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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

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as those as in the broodstock tanks, and the photoperiod was maintained at 24L:0D. Low aeration was provided. Immediately after hatching, three larvae per replicate were euthanized with tricaine methanesulfonate (MS-222) at an overdose of 0.02 mg/L. Another three larvae were collected and sampled from both treatments at 24, 48, 72, 96 and 120 h post-hatch, placed in a Petri dish fitted with a 1 cm square grid and digitally photographed under a dissecting microscope (5× magnification).

2.4. Effect of different zooplankton diets on early larval survival

This experiment evaluated the differences in the survival rate of larvae fed three different diets. Larvae hatched after 7 days of incubation were used in this trial. The first treatment received only *Euplotes* sp. (EU), the second was fed with the rotifers *B. rotundiformis*, *B. plicatilis* and *Paramecium* sp. (BP) filtered between 50 and 300 screens, and the third received a diet based on zooplankton collected from the wild (WZ). Larvae from two different fish pairs were pooled and randomly divided into three different tanks, and stocked at a density of 50 larvae per tank. New and enriched batches of zooplankton were replaced twice daily in the larval tank. All the treatments were maintained at a density of 10 ind./mL, according to Olivotto et al. (2005). The necessary amount of zooplankton were harvested, washed with filtered seawater, and housed separately in 10 L plastic containers under aeration and light (1500 ± 100 lx) for enrichment. Zooplankton were enriched at the recommended rate of 0.5 g/L for 12 h (Bio-Marine, Inc., 2013).

To estimate the mortality, the larvae were counted on 0, 5, and 10 days post-hatch (DPH). One unique survivor larvae was sampled in the 17th day of life. To monitor growth, at the same stage of development, three larvae from each treatment were selected randomly, photographed and the total length and total height were measured. The gut contents of these samples were examined with an optical magnifier with 10× magnification to determine what had been eaten.

2.5. Plankton collection and cultivation

The rotifers *B. plicatilis* and *B. rotundiformis* were obtained from a sterile culture containing 100,000 individuals, which was kept in gentle aeration at 26 °C. A 25% water exchange was performed daily, and the rotifers were fed with *N. oculata* paste at a concentration of 50,000 cells/mL and added to the tank twice daily. Wild plankton was collected daily ($n = 10$), approximately 45 min after high tide, using a plankton net with a 50 μm mesh and a 50 cm diameter at the Sebastian Inlet, located in Sebastian, Florida, USA. The contents were sieved using 600, 120 and 50 μm mesh. The plankton between 50 and 120 μm in size were retained and used as food for the larvae. After each collection, the composition of the wild plankton was determined by sampling 1 mL of the collection in triplicate using an optical microscope lens at 10× magnification. The averages of each and of all collections were calculated. The wild-collected feed consisted of 85% copepods (*Parvocalanus crassirostris*) (larger diameter from 40 to 120 μm), 10% diatoms (*Coscinodiscus* sp.) and 5% dinoflagellates.

The *Euplotes* sp. and *Paramecium* sp. were isolated from the rotifer cultures through 25 and 50 μm filtration screens and cultured using gentle aeration and 25% water exchanges every three days. The *Euplotes* sp. culture was maintained at 50,000 cells/mL using algae paste (*N. oculata*).

2.6. Statistical analysis

The assumptions of parametric statistics (normality and homogeneity of variances, assessed by Shapiro–Wilk and Levene's tests) were met and therefore non-transformed raw data was used in all tests. A p value of 0.05 was taken for significance in all statistical tests. Differences between groups were analyzed in terms of the larval length and height using one-way analysis of variance followed by a multiple comparison Scheffé's method. Results were expressed as the means ± standard deviation (SD). The larval survival was analyzed by descriptive statistics (frequency). The statistical package used for analysis was Statistica Statsoft™ V. 10.0.

3. Results

3.1. Breeding, incubation and hatching

Of the five couples observed, three couples produced frequent spawnings, while the other two did not produce eggs. Spawning pairs also demonstrated active courtship, and parental care behaviors. During courtship and spawning, the males displayed a gray head and pale yellow and black body coloration. Females displayed strong red and black colors until three days before spawning and became pale in body coloration for the next three days prior to spawning. Two days prior to spawning, the female abdomen became swollen. One day prior to spawning, the female urogenital region changed from brown to red. Simultaneously, the males exhibited behavioral changes and promoted burrow cleanliness by moving their pectoral fins. During this period, the males displayed heavy breathing by increasing opercular movements and moving horizontally forward within the PVC pipe. Spawning occurred at intervals of 7 to 10 days approximately at 15:00 to 15:30. During the spawning, the couples remained side by side inside the PVC pipe. After spawning, the females were evicted from the PVC pipe by the males and usually remained in front of or above the PVC pipe. Females fed normally during the reproductive period except for the day of spawning during which time they stopped eating, while males did not feed during the incubation period but remained active providing aeration to the fertilized eggs.

The two couples that did not produce eggs did not display courtship behavior and occupied different PVC pipes throughout the observation period. Furthermore, no color differences were observed in non-reproductive pairs, which presented intense red head coloration and black lines in the body region.

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Table 1
Number of spawning per pair, number of eggs spawned per clutch, with respective average and standard deviation.

Pair	Spawning				Mean	σ
	1°	2°	3°	4°		
A	225	240	100	175	185.00	63.11
B	150	125	120	160	138.75	19.31
C	45	180	180	–	135.00	77.94
Total					152.91	27.84

The number of spawned eggs was on average 152.91 ± 27.84 (Table 1), and the hatching rate was 98.5 ± 0.547%. All males were observed cleaning the dead eggs. Two days before hatching, some spawns lost up to 50% of the eggs due to the cleaning maintenance performed by the males. The newly laid eggs were spherical in shape, ranging between 0.4 and 0.7 mm, with an average of 0.55 ± 0.11 mm in diameter.

At 12 HPF, the eggs displayed a translucent golden color. Within 12 HPF, the initial formation of the adhesive filament and the embryonic capsule was observed (Fig. 1A). At 18 HPF, the embryonic capsule was fully formed (with a height of 2.2 ± 0.2 mm and a width of 0.5 ± 0.1 mm), with four protuberances at the lateral distal region and one at the cranial egg region (Fig. 1B). At 24 HPF, the presence of a large yolk sac and early head formation were observed (Fig. 1C). At 48 HPF, detachment and growth of the caudal portion of the embryo in relation to the yolk sac was observed (Fig. 1D).

Within 72 HPF, there was a reversal in the position of the head relative to the filament adhesive, and the auditory placodes were present (Fig. 1E). At 96 HPF, the eggs turned transparent yellow, retinal pigmentation in the cranio-lateral portion of the embryo was observed, and the cranium to body ratio increased (Fig. 1F). Rapid growth of the embryos occurred at 120 HPF, at which time the majority of the capsular content was full, the circulatory system was formed and a heartbeat was observed (Fig. 1G). Furthermore, the anal pore, notochord and gas vesicle also became visible. At 144 HPF, the length of the tail was folded behind the head (Fig. 1H). Furthermore, melanization developed in the caudal portion of the embryo, and the yolk sac was also partially absorbed. The rudimentary formation of the mouth and intestine had also occurred. Pectoral fins and gill arches were also observed. At this stage, all eggs that were exposed to the microscope light for observation of embryonic development hatched. At 168 HPF, the embryos moved their tails intensely (Fig. 1I). When there was incidence of light, it was possible to observe the eyes moving, showing the presence of an ocular reflex. At this time there was also a decrease in yolk sac size, and spontaneous hatching of the larvae occurred. This process lasted approximately 1.5 h.

3.2. Effect of the incubation period on early larval development

The newly hatched larvae were transparent with some melanophores located near the caudal peduncle. The larval eyes were black with a prominent red metallic sheen when observed under the light. Larvae from treatment 6D displayed positive phototaxis, while those from treatment 7D did not. There was no significant difference in the mean total length of the newly hatched larvae, which were an average 3.05 ± 0.50 mm and 2.95 ± 0.35 mm for treatments 6D and 7D, respectively, (df = 19; $p = 0.960$; F ratio = 1.938; calculated $F = 0439$). In contrast, the average body height of the fish from the 7D incubation was 0.48 ± 0.06 mm, significant higher than the average body height of 0.37 ± 0.12 mm of those fish incubated for six days (df = 19; $p = 0.018$; F ratio = 4.217; calculated $F = 8.269$). Animals from treatment 6D displayed a closed or semi-open mouth (Fig. 2A), while larvae from treatment 7D had open mouths and a prominent jaw (Fig. 2B). A very small yolk sac was observed until 2 DPH. Full caudal fin development was present in both treatments. All sampled larvae of the 6D displayed a curved body at 5 DPH, complicating their movement and measurement (Fig. 2C). Larvae in treatment 7D presented greenish content in the intestine, while in 6D no content was observed; however, both groups experienced high mortalities at 5 DPH of larviculture.

3.3. Effect of different zooplankton diets on early larval survival

The larvae from all treatments showed a critical mortality period at 4 and 5 DPH. The survival in treatments EU, BP, and PA were 8%, 7%, and 12%, respectively, at day 5. No larvae in the EU group survived after 5 DPH. At 8 DPH, 4% survival was observed in treatment BP and 2% for treatment WP. By 18 DPH, one larva from treatment BP survived and was used for analysis (Fig. 2E). This last surviving larva showed slow development, with growth of 1 mm after 7 DPH and 1 mm after 18 DPH, when the total length was 5 mm. Live food densities decreased throughout the day for treatments BP and WP, yet food was only found in the digestive tract of larvae in treatment BP. In treatment EU, no decrease in food density was observed.

4. Discussion

The main challenges for the development of marine ornamental aquaculture are sexing and obtaining suitable broodstock and inducing spawning (Olivotto et al., 2011). Embryonic development is directly related to reproduction, broodstock maintenance conditions, incubation, and the transition from endogenous to exogenous feeding (Olivotto et al., 2011). Components that affect egg quality include the endocrine status of the female during the growth of the oocyte in the ovary, the diet of the broodfish and the complement of nutrients deposited into the oocyte (Brooks et al., 1997).

The incubation and the embryonic development of *E. puncticulatus* were very similar to *Elacatinus figaro* (Shei et al., 2010). In both species, the complete formation of the embryo occurs at 7 DPH, which highlights the importance of maintaining *E. puncticulatus* incubation for at least 7 days.

The stimulus for the larva to feed not only depends on the size of the food but also its color, shape and aroma (Olsen, 2007). The mouth size is directly related to the

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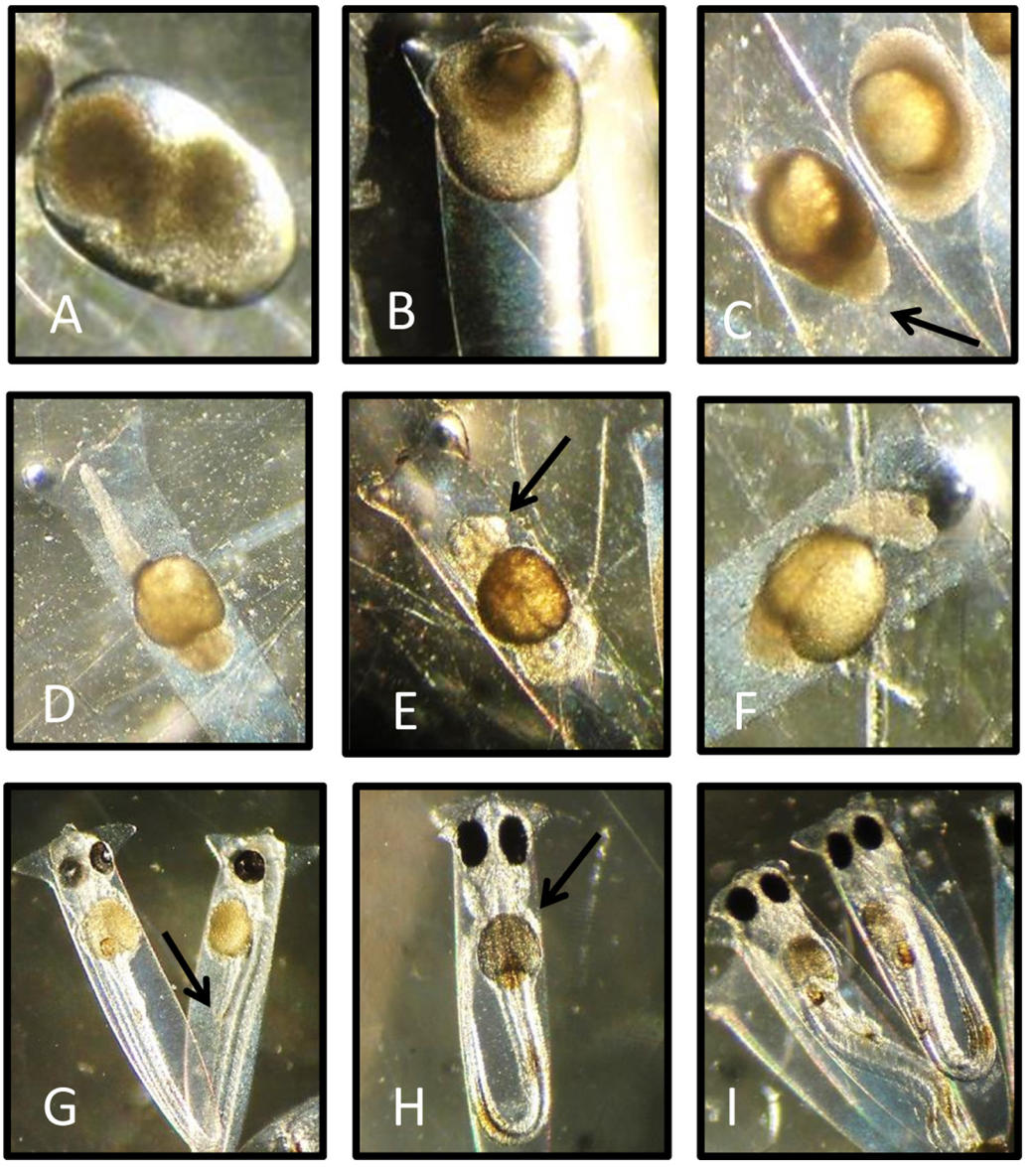

Fig. 1. Embryonic development of the red head goby, *Elacatinus puncticulatus*. (A) 12 HPF (hours post-fertilization), the early formation of the embryonic capsule (25× magnification); (B) 18 HPF, fully formed capsule and the germ ring reaching the vitelline pole (23× magnification); (C) 24 HPF, head constitution of the embryo (arrow) (22× magnification); (D) 48 HPF, significant body length increase (14× magnification); (E) 72 HPF, formation of the optical vesicle (arrow) (15× magnification); (F) 96 HPF, retinal pigmentation (26× magnification); (G) 120 HPF, anal pore formed (arrow) (10× magnification); (H) 144 HPF, development of pectoral fins (arrow) (15× magnification); (I) 168 HPF, larvae at hatching period (10× magnification). Note the empty embryonic capsule of newly hatched larva (left) and the strained tail of the larva in the center of the image (HPF—hours post-fertilization).

ability to catch food (Wittenrich, 2007). The biggest difference between treatments 6D and 7D was the formation of the mouth and jaw. The mouth opened immediately after hatching in treatment 7D larvae, at which point they could start to feed exogenously, which resulted in an increased survival. The period between the first feeding of the larva and the point at which larvae will inevitably die is fundamental to the mortality rate (Johnson and Katavic, 1986). The intense mobility observed in newly hatched 7D larvae suggests that they would have a greater ability to capture food and escape from predators (Ferreira et al., 2009). Copepods are small and fast swimming, which may have hindered successful food capture by the goby larvae. *Euplotes* sp. are spherical and very small (average of 100 μm of diameter) and also might not have been attractive to red head goby larvae. Despite the fact that the rotifer *Brachionus* sp. offers a low nutritional value to *E. figaro* (Souza, 2012) among the food items tested here, rotifers are an appropriate diet with regard to size, shape and ease of capture for *E. puncticulatus* larvae in its early stages of development.

In this study, enriching *Brachionus* sp. with commercial HUFA (Algamac®) was not enough to keep the animals properly nourished. According to Holt (2011), the levels of

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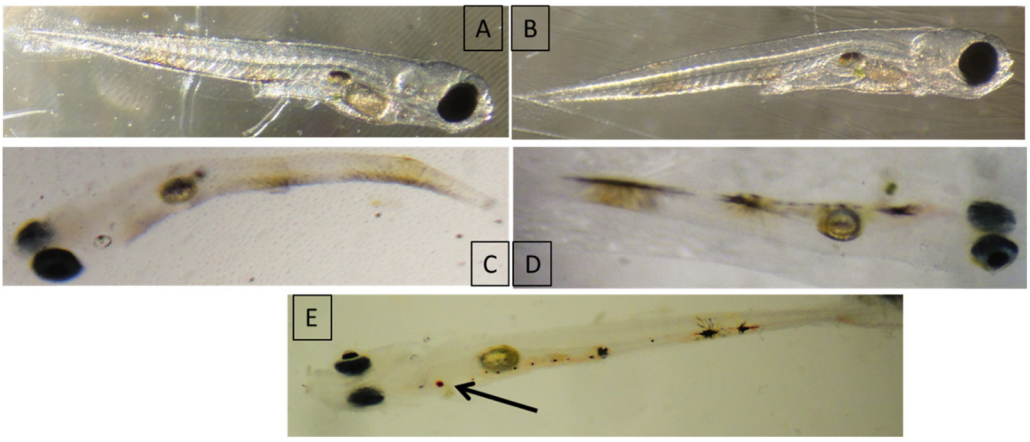

Fig. 2. Early larval development of the red head goby, *Elacatinus puncticulatus*. Note the closed mouth in newly hatched larvae in treatment 6D (A) (12× magnification) and the open mouth in treatment 7D (B) (10× magnification); (C) and (D) show the larvae at 5 DPH for treatments 6D and 7D, (9× and 7× magnification), respectively; (E) 18 DPH larvae (5× magnification). Note the presence of chromatophores on the dorsal portion of the larval body (arrow).

eicosapentaenoic (EPA, 20:5 $n-3$), docosahexaenoic (DHA, 22:6 $n-3$) and arachidonic (ARA, 20:4 $n-6$) fatty acids in the diet define the success of the physiological development of the nervous, sensory, genetic, immunological and homoeostatic balance systems. The high mortality that occurred at 5 DPH indicates that the larvae were not feeding properly. At 5 DPH, the yolk sac was completely absorbed, and no energy reserve was available for the larvae. The nutritional needs and food preferences of *E. puncticulatus* are critical because they feed naturally on parasites and cellular fish debris. The control of larval and broodstock nutrition will be essential for breeding this species in captivity. As no other studies on the species have been reported, these data may be used as a starting point for further studies to describe later larval development stages and cultivation of this and other species of the same genus.

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References

APPA, 2011. National Pet Owners Survey. American Pet Products Manufacturer Association (APPA), Greenwich, CT.

Bio-Marine, Inc., 2013. In: Bio-Marine, Inc. Aquafauna (Ed.), Feeding Protocols, Algamac-2000. , http://www.aquafauna.com/Protocols-AlgaMac-2000.htm#ROTIFER ENRICHMENT.

Brooks, S., Tyler, C.R., Sumpter, J.P., 1997. Egg quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries 7, 387–416.

Cortes, G.F., 2009. Produção e utilização de diferentes fontes de alimento vivo na fase inicial de larvicultura do neon gobi (*Elacatinus figaro*). In: Pós-graduação em Aquicultura. Universidade Federal de Santa Catarina, Florianópolis, SC.

Ferreira, A.V., Vidal, J.M.V., Andrade, D.R., Yasui, G.S., Mendonça, P.P., Mattos, D.C., 2009. Consumo de vitelo durante o desenvolvimento embrionário de melanotenia-maçã, *Glossolepis incisus* (Weber, 1907). Ciência Animal Brasileira 10, 721–729.

Fishbase, 2012. Elacatinus puncticulatus (Ginsburg, 1938). Fishbase, ⟨http://www.fishbase.org/summary/Elacatinus-puncticulatus.html⟩.

Gomes, M.B., 2010. Peixes recifais de ocorrência no Brasil: ameaças, atributos bioecológicos e percepção humana para a conservação. In: Pós-graduação em Ecologia. Universidade Federal de Santa Catarina, Florianópolis, SC.

Holt, J.G., 2011. Larval Fish Nutrition. John Wiley and Sons, Inc., Oxford, UK.

Johnson, D.W., Katavic, I., 1986. Survival and growth of sea bass (*Dicentrarchus labrax*) larvae as influenced by temperature, salinity, and delayed initial feeding. Aquaculture 52, 11–19.

Meirelles, M.E., Tsuzuki, M.Y., Ribeiro, F.F., Medeiros, R.C., Silva, I.D., 2009. Reproduction, early development and larviculture of the barber goby, *Elacatinus figaro* (Sazima, Moura & Rosa 1997). Aquaculture Research 41, 11–18.

Olivier, K., 2001. Globefish Research Programme. Rome, Italy.

Olivotto, I., Planas, M., Simões, N., Holt, G.J., Avella, M.A., 2011. Advances in breeding and rearing marine ornamentals. Journal of the World Aquaculture Society 42, 135–166.

Olivotto, I., Scott, A.H., Carnevali, O., Holt, J., 2006. Spawning, early development, and first feeding in the lemoonpeel angelfish *Centropyge flavissimus*. Aquaculture, 253.

Olivotto, I., Zenobi, A., Rollo, A., Migliarini, B., Avella, M., Carnevali, O., 2005. Breeding, rearing and feeding studies in the cleaner goby *Gobiosoma evelynae*. Aquaculture 250, 175–182.

Olsen, Y., 2007. Live food technology of cold-water marine fish larvae. In: Moksness, E., Kjorsvik, E., Olsen, E. (Eds.), Culture of Cold-Water Marine Fish. Blackwell Publishing Ltd, Oxford, UK.

Shei, M.R.P.S., Miranda-Filho, K.C., Rodrigues, R.V., Sampaio, L.A., 2010. Production of juvenile barber goby *Elacatinus figaro* in captivity: developing technology to reduce fishing pressure on an endangered species. Marine Biodiversity Records 3, 1–7, Special section.

Souza, M.F.S., 2012. Proteases alcalinas e manejo alimentar na larvicultura do neon gobi *Elacatinus figaro*. In: Programa de Pós-graduação em Aquicultura. Universidade Federal de Santa Catarina, Florianópolis, SC, pp. 63.

UNEP, 2008. Consultation Process on Monitoring of International Trade in Ornamental Fish. World Conservation Monitoring Centre. European Commission Directorate General E—Environment ENV.E.2.—Development and Environment, pp. 43.

Wabnitz, C., Taylor, M., Green, E., Razak, T., 2003. From Ocean to Aquarium: The Global Trade in Marine Ornamental Species. UNEP-WCMC, Cambridge, pp. 64.

Wittenrich, M.L.T.F.H., 2007. The Complete Illustrated Breeder's Guide to Marine Aquarium Fishes.