



Short communication

Captive spawning and embryonic development of marine ornamental purple firefish *Nemateleotris decora* (Randall & Allen, 1973)



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ARTICLE INFO

Article history:

Received 1 August 2013

Received in revised form 14 December 2013

Accepted 16 December 2013

Available online 31 December 2013

Keywords:

Microdesmidae

Nemateleotris

Breeding

Spawning

Embryo

Larvae

ABSTRACT

Courtship behaviour, captive breeding, embryonic and larval developments of *Nemateleotris decora* and its rearing with suitable live feed were described. Different age groups (60–100 mm TL) were stocked (glass aquaria, 500 L) in outdoor transparent roofed hatchery at water temperature $29 \pm 1^\circ\text{C}$ for pair formation. After 6 months of rearing, 4 pairs were formed. The size of the female ranged from 60 to 90 mm and males from 90 to 100 mm. Each pair was then stocked in 250-L perspex breeding tanks in the indoor breeding unit with a photoperiod of 14 L: 10 D by a 40-W bulb suspended at 20 cm above the water surface. The environmental parameters such as temperature, salinity, dissolved oxygen and pH, NO_2 , NO_3 and NH_3 were maintained at suitable levels and monitored once in 24 h. The pairs were daily fed with cooked meat of squid, shrimp, green mussel, raw fish egg mass at 10% of their body weight in four split doses and live adult artemia (10–15 per day). The pairs began to spawn after 6 months acquisition in the breeding tanks. Each spawning consisted of 400 to 500 eggs, which were elliptical in shape with a length of 1.1 ± 0.1 mm and a maximum width of 0.4 mm. The incubation period lasted for 96 h at a water temperature of $28 \pm 1^\circ\text{C}$, and most of the time, males guarded the eggs than did females. The embryonic development and colour changes of eggs during incubation were documented. The hatching percentage ranged from 93% to 98% at water temperature 29°C . The size of newly hatched larva varied between 1.9 ± 0.1 mm long and the mouth gape varied between 90 and 110 μm , and its behaviour was also documented. Larval rearing was carried out in 250-L rectangular tanks using phytoplanktons *Nannochloropsis oculata* and *Chlorella salina* (1:1 proportion at $1\text{--}5 \times 10^6$ cells ml^{-1}), along with different combinations of live micro zooplanktons: Diet I (*Brachionus rotundiformis* from 1 to 7 dph and *Brachionus plicatilis* from 8 to 18 dph), Diet II (ciliates *Euplotes* sp. from 1 to 7 dph and *B. rotundiformis* from 8 to 18 dph) and Diet III: *B. plicatilis* from 1 to 18 dph and Diet IV (Calanoid copepod *Acartia danae* nauplii from 1 to 7 dph and *B. rotundiformis* from 8 to 18 dph). In all diets, *Artemia* nauplii were fed from 19 to 40 dph to standardize its larval rearing and were provided with 24 h light. All the zooplankton species except copepods were enriched using Algamac 2000. Out of the four tested diets, Diet II showed maximum survival rate ($66\% \pm 0.23\%$) followed by Diet IV ($40\% \pm 0.40\%$), Diet I ($10\% \pm 0.61\%$) and Diet III ($3\% \pm 0.46\%$). The larvae metamorphosed to juveniles within 35 to 40 days of post-hatch (dph). This is the first scientific report on breeding of *N. decora* under captivity.

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1. Introduction

Ornamental fish production is an important component of aquaculture industry in several nations, and the marine ornamental aquarium trade has become an established sunrise industry over the last several decades. Out of the 1000 species of coral reef fishes traded (Green, 2003), only 51 have been cultured in captivity for aquarium trade (Arvedlund et al., 2000). However, the current reliance on wild collection of more than 90% of marine ornamental species has created concern for the long-term sustainability of the industry (Moe, 2003; Thlusty, 2002). The various collection methods and the widespread use of cyanide to stun tropical fish also harm coral reefs and marine ecosystems and ultimately threatens the food source. Therefore, in the last few

years, a number of studies were conducted on the reproduction of species that are most commonly used in aquarium trade for the purpose of rearing them in captivity (Holt, 2003; Madhu and Rema, 2005; Olivotto et al., 2003, 2004; Riley and Holt, 1993; Thresher, 1984). In this scenario, the development of aquaculture technology for coral reef species is one of the options to increase supply and reduce dependency on wild-caught counterparts (Olivotto et al., 2011a). However, the captive production of most of the coral reef species has proven challenging, mainly due to the complex reproductive biology, the small size of newly hatched larvae, the long larval phase and the lack of suitable-sized live feeds for first feeding, which leads to poor survival of larvae.

Nemateleotris decora, commonly known as purple firefish or elegant firefish and decorated firefish, belongs to the family Microdesmidae and subfamily Ptereleotrinae and is of keen interest for the aquarium trade especially due to their amazing colour with light pink to white body, dark purple rear end and purple red fins, peaceful behaviour towards

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tank mates, hardiness, elongate shape and small size. It is found in the Indo-Pacific Ocean, ranging from Philippines to Australia, Fiji to Ryoku islands, Mauritius to Samoa, New Caledonia, Maldives and Sri Lanka. As the name implies, the species also dart into holes when alarmed, and usually this prolific leapers are collected from deeper water of 25–75 m. This species is often found in monogamous pairs and feed on zooplankton, especially larvae of crustaceans and copepods (Froese and Paul, 2007). Apart from this species, its genus comprises other species such as *Nemateleotris magnifica* and *Nemateleotris helfrichi*. Out of these, *N. decora* is the top ranked fish in aquarium industry with an international market price of US\$35–40 per individual depending upon the size. A major impediment in larval rearing is the first feeding stage, when the larvae shift from endogenous yolk reserves to exogenous feeding (Olivotto et al., 2005, 2006a; Turingan et al., 2005). To overcome such critical periods, proper enrichment of live feeds is very much essential (Olivotto et al., 2003, 2006b). Most of the hatcheries commonly use species of rotifer and artemia as live feed organisms. However, the efficacy of such prey in the diet of marine fishes has been questioned (Holt, 2003), and their nutritional values are limited and must be enriched with HUFA supplements for the successful rearing of larvae under captive conditions (Olivotto et al., 2005, 2006b, 2011b). Moreover, prey capture success in the first stage of feeding and subsequent larval stages is influenced by many factors such as the development of suction-feeding mechanism (Lauder, 1983; Turingan et al., 2005), search behaviour (MacKenzie and Kiorboe, 1995), visual system and other sense organs (Job and Bellwood, 1996), swimming ability (Fisher et al., 2000), digestive system (Green and McCormick, 2001) and developments of complex of bones, muscles, ligaments and tendons in the head (Norton and Brainerd, 1993; Westneat and Wainwright, 1989), hyoid-mandible and opercular-mandible series (Hunt von Herbing, 2001; Turingan et al., 2005), which develop through ontogeny (Doi et al., 1997; Hunt von Herbing et al., 1996; Liu, 2001). Apart from these, size (Krebs and Turingan, 2003), colour (Checkley, 1982), density (Lasker, 1975) and swimming behaviour (Beck and Turingan, 2007) of non-elusive zooplankton prey organisms influences prey capture success and prey selectivity in marine fish larvae. Prey capture success and percentage of successful feeding strikes are low at first feeding by marine fish larvae (Hunter, 1981) but rises rapidly during early development (Braun, 1967; Houde and Schekter, 1980; Hunter, 1972) due to their improved ability to maneuver (Blaxter and Staines, 1971), feeding experience (Colgan et al., 1986; Coughlin, 1991, 1994; Meyer, 1986) and changes in mouth size, structure and feeding type (Liem, 1991). Because of these bottlenecks, the captive production of microdesmids are still in infancy when compared to clown fishes (Madhu and Rema, 2011; Madhu et al., 2011, 2012; Rema and Madhu, 2012; Rema et al., 2012) and other marine ornamental fishes. There is a few literature on the reproductive behaviour and spawning of members in Gobiidae family, most of which pertains to spawning strategies, embryonic and larval developments and parental care (Olivotto et al., 2005; Sonoda and Imai, 1971; Sunobe and Nakazono, 1987, 1989, 1995; Valenti, 1972; Wittenrich et al., 2007). However, descriptive scientific information on the early development of members in the Microdesmidae is scarce, and the only available information on its spawning behaviour was reported by Schiller (1990). As *N. decora* also plays an important role in the marine aquarium trade, the study was aimed to generate baseline information on its reproductive behaviour, spawning, egg morphology, embryonic and larval development and develop reliable captive breeding techniques and find out suitable live feed for the higher survivability of larvae and juveniles under captive conditions.

2. Materials and methods

2.1. Broodstock

N. decora ($n = 10$) having total length 60 to 100 mm and weight 1.3 to 2.8 g were obtained from a local aquarium dealers. As no sexual

difference was observed between specimens, all the fish were reared together in a 500-L glass aquaria in the outdoor ornamental fish breeding unit of Marine Hatchery in Central Marine Fisheries Research Institute, Kochi. The tank was provided with 5 pieces of 15-cm-long and 5-cm-diameter PVC pipes for a period of 5 months for pair formation at water temperature 29 ± 1 °C. Consequently, each pair formed was shifted to 250-L Perspex broodstock tanks in the indoor fish breeding unit after ensuring that one of the specimen is shorter and plump (presumptive female) and the second one is longer and slender (presumptive male). Three sides of each rearing tank were covered with marine underwater sceneries. The bottom of the tanks was provided with medium-sized coral sand at a thickness of 20 mm. Each breeding tank was provided with rough surfaced brown curved clay tiles as a substratum on which the fish could make nest to spawn and also for hiding purpose. All the tanks were filled with natural seawater after filtering through a 1- μ m filter bag. A central bucket filtration system and a canister filter with ultraviolet sterilization were also provided in each tank to ensure water recirculation. The environmental parameters such as temperature 28 ± 1 °C, salinity (31 to 33 ppt), dissolved oxygen (4.6 to 6.2 ml L^{-1}) and pH (8.0 to 8.4), $NO_2 < 0.01$ $\mu g L^{-1}$, $NO_3 < 0.05$ $\mu g L^{-1}$ and $NH_3 < 0.02$ $\mu g L^{-1}$ were maintained and monitored once in 24 h. Photoperiod was controlled during all observations at 14 L:10 D with a single 40-W bulb suspended at 20 cm above the water surface. The pairs were brought to reproductive state on feeding with cooked meat of squid, shrimp, green mussel, raw fish egg mass at 10% of their body weight in four split doses (10 am, 12 pm, 2 pm, 4 pm) and live adult artemia (10–15 per day) at 4.30 pm.

2.2. Reproductive behaviour and spawning

Pairs (referred as ND1–ND4) were monitored five times daily (9 am, 11 am, 1 pm, 3 pm and 5 pm) to observe the signs of reproductive behaviour and nest preparation. When one or both members of pair exhibited heightened activity accompanied with protrusion of the female's genital papilla, the behaviour of male and female were recorded. The clay tile was closely observed daily to ensure the deposition of eggs. On completion of spawning, the clay tile containing eggs was photographed with a Cannon Digital camera (PowerShot-G2, Pixel 5.0) for further analysis. Images were imported to Adobe Photoshop, and a grid containing 25 cells was imposed over the image. Every egg was counted in 12 consecutive spawns to gauge accurately the clutch size of each pair. The maximum breadth and length of the clutch was also noted.

2.3. Parental care and artificial incubation

Three types of experiments were conducted to analyze the influence of parental care on embryonic developments and hatching rate.

1. The egg clutches were allowed to remain in the spawning tank along with their respective parents up to 1 h before hatching. The clutches alone were then shifted to hatching tanks and kept in slanting position with steady stream of mild aeration up to the completion of hatching.
2. The egg clutches along with respective parents were shifted from spawning tanks to hatching tanks at 1 h before hatching, and the parents were allowed to remain up to the end of hatching.
3. The egg clutches alone from each pair were removed shortly after ensuring fertilization from the parental tanks to hatching tanks for subsequent incubation. The egg deposited clay tile was kept in a slanting position without parents in hatching tanks, and a steady stream of mild aeration was supplied to the eggs and also to mimic the fanning action of parents during incubation.

Six egg clutches from each of the four pairs ($n = 24$) were used, and all hatching tanks (100-L) were filled with water from respective parental tanks. In each experiment, water temperature of 29 °C was

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