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Influence of prompt first feeding on growth and survival of Clownfish *Amphiprion percula* larvae

K. V. Dhaneesh*, T. T. Ajith Kumar, S. P. Divya, S. Kumaresan and T. Balasubramanian

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608 502, Tamil Nadu, India

Abstract

The present study was conducted to evaluate the changes in the morphometric characteristics and survival rate of clownfish *Amphiprion percula* larvae with respect to different initial feeding times. The larvae started their initial feeding at 12 hr showed maximum survival (42%) and no survival was observed at 8th day when larvae started initial feeding after 24, 36 and 48 hr. Morphometric parameters such as Total length (TL), Weight (W), Head depth (HD), Body depth (BD) and Eye diameter (ED) were significantly higher in larvae which started their initial feed at 12 hr. The present study thus suggests that the first feeding of the clownfish *A. percula* larvae can be initiated before 12 hr, for better growth and survival.

Key words: Clownfish, Growth, Initial feeding, Larval rearing, Survival

Introduction

The hobby of marine ornamental fish keeping is more valuable. In the recent past, aquarium keeping has increased quiet a lot and more hobbyists are interested in this lucrative trade. Among marine ornamentals, clownfish is very popular among aquarists due to their aesthetic appeal and easy adaptability to captive condition. There are 30 species of clownfishes including two inter generic hybrids between *Amphiprion* and *Premnas* (Fautin and Allen, 1992; Allen et al., 2008, 2010; Mebs, 2009).

There are many reports on the successful rearing of various species of clownfishes from different parts of the world (Hoff, 1996; Wilkerson, 1998; Gopakumar et al., 1999; Ignatius et al., 2001; Ajith Kumar and Balasubramanian, 2010, Dhaneesh et al., 2011). The number of marine ornamental fishes reared in captivity today is more than 84 species, which come under the groups such as clowns, damsels, gobiids, cardinals and pseudochromids (Gopakumar, 2006). Of these, maximum number of breeding experiments have been conducted in the family Pomacentridae (26 species) which is noteworthy (Olivotto et al., 2003). Larval rearing of marine ornamental fishes is relatively complicated due to their small size and feeding problems, particularly first feed and peculiar water conditions required in the rearing system. As a result, only few species have been bred in captivity with good results.

First feeding of a fish larva is very crucial for its subsequent growth and survival. Most of the fish larvae show deformed growth and inability to swim and prey the feed if they don't initiate successful first feeding soon after the mouth opening (Dou et al., 2002). When yolk sac is exhausted, fish larvae will enter into exogenous feeding and must successfully establish the transition of exogenous nutrition or suffer progressive starvation (Blaxter and Ehrlich, 1974). During this critical period, at the time of first feeding, food availability and the larva's ability to consume it are the important factors for establishing successful exogenous feeding for larval survival, growth and recruitment of the next generation (Sanderson and Kupferberg, 1999).

In recent years, many studies have been carried out to examine the first and subsequent feeding behaviour and survival mechanisms of fishes; for example, Atlantic herring *Clupea harengus*, Gilthead sea bream *Sparus aurata*, Japanese flounder *Paralichthys olivaceu*, California halibut *Paralichthys californicus*, Japanese anchovy *Engraulis japonicas*, Spotted sand bass *Paralabrax maculatofasciatus* and Asian sea bass *Lates*

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*Corresponding Author

K. V. Dhaneesh
Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608 502, Tamil Nadu, India

Email: dhanee121@gmail.com

calcarifer (Yufera et al., 1993; Dou et al., 2002, 2005; Gisbert et al., 2004; Wan et al., 2004; Pena and Dumas, 2005; Kailasam et al., 2007). Due to the over exploitation from the wild for aquarium trade and environmental pressures, clownfishes have experienced a decline in the recent years. So far, information about the mechanism of larval survival strategy of the clownfish is still unclear. Therefore, improvement of the larval survival and juvenile quality is the need of the hour for sustainable aquarium trade and also conservation of the marine biodiversity. Further, characterization of starvation effects under culture conditions can be a valuable tool for evaluating the adequacy of the captive conditions and finding methods for avoiding mortality caused by starvation. The present study was carried out to investigate the consequence of delayed initial feeding on the growth, survival and to find out the most appropriate time to initiate the first feeding in captivity of clownfish *Amphiprion percula.*

Materials and Methods

Water quality

The experimental set up (Figure 1) was maintained with estuarine water, drawn from the Vellar estuary (Lat. 11° 29’ N; Long. 79° 46’ E), 1.5 km away from its mouth (Bay of Bengal). The water was filtered through cartridge (5µ particle), charcoal and UV filters. In addition two canister filters (Eheim, Germany), loaded with coarse fiber particle filter pad, activated carbon, zeolite, bioballs, ceramic rings and coral sands, were installed in the indoor water storage tank (capacity 3400 L hr^{-1}).

Figure 1. The experimental set up.

Temperature was measured using Mercury centigrade thermometer with 0.5^0C accuracy, salinity by Salinity meter (Ecoscan, Singapore), pH by pH pen (Eutech, Singapore), dissolved oxygen by D.O. probe (Ecoscan, Singapore), ammonia by Ammonium test kit (Merck, Germany) and light intensity by Lux meter (Lutron, LX-101, Taiwan).

Morphometric characters of the larvae

Figure 2 shows *A. percula* and sea anemone *Stichodactyla mertensii* with egg clutch. The eggs were hatched out in the parent tank itself and the larvae were gently collected using small glass bowls and introduced to the rectangular ash coloured larval rearing tanks (FRP) of 50 l capacity (45 x 37 x 30 cm). The stocking density of larvae was 3 no l^{-1} in twelve experimental tanks (95 ± 5 no. per tank). The newly hatched free swimming larvae measured 3.2-3.6 mm in length and the mouth size was 0.3-0.4 mm. The larva had transparent body, large eyes and small yolk sac. All the larvae used for the experimental set up, were collected from the same parents and maintained in the same rearing conditions. The initial feeding time was set at 12, 24, 36 and 48 hr after hatching. The larvae were initially provided with the micro algae *Nannochloropsis salina* followed by the wild plankton (15-20 no ml^{-1}) and then cultured rotifer *Brachionus plicatilis* (6-8 no ml^{-1}) up to 10 days. After 10 days, newly hatched *Artemia* nauplii were provided (6-8 no ml^{-1}) along with rotifers up to 20 days after hatching and the larvae were fed 3 times daily (09:00, 13:00 and 17:00 hr). Gentle aeration was provided to maintain dissolved oxygen and also to promote a homogeneous distribution of feed supplied.

Figure 2. *A. percula* and sea anemone *Stichodactyla mertensii* with egg clutch.

The larvae were monitored after transfer to the experimental tanks and mortality, if any, recorded at every 12 hr. All the experiments were carried out in triplicate under identical conditions. A total of 8 larvae from each triplicate of all the experimental tanks were sampled from 1st to 10th days for the measurement of total length (TL), head depth (HD), body depth (BD) and eye diameter (ED).

Measurement of total length (TL) alone was continued up to 18th day, when the larvae attained complete metamorphosis. The morphometric characters were measured with ocular micrometer (Erma, Tokyo) under light microscope (Novex, Holland). Weight was taken using an electronic digital balance (Sartorius, Germany) after blotting the larvae in a water absorbent paper. Morphometric measurements of the larvae, which initiated their first feeding after 24, 36 and 48 hr, could be taken only up to 8th, 5th and 3rd day respectively due to the complete mortality.

Yolk volume was measured using the formula $V = \pi / 6\ l\ h^2$, where l is yolk sac length and h is yolk sac height, which were measured by Ocular micrometer. The volume of oil globule was estimated from the formula $V = \pi / 6\ d^3$, where d is the oil globule diameter (Bagarinao, 1986).

Survival rate of larvae

The *A. percula* larvae were maintained in 50 l FRP tanks (3 larvae l^{-1}) and initial feeding was started at 12, 24, 36 and 48 hr after hatching. The experiment was continued up to 18 days after hatch and conducted in triplicates. Total survival rate was estimated at the end of the experiment and no sampling was conducted throughout the study period. Tanks were cleaned once in a day and dead larvae were removed by siphoning. Mortality was calculated daily by counting the dead larvae.

Statistical analysis

Data was analyzed using the SPSS statistical software. Karl Pearson's coefficient of correlation was performed with different variables such as TL, HD, BD, ED, W and the survival rate with different delayed initial feeding times. One way ANOVA was used to find out the significant variation among different initial feeding times.

Results

Influence of delayed initial feeding on morphometric characters of larvae

The body of newly hatched larvae was long and slender with a big sized yolk sac containing a single oil droplet. The different changes observed on the morphometric characters due to delayed feeding, started at different time intervals, are depicted in Figure 3 (a-e) and 4. Significant differences were observed in the various morphometric characters such as Total length (TL), Weight (W), Head depth (HD), Body depth (BD) and Eye diameter (ED) ($p<0.05$). The increase in total length, weight, head depth, body depth and eye diameter of the larvae were significantly higher in larvae which initiated their first feeding at 12 hr after hatch out.

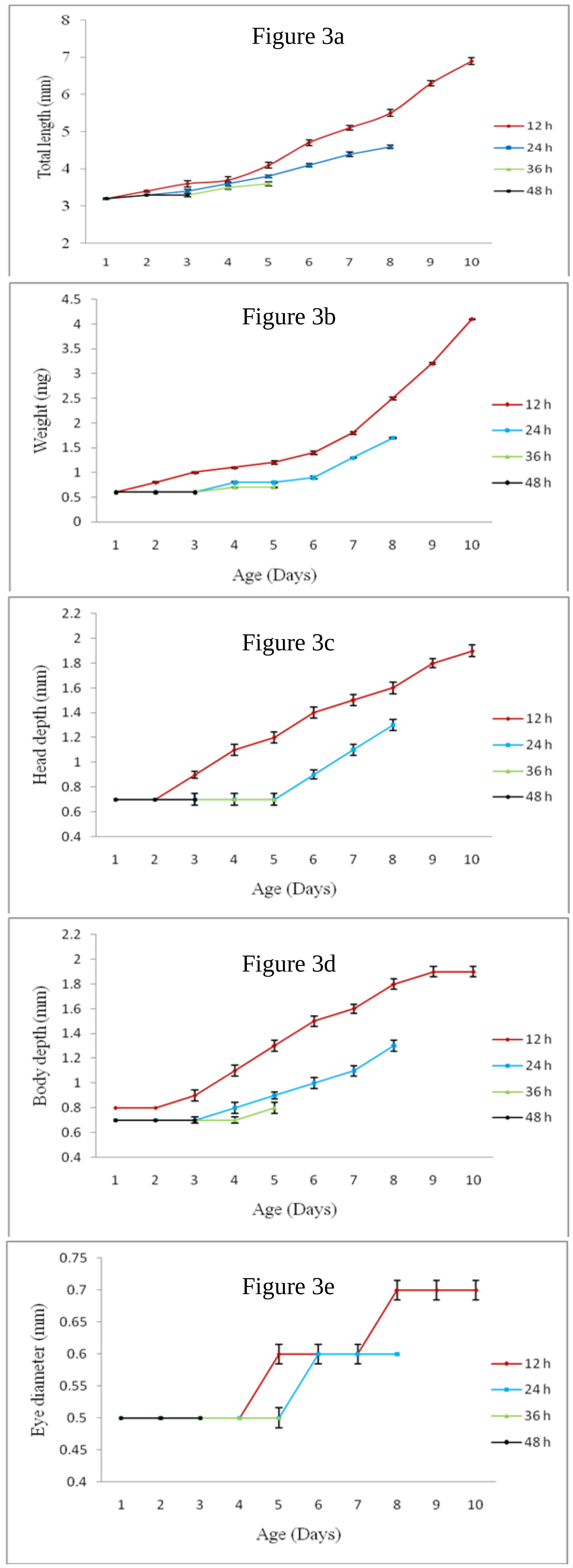

Figure 3. (a, b, c, d and e) Morphometric changes observed in *A. percula* larvae under different initial feedings.

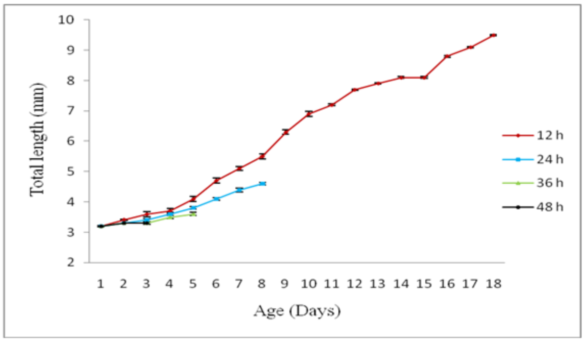

Figure 4. Growth (TL) of *A. percula* larvae under different initial feeding.

Using Karl Pearson's correlation coefficient, the morphometric characters such as TL, W, HD, BD and ED were analyzed and it was found that all the characters shown positive correlation with one another (at 0.01 level).

Water quality parameters such as temperature, salinity, pH, dissolved oxygen, ammonia and light intensity were monitored and maintained throughout the experiment as 28-30^{0}C, 22-24‰, 7.52-8.1, 4.5-5.5 mg l^{-1}, less than 0.01 ppm and 600-900 lux respectively. A 16 hr photoperiod was provided. The newly hatched percula larvae (TL 3.2 mm) had a yolk sac with a volume of 0.49 mm^3 and it was oval in shape due to the presence of spherical shaped oil globule. Gradual decrease in the yolk volume was observed after hatch out. The oil globule of newly hatched larvae had a volume of 0.349 mm^3. After started feeding on *Artemia* nauplii, the larvae showed steady growth which started its initial feeding at 12 hr. Due to the complete larval mortality within 8 days after hatch (24, 36 and 48 hr), it was difficult to compare the morphometric characters of those larvae fed with 12 hr onwards. Hence, it is concluded that the growth of the larvae strongly depends on the timing of first feeding.

Influence of delayed initial feeding on larval survival

The first feeding time also strongly affected the larval survival. After 18 days, the maximum survival (42%) was recorded where the larvae started their initial feeding at 12 hr. No survival was observed in the larvae which started their initial feeding after 24, 36 and 48 hr where complete mortality occurred on the 8th, 5th and 3rd day respectively.

Discussion

Newly hatched fish larvae will have large yolk to support the energy for developing the feeding ability and physiological mechanisms. In the present study, the maximum growth and survival rate of *A. percula* larvae were observed when it started its initial feeding at 12 hr after hatch. In many fishes, endogenous food source is utilized by the larvae for the development of physiological mechanisms before initiation of first feeding from exogenous source (Kailasam et al., 2007). The yolk absorption period for newly hatched larvae of Siamese gourami *Trichogaster pectoralis* was recorded at 4.5 days after hatching and in Green catfish *Mystus nemurus* it was at 3 days after hatching (Amornsakum et al., 1997; Amornsakum, 1999). In the present study, *A. percula* larvae with a total length of 3.2 mm and yolk sac volume of 0.349 mm^3 at the time of hatch out had ability to survive up to 24 hr. It is similar to other fish species such as lined sole *Achirus lineatus* with the TL of 1.9 mm which had yolk volume of 0.22 mm^3 at the time of hatching (Houde, 1974). The time span for yolk exhaustion is temperature dependent and species specific (Dou et al., 2005). The tolerance of larvae against the delayed initial feeding is influenced by the size of the egg and yolk sac (Kailasam et al., 2007). It is reported that the large sized egg can have large nutritional reserve which serves as a source of food for larvae for longer duration (Blaxter and Hempel, 1963).

Many researchers have reported that the morphometric characters are used to detect the degree of starvation of larvae (Yufera et al., 1993; Mookerji and Rao, 1999). Early studies have shown that many fish larvae were vulnerable to starvation due to delayed first feeding and starvation was a major cause of high mortality during the early life stages of the fishes both in nature and captivity (Yufera et al., 1993; Gisbert et al., 2004; Dou et al., 2005). The timing of starting first feeding and the ability to withstand starvation varies in different species (Gisbert and Williot, 1997; Dou et al., 2002; Gisbert et al., 2004). In some fishes, even a short period of food scarcity after yolk exhaustion can cause morphological deformities, abnormal feeding behaviours or nutritional problems of larvae, leading to drastic mortality and deformity in early stages (Gwak and Tanaka, 2001; Dou et al., 2002). Therefore, morphological characters and specific behaviours such as abnormal feeding during starvation are reliable and valuable criteria for evaluating the nutritional conditions and determining the optimal timing of first feeding of fish larvae.

In coral trout grouper *Plectropomus leopardus,* the larval survival and growth were affected if the feeding was delayed up to 3 days (Yoseda et al., 2003). In lined sole *Achirus lineatus,* survival and growth were affected if they failed to accept the first feed within 3-3.5 days after hatching and similarly in the bay anchovy *A. mitchilli* and the seabream *A. rhomboidalis* was regarded as within 0-2.5 days after hatch out (Houde, 1974).

Conclusion

In the present study, higher survival and growth rate were observed when the larvae started their initial feeding at 12 hr after hatching, while larvae showed low survival and growth rates with the initiation of the first feeding at 24, 36 and 48 hr after hatch out. It may be due to the starvation caused by the delayed feeding. It is suggested on the basis of larval survival rate that, the first feeding should be started before 12 hr after hatching.

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