Piaraactus mesopotamicus is hypoxia tolerant and performs antioxidant adjustments after rapid reoxygenation at low temperature

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Abstract

This study aimed to evaluate the effects of the different temperature interactions and reoxygenation rates on parameters of antioxidant defense and oxidative stress in pacu (*Piaractus mesopotamicus*) juveniles. The fish were exposed to 12 h of hypoxia (~2 mg O₂ L⁻¹) with subsequent rapid or gradual reoxygenation (with a return to normoxic levels in 0.5 or 5 h, respectively) in interaction with three different temperatures (~18, ~23, and ~28°C). A control group in normoxia (~7 mg O₂ L⁻¹) was also evaluated for each temperature, which was neither submitted to hypoxia nor reoxygenation, totaling nine treatments in triplicate. After 1 and 12 h of the end of the reoxygenation period, samples of gills and liver were collected to determine the total antioxidant capacity against peroxyl radicals (ACAP), lipid peroxidation (LPO), and protein thiol content (PSH). Results showed temporary changes in ACAP in both organs, where, in general, the lowest levels were found at 18 and 28°C at 1 and 12 h after recovery, respectively. A reduction of LPO levels in gills occurred in interactions between 18°C and rapid reoxygenation and 23°C and gradual reoxygenation after recovery (1 and 12 h). Liver LPO levels were higher at 23°C at 1 and 12 h after recovery. In general, gills PSH content was lower at 18 than at 23°C at 1 h after recovery. Liver PSH content was higher at 23°C after recovery (1 and 12 h). In conclusion, pacu juveniles are hypoxia tolerant and cope better with rapid reoxygenation at 18°C. Furthermore, the adjustments of the antioxidant system performed by the fish were sufficient for the partial resumption of homeostasis 12 h after recovery.

Introduction

Lack of oxygen is considered the main limiting factor for aerobic organisms due to releasing energy from mitochondria to tissues (Hermes-Lima and Zenteno-Savin 2002; Chen et al. 2017). Therefore, all organisms must deal with the harmful effects of oxygen, such as the increase in the concentration of reactive oxygen species (ROS) due to an imbalance between pro-oxidants and antioxidants. This imbalance results in oxidative stress on macromolecules such as DNA, proteins, and lipids (Lushchak 2011; Halliwell and Gutteridge 2015).

Dissolved oxygen (DO) concentration depends on environmental factors such as photosynthesis, aerobic respiration, and atmospheric oxygen diffusion (Boyd and Tucker 2014; Nitz et al. 2020b). In water, the solubility of oxygen is lower than in the atmosphere, and it is reduced when the water temperature increases. At the same time, the metabolic oxygen demand by organisms also increases (Campos et al. 2017). Therefore, an increase in water temperature related to the rise in oxygen consumption by organisms will enhance the flow of electrons in the respiratory chain, with consequent production of ROS (Halliwell and Gutteridge 2015; Nitz et al. 2020a).

This scenario is even more concerning for aquatic organisms, as they are more vulnerable to daily or seasonal variations in temperature and DO (Riffel et al. 2012). Variations in water temperature due to different periods of the day or seasons are common in temperate and subtropical regions (Pinto et al. 2017).
and they affect fish growth and health (Silva Júnior et al. 2021). More extreme fluctuations in oxygen concentrations, with a reduction in oxygen levels capable of causing physiological damage to animals, are called hypoxia. It may also adversely affect their behavior, immunology, growth, and survival (Copatti et al. 2019b; Nitz et al. 2020a).

In freshwater fish, optimal oxygen concentrations depend on the ability of each species to adjust to hypoxia (Toni et al. 2017). Tolerance to hypoxia may require reducing the metabolic rate, tolerance to metabolic by-products, and primarily avoiding or repairing cell damage caused by reoxygenation, which is the period following hypoxia (Bickler et al. 2007; Xiao 2015). Thus, evidence that hypoxia not only causes the activation of the antioxidant system but can also induce a situation of oxidative stress by the excessive formation of ROS in fish, as has already been widely reported in previous studies (Chandel 2010; Mustafa et al. 2011; Huang et al. 2015; Giraud-Billoud et al. 2019; Nitz et al. 2020a).

In addition to hypoxia, water reoxygenation is another crucial moment that requires metabolic adjustments in fish to maintain their homeostasis (Welker et al. 2012). Reoxygenation can trigger an intense leakage of electrons previously accumulated in the respiratory chain during hypoxia, leading to ROS overproduction (Ruuge et al. 1991; Lushchak et al. 2001). Consequently, this could result in lipid peroxidation (LPO), one of the most common cell damage processes. It starts due to the attack of ROS on unsaturated and polyunsaturated fatty acids (PUFAs), generating a chain reaction that triggers severe damage to plasma membranes with loss of their integrity and function (Madeira et al. 2013). Previously, Lushchak et al. (2001) have demonstrated the effects of reoxygenation on oxidative stress parameters in goldfish (Carassius auratus), reporting an increase in LPO levels in the liver and brain, respectively, at 1 and 14 h after reoxygenation. An increase in LPO in the liver and brain of darkbarbel catfish (Pelteobagrus vachelli) was also verified when the animals were exposed to hypoxia for 1.5, 4, and 6.5 h followed by rapid reoxygenation (20 – 30 min) (Zhang et al. 2016).

Changes caused by variations in temperature or DO levels have already been reported in the literature for several fish species (Boltaña et al. 2017; Islam et al. 2019; Welker et al. 2012; Chen et al. 2017; Copatti et al. 2019b). However, the interaction of these factors can trigger different responses with additive, synergistic, and antagonistic interactions (Maher et al. 2019), which may generate a stressful situation, damaging the fish's health. For example, high water temperature synergically with hypoxia may impair fish metabolism (McBryan et al. 2013), which could be avoided at lower temperatures (Pinto et al. 2019). In this sense, the liver and gills are complex organs that have a central role in fish physiology in freshwater teleosts (Copatti and Baldisserotto 2021; Vasconcellos et al. 2022). The gills are the first target of hypoxia and water reoxygenation since they are intimately in contact with the environment (Copatti et al. 2019). The liver has a high potential for ROS generation, counterbalanced by protective mechanisms to detoxify and repair organic molecules (lipids and proteins) (Riffel et al. 2012). Therefore, the liver and gills are relevant for evaluating oxidative stress responses in fish.

Pacu (Piaractus mesopotamicus), a native species of the Paraná and Paraguay River Basins (South America), inhabits subtropical regions of Brazil, Argentina, Paraguay, and Uruguay, where it is exposed to
low temperatures in winter (below 15°C) and high temperatures in summer (above 30°C) (Pinto et al. 2019). In these regions, pacu has been highly accepted by the consumer market (Aguilar et al. 2017). Its cultivation is highly valued in aquaculture systems where it is frequently exposed to conditions of high stocking densities, which can make the environment susceptible to hypoxia (Aguilar et al. 2017; Copatti et al. 2019; Nitz et al. 2020b). In a previous study, Nitz et al. (2020b) reported that exposure of pacu juveniles to hypoxia followed by gradual reoxygenation allowed the re-establishment of homeostasis of blood parameters. Still, these authors did not make any assessment of oxidative stress parameters.

We hypothesize that pacu is tolerant to hypoxia and withstand a rapid reintroduction of DO if kept at a low temperature (18°C), providing a less oxidizing condition (with less generation of ROS and reduction of antioxidant defenses). Therefore, this study aimed to evaluate antioxidant capacity in pacu juveniles after 12 h of hypoxia (~2 mg O₂ L⁻¹) and submitted to rapid (0.5 h) or gradual (5 h) reoxygenation in interaction with three different temperatures (~18, ~23 and ~28°C).

**Material And Methods**

The Animal Experimentation Committee approved this study of the Federal University of Rio Grande (FURG), Rio Grande, RS, Brazil, under process number P049/2017.

Fish and experimental conditions

One hundred and sixty-two pacu juveniles (61.7 ± 9.1g; 13.6 ± 0.63cm) acquired from a commercial fish farm in Ajuricaba, RS, Brazil, were used. Parasite-free juveniles were transported to the Continental Aquaculture Laboratory at FURG, where they were acclimated for ten days in a water recirculating aquaculture system (RAS) comprised of 3 tanks (250 L), with continuous aeration, photoperiod set to 12 h:12 h light: dark, and mechanical and biological filters. During this period and the acclimatization process to different temperatures, the fish were fed an extruded commercial diet (32% crude protein) until apparent satiety twice a day (09 a.m. and 04 p.m.). Feeding ceased 24 h before the start and during the experiments.

We carried out an experiment to verify the effects of interactions between low (~18°C), medium (~23°C), and high temperatures (~28°C) under normoxia (~7 mg O₂ L⁻¹) or rapid or gradual reoxygenation rates (0.5 and 5 h, respectively) after hypoxia (2.02 ± 0.06 mg O₂ L⁻¹ – procedure described below) on parameters of antioxidant defense and oxidative stress in gills and liver. The experiment was carried out in RAS composed of experimental units of 80 L useful volume with or without aeration according to the specifications of each treatment, as further detailed in the following paragraphs. Nine treatments were performed (three temperatures versus normoxia or two types of reoxygenation rates) and randomized in triplicate (n = 6 fish per tank; n = 18 fish per treatment).

Before starting the experiments, the water temperature of the tanks was gradually changed over two weeks, and the fish were acclimatized at the respective tested temperature for 30 days. The water
temperature was adjusted to low temperature (18.05 ± 0.09°C) by using a chiller with a thermostat, to medium temperature (23.10 ± 0.23°C) with the aid of an air conditioner, and to high temperature (27.80 ± 0.20°C) by using a heater with thermostat. The chillers and heaters were placed in the reservoir, and the RAS distributed the water. The temperatures were chosen based on Nitz et al. (2020b).

A control group was kept in normoxia (~ 7 mg O₂ L⁻¹) without exposure to hypoxia or reoxygenation, with exposure to the three different temperatures according to each experimental condition (~ 18, ~ 23, or ~ 28°C). An air blower was used in this treatment, which supplied each experimental unit individually through an aeration system. The other animals were exposed to the same temperatures evaluated in the control group. Initially, they were exposed to 12 h of hypoxia (2.02 ± 0.06 mg O₂ L⁻¹). Subsequently, two types of reoxygenation rates were immediately performed with a return to normoxic levels of ~ 7 mg O₂ L⁻¹: rapid (fast) reoxygenation, where return to normoxia was performed within 0.5 h; or gradual (slow) reoxygenation where the DO levels were gradually increased by 1 mg O₂ L⁻¹ per h over 5 h. Oxygen saturation was continuously measured with an oximeter. The DO levels were chosen since they are normal (normoxia) or sublethal (in hypoxia) values found in southern Brazil (Copatti et al. 2019). The 12 h hypoxia time and reoxygenation rates were chosen based on Nitz et al. (2020b).

The DO levels were reduced to hypoxia by regulating bubbling nitrogen gas (for deoxygenation; medicinal nitrogen) in the water through individual diffusers in each tank. Before starting the nitrogen injection, the aeration system was turned off, and ± 1 mg L⁻¹ DO was reduced every 30 min. After 2 h from the beginning of the infusion of pure nitrogen, the desired level of hypoxia (~ 2 mg O₂ L⁻¹) was reached, after which the experiment began. The maintenance of hypoxia was performed through constant monitoring (every 30 min) with an oximeter.

Water quality parameters were maintained during acclimation and experimental period for pH (7.5 ± 0.01), temperature (26.01 ± 0.4°C), and DO (6.58 ± 0.5 mg O₂ L⁻¹), nitrite (0.07 ± 0.02 mg N – NO₂ L⁻¹), total ammonia (0.15 ± 0.05 mg N – NH₃ L⁻¹), alkalinity and total hardness (135.0 ± 12.3 and 50.0 ± 0.03 mg CaCO₃ L⁻¹, respectively). Exceptions were considered when temperature and DO levels were changed to meet the proposed treatments.

Sample collection

Tissue collection co-occurred for all treatments. Thus, the experimental design was planned considering the different periods required for reoxygenation (rapid or gradual) and the existence of a control group not submitted to hypoxia or reoxygenation.

Three fish from each tank (n = 9 per treatment at each time) were randomly selected for tissue sampling (gills and liver) at 1 and 12 h after recovery (Nitz et al. 2020b). Before tissue collection, the fish were euthanized by immersion in benzocaine hydrochloride (500 mg L⁻¹). Tissue samples were then stored in
2 mL microtubes, flash-frozen in liquid nitrogen, and then stored in an ultra-freezer (−80°C) until the analysis of the oxidative stress parameters.

Antioxidant and oxidative stress parameters

Samples of the gills and liver were homogenized (1:5; w/v) in a Tris–HCl (100 mM, pH set in 7.75) buffer with EDTA (2 mM) and Mg²⁺ (5 mM) (Da Rocha et al. 2009). Afterward, the homogenates were centrifuged for 20 min (10,000 × g, 4°C); the supernatant was used for tissue analysis. The total protein concentration of the homogenized samples was determined in a spectrophotometer using the Biuret method (550 nm) with a commercial kit.

The total antioxidant capacity against peroxyl radicals (ACAP) was determined according to Amado et al. (2009), where a higher relative area means a lower antioxidant capacity and vice versa. The data were expressed as the relative area. According to Oakes and Van Der Kraak (2003), the LPO levels were measured by determining the content of thiobarbituric acid substances (TBARS). The data are expressed as nmol of equivalents TMP mg wet tissue⁻¹. Protein thiol (PSH) content was based on the method of Sedlak and Lindsay (1968), where the data are expressed as nmol SH mg protein⁻¹.

Statistical analysis

All results are expressed as mean ± SEM. The Levene and Shapiro-Wilk tests, respectively, previously tested the normality and homogeneity of the data. After, two-way ANOVA (first factor: temperature; second factor: DO or reoxygenation) tests were performed for each time (1 or 12 h), followed by Tukey's pair-wise comparisons. The significance level was 5% in all cases (p < 0.05).

Results

No mortality occurred during the acclimation or experimental period.

Total antioxidant capacity against peroxyl radicals (ACAP)

In the fish kept in normoxia or subjected to rapid reoxygenation, at 1 h after recovery, the gills ACAP levels were significantly lower (higher relative area) at 18°C compared to the other temperatures. Furthermore, gills ACAP was significantly lower (higher relative area) at 28°C than at 23°C in fish exposed to rapid reoxygenation at 1 h after recovery (Fig. 1a).

At 12 h after recovery, gills ACAP levels in the juveniles exposed to normoxia or rapid reoxygenation were significantly lower (higher relative area) at 28°C compared to 23°C and 18°C and at 23°C compared to 18°C. In addition, at this same 12 h period, at 28°C, gills ACAP was significantly lower (higher relative area) for animals subjected to rapid than gradual reoxygenation (Fig. 1b).

Liver ACAP levels were significantly higher (lower relative area), in this order, for gradual reoxygenation (5 h) > rapid reoxygenation (0.5 h) > normoxia treatments at 18°C at 1 h after recovery. Moreover, in this
same 1 h period, liver ACAP was significantly higher (lower relative area) at 23°C than at 28°C and 18°C and 28°C than at 18°C in the fish maintained in normoxia or subjected to rapid reoxygenation. Furthermore, on gradual reoxygenation, liver ACAP was significantly lower (higher relative area) at 28°C than 18°C at 1 h after recovery (Fig. 1c).

Regardless of the DO level or reoxygenation rates, liver ACAP was significantly lower (higher relative area) at 28°C than at other temperatures at 12 h after recovery (Fig. 1d). In addition, in this same 12 h period, liver ACAP was significantly higher (lower relative area) on rapid reoxygenation compared to the other treatments at 28°C.

Lipid peroxidation (LPO)

The LPO levels in the gills were significantly lower in the interaction between rapid reoxygenation and 18°C compared to other treatments at this same temperature or for different temperatures (23°C and 28°C) at this same reoxygenation rate at 1 h after recovery. During this same period (1 h), LPO levels in the gills were significantly lower in fish kept at 23°C than in those maintained at 18°C for the control group or at 28°C for the group subjected to gradual reoxygenation (Fig. 2a).

In all treatments, LPO levels in the gills were significantly higher at 28°C than at 18°C at 12 h after recovery. They were also significantly higher in the interaction between rapid reoxygenation and 23°C than between gradual reoxygenation and 23°C or between rapid reoxygenation and 18°C (Fig. 2b).

Liver LPO levels were significantly higher in the following order: gradual reoxygenation (5 h) > rapid reoxygenation (0.5 h) > normoxia at 18°C at 1 h after recovery. At this same 1 h period, liver LPO levels in the control group were significantly higher at 23°C than at other temperatures. Additionally, in general, at 28°C, liver LPO levels were significantly lower than at other temperatures at 1 h after recovery (Fig. 2c). Finally, in all treatments, liver LPO levels were significantly higher at 23°C than at other temperatures at 12 h after recovery (Fig. 2d).

Protein thiol (PSH)

Gills PSH content was significantly higher in fish exposed to gradual reoxygenation compared to the control group (normoxia) at 23°C at 1 h after recovery. At this same period (1 h), gills PSH content was significantly lower at 18°C than at 23°C in all treatments. Additionally, in the control group, gills PSH content was significantly higher at 28°C than at 18°C at 1 h after recovery (Fig. 3a). No significant differences were found in gills PSH content at 12 h after recovery (Fig. 3b).

In general, the liver PSH content of fish subjected to gradual reoxygenation was significantly higher and lower than the other treatments, respectively, in the temperatures of 23°C and 28°C at 1 h after recovery. In this same treatment (gradual reoxygenation) for this same period (1 h), the liver PSH content was significantly higher at 23°C than at other temperatures. Moreover, the liver PSH content of fish subjected to rapid reoxygenation was significantly higher than to other treatments at 18°C at 1 h after recovery. For
this period (1 h), liver PSH content was significantly higher at 28°C than at 18°C in the control group (Fig. 3c).

In all treatments, the liver PSH content was significantly higher at 23°C than at 28°C at 12 h after recovery. In addition, in the 12 h period, liver PSH content of fish subjected to rapid reoxygenation was significantly higher at 23°C than at 18°C (Fig. 3d).

**Discussion**

Fluctuations in DO levels are common in natural and captive conditions with high freshwater fish stocking densities (Copatti et al. 2019; Pontin et al. 2020). For survival under these conditions, animals often need to be able to tolerate hypoxia and reoxygenation (Nitz et al. 2020b). When oxygen flow returns during reoxygenation, several tissues become shocked as toxicity increases, causing oxidation of biomolecules and overproduction of ROS (Lushchak et al. 2001; Giraud-Billoud et al. 2019) and consequent loss of function or physiological homeostasis. In our study, there was no mortality, demonstrating that pacu is resistant to hypoxia and reoxygenation (rapid or gradual), regardless of the temperature evaluated. Furthermore, the oxidative stress parameters showed that pacu copes well with rapid reoxygenation under lower temperature conditions. However, these parameters may take longer (12 h) to stabilize, which confirms our study hypothesis.

The antioxidant defense system is composed of different enzymatic and non-enzymatic components that act as detoxifying agents for peroxyl radicals, representing the general antioxidant status of organisms (Amado et al. 2009). Our study observed changes in ACAP levels at both reoxygenation rates and under different thermal conditions. For example, gills and liver ACAP content were higher at the two highest temperatures (23°C and 28°C) at 1 h after recovery. As oxidative stress commonly occurs soon after the first moments of recovery (Welker et al. 2013; Johannsson et al. 2018; Giraud-Billoud et al. 2019), this behavior of the antioxidant defense system was expected. An increase in ACAP at elevated temperatures is related to a physiological adjustment generated to intercept increased ROS production (Lushchak 2011; Zebral et al. 2016).

Furthermore, the reduction in ACAP levels at 18°C at 1 h after recovery found in the different treatments (except for gradual reoxygenation) in our study may have been a result of the use of antioxidant components to contain the increase in free radical production in a recent hypoxia setting (Clanton 2007). This scenario seems to have been avoided in the current study in both organs (gills and liver) under conditions of intermediate temperature (23°C) and rapid recovery (0.5 h), as well as in the liver in the interaction between 18°C and gradual reoxygenation (5 h). Similarly, an elevation in ACAP levels in pacu gills exposed to hypoxia at 23°C was verified by Nitz et al. (2020a).

In addition, at 12 h after recovery, gills and liver ACAP content were lower at 28°C, although ACAP had a different behavior (12 h) for the organs evaluated. While for the gills, there was an increase in ACAP content in the gradual recovery compared to the rapid recovery, the opposite occurred in the liver at this
same temperature (28°C). Such alterations must have been organ-specific. Due to different metabolic demands, other tissues have oxygen tensions (Johannsson et al. 2018) that can trigger different oxidative responses. One organ can compensate for the increase in oxidative stress in another organ.

Overall, ACAP can provide an understanding of the resistance of each organism to the toxicity caused by ROS (Amado et al. 2009). ROS plays a fundamental role in activating the defense system. The success of each species depends on its ability to activate mechanisms that will serve as a shield to deal with the period of DO deprivation. This success also depends on the animals efficiently removing ROS in the subsequent period when oxygen is reintroduced (Onukwufo et al. 2016). Our study shows a reestablishment of ACAP levels in fish exposed to 18°C at 12 h after recovery, indicating that ACAP changes were temporary at this temperature.

When reperfusion occurs, organs are oxygenated, and, in parallel, there is an increase in ROS generation with LPO induction, protein oxidation, and DNA damage (Hermes-Lima and Zenteno-Savin 2002). On the other hand, according to Johannsson et al. (2018), reoxygenation generally does not cause new oxidative damage in hypoxia-tolerant species such as pacu. In our study, the best conditions for reducing gills LPO levels 1 and 12 h after recovery were observed at 18°C in fish subjected to rapid reoxygenation and at 23°C for those exposed to gradual reoxygenation. In general, the increase in temperature caused an increase in LPO levels in the gills, which could cause oxidative damage in fish that underwent rapid reoxygenation at an intermediate temperature (23°C). However, this would be the best temperature for fish that underwent gradual reoxygenation, and oxidative damage to the gills would only be linked to the highest temperature (28°C). On the other hand, liver LPO levels were higher at 23°C at 1 and 12 h after recovery. Similarly, Lushchak et al. (2005) also observed increased lipid damage (TBARS) in the liver of common carp (Cyprinus carpio) 14 h after rapid reoxygenation (up to 30 min) in fish maintained under hypoxia for 5.5 h.

A reduction in water temperature could also increase oxidative stress due to reduced enzyme activity and membrane fluidity (Tattersall et al. 2012), increasing LPO levels (Rossi et al. 2017). However, when exposed to low temperatures, ectothermic organisms commonly develop strategies such as home viscous adaptation to maintain the fluidity of biological membranes and other adjustments such as increasing the degree of lipid unsaturation and accumulation of PUFAs (Bagnyukova et al. 2007). However, this higher concentration of PUFAs increases the risks of oxidative stress since these substances are primary targets of ROS, which act by removing a proton from the conjugated double bond system. This event creates a peroxyl radical, which triggers the onset of LPO reactions (Abele and Puntarulo 2004). Therefore, an LPO may have occurred in the liver of the pacu maintained at the lowest temperatures tested in the current study (mainly 23°C). In contrast, in a previous study with Cyphocharax abramoides, a hypoxia-tolerant species, no change in LPO was observed after 3 h of hypoxia, regardless of the reoxygenation rate (Johannsson et al. 2018).

In addition to fatty acids, proteins are also affected by the overproduction of ROS, resulting in carbonylation, aggregation, fragmentation, amino acid modification, change in electrical charge and
inactivation of membrane enzymes, receptors, and transport proteins, and oxidation of sulfhydryl groups
(Lushchak 2011; Madeira et al. 2013). Although the modification of proteins by free radicals is not as
critical as LPO, it could lead to the loss of their functions and (mainly) their ability to communicate intra-
and intercellularly (Lushchak et al. 2005; 2011).

In our study, a reduction in the gills PSH content of juveniles was observed at 18°C at 1 h after recovery.
However, this tissue had no changes in PSH content at 12 h after recovery. In the liver, there was an
increase in PSH values at an intermediate temperature (23°C), which could have occurred in a stressful
situation as a protective reaction for the cells. Under stress, changes in thiol content can occur to remove
harmful components that are readily replaced by a disulfide (or -SS disulfide bridges) through enzymatic
reduction (Dickinson and Forman 2002). In addition, in the current study, fish must have triggered
protective response mechanisms in the interaction between 23°C and gradual reoxygenation, as
evidenced by the increase in liver PSH content at 1 and 12 h after recovery.

**Conclusions**

Fish demonstrated the ability to tolerate hypoxia and reoxygenation through the changes in several
enzyme activities. They cope better when subjected to rapid reoxygenation (0.5 h) at low temperatures
(18°C). In general, after hypoxia, oxidative damage was lower at 12 h after recovery, suggesting that the
antioxidant system adjustments were sufficient for restoring homeostasis during this period (12 h) in
pacu juveniles.

**Declarations**

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**Author contribution**

Lilian F. Nitz: executing the experiment, performing oxidative stress analysis, and writing the manuscript.
Lucas Pellegrin and Daniel Pinto: collaborated on the literature review, manuscript discussion, and
oxidative stress analysis. José Monserrat: collaborated with the planning of the experiment, oxidative
stress analysis, and discussion. Carlos E. Copatti: performed the statistical analysis, manuscript review,
and final text. Luciano Garcia: planning and financial support to the experiment, performed figures,
participated in the discussion, thinking of the manuscript, and final text.
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Data Availability

The data supporting this study’s findings are available from the corresponding author upon reasonable request.

Ethics approval

The Animal Experimentation Committee approved this study of the Federal University of Rio Grande (FURG), Rio Grande, RS, Brazil, under process number P049/2017.

Consent to participate

Not applicable.

Consent for publication

Not applicable

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the study reported in this paper.

References


Figures
Figure 1

Total antioxidant capacity against peroxyl radicals (ACAP) (relative area) in gills at 1 (A) and 12 h (B) after recovery and in liver at 1 (C) and 12 h (D) after recovery of pacu juveniles (*Piaractus mesopotamicus*) exposed for interactions between low (∼18 °C), medium (∼23 °C) and high temperatures (∼28 °C) under normoxia (∼7 mg O$_2$ L$^{-1}$) or after hypoxia (∼2 mg O$_2$ L$^{-1}$) followed by rapid or gradual reoxygenation rates (0.5 and 5 h, respectively). Data are presented as mean ± SEM (n = 9 fish per treatment). Different uppercase letters indicate statistically significant differences between temperatures at the same dissolved oxygen level (p < 0.05). Different lowercase letters indicate statistically significant differences between dissolved oxygen levels at the same temperature (p < 0.05).
Figure 2

Lipid peroxidation (LPO) levels in gills at 1 (A) and 12 h (B) after recovery and in liver at 1 (C) and 12 h (D) after recovery of pacu juveniles (*Piaractus mesopotamicus*) exposed for interactions between low (~18 °C), medium (~23 °C) and high temperatures (~28 °C) under normoxia (~7 mg O\(_2\) L\(^{-1}\)) or after hypoxia (~2 mg O\(_2\) L\(^{-1}\)) followed by rapid or gradual reoxygenation rates (0.5 and 5 h, respectively). Data are presented as mean ± SEM (n = 9 fish per treatment). Different uppercase letters indicate statistically significant differences between temperatures at the same dissolved oxygen level (p < 0.05). Different lowercase letters indicate statistically significant differences between dissolved oxygen levels at the same temperature (p < 0.05).
Figure 3

Protein thiol (PSH) content in gills at 1 (A) and 12 h (B) after recovery and in liver at 1 (C) and 12 h (D) after recovery of pacu juveniles (*Piaractus mesopotamicus*) exposed for interactions between low (~18 °C), medium (~23 °C) and high temperatures (~28 °C) under normoxia (~7 mg O₂ L⁻¹) or after hypoxia (~2 mg O₂ L⁻¹) followed by rapid or gradual reoxygenation rates (0.5 and 5 h, respectively). Data are presented as mean ± SEM (n = 9 fish per treatment). Different uppercase letters indicate statistically significant differences between temperatures at the same dissolved oxygen level (p < 0.05). Different lowercase letters indicate statistically significant differences between dissolved oxygen levels at the same temperature (p < 0.05).