - 3.5 mm in length and each had a transparent body, large eyes, visible mouth, and a small yolk sac. The mouth gape of the newly hatched larvae ranged from 300 to 365 μ. The larvae were fed with mixed culture of micro-algae *Chlorella marina* and *Nannochloropsis oculata* (1.5 x10⁶ cells/ml) in 1:1 proportion and the rotifer *Brachionus plicatilis* (6 to 8 no./ml) upto 10th day (Fig. 4).

From 11th to 14th day post-hatch, larvae were weaned on to newly hatched *Artemia* nauplii (4 to 6 no./ml) along with rotifer (6 to 8 no./ml) and mixed culture of micro-algae (1.5 x10⁶ cells/ml) and on 15th day to 17th day of post-hatch, the larvae were fed with newly hatched *Artemia* nauplii (4 to 6 no./ml). From 15th to 17th day of post-hatch, the size of the juveniles ranged between 10-12 mm and all attained bright reddish colour. Most of the fry resembled

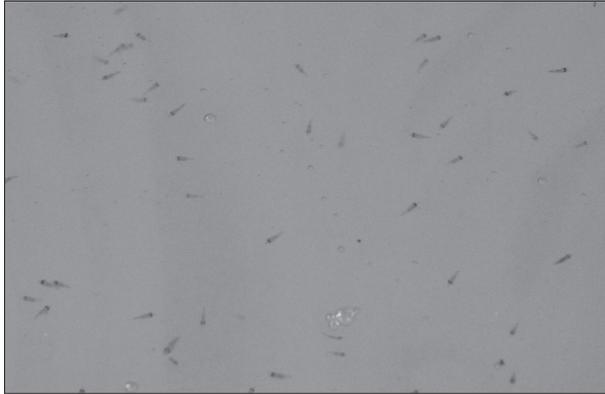


Fig. 4. Ten day old larvae of *A. frenatus* in green water system

juvenile fish and began to shift from partially pelagic to epibenthic, eating minced shrimp, fish flesh, mussel meat, clam meat and formulated diets. Daily 25% of water was replaced with filtered seawater.

Juvenile rearing

Most of the hatchery produced juveniles attained adult colouration and banding pattern at 30 days of post-hatch (Fig. 5).

Under hatchery conditions, with management of water quality and feeding, 85 to 90 % larval and juvenile survival were obtained. Three types of banding patterns were recorded in *A. frenatus* during its different life stages. All the juveniles exhibited three white bands (opercular, middle and tail band). In the



Fig. 5. Thrity days old juveniles of *A. frenatus*

sub-adult stage, the tail band completely disappeared and the mid body bar appeared feeble and subsequently disappeared. In the adult stage, all the fishes possessed only single broad white cross bar on head which was found just behind the eyes and it persisted throughout the life cycle. The body is usually black on sides with reddish snout, belly, dorsal fin and tail. The size of the female varied between 90 and 140 mm and that of male between 60 and 70 mm in total length. The growth studies of the hatchery produced juveniles, as well as the standardisation of larval rearing techniques are in progress. The juveniles produced under captivity will be made available to the farmers and traders through seed sale counter at CMFRI, Kochi.