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Reproductive behavior, embryonic and early larval development of the red head goby, *Elacatinus puncticulatus*

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ABSTRACT

The goals of this study are to provide a technical foundation for the production of the red head goby *Elacatinus puncticulatus* by evaluating its reproductive behavior and its embryonic and early larval development. Five pairs were kept under controlled conditions for thirty days. Courtship behavior, spawning period and the number of eggs produced were recorded. For the evaluation of embryo development, eggs were sampled at 12, 18, 24, 48, 72, 96, 120, 144 and 168 h post-fertilization(HPF). To test the influence of the incubation period on larval total length and height, eggs with six days (6D) of incubation and with seven days of incubation (7D) were subjected to flashlight illumination for 30 min to induce larval hatching. Another experiment evaluated the difference in larval survival with three different diets: *Euplotes* sp. (EU); rotifers *Brachionus rotundiformis* and *Brachionus plicatilis* and *Paramecium* sp. (BP); plankton collected from the wild (WP). The males displayed a gray head and pale yellow and black body coloration. Females exhibited strong red and black colors until three days before spawning, which occurred at intervals of 7 to 10 days. The hatching rate was 98–99%. The larvae total mean lengths and heights were 3.05 and 2.95 mm ($p > 0.05$) and 0.37 and 0.48 mm ($p < 0.05$) for treatments 6D and 7D, respectively. However, both groups exhibited high mortality at 5 days post-hatch (DPH). No larvae from the EU group survived after 5 DPH. At 8 DPH, 4% survivorship was found in treatment BP and 2% in treatment WP.

1. Introduction

The American Pet Products Association (APPA) revealed that 142 million freshwater fish and 9.6 million saltwater fish are kept for ornamental purposes in the United States (APPA, 2011). In recent years, there has been a large increase in the tendency to keep marine fish and corals in aquariums (Olivier, 2001). This increase has occurred because of the development of new technologies that facilitate the management of marine species in captivity and the decline in the price of these animals, which have become increasingly accessible to European and American markets. Most marine species marketed in the aquarium trade are collected in tropical and subtropical regions, particularly in coral reef areas, where the fauna displays a wide variety of colors and shapes. It is estimated that less than 10% of marine animals marketed for ornamental purposes originate from captive production (Wabnitz et al., 2003). The captive reared fish provide relief to the natural stock caused by fishery pressure and also offer other benefits of less aggressive behavior, the ability to readily feed on commercial dried feed, and reduced

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susceptibility to disease (Wittenrich, 2007).Currently, the major commercially cultivated species include the clownfish (*Amphiprion* sp.), gobies (*Gobiosoma* sp.), dottybacks (*Pseudochromis* sp.), seahorses (*Hippocampus* sp.) and Bangai cardinals (*Pterapogon kauderni*). Efforts are being made to develop technology for the breeding and rearing of at least 12 other species (UNEP, 2008).

The Gobiidae family consists of tropical species and represents 5% to 10% of all *Teleostei* species. They have great potential for the aquarium trade because of their bright colors, peaceful behaviors and ease of domestication and adaptation to commercial feed. The gobies of the genus *Elacatinus*, popularly called neon gobies, are small inhabitants of coral reefs. In addition to being ornamental, the neon gobies have the role of eliminating ectoparasites from larger fish and invertebrates (Gomes, 2010). Characteristics that favor their cultivation in captivity are the sex determination of *Elacatinus*, which is based on phenotypic characteristics and sexual behavior (Gomes, 2010), and the fact that they have demersal eggs and are considered prolific in captivity (Olivotto et al., 2011). However, there are few studies describing rearing protocols of *Elacatinus* sp., (Cortes, 2009; Meirelles et al., 2009; Shei et al., 2010; Souza, 2012).

The red head goby, *Elacatinus puncticulatus*, is a popular ornamental species because of the strong red and blue colors on the head and the yellow and black pigmentation of the body. This fish is native to the eastern side of the Pacific Ocean, occurring from North America to northeastern South America (Fishbase, 2012). Little information on the red head goby is available (Wittenrich, 2007) and therefore the goals of this study were to evaluate the reproductive behavior and embryonic and early larval development to provide a technical foundation for captive breeding of this species.

2. Materials and methods

This study was conducted through a partnership between the Laboratory of Research in Aquatic Organisms (LAPOA), Integrated Group of Aquaculture and Environmental Studies (GIA), Federal University of Paraná (UFPR), Curitiba, Paraná, Brazil, and the Vero Beach Marine Laboratory (VBML), Florida Institute of Technology, Vero Beach, Florida, USA. The study was conducted at the VBML.

2.1. Broodstock maintenance conditioning

Five pairs of *E. puncticulatus* formed six months before the start of the study were provided by Proaquatix®. Fish with a total length range between 3.5 and 5 cm were kept in individual 20 × 20 × 20 cm tanks interconnected to a recirculation system with a protein skimmer and biological filtration, a water heater and cooler. Two 4 cm long, 3 cm diameter PVC pipes were placed in each tank to provide a spawning habitat. Sand-filtered natural seawater from the Atlantic Ocean was maintained at a salinity of 33 g L^{-1} (±0.45), a temperature of 26 °C (±0.48), a pH of 7.9 (±0.04). The light intensity was maintained in 700 (±100 l×) and the photoperiod in 8L:16D. The levels of total ammonia, gaseous ammonia, nitrite and nitrate were maintained below 0.25 mg/L. Food (a mixture of shrimp, saltwater fish meat and micro-frozen and chopped crustaceans) was offered twice daily (8:30 and 15:30). One hour after each feeding, the bottoms of the tanks were siphoned to remove the excess food and feces.

2.2. Breeding, incubation and hatching

Observations of the reproductive behavior of red headed goby *E. puncticulatus* was observed three times a day (8:30 to 9:00, 11:30 to 12:00, 15:30 to 16:00) for thirty consecutive days (from September 17 to October 16, 2012) by the same observer. The courtship behavior, parental care and territorial behavior were recorded.

The spawned eggs were kept in the same aquarium as the broodstock so the male could perform cleaning and aeration until moments before hatching. Due to the difficulty of counting the eggs inside the PVC pipe, the total number of eggs was estimated. The PVC pipe was removed from the tank, while one of its exits remained closed to avoid water leak, and a flexible plastic ruler was introduced inside the pipe to measure the depth and length of the eggs mass. The total area of egg mass (TA) inside the PVC pipe was calculated by multiplying egg mass depth by the width. Then, the eggs were sampled (n = 10), and the individual area (IA) was measured using a 1 cm square grid under a dissecting microscope (10× magnification). To estimate the total number of eggs (NE), the area of egg mass (TA) was divided by IA (NE = TA/IA). Hatching occurred spontaneously and the hatching rate was estimated by counting the number of empty embryonic capsules after hatching. The incubation period of the eggs was recorded.

Embryo development was monitored using a dissecting microscope (10× magnification) and photographed regularly throughout the seven days of incubation. Three eggs per spawn from three different spawns were collected and sampled at 12, 18, 24, 48, 72, 96, 120, 144 and 168 h post-fertilization (HPF).

2.3. Effect of the incubation period on early larval development

The first experiment tested the influence of the incubation period on larval length and height. Different embryonic times were tested because of the significant loss of eggs on the last day of incubation and because it had been observed in the previous experiment that some larvae hatched before seven days of incubation. Eggs from two different pairs were pooled and tested after six days (6D) of incubation and, another group of larvae derived from the same two pairs but different clutches, after seven days of incubation (7D). They were subjected to flashlight illumination for 30 min to induce larval hatching. The newly hatched larvae from each treatment (n = 120) were randomly divided and transferred to three 20 L rectangular tanks (40 cm long × 25 cm wide × 20 cm deep) with the outside painted in black. The tanks were provided with frozen microalgae (*Nannochloropsis oculata*) and rotifers (*Brachionus rotundiformis*) previously enriched with commercial HUFA (Algamac®) as Olivotto et al. (2006). The physical–chemical water parameters were kept the same