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The effect of water temperature on muscle cellularity and gill tissue of larval and juvenile *Lophiosilurus alexandri*, a Neotropical freshwater fish

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ABSTRACT

The effect of temperature on muscle development and gill tissue were investigated in larval and juvenile of *Lophiosilurus alexandri*, a carnivorous Neotropical fish species. Larvae and juveniles were reared at temperatures of 23, 26, 29 and 32 °C for 15 and 35 days, respectively. The larvae were fed *Artemia* nauplii, while the juveniles received a formulated diet. In both stages, high temperatures resulted in increased fiber diameter of white muscle. Moreover, a pronounced effect of hypertrophy was observed in later stages of development. No alterations to minimum fiber diameter, and similar mosaic standards of muscle growth dynamics, were observed in larvae kept at 23–32 °C, suggesting that hyperplasia was not effected during early stages of development. Gills were analyzed by Degree of Tissue Change (DTC), which is based on the severity of lesions. Significant morphological changes were observed in gills when juveniles were kept at 32 °C, which was considered moderate tissue damage. Temperature modulates cellularity in *L. alexandri* larvae and juveniles, with pronounced alterations of hypertrophy of white muscle fibers in later stages of development when exposed to elevated temperatures. Higher temperatures may also induce muscle growth dynamics with increased recruitment of new white fiber, increased fiber diameter in the early stages and induced gill lesions.

1. Introduction

Temperatures are changing globally, and ecosystems and societies will need to continuously adapt to this moving target (Chavaillaz et al., 2016). Knowledge of how temperature effects animal development may help to understand the biology and methods of adaptation of animals to variation in temperature. Heterothermic animals have a given temperature range within which they can survival; however, this does not mean these temperatures are optimal growing conditions (He et al., 2015; Vinagre et al., 2015).

Water temperature is one of the most important abiotic factors that influence the growth (Barron et al., 2012; Eldridge et al., 2015), somatic development (Johnston, 2006; Martins et al., 2014), survival (Hansen and Falk-Petersen, 2001; Keckeis et al., 2001; Martinez-Palacios et al., 1996), metabolism (Khan et al., 2014, 2015; Wen et al., 2013) and behavior (Rijnsdorp et al., 2009) of fish. Fish possess optimum temperatures for development, which, for many species, can change with age and size (Árnason et al., 2009; Handeland et al., 2008; Otterlei et al., 1999). Moreover, temperature may influence the development of organs and their interaction with the environment, which can result in adaptations for the maintenance of homeostasis (Evans et al., 1987; Royas et al., 2013). In general, changes in water temperature cause increased energy expenditure for the maintenance of metabolism, thus decreasing the capacity for growth and increasing the efficiency of the transformation of food energy to net energy (Bermudes et al., 2010; Van Ham et al., 2003).

Fish growth is correlated with muscle development, and is affected by environment conditions, which can alter the rate of myogenesis, cellular organelle composition, gene expression patterns and the number and size distribution of muscle fibers (Periago et al., 2005; Portella et al., 2014). In general, abiotic factors, such as feeding management and food quality, can alter the growth of skeletal muscle in fish, including the duration and intensity of myotube formation in juveniles and adults (Johnston, 2006; Kojima et al., 2015; Leitão et al., 2011). In addition, temperature can affect the number and diameter of myotomal muscle fibers in larval fish (Assis et al., 2004; López-Albors et al., 2003).

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Chemical products, pollutants, and water quality, including parameters such as nitrogen concentration, have been implicated in altering gill morphology, and compromising the osmo-respiratory system due to direct contact between gills and the environment (Evans et al., 1987; Perry and Laurent, 1993). Morphological alterations of gills have been described for the Neotropical *Colossoma macropomum* and the tropical reef fish *Pomacentrus moluccensis* under different temperatures (Bowden et al., 2014; Royas et al., 2013).

Pacamã, *Lophiosilurus alexandri,* is an endangered Neotropical fish species of the order Siluriformes (Lins et al., 1997). The species was recently classified as Vulnerable, and is in need of improvements to its natural stock (ICMBio, 1876). A series of studies has been conducted in order to expand knowledge regarding reproduction, physiology and growth of the species (Costa et al., 2015; Santos and Luz, 2009; Takata et al., 2014). However, there is no information about the effects of temperature on muscle development and gills of *L. alexandri*. Therefore, the present study aimed to evaluate muscle cellularity and gill alterations in larval and juvenile *L. alexandri* reared under different temperatures.

2. Material and methods

All the methodologies used in the experiments involved in this study were approved by the Committee of Ethics and Animal Welfare of UFMG (CETEA-UFMG 065 – 2011).

2.1. Larval rearing conditions

Fertilized eggs of *L. alexandri* were collected from a 5000-L tank, with a sand substrate, containing two males and three females (total weight ranging from 1.5 to 5.0 kg). The fish were captured from naturally spawning broodstock in the São Francisco river using a fishing net, and then adapted to laboratory conditions at Federal University of Minas Gerais, Brazil (LAQUA). Fish reproduction occurred at 28 °C. Eggs and larvae in the lecithotrophic stage were incubated and reared, respectively, in aerated tanks at temperatures between 28 and 29 °C (Costa et al., 2015). At the commencement of exogenous feeding, the larvae were quantified to initiate the period of rearing.

Larvae of *L. alexandri* at eight days post-hatching (11.9 ± 0.7 mm total length; 16.8 ± 1.2 mg) were used to initiate the feeding trial. Four water temperatures (23, 26, 29 and 32 °C) were tested to evaluate muscle cellularity of the larvae over a period of 15 days. Larvae were stocked at a density of 30 larvae L^{-1} in 12 1.5-L aquaria (three per temperature), with aeration supplied by a porous air stone without causing water turbulence. In order to reach the predetermined temperatures in each treatment, the tanks were placed in thermostatic baths and the temperature gradually adjusted by 1 °C every two hours over the course of one day to the temperatures of the corresponding treatments. The specific growth rate [SGR = (ln final total length − ln initial total length/time)*100] was calculated.

All treatments had a photoperiod of 10 L/14D with the light intensity on the water surface being 150 lx (Digital Lux Meter, model: ITLD 260). Dissolved oxygen was checked every day and the concentration maintained above 5.0 mg L^{-1} throughout the experiment (Portable Waterproof Microprocessor Dissolved Oxygen Meter – HI 9146, Hanna Instruments). Total ammonia nitrogen (TAN) and pH were 0.016 ± 0.015 mg L^{-1} and 8.75 ± 0.03, respectively, and were checked every three days according to APHA (2005) using a Waterproof Tester (Hanna Instruments).

Larvae were fed *Artemia* nauplii three times a day at 0900, 1300 and 1700 h in increasing amounts during development: 1300 *Artemia* nauplii $larvae^{-1}$ during the first five days of feeding; 1950 *Artemia* nauplii $larvae^{-1}$ from the sixth to the 10th day; and 2600 *Artemia* nauplii $larvae^{-1}$ from the 11th to the 15th day. For feeding, the prey concentration was estimated and divided equally among meals.

To feed the larvae, *Artemia* cysts were hatched daily. The nauplii were then concentrated into a small volume of brackish water (10‰) for quantification and subsequent larval feeding. This strategy was designed to avoid increasing the salinity of the water in the aquaria. Each day, before the first and the last feedings, the aquaria were siphoned to remove waste, with the volume of water withdrawn being replaced with water at the same temperature.

2.2. Juvenile rearing conditions

Juveniles of *L. alexandri* measuring 12.6 ± 0.5 cm and weighing 30.6 ± 3.6 g were subjected to four water temperatures, 23, 26, 29 and 32 °C, with four replicates per temperature. To reach the predetermined temperature of each treatment, the tanks were placed in thermostatic baths and the temperature gradually adjusted over the course of two days to reach the corresponding treatment temperature (23, 26, 29 or 32 °C).

Juveniles were stocked in 16 tanks at a density of three juveniles per 9-L tank. The experiment lasted 35 days. Juveniles were fed twice daily until apparent satiation (0800 and 1700 h) with formulated diet containing 44% crude protein, 8% lipids, 14% fiber and 14% mineral matter. The amount of food offered was monitored and any excess was removed 30 min after feeding for corrections to diet consumption and to maintain water quality. During this process, the excess food was siphoned out and the water replaced with water at the corresponding treatment temperature. The specific growth rate [SGR = (ln final total length − ln initial total length/time)*100] was calculated.

All the treatments had a photoperiod of 10 L/14D and dissolved oxygen maintained above 6.5 mg L^{-1} (Portable Waterproof Microprocessor Dissolved Oxygen Meter – HI 9146, Hanna Instruments). The mean values for total ammonia nitrogen (TAN) and pH were 1.70 ± 0.99 mg L^{-1} and 7.47 ± 0.33, respectively, and were checked every three days according to APHA (2005) using a Waterproof Tester (Hanna Instruments).

2.3. Quantification of muscle cellularity and gill histology

At the end of the experiments, samples of 10 larvae and nine juveniles from each treatment were collected for quantification of muscle cellularity and gill alteration; only juveniles were analyzed for gill histology. Fish were sacrificed by an overdose of eugenol (80 mg L^{-1}) and their branchial arches fixed in Bouin's liquid for 8–12 h and conserved in 70% alcohol solution. Muscle samples were fixed in Karnovsky solution (2.5% glutaraldehyde and 2% paraformaldehyde in phosphate-buffered saline 0.1 M, pH 7.3) for 18–22 h at 4–8 °C.

After fixation, muscle samples were embedded in glycol metacrylate plastic resin (Historesin® Leica, Germany) while branchial arches (gills) were embedded in paraffin. The samples were sectioned at a thickness of 3–5 μm and stained with toluidin blue–sodium borate for muscle and haematoxylin–eosin for gills.

In order to quantify both hyperplasia and maximum hypertrophy, white muscle cellularity was studied in the deep sections of myotomes, constituting a dorsal quadrant of white muscle, which included both newly recruited and older muscle fibers. The individual outlines of 150 and 300 deep white muscle fibers were drawn for larvae and juveniles, respectively, and the individual equivalent diameter (D) of white fibers was determined (D of a circle whose area is the same as that of the muscle fiber; hereafter referred to as “fiber diameter”). Mean and median diameters were evaluated, and the mean of the ten largest (D of the 10 largest) and the ten smallest (D of the 10 smallest) fibers, and the largest fiber diameter (D maximum) and the smallest fiber diameter (D minimum), were calculated (hereafter referred to as “maximum diameter” and "minimum diameter", respectively) in order to quantify the maximum hypertrophy of the white muscle fibers and hyperplasia (Alami-Durante et al., 2000, 2010a, 2010b). White muscle cellularity is length-dependent in trout (Weatherley et al., 1980), as in other teleosts (Weatherley et al., 1988), and so data fiber diameter was normalized by *ln* of the final total length (TL) of each fish in order to take into account