World Journal of Fish and Marine Sciences 2 (5): 410-415, 2010
ISSN 2078-4589

Spawning Behaviour and Embryonic Development of Regal Damsel Fish, *Neopomacentrus cyanomus* (Bleeker, 1856)

Subodh Kant Setu and T.T. Ajithkumar

Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India

Abstract: The regal damsel fish, *Neopomacentrus cyanomus* (Bleeker, 1856) were reared using estuarine water to study their captive spawning, behaviour, egg deposition and embryonic development. The spawning was recorded year round with an average reproductive cycle between 10-15 days. The eggs were capsule shaped, transparent, measuring 0.8-1.0 mm in length and 0.2-0.4 mm in width. Eggs were adhesive in nature and attached with substratum by fillial membrane. Uniparaental care was observed. Hatching takes place during dusk period on/after 96 hr post fertilization based on environmental condition.

Key words: Regal damsel fish · Uniparental care · Embryonic development · Spawning behaviour

INTRODUCTION

Aquarium keeping is a popular hobby of the world and is second only to photography [1]. In recent decades, marine ornamental fish keeping emerged worldwide due to advancement in filtration system, aquarium keeping etc. Currently, these ornamental fishes are collected from its natural habitat is coral reef ecosystem and sold worldwide. Pomacentridae fishes are the most sought after group in the ornamental fish trade. Increasing pressure on natural populations of coral reef animals, due to their expanding popularity in the aquarium trade has stimulated interest in developing breeding and culture techniques for these fishes [2]. For the development of hatchery production techniques, it is necessary to understand the entire ontogeny of the particular fish. Ontogeny of fish can be divided into several periods such as embryonic, larval, juvenile, young, adolescent and adult [3, 4]. Further, Moyle and Cech [5] divided the period that begins at fertilization into two phases; first is the egg cleavage phase and the second is the embryo phase.

Fishes are exceptional among vertebrates because of their unparalleled variability of reproductive and social patterns [6,7]. Among fishes, family Pomacentridae have diverse variability in breeding and its social patterns. The family includes damsel and clown fishes. Damsel fishes show protogynous type of hermaphroditism, in which, at primary stage, all adolescents are female and later they use to change their sex depending on the colony conditions. This condition is said to occur frequently in polygynous mating systems [8]. Damsel fishes are generally omnivorous, feeding on algae, zooplankton and a wide variety of other invertebrates [9]. In damsel fishes, spawning takes place externally. Tanaka [10] reported that 52 species of Pomacentrids spawn in tank conditions. In the Indian perspective, only very less number of ornamental fish species bred in captivity and much attention has not paved on reproduction and embryonic developmental studies. In India regal damsel fish is very popular in marine ornamental fish trade. Therefore, for captive breeding and hatchery production of regal damsel fish, it is necessary to observe breeding behaviour and embryonic development. Hence, the present study has been undertaken.

MATERIALS AND METHODS

Fifteen number of sub-adult healthy regal damsel fish were procured from the ornamental fish suppliers and brought to the marine ornamental fish hatchery at the Centre of Advanced study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India. They were accommodated in a quarantine tank for a week and later shifted to the rearing tank. All the fifteen fish size were ranging from 40 mm to 60 mm in total length. Since damsel fishes are polygamous, they were reared in three different cement tanks with 1000 l water holding capacity in five numbers each. For rearing the fishes estuarine water was pumped from the Vellar estuary (Lat. 11° 29'N;

Corresponding Author: Subodh Kant Setu, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India, E-mail: subodhsetu@gmail.com.

Long. 79° 46' E) during high tide periods and allowed them for settlement in cement tanks (sump), overnight. After settling the dust particles, the water was passed through a sand filter and stored in over head tank. The stored water was further treated with UV ray for use in fish tanks. In the tank, earthen pots and tile pieces were provided, so that the fish could hide or get shelter and also, it served as substratum for laying the eggs. Under water filtration setup was kept for maintaining the good water quality. The fishes were fed thrice a day with different feeds such as live *Acetes* spp., boiled clam, oyster and polycheate worms. Water quality parameters such as temperature, salinity, pH, dissolve oxygen and photoperiod were maintained as 25-30°C, 22-26 ‰, 7.5-8.1, 4.5-6.8 mg/l, 11 hr light and 13 hr dark, respectively. After four months of rearing in the spawning tank, the fishes started to lay eggs with a brief courtship. Number of eggs in a clutch was estimated by counting the number of eggs in a known portion of the clutch area and multiplying it with the total area of the egg clutch [11]. Embryos were kept in parental tank for incubation and for developmental studies. The embryo samples were collected by scrapping with ink filler and the time was recorded when 60% of the embryos were ready to attain cleavage, blastulation, gastrulation, organogenesis, pre-hatching and larval stage clearly. The development stages were observed under light microscope and sequenced based photographs were taken using digital camera. The developmental stages were also referred to the previous studies [12-14].

RESULTS

During the present course of study it was observed that, 6 to 48 hours before spawning, male fishes changes its body colour and retained it until spawning. Courtship began two days before spawning with the initiative taken by male and they used to chase the females. A male dives vertically to attract the female and it was also observed that they have given gentle touch on operculum by using the mouth. At the time of spawning, female belly become bulged. On the breeding day, male used to clean the substratum in which they could lay the eggs by rubbing it with their pelvic fins and picking off any loose particle or algae with their mouth. During this period, the females entered the nesting site several times, but did not spawn. After satisfaction, female entered the nesting site with a distended venture and participated in a brief courtship consisting of side to side quivering motions and contact with the males. The male butted the female in the genital region and slapped them on the head with their caudal fin. Spawning on clean site usually occurred during dusk period (5-8 pm) mostly, just as the lights were turned off and took 30-90 min with 2 to 10 min interval (Fig. 1).

Regal damsel fishes were showing uniparental care. Only, the male fish guard and fan the egg clutch (Fig. 2) and it was observed that during the process, the male removed the dead and unfertilized eggs from the clutch. After spawning, male was not allowing any other fish to enter into the nest area including their female partner. The eggs were demersal, adhesive, capsule shaped (elliptical), transparent, chorion measuring 0.8-1.0 mm in length and 0.2-0.4 mm in width. The number of eggs in clutch varied between 2200±560. The surface of the egg capsule was smooth and one end of the egg capsule was identified as animal pole, contained some glutinous substance to adhere itself to the lining materials.

The embryonic development of regal damsel fish from fertilization to hatching has been classified into 14 stages as follows.

Stage 1: The animal pole was characterized by capsule shape which attached to the egg laying materials, while the vegetal pole contained yolk and different sizes of fat globule Sixteen blastomeres appeared in between 130-140 minutes after fertilization. The blastomeres were smaller and equal in size. One end of the capsule was attached to the substratum by filial membrane (Fig. 3).

Stage 2: This stage reached after 180-190 minutes of post fecundation. Four rows of eight cells were observed in this stage and total number of cells reached thirty two (Fig. 4).

Stage 3: This event happened in between 480-540 minutes and this stage is called blastula. The embryo formed a space called blastocoel which was clearly separated from the yolk. In this stage, dense cells were observed (Fig. 5).

Stage 4: The blastomeres moved downwards from the animal pole to cover a part of the yolk called epiboly. The blastomeres started moving inward to form three germ layers called gastrulation. It took fourteen hours (Fig. 6).

Stage 5: The neural tube was formed and the yolk was enclosed within the germ ring leaving a small yolk plug outside (early yolk plug stage). This event appeared at 21hours after post fecundation (Fig. 7).

Stages 6: In this stage, yolk plug was more prominent and on the margin melanophores appearance was started. This stage reached on 24 hours after post fecundation (advanced yolk plug stage) (Fig. 8).

Fig. 1 Spawning of regal damsel fish

Fig . 2 Fanning and guarding the egg clutch

Fig. 3. 2 hr 10 min

Fig. 4. 3 hr

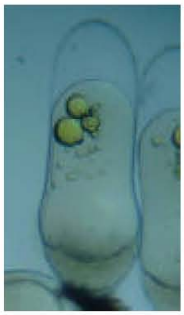

Fig. 5. 8 hr

Fig. 6. 14 hr

Fig. 7. 21 hr

Fig. 8. 24 hr

Fig. 9. 28 hr

Fig. 10. 34 hr

Fig. 11. 38 hr

Fig. 12. 40 hr

Fig. 13. 48 hr

Fig.14. 68 hr

Fig. 15. 82 hr

Fig.16. 95 hr 30min

Fig. 17. Newely hatched larva

Fig. 3 to 16 showing embryonic developmental stage and fig. 17 showing newly hatched larva

Stage 7: In this stage, head and tail formation was started. On the head region black spot like appearance, the primary sign of eye formation was observed. The embryo took 28 hours to reach this stage (Fig. 9).

Stage 8: The head with two optic buds was clearly apparent and mouth was noticeable but not clear. The body was transparent having muscular structure. It took about 34 hours after post fecundation (Fig. 10).

Stage 9: The optic buds were prominent, head and trunk began to differentiate and on the head region, melanophores started to appear. The embryo took 38 hours to reach this stage (Fig. 11).

Stage 10: The melanophores were observed on the optic lens, tail bud and on the surface of head. Head and trunk began to differentiate and movement started on tail region. The embryo took about 40 hours (Fig. 12).

Stage 11: At this stage, the heart began to beat and the skeletal muscle was found along the body length. Melanophore pigmentation became slightly dark on head. The embryo took about 48-52 hours from fertilized egg to reach this stage (Fig. 13).

Stage 12: The embryo size was growing and the envelope decreased. The head and tail were clearly separated from the yolk. High pigmentation was seen on the head region whereas less, on the tail. The head and tail were attached with embryo. This embryonic event took place between 68-70 hours after post fecundation (Fig. 14).

Stage 13: The embryo became large and filled most of the envelope area. Heart beating and frequent tail movement was also observed. Melanophores became deep in the entire region. This stage took place after 82hours from fertilization (Fig. 15).

Stage 14: The embryo was free from the envelope, to become a larva. Pectoral fins were well developed and the vertical fin fold had partially separated into dorsal and caudal fins. The movement of embryo was very violent inside the envelope and it started to break the envelope. This stage took about 96 hours after fertilization (Fig. 16).

Newely Hatched Larva: This was the just hatched out larva containing large yolk sac and melanophores were prominent on the ventral margin of the body and on forehead. The eye and mouth were not well developed and there was no opening observed in mouth to engulf the feed. The body was transparent and the total length varied between 1.8-2.2 mm in length (Fig. 17).

DISCUSSION

In the present study, natural spawning was observed under captive condition and it started with male initiation as observed in *Chrysiptera parasema* by Olivotto *et al.* [15]. In a few damsel fish species, usually one female and one male take part simultaneously in spawning eventhough more functional females are present in the colony with occasional polygamy [16, 17]. Goulet [16, 18] reported that the females of yellowbelly damselfish, *Amblyglyphydodon leucogaster* mated with different males at different spawning times. In the present study, it was observed that the regal damsel, *N. cyanomus* spawned during dusk period whereas according to Manica [19], the scissor tail sergeant major, *A. sexfasciatus* spawning took place in the morning until sunrise.

Like other Pomacentrids, *N. cyanomus* male parent also exhibited culling by removing the unfertilized or dead embryos and this was done to protect the other embryos. Removal of dead and unfertilized eggs was also reported in other *Amphiprion sebae* [20]. All the eggs in the nest were encased in a flexible, transparent, conical capsule and adhesive in nature, as reported in the other damsel fish, *C. parasema* [15]. The eggs were telolecithal with a mass of yolk and the size of the yolk was reduced gradually when the cleavage proceeded. During the embryonic development, the reduction of yolk size was also reported in *A. ocellaris* and the same is the basic pattern in teleost fishes [14].

In the present study, it was observed that from 0 hr to 24 hrs after fertilization cleavage phase started. In clown fish, *Amphiprion polymnus* and *A. ocellaris* also 0 hr to 24 hrs was the cleavage phase [13, 14]. One hour after fertilization, the process of segregation of cytoplasm and yolk was almost completed whereas in *Abudefduf sexfasciatus*, it took only 50 minutes after fertilization [21] and it could be due to the effect of temperature and other environmental parameters.

The newly deposited eggs were 0.8-1.0 mm in length and 0.2 - 0.4 mm in width with the presence of different size of fat globules. This supports the findings of the previous study regarding embryonic development and presence of fat globules. In *N. cyanomus*, the yolk had different sizes of fat globules dispersed in the vegetal

pole. Dispersal of oil globules of damsel fish was different from that of Medaka fish, *Oryzias latipes* where, they were mainly found near the vegetal pole when mitosis began [22].

The egg attained the 8, 16, 32, 64 blastomere stages after 2 hrs of fertilization whereas in *A. ocellaris*, it was reported after 3 hrs of fertilization. In *A. sexfascitus*, 32 blastomere was formed in 2 hrs 45 min [21]. Allen [23] reported that *A. chrysopterus* took 3 hrs to reach 8-cell stage, 5 hrs to 32-cell stage and 10 hrs to the blastula stage. The early developmental stage varied depending on egg quality, brood fish health status, species nature [24] and temperature [25, 26]. Complete hatch out took place on the 4th day of post fecundation and some time it was observed on the 5th day and this may be due to the prevailing environmental parameters particularly the surrounding temperature. In the present study, during incubation, the temperature varied between 26°C and 29°C. Temperature variations during the embryonic development were also studied and reported in different Pomacentrids like in sergeant major, *A. sexfasciatus*, 25±1°C [21], Saddleback clown fish, *A. polymnus*, 25-28°C[13], False clown fish, *A. ocellaris*, 27-28°C [14].

At the basal part of the egg, a small end of the capsule is a mass of adhesive threads that anchors the egg to the substrate [15]. The chorion is highly adhesive but does not adhere to the zona radiata of other oocytes in the clutch and this specific feature provides egg distribution over the substrate only in one layer and excludes the possibility of depositing the eggs as a second layer [21]. Due to this mechanism, the parent, while fanning the eggs, provides aeration or oxygen which prevents spoilage of the eggs. It was also evident from the study that, development of egg also varies with surrounding oxygen content [27]. The spawning behaviour and embryonic development of regal damsel fish provides a superior initiative on developmental stages and this information helped the mass production of this tropical coral paradise fish in captivity.

In conclusion, the technological breakthrough in marine ornamental fish rearing has increased demand of marine ornamental fishes with negative repercussions on coral reef ecosystem. For sustainable development of marine ornamental trade and to conserve the nature, captive breeding is the alternate solution. Behavioural and embryological study will be helpful for the hatchery production of this charming regal damsel fish. The information contained herein may represent to other damsel fishes.

ACKNOWLEDGEMENT

The authors are thankful to the Director of Centre of Advanced Study in Marine Biology and authorities of Annamalai University for the facilities. Department of Science and Technology, Government of India, New Delhi also acknowledged for the financial assistance.

REFERENCES

1. Alava, V.R. and L.A.O. Gomes, 1989. Breeding marine aquarium animals: The anemone fish. The Naga, ICLARM Q., pp: 12-13.
2. Rajasekar, J., S.K. Setu, T.T. Ajithkumar and T. Balasubramanian, 2009. Development of hatchery technology for marine ornamental fishes with special reference to in captivity. World J. Agric. Sci., 5(4): 466-469.
3. Balon, E.K., 1981. Additions and amendments to the classification of reproductive styles in fishes. Environ. Biol. Fish, 6: 377-389.
4. URHO, L., 2002. Characters of larvae – what are they? Folia Zool., 51: 161-186
5. Moyle, P.B. and J.J.R. Cech, 2004. Fishes: An introduction to ichthyology, 5th ed. Pearson, Prentice-Hall, Inc., Upper Saddle River, NJ., pp: 726.
6. Taborsky, M., 1994. Sneakers, satellites and helpers: Parasitic and cooperative behaviour in fish reproduction. Adv. Study Behave., 23: 1-100.
7. Taborsky, M., 1999. Conflict or cooperation: What determines optimal solution to competition in fish reproduction? In: Behaviour and Conservation of Littoral Fishes (Eds. R. Oliveira., V. Almada and E. Gonclaves). Lisbon: ISPA: pp: 301-349.
8. Warner, R.R., 1984. Mating Behavior and Hermaphroditism in Coral Reef Fishes. Am. Sci., 72: 128-136.
9. Allen, G.R., 1975. Damsel fishes of the South Seas. T.F.H. Publications Inc. New Jersey, pp: 240.
10. Tanaka, Y., 1998. Reproductive behaviour and morphology of eggs and larvae of damselfish. J. The School of Marine Science and Technology. Tokai University, 45: 167-179.
11. Danilowicz, B.S., 1995. The role of temperature in spawning of damselfish *Dascyllus albisella*. Bull. Mar. Sci., 57(3): 624-636.
12. Chen, Y.F., 2005. Induced ovulation and embryonic development of ocellated puffer, *Takigugu ocellatus*. J. Appl. Ichthyo., l(21): 136-140.

13. Rattanayuvakorn, S., P. Mungkornkarn, A. Thongpan and K. Chatchavalvanich, 2005. Embryonic development of saddleback Anemonefish, *Amphiprion polymnus*, Linnaeus (1758). Kasetsart J. Nat. Sci., 39: 455-463.
14. Liew, H.J., M.A. Ambak, A.B. Abol-Munafi and T.S. Chuah, 2006. Embryonic development of clownfish *Amphiprion ocellaris* under laboratory conditions. J. Sustain. Sci & Mngt., 1(1): 64-73.
15. Olivotto, I., M. Cardinali, L. Barbaresi, F. Maradonna and O. Carnevali, 2003. Coral reef fish breeding: the secrets of each species. Aquaculture, 224: 69-78.
16. Goulet, D., 1995. Temporal patterns of reproduction in the Red sea damsel fish *Amblyglyphydodon leucogaster*. Bull. Mar. Sci., 57(3): 582-595.
17. Schwarz, A.L., 1995. Social organization and behavior in *Dascyllus reticulates* (Pisces:Pomacentridae) in two contrasting habitats. Bull. Mar. Sci., 57(3): 706.
18. Goulet, D., 1997. Reproductive behavior and spawning success of the female *Amblyglyphydodon leucogaster* (Pisces: Pomacentridae) from the red sea. Env. Biol. Fish., 50: 49-60.
19. Manica, A., 2003. The effect of brood size and age on partial filial cannibalism in the scissortail sergeant. J. Fish. Biol., 63: 37-47.
20. Ignatius, B., G. Rathore, I. Jagdish, D. Kandasmi and A.C.C. Victor, 2001. Spawning and larval rearing technique for tropical clown fish *Amphiprion sebae* under captive condition. J. Aqua. Trop., 16(3): 241-249.
21. Shadrin, A.M. and N.G. Emel'yanova, 2007. Embryonic-larval development and some data on the reproductive biology of *Abudefduf sexfasciatus* (Pomacentridae: Perciformes). J. Ichthyol., 47(1): 67-80.
22. Iwamatsu, T., 1994. Stages of normal development in the medaka, Oryzias latipes. Zool. Sci., 11: 825-839.
23. Allen, G.R., 1974. The Anemonefishes. The classification and biology. T. F. H. Publication, Inc. USA, pp: 352.
24. Kjorsvik, E., K. Hoehne-Reitan and K.I. Reitan, 2003. Egg and larval quality criteria as predictive measures for juvenile production in turbot (*Scophthalmus maximus* L.). Aquaculture, 227: 9-20.
25. Falk-Petersen, I.B., 2005. Comparative organ differentiation during early life stages of marine fish. Fish Shellfish Immun., 19: 397- 412.
26. Ajithkumar, T.T., S.K. Setu, P. Murugesan and T. Balasubramanian, 2010. Studies on captive breeding and larval rearing of clown fish, *Amphiprion sebae* (Bleeker, 1853) using estuarine water. Indian J. Mar. Sci., 39(1): 114-119.
27. Yamoto, T., 1975. Stage in the development. Accessed 21 July, 2002. http://www.bioll.bio.nagoya-u.ac.jp:800/stages.html.