SPAWNING AND LARVAL REARING TECHNIQUE FOR TROPICAL CLOWN FISH *AMPHIPRION SEBAE* UNDER CAPTIVE CONDITION

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ABSTRACT

Research on breeding and larval rearing of marine ornamental fishes are in its infancy. For the first time in India, successful breeding and larval rearing of Tropical clown fish *Amphiprion sebae* was accomplished at Regional Centre of Central Marine Fisheries Research Institute, Mandapam Camp. Adult pairs of clown fishes along with sea anemones collected from the in-shore waters of Gulf of Mannar were maintained in one tonne glass aquarium fitted with bio-filters. The fishes were fed with polychaete worms, clam and fish meat. After three months of maintenance natural spawning took place and the fish deposited its eggs on an asbestos substratum placed in the tank. The salinity and temperature of the water media was in the range of 28–32°C and 33–35 ppt respectively. In each spawning the females lay about 300 to 600 eggs, and the incubation period was 6–7 days. Brood stock maintenance, spawning behaviour, and egg development were described. Hatching was effected in a 250 l fibreglass tank containing *Chlorella* conditioned seawater along with rotifer *Brachionus plicatilis*. Hatching was successful up to 70% and the newly hatched larvae measured 4–5 mm. Sequential description of the larval development and the feeding regime are detailed. The larvae metamorphosed into adults on the 15th day, and were transferred to 1 ton fibreglass tank containing sea anemones, and the juveniles got accommodated in the anemone within a day. Mortality of clown fish larvae at different developmental stages was observed especially on the 2nd and 7th day. The reason attributed to the mortality was non-availability of right sized feed and their nutritional insufficiency in terms of essential fatty acids. Two types of combination of live feed i.e. rotifer and artemia; rotifer and copepods were tried. Higher survival and growth of larvae was observed in the combination of rotifer and copepod, which signifies the suitability of copepods to artemia.

Key words: Clown fish, *Amphiprion sebae,* Larval rearing, Live feeds.

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Recently the demand for marine ornamental fishes has gained a thrust among aquarium hobbyists due to their multitudinal colour and beauty. The annual worldwide market for ornamental marine reef fish has shown a steady increase over the past few years. This trade is almost dependent on ornamental fishes captured from the wild. This has resulted in considerable pressure and may lead to over-exploitation of natural population, damage to coral reef and environmental degradation. Therefore research on the commercial rearing of these fishes is an imminent necessity to save this fragile ecosystem. However till date efforts in this direction have been extremely limited to very few coral fishes like damsels, neon gobies etc. and that too only in temperate conditions (Hunziker, 1990; Danillowicz and Brown, 1992).

The major constraint in rearing of any marine fish larvae is the heavy mortalities at different stages of development due to various factors. The size and the nutritional quality of live feed are the two major factors affecting the survival of larvae. Nutritional quality of live feed depends on its unsaturated fatty acid contents. To improve the nutritional quality of the live feed, various enrichment methods have been tried (Watanabe, 1983). This improvement has resulted in better survival of marine fish larvae.

Clown fishes are very popular among fish hobbyists due to its aesthetic appeal and its easy adaptability to captive conditions. The present study was aimed to develop a commercially viable technique for raising the common species of clown fish (*Amphiprion sebae*) in large numbers. Clown fish hatcheries can be set up in selected areas to promote exports adopting the technology developed by the institute. This will provide valuable foreign exchange generation and also minimise the destruction to natural environment occurring during the collection of wild stock.

The approach was to develop suitable rearing methods for better survival of clown fish larvae, to determine the suitable combination of enriched rotifer, copepods and artemia as feed. The detailed description of the spawning behaviour, spawning, egg development, feeding and larval rearing are discussed in this paper.

MATERIALS AND METHODS

Brood stock: Adult pairs of clown fish along with sea anemone were collected by divers from the in-shore areas of Gulf of Mannar and transported to the marine aquarium facility of the institute. They were domesticated for 3 months in 1-ton glass aquarium (Temperature: 28–32°C; Salinity: 33–35 ppt), fitted with biological filters. The fishes were fed with different feeds such as fish and bivalve meat, polycheate worms regularly twice a day. The excess feed was removed daily to avoid water

spoilage. Several pieces of asbestos sheet were placed in the tanks as substratum for deposition of eggs.

Hatching: Hatching was done in a separate 100 l FRP tanks. Preparation of the hatching tanks began one day prior to the transfer of fully developed eggs. The unicellular algae, dominated by *Chlorella* sp. were added to the tank as a water conditioner. In addition, rotifers *Brachionus* sp. (below 100 μ) were added to the tank at a concentration of 10–15 nos/ml of seawater.

Fertilised eggs were maintained in the broodstock tank along with parents till 5th day. On the 6th day of incubation, the substratum with the fully developed eggs were transferred in the evening hours to the conditioned hatching tank. Extreme care was taken not to expose the eggs to bright light and air during transfer. Artificial fanning was done with the help of air stones to provide gentle movement of eggs.

Culture of live feeds

Rotifers: Marine rotifers, *Brachionus plicatilis* were obtained from the live feed culture laboratory and enriched with cod liver oil and raw egg (Ruangpanit, 1993). Briefly, 50 ml of cod liver oil and 2 egg yolks were blended together and a homogenous emulsion was prepared. Emulsion was added at the rate of 1 ml/l of rotifer culture having a density of 500–1000 nos/ml. The enrichment process lasted for six hours. The enriched rotifers were collected in a sieve washed with seawater and used as feed for the larvae.

Copepods: The production of copepods by batch method was done (Stottrup and Norsker (1997)). Adult copepods were collected by sieving the raw sea water through a sieve of 250 μ mesh and inoculated to a 250 l outdoor FRP tank at a concentration of 5–8 nos/ml of tank water. The microalgae *Chlorella* sp. and *Nanochloropsis* were used as feed for the rotifers at a concentration of 0.1 lakh cells/ml of seawater. Continuous aeration was provided to the outdoor tanks. After 10 days, copepod nauplii were ready for harvest.

Artemia: Artemia cysts were hatched in transparent containers provided with vigorous aeration for 24 hrs. Thereafter nauplii were ready for harvest.

Larval rearing: After 3 to 4 hours of hatching, the floating larvae were carefully transferred to 250 l larval rearing tank, prepared in a similar manner as the hatching tank. The tank was provided with mild aeration and 25% water exchange on every third day along with bottom cleaning to avoid excessive build up of organic load.

Water quality parameters like temperature, salinity, pH and dissolved oxygen were monitored every alternate day (Table 1). Density of unicellular

Table 1: Feeding and water management strategies

Day of hatching	1	5	10	15	20	25	30
Water Exchange	←	25% every third day					→
Bottom cleaning	←	every third day					→
Type of feed		Feeding scheme					
Enriched rotifer	< 100 μ	> 100 μ					
Copepod Nauplii		> 250 μ					
Artemia Nauplii							
Adult copepod							
Adult Artemia							
Minced meat							

Water quality parameters

Temperature (°C)	26.5—33.4
Salinity (ppt)	28.2—34.9
pH	8.1—8.5
DO2 (ml/l)	3.6—5.6
NH3	insignificant

algae and rotifer were periodically measured and supplemented when necessary. Feeding was changed after 5–6 days to larger rotifers and copepods of size above 250 μ at the rate of 5–8 nos/ml. Size of copepods fed to the larvae was increased to 400 μ from 11th day onwards. On 15th day the larvae attained a size of 1.0–1.5 cm and were again transferred to 1 ton capacity FRP tanks containing sea anemone. The tank water was saturated with *Chlorella*, copepods, rotifers and artemia of all sizes. By 30th day minced fish, prawn and clam meat were provided to the juveniles.

RESULTS

Spawning and egg development: Clown fish pairs fed with minced fish meat did not spawn, whereas the other pairs fed with clam meat and

supplemented by live polycheate worms matured faster and just prior to spawning, the pair exhibited typical courtship behaviour of chasing each other and cleaning the asbestos substratum. Spawning was observed in morning hours between 0800 to 1000 hours. Female first lays capsule-shaped eggs on the cleaned substratum in nearly rounded patch. The male subsequently fertilises the eggs. Thereafter, mostly the male fish gently fans the cluster of eggs at regular intervals. The dead eggs and the unfertilised eggs were selectively removed by the parents during the course of incubation. Incubation period lasted for 6 to 7 days.

Spawning commenced after 3 months of rearing (water temperature: 28°C, salinity 33 ppt) and occurred on every 10th day during morning hours until the 4th month. In each spawning 300 to 600 eggs were laid by a single female. The eggs were 2–3 mm in length and 1 mm in diameter and were adhered to substrate by stalk. For the first two days, the eggs were pale yellow/orange in colour, later turned to dark brown. As embryonic development progressed the eggs turned silvery on 6th day due to the development of large eyes.

Hatching and larval rearing: After incubation of 7 days, the hatching took place invariably in darkness between 1800 and 2000 hrs at 28–30°C and 33 ppt salinity. Approximately 70% of the fertilised eggs hatched each time. The newly hatched larvae measured 4–5 mm in size and had a transparent body, large blue eyes, open mouth (250 μ) and a small dark yolk sac. Microscopic examination of the newly hatched larvae revealed a fully developed heart, blood vessels and a network of nerves and ganglions. The fins were in the process of development. Immediately after hatching the larvae were floating on the surface vertically. After 3–4 hours the hatched larvae were transferred to rearing tanks (250 l). The larvae were fed with enriched rotifers of below 100 μ size. At this stage a considerable reduction in larval number was observed. On the 6th day the larvae started feeding on copepod nauplii (> 250 μ) and larger rotifers (> 100 μ). The larvae grew to a size of 6–8 mm at the end of 7th day. During the growth, the depth increased faster than the length of the larvae. Larvae were tan in colour with large silvery and prominent eyes. Larvae were swimming freely in the water column, with fully developed fins. However the caudal fin movement was jerky.

On 10th day, the larvae started accepting the Artemia nauplii. Slight orange pigmentation started appearing near the dorsal part of the body. On the 12th day, two white bands appeared and later the bands became prominent and the colour of the lips changed to yellow. By 15th day the juvenile attained all colouration pattern of an adult fish. The fishes also showed a significant change in swimming behaviour. Instead of swimming in the water column, the fishes started going to the bottom of the tank and touching their ventral portion of their body with tank bottom. When

this behaviour was observed, the juveniles were transferred to 1 ton FRP tanks having few sea anemones. Within a day all the juveniles got accommodated in the anemones, and were accepting artemia and minced earthworms. On 30th day they started feeding on minced fish, prawn and clam meat.

DISCUSSION

This is the first time that the complete larval rearing of *Amphiprion sebae* has been achieved following natural spawning of broodstock in captivity in Indian waters. This study was also one of the first successful attempts on breeding and larval rearing of any marine ornamental fish in India.

There are few reports on successful larval rearing of marine ornamental fishes such as *Amphiprion clarkii*, *A. percula* (Alava and Gomes, 1989; Malpass, 1996; Allen, 1998), *Dascyllus albisella* and *D. aruanus* (Danillowicz and Brown, 1992) in temperate waters.

In the present study it was observed that fishes fed with clam meat and supplemented by marine polycheate attained sexual maturity within 3 months of maintenance and spawned repeatedly when compared with those pairs fed with minced fish meat alone. This indicates that these worms apart from having high level of EFA certain other compounds that influence spawning are also available. Pairing of adult male and female fish was permanent in nature. Introduction of new fish does not alter the original pair and instead the new fish is driven out from the host anemone. The eggs were laid always on flat and smooth substratum during morning hours on contrast to evening hours reported by others (Alava and Gomes, 1989; Malpass, 1996). The egg diameter, egg length, incubation period, size of newly hatched larvae were similar to those reported earlier. Mortality observed on 2nd day was due to inability of the larvae to take the feed. This may be due to the larger size of the live feed supplied (> 150 μ) which the larvae are unable to ingest as in other marine fishes. The mouth gap of larvae was approximately 200–250 μ in size and feed size required for this mouth size could be below 100 μ. The heavy mortalities on 2nd day during the first three attempts were attributed to the feed size since the right sized (100 μ) live feed was not readily available coinciding with the hatching. Later use of small sized enriched rotifer increased the survival percentage at this stage of development. Bacteriological examination of the dead larvae at this stage of mortality revealed no significant pathogen as the aetiological agent of mortality. This confirms that the feed was the sole factor responsible for the mortality at this stage.

Sudden mortality of around 30% was also observed on 7th day during initial attempts. Investigations revealed the under development of the

digestive tract in the dead larvae which may be due to the deficiency of HUFA in the feed.

The rearing of majority of marine fish larvae is done mainly with live feed like rotifer and artemia. The critical factor for their dietary value is their content of n-3 HUFA (Rainuzzo *et al.*, 1997). Recent studies have demonstrated that supplementation of dicosahexaenoicacid in live feed of marine fish larvae improved growth and survival and stress resistance (Watanabe, 1993). Other recent studies have also shown that HUFA, eicosapentaenoic acid (EPA: 20:5n-3) and dicosahexaenoic acid: DHA, 22:6n-3) are essential dietary components for marine fish larvae (Webster and Lovell, 1990; Sorgeloss *et al.*, 1988) these essential fatty acids (EFA) must be supplied in diet to ensure good growth and survival of marine fish larvae.

To improve the nutritional quality of rotifer, an enrichment method described by Watanabe *et al.*, 1983 was followed, which resulted in improved survival and growth of larvae at the 7th day stage.

Attempts with Artemia as feed after 7 days were not encouraging. Although the larvae were consuming the feed, the digestibility of Artemia was very poor. Microscopic examination of the dead larvae showed presence of large quantities of undigested Artemia nauplii. Early stages of many marine fish larvae do not have a well developed digestive system and may benefit from exogenous supply of enzymes from live food organism (Delbare *et al.*, 1996).

Evidence that copepods may be preferable to Artemia comes from Pederson (1984), who examined digestion in first feeding of Herring larvae and found that copepods passed more quickly through the gut and were better digested than Artemia. As per the earlier studies, marine copepods like *Tigriopus* sp. are rich in HUFA content as compared to other live feeds (Watanabe *et al.*, 1993). Copepods have high protein content (44–52%) and a good amino acid profile with exception of methionine and histidine. Copepods also contain higher level of digestive enzymes, which may play an important role in larval nutrition (Delbare *et al.*, 1996). In the present study marine copepods were used extensively to provide the required EFA to fish larvae after 7 days. This ensured a high survival and growth rate of larvae (Table 2). This show the qualitative

Table 2: Feed combinations and percentage survival of clown fish larvae

Feed combinations	Percentage survival
Rotifer and Artemia nauplii	5–10%
Enriched rotifer and Artemia nauplii	10–15%
Rotifer and copepod nauplii	45–50%
Enriched rotifer and copepod nauplii	50–65%

suitability of marine copepods in the larval rearing of marine fishes and can be used as an alternative source of zooplankton for marine larviculture.

Larval rearing done under different light condition and tank revealed that light and colour of the rearing tanks are the important factors influencing the larval metamorphosis and survival. Larvae reared under complete darkness and glass aquarium tanks without covering resulted in very poor survival whereas it was high in normal light condition in blue coloured FRP tanks indicating that these larvae are essentially visual feeders, and need suitable background to locate the feed and consume it.

Although it is felt that there is need for improvement, the larval rearing method described for clown fish is successful, and this in itself has implications for future tropical ornamental fish culture. This method could be tried not only for marine ornamental fish culture, but also for other marine food fishes, as it is simple and cost effective. Improvement of this rearing technique will result in successful rearing of more species of fishes in a cost efficient manner and will lead to commercial production of variety of marine ornamental fishes. This will not only lead to promotion of viable industry but also save the fragile ecosystem from the present destructive fish collection methods.

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