

Studies on captive breeding and larval rearing of clown fish [a¹], *Amphiprion sebae* (Bleeker, 1853) using estuarine water

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An attempt was made to study the captive breeding and larval rearing of *Amphiprion sebae* by using estuarine water. Sub-adults of the anemone fish [a²], *A. sebae* were procured from the traders with the size range of 4-6cm and acclimatized to captive condition. After 2 months of acclimatization [a³], pair was formed. At the end of 4th month, the fishes were spawned. After 6-8 days of incubation hatching took place and the larvae metamorphosed in 15-18 days. Rotifer, *Brachionus plicatilis* and *Artemia* nauplii were fed to the larvae gave a maximum survival rate. Total larval survival in the present study was 55%. The babies reached the marketable size in 3 months.

[Keywords: Clown fish [a⁴], *Amphiprion sebae*, estuarine water, captive breeding, larval rearing]

Introduction

The marine ornamental fish trade is a rapidly growing sector that relies almost exclusively on the collection of these animals from coral reef ecosystem¹. Unlike freshwater ornamental fishes, only a few species of marine ornamentals have been cultured in captivity. Collection of these organisms from wild is done extensively in Indian waters, which has raised concern that the continuous harvest of these fishes will not only affect the target species but also to have irreversible impact on the components of the entire reef ecosystem². Among the marine ornamental fishes, [clown fishes [a⁵] are considered to be most popular attractions of aquarists, and they are important in the aquarium trade in view of their bright colour, interesting display behavior and their ability to adapt in captive condition³. Among the 28] [a⁶] clown fish species, *Amphiprion sebae* is the commonly occurring species in South east coast of India and they are continue to be the most sought-after marine tropical fish⁴ [a⁸]. In the past, few studies^{5,6,7} have been made elsewhere on captive breeding of clown fish [a⁹] *A. sebae* using sea water but as an oddity, presently an attempt has

been made to study the captive breeding and larval rearing of *A. sebae* using estuarine water.

Materials and Methods

Species description

Colour pattern is the most important feature for identifying anemone fishes [a¹⁰]. Dark brown to blackish with two milky white bars, the mid-body bar slanting slightly backwards and extending onto rear part of dorsal fin, snout, breast [a¹¹] and belly often yellow orange, tail yellow or orange but some times entirely dark brown to blackish on the body (except for milky white bars), orange colour on the snout, breast [a¹²] and belly are the identifying characters of this fish⁴ (Fig. 1)

For the present study, estuarine water was drawn from the Vellar estuary (Lat.11° 29'N; Long. 79° 46' E) with the help of 5 Hp pump during the high tide and allowed to settle in a sump overnight. Then the clear water was passed through sand and U.V filters and finally stocked in a storage tank from where water was taken for hatchery use.

Brood stock management

A. sebae with the size range of 4-6 cm was procured from the ornamental fish traders along

with host anemone, *Stichodactylus* [a¹³] *haddoni*⁸. After acclimatization, they were keenly observed for an [a¹⁴] injury or any sign of diseases. After making sure, 10 numbers of uniform size [a¹⁵] fishes and 5 anemones were transferred to a 5 ton cement tank (conditioning tank) filled with 2 ton water where a under water filtration system was provided, which was made using activated carbon, ceramic ring and coral sand. The fishes were fed thrice a day with different feeds such as live Acetes, trash fish, meats of clam, mussels, squids etc. After 2 months, one pair measuring 5-7 cm grew ahead of others and that was transferred to [a¹⁶] 1 ton FRP tank (spawning tank). The photoperiod [a¹⁷] maintained 12 hr. light and 12 hr. darkness using artificial light. The submerged objects such as tile, earthen pot, PVC pipe etc., were placed in the bottom of the tank on which the fishes were



Fig. 1—Spawning pair *Amphiprion sebae*



Fig. 2—Egg clutch along parents

spawned. Spawning took place on 4th month after a brief courtship (Fig. 2). Embryonic development of eggs was studied on daily basis (Fig. 3). The water quality parameters such as temperature, salinity and pH were measured regularly using [a¹⁸] standard methodologies.

Larval rearing

Eggs were allowed to hatch in the spawning tank itself. Two hrs. after hatching, the larvae were collected gently without much disturbance and transferred to the larval rearing tank (FRP 250 lit) with 8-10 nos/l. Photoperiod was maintained 12 hrs. darkness and 12 hr. light. As the larvae have yolk sac, on first day, they were not fed. On 2nd day onwards algal enriched rotifer, *Brachionus plicatilis* was introduced thrice a day and maintained at the concentration of 8-10 nos/ml. The waste particles settled in the bottom were removed without disturbing the larvae and the amount of siphoned out water was replaced with fresh filtered estuarine water. From 10th day onwards, newly hatched *Artemia* nauplii were given as feed. After metamorphosis, adult artemia, boiled squashed clam and mussel meat were given. The feeding regime followed in the present study is given in Table 1.

Live feed culture

The rotifer, *Brachionus plicatilis* (SS) with 70-239 μm dia was used as initial feed to the larvae. Rotifers were raised with the help of micro algae, *Chlorella* spp., *Nanochloropsis* spp. and *Isochrysis* spp. The stock culture of micro algae was maintained using Conway medium and for the mass culture, agriculture fertilizers such as ammonium sulphate, super phosphate and Urea at the ratio of 10:4:2 were used. The algae were cultured in 300 lit cylindrical FRP tanks and used as feed for rotifers and the same was harvested using 50 μm nylon net, when it reached the density 150 ind/ml.

Results

Brood stock development

For the successful brood stock development, water quality parameters were maintained in the



Fig. 3—Embryonic development and newly hatched larvae

Table 1—Feeding regime followed in larval rearing

Days	Morning		Afternoon		Evening	
	Rotifer/ml	Artemia/ml	Rotifer/ml	Artemia/ml	Rotifer/ml	Artemia/ml
1	No feeding					
2	10-15	-	8-10	-	6-8	-
3	10-15	-	8-10	-	6-8	-
4	10-15	-	8-10	-	6-8	-
5	10-15	-	8-10	-	6-8	-
6	10-15	-	8-10	-	6-8	-
7	10-15	-	8-10	-	6-8	-
8	12-16	-	10-12	-	8-10	-
9	12-16	-	10-12	-	8-10	-
10	8-10	6-8	6-10	4-6	4-8	2-4
11	8-10	6-8	6-8	4-6	4-8	2-4
12	8-10	6-8	6-8	4-6	4-8	4-6
13	6-10	6-10	6-8	4-8	4-8	4-8
14	6-10	6-10	6-8	4-8	4-8	4-8
15	6-10	6-10	6-8	4-8	4-8	4-8
16	4-8	6-10	6-8	4-8	4-8	4-8
17	4-8	10-15	6-8	8-12	4-8	8-12
18	Nil	10-15	Nil	8-12	Nil	8-12
19	Nil	16-18	Nil	12-16	Nil	12-16
20	Nil	16-18	Nil	12-16	Nil	12-16

optimum level. The temperature in the spawning tank was maintained at $28 \pm 2^\circ\text{C}$, salinity at 22-24‰, dissolved oxygen 4.5-5 mg/l and pH 7.5-8.2. Regular water exchange (20-40%) was given depending on the water quality. After a period of four months rearing in the above said conditions, fishes were started spawning.

Behavioral observation

During feeding, it was noticed that the male gives opportunity to female to take feed. Among

the 10 fishes in the conditioning tank, one pair grew ahead of others in 2 months. After the removal of the same, another pair was formed within ten days. [The fish was spawned in 2 months time and thereafter the same was stocked in spawning tank] [a²⁰]. The courtship began a week before spawning with the initiatives taken by the male, which includes nest site selection and cleaning the area. On the day before spawning, male actively cleaned the site by rubbing his body and pickling [a²¹] off any

loose particles of algae or faecal matter with his mouth. Then the female entered into the nesting site and laid eggs subsequently. Spawning took place during morning hours. The eggs were fertilized by male and the spawning process lasted for about 45 min. to 1 hr. It was observed that guarding of egg clutch actively done by male and on a few occasions female too. Fanning of the eggs was done mostly by male and rarely by female with the help of their pectoral fins and the tail. Fanning was more frequent on the day of hatching and the time was varied from 10 to 90 seconds. The number of eggs in a clutch was found to be initially less in the first spawning and in subsequent it was high and ranged between 400-2000 nos. The spawning frequency varied from 07 to 15 days. The fish spawned throughout the year with 2.4 nets per month except during December. Maximum spawning was observed in summer, where 4 spawning were achieved.

Hatching

Before hatching, the eggs became dark silvery in colour and chorion membrane became pliable. During this period, the movements of embryo were more frequent, violent and fanning duration also increased. Initially the tail was wrapped completely around the egg, reaching its distal end of the chorion. The colour of the eggs was pale orange and then turned slightly brown to black. On the day of hatching, the colour became silvery due to development of eye inside the embryo. Hatching took place 6-8 days post fecundation and the newly hatched larvae possessed a small yolk sac.

Larval development

The size of the newly hatched larvae varied between 2 and 2.9 mm in total length and mouth gap varied from 200-250 μm . The yolk sac of newly hatched larvae was small, orange, slightly elongated and had a mid body ball. After a day, it became active and swam to the surface. The larvae were fed with *B. plicatilis* and maintained the same with a density of 08-10 nos/ml in the larval rearing tank. On 3rd and 4th day, the body of the larvae was deeper and round in shape. From 10th day onwards, the larvae were fed with newly hatched *Artemia* nauplii at different concentrations along with rotifer (Table 1). On 15th to 18th day, the larvae were entered into metamorphosis. The average length of larvae after metamorphosis was 4-5 mm. There was distinct pigmentation appeared on 18th day and from 20th day onwards complete miniature of adult *A. sebae* fishes could be seen. On 25th day the juveniles settled with [a²²] sea anemone (Fig. 4). The young ones attained marketable size in three months of rearing.

Discussion

Increasing demand of marine ornamental fishes may leads to depletion of wild stock [a²³], further, land based cultivation of coral reef species could alleviate the growing market demands⁹ and thereby it helps to increase the wild stock and also employment generation. The rearing of anemone fishes [a²⁴] in captivity involves less challenge, compared to other marine ornamental fishes due to their easy acceptance of fresh, frozen and dried feeds³.

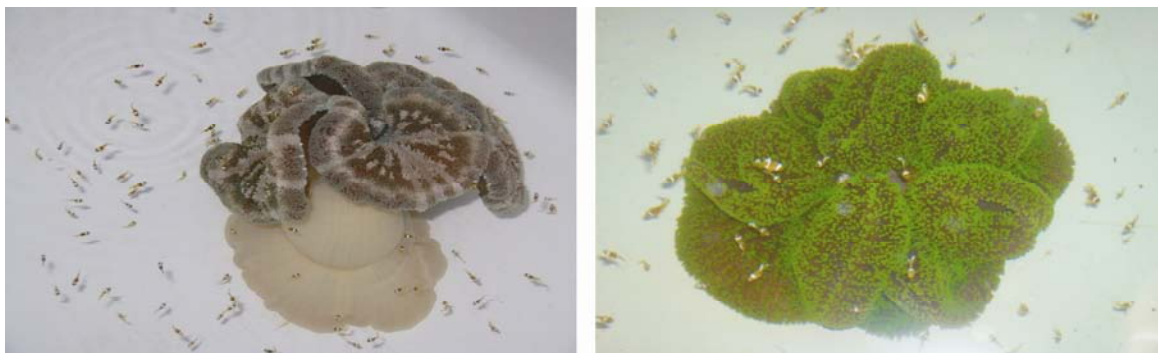


Fig. 4—Hatchery produced young ones

Breeding was reported for 26 different species of the Pomacentridae family which is worthy of note¹⁰. Earlier, many studies have been done on breeding of clowns using seawater^{3, 4, 5, 6, 7, 11, 12, 13}.

In the present study, estuarine water was used for breeding and larval rearing. Like many Pomacentrid fishes, the clown fishes [a²⁵] deposit demersal adhesive eggs on hard substrates, usually in sheltered areas. The eggs are elliptical and get attached by adhesive filaments. Although the early life histories [a²⁶] of many reef fishes are known¹⁴, the embryological and larval descriptions for many reef fishes are not studied thoroughly. In the present study, eggs were laid always during morning hours but in other species of clown fish [a²⁷] it was reported during evening hours⁵ and it may be due to some environmental factors. According to Olivotto *et al.*¹⁰ the male *Chrysiptera parasemna*, a Pomacentridae fish, guarded and fanned the egg clutch but in the present observation both the sexes done the process. During fanning, they removed the unfertilized eggs as well as rotten eggs to protect other healthy eggs by eating them. The fishes were also showing biparental care like a Pomacentridae fish, *Acanthochromis polycanthus* as reported by Kavangh¹⁵.

The fecundity rate, clutch size and spawning frequency were depends on several factors such as feed quality, brooder condition and environmental parameters etc. There was no variation in egg size as reported earlier⁷. According to Ignatius *et al.*⁷, the size of the newly hatched larvae varied between 4-5 mm in length but presently it varied between 2.4-3.1 mm and it may be due to the size of parents, nature of egg and environmental parameters. According to Lubzens *et al.*¹⁶ the rotifer, *B. plicatilis* is an essential food for marine larvae. Various studies¹⁷ done elsewhere reported that PUFA's enriched diet is extremely important for optimal nervous system function during the early larval stages but in the present study, rotifers were enriched with only micro algae. Since the micro algae contain DHA (Docosahexaenoic acid), no extra enrichment process was done.

Ignatius *et al.*⁷ studied on the spawning and larval rearing of *A. sebae* using sea water with salinity range of 33-35‰. In the present study, the fishes were bred in estuarine water with the salinity range of 22-24‰ and the larval survival rate was 55%. The larvae attained all pigmentation patterns on 20th day, but according to Ignatius *et al.*⁷, it should have happened on 15th day. The juvenile settlement with sea anemone was observed on 25th day. Hence, [a²⁸] the delay is attributed to the low saline water. Live feed and proper maintenance of water quality are indispensable to successful rearing of a reef fish *A. sebae* in captivity using estuarine water. The results of the present study will felicitate mass scale production of *A. sebae* by using low saline waters.

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