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# Fish injuries resulting from transient operating conditions in a Brazilian hydropower plant: morphological, physiological and biochemical evaluation in *Pimelodus maculatus* (Siluriformes: Pimelodidae)

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This study investigated injuries sustained by *Pimelodus maculatus* caused by transient operating conditions in a Brazilian hydroelectric power plant. Fish (n = 43) that inhabited the tailrace and appeared on the water surface after changes in the operating conditions were evaluated (IF). Additionally, fish (n = 10) captured using longline in the vicinity of the tailrace were used for comparisons (NF). Blood samples were evaluated for cortisol, glucose, and lactate concentrations. Tissue samples (gill, liver, brain, and muscle) were analyzed for oxidative stress biomarkers (TBARS, protein carbonyl content, and catalase activity) and histology (gill, liver, and spleen). The most prominent findings in IF were barotraumas with intra-abdominal hemorrhage and swim bladder rupture. Cortisol and glucose concentrations were lower for IF when compared to NF. IF also suffered from oxidative stress, as indicated by increased TBARS in the liver and brain, as well as increased protein carbonyl content in the gills and liver. The main histological alterations induced by the adverse conditions were circulatory disorders, such as telangiectasia and gill aneurysm, hepatic congested veins, and hemorrhage in the liver and spleen. The abrupt drop of pressure resulting from changes in the operating conditions of the turbines caused barotraumas and oxidative stress, which were the leading causes of fish mortality

Keywords: Barotrauma, Blood parameters, Hemorrhage, Oxidative stress, Uruguay River.

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Este estudo investigou os efeitos induzidos por condições transitórias de operação em uma usina hidrelétrica brasileira em *Pimelodus maculatus*. Peixes (n = 43) no canal de fuga que apareceram na superfície da água após mudanças nas condições operacionais foram avaliados. Adicionalmente, peixes (n = 10) capturados a jusante da barragem com espinhel foram utilizados para comparações (NF). Amostras de sangue foram avaliadas para as concentrações de cortisol, glicose e lactato. Amostras de tecidos (brânquia, fígado, cérebro e músculo) foram analisadas para biomarcadores de estresse oxidativo (TBARS, conteúdo de proteína carbonil e atividade da catalase) e histologia (brânquia, fígado e baço). Os achados mais proeminentes dos peixes afetados (IF) foram barotraumas com hemorragia intra-abdominal e ruptura da bexiga natatória. As concentrações de cortisol e glicose foram menores para IF quando comparadas aos NF. IF também sofreram estresse oxidativo representado pelo aumento de TBARS no fígado e cérebro, e aumento do conteúdo das proteínas carboniladas nas brânquias e fígado. As principais alterações histológicas induzidas pelas condições adversas foram distúrbios circulatórios, como telangiectasia e aneurisma nas brânquias, congestão das veias hepáticas, e hemorragia no fígado e baço. A queda abrupta da pressão resultante de mudanças nas condições operacionais das turbinas causou barotrauma e estresse oxidativo, que foram as principais causas da mortalidade dos peixes.

Palavras-chave: Barotrauma, Estresse oxidativo, Hemorragia, Parâmetros sanguíneos, Rio Uruguai.

## INTRODUCTION

Fish passage through turbines from upstream to downstream is considered one of the major causes of fish mortality at hydroelectric power plants (HPPs) in the Northern Hemisphere (Cada, 2001; Brown *et al*., 2014). In Brazilian dams, however, fish mortality related to HPP operation is mainly associated with fish movements from downstream to upstream where fish can enter the tailrace and draft tube during transient operating conditions of the turbine, such as shutdown, startup, and synchronous condenser states (Silva *et al*., 2018). In all cases, the leading causes of fish mortality and injuries are strike (mechanical shock against building structures, as well as runner blades), shear stress (friction between two water bodies at different velocities), cavitation (bubble formation that causes shock waves at high pressure), and abrupt pressure changes under the runner (Beirão *et al*., 2016; Carvalho, Marques, 2018).

Rapid decompression in the turbines causes the fish to suffer internal and external tissue injuries, known as barotrauma. Barotrauma includes various injuries such as hemorrhage, embolism, stomach eversion, exophthalmia, swim bladder, and other organ rupture (Algera *et al*., 2020).

When fish undergo external adverse conditions, such as passage through turbines or rapid decompression, physiological and biochemical responses are triggered, along with compensatory or adaptive mechanisms, in an attempt to overcome the stressor. For instance, the stress hormone cortisol triggers physiologic responses like increasing metabolic parameters, such as glucose and lactate, to recover fish homeostasis (Barton, Iwama, 1991; Trushenski *et al*., 2010). Also, fish can activate their antioxidant defense systems by producing catalase enzymes, to overcome oxidative cell damage, such as lipid and protein peroxidation, measured as TBARS (thiobarbituric acid reactive substances), and protein carbonylation, respectively (Velisek *et al*., 2011). Through histology, it is also possible to verify fish health because tissue changes express stress-induced conditions (Copper *et al*., 2018). Thus, evaluating blood parameters, oxidative tissue stress, and histology are tools for monitoring and understanding the health of downstream fish captured after abrupt decompression exposure at HPPs, as well as turbine-passed fish.

In Brazil, catfish comprise one of the groups most frequently found downstream during mortality episodes owing to transient operating conditions of HPPs (Silva *et al*., 2018). *Pimelodus maculatus* (Lacepède, 1803) is a yellow-mandi catfish widely distributed in several South American hydrographic basins, including the Uruguay River (Zaniboni Filho, Schulz, 2003), where Machadinho HPP is located. This is the fish species most frequently identified with lethal injuries after switching the turbine operating regime at this HPP, as in other dams in Brazil (Andrade *et al*., 2012).

Although several studies reported fish mortality at HPPs, the physiological and biochemical responses are unclear because they commonly focus on macroscopic lesions. Therefore, this study aimed to investigate the injuries of *P. maculatus* exposed to abrupt decompression inside the draft tube at Machadinho HPP by evaluating morphological, physiological, and biochemical parameters.

## MATERIAL AND METHODS

**Study area and fish sampling.** The Machadinho HPP is located on the upper Uruguay River (27°31'31"S and 51°47'07"W). It borders Santa Catarina and Rio Grande do Sul states in southern Brazil. It has three Francis turbines, each with a 380 MW output, totaling 1,140 MW of installed capacity. From September 2019 to June 2021, the tailrace was inspected 11 times during changes in the operating regimes (Tab. 1). The inspections consisted of observing fish that appeared on the water surface for approximately 20 min. When sighted floating with erratic swimming, *P. maculatus* individuals were sampled (n = 43) for immediate analysis. The species was also captured in the immediate vicinity of the tailrace (n = 10) using longline gear for comparative analyses. A voucher specimen was deposited in the Ichthyological Collection of the Universidade Estadual de Londrina, Brazil (MZUEL 9599).

For convenience, the term "Injured Fish (IF)" refers to fish collected on the water surface during tailrace inspections, whereas "Naïve Fish (NF)" refers to those captured using longline in the proximity of the tailrace.

**Sampling and analyses.** Fish were measured, weighed, and then anesthetized using 50 mg $L^{-1}$ eugenol. Blood was sampled from the caudal vein for glucose (Accu Cheek Active, Roche, Germany), lactate (Accutrend Plus, Roche, Germany), and cortisol analyses. A 3.0 mL syringe with anticoagulant (EDTA, 10%) was used, and the collected blood was stored in 2 mL sterile microtubes. Cortisol determination used plasma from centrifuged blood (2,500 x *g*, 15 min, 4 ºC), maintained frozen in liquid nitrogen

**TABLE 1** | Transient operating conditions of turbines at the Machadinho hydroelectric power plant (HPP) where catfish sustained injuries evaluated in this study.

| Transient operating condition | Description |
|---|---|
| Startup | The turbine runner goes from rest to nominal speed in seven min (slow startup scheme), the unit is synchronized to the grid, and the load is increased (load acceptance). |
| Synchronous condenser to generating mode | At the synchronous condenser mode, the turbine is operated in air conditions rotating at the synchronous speed driven by the generator acting as a motor. Depending on the energy demand, the turbine goes from synchronous condenser mode to generating mode. The scheme adopted by the Machadinho HPP to return to generating mode involves a sequence of shutdown and slow startup. |
| Shutdown | The turbine runner comes to stop after working as synchronous condenser, by opening the deaeration valve to expel air from the runner to the atmosphere and break the runner by friction with water under the runner. |

(-180 ºC) until analyses, and measured in duplicate using ELISA kits (Arbor Assays Cortisol Immunoassay, Arbor Assays, USA). After blood sampling, fish were euthanized with an overdose of the anesthetic (100 mg $L^{-1}$ eugenol) and submitted to necropsy. Necropsy consisted of external and internal macroscopic analysis.

Gill, liver, brain, and muscle samples were collected and stored in liquid nitrogen (-180 ºC) until required for biochemical analyses. Levels of the biomarkers thiobarbituric acid reactive substances (TBARS) and protein carbonyl, as well as catalase activity, were measured using the methods described by Reznick, Packer (1994), Buege, Aust (1978), and Nelson, Kiesow (1972), respectively with some modifications as detailed in Thiel *et al*. (2020). For IF, biochemical and hematological analysis were carried out with 30 randomly sampled fish.

Gill, liver, and spleen tissues sampled for histology were fixed in 10% buffered formalin solution for 24 h, washed in 70% alcohol, and stored in 70% alcohol until analysis. Next, tissues were dehydrated in a series of alcohol solutions (70, 80, 90, and 100%) and embedded in paraffin following a routine technique. Subsequently, 5 µm thick cross-sections were taken using a Leica RM2255 rotary microtome (Leica Microsystems, Germany). Then, each tissue was stained with hematoxylin and eosin using the automatic Leica AutoStainer XL (Leica Microsystems, Germany). Images were captured under a light microscope (Leica DM3000 LED, Leica Microsystems, Germany) with a connected camera and analyzed with Leica Application Suite 3.4.0 (Leica Microsystems, Switzerland).

**Statistical analyses.** Comparisons among groups were made by one-way ANOVA followed by Tukey-Kramer test after checking for homoscedasticity (Levene's test) and normality (Shapiro-Wilk test). Outliers were detected and removed from the analysis using the Tukey outlier detection test ($k$ = 2.1). The Kruskal-Wallis test was performed when assumptions were violated. The frequencies of lesions were compared between groups using Fisher's test. All analyses were performed using Statistica Software 7.0 (Stat Soft, USA) at a 0.05 significance level.

## RESULTS

*Pimelodus maculatus* sampled during inspections of operating regime shifts showed 26 types of injuries, and the percentage of occurrence varied with the operation performed (Tab. 2). Cortisol ($F_{1,39}$ = 31.4; $P$ < 0.05) and glucose ($F_{1,32}$ = 15.1; $P$ < 0.05) levels were lower in IF, while no changes were observed in lactate concentrations ($F_{1,33}$ = 2.2; $P$ > 0.05) (Tab. 3).

IF presented higher levels of TBARS in the liver ($F_{1,39}$ = 7.7; $P$ < 0.05) and brain ($F_{1,38}$ = 6.9; $P$ < 0.05), increased protein carbonyl content in the gill ($F_{1,33}$ = 371.3; $P$ < 0.05) and liver ($F_{1,38}$=5.4; $P$ < 0.05), and lower protein carbonyl content in the muscle ($F_{1,37}$ = 8.4; $P$ < 0.05). Catalase activity was reduced in the brain ($F_{1,34}$ = 20.1; $P$ < 0.05) of IF (Tab. 4).

**TABLE 2** | Types of injuries and their frequency of occurrence (%) as determined following necropsy of *Pimelodus maculatus* collected in the tailrace at the transient operating regimes of machine shutdown (n = 16), startup (n = 22), and synchronous condenser (SC) to generating mode (n = 5).

| Injury | Shutdown | Start-up | SC to generating mode |
|---|---|---|---|
| Branchial arch rupture | – | 4.5 | – |
| Swim bladder rupture | 93.8 | 90.0 | 80.0 |
| Intestine rupture | – | 4.5 | – |
| Spleen ruptured | – | – | 20.0 |
| Intra-abdominal hemorrhage | 68.8 | 72.7 | 100.0 |
| Branchial hemorrhage | 12.5 | 27.3 | 20.0 |
| Eye hemorrhage | 6.3 | – | – |
| Subcutaneous hemorrhage | 12.5 | 13.6 | – |
| Abdominal distension | 68.8 | 54.5 | 100.0 |
| Over-inflated swim bladder | 6.3 | 4.5 | 20.0 |
| Subcutaneous emphysema | 6.3 | 4.5 | – |
| Grazes/Laceration | 18.8 | 9.1 | – |
| Stomach eversion | 12.5 | 18.2 | 20.0 |
| Exophthalmia | 18.8 | 9.1 | 20.0 |
| Viscera extrusion through the urogenital opening | – | 4.5 | 60.0 |
| Eye loss | 6.3 | – | – |
| Venous embolism | 6.3 | 18.2 | – |
| Embolism in kidney | – | 13.6 | – |
| Hemorrhagic fins | 12.5 | 13.6 | – |
| Hemorrhagic kidney | – | 27.3 | – |
| Hemorrhagic liver | 12.5 | 22.7 | – |
| Hemorrhagic spleen | 43.8 | 22.7 | – |
| Opercular fracture | 6.3 | 4.5 | – |
| Spine fracture | – | 4.5 | – |
| Fins fracture | 6.3 | 4.5 | – |
| Head fracture | 12.5 | 18.2 | – |

**TABLE 3** | Hematological responses of *Pimelodus maculatus* captured using longline in the vicinity of the tailrace (NF) or collected in the tailrace during transient operating conditions (IF) of the Machadinho hydroelectric power plant (HPP). Data are reported as mean ± standard deviation. Different letters indicate significant differences ($P < 0.05$) among groups for each biochemical parameter.

| Blood parameter | NF | IF |
|---|---|---|
| Cortisol (ng mL$^{-1}$) | 39.9 ± 16.9$^{a}$ (n = 10) | 13.2 ± 11.4$^{b}$ (n = 30) |
| Glucose (mg dL$^{-1}$) | 86.1 ± 55.7$^{a}$ (n = 10) | 21.6 ± 11.5$^{b}$ (n = 23) |
| Lactate (mmol L$^{-1}$) | 8.2 ± 3.9$^{a}$ (n = 10) | 5.7 ± 4.6$^{a}$ (n = 24) |

**TABLE 4** | Biochemical responses in different tissues (gill, liver, brain, and muscle) of *Pimelodus maculatus* captured using longline in the vicinity of the tailrace (NF) or collected in the tailrace during transient operating conditions (IF) of the Machadinho hydroelectric power plant (HPP). Data are reported as mean ± standard deviation. Different letters indicate significant differences ($P < 0.05$) among groups for each parameter and tissue. TBARS = thiobarbituric acid reactive substances.

| Biochemical parameter | Fish group | Tissue | | | |
|---|---|---|---|---|---|
| | | Gill | Liver | Brain | Muscle |
| TBARS levels (µmol mg$^{-1}$ protein) | NF | 554.9 ± 36.7$^{a}$ (n = 10) | 147.3 ± 107.8$^{a}$ (n = 10) | 30.4 ± 19.2$^{a}$ (n = 10) | 3.7 ± 2.9$^{a}$ (n = 8) |
| | IF | 475.1 ± 331.9$^{a}$ (n = 26) | 630.5 ± 543.3$^{b}$ (n = 30) | 46.4 ± 15.7$^{b}$ (n = 30) | 3.9 ± 2.9$^{a}$ (n = 27) |
| Protein carbonyl content (µmol mg$^{-1}$ protein) | NF | 137.5 ± 19.8$^{a}$ (n = 9) | 127.6 ± 36.5$^{a}$ (n = 10) | 59.2 ± 48.9$^{a}$ (n = 10) | 32.6 ± 21.7$^{a}$ (n = 10) |
| | IF | 398.0 ± 38.5$^{b}$ (n = 25) | 168.1 ± 50.2$^{b}$ (n = 29) | 74.6 ± 46.9$^{a}$ (n = 30) | 18.6 ± 8.3$^{b}$ (n = 28) |
| Catalase activity (µmol min$^{-1}$ mg$^{-1}$ protein) | NF | 100.4 ± 74.3$^{a}$ (n = 10) | 334.2 ± 249.9$^{a}$ (n = 10) | 122.2 ± 54.9$^{a}$ (n = 10) | 31.9 ± 29.5$^{a}$ (n = 10) |
| | IF | 71.4 ± 59.1$^{a}$ (n = 30) | 488.2 ± 474.3$^{a}$ (n = 30) | 56.6 ± 29.5$^{b}$ (n = 30) | 35.7 ± 23.3$^{a}$ (n = 30) |

Four types of gill lesions were detected in histological analyses (Fig. 1). Epithelium detachment (Fig. 1B), telangiectasia (Fig. 1C), and interlamellar hyperplasia (Fig. 1D) were higher ($P < 0.05$) in IF (Tab. 5). Branchial aneurysm (Fig. 1A), telangiectasia (Fig. 1C), and interlamellar hyperplasia (Fig. 1D) were not found in NF. The presence of congested veins and hemorrhage were identified in the liver and spleen of IF, but it was only significant ($P < 0.05$) in congested veins at the liver (Tab. 6; Figs. 2A–B). Melanomacrophage centers were found in the liver and spleen of all fish and their presence was no different ($P > 0.05$) between IF and NF (Tab. 6; Figs. 2A, C). Mononuclear inflammatory infiltrates were higher ($P < 0.05$) in NF spleen (Tab. 6: Fig. 2D). They were not found in the liver of NF or IF.

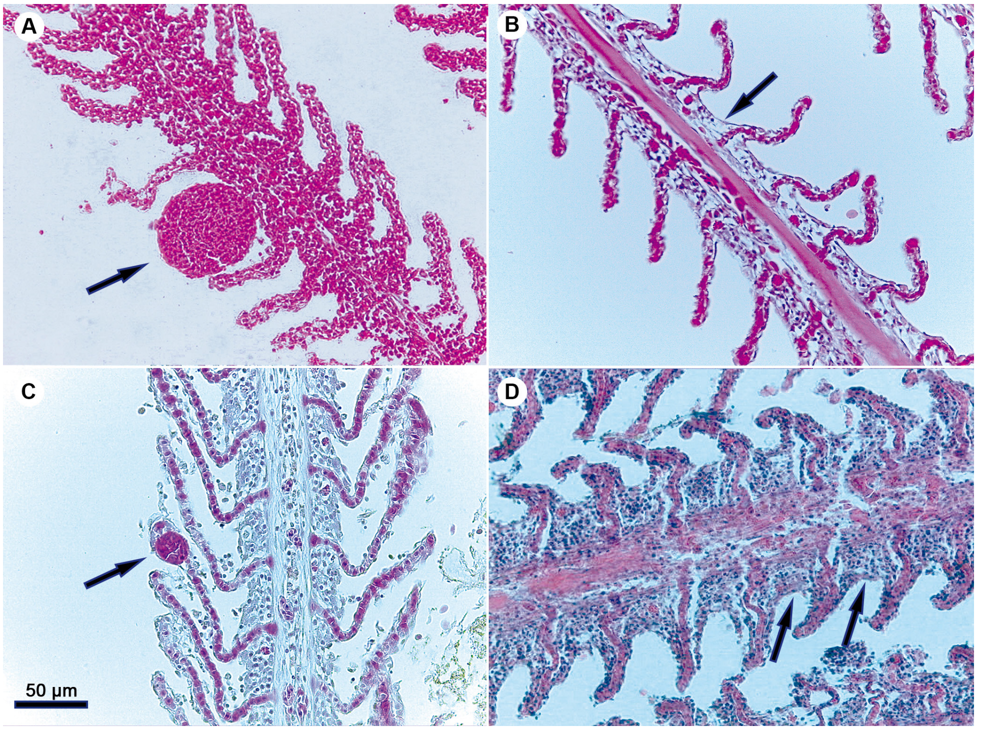


**FIGURE 1** | Histological gill alterations in *Pimelodus maculatus* collected during transient operating conditions (magnification: × 400). Arrows indicate the following lesions: **A.** Aneurysm; **B.** Epithelium detachment; **C.** Telangiectasia; and **D.** Interlamellar hyperplasia.

**TABLE 5** | Frequency of occurrence (%) of histological gill lesions found for *Pimelodus maculatus* captured using longline in the vicinity of the tailrace (NF) or collected in the tailrace during transient operating conditions (IF) of the Machadinho hydroelectric power plant (HPP). Different letters indicate significant differences ($P < 0.05$) among groups for each lesion.

| Fish group | Aneurysm | Epithelium detachment | Telangiectasia | Interlamellar hyperplasia |
|---|---|---|---|---|
| NF | 0.0[a] | 20.0[a] | 0.0[a] | 0.0[a] |
| IF | 28.1[a] | 71.9[b] | 46.9[b] | 87.5[b] |

**TABLE 6** | Frequency of occurrence (%) of histological liver and spleen lesions found in *Pimelodus maculatus* captured using longline in the vicinity of the tailrace (NF) or collected in the tailrace during transient operating conditions (IF) of the Machadinho hydroelectric power plant (HPP). Different letters indicate significant differences ($P < 0.05$) among groups for each lesion.

| Tissue | Fish group | Melanomacrophage Centers | Mononuclear inflammatory infiltrate | Congested veins | Hemorrhage |
|---|---|---|---|---|---|
| Liver | NF | 100.0[a] | 0.0[a] | 0.0[a] | 0.0[a] |
| | IF | 100.0[a] | 0.0[a] | 70.3[b] | 24.3[a] |
| Spleen | NF | 100.0[a] | 100.0[a] | 0.0[a] | 0.0[a] |
| | IF | 100.0[a] | 64.5[b] | 29.0[a] | 12.9[a] |

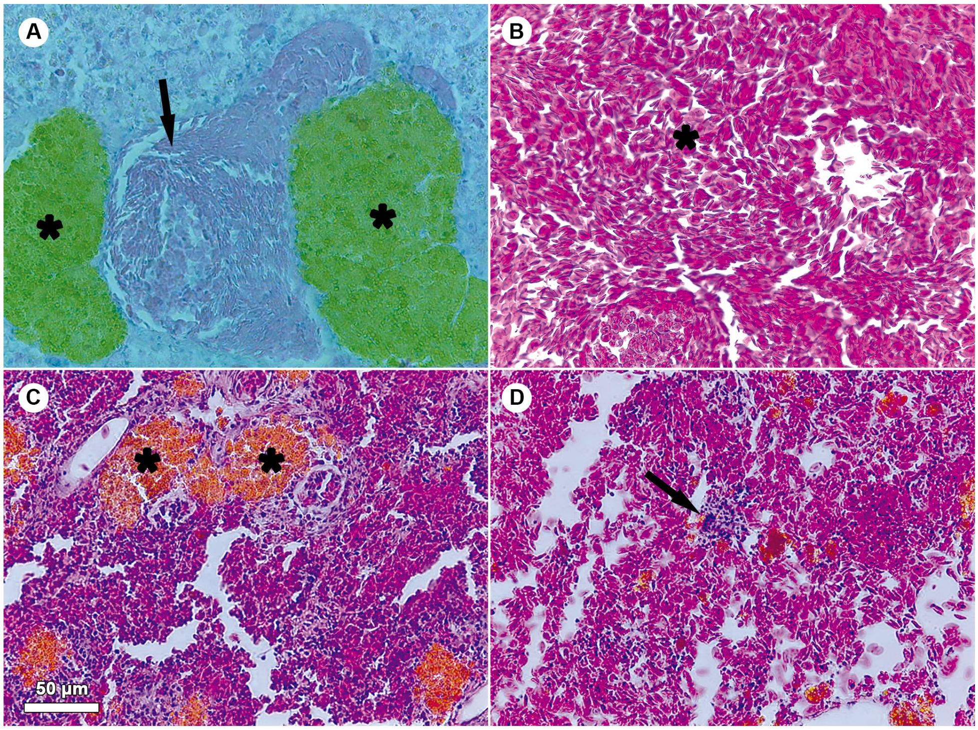


**FIGURE 2** | Histological alterations in liver and spleen of *Pimelodus maculatus* collected during transient operating conditions in the tailrace of Machadinho HPP (magnification: × 400). Arrows and asterisks indicate the following lesions: **A.** Melanomacrophage centers (asterisk) and congested vein (arrow) in the liver; **B.** Blood cells indicating hepatic hemorrhage (asterisk); **C.** Melanomacrophage centers in the spleen (asterisk); **D.** Mononuclear inflammatory infiltrate in the spleen (arrow).

## DISCUSSION

Hydrostatic pressure variation on the turbine runner during transient operating conditions causes fish to decompress and, consequently, suffer from barotrauma (Beirão *et al*., 2016). This is the same kind of pressure drop experienced by turbine-passed fish during steady state operating conditions, typical of partial load or full load operation at hydropower plants worldwide (Cada *et al*., 2006; Brown *et al*., 2014).

*Pimelodus maculatus* collected in the tailrace after changing the operating condition were found with various barotraumas, such as swim bladder rupture, hemorrhage, embolism, stomach eversion, and exophthalmia. Bone fractures as result of mechanical shocks were also observed, as described for other species (Brown *et al*., 2009; Algera *et al*., 2020).

The swim bladder controls fish buoyancy in the water column, acting as a gas reservoir, and its retention capacity depends on fish morphology (Stephenson *et al*., 2010; Pflugrath *et al*., 2012). *Pimelodus maculatus* is a benthic and physoclistous species that exchanges gas through the circulatory system. It often maintains negative buoyancy by retaining a little air in the swim bladder (Silva *et al*., 2018). Therefore, when this fish undergoes rapid decompression, the dissolved gases boil (Henry's Law) and expand (Boyle's Law), causing the swim bladder to overinflate and burst (Brown *et al*., 2009), a condition that affected 90% of the IF evaluated in this study.

Hemorrhage is a frequent finding that results from gas embolism, *i.e.*, bubble formation in blood vessels. It increases blood volume and reduces blood density, causing organ rupture and blood loss, leading to death (Brown *et al*., 2012, 2014). More than 70% of IF presented widespread bleeding, which extended to the peritoneal cavity, liver, spleen, kidney, gills, eyes, skin, and fins.

Adaptive stress responses, classified as primary and secondary, follow a pattern in most fish, and several of these responses can be analyzed through blood (Wandelaar-Bonga, 1997; George *et al*., 2013). Among the primary responses, cortisol is the most important corticosteroid hormone produced during stressor events. The hypothalamic-pituitary-interrenal axis controls the release of cortisol that acts mainly on the gills, intestine, and liver, regulating metabolic energy and hydromineral balance (Barton, Iwama, 1991). Secondary responses are triggered by primary responses and are mainly metabolic in origin. The release of glucose from the liver by glycogenolysis, known as hyperglycemia, is a metabolic response mediated by catecholamines, acting as a substrate for energy production. Also, in anaerobic situations, lactate is released as an energy source for cellular metabolism (Olsen *et al*., 1995; Polakof *et al*., 2012).

The reference values of blood stress parameters are unknown for fish exposed to pressure drops. However, it is possible to infer those values from fish farming studies. Barcellos *et al.* (2003), for example, have found increased cortisol (seven times) and glycose (three times) levels in the juvenile silver catfish *Rhamdia quelen* (Quoy & Gaimard, 1834) after harvesting. In juvenile cobia *Rachycentron canadum* (Linnaeus, 1766) exposed to air, cortisol, glucose, and lactate have increased six, three, and eight times, respectively (Trushenski *et al*., 2010).

The cortisol and glucose concentrations in fish under stress rise to establish homeostasis (Iversen *et al*., 2009). Accordingly, we were expecting higher cortisol and glucose levels in IF compared to those in NF owing to the severity and irreversibility of the trauma herein described. Notably, even fishing is a stressor able to drive both stress indicators away from the baseline in NF. However, systemic response did not occur in IF, possibly because of hemorrhage. Typically, cortisol travels through the bloodstream signaling the onset of a stressful situation (Wandelaar-Bonga, 1997). However, the severe bleeding found in most IF (70%) blocked the activation of physiological responses that would otherwise have increased cortisol and glucose levels. Therefore, in the present study, neither cortisol nor glucose was suitable as a stress marker in the assessment of fish exposed to pressure drop.

To the best of our knowledge, the present study is the first to perform biochemical analyses of oxidative stress parameters on fish affected by HPP operation, a common approach in ecotoxicological studies (Parvez, Raisuddin, 2005; Menezes *et al*., 2011; Toni *et al*., 2011). Oxidative stress is the imbalance between the generation of oxidant compounds and the action of antioxidant defense systems. A deficiency in the antioxidant system or insufficient ability to repair oxidative damage can increase reactive oxygen species and cause cell death (Barbosa *et al*., 2010; Halliwell, Gutteridge, 2015).

Lipid peroxidation, measured as TBARS has been reported as the one of the main factors responsible for the loss of cellular functions under oxidative stress conditions (Almroth *et al*., 2005; Barata *et al*., 2005; Menezes *et al*., 2011). Lipid peroxidation acts as a mechanism of cell injury caused by oxygen free radicals in biological membranes. These reactions produce lipid hydroperoxides which break down the double bond of unsaturated fatty acids and damage the cell membrane (Barata *et al*., 2005). The formation of lipid hydroperoxides can, in turn, further oxidize proteins, causing an excessive formation of carbonylated proteins, a biomarker for protein oxidation assessment (Lackner, 1998; Almroth *et al*., 2005).

TBARS levels have been reported to increase in the gills, liver, and muscle of lambari *Astyanax jacuhiensis* (Cope, 1894) (= *A. lacustris* (Lütken, 1875)) in the presence of five pesticides in the water (Loro *et al*., 2015). Also, the increased levels of TBARS in the gill, liver, and kidney previously demonstrated that *Prochilodus lineatus* (Valenciennes, 1837) is living under stressful field conditions in the Salado River basin (Santa Fé, Argentina) (Cazenave *et al*., 2009). Similarly, increased TBARS levels in the liver (328%) and brain (52%) of IF in the present study showed that changes in the operating conditions of HPP turbines also caused cellular damage to *P. maculatus* tissues as a direct result of stress and trauma.

IF also showed increased protein carbonyl levels in the gills (190%) and liver (32%). Similarly, increased protein carbonyl content was reported in the gill (200%), liver (111%), and kidney (190%) of *Channa punctata* (Bloch, 1793) exposed to the pesticide deltamethrin (Parvez, Raisuddin, 2005), as well as the liver of silver catfish exposed to herbicides (Ferreira *et al*., 2010; Menezes *et al*., 2011). Unexpectedly, IF muscle showed a reduction of protein carbonyl content (44%). In this case, exhaustion caused by the attempt to escape from the longline could explain the higher oxidative stress in NF muscle compared to that in IF. Protein carbonylation is induced by reactive oxygen species (ROS), which are produced in low amount during muscle contractility (Dalle-Donne *et al*., 2003). Under strenuous activity, however, ROS accumulate in the muscle, increasing oxidative stress (Duberstein *et al*., 2009).

Organisms have developed antioxidant defense systems to minimize oxidative damage to cellular components, as determined through the activity of antioxidant enzymes, such as catalase (Barata *et al*., 2005; Monteiro *et al*., 2006). Catalase is an enzyme that belongs to the first line of antioxidant defense and converts hydrogen peroxide into water and oxygen (Halliwell, Gutteridge, 2015). Catalase and other antioxidant enzymes can increase, decrease, or remain unchanged, depending on the intensity and duration of the stressor event, or even vary according to fish species (Ballesteros *et al*., 2009).

Catalase activity was reported to decreased in the liver of *R. quelen* infected with *Aeromonas hydrofila*. This was attributed to the low capacity of this species to neutralize the hydrogen peroxide produced by high TBARS, contributing, in turn, to the oxidative liver damage (Baldissera *et al*., 2017). A similar trend in these biomarker levels was observed in the brain of IF, where high TBARS levels occurred, along with a decrease in catalase activity, indicating oxidative brain damage.

The brain has very low antioxidant defenses compared to other tissues, *e.g*., the liver (Halliewell, Gutteridge, 2015). In addition, the brain is characterized by high oxygen consumption and lipid-rich content; therefore, it is highly susceptible to oxidative stress. In IF, decreased catalase activity and increased TBARS in the brain indicate the induction of oxidative damage and further indicate that such damage may be related to the severity of the lesions. It is postulated that catalase levels are low in all brain regions compared to the liver. Owing to the intrinsic characteristics of the central nervous system and its metabolism, several antioxidant defense systems are, however, present in the brain, making it more susceptible to damage caused by oxidant species (Halliwell, Gutteridge, 2015; Streck *et al*., 2013).

Two fish species, *Oreochromis niloticus* (Linnaeus, 1758) and *Clarias gariepinus* (Burchell, 1822), reported to be chronically exposed to metal concentrations in water and sediments, showed marked increases in catalase activity and TBARS levels in their liver and gills (Moussa *et al*., 2022). Since these species have antioxidant defense capacity against different stressors, such findings suggest these organs are good indicators of pollution. In the present study, our analyses showed an increase in TBARS and protein carbonyl content in the liver, but no change in catalase activity. Perhaps the time required for an increase in catalase expression in this tissue was not sufficient to generate antioxidant responses to trauma. Thus, our data provide new information on the use of oxidative markers in IF, mainly catalase activity, TBARS levels and protein carbonyl content in the different organs analyzed.

For *P. maculatus*, no changes in catalase activity were observed in the liver and gills, even with an increase in TBARS levels and protein carbonyl in the liver. The lack of antioxidant enzyme response contributes to more substantial lipid and protein oxidation and, consequently, more severe oxidative damage (Toni *et al*., 2011). Toni *et al*. (2011) also did not observe changes in catalytic activity of in *Cyprinus carpio* (Linnaeus, 1758) exposed to tebuconazol, even with the increased levels of TBARS and protein carbonyl content.

Histology is a widely used tool to determine fish health and physical condition because tissue changes express conditions induced by stress, pathogens, inadequate nutrition, improper water quality, or injuries suffered (Copper *et al*., 2018). However, this approach is rarely applied in investigating the possible causes of fish mortality at HPPs (Brown *et al*., 2009). Our evaluation of IF histology revealed several lesions related to circulatory disorders, such as aneurysm and telangiectasia in the gills, congested veins in the liver, and hemorrhage in the liver and spleen.

Aneurysms and telangiectasia in the gill are related to physical or chemical damage, usually linked to trauma, intense handling, or chemical contaminants (Flores-Lopes, Thomaz, 2011). These circulatory injuries are associated with an increase in blood flow increase owing the dilatation of blood capillaries, starting with blood congesting the extremity of the secondary lamellae (telangiectasia) and then developing into an aneurysm, congesting all lamellae (Strzyżewska-Worotyńska *et al*., 2017). In most cases, these are irreversible lesions, and when many lamellae are affected, the respiratory capacity of fish is compromised (Roberts, 2002). Among the hemorrhagic findings from the necropsy of the injured fish, both aneurysm and telangiectasia resulted from abrupt hydrostatic pressure variation.

Congested veins are indicative of blockages in blood passage from the hepatic artery and spleen to the central vein, requiring greater effort from the heart to pump blood (Kostić *et al*., 2017). However, because of abdominal and local bleeding in organs like the liver and spleen, even greater pumping could not supply the circulatory loss in severe hemorrhage.

The liver and spleen of NF and IF presented melanomacrophage centers, which are typically located in the hematopoietic tissues of these organs, as well as kidneys (Wolke, 1992). Therefore, it is implausible that they were associated with the injuries caused by changes in the operating conditions. The melanomacrophage centers are associated with inflammatory processes, metabolizing toxic compounds by phagocytosis, and iron cycling; accordingly, they have been considered suitable water quality biomarkers of environmental pollution (Agius, Roberts, 2003; Sayed, Younes, 2017). Some studies have related the higher incidence of melanomacrophage centers in *P. maculatus* liver to water contaminants (Brito *et al*., 2012; Paulino *et al*., 2014).

Mononuclear inflammatory infiltrates, which include first-line defense cells such as macrophages, lymphocytes, and plasmocytes (Manrique *et al*., 2017), were observed in the spleen of most fish evaluated, indicating that changes in the operating conditions did not trigger the inflammatory process. Araújo *et al*. (2019) have also found mononuclear inflammatory infiltrates in the liver of *P. maculatus* collected in the Funil's HPP (Rio de Janeiro, Brazil), suggesting that these infiltrates likely result from sublethal concentrations of pollutants.

Morphological changes were also found in IF gills, such as interlamellar hyperplasia and detachment of the lamellar epithelium. Excessive cell proliferation, such as that which occurs in hyperplasia, is an adaptive response that increases the distance between the external environment and the blood, reducing the contact surface area with water. As this process intensifies, the diffusion of respiratory gases reduces causing hypoxia and death (Mallatt, 1985; Sepici-Dinçel *et al*., 2009). The detachment of the lamellar epithelium can lead to osmoregulatory disorders, also caused by the difficulty in carrying out gas exchange (Pereira *et al*., 2014).

*Pimelodus maculatus* inhabiting the tailrace when exposed to the transient operating conditions showed barotrauma as the main cause of mortality, with swim bladder rupture and hemorrhage being the most recurrent traumas. Diffuse hemorrhage was the most critical effect because it caused circulatory disorders that promoted gill lesions, such as aneurysms. Furthermore, liver and spleen lesions, such as congested veins and blood circulation blockage, inhibited the activation of hormonal cascade essential for fish recovery and oxygen transport. In addition, lipid and protein oxidative damage to all analyzed tissues probably prevented the synthesis of antioxidant enzymes that would have otherwise helped in fish recovery, thus causing irreversible cell damage.

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**Janaína dos Santos Pedron:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing-original draft, Writing-review and editing.
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**Alexandra Latini:** Conceptualization, Methodology, Validation, Writing-review and editing.
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## ETHICAL STATEMENT

Collecting permits were issued by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) through license number 70928-1 and subsequent renovations. All procedures complied with the Ethics Committee on the Use of Animals from the Universidade Federal de Santa Catarina (protocol number 7124290719).

## COMPETING INTERESTS

The author declares no competing interests.

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