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Spawning, embryonic development and larval culture of redhead dottyback *Pseudochromis dilectus* Lubbock, 1976 under captivity

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ABSTRACT

Reproductive behaviour, captive breeding, embryonic, larval development and suitable live feed for *Pseudochromis dilectus* were found out. Fishes ranging in total length 80–120 mm (male) and 70–80 mm (female) were reared in FRP tank (1000-L) for six months to develop pairs. The pairs laid ball-shaped egg mass (25 to 35 mm diameter) which consisted of 400 to 500 spherical eggs/pair/spawning (n = 18).The size of the individual egg varied between 1743 and 1919 μm and all the eggs were interconnected by fine sticky threads. The egg ball was white or transparent on the first and second day, black on 3rd day and silvery on 4th day of incubation. The hatching rate varied between 91 and 95%, and the peak hatching took place under complete darkness on completion of 96 h incubation. Total length of the newly hatched larvae varied between 5.1 and 5.3 mm with mouth gape 150 to 160 μm. First feeding started at 10 to 12 h after post hatch. The study concluded that *Euplotes* sp. (0 to 5 dph), enriched rotifer (6–15 dph) and microalgae enriched *Diaphanosoma celebensis* (16 to 30 dph) can be used as effective feed for high survival of larvae of *P. dilectus*. On 20 dph the metamorphosis initiated and became juvenile stage at 30 dph (82%). At 45 dph, all the larvae transformed to juvenile and shifted from pelagic to epibenthic in the aquarium with denser body having reddish colouration.

Statement of relevance: Pseudochromis dilectus (Family: Pseudochromidae) is a species of keen interest in marine aquarium trade and has distribution in the Western and Central Indian Ocean to Sri Lanka. As its males are colourful than the female, males are selectively exploited from the nature for aquarium trade which is also causing threat to natural population thereby reducing its availability. Hence captive production is a potential solution for reducing pressure on the natural stocks. However no scientific studies were conducted in *P. dilectus* with an objective to captive breeding. Hence the present study was aimed to generate baseline information on its reproductive behaviour, spawning, egg morphology, embryonic and larval development of *P. dilectus* and also to find out suitable live feed for higher survivability of larvae, their metamorphosis and juvenile production under captive conditions with a view to develop a reliable captive breeding and rearing techniques for its mass scale production.

1. Introduction

Marine ornamental fish plays an important role in interior decoration, and its culture is a rewarding industry as aquarium keeping has become the second popular hobby in the world. As a result, the ornamental fish trade is a global multi-million dollar industry with a world export value of US$ 362 million and corresponding imports valued US$ 362 million (FAO, 2014). Over the years since 1985, its international trade showed an increasing trend (Moorhead and Zeng, 2010) with an average growth rate of approximately 14% per year with an estimated wholesale trade of nearly US$ 1 billion and retail trade of about US$ 3 billion (Olivotto et al., 2005). However the marine aquarium trade relies predominantly on wild collected specimens (90%) by way of indiscriminate collection methods which created a negative repercussions on coral reefs and marine ecosystems (Olivotto et al., 2003), and most marine aquaria are stocked from the wild-caught specimens (Wabnitz et al., 2003). Increasing pressure on natural populations of reef dwelling organisms due to their expanding popularity in the aquarium trade has stimulated interest in the breeding and culture of marine tropical fish (Holt, 2003; Olivotto et al., 2003, Olivotto et al., 2011). Thus captive production of most demanded group would certainly help to relieve the fishing pressure on coral reefs (Tlusty, 2002; Pomeroy et al., 2006). Moreover, the hatchery produced juveniles are proved to be hardier, less susceptible to diseases and survive better in aquaria than their wild caught counterparts (Dawes, 1999; Ogawa and Brown, 2001; Ziemann, 2001; Olivier, 2003; Wabnitz et al., 2003; Wittenrich, 2007; Anon, 2011a). In the marine aquarium trade, the species under the genus *Pseudochromis* are the most sought-after group of dottybacks due to their contrasting colours, tiny size, hardiness and adaptability to live in aquaria (Thresher, 1984).

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The redhead dottyback *P. dilectus* (Family: Pseudochromidae) is a species of keen interest in the marine aquarium trade. This family comprises 4 sub-families, 16 genera with over 120 species, and the subfamily Pseudochrominae alone consists of 5 genera with more than 70 species and are distributed throughout the tropical and subtropical Indo-West Pacific (Nelson, 2006). Though collection of these Indo-Pacific coral reef dwelling species from the wild is difficult due to their occurrence in crevices and deeper waters, the dottyback fish truly are a joy to keep in aquarium on account of their brilliant colour and intriguing personality (Debelius and Baensch, 1994). The prices are also very high when they are available in the market because of their rare availability. As a result currently there is a heavy reliance on wild caught Pseudochromids to satisfy the demand. Due to the importance of dottyback in aquarium trade, various research are ongoing in the aspects of their sex change (Wittenrich and Munday, 2005), reproductive behaviour, breeding and juvenile production of *Pseudochromis fridmani* (Brons, 1996; Moe, 1997) and *Pseudochromis flavivertex* (Brons, 1996; Olivotto et al., 2006a). In India and Sri Lanka, *P. dilectus* are exploited from their natural source for aquarium trade and due to heavy reliance on nature, it also showed a decline in its population from 2008 onwards (Jonathan, 1993; Anon, 2011b). Moreover scientific information on its reproductive behaviour and early development is scarce though it can be reliably reared in captivity in large quantities. Therefore, the present study was aimed to generate baseline information on reproductive behaviour, spawning, egg morphology, embryonic and larval development, metamorphosis and juvenile production of *P. dilectus* under captive conditions, and also to find out suitable live feed for higher survivability of larvae with a view to develop a reliable captive breeding and rearing techniques for its mass scale production.

2. Materials and methods

2.1. Pair formation

The fishes (n = 30) ranging from 70 to 120 mm total length, of which 15 with greenish colour having total length 70–80 mm (presumptive female) and the rest having 80–120 mm total length with reddish-orange and greenish tinge (presumptive male) were collected from the pet shop, Mandapam, India, and stocked (1:1 ratio) in 1 ton FRP tank for pair formation. The experimental tanks were provided with biological filter and kept in the outdoor hatchery where an incident light intensity of 2500 to 3000 lx was available during day time. Each tank was provided with 1.5 in. PVC pipes having 20 cm length for hiding. The fishes were daily monitored to observe their interaction and ensure pair formation. The environmental parameters such as temperature 29 ± 1 °C, salinity (30 to 32‰), dissolved oxygen (5.2 to 6.2 mL^{-1}) and pH (8.2 to 8.4), NO_2 < 0.01 μg L^{-1}, NO_3 < 0.05 μg L^{-1}, NH_3 < 0.02 μg L^{-1} were maintained and monitored once in 24 h. The fishes were fed with wet feeds such as meat of shrimp, squid and green mussel at 15% of their body weight (BW) thrice per day (11:30, 13:30, 15:30) and pellet feed (Trade name Varna,CMFRI, Cochin, India) at 5% of their BW once in the morning (09:30).

2.2. Broodstock management

Six healthy pairs formed were carefully removed from the pair formation tank by closing both entrance/exit of PVC pipes at a time so that each pair was retained without breaking their pair bond and then transferred to separate 500-L perspex tanks (one pair/tank) (Fig.1) for broodstock development in the indoor ornamental fish breeding unit. The pairs were fed with wet feeds such as meat of mussel, shrimp, squid and clam at the rate of 10% of their body weight in split doses 4 times per day (09:30, 11:30, 13:30, 15:30) and also provided live feeds *viz.*, adult *D. celebensis* (15–20/day) fed with mixed microalgae such as *Tetraselmis chuii* (10×10^4 cells mL^{-1}), *Chaetoceros calcitrans* (10×10^4 cells mL^{-1}), *Nannochloropsis oculata* (10×10^5 cells mL^{-1}), *Chlorella salina* (10×10^5 cells mL^{-1}), *Isochrysis galbana* (10×10^5 cells mL^{-1}) in 1:1:2:2:2 proportion respectively and *Artemia* nauplii (10–15/day) after enrichment with vitamins, minerals and fatty acids. The environmental parameters were maintained as in the case of pair formation experiments whereas the photoperiod was controlled at 14L:10D with double 40 W bulb suspended at 20 cm above the water surface of each tank throughout the period of study for broodstock management and breeding. Water was recirculated to ensure water movement, and water quality was maintained with the aid of filter system and monitored once in 24 h. Each broodstock tank was provided with tiles and PVC pipes for hiding purpose.

Fig. 1. A pair of *P. dilectus*.

2.3. Courtship behaviour and spawning

The reproductive behaviour and nest preparation after establishment of pair bond of each selected pair were observed. The pre-spawning and courtship behaviour of male and female were recorded directly and videographed using Canon camera EOS700D with zoom lens when one or both members of pair exhibited heightened activity accompanied with a plumpy abdomen in female. The cave made by male and the hiding materials were closely observed four times daily (09:30, 11:00, 13:00, 15:00) to ensure the deposition of eggs and document the time of spawning. To account the number of eggs per spawning, three egg balls from each pair (n = 18) were preserved in 5% formalin solution after 3 h of spawning and number of eggs were counted after spreading the egg ball and also photographed with a Cannon Digital camera (PowerShot-G2, Pixel 5.0) fitted to Trinocular microscope (Zeiss, Germany) at 5× and also using Stereo Zoom microscope (Leica S8 APO) with software IM50 for further analysis. Images were also imported to Adobe Photoshop (Version CS3) and a grid containing 25 cells imposed over the image. The size of the egg ball was also noted.

2.4. Parental care during incubation

Parental care exhibited by male and the attitude of female towards the egg ball during incubation period were also observed in all breeding pairs.

2.5. Sampling of embryos

After spawning, samples of 10 ± 2 eggs were randomly removed at 7, 7.30, 8, 9, 10, 11, 13, 15, 18, 24, 48, 72 and 96 h of post fertilization (hpf) from the egg balls of each pair. The main developmental stages were photographed under Trinocular microscope (Zeiss, Germany) at