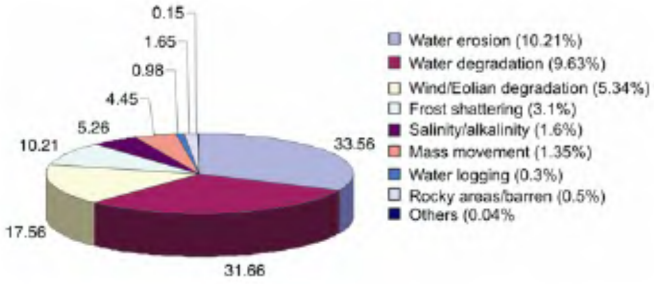

Figure 7. Processwise status of land degradation in India (mha).

of the TGA) under land degradation, followed by J&K (12.79% of TGA), Maharashtra (12.66% of TGA) and Gujarat (12.72% of TGA).

The boundaries of arid, semi-arid and dry subhumid regions of the country were superimposed on the DSM of India to find out the area under desertification. The processwise area under desertification for the country is given in Table 3.

In India, the total area under desertification is 81.45 mha. Water erosion (26.21 mha), wind erosion (17.77 mha), vegetal degradation (17.63 mha) and frost shattering (9.47 mha) are the major processes of desertification.

Nearly one third of the country's land area (32.07%) is undergoing processes of land degradation. There are about eight major processes of land degradation active in the country. Water erosion is the most pronounced process, followed by vegetal degradation and eolian processes. Total area under land degradation is 105.48 mha. Area-wise Rajasthan, J&K, Gujarat and Maharashtra have high proportions of land undergoing degradation. 81.45 mha land area of the country is undergoing the process of desertification. This study provides baseline data on desertification/land degradation for the country and will be useful for future monitoring of desertification.

1. UNEP, A Status of Desertification and Implementation of the United Nations Plan of Action to Combat Desertification. Report of the Executive Director, Nairobi, United Nations Environment Programme, 1992.
2. NBSS&LUP, Agro-ecological subregions of India for planning and development, NBSS&LUP Publication, ICAR, Nagpur, 2001.
3. MoEF, National Action Programme to Combat Desertification. Status of Desertification, Ministry of Environment and Forests, Govt of India, September 2001, vol. 1, p. 293.
4. Ajai *et al.*, Desertification Monitoring and Assessment using Remote Sensing and GIS: A Pilot Project under TPN-1 UNCCD SAC/RESIPA/MESG/DMA/2007/01, 2007.

ACKNOWLEDGEMENTS. We thank Dr R. R. Navalgund, Director, Space Applications Centre (SAC) for his encouragement and guidance. We are grateful to Dr V. S. Hegde, Director, EOS and Dr J. S. Parihar, Mission Director, EOAM, SAC for providing all support and guidance. We thank all the collaborating agencies for their necessary support and help. We also thank Mr Rui Zheng, UNCCD, Bonn for providing all necessary information and support from time to time.

Received 24 March 2009; revised accepted 14 October 2009

Broodstock development, spawning and larval rearing of the false clown fish, *Amphiprion ocellaris* in captivity using estuarine water

T. T. Ajith Kumar* and T. Balasubramanian

Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai 608 502, India

Broodstock development, spawning and larval rearing of the false clown fish, *Amphiprion ocellaris* is studied under captive condition. *A. ocellaris* spawns in seawater and has also been bred in captivity using estuarine water. Continuous aeration and 10–20% water exchange was provided on alternate days. Temperature, salinity, pH, dissolved oxygen and ammonia were maintained at optimum levels. The fish were fed twice a day with various feed combinations like prawn/mussel/squid meat and live *Acetes* spp. At the age of six months in the hatchery, the fish attained 6.5–8 cm length and 14.6–15 g weight at the time of first spawning and laid eggs. The newly laid eggs were orange in colour, translucent and capsule shaped. The parents were allowed to remain in the spawning tank till hatching, which took place after 6–8 days and the hatching success rate was 90–95%. The newly hatched larvae measured 3–4 mm in length and were transferred into separate rearing tanks. They were fed with rotifers and *Artemia* nauplii and 21 days after hatching, the juveniles shifted from pelagic to epibenthic mode of life. The young ones were fed with different wet feed and they attained marketable size within three months. This is the first report on hatchery production of false clown fish, *A. ocellaris* both in India and elsewhere using estuarine water.

Keywords: *Amphiprion ocellaris*, broodstock maintenance, estuarine water, juvenile production, larval rearing.

IN recent years, there has been a surge in the trade of tropical ornamental fishes and at the same time indiscriminate exploitation has also led to negative repercussions on coral reef ecosystem. For the last two decades, the marine aquarium fish trade has witnessed steady growth[1,2]. At present, the wholesale value of the global ornamental fish trade is estimated at around 6 billion US$. According to the Global Marine Aquarium Database (GMAD), the annual global trade varies between 20 and 24 million fishes, and 9 and 10 million for other invertebrates[3]. About 90% of the freshwater fishes are farmed and the rest are collected from the wild. But in the case of marine ornamental fishes, about 95% are from the wild, whereas 5% are captive-bred[4].

*For correspondence. (e-mail: tt_ajith@yahoo.co.in)

As many as 400 species of marine ornamental fishes belonging to 175 genera coming under 50 reef families are known to occur in the Indian seas. Culture practices of marine ornamental fishes in an eco-friendly way have been well accepted to increase the supply of the same by reducing the pressure on wild populations and producing juveniles of a wide variety of species round the year. In addition, hatchery bred fishes are hardier in nature, grow better in captivity and survive well. The high value at their young age is the key advantage of marine ornamental aquaculture. More than 84 species of marine ornamental fishes which come under groups such as clowns, damsels, gobiids, cardinals and pseudochromids can be reared in captivity[5]. Of these, breeding has been reported only for 26 pomacentrids[6].

Clown fishes belong to the diverse and well-distributed family Pomacentridae. There are 28 known species belonging to two genera: *Amphiprion* and *Premnas*. They are beautiful tropical marine aquarium fishes suited for home aquaria, hence they are in great demand in the international market. They are morphologically and taxonomically well-distinguished from the damsel fishes due to their dependency on sea anemone for their protection. The popularity of clown fish among the aquarists is due to their small size, hardy nature, attractive colour, adaptability in captivity and interesting behaviour display in association with anemone. Though India holds rich stock of innumerable varieties of ornamental fishes of colourful design, the technique for sustainable harvest and culture using the easily manageable estuarine water has not been perfected.

A. ocellaris, the false clown fish is mainly found in association with sea anemone species such as *Heteractis magnifica*, *Stichodactyla gigantean* and *S. mertensii* as part of their symbiotic relationship[7,8]. The recorded maximum size of this fish is about 11 cm with bright orange colour and three white bars on the head and body. Females are larger than the males. It has 11 dorsal spines and 17 pectoral fin rays, which help to distinguish from the closely related *Amphiprion percula*[9].

Six numbers of *A. ocellaris* with size ranging from 4.5 to 6.0 cm length and 12.5 to 13 g weight along with sea anemone *Heteractis magnifica* and *Stichodactyla mertensii* were procured from ornamental fish traders and transported to the hatchery at the Centre of Advanced Study in Marine Biology, Annamalai University. They were domesticated in 5 tonne capacity cement tank filled with 2 tonne UV-treated estuarine water with salinity range of 22–24 g/kg. An Eheim brand canister filter loaded with activated carbon, bio-balls, ceramic rings and coral sands was installed in the tank with pumping capacity of 3400 l/h. The schematic diagram of experimental set-up is given in Figure 1. Continuous aeration and 10–20% water exchange were provided on alternate days. The fishes were fed thrice a day with different feed combinations such as prawn, mussel, squid meat and live *Acetes* at 5% body weight. Algal accumulation and excess feed were siphoned out to avoid water spoilage.

A spawning tank of 750 l water holding capacity was used. Bottom of the tank was provided with broken coral pieces, dead shells and live rocks to imitate natural environment. A locally made, low-cost underwater filtration setup using activated carbon, ceramic rings and coral sand was also kept in the tank. Because this spawning tank was kept in a closed system, artificial light was fixed at the top of the tank to maintain the light intensity of 2500–3000 lux for 12 h/day.

Stock culture of marine microalgal species such as *Chlorella marina* and the rotifer *Brachionus rotundiformis* were obtained from the live feed culture laboratories and mass cultured by using agricultural fertilizers. *Artemia* cysts were allowed to hatch in FRP (fibreglass reinforced plastic) container with vigorous aeration and artificial light for 24 h. The rotifers and *Artemia* nauplii were enriched with microalgae and used as larval feed.

After 4–6 h of hatching, the floating larvae of *A. ocellaris* were transferred to 250 l larval rearing tank. The tank was provided with mild aeration and 10–15% water exchange was made daily along with bottom cleaning to avoid excessive build up of organic load. Water quality parameters such as temperature, salinity, pH and dissolved oxygen were maintained at optimum levels (28 ± 2°C, 22–24‰, 7.5–8.1 and 4.5–5 mg/l). The larvae were fed with rotifer, *B. rotundiformis* from the 2nd day after hatching (dh) till the 10th dh at the density of 5–10 no./ml. From the 11th dh onwards, the larvae were weaned on newly hatched *Artemia* nauplii enriched with *Chlorella* at the rate of 6–8 no./ml. Density of rotifers and *Artemia* was periodically measured and supplemented, when needed.

Among the six fishes, after a period of two months, one pair outgrew others and settled on the sea anemone, *Stichodactyla mertensii* (Figure 2). They became the potential spawning pair and were transferred from conditioning

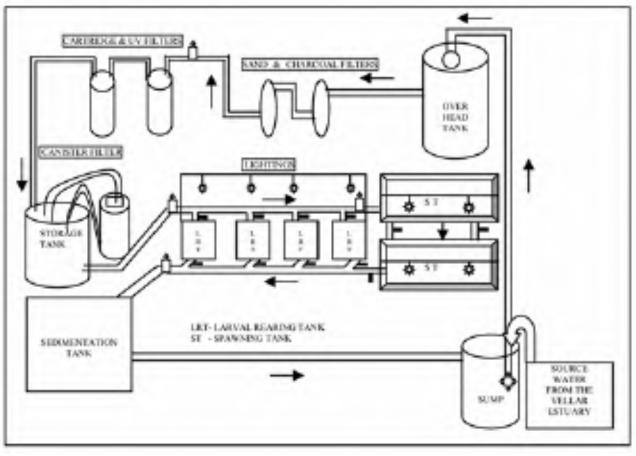

Figure 1. Schematic of the hatchery set-up.

tank to spawning tank. As behavioural changes are a vital part of monitoring the health of fishes and also their readiness to lay eggs, the potential spawning pair was kept under close observation. After four months of rearing in the spawning tank, the spawning pair exhibited typical courtship behaviour and the male attracted the female by extending his fins, biting and cleaning the bottom of the tank to make a nest. Spawning was observed in the morning between 07.00 and 10.00 hours.

When spawning commenced, the female pressed its body towards the substrate and slowly moved in a rowing fashion using pectoral fins. While spawning was about to occur, the male chased the female to the nest. The female passed several times over the nest and eventually laid orange colour eggs measuring 1.4–2.9 mm length and this process actively lasted for 45–60 min. The male then continued the process as he passed over the eggs and fertilized them. The fertilized eggs were orange in colour. The number of eggs varied from 400 to 800. It was initially less but increased in subsequent spawnings. The male took the responsibility of attending to the eggs, which included fanning the eggs and eating eggs that were infertile or infected by fungus. Incubation lasted for 6–8 days depending on water temperature. During rainy season, incubation extends by a maximum of nine days due to water temperature and salinity (below 22 g/kg) of estuarine water dropping down. The embryonic development is shown in Figure 3.

The fish spawned throughout the year except during December and produced an average of 2.4 nests per month. The frequency of spawning showed significant relationship with temperature and increased during summer months. Most spawnings were noticed after 1–5 lunar days, indicating the correlation of spawning with lunar periodicity.

During incubation, the eggs underwent several distinct colour changes orange on first day, blackish from third day onwards and then silvery just prior to hatching. The silvery colouration with distinct eyes is usually a good indication that the eggs will hatch within a night. Hatching took place during dusk between 19.00 and 21.00 hours at 25–32°C. On an average, 90–95% of the fertilized eggs were hatched for each spawning, indicating the suitability of rearing facility. The newly hatched larvae measured 3–3.5 mm in size and 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. After 3–5 h, the larvae were transferred to larval rearing tanks (250 l, FRP) and from the second dh onwards, they were fed with algal enriched rotifer *B. rotundiformis* up to the 10th dh.

The larvae grew to an apparent size at the end of the 10th dh. From the 11th dh onwards, they were weaned on newly hatched *Artemia* nauplii. A low intensity light was provided in the larval tank for 12 h. Orange colour pigmentation started appearing on the 16th dh and on the 21st dh, metamorphosis occurred in the entire batch of larvae and almost all the fries attained full colouration pattern of adult fish. Juveniles started to shift from pelagic to epibenthic mode of life. On the 25th dh, all the juveniles were transferred to 500 l FRP tank containing sea anemone, *Heteractis magnifica* (Figure 4). Within a day or two, the juveniles got acclimatized with the anemone and were accepting minced shrimp, mussel meat and frozen *Artemia*. With this feeding regime, the survival and growth of larvae were hastened and about 50–55% of juvenile survival was obtained for each spawning. The young ones attained the marketable size (3 cm) after 3 months period.

There are many studies which report the successful culture of *Amphiprion* species using seawater[7,10–12]. But, the present study is the first successful attempt on broodstock development, breeding and larval rearing of false clown fish, *A. ocellaris* using estuarine water with salinity range of 22–24 g/kg. When suitable husbandry and conducive environment parameters are provided, as done in the present study, many *Amphiprion* species undergo gonadal maturation in captivity[13]. The most remarkable findings of the present work is that a typical reef fish, *A. ocellaris* can indeed be successfully reared in captivity by using low saline water, as long as the physicochemical characteristics, photoperiod and food chain are optimized. In this context, water quality, suitable diet and lighting cycle are indispensable for successful rearing and development of potential breeding pair in short-term period which is quicker than the previous report on clown fish breeding[14].

The female laid eggs on a suitable nest selected by the male and size of the egg ranged from 1.4 to 2.9 mm length which is smaller than the earlier reports and this might be ascribed to the salinity variations. But the number of eggs laid was less than the fish lining in running seawater (7200–24,000 eggs/pair/year)[15]. The newly hatched larvae were fed with algae as first feed and later with rotifers and finally *Artemia* nauplii. Algal enriched rotifers and *Artemia* nutrition represents the underlying

Figure 2. Spawning pair – *Amphiprion ocellaris* on *Stichodactyla mertensii*.

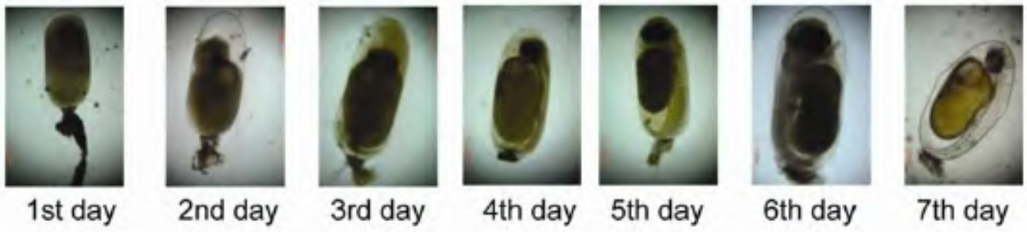

Figure 3. Different stages of embryonic development of *A. ocellaris*.

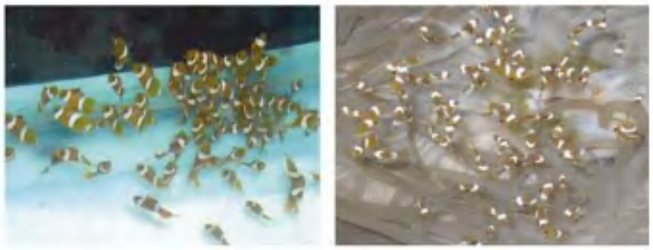

Figure 4. Hatchery produced young ones of *A. ocellaris*.

foundation for successful larval rearing. This feed composition provides fatty acids such as PUFA, HUFA and EFA along with the regular diet[16,17].

Higher mortality of larvae was noticed on the 2nd day throughout the study period when the larvae were fed with rotifers. This indicates that initially the larvae were unable to ingest the rotifers but slowly adapted to the supplement feed and stabilized. Similarly, initial mortality was noticed in studies conducted elsewhere by using seawater also[12]. To reduce the harmful bacterial growth and organic load of decayed materials of feed supplied, 25% water exchange was performed in the larval tank. The quantum of water used to produce the clown fish is similar to the work done using seawater. Studies are also underway to further reduce initial mortality and increase survival rate of larvae. This could be possible by developing suitable continuous water cycling systems and substituting with appropriate initial dietary composition.

This study demonstrates the successful hatchery production protocol for the clown fish, *A. ocellaris* under captive conditions using estuarine water in all the stages.

1. Sadovy, Y., A preliminary assessment of the marine aquarium export trade in Puerto Rico. Proceedings of the 7th International Coral Reef Symposium (ed. Richmand, R. H.), Guam, University of Guam Press, 1992, vol. 2, pp. 1014–1022.
2. Wabnitz, C., Taylor, M., Green, E. and Razak, T., *From Ocean to Aquarium UNEPWCMC*, Cambridge, UK, 2003, p. 66.
3. Gopakumar, G., *Development of a Sustainable Trade on Marine Ornamental Species from India*, INDAQUA, Chennai, 2007, pp. 1–14.
4. Ajith Kumar, T. T., Nithya Jeniffer, P., Murugesan, P. and Balasubramanian, T., Marine ornamentals in India: Challenges and opportunities for sustainability. *Fish. Chimes*, 2007, **27**, 44; 51.
5. Gopakumar, G., Culture of marine ornamental fishes with reference to production system, feeding and nutrition. In *Ornamentals Kerala* (eds Kurup, *et al.*), Souvenir, Department of Fisheries, Kerala, 2006, pp. 61–70.
6. Olivotto, I., Cardinali, M., Barbaresi, L., Maradonna, F. and Carnevali, O., Coral reef fish breeding: the secrets of each species. *Aquaculture*, 2003, **224**, 69–78.
7. Allen, G. R., *The Anemone Fishes: Their Classification and Biology*, T. F. H. Publications, Inc, Neptune City, New Jersey, 1972, p. 288.
8. Fautin, D. G. and Allen, G. R., *Field Guide to Host Anemones and Anemone Fishes*, Western Australia Museum, Perth, 1992, p. 145.
9. Nelson, J. S., Phang, V. P. E. and Chou, L. M., Survival and growth of the anemone fish *Amphiprion ocellaris*: a transfer experiment. *J. Fish. Biol.*, 1996, **48**, 1130–1138.
10. Alva, V. R. and Gomes, L. A. O., Breeding marine aquarium animals: the anemone fish. The NAGA ICLARM quarterly, 1989, pp. 12–13.
11. Malpass Jr, D., Raising *Amphiprion percula*, Tropical fish hobbyist, 1996, pp. 56–61.
12. Boby, I., Gaurau, R., Jagdish, I., Kandasmi, D. and Victor, A. C. C., Spawning and larval rearing technique for tropical clown fish *Amphiprion sebae* under captive condition. *J. Aqua. Trop.*, 2001, **16**, 241–249.
13. Melville, K. and Griffiths, S., Recent developments in disease management. Bulletin of the Aquaculture Association of Canada, 1997, Selected Pub. 2.
14. Madhu, K., Brood stock development of clown fishes. In *Recent Advances in Seed Production and Grow out Techniques for Marine Finfish and Shellfish* (eds Gopakumar, G. *et al.*), Regional Centre of Central Marine Fisheries Research Institute, Mandapam, 2006, pp. 301–309.
15. Rema Madhu, R., Larviculture of clown fishes. In *Recent Advances in Seed Production and Grow out Techniques for Marine Finfish and Shellfish* (eds Gopakumar, *et al.*), Regional Centre of Central Marine Fisheries Research Institute, Mandapam, 2006, pp. 310–320.
16. Watanabe, T., Importance of decosahexaenoic acid (DHA) in marine larval fish. *J. World Aquacul. Soc.*, 1993, **24**, 152–161.
17. Webster, C. D. and Lovell, R. T., Responses of stripped bass larvae fed brine shrimp from different sources containing different fatty acid composition. *Aquaculture*, 1990, **90**, 41–61.

ACKNOWLEDGEMENTS. We thank Annamalai University for providing facilities and the Centre for Marine Living Resources and Ecology (MoES), Cochin for financial support.

Received 5 February 2009; revised accepted 22 September 2009