Breeding and seed production of Clown fishes under captivity

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Introduction

Ornamental fish production for the aquarium industry is a multimillion dollar industry in the world. Aquarium keeping is amongst the most popular of hobbies with millions of enthusiasts worldwide. Although most fish kept in aquariums are from freshwater, the acquisition of marine ornamental fish has greatly increased in recent years and is also popularized through children's movies by starring charismatic colourful fishes and other creatures. Recent advances in fish husbandry and aquarium gadgets and technology have further facilitated the hobby. In India, marine ornamental fish trade is an emerging area during the last two decades and the industry is further advancing. Along with it ancillary business such as aquarium making, aquarium plants and live feed production, grow- out culture etc. are also flourishing.

India has about 200 varieties of marine ornamentals, of which more than 50 have export potential. Among these, the clown fishes or anemonefishes belonging to the family Pomacentridae, comprising of genera Amphiprion and Permnas have always been the most popular and sought after group. Altogether 28 species of clown fishes were reported from the different geographical locations of the world. The members of the family Pomacentridae commonly known as damselfishes and anemonefishes are a diverse group of marine fishes found in tropical seas, also have very high demand in marine ornamental fish trade. The family comprises 29 genera and 350 species inhabiting in the coral reef ecosystems. Pomacentrids have been divided into four subfamilies: Amphiprioninae, Chrominae, Lepidozyginae and Pomacentrinae. Under the genera Amphiprion, 29 species and a single species, the maroon clown Premnas biaculeatus have been reported under the genus Premnas.

Anemone fishes or clown 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 long ranked as one of 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 to artificial diet and their fascinating display behavior and symbiotic relationship with the sea anemones. Clownfishes are the most longstanding and intensively cultured family of marine ornamentals and are the best ranked in marine aquarium trade. They were the first reef fish species bred successfully in captivity. However, large scale culturing of clownfish has not always been successful technically due to the lower larval survival which usually go through one or many larval stages, start out at a very small size, and are extremely sensitive to external factors. The clown fish species most studied is A. ocellaris. The technique used for this fish has been used for a long time to establish the protocol or guidelines for the breeding of other clownfish such as A. chrysopterus, A. clarkii, A. percula, A. melanopus and Premnas biaculeatus.

From the Indian waters 15 species in the genera Amphiprion and the single species of the genus Premnas have been reported (Madhu and Madhu 2000). Most of the traded marine ornamental fishes are being collected from the wild and hence there is a great concern regarding the depletion of the stocks due to over exploitation as well as the destruction of reef habitat and damaging collection methods all over the world. Recent studies shown that captive bred clownfish are generally hardier, disease free, and are better adjusted to life in aquaria than their wild-collected specimens, and as a result the demand for the captive bred fishes are increasing. For the breeding of clown fishes under captive conditions, few important steps are to be followed. These include selection of suitable broodstock, setting up the tank, broodstock feeding, maintenance of high water quality, provision of suitable environmental parameters, creating suitable condition for spawning and system for raising the larvae and juveniles.

Transportation of broodstock

For the captive mass production of clown fishes, the basic requirement is to have a sufficient number of broodstocks or breeding pairs which can either be collected form the coral reef habitat or can be purchased from the pet shop depending upon the availability. In the wild, the clown fishes generally occupy in social groups centered in a host sea anemone with a sexually active pair of adults and one to three juvenile or sub adult fish and the female is larger than the male. The clown fishes naturally exhibit monogamous pair formation and these pairs are to be collected for broodstock development and breeding programme. In case such mated pairs are not available, fish of different size groups can be collected and allowed to form pair under captive conditions. In order to make breeding pairs form the juveniles groups, many social groups of clown fishes can be collected from the wild and transported to the laboratory. During transportation, the fish and the sea anemones should be kept in separate transportation containers.

Pair formation

For pair formation, five fishes of each sex of different size groups need to be stocked together along with a host anemone in a 500 L FRP tanks fitted with biological filter to reduce the aggression. Pair formation tanks have to be maintained in the hatchery with a light intensity of 2500 to 3000 lux as the anemones require light for survival under laboratory conditions. The fish and anemones should be fed twice a day with wet feeds like shrimp, mussel and clam meat at the rate of 15% of their body weight and live feeds like Brachionus plicatilis, artemia nauplii and adult artemia. Environmental parameters such as temperature (26 to 29ºC), salinity (33 to 35 ppt), dissolved oxygen (4.6 to 6.2 ml/L) and pH (8.1 to 8.4) are to be maintained in all rearing tanks.

Sex change and pairing

As the clown fishes are protandrous (male first) sequential hermaphrodites, a pecking order is established in which the female is dominant, the male is subordinate to the female, and all the other juveniles are subordinate to the adult male and female. Thus generally all clown fish individuals start out as males and change into females when they reach larger sizes or under the situation of loss of mate. The male and female form a monogamous pair bond that lasts until one member of the pair dies. If the female dies first, the largest male rapidly changes sex into a female and the second largest or dominant juvenile becomes an active male and that pairs up with the newly transformed female. By utilizing this adaptation, pairs of clown fishes can be developed under captive condition by creating social systems. After a period of 3 to 4 months of rearing for pair formation, in each tank one pair grew ahead of others and becomes the spawning pair. As the newly formed pairs will be very aggressive and spending time for fleeing the other subordinates rather than reproductive activity, it is very essential to stock each breeding pairs in separate tanks.

Tank set-up for broodstock

A clownfish broodstock/ spawning tank should be of 250 to 500 L capacity with a single healthy pair and host sea anemone. An ideal tank would be a 3 ft x 2 ft x 2 ft with a layer of coral sand at the bottom, few live rocks, bright lighting and good filtration, preferably with an efficient protein skimmer to reduce the ammonia and organic materials in the system. A trickle filter could be used with regular water changes to keep the nitrates low enough for the anemone to do well. Since the gonad development and spawning of clown fishes are influenced by moon phases, the broodstock/ spawning tanks should be kept at a place where the fish receive regular day/ night light cycle (moon phase). Anemone is generally not required to breed clownfish under captive condition. But generally the clown fish select a nest site adjacent to the sea anemone for deposition of eggs. Moreover an added benefit of having an anemone is that it may release compounds that help to protect the eggs or even chemically induce immunity that clownish have with the anemone.

Broodstock development and maintenance

After the pairs are formed, they are transferred to glass aquaria for broodstock development. Depending upon the production capacity and seed demand, several pairs can be maintained in commercial hatcheries. The broodstock are fed with meat of green mussel, shrimp, clam and fish egg, along with supplemental formulated feeds enriched with vitamins, minerals and algal powder at the rate of 10% of their body weight and fed during day time at an interval of 3 h. Apart from these, the broodstock are also fed with enriched rotifer 800 to 1000 nos/ml and artemia nauplii

(200-400 nos/ml) and adult artemia (3 to 5 nos/ ml) every day.

Enrichment of rotifer and artemia

Three litres of enrichment medium has to be prepared using microalgae (Chlorella salina, C marina, Nannochloropsis oculata @1x106 cells/ ml) for enrichment. To this, an emulsion prepared with homogenized cod liver oil (5 g), vitamin A (0.1%), vitamin D (0.2%), vitamin E (0. 3%) and vitamin K (0.1%) has to be added. The rotifers are released to this enrichment medium @ 800 to 1000 nos/per ml along with 50 mg/L of bakers' yeast for 12 to 24 h. The rotifers thus enriched are harvested, washed and used for feeding the fishes.

The artemia nauplii (instar II stage) harvested through 100μ bolting silk cloth after completion of about 16 hours of hatching, are released @200-400 nos/ml to the 5 L plastic circular tub containing 4 L of mixed algal water: N. oculata, P. lutheri, C. marina and C. salina (105 cells/ml), I. galbana, D. inornata, C. pleoides (104 cells/ ml) for bioencapsulation and maintained at optimum environmental parameters. To this, 8 g of cod liver oil with fat soluble vitamin: vitamin A(0.2%), vitamin D(0.1%), vitamin E (0. 6%) and vitamin K (0.3%) are added. The enriched artemia are harvested and fed to the fish after 12 to 24 h of enrichment.

Feeding with enriched live feeds

After enrichment, the rotifers and artemia were harvested, washed and released to 4 L of bio-filtered seawater containing mixed culture of micro algae: N. oculata, P. lutheri, I. galbana, D. inornata, C. pleoides and C. marina (104 to 106 cells/ ml) in 5 L capacity transparent tub with mild aeration. The enriched rotifer and artemia were given in split dose (10-11am and 3.0 to 4.0 pm daily).

Water quality maintenance

Maintenance of water quality is the most critical factor for breeding of clown fishes or any marine fishes under controlled conditions. As a measure for this, the sea water need to be filtered through a series of sand filters before being taken to the rearing tanks. The temperature in all the breeding tanks has to be maintained between 26 to 30°C, dissolved oxygen (4.8-6.3 ml/L), pH (8.0-8.4), salinity (32-35 ppt) and the water should be recirculated to ensure water movement and good quality during the rearing period. Once in a week 25% of the water should be exchanged to avoid stress like a rapid increase in plasma corticol concentration, depression of gonadal streroidogenesis, and subsequent development of gonadal atresia.

Egg deposition

The clownfish have attached eggs and are known to spawn on rough surfaces near the host anemone. Hence it is essential to provide suitable substratum preferably tiles or earthen pots or shells of edible oyster or PVC pipes for the egg deposition which will also be helpful for the transfer of deposited egg to hatching tank without any mechanical injury.

Breeding behaviour

After broodstock rearing, each pair will start breeding within a period of 4 to 6 months under captive condition. Few days prior to spawning, the male select a suitable site near to the anemone for egg laying and it clears algae and debris with its mouth and on the day of spawing both the parents spent considerable time for cleaning of the site which indicates the imminent at within few hours. Under laboratory conditions, the spawning can be obtained between 0500 h to 1530 hrs during day time and it lasts for one to one and a half hours. Each female lays 300 to 1000 capsule shaped eggs at every 12 to 15 days interval depending on the species and size of the fish. The egg size ranges between 1.5 mm and 3.0 mm in length with a width of 0.8 to 1.84 mm and remain adhered to the substratum with a stalk. At an average two spawnings/ pair/ lunar month result in an estimated annual fecundity of 7200 to 24000 eggs/ breeding pair/ year under laboratory conditions.

Parental care

As parental care is inevitable for hatching out of the larvae, the parents should be allowed to remain in the tank itself till hatching. During incubation period, both the parents carefully look 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. The newly spawned eggs are white to bright orange in colour for initial two days and as the embryo develop; these are turned to black on days 3 to 6 and later to silvery on day 7 to 8 of incubation. At this stage the

glowing eyes of the developing larvae inside the egg capsule is clearly visible when viewed from a short distance. Male assumes all the responsibility of caring for the eggs and spent a higher percentage of time at the nest than the females, which then increase gradually up to 70% of time as the day of hatching approaches. When incubated at 27 to 29º C, hatchling was emerged on day 8 and the peak hatching took place shortly after sunset.

Egg hatching and larval rearing

On the expected day of hatching, two hours before sunset, the eggs along with substratum were transferred from the parental tank to hatching tanks (100 L) and provided with complete darkness for accelerating the hatching. The larvae broke the egg capsule and the tail of the hatchling is emerged first and the hatching occurred soon after sunset and the peak hatching took place between 1900 to 2030 hrs. The newly hatched larvae measured 3 to 4 mm in length with 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 larval rearing was carried out under green water system and feeding with super small rotifer B. rotundiformis and newly hatched artemia nauplii. The larval period of clown fishes generally last for maximum of 20 days and then after most of the fry resembled juvenile adult fish and began to shift from partially pelagic to epibenthic and started eating minced shrimp, fish flesh, musselmeat, clam meat and formulated diets.

Larval feeding

The successful feeding strikes are low at first feeding but rises rapidly during early development in clown fishes. At this stage provision of suitable size and nutritionally adequate enriched feed in high density is important for their survival. Larvae have only little quantity of yolk material and it starts feeding within few hours after hatching. As the mouth gape of clown fish larvae is between 80-123 μ, the larvae need to be fed with live feeds measuring less than 100μ for its active feeding. The rearing tanks need to be provided 24 hrs with light up to 15 days of post hatch (DPH). During this time the larval tank must be kept very clean with the bottom siphoned off by removing dead larvae, detritus and faeces twice a day from the bottom. Water exchange has to be done at a rate of at least 25% per day.

Feeding schedule of larvae of clown fishes can be performed in two stages: Stage 1: covered the rotifer with algae feeding phase from Day 1 to day 8; Stage 2: the newly hatched artemia and rotifer with algae feeding phase from day 9 to day 20. For the successful prey capture of larvae, 50-100 numbers ml-1 rotifer (B. rotundiformis) of 60 to 100 μ size need to be provided after enrichment with vitamins and fatty acids. As the larvae attains successful prey capture within two days, the density of rotifer in the larval rearing tank need to be reduced to 30-50 nos. ml-1 from day 3 to 8. From day 9 onwards the larvae were weaned onto newly hatched Artemia nauplii (5-10 nos/ml) along with rotifer (SS and S type) (20-30 nos/ml) whereas algal concentration should be same as the prey capture step till day 20 of post hatch. The clownfish have a larval period between 10 and 20 days. After 20 days of rearing the larvae develop the adult striped colouration and metamorphose to juveniles and shift from partially pelagic to epibenthic and look like miniature adults. From metamorphosis onwards, the clownfish actively swim on the bottom of the tank and settle in the host sea anemone. Up to 20 days, the rearing can be carried out in the same tank and on completion of metamorphosis, the juveniles should be graded into several groups and stocked in separate tanks in which biological filtrations system need to be was provided.

Copepod as a live feed

Survival can be significantly made higher when larvae were fed with copepods. The higher omega-3 fatty acids found in copepods appear to be important for survival of larvae under more stressful conditions. But mass production of copepod is often collapsed due to several factors. Hence dependence on copepod for larval rearing is unreliable until and unless a copepod mass production technique is standardized.

Rearing conditions

The maintenance of water quality is a critical factor in larval rearing of clown fishes or any marine fishes under controlled condition. As a measure for this, the sea water needs to be filtered through a series of sand filter tanks before being taken to the larval rearing tank. However during larval rearing it was found that the period from 3rd to 8th day of post hatching (dph) was very critical may be due to the alteration or change

in feeding (exogenous) whereas once the larvae completed 8 days after hatching, no further mortality was observed. During the larval rearing period, in all tanks, the environmental parameters were maintained to their optimum level with pH ranging from 8.0 to 8.4 water temperature 26 - 30° C, dissolved oxygen 5.5 - 7.8 (mg/L), salinity 33-35 ppt, NH4+ /NH3 and NO2 values at 0 mg per L and NO3 levels below 0.2 mg /L. Daily the tanks were cleaned with cotton and magnetic tank cleaner to remove the dust and slimy coating forming inside the tank and one fourth water is replaced with same amount of filtered sea water along with enriched rotifer and artemia and micro algae.

Light intensity

Head-butting syndrome was another the critical problem encountered during larval rearing due to the immature development of the retina and subsequent hitting of larval head to the sides of the tank. In order to reduce this, two major measures taken were i) all the 4 sides of the tanks were covered with black cloth or painted black to avoid reflection of the light ii) a low intensity light provided by hanging 2 nos. of 60 watt bulb or night lamp at a height of 15-20 cm from the surface of water level in rearing tank for 24 hours from day 0 to day 20 which enable the larvae to detect and capture its feed and it also helped them to swim towards the surface at night rather than sinking to the bottom which otherwise show high overnight mortality. The type of lighting is not critical and can be from any source of light, i.e. fluorescent or metal halide etc. The reason for having a light is that the larvae are visual predators and require light to hunt for their live food. In addition to these, all the larval tanks need to be covered with net cloth during the night time to prevent the entry of insects.

Problems in larval feeding

In general, the mortality of larvae were reported due to over eating,intestinal blockage, ingestion of air bubbles or bacterial problems. Though Artemia is in regular use for larval rearing of marine fishes, there is one serious concern with introducing unhatched cysts along with the Artemia nauplii to the larval rearing tank and these cysts are often eaten by the larvae and will cause intestinal blockage. Hence care must be taken to separate all the empty cysts from the newly hatched artemia before being added to the larval tanks. It is also equally important to add newly cultured or hatched live food every day because the nutritional value of the live food that remains in the tank will decrease very quickly. The nutritional quality of rotifer also depends upon the quality of feed offered. Hence every day, after water exchange from the larval rearing tanks, new rotifers and or Artemia must be added. The healthy larvae will appear to have a round body and swimming in a close horizontal position. Unhealthy larvae will tend to either buzz around on the surface at 45 degree angle. For the first two days there will be some loss of larvae, if the larvae have been transferred using the siphon method. From day three to eight the larvae will grow very fast. The densities of live feed can be reduced as the larvae have become proficient at food capture.

Juvenile rearing

On days 19-20 of post hatch, the larvae became juvenile and shift from pelagic to epibenthic stages, and look like miniature adult fish. The rate at which the young fish grow depends on the size of the rearing tank, stocking density, quality and quantity of food given and the water temperature. As the clownfish exhibit social hierarchy, dominant clownfish will grow faster and will suppress the growth of the fish below. However, this can overcome by growing the fish altogether in a large tank with sufficient host anemones or culling the juveniles to several groups in different juvenile rearing tanks of 250 to 1000 L capacity. At this stage, the stocking density has to be reduced to 90 -100 numbers of juveniles (size range between 8-10 mm) with single host sea anemone in glass or perspex tank at 100 L capacity for initial 1 to 2 months rearing. During juvenile stages, the fishes show different banding pattern and growth rate, and on attaining a size of 24 to 35 mm in total length (TL), the stocking density need to be reduced to 30 to 50 number with single sea anemone in 100 L tank with 80 L bio filtered sea water until marketing. In the case of each 500 L FRP tanks, 130 to150 juveniles can be reared with 1 to 3 anemones.

Feeding

In the juvenile rearing, a survival of 100% was obtained through feeding with different wet feeds at the rate 15 to 20% of body weight. Apart from these, artemia nauplii 10-15 numbers/ ml and ro-

tifer (B. plicatilis) 50- 55 nos./ml were given after enrichment with brown algae (104 cells/ml) and green algae (106 cells/ml) with cod liver and fat soluble Vitamin A, D, E, K, twice a day which helped to retain the colour of fishes and provided adult artemia (2-4 nos/ml). Through this feeding schedule, the larvae will attain 10 to 12 mm within 30 days of post hatch and the juveniles reach 25 mm to 35 mm within 60 days and attain marketable size within 6 months after post hatch. Once in a week, one third water need to be decanted and refilled with same quantity of filtered sea water in all juvenile rearing tanks. With these feed management procedures, 90-95% of larval survival can be obtained under captive conditions in each spawning.

Packing and Transportation

Fishes are starved for about 2-3 days before being exported. A small amount of freshwater is added to the packing water and chemicals may be added to tranquilize for longer journeys. Packing starts just prior to the transportation. Fishes are packed with oxygen and a little water either singly in double polythene bags to ensure that fish are not stranded without water. Polythene bags are packed in cardboard boxes for short journeys and for long journeys they are packed in Styrofoam boxes with some ice to keep the temperature down. Layers of paper may be inserted between plastic bags in the box to avoid catching sight of aggressive species. Packaging methods have improved considerably over the years mainly due to feed back from the customers and many exporters now guarantee almost 100% survival for most destinations provided that good connecting flights is available. Regulating the standards of the holding facilities and of standards of packing is important to ensure minimum mortality of fish at holding facilities and in transport.

Marine ornamental fishes bred in India

Central Marine Fisheries Research Institute (CMFRI) has taken initiatives on culture of marine ornamental fishes with objectives to generate scientific knowledge on ornamental fish maintenance, behaviour, influence of social status on sex change, pair formation, breeding, influence of lunar periodicity in spawning, parental care, egg incubation and hatching, developments of egg, larvae, and juveniles. These investigations have resulted in the development of hatchery technology for 20 species of marine ornamental fishes such as clown fishes True pecula/ clown anemone fish Amphiprion percula (Madhu and Rema, 2000,2002); Common Clown/ False clown aenemone fish A. ocellaris (Rema et al.,2012); Yellow Skunk Clown A. sandaracinos (Rema and Madhu, 2012); Tomato clown A. frenatus (Madhu et al, 2011), Clark's Anemonefish A. clarkii, Maldives Anemonefish A. nigripes (Madhu and Rema Madhu,2006; Madhu et al., 2006a,b,c; Rema Madhu, et al., 2007; Madhu et al., 2008, Madhu and Rema , 2011), Pink anemone fish A. perideraion (Anil et al.,2012), redsaddle back anemone fish Amphiprion ephippium, Sebae clown A. sebae (Gopakumar, et al.,2007, 2009); and Maroon clown/ Spine cheek anemone fish Premnas biaculeatus (Madhu et al., 2012) and dotty back Pseudochromis dilectus (Redhead Dottyback) were bred. The species such as damsels Three spot damsel Dascyllus trimaculatus; Striped damsel D. aruanus; Blue damsel Pomacentrus caeruleus; Sapphire or Peacock Damsel P. pavo; Yellow tail damsel Neopomacentrus nemurus; Filamentous tail damsel N. filamentosus; Sapphire devil Chrysiptera cyanae; One spot damsel C. unimaculata and Green chromis Chormis viridis (Gopakumar, et al.,2007,2009, Syda Rao et.al., 2010) for the first time in India.

Conclusion

Considering the commercial importance of anemonefishes, it is very essential to develop the breeding techniques for mass scale production under captive condition. In order to produce its seeds, healthy broodstocks need to be reared for pair formation and breeding. As the clown fishes are protandrous and breed two times per month, provision of suitable feed and maintenance of environmental parameters are the important management practices for obtaining consistent breeding under captivity.