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Breeding and Rearing of Regal Damselfish *Neopomacentrus cyanomos* (Bleeker, 1856): The Role of Green Water in Larval Survival

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Abstract: Recent developments in the marine ornamental fish trade have drastically encroached on the coral ecosystem. To ensure the sustainable development of the aquarium industry, it is necessary to develop hatchery technology for different marine ornamental organisms. In this context, we investigated the breeding success of regal damselfish, *Neopomacentrus cyanomos*, in low salinity water and studied the role of green water in larval survival. Since the regal damselfish is polygamous, an experiment on breeding group formation was made. We observed that it took groups of 2 fish 7.6 months to spawn, whereas groups of 6 fish bred in 3.3 months. Before spawning, males changed body colour on their dorsal side to attract a partner. In the rearing tanks, eggs were laid in an earthen pot and the male guarded the clutch until hatching, not allowing his female partner to enter the nesting area during this time. Hatching generally took place 4 days after fertilization but sometimes occurred on the fifth day. Larvae were reared using 2 experimental setups in order to examine the importance of green water to larval survival. The water quality, photoperiod and feed supplied were similar in both setups. However, in setup I, clear water was provided and in setup II green water was provided and the larval survival rate was 0% and 20% respectively. The larvae metamorphosed on day 35 post-hatching. The results presented here provide additional information about the importance of green water in damselfish larval rearing.

Key words: Estuarine water %Green water % Larval rearing % Larval survival % Regal damselfish

INTRODUCTION

Ornamental fishes are the most attractive organisms in aquaria worldwide and the household demand for aquariums has recently increased significantly. The values of the wholesale ornamental trade and retail trade are estimated at US$ 1 billion and approximately US$ 3 billion, respectively [1]. A recent report by the United Nations Environmental Programme estimated that 1,471 species are traded worldwide, which equates to between 20 and 24 million ornamental fishes, 11-12 million pieces of coral and 9-10 million invertebrates [2].

The available literature states that the retail value of one kilogram of reef fish destined for the aquarium trade may be worth USD 500 to USD 1,800 while marine fish intended for human consumption can be priced between USD 6 and USD 16.50 per kilogram [3, 4]. The ornamental fish industry is a possible contributor to the economic growth of developing countries, which may face future challenges regarding the sustainability of the trade and its environmental safety. These animals are continuously collected from reefs worldwide for trade purposes. Destructive fishing practices such as cyanide and cage fishing, selective harvesting and overexploitation are major threats that have raised concern that the continuous harvest of these organisms will affect not only the target species but also irreversibly damage the entire reef ecosystem.

Most popular among aquarists are the pomacentrid fishes [5], which include clown and damselfishes, number

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approximately 369 known species [6], inhabit tropical and subtropical seas and mostly occur in Indo-Pacific regions [7]. These fishes are preferred because of their tiny size, often bright colour and ability to adapt in captive conditions. They are mostly omnivorous, feeding on algae, zooplankton and a wide variety of other invertebrates[8 - 10]. One of the important marine ornamental fishes, the regal damselfish, *Neopomacentrus cyanomos*, is the most common damselfish. It is characterized by an ovate to elongate, laterally compressed body and is metallic green to black or brown, with a distinctive white spot at the end of the spiny dorsal fin base. A dark blue blotch is found behind the opercle at the origin of the lateral line and another appears at the upper part of the pectoral fin base. They deposit demersal adhesive eggs on hard substrates, usually in sheltered areas [11] which are elliptical and attached by adhesive filaments.

Reports on damselfish larval rearing are very limited [12-16]. Up to now, 90 species of marine ornamental fishes, 30 of these Pomacentridae, have been bred in captivity; however, the mass-scale production of these species is still at a nascent stage. In the present study, we attempted brood stock development, captive breeding and larval rearing of a coral reef fish, *Neopomacentrus cyanomos* (Bleeker, 1856), in low salinity water and observed the role of microalgae in larval growth and survival.

MATERIALS AND METHODS

Breeding Group Formation: Fifteen sub adults of *N. cyanomos* measuring approximately 3.5-4.5 cm were procured from traders in August 2007 and allowed to acclimatize for 10 days in estuarine water conditions. Later, the healthy fishes were accommodated in 3 tanks (labelled A, B and C) with a stocking density of 2, 4 and 6 respectively. All 3 tanks were provided with an earthen pot, tiles where the fishes could hide or lay their eggs and one locally made underwater filtration system. Cycles of 12 h light and 12 h darkness were provided uniformly in all 3 tanks. The temperature was maintained at 26-30°C, salinity at 22-24 ppt, dissolved oxygen at 5.5 to 6.5 mg/L and pH at 7.5-8. The fishes were fed a diet consisting of boiled oyster, clam, prawn and live *Acetes* spp. 3 times a day.

Behavioural Observations: Behavioural studies were conducted during the experimental period. Territorial behaviour, feeding, courtship, spawning and uniparental care of egg clutch were monitored. These observations were made during the photoperiod only.

Sampling of Eggs and Larvae: To study the embryonic development, a total of 10 ± 2 eggs were collected from 3 different clutches at different periods and the developmental stages were observed. The embryos were collected 8, 24, 48, 82 and 96 h into the incubation stage. The development stages were referred with the previous studies[17, 30]. The experiment was replicated to monitor the difference in embryonic development at different spawning stages. The number of eggs in a clutch was also estimated by counting the number of eggs in a known portion of the clutch area and multiplying it by the total area of the egg clutch [28]. An oculometer was used to determine the size of each egg. In the larval developmental component of the study, 4 ± 2 larvae were collected 1, 2, 4, 6, 8, 12, 25 and 35 days post-hatching for examination of morphological changes.

Live Feed Culture: Different species of zooplankton such as copepod *Acartia* spp. rotifer *Brachionus plicatilis* and ciliate *Euplotes* spp. were cultured using the microalgae *Nannochloropsis oculata* at 24-26 ppt salinity and 25-28°C. Newly hatched super small *Artemia* spp. nauplii (INVE Technologies, Belgium) were introduced in the larval tank from day 21 Post-hatching onwards.

Larvae Collection and Rearing: Two hours before hatching, the egg clutch was shifted to a 5-L bucket filled with filtered estuarine water without exposing and placed in a dark room to hatch. Gentle aeration, to imitate fanning by the parents, was provided near the egg clutch to keep it alive. The average hatching rate was estimated for the different clutches. The new hatchlings were transferred to oval-shaped, 100-L capacity larval rearing tanks made of fibreglass reinforced panels.

Effect of Green Water on Larval Survival Rate: Egg clutches obtained from 2 different pairs were used to study the role of green water on larval survival. About 100 ± 10 larvae were accommodated in each tank with the clear water (setup I) and provided with algal-enriched ciliate, *Euplotes* sp. (20 individuals/mL), copepod *Acartia* spp. nauplii (8 /mL) and from day 5 onwards, rotifer, *B. plicatilis* (9 /mL). In setup II, 100 ± 10 larvae were stocked in each tank and the feed was enriched with the microalgae *Nannochloropsis oculata*, provided at 60,000 cells/mL concentration. The larvae were fed according to the setup I feeding regime. From day 21 onwards, *Artemia* nauplii individuals provided at a concentration of 4 individuals/mL. Each setup was replicated 3 times to confirm the findings. Larval survival was statistically analysed using SPSS and Excel software.

RESULTS

Breeding Group Formation: During the brood stock development period the fishes spawned after 7.6, 4.6 and 3.3 months rearing in tanks A, B and C, respectively. This observation reveals that polygamous fish can also breed when kept as a potential pair.

Behavioural Observation: It was observed that the bigger fishes always dominated and occupied the earthen pots, which they defended aggressively. The bigger fish also fed first. Courtship began 2 days before spawning, with the male initiating by displaying a range of behaviours that included vertical dives, violent swimming motions and clinging to the bottom of the rearing tank. Before spawning the male fish also changed body colour. On the day before spawning, the male cleaned the nest site by rubbing it with his pelvic and caudal fins and picking off any loose particles with his mouth. During this period the female entered the proposed nesting site several times but did not spawn. On the day of spawning, the female entered the nest and participated in a brief courtship ritual which consisted of side to side quivering motions and the male gently touching the operculum and posterior part of the female's body. Spawning, which took 30-90 min at an interval of 2-10 min, almost always occurred at dusk but on a very few occasions was also observed in the afternoon. The newly laid eggs were immediately fertilized by the male (Fig. 1a), who usually guarded (Fig. 1b), fanned (Fig. 1c) and culled the egg clutch. During the incubation period the male did not allow his female partner to enter the nesting area. The number of eggs in a clutch varied between 2200 ± 560, with an average diameter of 0.71 mm. After hatching neither parents cared for the larvae.

Embryonic and Larval Development: The newly laid eggs were elliptical, milky white coloured, adhesive and measured 0.8-1.0 mm and 0.2-0.4 mm in length and width, respectively. The surface of the egg capsule was smooth and one end of the egg, identified as the animal pole, contained a glutinous substance to adhere itself to the lining materials. The eggs hatched after 96 h of incubation at 26-29°C, often during the dusk period. At 8 hrs the blastula stage was observed, with blastocoels formed inside the embryo and dense cells were apparent (Fig. 2a). At 24 h, organogenesis occurred and melanophores appeared (Fig. 2b). At 48 h the heart was developed, skeletal muscle was noted, the head was formed and melanophore pigmentation was observed on the head region (Fig. 2c). At 82 h, the embryo became large and filled most of the envelope area and the melanophores embedded deep into the entire region (Fig. 2d). At 96 h the embryo was free of the envelope and become a larva. Pectoral fins were well developed and a single fin fold was observed. When the movement of the embryo became very violent it started breaking the envelope (Fig. 2e), which took place during the dusk period. In the present study, the total hatching rate was calculated to be 92 ± 5 %.

The newly hatched larvae had large yolk sacs and the melanophores were prominent on the ventral margin of the body and forehead. The eyes and mouth were not well developed and no opening was observed in the mouth to engulf the feed. The body colour was transparent, the standard length (*L*s) varied between 1.8-2.2 mm (Fig. 3a) and they were pelagic and planktonic. Within 24 h the mouth had opened, the eyes were heavily pigmented and the yolk was largely exhausted. The mouth opening of the 2-day-old larvae (Fig. 3b) ranged from 18.4 µm to 24.1 µm. Complete yolk absorption occurred on day 8 post-hatching (Fig. 3e). On day 12, the caudal fin had assumed a round shape and started to furcate thereafter. On day 25 the body colour darkened slightly (Fig. 3g) and on days 28 to 32 the larvae were on the verge of entering metamorphosis and started to settle down. The juveniles obtained their colouration from day 30 onwards. The larvae resembled adults on day 35 (Fig. 3h) but only attained full adult colouration on day 40 (Fig. 4).

Role of Green Water on Larval Survival: In setup I, all the larvae died gradually, whereas in setup II 20% survival was observed. In setup I the larval survival rate was defined by age (12 days post-hatch) and the regression equation obtained was: -6.821 × age (day post-hatch) + 96.09 ($R^2 = 0.907$). In setup II, survival rate was defined at 40 day post-hatch and the regression equation obtained was: -1.427 × age + 65.43 ($R^2 = 0.700$). The survival rate of the larvae between the 2 experimental setups varied significantly ($F = 4.9$, $P < 0.05$). In both setups, the highest mortality was observed on days 2, 5, 10 and 12. Less mortality was observed in setup II after day 30 post-hatch. Experimental setup II indicated that green water is necessary for the initial stages of larval development in captivity. The larval survival rate in both culture conditions are shown in Figure 5.

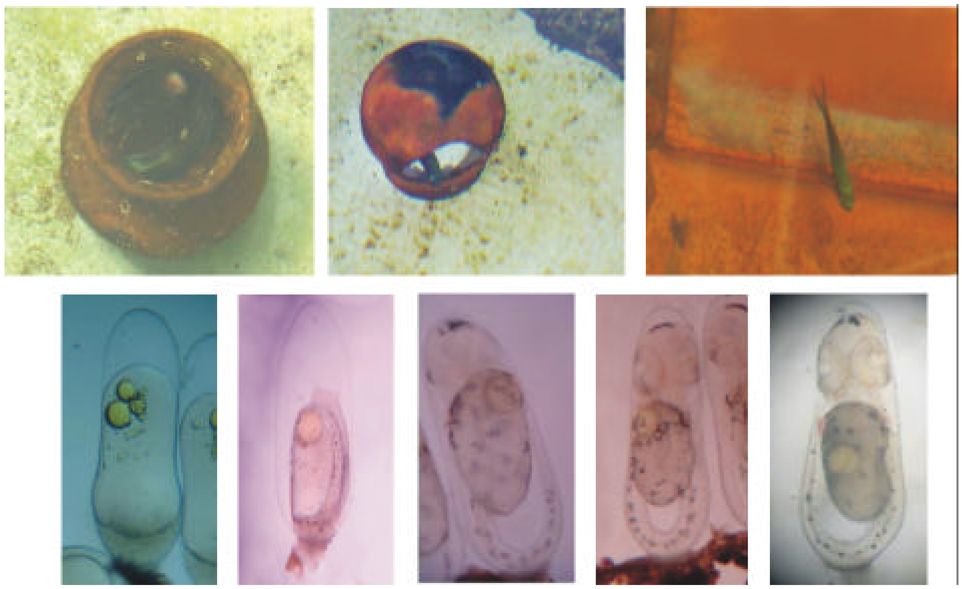

Fig. 1a: Spawning, b. Male guarding the clutch, c. Male fanning the clutch

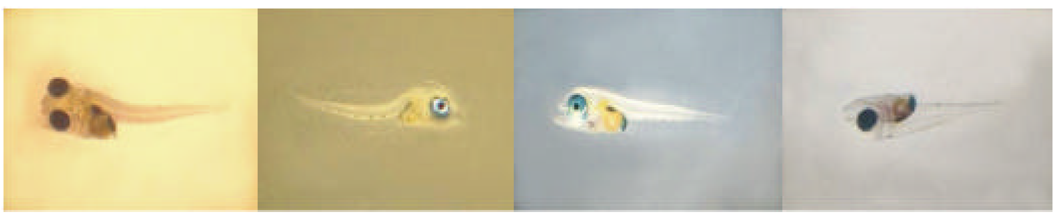

Fig. 2a: 8-h-old embryo, b. 24-h-old embryo, c. 48-h-old embryo, d. 82-h-old embryo, e. 96-h-old embryo

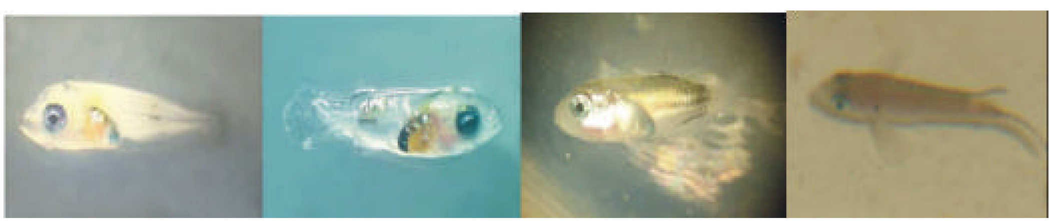

Fig. 3a: 1-day-old larva, b. 2-dayold larva, c. 4-day-old larva, d. 6-day-old larva, e. 8-day-old larva, f. 12-day-old larva, g. 25-day-old larva, h. 35-day-old juvenile

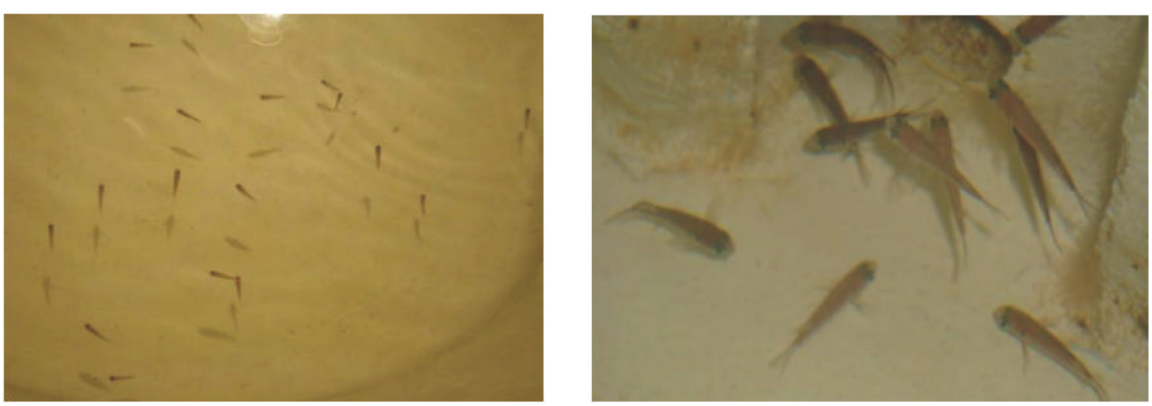

Fig. 4a: 40-day-old juveniles, b. 50-day-old juveniles

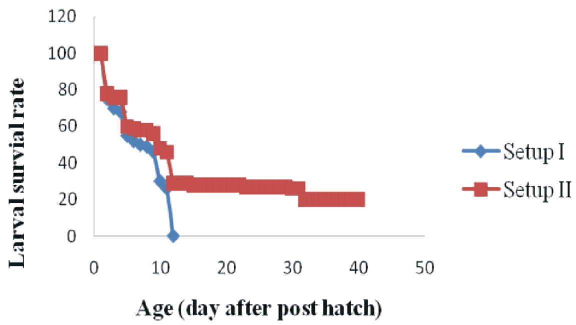

Fig. 5: Survival rates of *N. cyanomos* larvae in 2 experimental setups

DISCUSSION

The increasing demand for marine ornamental fishes is currently driving research on breeding and larval rearing, suitable husbandry practices, appropriate environmental variables and proper feeding. The brood stock development pattern and breeding observed in the present study were similar to the general patterns of the Pomacentrid fishes. The majority of our attention was focused on the selection of healthy fishes for brood stock development as stated by Melville and Griffiths [18]. While earlier reports on damselfishes have shown that they adopt a protogynous sexual pattern with a polygamous mating system [19], it was evident in the present study that the species could breed in captivity when kept as a proper pair. Six individuals is a suitable group size for commercial production because the fish can breed very early and continuously spawn. We also observed that the fishes spawned naturally in a 500-Lwater holding tank. In captive conditions, the major considerations are the size of the rearing tank [20] and suitability of the substrate and environment [16].

Larval rearing of marine ornamental fish is convoluted by the small mouth gape of larvae, poor knowledge of the appropriate feed and specific conditions. Water quality management is of great importance in aquaculture systems as it plays a vital role in the growth, survival and healthiness of brooders, larvae and juveniles. In the present study, suitable water quality parameters were standardized and followed for further rearing.

The present study revealed that the breeding behaviour of regal damselfish consists of 5 major steps, including: establishment of territory, pair formation, nest preparation, courtship and spawning, fertilization and parental care, similar to other pomacentrid fish [21]. It was also observed that the male cleaned the nesting site and defended it from other fishes, an observation that has also been recorded by several authors [22 -25].

Spawning began at dusk whereas in other members of Pomacentridae it was reported during early morning [16, 26]. As with other species of Pomacentridae, the male guarded the egg clutch and would not allow the female partner to enter the nesting area [16, 27, 28]. The incubation period generally spanned 4 days but sometimes extended to the fifth day, which may be due to the environmental conditions [29, 30]. Like other marine fish larvae, regal damselfish larvae were pelagic, had a large yolk sac, their eyes and mouth were not developed and they varied in size from 1.8 to 2.2 mm *L*s. On day 2, the mouth gape ranged from 18.4 to 24.1 µm.

In the natural habitat, marine larvae usually feed on a wide variety of micro-zooplankton including protozoa, dinoflagellates and especially, copepod eggs and nauplii [31]. In this study the copepod *Acartia* spp. ciliate *Euplotes* spp. rotifer *B. plicatilis* s.s. and newly hatched *Artemia* nauplii were used as live feed. Use of ciliates, *Euplotes* spp. as larval feed was described by Moe [32] and Olivotto *et al.* [1]. Complete yolk exhaustion was observed on day 8 whereas in other damselfish it took only 24 h. From day 28 day onwards, the larvae started to settle down.

In the present study, our experiment on the suitability of green water for larval rearing in initial stages revealed a better survival rate (20%) than obtained in the clear water (0%), as was reported for other Pomacentridae larvae [13]. Several available reports detailing the use of phytoplankton in rearing tanks indicated that it gave better survival rates than clear conditions [33, 34]. Earlier reports related to damselfish larval rearing showed that the larval survival rate of *Neopomacentrus filamentosus*, *N. nemurus* and *Pomacentrus caeruleus* was only 3-4% (15) and in *Chrysiptera parasema* it was 25% [16] whereas in the present study the survival rate was 20%, which may be attributed to the green water. In this study the larvae metamorphosed 35 days post-fertilization, a delay which may be due to low saline water, whereas in other damselfish the reported larval duration is only 25 days[16, 35].

Hence a detailed study to determine what modifications should be made to improve the larval rearing system is necessary to improve the survival for sustainable aquarium trade.

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