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Embryonic Development of Percula Clownfish, *Amphiprion percula* (Lacepede, 1802)

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Abstract: The Percula clownfish, *Amphiprion percula* (Lacepede, 1802) were reared in marine ornamental fish hatchery by using estuarine water to study their spawning behaviour, egg deposition and embryonic development. The spawning was recorded year round with the reproductive cycle between 14-21 days. The eggs were adhesive type, capsule shaped and bright orange in colour measuring 2.0-2.3 mm length and 1.0-1.2 mm width containing fat globules. The process of embryonic development was divided into 26 stages based on the morphological characteristics of the developing embryo. The time elapsed for each embryonic developmental stage was recorded. Hatching took place 151-152 hours after fertilization.

Key words: Percula clownfish · Captive condition · Morphology · Embryonic development

INTRODUCTION

The anemonefish, *Amphiprion percula* is a tropical coral reef fish belonging to the family Pomacentridae and sub family Amphiprioninae and they are one of the most popular attractions in the marine ornamental fish trade. Totally, 28 species of anemonefishes are under two genera *Amphiprion* and *Premnas* [1-3]. Compared to other damsel fishes, these fishes have some remarkable behavioural characteristics such as symbiotic association with sea anemones [2-4], formation of a group consisting of monogamous pair and varying number of sub-adults or juveniles [4-9].

Breeding patterns and behavioural aspects of many tropical and sub tropical Pomacentrid fishes have been well documented. The description of eggs, embryological development, larval rearing and juveniles production are available for relatively few Pomacentrid fishes and such reports are lacking in Indian waters. Since the studies on reproduction and their embryonic development are not much popular in percula clownfish, this study will provide basic information on its different stage of development to enhance the captive production.

MATERIAL AND METHODS

Ten numbers of sub-adult *Amphiprion percula* and five numbers of sea anemones, *Heteractis magnifica* were procured from the ornamental fish suppliers and transported to the hatchery at Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India. For the better health and survival, the fishes and anemones were packed in individual polythene bags filled with sufficient oxygen. After transportation, the fishes and anemones were accommodated in a quarantine tank for a week and later transferred to acclimatization tank. The fishes were retained their actual health condition during quarantine period and it allows them to adapt and it also helpful to reduce the stress associated with captivity. All the ten fishes were ranging from 50 mm to 60 mm in length. During acclimatization, after three months of rearing, one pair grew ahead of others and became the spawning pair in which the female made dominance over male and the same was shifted to the 750 l ash coloured rectangular FRP tank, spawning tank, containing 600 l of water along with its host anemone. Followed by this, other pair was formed and the size of the fishes ranged between 50-65 mm at the time of introduction into the spawning tanks.

The marine ornamental fish hatchery was maintained with estuarine water, which is taken from the Vellar estuary, (Lat. 11° 29' N; Long 79° 46' E) 1.5 Km away from the mouth (Bay of Bengal). The water was filtered through sand (5µ particle), charcoal and UV filters. An 'Eheim' brand canister filter was installed in the indoor water storage tank with a pumping capacity of 3400 l/hr and the same was loaded with coarse fiber particle filter pad, activated carbon, ceramic rings, zeolite, bioballs and coral

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sands respectively. The fishes and sea anemones were fed with different feeds such as boiled shrimp, bivalve meat, frozen adult *Artemia* and live *Acetes* spp. thrice a day. The water has been changed from the tank at the volume of 20 % at once in a week. During the study period, the temperature, salinity, pH, D.O. and light intensity were maintained at the following level. 25-29.9ºC, 22-26 ‰, 7.51-8.1, 4.13-6.94 mg/l and 600-900 lux respectively. Tanks were illuminated by 40-W fluorescent tube light suspended from 45 cm above the tank water level. The photoperiod of each tank were maintained at 12 hrs light and 12 hrs dark throughout the study period. In each spawning tank, a separate locally made underwater filtration setup was installed to maintain good water quality.

Each spawning tank was provided with white coloured tiles, dead coral pieces and live rocks as substratum for egg deposition which imitates the natural environment. After 3.5 months of rearing in the spawning tank, the fishes started to lay eggs. The total number of eggs per clutch was estimated by counting all eggs in 1 cm^2 and then multiplying with deposition area [8-10].

In the present study, the fertilized eggs samples were collected by scraping using 'Ink filler' from the spawning tank during different periods. The samples were taken immediately after fertilization and followed on each day at the same time till hatching. The developmental stages were observed under a light microscope and sequenced based on morphological features and taken photos by using digital camera. The length and width of the egg were measured. The eggs were continuously monitored for studying the daily changes in embryonic development.

RESULTS

At the time of spawning, the abdomen of females became larger. The spawning was happened in between 9 am and 1 pm, mostly during morning hours. Female laid capsule shaped eggs on the cleaned substratum in nearly rounded or oval patch, followed by the male subsequently fertilized the eggs. The spawning lasts for one hour to one hour and forty five minutes. The colour of newly laid eggs was varied from bright orange to yellowish. The eggs were adhesive, covering with transparent chorion with narrow perivitalline space. The eggs were measuring 2.0-2.3 mm length and 1.0-1.2 mm width. The surface of egg capsule was smooth. The number of eggs per spawning was approximately 400-700 eggs depend upon the size of the female. Initially the number of eggs was less and latter spawning it was increased.

Fig. 1: Spawning pair of *Amphiprion percula*

Fig. 2: *Amphiprion percula* with egg period

Fig. 3: Morphological changes occurring in the eggs during incubaltion period

One end of the egg capsule was identified as animal pole, contained some gelatinous substance to adhere itself to the substratum. Newly laid eggs have yolk in light yellow colour with large fat globules and the colour was more intense as the eggs became older. The fertilized eggs took 7-8 days for hatch and the hatching time often occurred at 9.00 pm-11.00 pm, particularly during dusk period. During the incubation, the major role in parental care was played by the male, which mainly involves fanning and mouthing the eggs. Fanning was done by fluttering the pectoral fins, which created a cooling effect to the clutch that reduced the damage of eggs. The unfertilized, dead eggs and dust particles were also removed by the process of mouthing.

The embryonic development of the percula clown fish from fertilization to hatching was classified into 26 stages as follows.

Stage 1: It was a one-celled stage. This stage was specified as zygote or immediately after fertilization, having one uncleaved cell. The cytoplasm of fertilized egg was clear. The animal pole was characterized by its half circle shape which attached to the egg laying materials while the vegetal pole contained yolk and different sizes of fat globules dispersed in it (Fig.4).

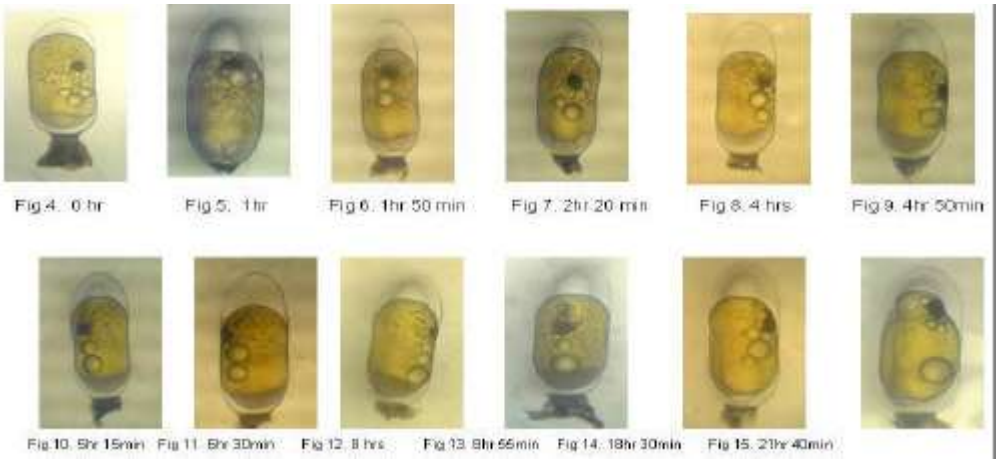

Fig. 4-15: Embryonic development of *Amphiprion percula* (stages 1 to 12)

Stage 2: The first cleavage started by dividing the blastodisc into two blastomeres. This event appeared in the first hour after fertilization with meroblastic type. Two blastomeres were observed at the animal pole containing only half size of the original cell. Their cytoplasm was clear. The fat globules were very small and moved towards vegetal pole (Fig.5).

Stage 3: Four equal blastomeres resulting from the second mitosis appeared on vertical plane. This event happened at one hour and fifty minutes after fertilization. Each blastomere was smaller in size and fat globules were observed in the yolk (Fig.6).

Stage 4: Eight blastomeres were seen after 2 hours and 20 minutes of fertilization. The blastomeres were smaller and equal in size (Fig.7).

Stage 5: Sixteen blastomers appeared 4 hours after fertilization (Fig.8).

Stage 6: The blastomeres became overlapping due to the limited confined space with in the capsule. This was happened after four hours and fifty minutes after fertilization (Fig.9).

Stage 7: The blastomeres extended more laterally and found as a flat layer about five hours and fifteen minutes after fertilization (Fig.10).

Stage 8: The blastomeres became much smaller and have equal size, about six hours and thirty minutes after fertilization (Fig.11).

Stage 9: The blastomeres were very small and the fat globules which present in the yolk were moved towards the animal pole after eight hours from fertilization. (Fig.12)

Stage 10: The embryo was developed and called morula. The blastomeres were very small about eight hours and fifty five minutes after fertilization (Fig.13).

Stage 11: It is the gastrula stage in which the blastomeres were extended towards the vegetal pole. The number of fat globules was decreased. It took about 18 hours and 30 minutes after fertilization to reach this stage (Fig.14).

Stage 12: The blastomeres moved more from animal pole to cover the part of yolk called epiboly. The fat globules are started to disappear. It took about 21 hours and 40 minutes after fertilization (Fig.15).

Stage 13: The embryo began to form head and neural ectoderm. The outer layer of the embryo formed the longitudinal ridges close to the yolk after 27 hours and 35 minutes from fertilization (Fig.16).

Stage 14: The longitudinal neural tube was formed and attached to the yolk. The optic buds were also seen. It took about 30 hours and 25 minutes after fertilization to reach this stage. (Fig.17)

Stage 15: The head was clearly visible. The body was transparent having no muscular structure. It took about 34 hours and five minutes after fertilization (Fig.18).

Fig. 16-27: Embryonic development of *Amphiprion percula* (stages 13 to 24)

Stage 16: The embryo completely turned itself. The body was still attached to the yolk sac but the tail had showed movement. The melanophores were found on head and yolk. It took about 42 hours and 5 minutes to reach this stage after fertilization (Fig.19).

Stage 17: The tail became separated from the yolk and moved freely but the body still attached to the yolk. The heart began to beat at this stage. The melanophores were increased in the entire body especially in the head region and eye lens was visible. The embryo took about 48 hours and 20 minutes from fertilization (Fig.20).

Stage 18: The body length distinctly increased and the tail moved freely. The mouth and the lens were evident. The blood circulation through the vessels could be observed indicating the functioning of circulating system of the embryo. It took about 55 hours and 10 minutes after fertilization to reach this stage (Fig.21).

Stage 19: The head and tail were clearly separated from the yolk. The large eyes contained brown pigments and their lens were prominent. High pigmentation observed in the head but less in the tail region. The head and tail of the embryo extended to attach the capsule. The blood circulation was clearly visible through vessels. It took about 64 hours and 15 minutes from fertilization to reach this stage (Fig.22).

Stage 20: The embryo was growing while the yolk was decreasing. The forming organs were getting enlarged and the yolk was covered with body. The head was enlarged which have prominent big eyes and brown pigments. It took about 75 hours and 30 minutes from fertilization (Fig.23).

Stage 21: The embryo was further enlarged and occupied most of the space in the capsule. Its movement in the capsule was continuous. The melanophores were abundant in the head region. The pectoral fin was quite large. It took about 98 hours and 25 minutes from fertilized egg to reach this stage (Fig.24).

Stage 22: The yolk sac became quite small and covered by the abdomen of the embryo. The head occupied one third of the capsule space. The melanophores are distributed throughout the body. Fins and eyes are well developed. It took about 109 hours and 20minutes from fertilization (Fig.25).

Stage 23: The embryo showed vigorous movement inside the egg capsule. It took about 119 hours and 55 minutes from fertilization to reach this stage (Fig.26).

Stage 24: The capsule is fully occupied by the embryo and the size of the yolk gradually decreased. It took about 127 hours and 15 minutes from fertilization (Fig.27).

Stage 25: The embryo began to hatch by moving itself vigorously to break the capsule. The eyes were found glowing and rotating itself. It took about 151 hours and 15 minutes from fertilization before hatching took place (Fig.28).

Fig. 28,29: Embryonic development (stages 25 to 26) and newly hatched larvae of *Amphiprion percula*

Stage 26: The embryo was free from the capsule and became a larva. The dorsal fin, caudal fin and anal fin were continuous in a longitudinal line. The total length of larva was about 3.2 mm. It took about 152 hours and 20 minutes after fertilization to become a larva (Fig.29).

DISCUSSION

Pomacentrid fishes lay demersal eggs attached to the submerged objects. The shape of eggs was varying from ovate to capsule shaped in different species [9-11]. The eggs of *Amphiprion chrysopterus* was 2.4 X 0.9 mm [3] and Hoff [1] reported the length of anemonefish eggs ranged from 2.0 to 2.4 mm. The size of *Amphiprion percula* eggs in the present study is 2.0-2.3 mm length and 1.0-1.2 mm width.

The spawning activity was lasted for more than one hour and the female laid eggs in circle or oval shape clutches. Usually the male fertilizes the eggs immediately after the female complete the process, hence the time of fertilization varies within the clutch. Similar observations were recorded in wild populations of *Amphiprion chrysopterus [4]*. Therefore when spawning is completed, the clutch contains eggs are randomly fertilized at different times. So when samples are taken after spawning, it will be difficult to ascertain the exact time of fertilization. The developmental rate of the fertilized egg is profoundly varied with temperature and also with oxygen content of water [12-13].

In the present study, the hatching started about after 152 hours and 20 minutes of fertilization. The cleavage pattern of *Amphiprion percula* is same as those of other anemonefishes [14-17]. In *Amphiprion percula,* the yolk had different sizes of fat globules dispersed in the vegetal pole, which was similarly reported in *Amphiprion polymnus* [12-14].

Delsman (1930) studied *Amphiprion percula* eggs and larvae from the Java Sea. He described the morphological characteristics and correlated roughly with the time after fertilization but did not categorize them into different stages. Hoff [4-5], studied the egg development of the anemonefish, *Amphiprion ocellaris.* There are variation in time and stages of embryonic development among different genus and species of fishes. Several factors such as photoperiod were also known to affect their growth and development [6].

Sreeraj [12] made a detailed embryological development study of seven species of Pomacentrid fishes such as *Amphiprion sebae, Pomacentrus caeruleus, Pomacentrus pavo, Neopomacentrus cyanomos, Neopomacentrus nemurus, Neopomacentrus sindensis* and *Dascyllus carneus.* The embryological development of Percula clownfish provides a better idea on their developmental stages and this information helped the mass production of this tropical coral reef ornamental fish in captivity.

CONCLUSION

In recent years, the surge in the trade of tropical marine ornamental fishes has increased considerably and at the same time indiscriminate exploitation has also led to negative repercussions on coral reef ecosystem. The tank reared ornamental fishes is the final solution for a long term sustainable trade and the way to marine biodiversity conservation. Development of culture technology is well accepted as an environmentally sound way to increase the supply of hatchery bred marine ornamentals by reducing the pressure on wild population. Besides this, intensive research in development of reliable system for broodstock maintenance, breeding and larval rearing can result in the successful hatchery production of many more species belonging to the family Pomacentridae. Further studies on embryological development of marine ornamental fishes will give an enhanced improvement to learn their developmental stages and this should be helpful for the commercial production of this elegant fish.

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