ADAPTATIONS OF THE PIAVA (*Leporinus obtusidens*) JUVENILE EXPOSED TO HYPOXIA*

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ABSTRACT

The piava (*Leporinus obtusidens*) is a migratory freshwater fish. There is relatively little information on the hematological and morphological adaptations of tropical freshwater fish when subjected to chronic and sublethal concentrations of dissolved oxygen (DO). The aim of this study was to verify the existence of adaptations of the gills and blood in juvenile piava exposed to different concentrations of DO. The juveniles used in this experiment were cultured for 70 days at different DO concentrations (1.74 ± 0.04, 3.54 ± 0.06, 5.34 ± 0.05 and 7.34 ± 0.02 mg L^{-1}) and kept at mean water temperature of 26.4°C. Individuals subjected to conditions of severe hypoxia (1.74 mg L^{-1} DO) showed a significant increase in the number of erythrocytes (P<0.05) and a decrease in erythrocyte corpuscular volume (P<0.05), but no changes were observed in gill lamellae. This study concludes that *L. obtusidens* juveniles undergo hematological change when exposed to conditions of severe hypoxia.

Keywords: Dissolved oxygen; hematocrit; erythrocytes; gills; blood

ADAPTAÇÕES DE JUVENIS DE PIAVA (*Leporinus obtusidens*) EXPOSTOS À HIPÓXIA

RESUMO

A piava (*Leporinus obtusidens*) é um peixe migrador de água doce. São relativamente escassas as informações de adaptações hematológicas e/ou morfológicas apresentadas por peixes tropicais de água doce quando submetidos à concentrações crônicas e subletais de oxigênio dissolvido (OD). O objetivo deste trabalho foi verificar a existência de adaptações branquiais e sanguíneas em juvenis de piava submetidas a diferentes concentrações de oxigênio dissolvido. Para tal, juvenis foram cultivados durante 70 dias em diferentes concentrações de oxigênio dissolvido (1,74 ± 0,04, 3,54 ± 0,06, 5,34 ± 0,05 e 7,34 ± 0,02 mg L^{-1}) a uma temperatura média da água de 26,4°C. Indivíduos submetidos às condições de hipóxia severa (1,74 mg L^{-1} de OD) apresentaram aumento significativo do número de eritrócitos (P<0,05) e redução no volume corpuscular dessas células (P<0,05). Não foram observadas modificações morfológicas nas lamelas branquiais. É possível concluir que juvenis de *L. obtusidens* apresentam adaptações hematológicas quando submetidos à condição de hipóxia severa.

Palavras chave: Oxigênio dissolvido; hematócrito; eritrócitos; brânquias; sangue

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INTRODUCTION

Hematological parameters are a useful tool for understanding some of the physiological strategies used by the fish when they are exposed to hypoxia conditions.

An increase in the hematocrit and the number of erythrocytes (red blood cells - RBC) are some of the classic changes observed in fish subjected to hypoxia (VALENZUELA *et al*., 2002; BALDISSEROTTO *et al*., 2008; BALDISSEROTTO, 2009). Increases in RBC are due to the fact that most of the oxygen transported in the blood is attached to hemoglobin encapsulated inside these cells (BRAUNER and VAL, 2006). The spleen may also promote a rapid response to hypoxia conditions by contributing with additional RBC (VALENZUELA *et al.,* 2002). Some species of tropical fish can adapt to local conditions of low dissolved oxygen (DO) levels via morphological modifications of the gills; an increase of respiratory surface area allows for higher oxygen uptake (CHAPMAN and HULEN, 2001; CHAPMAN *et al*., 2002, 2008; CRAMPTON *et al*., 2007; ONG *et al*., 2007).

Leporinus obtusidens (Anostomidae) is a large migratory freshwater fish that can reach weights exceeding 7.5 kg (TAITSON *et al*., 2008) and is found in the South American basins of the Paraná, São Francisco and Uruguay rivers (OLDANI and OLIVEROS, 1984; TABLADO *et al*., 1988; SATO *et al*., 2000; ZANIBONI-FILHO and SCHULZ, 2003). *Leporinus obtusidens* is an omnivorous species (HAHN *et al*., 1998) and it is of great commercial importance for the quality and flavor of its meat.

The relative lack of information about the response of South American freshwater species to different levels of DO hinders the establishment of criteria for adequate cultivation conditions. It is known that prolonged exposure to stressful levels of DO can affect the physiological processes of fish, interfering with feeding rates, and limiting growth (KARIM *et al*., 2002; WILHELM FILHO *et al*., 2005) and reproduction (DABROWSKI *et al*., 2003).

This study aims to assess whether hematological and morphological adjustments in juvenile *L. obtusidens* are made in response to different concentrations of DO.

MATERIALS AND METHODS

Piava juveniles (*L. obtusidens*) were obtained from the hormonal induction of the wild breeding stock from LAPAD/UFSC, captured in the Upper Uruguay River basin and maintained and bred in captivity. The juvenile piava (9.22 ± 1.76 g and 10.19 ± 0.64 cm) were exposed to four different concentrations of DO: 1.74 ± 0.04, 3.54 ± 0.06, 5.34 ± 0.05 and 7.34 ± 0.02 mg L^{-1}; tested in triplicate. These values are equivalent to 21, 47, 70 and 92% of the oxygen saturation, respectively.

Seventy-liter fiberglass tanks were stocked with 20 fish per tank. The fish were reared over 70 days of experimentation and kept at an average temperature of 26.4°C. Piava juveniles were fed twice daily (08:00 and 16:00 hours) with commercial feed (42% crude protein (CP) and 3,800 kcal kg^{-1} of digestible energy) until apparent satiation during almost 10 minutes. To avoid gas exchange between atmospheric air and water, each tank water surface were covered with plastic. Survival was evaluated at the end of each day. The fish were cultivated in a closed recirculating system, using mild saline water (NaCl at 1 ppt), and water was renewed 250% per day in each fiberglass tanks. Dissolved oxygen concentrations were maintained through aeration and incorporation of nitrogen gas.

Dissolved oxygen were monitored every hour from 08:00 to 22:00 hours (15 times per day); temperature were monitored three times per day (08:00, 12:00 and 16:00 hours), and pH and conductivity were measured twice daily (08:00 and 16:00 hours). Measurements of oxygen and temperature were taken using YSI model 550A (Yellow Springs Instruments Company, OH, USA), and pH and conductivity were evaluated using a YSI model 63 multiparameter probe (Yellow Springs Instruments Company, OH, USA). Hardness and concentrations of total ammonia, nitrite and alkalinity were evaluated once a week using the colorimetric titration method (Alpha Tecnoquímica, SC, Brazil). All the water quality parameters were taken from the center of the tank between the inlet and outlet of water.

At the end of 70 days of cultivation, five fish from each tank were randomly selected and anesthetized with 100 mg L^{-1} of eugenol. These fish were individually weighed, and then 1 mL of blood was collected with the aid of needles and syringes with EDTA, via caudal puncture.

The material collected was used to determine the hematocrit (Ht), obtained through the micro hematocrit technique by centrifuging the blood at 2,800 rpm for five minutes. Prior to blood dilution in a 1:100 saline solution, the number of RBC from this same sample was counted in a Neubauer chamber. The mean corpuscular volume (MCV = Ht x 10/RBC) was then calculated. The protocol used to handle fish was previously approved by ethics committee (CEUA-UFSC) by number PP00788. The fish were sacrificed with an overdose of eugenol (300 mg L^{-1}), to determine the spleen-somatic index (SSI = spleen weight x 100/fish weight) using an electronic balance (0,001 g). After 24 h of immersion in 10% phosphate-buffered formalin fixative, the second left gill arch was cut away and then routinely processed for paraffin embedding. The gill arches were serially sectioned in 4 µm increments and then stained with hematoxylin and eosin. The slides were viewed using a LEICA DM3000LED light microscope with ocular lens ruler (10 mm) to determine the total length (L) (measured from the edge adjacent to the filament to the most distal point of the lamellae from the filament) and width (W) (measured parallel to the filament at the base of the lamellae from one edge to the other) of the lamellae. Measurements were obtained using the methodology described by ONG *et al*. (2007).

The variables Ht, RBC, MCV, SSI, L and W were subjected to an analysis of variance (ANOVA), followed by the Tukey test (P<0.05). All analyses were performed using version 8.0 of the statistical program SAS (SAS Institute Inc., NC, USA).

RESULTS

Survival rates were 100% for all treatments. The mean values of Ht, L and W were similar (P>0.05) for fish kept at different DO concentrations. The highest RBC and the lowest MCV were observed in fish exposed to severe hypoxia (1.74 mg L^{-1}) (P<0.05). The SSI values were lower in fish kept in severe hypoxia (1.74 mg L^{-1}) than in those kept at 3.54 mg L^{-1} DO, although similar values were observed in fish kept in normoxia (Table 1).

The water quality parameters were similar in all treatments, showing the following mean values (± SE): temperature: 26.44 ± 0.30ºC; pH: 6.47 ± 0.19; electrical conductivity: 1,821.17 ± 89.79 µS; total ammonia: 0.40 ± 0.08 mg L^{-1}; nitrite: 0.44 ± 0.12 mg L^{-1}; hardness: 102.67 ± 2.51 mg L^{-1} $CaCO_3$; alkalinity: 18.49 ± 1.26 mg L^{-1} $CaCO_3$.

Table 1. Mean values (± SE) of hematological parameters and morphological gill characteristics in *Leporinus obtusidens* submitted for 70 days at different oxygen concentrations.

Dissolved oxygen (mg L^{-1}) [%]	**Ht** (%)	**RBC** (10^5 µL^{-1})	**MCV** (µ^3)	**SSI** (%)	**L** (µm)	**W** (µm)
(1.74±0.04) [21.50±1.34]	31.54 ± 7.33a	6.78 ± 0.95a	46.11 ± 4.50b	0.019 ± 0.001b	64.79 ± 14.77a	10.06 ± 1.12a
(3.54±0.06) [47.43±0.67]	31.79 ± 6.57a	4.87 ± 0.07b	65.35 ± 13.75ab	0.027 ± 0.002a	54.29 ± 7.81a	10.70 ± 1.95a
(5.34±0.05) [70.35±0.33]	33.47 ± 1.88a	4.48 ± 0.09b	74.68 ± 4.34a	0.024 ± 0.002ab	61.95 ± 8.07a	11.79 ± 1.18a
(7.34±0.02) [91.77±1.19]	34.99 ± 3.22a	5.05 ± 0.17b	69.41 ± 8.63a	0.023 ± 0.004ab	61.75 ± 1.55a	11.67 ± 2.12a

Ht = hematocrit; RBC = number of erythrocytes; MCV = mean corpuscular volume; SSI = spleen somatic index; L = total length of the lamellae; W = width of the lamellae. Different letters in the same column indicate significant differences as determined by the Tukey test. (P<0.05).

DISCUSSION

Leporinus obtusidens juveniles undergo a hematological adaptation to hypoxia by increasing their RBC numbers when subjected to low DO concentrations (1.74 mg L^{-1}). This same response to hypoxia has been observed in other fish species, such as *Astronotus ocellatus* (MUUSZE *et al*., 1998), *Piaractus mesopotamicus* (GÓMEZ-MANRIQUE *et al*., 2009), *Astatoreochromis alluaudi* and *Haplochromis ishmaeli* (RUTJES *et al*., 2007). VALENZUELA *et al*., (2002) also showed a reduction in spleen size associated with an increased number of RBC in *Oncorhynchus mykiss*. In this study, we observed a reduction of the SSI and an increased number of RBC for fish kept in severe hypoxia.

As a result of the increased number of RBC, the hematocrit is expected to increase, or the MCV is expected to decrease. Some authors suggest that a reduction of RBC volume favors rapid oxygenation of hemoglobin. This oxygenation is a result of an increased relationship between the surface area and volume of erythrocytes, thus improving their ability to transport oxygen (WELLS *et al*., 2005; TAVARES-DIAS *et al*., 2008). In this study, hematocrit values remained similar in juvenile *L. obtusidens* exposed to different concentrations of oxygen. However, MCV showed a reduction as a result of varying the concentration of DO.

Some fish species showed a morphological remodeling of the gills in response to hypoxia; *Gymnocypris przewalskii* demonstrated a gradual lengthening of lamellae when subjected to values of 0.3 mg L^{-1} DO for 24 hours (MATEY *et al*., 2008), and *Pseudocrenilabrus multicolor victoria* demonstrated a greater average gill filament length when kept for a minimum of one year at a concentration of 1.3 mg L^{-1} DO (CHAPMAN *et al*., 2008).

Leporinus obtusidens juveniles showed no morphological changes to total length and width of the gill lamellae in response to reductions in oxygen values. It is possible that the hypoxia intensity and exposure time used in this study were not sufficient for the species to manifest any such adaptive response.

According to NILSSON (2007), the apoptosis and mitosis mechanisms in morphological remodeling of the gills represent high energy costs.

Morphological adaptation would therefore be less used by species facing hypoxic conditions for short periods of time. Additionally, an increase in respiratory surface area allows for greater uptake of oxygen and, also would increase the flow of ions and water through the gills. These augmentations result in additional osmoregulation costs and make fish more vulnerable to pathogens and toxic substances (SOLLID and NILSSON, 2006).

CONCLUSION

Leporinus obtusidens juveniles showed hematological adaptations to hypoxia, with an increased number and reduced volume of RBC when oxygen concentrations were maintained at 1.74 mg L^{-1}. When the fish were kept in water with oxygen concentrations equal to or higher than 3.54 mg L^{-1}, they showed no hematological changes. These results may suggest a condition of comfort for *L. obtusidens*.

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