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BROOD STOCK BREEDING AND GROWTH DEVELOPMENT OF CLOWN FISH LARVAE OF *AMPHIPRION OCELLARIS* (CUVIER, 1830) UNDER LABORATORY CONDITION

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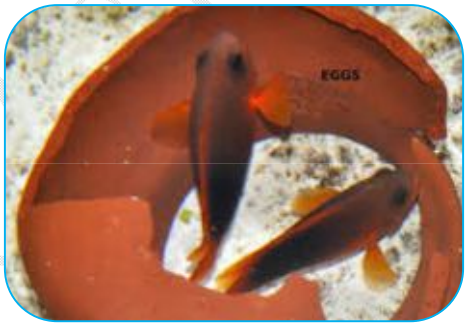
ABSTRACT:



Marine ornamental species are considered as the highest valued products that can be harvested from tropical marine ecosystems, and trade in ornamentals supports employment in predominantly rural, low-income coastal communities, as well as providing a strong economic incentive for coral reef conservation. In the present study, the growth rate and reproductive of Amphiprion ocellaris by using Western water in host Heteractis magnifica Anemone were investigated. A. ocellaris were procured from the traders with the size range of 5.0-6.5cm and acclimatized to observed condition. After 3 months of acclimatization, the pair was formed. At the end of the 6th month, the fishes were spawned. After 6-8 days of incubation hatching took place and the larvae metamorphosed during 18-23 days. Artemia nauplii fed to the larvae gave a maximum survival rate. Total larval survival in the present study was 60%. The offspring reached the marketable size in 6 months. Live food is the most important factor in production of this species and enrichment of their live prey with probionts improve the nutritional value of a fish's diet.

KEYWORDS: *Clown fish,Amphiprion ocellaris, brackish water, growth rate, captive breeding.*

INTRODUCTION

Ornamental fish are the most wonderful entertainment product given by the nature to human beings. They are capable of reducing our stress and anger very easily. These decorative fishes are very helpful for us when we find peace and relief in the developing world of science.The increasingly popular aquarium hobby is filling the rapid growth of the aquatic ornamental industry, specifically the trade of marine ornamental species, due to their trust attractive colour, behaviour and adaptive nature. Netherless, there is a higher demand on wild caught marine ornamental to satisfy consumer demand. There are a number of promotional schemes from various government agencies, which would boost up aspiring entrepreneurs. The technology is quite simple and easy to take up and attain success. The clownfish has become a popular aquarium pet. Nemo and his dad, Marlin, ocellaris are clownfish, also called false clownfish or clown anemone fish. Anemone fish are so-named for the sea anemones in which they make their homes. There are 28 species of anemone fish and they come in many colors, such as pink, red, yellow, black, brown and multi-colored stripes. Their color, size and length varies according to the nature and temperature of the existing water. They live in the warmer waters of the Pacific Ocean and

Indian Ocean. They are also found in northwest Australia, Southeast Asia, Japan and Indo-Malaysian region.

India has about 200 varieties of marine ornamentals, of which more than 50 have export potential. Among these, the clown fishes or anemone fishes belonging to the family Pomacentridae, comprising of genera *Amphiprion* and *Permnas* have always been the most popular and sought after group. Clark's anemone fish inhabit coral reefs, sheltered lagoons and steep edges of reefs up to a depth of 60 meters. This species is always found living within the tentacles of sea anemones (Class :Anthozoa). Although they are often found near anemones such as *Entacmaeaquadricolor*, *Heteractis magnifica* and *Stichodactyla gigantea*, they are known to naturally associate with all 10 of the anemone species known to host clown fishes. Clark's anemone fishes are colorful, although the exact pattern shows considerable geographical variation. These are used to defend its host anemone from invading fish and can inflict harm to humans if the fish is provoked. On rare occasions, these anemone fish will develop an interesting mutation within their eyes. A pale blue crescent will appear in the upper part of the iris, often referred to as "Pearl Eyes". Individuals with this trait are highly desirable within the aquarium trade. (Allen, 1997; Fautin and Allen, 1992). In this present study we will detail, see about the growth rate and captive breeding larvae of the clown fish *A. ocellaris*with the suitable environment.

MATERIALS AND METHODS

Species identification

Clownfish are small sized, 10–18 centimeters (3.9–7.1 inches) and depending on species, they are overall yellow, orange or a reddish or blackish color and many shows white bars or patches. Within a species there may be color variations, most commonly according to distribution, but also based on sex, age and host anemones. Clownfish are found in warmer waters of the Indian and Pacific oceans and the Red Sea in sheltered reefs or in shallow lagoons. For the present study, brackish water was drawn from the Vellar estuary (Lat.11 29'N; Long. 79 46' E) with the help of 5 Hp pump during the high tide and allowed to settle in a sump overnight. Then the clear water was passed through sand and U.V filters and finally stocked in a storage tank from where water was taken for hatchery use.

Brood stock management

A.ocellaris with the size range of 3.5-6 cm was procured from the ornamental fish traders along with host anemone, *Heteractis magnifica* anemone. After acclimatization, they were fanatically observed for an injury or any sign of diseases. After making sure, 10 numbers of uniform size fishes and 5 anemones were transferred to a 5 ton cement tank filled with 2 ton water where a under the water filtration system was provided, which was made using activated carbon, ceramic ring and coral sand. The fishes were fed thrice a day with different feeds such as live artificial pellets and *Artimia.* After 3 months, one pair measuring 6-7.5 cm grew ahead of others and that was transferred to 1 ton FRP tank (spawning tank). The photoperiod maintained 12 hours light and 12 hours darkness using artificial light. The submerged objects such as tile, earthen pot, PVC pipe, etc., were placed in the bottom of the tank on which the fishes werespawned. Spawning took place on the 4thmonth after a brief courtship. The embryonic development of eggs was studied on a daily basis. The water quality parameters such as temperature, salinity and pH were measured regularly using standard methodologies.

Growth rate

Eggs were allowed to hatch in the spawning tank itself. Two hours after hatching, the larvae were collected gently without much disturbance and transferred to the larval rearing tank (FRP 250 lit) with 6-10 nos/l. Photoperiod was maintained 12 hours darkness and 12 hours light. As the larvae have yolk sac, on the first day, they were not fed. On the 2nd day onwards algal enriched *Artemia* nauplii was introduced thrice a day and maintained at the concentration of 6-10 nos/ml. The waste particles settled in the bottom were removed without disturbing the larvae and the amount of siphoning out the water was replaced with fresh filtered estuarine water. From 10th day onwards, newly hatched *Artemia*

nauplii were given as feed. After metamorphosis, adult Artemia, boiled squashed clam and mussel meat were given. The feeding management followed in the present study is given in Table:1

Table:1 Feeding management followed in Growth rate

Days	Morning		Afternoon		Evening	
	Artimia/ml	Artificial pellets/ml	Artimia/ml	Artificial pellets/ml	Artimia/ml	Artificial pellets/ml
1	No Feeding					
2	-	13-18	-	10-15	-	8-12
3	-	13-18	-	10-15	-	8-12
4	-	13-18	-	10-15	-	8-12
5	-	13-18	-	10-15	-	8-12
6	-	13-18	-	10-15	-	8-12
7	-	13-18	-	10-15	-	8-12
8	-	13-18	-	10-15	-	8-12
9	-	13-18	-	10-15	-	8-12
10	-	15-20	-	12-14	-	14-16
11	-	15-20	-	12-14	-	14-16
12	10-12	12-16	8-10	10-13	6-10	5-8
13	10-12	12-16	8-10	10-13	6-10	5-8
14	10-12	12-16	8-10	10-13	6-10	5-8
15	10-12	12-16	8-10	10-13	6-10	5-8
16	15-18	9-12	8-10	7-10	6-10	4-6
17	15-18	9-12	12-15	7-10	8-12	4-6
18	15-18	9-12	12-15	7-10	8-12	4-6
19	15-18	6-8	12-15	5-7	8-12	2-4
20	20-22	6-8	16-19	5-7	10-14	2-4
21	20-22	6-8	16-19	5-7	10-14	2-4
22	20-22	Nil	16-19	Nil	10-14	Nil
23	23-25	Nil	20-22	Nil	15-20	Nil
	23-25	Nil	20-22	Nil	15-20	Nil

RESULTS

Brood stock development

The physic- chemical parameters for the successful brood stock development were recorded. The temperature in the spawning tank was maintained at 28 ± 2°C, salinity at 22-24%, dissolved oxygen 4.5-5 mg/l and pH 7.5-8.2. Regular water exchange (20-40%) was given depending on the water quality. After a period of four months rearing in the above said conditions, fishes were started spawning.

Behavioral study

All through feeding, it was noticed that the male gives the prospect to female to take feed. Along withthe 10 fishes in the conditioning tank, one pair grew ahead of others in 3 months. Later deletion of the similar, another pair was produced within ten days. The fish was spawned in 3 months and thereafter, the same was stocked in the spawning tank. The courtship began a week earlier than spawning with the initiatives taken by the male, which includes nest site selection and cleaning the area. On the day before spawning, male actively cleaned the site by rubbing his body and picking off loose particles of algae or fecal matter among his mouth. Then the female entered into the nesting site and lay eggs consequently. The eggs were fertilized by male and the spawning process lasted for about 45 minites to 1 hours. It was observed that guarding of egg clutch actively done by male and on a few occasions female too. Fanning of the eggs was done mostly by male and rarely by female with the help

of their pectoral fins and the tail. Fanning was more frequent on the day of hatching and the time was varied from 10 to 90 seconds. The number of eggs in a clutch was found to be initially less in the first spawning and in the subsequent it was high and ranged between 350-2500 nos. The spawning occurrence varied from 8 to 15 days. Spawning took place during morning hours. Maximum spawning was observed in summer, where 4 spawning were achieved. The fish spawned throughout the year with 2.4 nets per month except during December.

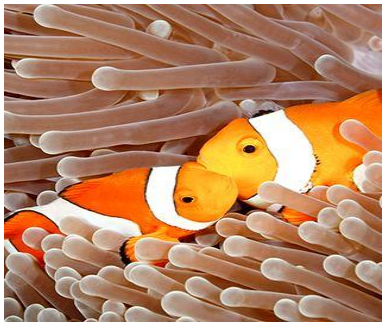


Fig 1:Brooder stage of *Amphiprion ocellaris*

Hatching

Before hatching, the eggs became dark silvery in colour and chorion membrane became pliable. During this period, the movements of embryo were more frequent, violent and fanning duration also increased. Initially the tail was wrapped completely around the egg, reaching its distal end of the chorion. The colour of the eggs was pale orange and then turned slightly brown to black. On the day of hatching, the color became silvery due to the development of eye inside the embryo. Hatching took place 6-8 days post fecundation and the newly hatched larvae possessed a small yolk sac.

Fig 2: *Amphiprion ocellaris* with their eggs

Larval development

The yolk sac of newly hatched larvae was small, orange, slightly extended and had a mid body ball. The size of the newly hatched larvae varied between 2 and 2.9 mm in total length and mouth gap varied from 200-250 µm. After a dayit became active and swam to the surface. The larvae were fed with *Artemia* and maintained the same with a density of 08-10 nos/ml in the larval rearing tank. On 5th and 6th day, the body of the larvae was deeper and round in shape. Since 12th day beyond, the larvae were fed with newly hatched *Artemia* nauplii at different concentrations beside with rotifer. On15th to 18th day, the larvae were entered into metamorphosis. The average length of the larvae after metamorphosis was 4-5 mm. There was distinct pigmentation appeared on 20th day and from 22th day onwards complete miniature of adult *A. ocellaris*fishes could be seen. On 25th day the juveniles settled with sea anemones. The young ones attained a marketable size in three months of rearing.

DISCUSSION

False clownfish, *Amphipriono cellaris,* is an important attractive fish for the aquariumindustry because of its body coloration, swimming activities, and its symbiotic relationshipwith anemone (Yasir and Qin, 2007).However, the color of farmed clown fish is lessbeautiful than their congener source from natural (Allen, 1991).Tanaka *et al.,* (1992) that the carotenoid content in the integument has the possible to change the clown fish from yellow orange to orange pinkish when the fish were moved from wild to interior tanks.In the present study, brackish water was used for growth rate and captive breeding. Like many Pomacentrid fishes, the clown fishes deposit demersal adhesive eggs on hard substrates, usually in sheltered areas. The eggs are elliptical and get attached by adhesive filaments. Although the early life histories of many reef fishes areknown, the embryological and larval descriptions for many reef fishes are not studied thoroughly.

In the present study, eggs were laid always during the morning hours, but in other species of clown fish it was reported during evening hours and it may be due to some environmental factors.The major technological aspects of clownfish rearing program are the successful development of broodstock, methods of hatching the eggs, development of a biological detoxifying filtration system for larval rearing and appropriate larval feeding schedule.Brood stock nutrition is an important factor governing egg production and larval survival (Izquierdo*et al*., 2001). An improvement in broodstock nutrition and feeding has been shown to improve not only egg quality but also enhance seed production. Gonadal development and fecundity were affected by certain essential nutrients (Izquierdo*et al.*, 2001). Dietary protein and lipid play major roles in growth and reproductive performance.

The fecundity rate, clutch size and spawning frequency were depends on several factorsThe tolerance of this species to environmental factors change makes this fish the best between other clown fish and the variation color either improved their attrition.The time and stages of embryonic development and larvae of *A. ocellaris* showed variation as per the environmental conditions. The developmental rate of fertilized egg and its hatchability of clownfishes varied with temperature and dissolved oxygen content of water (Allen,1972; Fautin and Allen,1997) and also in other teleost (Delsman,1930). In the present study, 95-100% hatchability was obtained at 168 hours.Ignatius *et al.,*2001 studied on the spawning and larval rearing of *A. sebae* using sea water with salinity range of 33-35%. In the present study, the fishes were bred with the salinity range of 20-25% and the larval rearing range was 58%.

CONCLUSION

Briefly, with the increasing demand for the captiveproduced marine ornamental fishes, particularly clownfish, *A. ocellaris* was successfully reared in captivity using brackish water. This achievement will assure success in raising subsequent generations by the aqua culturists for the prolonged existence of aquarium keeping of this species. Persian Gulf, having excellent relation with the Indian Ocean, is one of the vital niches of fishes and the specific situation of this Gulf makes its fishes popular. The *A.ocellaris* clown fish which originates in this gulf has the best survival rate and health than the other areas. Live food is the most important factor in production of this species and enrichment of their live prey with probionts improve the nutritional value of a fish's diet.

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