Reef fish Breeding and Hatchery Production Using Brackishwater, A Sustainable Technology with Special Reference to Clark's Clownfish, Amphiprion Clarkii (Bennett, 1830)

Swagat Ghosh, T. T. Ajith Kumar, K. Nanthinidevi, and T. Balasubramanian

***Abstract*—The present study on spawning and larval rearing of reef associated clownfish, *Amphiprion clarkii* was investigated under captive condition. Fishes 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 up to eight days for incubation. Spawning, embryonic development, hatching success, larval survival and juvenile production were studied 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 to 17th day, an indication of metamorphosis. The complete metamorphosis was occurred on 25th day 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 brackishwater, by removing the existing foremost technological snag of rearing them in running seawater.**

***Index Terms*—Amphiprion clarkii, brackishwater, brood stock, larval development, juvenile.**

I. 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 [1, 2].

Among the coral associated fishes, clownfishes belonging to the family, Pomacentridae and subfamily Amphiprioninae are abundant [3] and about 30 species have been recognized under two genera, *Amphiprion* and *Premnas* [4,5]. These fishes have some remarkable behavioural characteristics such as symbiotic association with sea anemones [6-8], formation of a group consisting monogamous pairs and protandrous hermaphrodites [7, 9-11] and their adaptability to live in captivity, easiness to be fed with artificial diets and their fascinating display behaviour [12]. 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 sea anemones [8]. This species is popular aquarium fish and can be bred and reared in captivity [7].

The hatchery bred fishes are hardier in nature, grow better in captivity, survive well and fetch attractive prices. However, rearing of a reef-associated fish in low saline water, particularly brackishwater 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 [13], which has been attributed in the present study by providing proper water treatments and filtration systems. The reason for brackishwater culture was, once the technology will be perfected, it could be reached to the coastal folk where the wide range of estuarine and mangrove water areas can be utilized by setting up of backyard hatcheries as livelihood option. The aim of this study was to develop suitable rearing facilities, adopting low cost methods for improving the survival and growth of *A. clarkii* larvae and juveniles.

II. Materials and Methods

A. Brood stock Development, Acclimatization in Brackishwater 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, Chennai and transported to the hatchery at Centre of Advanced Study in Marine Biology, Annamalai University, Tamil Nadu. The duration of the study was April, 2009 to March, 2010. The fishes were acclimatized in quarantine tank for ten days with gradually mixing of freshwater and the salinity was maintained at 26 psu and later they were shifted to the conditioning tank filled with two tons of filtered brackishwater. After three months rearing, pair formation occurs (total length - 70 to 100 mm) and each pair were separated and introduced into 750 l capacity spawning tanks [Fiberglass Reinforced Plastics (FRP)] along with their host anemone (Fig. 1.). The tanks were equipped with under water biological filtration

Manuscript received January 2, 2012, revised January 28, 2012. Authors are grateful to the authorities of Annamalai University for providing facilities and the Ministry of Environment & Forests, Govt. of India for financial support.

Swagat Ghosh, T. T. Ajith Kumar, K. Nanthinidevi and T. Balasubramanian are with the Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608502, TamilNadu, India (email: swagotor@gmail.com; ajith87@gmail.com; vani.nandi@gmail.com).

system made up of ceramic rings, activated carbons and coral sands. The fishes were fed with boiled oyster meat and prawn thrice a day at 08:00, 13:00 and 16:00 hrs [12]. One hour after feeding, uneaten food particles and fecal matter were siphoned out to avoid water ruining. During the feeding time, aeration and filtration systems were switched off.

Fig. 1. Spawning pair of *A. clarkii* with sea anemone *S. mertensii* and egg deposition

Before spawning, the pairs exhibit their courtship behaviours, where the male showed morphological and behavioural changes such as fin erection, chasing, clutch preparation, 'signal jumping' and biting the anemone. It was continued with swimming motions and finally, extension of anal, dorsal and pelvic fins accompany the aggressiveness of the male. Fishes were layed eggs in the side of the tank and ceramic tiles and they eggs were allowed to hatch in the spawning tank itself and the parents were retained the same tank.

B. Day's Wise Embryonic Development

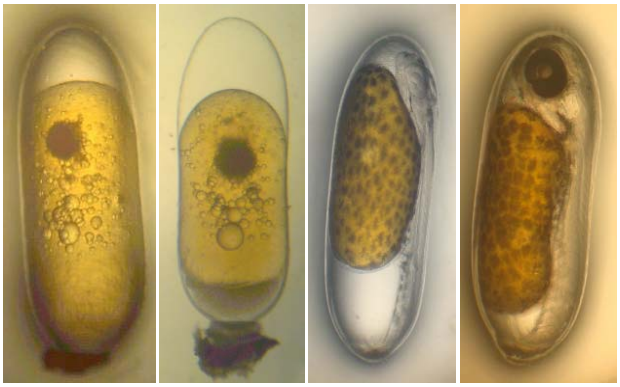
The eggs were sampled randomly (5 eggs/day) to document the embryonic development. The eggs were placed into sterile glass slide with UV filtered brackishwater. Each egg was placed on a slide to observe the morphological development [14]. The photographs were aken, using a digital camera (Canon, China) with a light microscope (Novex, Holland) from day 1st to 8th for documenting major morphological and functional changes of the embryos. Milky white eggs found in the sample were considered as dead or unfertilized and were removed. The eggs took 7 - 8 days for hatching, mainly depends on surrounding temperature.

C. Culture of Live Feeds Micro-Algae

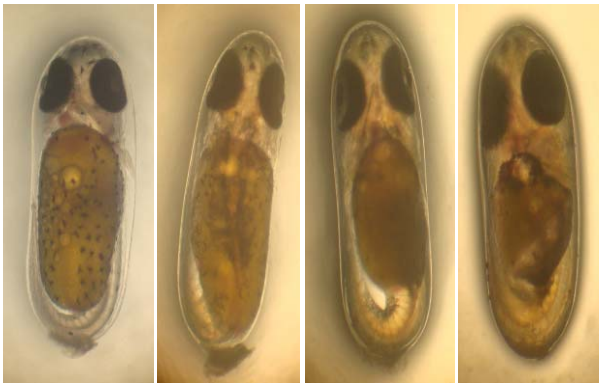
For the stock culture of marine micro-algal species, *Nannochloropsis salina* was collected from private hatchery and developed using Conway medium and agricultural fertilizers for mass culture. The harvested algae were used as enrichment for rotifers, wild plankton and newly hatched *Artemia* nauplii.

Rotifers

B. rotundiformis and *B. plicatilis* were also collected from the private hatchery and mass cultured using micro-algae as feed. The rotifers were harvested on the third day when they reached at a concentration of 20-25 nos/ml.



2.a 2.b 2.c 2.d



2.e 2.f 2.g 2.h

Fig. 2. 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.

3.a (up) and 3.b(below)

Fig. 3. Hatchery produced youngones of *A. clarkii* (3.a and 3.b).

Artemia cysts (Supreme plus, Golden West Artemia, U.S.A.) were procured from aqua shops and allowed to hatch in 250 l capacity FRP cylindrical tank with transparent

bottom. Vigorous aeration and an artificial light for 24 hrs were provided. The *Artemia* cysts hatched out after 18 - 24 hrs and the nauplii were enriched with micro-algae for an hour and used as larval feed.

D. Larval Rearing with Algae and Rotifers

Colours of the eggs became silvery from orange and black 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* Photoperiod was maintained 12 hrs light and 12 hrs dark [15]. 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 larvae l^{-1}. 10% of water exchange was done from the second day onwards along with bottom cleaning to avoid excess build up of organic load. No additional algae were added during water exchange. Water quality was maintained as in the parent's tank. The newly hatched larvae were initially fed with cultured rotifer, *Brachionus rotundiformis* (Lorica length range - 100 to 210 mm) during 2nd to 4th day and *B. plicatilis* (Lorica length range - 130 to 340 mm) from 5th to 10th day (Fig. 7).

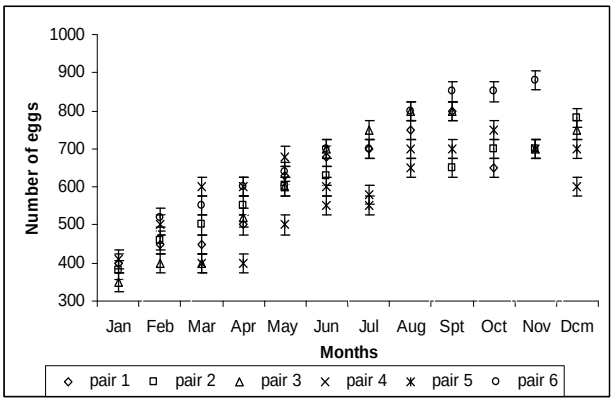


Fig. 4. Egg laying capability of *A. clarkii* during several months (different symbol and bars indicates the mean value and standard error, respectively).

E. Juvenile Production

After rearing 30 days in the same setup, the juveniles were collected and stocked in 500 l glass tank containing sea anemone, *S. mertensii* and (Fig. 3.a & 3.b) they were fed with squashed mussel, frozen *Artemia* and *Acetes* sp.

III. Data Analysis

Statistical analysis was done with the SPSS statistical software and significance level of $P<0.05$ was used. One way ANOVA was performed with different variables namely survival, number of eggs, rotifers and *Artemia* nauplii at morning and evening feeding.

IV. Results

A. Spawning Behaviour, Mating and Egg Incubation

The fishes developed as pairs within three months interval and they were taken from the conditioning tank and kept in the spawning tank along with the sea anemone. All the pairs were found spawning within the next three months rearing, after brief courtship viz. males attracted the females by extending fins, biting and chasing. The fishes showed their readiness to spawn by extending their peculiar activities which were documented. After cleaning egg laying substratum, the female pressed its body towards the tank wall and slowly moved in a rowing fashion using pectoral fins. The female made several passes over the nest and eventually laid yellowish orange colour eggs and this process actively lasted for 50 - 60 minutes. Spawning was observed during morning hours in between 7 - 9 a.m. and it lasted for 50 - 60 minutes. The fecundity was 513 ± 54 (Fig.4) and it was 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 and third day onwards, brownish which continued as dark brown and finally with silvery colour. The unfertilized eggs were selectively removed by the parent and it was observed that the male spent maximum time near the clutch during the course of incubation which lasted for 7 - 8 days. During the study period, the fishes spawned frequently i.e. twice a month, throughout the year, except one month.

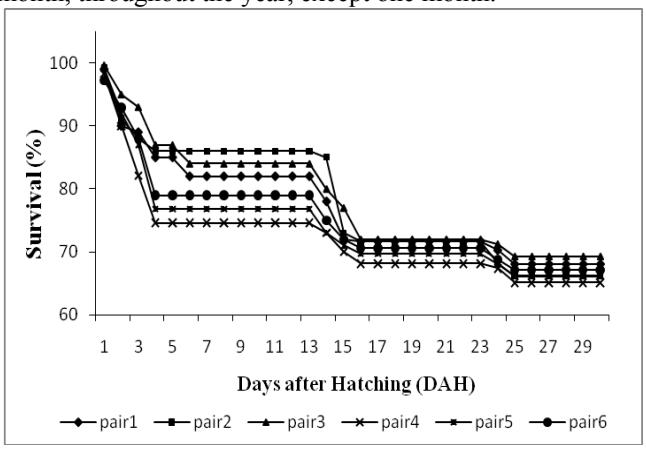


Fig. 5. Survival of juveniles (0-30 DAH) of *A. clarkii* during culture period (different symbol and bars indicates the mean value and standard error, respectively).

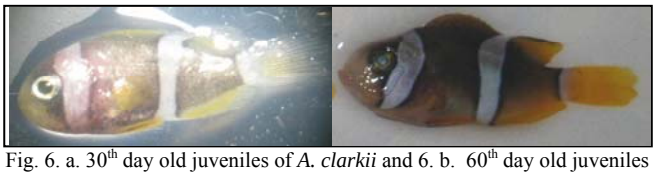


Fig. 6. a. 30th day old juveniles of *A. clarkii* and 6. b. 60th day old juveniles of *A. clarkii*.

B. Hatching and Larval Rearing

Hatching took place invariably in darkness between 1800 - 2000 hrs. The eggs underwent several distinct colour changes from orange (1st and 2nd days) to dark brown (3rd day to 7th day) to silvery (8th day) (Fig. 2. a-h). When the eggs become silvery they would hatch out within 12 hrs. Hatching took place during dusk and 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 the stocking density of 5 individual's l^{-1}. Milky pigment colour band started appearing on 15 - 17th days of post hatch.

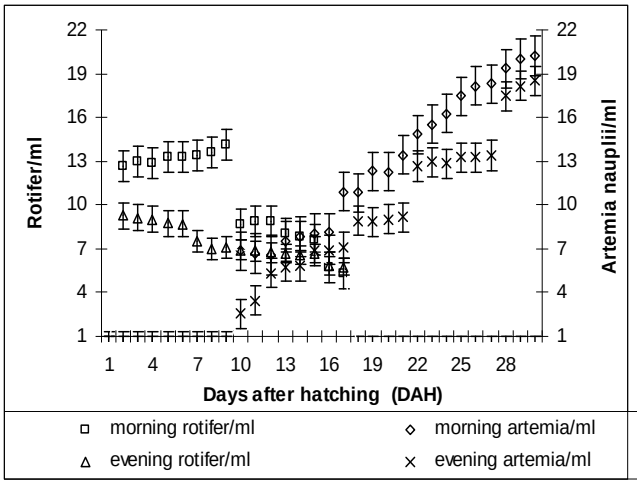


Fig. 7. Feeding behaviour of *Amphiprion clarkii* (0-30 DAH) during morning and evening /day with initial rotifers and later Artemia nauplii (different symbol and bars indicate the mean value and standard error, respectively).

On completion of 22nd day, almost all the fries attained full body colouration of an adult fish and from 25th day onwards, all the fins got their peculiar yellow colour and the total metamorphosis took place in this time. This stage is considered as the completion of metamorphosis. Followed, the fries were transferred to the grow-out tanks, having sea anemones. The survival of larvae was about 53.67±3.93 (mean± SD) %, in all the spawning (Fig.5).

C. Juvenile Production

After 30 days of rearing, the juveniles attained the size of 0.8 - 1.0 cm (Fig. 6.a) and later the entire batch was transferred to 1000 l FRP growout tank with 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 (2 cm) after 60 days of rearing from the post hatch (Fig. 6.b). Variable growth of the juveniles was commonly observed and they were grouped by grading based on their sizes.

V. Discussions

Rearing of clownfishes in hatchery conditions involves minimum challenges as compared to other marine fishes (Fig.8). There are many breeding experiments conducted successfully on different species of clownfishes using the running seawater [12, 15-16]. But quite contrary, 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 brackishwater. The spawning frequency and fecundity of these fishes in both seawater and brackishwater was studied and which proved that there is no significant change between them [17]. Hence, this technology can be extended to the coastal community of other regions including mangrove, estuarine and backwaters for the establishment of back ward hatchery for their livelihood development. At the initiation of the work, priority was given on the selection of healthy sub adults for developing the broodstock and provided with suitable rearing facilities for natural spawning. If suitable husbandry and environment are provided, many species of fishes will undergo gonadal maturation in captivity [18]. Good quality of eggs could be obtained only based on the balanced nutrition provided to their parents. After hatching, the major portion of the yolk in clownfish larva is normally exhausted during the initial stages of development; so it is essential that the larvae are to be supplemented with suitable live feed on the second day onwards. In the present study, different live feeds such as micro-algae, rotifer and *Artemia* nauplii were given as larval feed. Infact, rotifer and *Artemia* nutrition represents the underlying foundation for the successful rearing of larvae.

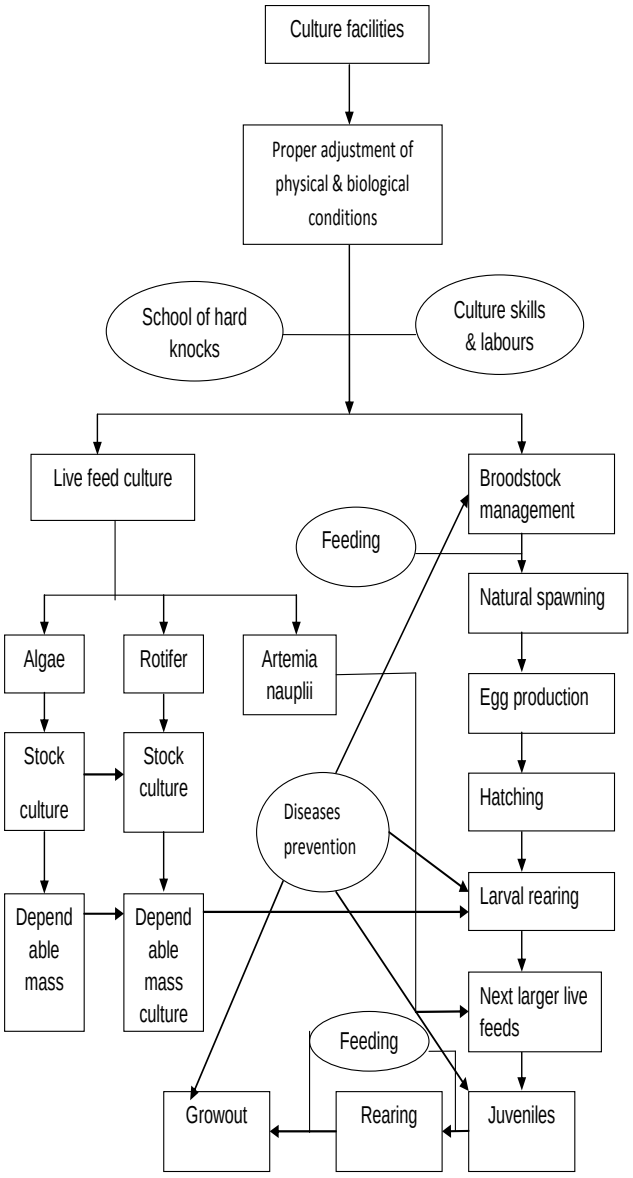


Fig. 8. Imperative hatchery equipment of successful clownfish (*Amphiprion clarkii*) production in brackishwater.

The first indication of the spawning readiness is that the male swims up and down in front of female and this behaviour is called "clownfish waggle" [19] as reported in other species of clownfishes and the same behaviour was observed in the present study also. Spawning usually occurs in anemonefishes during morning hours as reported by Thresher [20] and after spawning, the male takes responsibility of attending the eggs while the female acts as the supervisor of her male [21], 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. Even though the brood fishes were maintained in the brackishwater, the fecundity and spawning frequency were similar to those kept in running seawater [12, 15-16]. After fertilization, the parents especially the 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 [12, 19], thus using low saline water have to influence in 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 hatchout within 12 hours. These observations are also similar to those reported for other clown species [22]. 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 [15]. In this context, in the present study, 12 hrs light and 12 hrs dark periods was adopted, which is suitable for successful broodstock development in *A. clarkii*.

After hatching to reach metamorphosis, the time taken was 12-15 days for *A. sebae* [12]; 9-10 days for *A. ocellaris* [23], 11-12 days for *Premnas biaculeatus* and 12-15 days for in *A. chrysogaster* [19]. But the in present study, complete metamorphosis was observed on 25th day. This was the major difference observed in the present study, which needs detail investigation with comparative study along with seawater system.

VI. Conclusion

Briefly, with the increasing demand for the captive-produced marine ornamental fishes, particularly clownfish, *A. clarkii* was successfully reared in captivity 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. Production of clownfish in brackishwater areas along the Indian coastal region would most likely be best option for livelihood development. However, further studies on the adaptation processes to decreasing salinity are required in order to truly understand the potential for clownfish production in low saline brackishwater areas and this will help the coastal fisher folk to enhance their livelihood through setting up of backyard hatcheries and significantly, it will help conserve the precious marine biodiversity.

References

[1] Y. Sandovy "A preliminary assessment of the marine aquarium export trade in Puerto Rico". In: *Proceedings of the 7th International coral Reef symposium (ed. Richmand, R. H.)* Guam, University of Guam press. Vol. 2, pp. 1014-1022, 1992.

[2] C. Wabnitz, M. Taylor, E. Green, T. Razak. "From ocean to aquarium" UNEPWCMC, Cambridge, U.K. pp. 66. 2003.

[3] D. G. Fautin and G. R. Allen. "Field guide to host anemones and anemone fishes". *Western Australia Museum*, Perth pp. 145. 1992

[4] G. R. Allen , J. A. Drew, and L. Kaufman. "*Amphiprion barberi*, a new species of anemonefish (Pomacentridae) from Fiji, Tonga, and Samoa". *Aqua: International Journal of Ichthyology*, vol.14, no. 3, pp. 105-114, 2008.

[5] R. N. Mariscal. "An experimental analysis of the protection of *Amphiprion xanthurus* Cuvier and Valenciennes and some other anemonefishes from sea anemones". *J. Exp. Mar. Bio. Ecol.*, vol. 4, pp. 134-149, 1970.

[6] G. R. Allen. "The Anemone fishes: Their classification and biology". *T. F. H. Publications, Inc; Neptune City*, New Jersey pp. 288. 1972.

[7] D. G. Fautin and G. R. Allen. "Anemone fishes and their host sea anemones". *Western Australian Museum*. pp. 160, 1997.

[8] H. W. Fricke and S. Fricke. "Monogamy and sex change by aggressive dominance in coral reef fish", *Nature*, vol. 266, pp. 830-832, 1977.

[9] J. T. Moyer and A. Nakazono. "Protandrous hermaphroditsm in six species of the anemone fish genus *Amphiprion* in Japan". *Jap. J. Icthyol* 25, 101-106, 1978

[10] R. M. Ross. "Territorial behaviour and ecology of the anemone fish *Amphiprion melanopus* on Guam". *Z. Tier psychol.*, vol. 46, pp. 71-83, 1978.

[11] B. Ignatius, G. Rathore, I. Jagdish, D. Kandasmi and A. C. C. Victor. "Spawning and larval rearing technique for tropical clownfish *Amphiprion sebae* under captive condition". *J. Aqua. Trop* vol. 16, no. 3, pp. 241-249, 2001.

[12] J. L. Overton, M. Bayley, H. Paulsen and T. Wang. "Salinity tolerance of cultured Eurasian perch, *Perca fluviatilis* L.: Effects on growth and on survival as a function of temperature". *Aquaculture* vol. 277, pp. 282-286, 2008.

[13] H. J. Liew, M. A. Ambak, A. B. Abol-Munafi. "Embryonic development of clownfish *Amphiprion ocellaris* under laboratory conditions". *J. Sus. Sci. Manag. Vol.* 1, no.1, pp.64-73, 2006.

[14] I. Olivotto, M. Cardinali, L. Barbaresi, F. Maradonna and O. Carnevali. "Coral reef fish breeding: The secrets of each species". *Aquaculture.* Vol. 224, pp. 69 -78, 2003.

[15] V. R. Alva and L. A. O. Gomes. "Breeding marine aquarium animals: The anemone fish". *The Naga Iclarm Quarterly*, 12-13, 1989.

[16] J.D. Wilkerson. "Clownfishes. A guide to their captive care, breeding and natural history". *1st ed. Microcosm Ltd.,* Shelburne, VT. pp.240, 1998.

[17] M. Arvedlund, I. Bundgard and L. E. "Nielson Host imprinting in anemone fishes (Pisces: Pomacentridae): Does it dictate spawning site preferences?". *En. Biol. Fishes.* vol.58, pp. 203-213, 2000.

[18] G. Gopakumar, R. M. George and S. Jasmine. "Hatchery production of the clown fish *Amphiprion chrysogaster*. Perspective in Mariculture". *J. Mar. Biol. As. In*. pp. 305-310, 2001.

[19] K. Melville and S. Griffiths. "Recent developments in disease management". *Bull. Aqua. Asso. Can.,* selected pub. Vol. 2. Pp19, 1997.

[20] R. E. Thresher. "Reproduction in reef fishes". *T. F. H publication*, Neptune city, pp. 399, 1984.

[21] J. M. Satheesh. "Biology of the clown fish, *Amphiprion sebae* (Bleeker) from Gulf of Mannar (South east coast of India)". Ph. D thesis, Annamalai University, India 12-140, 2002.

[22] K. Madhu, R. Madhu, L. Krishnan, C. S. Sasidharan and K. M. Venugopalan. "Spawning and larval rearing of *Amphiprion ocellaris* under captive conditions". *Mar. Fish. Info. Ser., T & E Ser.*, pp. 188, 2006.

T.T. Ajith Kumar received his M.Sc. degree in Marine Biology and Oceanography in 1993 and Ph.D. in 2001 from the Centre of Advanced Study in Marine Biology, Annamalai University. He is working as Assistant Professor in this centre. His research interests include marine ornamentals, technology development and extension.

Swagat Ghosh received his M.Sc. degree in Marine Biology and Oceanography in the year the 2008 and the M. Phil. at 2010 from the Centre of Advanced Study in Marine Biology, Annamalai University and he is doing Ph.D. in this centre. His research interests include marine ornamentals fish breeding and culture.