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1169 Spawning and larval rearing of *Amphiprion ocellaris* under captive condition.

Marine ornamental fishes have gained much popularity all over the world. India is endowed with a variety of marine ornamental fishes distributed in our coral reef areas which offers vast scope for the development of a domestic as well as export trade.

Clown fishes or sea anemone fishes

Among the different marine ornamental fishes, the genera *Amphiprion* and *Premnas* belonging to the family Pomacentridae and sub family Amphiprioninae commonly known as clown fishes or sea anemone fishes are the most popular attractions all over the world because of their tiny size, hardiness, attractive colour features, peaceful nature, high adaptability to live in captivity, acceptability of artificial diet and their fascinating display behaviour and symbiotic relationship with the sea anemones. But most of the traded salt water ornamental fishes are being collected from the wild and hence there is a great concern regarding the depletion of the stocks due to over expoitation as well as the destruction of reef habitat and damaging collection methods. Hence investigations should be focused on the development of hatchery technology and the production can lead to hatchery produced ornamental fishes trade of marine which is long term sustainable. Efforts made in this line in CMFRI, Kochi which resulted in the successful breeding and mass scale production of false clown *Amphiprion ocellaris*, one of the most demanded species among the clown fishes.

Pair formation

In order to make breeding pairs, many social groups of *A. ocellaris* were collected from the wild and transported to the laboratory in live condition. During transportation, the fishes and sea anemones were kept in separate plastic transportation bags. Five fishes of each sex of different size groups were stocked together along with single sea anemone *(H. magnifica)* in 500-liter FRP tanks fitted with biological filter in order to reduce the level of aggressive behaviour. The pair formation tanks were maintained in the hatchery where an incident light intensity of 2500 to 3000 lux was available as the sea anemones require sunlight for its better survival under labortory condition. The fishes and anemones were fed two times per day with wet feeds such as meat of shrimp, mussel and clam at the rate of 15% of their body weight and live feeds like

Brachionus plicatilis and *Artemia nauplii.* In all the tanks, the range of environmental parameters maintained were : temperature : 26 to 29^{O} C, salinity : 33 to 36 ppt, dissolved oxygen : 4.6 to 6.2 ml/l and pH : 8.1 to 8.9 After a period of 3 to 4 months rearing, one pair grew ahead of others in each tank and became the spawning pair and the functional female and male (the two largest specimens of the colony) live together as pairs in which the female dominates male and the sub adults are dominated both by the female and male. The standard length of the female varied between 89 to 100 mm (presumptive female) and that of male varied between 40 to 60 mm (presumptive male).

Brood stock development and maintenance

The pairs developed through pair formation was then transferred to seperate glass aquaria to develop as brook stock in 500 liters capacity tank along with single befitting host anemone. Depending upon the production capacity and seed demand, several pairs can be maintained for commercial hatcheries. The broodstocks were fed with wet feeds such as mussel meat and shrimp, clam meat, fish egg mass and also provided formulated feeds enriched with vitamins, minerals and algal powder at the rate of 10% of their body weight and supplied at an interval of every 3 hrs during day time, whereas in the night, they were fed with live feeds such as rotifers and *Artemia* nauplii at the rate of 3,5 Nos./ml respectively after enriching the same with a mixed culture of micro algae such as *Nannochloropsis oculata, Pavlova lutheri, Isochrysis galbana, Dicrateria inornata, Chromulina pleoides, Cholrella marina* and *Chaetoceros gracilis* which apparently improved egg quality and hatchability than the brooders fed with non enriched live feeds. The temperature in all the breeding tanks were maintained between 26 to 29^{O}C, and level of dissolved oxygen (4.8 to 6.3ml/l), pH (8.0 to 8.9), salinity (32 to 36 ppt) and the water is recirculated to ensure water movement and provided good water quality with the aid of a specially devised filter system during the period of rearing. 25% of the water was exchanged weekly to avoid stress like a rapid increase in plasma corticol concentration, depression of gonadal streroidogenesis, and subsequent development of gonadal atresia. Each brood stock tank was provided with tiles or earthen pots for egg deposition so that the substratum itself along with egg could be transferred to hatching tank and it also helped to minimize the mechanical injury which may happen during transferring of newly hatched

larvae to the larval rearing set up.

Breeding

Each pair started breeding within a period of 4 to 6 months rearing under captive condition. Few days prior to spawning, the male selected a suitable site near to sea anemone for laying the egg and cleared algae and debris with its mouth. On the day of spawing both the parents spent considerable time for the celaning of site which indicated that spawning may occur within few hours. Under laboratory condition, the spawning was noticed between 0500 to 1530 hrs during day time and the spawning lasted for one to one and a half hour. Each female laid 300 to 1000 capsule shaped eggs at every 12 to 15 days interval depending on the size of fish and previous experience. The egg size ranged between 1.5 to 3.0 mm in length with a width of 0.8 to 1.84 mm and adhered to the sides of earthen pot with stalk. An average of two spawnings per lunar month per pair resulting in an estimated annual fecundity of 24000 eggs per breeding pair per year can be obtained under laboratory condition.

Parental care and eggs developments

As parental care is inevitable for hatching the parents were allowed to remain in the parental tank itself till hatching. During incubation period, both the parents carefully looked after the eggs during day time and it involved two basic activities *viz.* fanning by fluttering the pectoral fins and mouthing to remove the dead or weakened eggs and dust particles, but no nocturnal care was noticed. The newly spawned eggs were white in colour for initial two days and as the embryo developed, these turned to black on 3 rd to 6 th day and later turned to silvery on 7th to 8th day of incubation. At this stage the glowing eyes of the developing larvae inside the egg capsule was clearly visible when viewed from a short distance. Male asumed nearly all responsibilities of caring for the eggs and spent a higher percentage of time at the nest than the females, which increased gradually up to 70% of time as the day of hatchling approached. When incubated at a water temperature range of 27 to 29^{O} C, the hatching emerged on 8th day of incubation and peak hatching took place shortly after sunset.

Egg hatching and larval rearing

On the expected day of hatching, two hours before sunset, the parents and eggs along with substratum were transferred from the parental tank to hatching tanks (100 liters) which were provided with complete darkness for accelerating the hatching. The larvae broke

the egg capsule and the hatchlings emerged tail first and the hatching occurred soon after sunset and the peak hatching took place between 1900 to 2200 hrs under darkness. Soon after hatching, the parents were transferred to breeding tank. The newly hatched larvae measured 3 to 4mm in length and each had a transparent body, large eyes, visible mouth, and a small yolk sac and remained at the bottom of the tank for a few seconds and soon after became free swimming. The mouth gape of the newly hatched larvae measured 170 to 210µ. The larval rearing was carried out under green water system . The larvae were initially fed with micro algae and later on they were also fed with super small rotifer *B. rotundiformis* and newly hatched *Artemia* nauplii. First day onwards the larvae were fed with mixed culture of microalgae and rotifer *B. rotundifrmis* (6 to 8 nos/ml) upto 8th day. From 9th day onwards the larvae were weaned onto newly hatched *Artemia* nauplii (4 to 6 nos/ml) along with rotifer and mixed culture of micro algae and on 12th day to 17th day of post hatch the larvae were fed with newly hatched *Artemia* nauplii. The first sign of pigmentation appeared on 9th to 10th day of post hatch. At 15 to 17th day of post hatch, most of the fry resembled juvenile adult fish and began to shift from partially pelagic to epibenthic (Fig. 1) and started eating minced shrimp, fish flesh, mussel meat, clam meat and formulated diets. The range of environmental parameters maintained were salinity (32 to

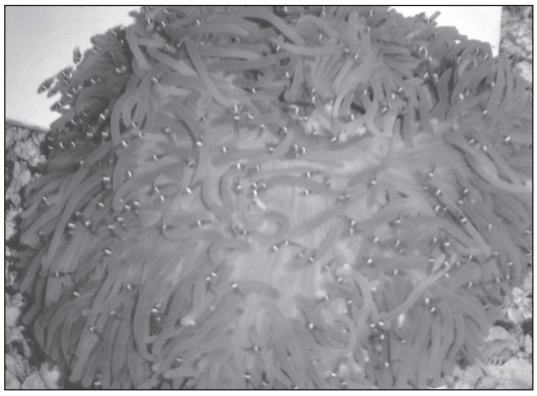

Fig. 1 Juveniles of *Amphiprion ocellaris* (15 days old) settling in sea anemone *Heteractis magnifica*

35ppt), temperature (24 to 28^{O}C), dissolved oxygen (5.3 to 6.8 ml/1) and pH (8.1 to 8.9) With this feeding schedule and environmental conditions, the larval survival and growth were hastened and 90 to 95% survival was obtained at each spawning. The juveniles were fed with different wet feeds at the rate 20% of the body weight and attained marketable size within three to 4 months. Culling of the juveniles were started from 40mm size onwards to reduce aggressiveness and competition for food and space. Twenty five to 50 numbers of juveniles (50 mm size) can

be stocked in a 250 litre FRP or glass tank with a single host anemone (200 to 300 mm dia) to ensure maximum survivability (Fig. 2).

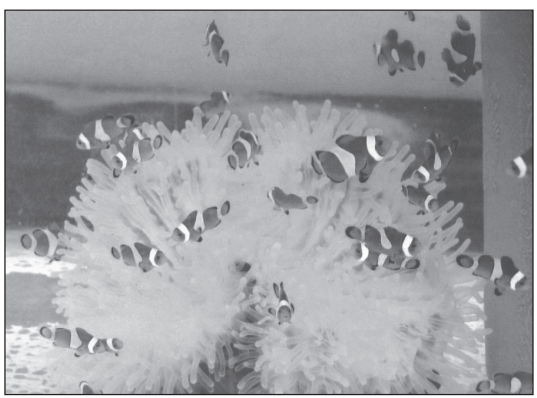

Fig. 2 Three months old hatchery produced juveniles of *Amphiprion ocellaris*

The major technological hurdles of anemone fish breeding is the selection of suitable breeding pairs. As these fishes are protandric hermaphrodites, maintaining good water quality and ensuring slight water circulation is also found very essential for better survivability of larvae. For this , aeration was provided at four corners of the tank through the PVC columns covered with 200 micron bolting silk cloth to avoid thrashing of delicate larvae. One of the critical problems encounterred during the larval rearing was the "head-butting syndrome". In order to reduce this, measures have been taken to avoid reflection of light inside the tank and a low intensity light (15W) has been providedd to the larvae during day and night to locate the feed and it also helped to keep the larvae swimming towards the surface at night rather than sinking to the bottom which otherwise show high overnight mortality. It was found that larval period from 5th and 8th day of post hatch was critical period and mortality may occur during this period due to change in feeding behaviour. At this stage provision of nutritionally adequate food in optimum quantity is of vital importance to overcome the critical stage. Through various experimental tarils all these hurdles have now been overcome and a production protocol has been developed for the mass scale production of *A. ocellaris* under captive conditions.

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