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Thermal influence on the embryonic development and hatching rate of the flameback pygmy angelfish *Centropyge aurantonotus* eggs

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Summary

The flameback pygmy angelfish *Centropyge aurantonotus*, highly appreciated and valued by the aquarium market, is heavily harvested and traded. Temperature is one of the abiotic factors that has the most influence on fish development, especially in the early stages of life. For captive production, it is essential to know the appropriate environmental parameters for each species. In this sense, this study aimed to evaluate the influence of temperature on the embryonic development and hatching rates of *C. aurantonotus* incubated at six different temperatures (20, 22, 24, 26, 28, 30°C). Embryonic development events were very similar in terms of morphological and chronological characteristics compared with other species of the genus *Centropyge*. Incubation time was inversely proportional to temperature. The treatment at 22°C required twice the time of that required by 30°C treatment for hatching to occur. The best incubation temperature range was 24–28°C. Values below 22°C and at 30°C showed lower hatching rates compared with other treatments. Based on these results, the recommended temperature at which to incubate *C. aurantonotus* eggs is between 24–28°C.

Introduction

The genus *Centropyge*, known as the 'dwarf angel fish', is the largest within the Pomacanthidae family, with 34 species described (Gaither *et al.*, 2014). The flameback pygmy angelfish *Centropyge aurantonotus*, the only representative of this genus from Brazil, distributes from São Paulo, southeast Brazil to southeast of the Caribbean (FISHBASE, 1974). Due to its bright colours, small size in adulthood and resistance to captive conditions, fish from this genus are highly valued by the aquarium market (Pyle, 2003) and therefore are heavily harvested and traded. These characteristics make the flameback pygmy angelfish a potential candidate for aquaculture.

Apart from the transition from endogenous to exogenous feeding, one of the major obstacle difficulties in captive rearing of these fishes is to improve early larval performance by optimizing environmental conditions for embryonic development.

Many studies have shown that temperature strongly affects ontogeny (Gracia-López *et al.*, 2004; Yang and Chen, 2005; Uehara and Mitani, 2009; Shi *et al.*, 2010) and directly influences the larval condition at hatching with respect to the amount of endogenous resources remaining in the yolk sac (Kamler, 2008). The effect of temperature on ontogeny is determined by the rate of enzymatic reactions (Blaxter, 1969) that will directly influence the rate of development.

Therefore, the present study evaluated the influence of temperature on the embryonic development and hatching rates of the flameback pygmy angelfish incubated at different temperatures.

Material and methods

The study was carried out at the Laboratory of Marine Fishes and Ornamentals (LAPOM), Universidade Federal de Santa Catarina (UFSC), Florianópolis, Santa Catarina, Brazil. Wild adult fish were caught in Guarapari, Espírito Santo (SISBIO/ICMBio authorization no. 46575-3) and sent to the laboratory in which six pairs were formed. All experiments were carried out with proper authorization of the Ethics and Animal Use Committee CEUA, UFSC no. 8650270218.

Each pair of *C. aurantonotus* (males ~7 cm, females ~5.7 cm) was kept in 1000 L tanks in a closed recirculation system with mechanical filter (bag – 5 microns), biological filter (ceramic medium) and a 55 W UV filter. Temperature was maintained between 24.2–28.5°C, salinity 33 ppm, pH 8.2, NO_2^- and NH_3 < 0.025 ppm. The photoperiod was set at 14 h light:10 h dark.

Eggs were obtained through natural spawning and collected with an external egg collector made of PVC pipe with a mesh of 250 µm, located at the water outlet of the breeding tanks. Newly fertilized eggs were distributed in the experimental units (50 eggs per unit – 0.1 eggs/ml), which consisted of plastic containers with a volume of 500 ml. The water used in the experiment (salinity 33 ppm) was previously sterilized with sodium hypochlorite for 4 h, and neutralized with sodium thiosulfate.

Eggs were incubated without water exchange (static method) or aeration. All experimental units had an initial water temperature of 26°C that was gradually adjusted to 20, 22, 24, 26, 28, 30°C, by immersing them in a water bath using heating thermostats (± 0.3°C). All temperatures were reached within 2 h.

Each treatment had four replicates. Three of these were for evaluation of hatching rates and one for observation of embryonic development. Observation was performed at intervals that varied according to the ontogenetic phase. Because of their rapid cell division, at the end of egg distribution in the experimental units, the embryos already had eight cells. From this point to the closure of the blastopore, observation was made at intervals of 15 min and from closure of the blastopore until hatching, at intervals of 1 h. During observation, eggs were collected using a pipette and placed on a glass slide ($n = 5$) and immediately photographed with a ×10 objective optical microscope (Leica, DM750). After these observations, eggs were discarded.

Incubation time was defined as the period between egg activation up to 50% fertilized eggs hatched (Kamler, 2002). The total hatching rate was determined as the percentage of fertilized eggs that hatched.

After transformation of data into sine–arcs in percentages, the differences between the means of hatching rate were tested through one-way analysis of variance (ANOVA). All statistical tests were analyzed using Statistica software version 7.0 and data were presented as mean ± standard deviation.

Table 1. Stages of the embryonic development of the flameback pygmy angelfish *Centropyge aurantonotus* at different temperatures, in hours, from 64 cells up to hatching

Development stage	20°C	22°C	24°C	26°C	28°C	30°C
64-cell	2.45	2.03	1.55	1.08	0.63	0.38
128-cell	2.80	2.30	1.90	1.50	0.87	0.75
20% epiboly	–	7.65	7.20	6.82	6.45	5.90
50% epiboly	–	11.88	9.83	7.52	7.03	6.70
90% epiboly	–	14.20	11.52	8.20	7.50	7.03
Blastopore closure	–	14.45	11.85	8.42	7.68	7.25
Six somites	–	14.87	12.10	9.55	8.08	7.70
Melanophores	–	17.15	13.02	10.75	9.25	8.11
Kupffer vesicle	–	17.25	13.18	10.90	9.38	8.25
Head and tail differentiation	–	17.25	13.18	10.90	9.38	8.25
Hatching	–	25.13	20.09	15.82	13.59	12.58

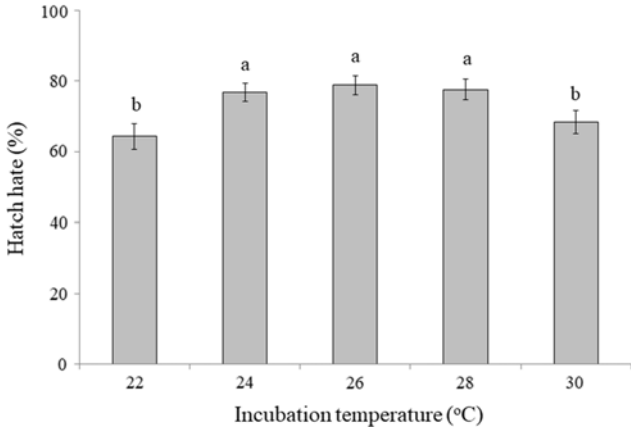

Figure 1. Hatching rates of the flameback pygmy angelfish *Centropyge aurantonotus* eggs incubated at six temperatures (mean ± standard deviation (SD); $n = 50$). [a,b]Distinct letters denote significant difference ($P < 0.05$).

Results and Discussion

Fertilized eggs of *C. aurantonotus* are transparent, spherical and pelagic, measuring between 0.64 and 0.72 mm in diameter and containing a single globule with a diameter of 0.16–0.18 mm. The fertilization rate of the eggs was 95%. Due to the rapid cell division and embryonic development after fertilization, evaluation of the cleavage patterns as well as the number of blastomeres for each treatment in the early stages of division could not be assessed. Therefore, especially at higher temperatures, it was only possible to begin observation for all treatments when the embryos were in the 64-cell phase. Eggs exposed to 20°C showed compromised cell divisions (irregular divisions) and, from 8 h after fertilization, it was possible to verify 100% of non-viable embryos. The main events occurring during embryonic development, ranging from 64 cells to hatching, are described in Table 1.

Centropyge aurantonotus is found in both tropical and subtropical areas of the Atlantic Ocean (IUCN, 2015) (Pyle *et al.*, 2010), in which there is a considerable temperature variation. In the present study, the development of *C. aurantonotus* embryos was shown to be strongly influenced by temperature. Higher hatching rates (around 80%) were obtained at 24, 26 and 28°C (Fig. 1), which correspond to the temperatures in the natural habitat of this species, therefore this range of temperature should be more suitable for incubation in laboratory conditions. At this range, incubation time and embryonic development events were similar to those found in species of the same genus, such as *C. ferrugatus* (eggs hatched 16 h, 10 min AF at 26°C; Hioki *et al.*, 1990); *C. argi*, (hatched 18 h after fertilization (AF) at 24°C; Holt and Riley, 2001) and *C. flavissimus* (hatched at 15 h AF at 26°C; Olivotto *et al.*, 2006).

Temperature directly influences all aspects of embryonic development (Fielder *et al.*, 2005). In general, increasing the temperature accelerates embryonic development. However, this increase occurs within a specific thermal range acceptable for each species and, for *C. aurantonotus*, this was between 24–28°C. Treatments with temperatures beyond this range, presented suboptimal results in the hatching rate. For example, eggs exposed to 20°C showed compromised cell divisions (irregular divisions) and all embryos died after 8 h post fertilization (Fig. 2).

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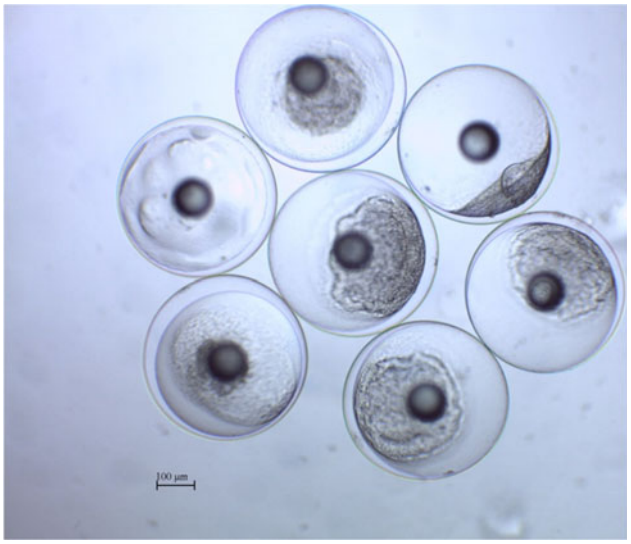

Figure 2. Flameback pygmy angelfish *Centropyge aurantonotus* eggs exposed at 20°C, with irregular divisions (anomalies).

In conclusion, water temperature influenced the embryonic development of *C. aurantonotus*. Embryonic development events were very similar in morphological and chronological characteristics to other species of the genus *Centropyge*. Temperatures below 22°C and above 30°C were not recommended for incubation of *C. aurantonotus* eggs due to lower hatching rates compared with other treatments.

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Conflicts of interest. The authors certify that they have nothing to disclose.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guidelines on the care and use of laboratory animals. Treatment of the animals was approved by Committee of Ethics and Use of Animals (CEUA) UFSC no. 8650270218.

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