

## Seed Production of Clown Fishes

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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 acquisitions of marine ornamental fish has greatly increased in recent years and are also popularized through children's movies by starring charismatic colorful fishes and other creatures. Recent advances in fish husbandry and aquarium gadgets and technology have further facilitated the hobby. The vast array of organisms that make up the generic ornamental aquarium fish trade is staggering. In India, marine ornamental fish trade is an emerging field during the last two decades and day by day the industry is advancing. As the industry is growing, it also fosters additional sideline business such as aquarium making, aquatic plants and live food production, grow-out culture there by providing job opportunity and livelihood options.

India is a repository of more than 200 varieties of marine ornamentals of which more than 50 are very bright and have export potential. Among these, the clown fishes or anemonefishes belonging to the family Pomacentridae, comprising of genera *Amphiprion* and *Premnas* have always been the most popular and sought-after group of marine ornamentals due to their beautiful colour, small size, hardiness, longevity, proclivity to live in association with sea anemone, interesting display behaviour and adaptability to life in captivity. Altogether 28 species of clown fishes were reported from the different geographical locations of the world (Allen, 1975). The members of the family Pomacentridae commonly known as damselfishes and anemonefishes are a diverse group of marine fishes found in tropical oceans, and have very high demand in the marine ornamental fish trade. The family includes 29 genera and 350 recognized species living mainly in coral reef environments. Pomacentrids have been divided into four subfamilies: Amphiprioninae, Chrominae, Lepidozyginae and Pomacentrinae. Under the genera *Amphiprion*, so far 29 species and 1 species under the genera *Premnas* have been reported world wide. The maroon clown *Premnas biaculeatus* is the sole member in the genus *Premnas*. Clownfishes are the most longstanding and intensively cultured family of marine ornamentals world wide and are long ranked in the marine aquarium trade.

### Anemone fishes or Clown 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 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. Clownfish were among the first reef fish species bred successfully in captivity and the breeding under captive condition was real successful. However large scale culturing of clownfish has not always been successful technically due to the less 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 complete study of the biological cycle of many species of clown fishes has been mostly acquired in the last 20 years, especially regarding the subject of aquaculture (Jacquin 1975, Hoff, 1996 and Moe, 1992). The clown fish species most studied is *A. ocellaris* (Alayse, 1984). 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* (Allen, 1975), *A. clarkii*, (Hoff, 1996 and Moe, 1992), *A. percula*, *A. melanopus* and *Premnas biaculeatus* (Job *et al.*, 1997).

Survey of the clown fish resources of the Indian waters revealed that out of 29 clown fish species in the genera *Amphiprion* and one species of *Premnas* reported so far from the different geographical locations of the world, Andaman and Nicobar islands and Lakshadweep islands harbours 15 varieties of sea anemone fishes (Madhu and Madhu 2000). 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 exploitation as well as the destruction of reef habitat and damaging collection methods all over the world. More over recent studies showed that captive bred clownfish are generally hardier, more disease free, and in general better adjusted to life in aquaria than their wild-collected specimens, and as a result the demand for the captive bred fishes are increasing (Wabnitz *et al.*, 1992). Hence investigations should be focused on the development of hatchery technology and the production can lead to the trade of hatchery produced ornamentals which is long term sustainable and the captive propagation will also help to sea ranch the species in depleted area for stock enhancement. For the breeding of clown fishes under captive condition, 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 from 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. Invariably the female was somewhat larger than the male. In nature, the clown fishes are showed monogamous pair formation, and these pairs are need to be collected for broodstock development and breeding programme. In case such mated pairs are not available, the fishes having different size groups can be collected and made to pair under captive condition through pair formation. In order to

make breeding pairs from the juveniles groups, many social groups of clown fishes can be collected from the wild and transported to the laboratory in live condition. During transportation, the fishes and sea anemones should be kept in separate plastic transportation bags.

### **Pair formation**

For the pair formation, five fishes of each sex of different size groups need to be stocked together along with single host sea anemone in a 500 L FRP tanks fitted with biological filter to reduce the aggression. The pair formation tanks need to maintain 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 laboratory condition. The fishes and anemones should be 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*, artemia nauplii and adult artemia. Environmental parameters such as temperature 26 to 29° C, salinity 33 to 35 ppt, dissolved oxygen 4.6 to 6.2 ml/L and pH 8.1 to 8.4 are need 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 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 through creating social systems. After a period of 3 to 4 months rearing in the pair formation, in each tank one pair grew ahead of others and became 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 broodstock tanks.

### **Tank set up for broodstock**

A clownfish broodstock or spawning tank should be 250 to 500 L capacity with single healthy pair and host sea anemone. An ideal tank would be a 3ft x 2 ft x 2 ft with a layer of coral sand on the bottom, a few live rocks, bright lighting and good filtration, preferably an efficient protein skimmer to reduce the ammonia and organic materials from the fish. 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 broodstocks/ spawning tanks need to be kept in an apt place where the fish receive a regular day/night lighting cycle (moon phase). An 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. More over 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 clownfish have with the anemone. Hence provision of suitable host sea anemone in the broodstock tank will help the fish to feel as comfortable as in the wild and this will however certainly makes the task much easier in the long run.

### **Broodstock development and maintenance**

The pairs formed through pair formation should then be transferred to separate glass aquaria for broodstock development. Depending upon the production capacity and seed demand, several pairs can be maintained for commercial hatcheries. The broodstocks need to be fed with wet feeds such as meat of green mussel, shrimp, and clam and fish egg mass, and can also be 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. Apart from these, the broodstocks were 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. Provision of enriched live feeds which apparently improved egg quality and hatchability than the brooders fed with non enriched live feeds.

### **Enrichment of rotifer**

Three litres of enrichment medium has to be prepared using microalgae (*Chlorella salina*, *C marina*, *Nannochloropsis oculata*  $1 \times 10^6$  cells/ ml) in five litre capacity circular transparent tubs for enrichment. To this, an emulsion prepared with homogenizing 5 gm of cod liver oil, 0.1% vitamin A, 0.2% vitamin D, 0.3% vitamin E and 0.1% vitamin K has to be added. The rotifers harvested from the mass culture tank has to be released to this enrichment medium at the rate of 800 to 1000 nos of rotifer per ml along with 50 mg/L of beakers yeast for 12 to 24 hr. The rotifers thus enriched were harvested, washed and used for feeding the fishes.

### **Enrichment of artemia**

The artemia nauplii (instar II stage) were harvested through  $100\mu$  bolting silk cloth after completion of about 16 hours of hatching, and 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* ( $10^5$  cells/ml), *I. galbana*, *D. inornata*, *C. pleoides* (104 cells/ ml) for bioencapsulation and maintained optimum environmental parameters. To this, 8 gm of cod liver oil with fat soluble vitamin: 0.2% vitamin A, 0.1% vitamin D, 0.6% vitamin E and 0.3% vitamin K were added. The artemia were harvested and fed to the fish at 12 to 24 hours 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* ( $10^4$  to  $10^6$  million cells/ml) in 5 L capacity transparent tub with mild aeration. The enriched rotifer and artemia were given in split dose (10-11 a.m. and 3.0 to 4.0 p.m. daily).

### **Water quality maintenance**

The maintenance of high water quality is possibly the critical factor for the breeding of clown fishes or any marine fishes under controlled condition. 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 need to maintained between 26 to 30°C, and level of dissolved oxygen (4.8 to 6.3 ml/L), pH (8.0 to 8.4), salinity (32 to 35 ppt) and the water needs to be recirculated to ensure water movement and provided good water quality with the aid of a specially devised filter system during the period of rearing. Once in a week 25% of the water should be exchanged to avoid stress like a rapid increase in plasma cortisol concentration, depression of gonadal steroidogenesis, and subsequent development of gonadal atresia.

### **Egg deposition**

The clownfish have attached eggs and are known to spawn on rough surfaced substrata near to the host sea anemone. Hence it is very essential to provided 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 without any mechanical injury to hatching tank.

### **Breeding behaviour**

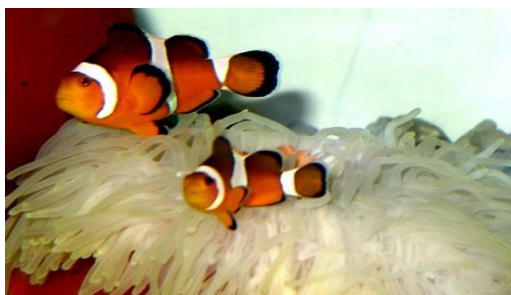
After broodstock rearing, each pair will start breeding within a period of 4 to 6 months rearing under captive condition if the broodstocks are provided nutritious food and provided suitable rearing conditions. Few days prior to spawning, the male selected a suitable site near to sea anemone for laying the egg and cleared algae and debris with its mouth and on the day of spawning both the parents spent considerable time for the cleaning of site which indicated that spawning may occur within few hours. Under laboratory condition, the spawning can be obtained between 0500 hrs to 1530 hrs during day time and the spawning lasted for one hr to one and a half hour. Each female lays 300 to 1000 capsule shaped eggs at every 12 to 15 days interval depending on the species of clown fish, size of fish and previous experience. Generally the egg size of clown fishes ranges between 1.5 mm to 3.0 mm in length with a width of 0.8 to 1.84 mm and adhered to the provided substratum with stalk. An average of two spawning per lunar month per pair resulting in an estimated annual fecundity of 7200 to 24000 eggs/ breeding pair/ year can be obtained under laboratory condition.

### **Parental care**

As parental care is inevitable for hatching out of the larvae, the parents should be allowed to remain in the parental 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 were white to bright orange in colour for initial two days and as the embryo develop; these were turned to black on 3rd to 6th day and later turned to silvery the colour of the larvae's large eyes 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 assumed nearly all responsibility 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 hatching approached. When incubated at a water temperature range of 27 to 29° C, the hatchling emerged on 8th day of incubation and peak hatching took place shortly after sunset.

Captive pairing, breeding and embryonic development of clown fishes



Pair of *A. percula*



Pair of *A. percula* with egg clutches



Pair of tomato clown



Pair of *A. clarkii*

### 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 hatchling emerged tail first and the hatching occurred soon after sunset and the peak hatching took place between 1900 to 2030 hrs under darkness. 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 larval rearing can be 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 one of the important factor for their survival as the larvae will be able to accept small size organism due to the small mouth gape, and if they do not encounter and successfully capture food before depleting their energy reserves, the larvae may starve and it

will eventually lead to mortality. More over many of the larvae had 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  $\mu$ , the larvae need to be fed with live feeds measuring less than 100 $\mu$  for its active feeding. All the rearing tanks need to be provided 24 hrs 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 dead larvae, detritus and faeces twice a day. This is important because any decaying matter on the bottom will encourage potentially harmful bacterial growth and will lower the oxygen levels and deteriorate the water quality. Water changes will also need to be performed 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<sup>st</sup> to 8<sup>th</sup> day. Stage 2: the newly hatched artemia and rotifer with algae feeding phase from 9<sup>th</sup> to 20<sup>th</sup> days. For the successful prey capture of larvae, 50-100 numbers ml<sup>-1</sup> supper small rotifer (*B. plicatilis*) having size 60 to 100  $\mu$  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<sup>-1</sup> from 3<sup>rd</sup> to 8<sup>th</sup> day. From 9<sup>th</sup> day 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 20<sup>th</sup> day 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 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 high water quality is possibly the critical factor when larval rearing of clown fishes or any marine fishes is done 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 3<sup>rd</sup> to 8<sup>th</sup> 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 33-35ppt, NH<sub>4</sub><sup>+</sup> /NH<sub>3</sub> and NO<sub>2</sub> values at 0 mg per L and NO<sub>3</sub> 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 the 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 have been taken that 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 needs to be 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 0 day to 20th day which enabled 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 over night 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 prey. 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 was 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 un hatched 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 well rounded body and should be swimming in a close horizontal position. Unhealthy larvae will tend to either buzz around on the surface at a 45 degree angle. For the first two days there will be some losses 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 food can be reduced as the larvae have become proficient at food capture.

### **Juvenile rearing**

On 19-20 dph, the larvae became juvenile and shift from pelagic to epibenthic stages, and look like a miniature of adult fishes. 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. This can largely overcome however by growing the fish up all together in a large tank with sufficient host anemones or culling the juveniles to several groups in different juvenile rearing tanks of size 250 to 1000 L capacity fitted with biological filters. At this stage, the stocking density need 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 to 150 juveniles can be reared with 1 to 3 sea anemones.

Larvae and juvenile of *A. ocellaris*



30 days old juvenile



5 months old juveniles

### Juvenile feeding

In the juvenile rearing, a survivability 100% were obtained through feeding with different wet feeds: mussel meat, prawn muscle, fish eggs and minced flesh of trash fish at the rate 15 to 20 % of body weight. Apart from these, artemia nauplii 10-15 numbers/ ml and rotifer (*B. plicatilis*) 50 - 55 nos. /ml were given after enrichment with brown algae ( $10^4$  cells/ml) and green algae ( $10^6$  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 reaches 25 mm to 35 mm within 60 days and reaches 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 survivability can be obtained under captive condition in each spawning.

### Quarantine

Newly acquired fish or shrimp may carry disease and may infect valuable, healthy, broodstock. They are therefore kept separately, in a tank or system, for three to four weeks where they are closely observed and treated with medications for possible disease outbreaks.

## Packing and Transportation

Fishes are starved for about 2-3 days before being exported. A small amount of fresh water 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 feedback 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 clown 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 27 species of marine ornamental fishes of which 12 are from clown fishes such as *Amphiprion percula* (True percula/ clown anemone fish) Madhu and Rema, 2000,2002; *A. ocellaris* (Common Clown/ False clown anemonefish) Rema *et al.*, 2012; *A. sandaracinos* (Yellow Skunk Clown) Rema and Madhu, 2012 ; *A. frenatus* (Tomato clown) Madhu *et al.*, 2011, *A. clarkii* (Clark's Anemonefish ), *A. nigripes* (Maldives Anemonefish ) (Madhu and Rema Madhu, 2006; Madhu *et al.*, 2006a,b,c; Rema Madhu, *et al.*, 2007; Madhu *et al.*, 2008). Madhu and Rema , 2011.; *A. perideraion* (Pink anemone fish)(Anil *et al.*, 2012), *Amphiprion ephippium* (redsaddle back anemone fish), *A. sebae* (Sebae clown)(Gopakumar, *et al.*, 2007, 2009); *A. akkalopisos* (Skunk anemone fish), *A. ocellaris – Black ocellaris* clown and *Premnas biaculeatus* (Maroon clown/ Spine cheek anemone fish) Madhu *et al.*, 2012. and also achieved success in hybridization and designer clown fish production under captivity for the first time in India. The public awareness created through training, awareness programme, exhibitions has resulted in the emergence of several ornamental fish trade shops all over the country. In the near future India can emerge as one of the leading international traders of marine ornamental fishes through hatchery production.

## Mass scale seed production and seed supply

Mass scale seed production of marine ornamental clownfishes are ongoing under ICAR-Mega seed project and seed counters are operational at Kochi, Vizhinjam and Mandapam and larvae, metamorphosed juveniles, seeds juveniles and brood stock pairs are supplied to farmers, hobbyist and entrepreneurs based on the rate fixed by the institute.

## 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.

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