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Hatchery Production of Clark's Clownfish, *Amphiprion clarkii* (Bennett, 1830) Using Brackishwater

Swagat Ghosh[+], T. T. Ajith Kumar, K. Nanthinidevi and T. Balasubrananian

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamil Nadu, India

Abstract. Using the filtered brackish water as a source for broodstock maintenances, spawning and larval rearing of a reef associated clownfish, *Amphiprion clarkii* was investigated under captive condition, for the first time in aquaculture vista. Sub-adult of *A. clarkii* and sea anemones were obtained from the traders and maintained in a conditioning tank. After pair formation, they laid eggs in the spawning tank, which were sticky, capsule shaped and yellow orange in colour. The eggs were allowed to remain in the same tank till hatching, which took 7 - 8 days for incubation and the percentage of hatching was up to 93.37±3.52 (mean±SD) %. Spawning, embryonic development, hatching success, larval survival and juvenile production were noted in detail. Optimum water quality parameters were also standardized for the hatchery operations. The size range of newly hatched larvae measured 3.5 to 3.8 mm in length and they were transferred to separate larval rearing tanks. The first white band was prominent on the body between 15 - 17th days, an indication of metamorphosis. The complete metamorphosis was occurred 25th days after hatching. The larvae were initially fed with micro-algae, rotifers, *Artemia* nauplii and later they accepted frozen *Artemia* and squashed boiled meat of oysters and clams. The present findings have shown the prospective sign for captive breeding of a highly demanded aquarium fish using brackish water, by removing the existing foremost technological snag of rearing them in running seawater.

Keywords: *Amphiprion clarkii*, Brackishwater, Brood stock, Larval development, Juvenile

1. Introduction

Marine ornamental fishes are one of the most popular attractions world-wide, due to their adaptability to live in confinement. The tropical ornamental fish has increased a thrust among aquarists due to their multitudinal colour and gorgeousness. In the last two decades, marine aquarium fish trade has been witnessing continuous steady growth, involving major movements of wild reef fishes all over the world (Wabnitz *et al.,* 2003).

Among the coral associated fishes, clownfishes belonging to the family, Pomacentridae and subfamily Amphiprioninae are abundant and about 30 species have been recognized under two genera, *Amphiprion* and *Premnas* (Allen *et al.,* 2010). These fishes have some remarkable behavioural characteristics such as symbiotic association with sea anemones (Fautin and Allen, 1997), formation of a group consisting monogamous pairs and protandrous hermaphrodites (Ross, 1978). Their adaptability to live in captivity, easiness to be fed with artificial diets and their fascinating display behaviour (Ignatius, 2001) and symbiotic relationship with the sea anemone are the special features. Usually *A. clarkii* has black colour with variable amounts of orange on head, ventral parts and fins and three milky white bars on head, body and base of caudal fin. *A. clarkii* has the symbiotic relationship with ten types of symbiotic sea anemones (Fautin and Allen, 1997) but it prefers *S. mertensii* in captivity. This species is popular aquarium fish and can be bred and reared in captivity.

[+] Corresponding author.
E-mail address: (swagotor@gmail.com).

The hatchery bred fishes are hardier in nature, grow better in captivity, survive well and fetch attractive prices. However, grow-out of a reef-associated fish in low saline water, particularly brackish water is a new concept and it has effects on osmoregulatory processes due to slight salinity changes and its impact on growth and feed conversion efficiency (Overton, 2008), which has been attributed in the present study by providing proper water treatments and filtration systems. The main reason for brackishwater culture was technology could be reached to the coastal fishermen where the wide range of brackish water areas can be utilized for livelihood option for the fisher folks by this technique. The aim of this study was to develop a suitable rearing method for improving survival and enhancing growth of *A. clarkii* larvae and juveniles.

2. Material and methods

2.1. Broodstock origin and spawning

Twelve similar size fishes of *A, clarkii* along with six sea anemones, *S. mertensii* were obtained from the ornamental fish traders at Kollathur ornamental fish market, Chennai and transported to the hatchery at Centre of Advanced Study in Marine Biology, Annamalai University, Tamil Nadu. The fishes were acclimatized in quarantine tank for ten days with gradually mixing of fresh water and salinity maintained at 26 psu and later sifted to the conditioning tank filled with two tons of filtered brackish water. After pair formation of fishes (size range, total length - 70 to 100 mm), each pair were separated in 750 l capacity spawning tanks Fibreglass Reinforced Plastics (FRP) transferred along with their host anemone. The fishes were fed with boiled oyster meat and prawn thrice a day at 08:00, 13:00 and 16:00 hrs (Ignatius, 2001). One hour after feeding, uneaten food particles and fecal matter were siphoned out to avoid water ruining. Before spawning, the pairs show their courtship behaviours which are precise in the male showed morphological and behavioural changes such as fin erection, chasing, clutch preparation, 'signal jumping' and biting the anemone. Swimming motions and finally, extension of anal, dorsal and pelvic fins accompany the aggressiveness of the male. In the present study, it was observed that they preferred the egg laying substratum as in the side of the tank followed ceramic tiles. The eggs were allowed to hatch in the spawning tank itself.

2.2. Embryonic development

The eggs were sampled randomly (5 eggs/day) to document the embryonic development. The eggs were placed into sterile glass slide with UV filtered brackish water. Each egg was placed on a slide to observe the morphological development (Ignatius, 2001). The photographs were taken with a digital camera (Canon, China) with a light microscope (Novex, Holland) from day 1 to 8^{th} day for documenting major morphological and functional features of embryos. Milky white eggs found in the sample were considered as dead or unfertilized and were removed.

2.3. Larval rearing

The colour of the eggs became silvery; an indication for hatching and attention was paid to monitor the hatchouts. The larval rearing tank was filled with 15 l of micro alga, *Nannochloropsis salina* (1.0×10^6 cells/ml) with mild aeration. At the same time of larval transfer, 30 l of water from the parent's tank was also collected to provide same water and the tank was maintained with 45 l water. Photoperiod was maintained 12 hrs light and 12 hrs dark (Overton, 2008). The larvae were collected using glass bowl (500 ml) without much disturbance and accommodated in the larval rearing tanks with the stocking density of 5 fish l^{-1} and 10% of water exchange from the second day onwards along with bottom cleaning to avoid excess build up of organic load. Water was maintained as in the parent's tank. The larvae were fed with cultured rotifer, from 2^{nd} onwards cultured *Brachionus plicatilis* were provided up to 10^{th} day and 11^{th} day after hatching. After 11^{th} day newly hatched *Artemia* nauplii were introduced with the mixing of rotifers and slowly weaned the rotifers and at the 17^{th} or 18^{th} day rotifers were fully stopped the larvae was fed with newly hatched *Artemia* nauplii enriched with *N. salina*. Juveniles were collected and stocked in 500 l glass tank containing sea anemone, *S. mertensii* and the juveniles (Figure. 1.a & 1.b) were fed with squashed mussel, frozen *Artemia* and *Acetes* spp. *B. plicatilis* was mixed with algae with mild aeration for enrichment and after one hour they were filtered and used as a larval feed. The newly hatched *Artemia* nauplii were enriched with micro-algae for an hour and used as larval feed.

3. Results

3.1. Spawning behaviour and egg incubation

Spawning was observed during morning hours in between 7 - 9 a.m. lasted for 50 - 60 minutes. The number of eggs in one successful spawning was 513 ± 54. Spawning of eggs has less initially but increased in the subsequent spawning and the size of the eggs ranged from 2.2 - 2.5 mm. The maximum parental care exhibited by the males was fanning and mouthing the eggs which are the peculiar characters of the clownfish. On 1st day, the eggs looked yellowish orange; 2nd day dark orange; third day onwards, brownish which continued as dark brown and finally ended in silvery colour. The unfertilized eggs were selectively removed by the parent during the course of incubation which lasted for 7 - 8 days, depending on the surrounding environmental conditions. During the study period, the fishes spawned frequently i.e. twice a month.

3.2 Hatching and larval rearing

Hatching took place invariably in darkness between 1800 - 2000 hrs. The eggs underwent several distinct colour changes from orange (first two days) to dark brown (3rd day to 7^{th} day) to silvery (Figure. 1. a-h) When they become silvery the eggs would hatch out within 12 hrs of the colour change. Hatching took place during dusk and move this to incubation section. The average hatching success was 93.37±10.52 (mean± SD) %.

The newly hatched out larvae had a transparent body, large eyes, open mouth and a small yolk sac. Immediately after hatching, the larvae were found floating on the surface vertically with up head position and larval size ranged between 3.5 - 3.8 mm in length. After 3 - 5 hrs, the larvae were transferred to 50 l FRP larval rearing tanks with algae enriched rotifer (Figure. 2) the stocking density of 5 fish l^{-1}. Milky pigment colour band started appearing on 15-17^{th} days of post hatch and complete *Artemia* nauplii feeding started (Figure. 3). On completion of 22^{nd} day, almost all the fries attained full body colouration pattern of an adult fish and from 25th day onwards all fins got their peculiar yellow colour in the ventral region, pelvic fins and caudal fin and the total metamorphosis took place in this time. Following this, fries were transferred to the grow-out tanks, containing sea anemones. The survivals of larvae were about 53.67±3.93 (mean± SD) %, in all spawning (Figure.4).

3.3 Juvenile rearing

After 45 days of rearing, the juveniles attained the size of 0.8 - 1.0 cm (Figure. 5.a.) and later the entire batch was transferred to 1000 l FRP growout tank containing the sea anemone, *S. mertensii*. Within a day, all the juveniles got acclimatized with the anemones and accepted the minced frozen *Artemia*, squashed boiled oyster and prawn meat and live *Acetes* sp. The young ones attained the marketable size (3cm) after 90 days of rearing from the post hatch (Figure. 5.b.). Variable growth of the juveniles was commonly observed and they were grouped by grading based on their sizes.

4. Discussion

Rearing of clownfishes in hatchery conditions involves minimum challenges as compared to other marine fishes. There are many breeding experiments, conducted successfully on different species of clownfishes using the running seawater (Gopakumar *et al.,* 2001). But quite contrary to this, the present study is one of the first successful attempts on the broodstock development, spawning, larval rearing and juvenile production of *A. clarkii* in captivity using the brackish water.

The first indication of the spawning readiness is that the male swims up and down in front of the female and this behaviour is called “clownfish waggle” (Gopakumar *et al.,* 2001) as reported in other species of clownfishes and the same male behaviour was observed in the present study. Spawning usually occurs in anemonefishes during morning hours as reported by Thresher (1984) and after spawning, the male takes responsibility of attending the eggs while the female acts as the supervisor of her male (Satheesh, 2002) as observed in the present findings. Fecundity rate, clutch size and spawning frequency depend on several factors such as feed quality, brooder health and environmental parameters. Though the brood fishes were maintained in the brackish water in the present study, the fecundity and spawning frequency were similar to those kept in running seawater (Gopakumar *et al.,* 2001). After fertilization, the parents specially male took

care of the eggs by fanning with their pectoral fins and cleaned the clutch area by gently mouthing them without disturbing and this process was continued until hatching. Similar observations were reported in other clown species (Gopakumar *et al.,* 2001) thus using brackish water in our study did not change the parental behaviour of *A. clarkii*.Development of eggs was observed through colour changes, in the clutch. Silvery colouration with distinct visible eyes is usually a good indication, for the hatch out within 12 hours. These observations are also similar to those reported for other clown species (Madhu *et al.,* 2006). There are variations in time and development of embryos among different genus and species of fishes. Several factors such as photoperiods are known to affect the growth and development of clownfish by Olivotto *et al.* (2003). In this context, present study also followed the 12 hrs light and 12 hrs dark periods (Arvedlund *et al.,* 2000) is optimal for the successful broodstock development in *A. clarkii*.

According to Ignatius et al. (2001), the days to reach metamorphosis varied between 12-15 days for *A. sebae*; 9-10 days for *A. ocellaris* (Olivotto *et al.*, 2003)., 11-12 days for *Premnas biaculeatus* (Madhu, *et al.,* 2006) and 12-15 days for in *A. chrysogaster*, it was reported by Gopakumar (2001). But the in present study, complete metamorphosis was observed in between 25 days.

Briefly, with the increasing demand for the captive-produced marine ornamental fish, particularly clownfish, *A. clarkii* was successfully reared in captivity by using brackish water. This achievement will assure success in raising subsequent generations by the aquaculturists for the prolonged existence of aquarium keeping of this species. Interest in production of clownfish in brackish water areas such as the Indian coastal region would most likely be best served by the clownfish that is adapted to the local brackishwater conditions. However, further studies into the adaptation processes to decreasing salinity are required in order to truly understand the potential for clownfish production in low saline brackish water areas and this will help the coastal fisher folk to enhance their livelihood through trade their hatchery bred ornamental fishes and significantly, it will help conserve the precious marine biodiversity.

5. Acknowledgement

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1.a. 1.b. 1.c. 1.d 1.e 1.f 1.g 1.h

Fig. 1: Embryonic development of *A. clarkii,* (2 a) Just laid egg, (2 b) 1st day, (2 c) 2nd day, (2 d) 3rd day, (2 e) 4th day, (2 f) 5th day, (2 g) 6th day & (2 h) 7th day.

2.a 2. b

Fig.2: Hatchery produced youngones of *A. clarkii* (2.a & 2.b).

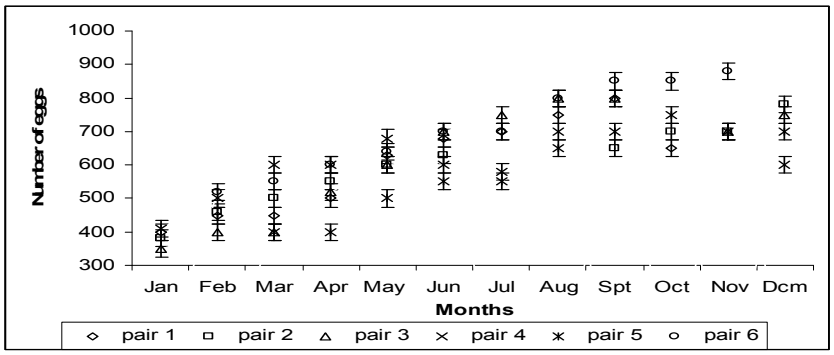


Fig.3: Egg laying capability of *A. clarkii* during several months (different symbol and bars indicates the mean value and standard error, respectively).

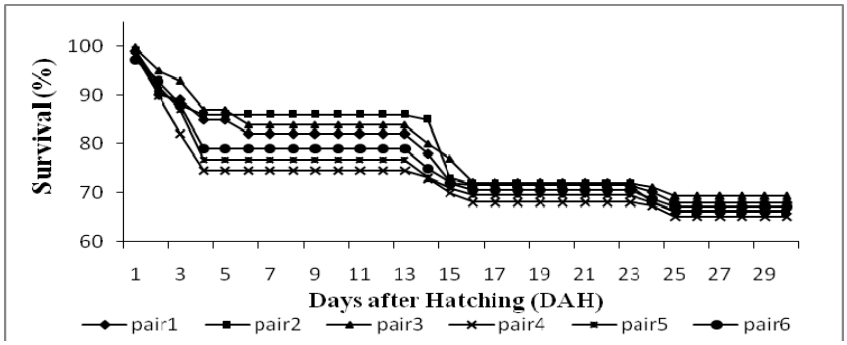
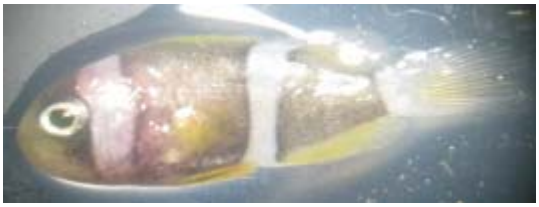


Fig.4: Survival of juveniles (0-30 DAH) of *A. clarkii* during culture period (different symbol and bars indicates the mean value and standard error, respectively).



5. a

5.b

Fig.5: a. 30th day old juveniles of *A. clarkii* and 5. b. 60th day old juveniles of *A. clarkii*.