ECOTOX BRASIL
SOCIEDADE BRASILEIRA DE ECOTOXICOLOGIA

Ecotoxicol. Environ. Contam.,v. 17, n. 2, 2022, 99-114
doi: 10.5132/eec.2022.02.11

EEC

Original Article

How Can Different Land Uses Impact Aquatic Organisms? An Evaluation of Metabolic Alterations During Embryonic Development of Freshwater Fish, *Rhamdia quelen*

Camila Maria Toigo de Oliveira Piffero[1], Silvia Romão[2], Luisa Helena Cazarolli[2], Cristian Zwetzch do Nascimento[3], Thaís Maylin Sobjak[4]; Lucélia Donatti[5]; Maria Rosa Dmengeon[5]; Ana Tereza Bittencourt Guimarães[1,6]*

[1] Conservation and Management of Natural Resources Postgraduate Program, Universidade Estadual do Oeste do Paraná, Cascavel, Paraná, Brazil.

[2] Graduate Program in Agroecology and Sustainable Development, Universidade Federal da Fronteira Sul. Laranjeiras do Sul, Paraná, Brazil.

[3] Fingerling production unit, Cooperativa Agroindustrial Consolata, Nova Aurora, Paraná, Brazil.

[4] Department of Morphology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

[5] Adaptive Biology Laboratory, Department of Cell Biology, Universidade Federal do Parana, Curitiba, Parana, Brazil.

[6] Biosciences and Health Postgraduate Program, Universidade Estadual do Oeste do Paraná, Cascavel, Paraná, Brazil. .

Received September 29, 2022; Accept December 9, 2022

Abstract

Chemical compounds used in agricultural activities are constantly leaching to water bodies where they can potentially cause damage to non-target organisms. In this study, we attempt to understand how water from different localities with distinct land uses may alter the metabolism and neurotoxicity during the development of fish. Embryonic development of *Rhamdia quelen* was used as a quality indicator of two different aquatic environments, a stream considered impacted (IMP) and another as reference (REF) in south of Brazil. The eggs were exposed to water collected from the reference and impacted streams during the initial period of development (Period 1 - 12, 24h) and, during the larval development (Period 2 - 48, 72h). During each period, the samples were evaluated regarding the area of eggs and larvae, the energy metabolism (hexokinase – HK, phosphofructokinase – PFK, lactate dehydrogenase – LDH, citrate synthase – CS, malate dehydrogenase – MDH), the antioxidant defense system (catalase – CAT, glutathione peroxidase – GPx, glutathione reductase – GR, glutathione S-transferase – GST), oxidative stress (lipid peroxidation - LPO) and cholinergic system (cholinesterase – ChE). The total areas of larvae developing in the waters from the IMP river were smaller than REF larvae. Changes in the activity of energy metabolism enzymes were observed in animals exposed to the impacted stream water, with induction of HK, PFK, LDH, and MDH, and inhibition of CS during the initial developmental period, and during the larval stage was detected the inhibition of HK, CS, LDH and MDH activities. The antioxidant defense system (CAT, GPX, GR, GST) also presented changes in enzymes activity in both periods, despite the absence of difference in lipid peroxidation between the groups. The cholinesterase enzyme showed inhibition in activity. It is noteworthy the significant change in the physiology of animals in larval phase, with the formation of the opercular opening and, therefore, a greater contact with the external environment. The constant use of these compounds can trigger harmful effects to fishes in the early stages of development and alter the body's metabolic and defense enzymatic activities.

Keywords: agriculture; antioxidant system; energetic metabolism; silver catfish; early life stage; xenobiotics.

*Corresponding author: anatbguimaraes@gmail.com

INTRODUCTION

In Brazil, it is possible to notice a heterogeneous scenario concerning landscapes, with the constant conversion of forests into agricultural areas. This change was intensified between 1985 and 2017 when 38% of the Brazilian territory was modified from native forests to cattle ranching and agriculture activities (Souza *et al.* 2020). Recently, agriculture occupies 31% of the national territory with large-scale production (Mapbiomas 2021; Souza *et al.* 2020), resulting in negative impacts on erosion control, water flow regulation, a decline in habitat quality, carbon storage, and pollination (Gomes *et al.* 2021).

The productivity success in the agricultural sector is accompanied by the increased use of fertilizers and pesticides (Wu *et al.* 2017; Zhuo *et al.* 2018). These chemical compounds are deposited into the soil and then leach out, reaching water bodies where they cause negative effects, such as cellular damage and even genotoxic effects in non-target aquatic organisms, causing a decrease in fitness and its populational decline (Grivennikova and Vinogradov 2006; Valko *et al.* 2006; De Castro Marcato *et al.* 2017).

The Lower Iguaçu River basin is located in the State of Paraná, in a region composed of two opposing physiographic scenarios. One is recognized as one of the most agriculturally productive areas of Brazil, being a reference in agricultural production for soybean, corn, and wheat (SEAB 2021). On the other hand, this hydrographic basin in southern Brazil presents a wide and exuberant Atlantic Forest conservation area, the Iguaçu National Park, where the Iguaçu Falls are located. This conserved area of the physiography has sustained the high endemism rate of its aquatic and terrestrial fauna (Ghisi *et al.,* 2020). Considering the importance of the endemic fauna of the Iguaçu River basin, ecological studies to understand the animal health are crucial in this context of landscape changes.

In the Lower Iguaçu River basin is found the *Rhamdia quelen* fish, which has been considered a useful bioindicator because it is an abundant native species with wide geographical distribution in South America, and promising in the consumer market (Carneiro *et al.* 2002; Zaniboni-Filho 2004). Adults and juveniles of *R. quelen* have been used as bioindicators of ecotoxicological assays (Pimpão *et al.*, 2012; Baldissera *et al.* 2021; Marins *et al.* 2021), and in recent decades fish eggs and embryos have received more attention from the scientific community as an alternative replacement for the acute fish test and to understand the xenobiotic impact on embryonic development (Pimpão *et al.*, 2012, Gomes *et al*., 2021).

The embryonic stage of life is a critical period of development when the organism is more susceptible to the effects of the chemicals (Malafaia *et al.*, 2020). Therefore, exposure of organisms during earlier stages of development to environmental contaminants may significantly increase the understanding of diseases affecting subsequent stages of life (Gomes *et al.,* 2021). Researchers have demonstrated the effects of exposing *R. quelen* embryos and juveniles to certain insecticides, as for example the inhibition of acetylcholinesterase activity in the brain, the redox imbalance in different tissues (Marins *et al.,* 2021), and the occurrence of morphological deformities (Azevedo-Linhares *et al.,* 2018). These disorders caused by xenobiotics in the earlier development stages can promote the decline of a species and an imbalance in ecological relations (Modesto *et al*., 2018).

This study attempts to understand how the change in land use and water quality of the streams in the region can affect the metabolism during embryonic development of native species. Therefore, the aim was to evaluate the metabolic and neurotoxicity effects of the *R. quelen* embryos exposure to the waters of two streams with different land uses, one of them located in a Conservation Unit (Iguaçu National Park), considered a minimally impacted environment and, the other stream in a scenario surrounded by monoculture plantations and considered to have a high potential for environmental impact.

METHODOLOGY

Study Area

The study was carried out in the western region of Paraná in two third-order streams of the Hydrographic Unit for Water Resources Management Lower Iguaçu (HUWRM). The geographic locations where water samples were collected presented different environmental characteristics. The Manoel Gomes River (25°09.723’ S, 53°49.768’ W) was considered as reference (REF) because it is in the Iguaçu National Park (INP), the largest conservation unit in the Interior Atlantic Forest domain. In the upstream portion of the reference point there is a total of 2,276.698 ha, being 97,13% of the total represented by forest, 1,92% by urban area, 0,83% by farming area and 0,13% by roads (Global Forest Watch 2021).

The Tormenta River (25°28.59’S, 53°22’ W, Fig. 1) runs through a large rural área. In the upstream portion of the basin there is a total area of 9,923.319 ha, being 71,72% represented by farming area, 26% by forest, 2,1% by silviculture 0,08% by buildings and 0,11% by other categories (e.g. dams and roads), thus the large majority can be considered part of the agricultural activities (Global Forest Watch, 2021). This stream was considered impacted (IMP) due to the characteristics of soil use in its micro basin, as well as in its sediments with 6.32 ppb of organophosphates (Nimet *et al*. 2017).

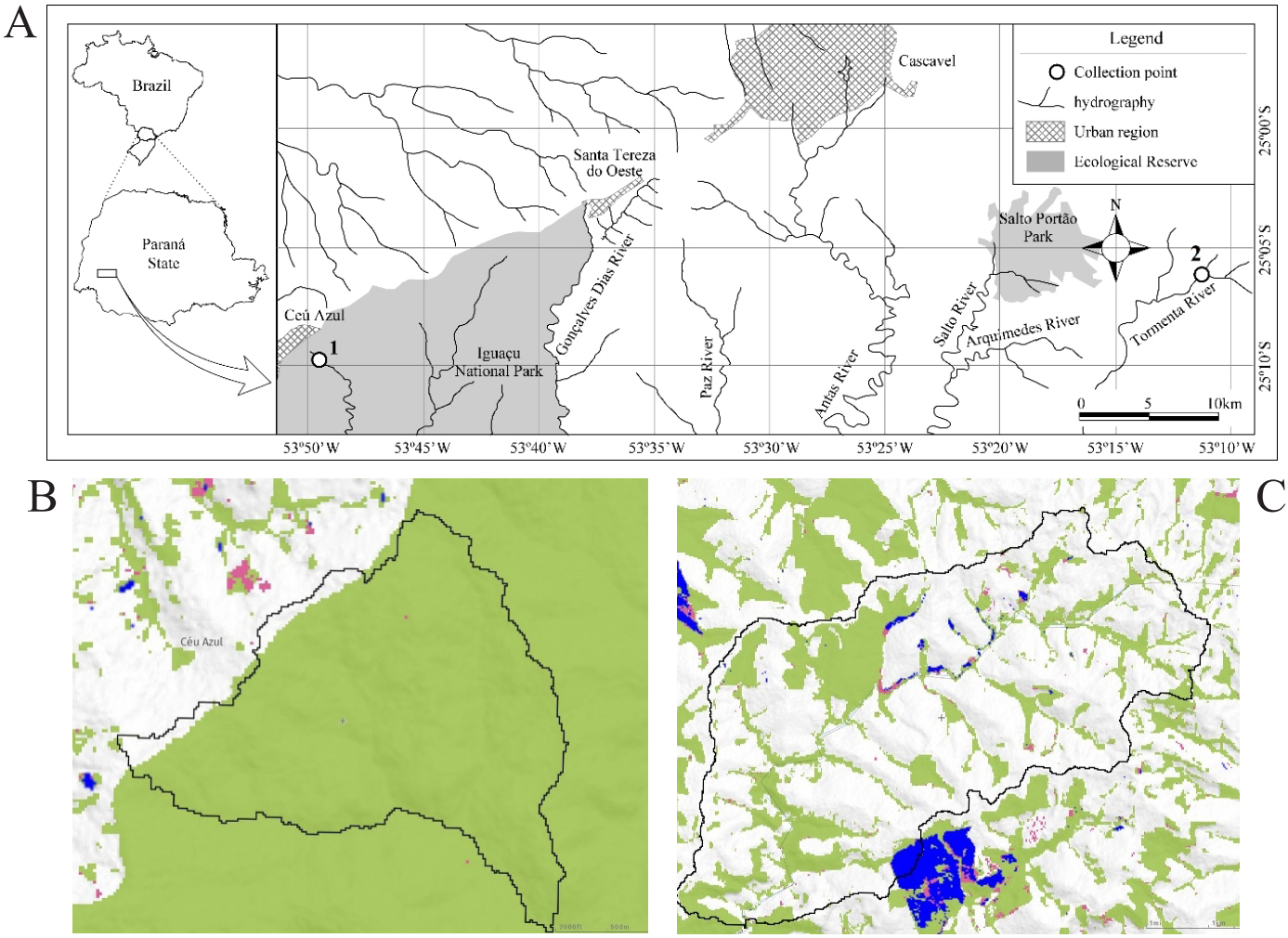

Figure 1 – A) Map of the study area with the location of Manoel Gomes River, in Céu Azul (Paraná, Brazil) (1), considered as reference (REF), and Tormenta River, in Cascavel (Paraná, Brazil) (2), considered as impacted (IMP). The highlight at the points indicates the studied area of the watershed. B) Vegetation cover (Seasonal Semideciduous Forest) present in the Manoel Gomes River basin (REF). C) Vegetation cover (Araucaria Forest) in the Tormenta River basin (IMP) - Global Forest Watch (2021).

Three sediment samples were collected from each of the places for chromatographic analyses, to assess contamination by pesticides following the methodology proposed by Fernandes *et al.* (2013).

The water samples (n=9) were collected in March 2018 (summer), always in the morning (8 – 10 p.m.) and from random points across a 25m section of the rivers, to evaluate both physical (temperature) and chemical (dissolved oxygen, pH, turbidity, ammonia and nitrite) aspects of the local waters. After that, 120 L of water samples were transferred to the Pathology Laboratory at Universidade Federal da Fronteira Sul – UFFS, and filtered in 300 mesh size (47 mm) to remove zooplankton and other debris, then stored in reservoirs for two days with constant aeration before the start of the experiment.

Experimental design

The *R. quelen* oocytes fertilization procedure was undertaken according to Pereira *et al.* (2006). Reproducers were acquired from an organic farming fish farm, in the municipality of Laranjeiras do Sul, Parana State, during the reproductive period of this species (Gomes *et al.* 2000). The collected fish were maintained in 100 L tanks for 24h with constant aeration at room temperature. The hormonal induction procedures using carp pituitary extract (CPE – male: 2.5 mg CPE kg $^{-1}$; female: 1 mg CPE kg^{-1}) for spawning and subsequent extrusion of oocytes were undertaken according to Woynarovich (1983). Oocytes and sperm were chosen from only one female and one male to ensure the homogeneity of the offspring from just one couple.

After extrusion of the oocytes, sperm were added at an average ratio of 89,000 sperm to one oocyte for fertilization. After that, 2 mL of eggs (~250 eggs) were distributed in polyethylene containers with 450 mL of water collected from either the REF or IMP streams, with six containers for each

group prepared for each development period (12, 24, 48 and, 72h). The experiment was semi-static, with total water renewal from the respective streams every 24 hours and constant aeration. The variables temperature, pH, Nitrate (NO^{3-}), Nitrite (NO^{2-}), and Ammonia (NH_3) were evaluated every 6 hours and controlled as recommended by Gomes *et al.* (2000).

The biological material samples were collected at each developmental time point, being calculated the percentage of living organisms (% viability) and morphometric analysis.

Biometric analyses

For these biometric analyses, eggs and larvae samples fixed (2 hours) in 10% formalin and preserved in alcohol 70° were placed on a slide, and image captures were taken with the aid of the Optical Photomicroscope Olympus BX 60 with Olympus DP71 digital camera. After obtaining the images of 10 individuals from each experimental group, the larvae with 72 h had their total areas measured with the aid of ImageJ software (Rasband 2015-2018).

Biochemical analysis

At each experimental time, all living eggs and larvae (~ 133 individuals) were collected from each replicate and were homogenized in 1 mL of Tris-HCl buffer pH 7.4, centrifuged at 12,000 g for 12 min, at 4 °C, and the supernatant was frozen at -80 ºC for further analyses. The protein quantification of the samples was determined through the Bradford method, using bovine serum albumin as standard (Bradford, 1976) and read at an absorbance of 595 nm (EPOCH – Biotek/Agilient).

Energetic metabolism

The evaluation of the hexokinase enzyme activity (HK, EC 2.7.1.1) was made in a reaction system of imidazole buffer (pH 7.4), 20 mM glucose, 2 mM ATP, 10 µM $MgCl_2$, 0.4 mM $NADP^+$, 1 mM DTT, 2 mM KCl, 0.3U G-6-PDH, and deionized H_2O. The enzymatic activity was measured at 340 nm and expressed at µmoles of NADPH. min^{-1}. mg of $protein^{-1}$ (Baldwin *et al.* 2007).

The phosphofructokinase enzyme (PFK, EC 2.7.1.11) was evaluated in Tris-HCl buffer pH 8.2, deionized water, 10 mM $MgCl_2$, 1 mM ATP, 0.15 µM NADH, 2 mM AMP, 1 U/ml glycerophosphate dehydrogenase, aldolase 1.2 U/mL, 10 U/L triosephosphate isomerase, and 5.0 mM fructose-6-phosphate, and the enzymatic activity was measured at 340 nm, the absorbances transformed and expressed in µmoles of NAD^+. min^{-1}. mg of $protein^{-1}$ (Baldwin *et al.* 2007).

The enzymatic activity of lactate dehydrogenase (LDH, EC 1.1.1.27) was determined in a reaction system composed of a 50 mM buffer Tris-HCl (pH 7.4), 1.0 mM sodium pyruvate, 100 mM KCL, 0.25 mM NADH and deionized water, the enzymatic activity was measured at 340 nm and expressed in µmoles NAD^+. min^{-1}.mg of $protein^{-1}$ (Thuesen *et al.* 2005).

The enzymatic activity of citrate synthase (CS, EC 4.1.3.7) was performed in media reaction of 50 mM Tris-HCL buffer (pH 7.4), 100 mM KCl, 1.0 mM EDTA, 200 µM DTNB (in tris), 200 µM acetyl-CoA, deionized water and 500 µM oxaloacetate. The enzyme activity was measured at 412 nm and expressed in µmoles of CoA-SH.min^{-1}.mg of $protein^{-1}$ (Saborowski & Buchholz, 2002).

Malate dehydrogenase (MDH, EC 1.1.1) was evaluated in a reaction media of 50 mM Tris-HCl buffer pH 7.4, 0.4 mM oxaloacetate, 20 mM $MgCl^2$, 150 µM NADH and deionized water, the absorbance was measured at 340 nm, and enzyme activity was expressed in µmoles of NAD+. min^{-1}.mg of $protein^{-1}$ (Childress and Somero 1979).

Antioxidant system

The catalase activity (CAT, EC 1.11.1.6) was measured by a decrease in absorbance at 240 nm in a reaction media composed of 1.0 M Tris-HCl buffer, 5.0 mM EDTA pH 8.0, 30 mM H_2O_2. The unit for expression of catalase activity was mmoles of degraded H_2O_2 min^{-1}.mg of $protein^{-1}$ (Aebi, 1984).

The activity of glutathione peroxidase (GPx, EC 1.11.1.9) was evaluated according to the technique proposed by Flohé and Günzler (1984). The enzymatic activity was measured at 340 nm in a reaction system of 100 mM phosphate buffer (pH 7.0) 1 mM EDTA, 2 mM GSH, 0.15 mM NADPH, 0.2 U purified glutathione reductase, 0.5 mM t-butyl hydroperoxide and expressed in µmoles of $NADP^+$.min^{-1}.mg of $protein^{-1}$.

Glutathione reductase activity (GR, EC 1.8.1.7) was evaluated in reaction system of 100 mM phosphate buffer (pH 7.0) 1 mM EDTA, 0.66 mM GSSG, 0.075 mM NADPH, measured at 340 nm and expressed in µmoles of oxidized $NADP^+$.min^{-1}.mg of $protein^{-1}$ (Sies *et al.* 1979).

The evaluation of the activity of the glutathione S-transferase enzyme (GST, EC 2.5.1.18) was carried out in a reaction system composed of potassium phosphate buffer pH 6.5, 1.5 mM GSH, 2 mM CDNB in 1 mL of ethanol, measured at 340 nm, and the results were expressed in µmoles of thioether.min^{-1}.mg of $protein^{-1}$ (Keen and Jakoby 1976).

Cell disorders

The measurement of lipid peroxidation was evaluated by the reaction product of the thiobarbituric acid (TBARS) with malondialdehyde (MDA), being measured in a spectrophotometer at 535 nm, compared with a standard malondialdehyde curve and expressed in nmol of MDA.mg of $protein^{-1}$ (Buege and Aust 1978).

To evaluate the neurotoxic effect, the activity of cholinesterase enzymes (ChE, EC 3.1.1.7) was analyzed using acetylthiocholine (ATC) as substrate and 5,5'-diethyl-bis-(2-nitrobenzoic acid) (DTNB) as a color reagent. Absorbance was measured at 405 nm and expressed in nmol of hydrolyzed acetylthiocholine.min^{-1}.mg of $protein^{-1}$ (Ellman *et al.*1961, modified for microplates by Silva de Assis 1998).

Statistical analysis

The data of local abiotic variables were evaluated using descriptive statistics and comparing between streams using Mann-Whitney-U test, because the assumptions of normality (Shapiro-Wilk test) and homocedasticity (Bartlett test) were not accepted. The data of abiotic variables were evaluated using descriptive statistics and compared to the reference values defined for the cultivation of *R. quelen* (Graeff *et al.* 2008; Amaral Junior *et al.* 2015; Galdino 2009; Gomes *et al.* 2000).

The percentage of living eggs and larvae throughout the experimental period (12, 24, 48 and 72h), defined as viability (%), were compared using the Pearson Chi-Square Test for Independence (α=0,05). The data of the larval areas were analyzed using non-parametric Mann-Whitney-U test.

Since the variables related to biochemical analyzes of energetic metabolism, antioxidant system activity and cell disorders were obtained from a completely randomized design, the Gaussian distribution pattern (Shapiro-Wilk test) and homoscedasticity of variances (Bartlett test) in each analyzed condition were assumed. Thus, the variables were analyzed using the two-way ANOVA, assuming Streams and Development period as fixed factors. With statistical significance ($p<0.05$), the Least Significant Difference (LSD-Fisher) test was performed. The interpretations will be demonstrated between the periods of 12 to 72 h of development, considering that between 12 and 24h of development is the period from the initial development until the eclosion of eggs (Period 1); 48 and 72h will be considered as representative of larval phase (Period 2). All performed analyses assumed a significance level of 0.05, being performed with the aid of the *ExpDes.pt* (Ferreira *et al.* 2013) and *ggplot2* (Wickham 2009) in the program R (R Core Team 2019).

RESULTS

According to chromatographic analyses, none of the places presented pesticide contamination at the moment of the sample (Supplementary Material). Nonetheless, statistic differences were observed in regard to variables between the places, showing that the water temperature and turbidity were higher in Tormenta river (IMP, $p=0.0001$ and $p<0.0001$, respectively), as well as lower dissolved oxygen concentration when compared to Manoel Gomes river ($p<0.0001$). Ammonia, pH, and nitrite presented no differences between the streams ($p>0.05$; Table 1).

Throughout the entire experiment, the abiotic variables analyzed were within the standards indicated as ideal for *R. quelen* cultivation in a fish farm in Brazil (Table 2) and in agreement with the values in Class II stated by the CONAMA n° 357/2005, suitable to animal growth.

Tab. 1 – Median and interquartile interval of abiotic local variables evaluated during water sampling. P-value of Mann-Whitney-U test.

Sample	Manoel Gomes	Tormenta	p-value
Temperature (°C)	15.6 [14.9 - 17.5]	18.3 [17.6 - 19.2]	**0.0001**
DO ($mg.L^{-1}$)	9.8 [9.5 - 10.4]	8.7 [8.1 - 9.1]	**< 0.0001**
pH	7.2 [6.9 - 7.4]	7.0 [6.4 - 7.5]	0.1838
Turbidity (NTU)	9.0 [8.3 - 14.7]	19.8 [15.7 - 23.8]	**< 0.0001**
Ammonia ($mg.L^{-1}$)	0.000 [0.000 - 0.000]	0.000 [0.000 - 0.000]	0.9251
Nitrite ($mg.L^{-1}$)	0.000 [0.000 - 0.001]	0.000 [0.000 - 0.001]	0.8304

Tab. 2 – Means of abiotic variables evaluated over the 72 hours of experimentation, and reference values for cultivation of *R. quelen.*

		12h	24h	48h	72h	Reference values*	CONAMA N° 357/2005
Temperature (°C)	Manoel Gomes	26.63	25.90	25.53	25.50	24 to 32°C	-
	Tormenta	26.20	25.77	25.83	25.50		
pH	Manoel Gomes	7.53	7.07	7.37	7.48	$4 \leq x \leq 8.5$	$6 \leq x \leq 9$
	Tormenta	7.24	7.15	7.02	7.30		
DO ($mg.L^{-1}$)	Manoel Gomes	7.23	7.77	7.47	6.90	> 5.00	> 5.00
	Tormenta	7.27	7.37	7.00	7.03		
Nitrite ($mg.L^{-1}$)	Manoel Gomes	0.04	0.05	0.03	0.04	$0.5 < x < 1.0$	< 1.0
	Tormenta	0.12	0.08	0.05	0.08		
Nitrate ($mg.L^{-1}$)	Manoel Gomes	1.30	0.8	1.30	1.20	< 2.00	< 10.0
	Tormenta	1.90	1.1	1.80	1.70		
Ammonia ($mg.L^{-1}$)	Manoel Gomes	0.00	0.03	0.04	0.09	< 0.1	<3.7*
	Tormenta	0.002	0.004	0.02	0.04		

*Reference values: Temperature - (Andrews et al. 1972; Chippari-gomes et al. 1999)/ pH – (Alabaster et al.,1988; BRASIL 2005, Reis and Mendonça, 2009) / DO- dissolved oxygen - (Gomes et al. 2000)/ nitrite - (Amaral Junior et al. 2015) / nitrate and ammonia (Graeff et al. 2008). * The CONAMA resolution has presents only total ammoniacal nitrogen reference.

Biometric data

The animals showed similar survival, with a similar percentage of living individuals from both groups over time (Chi-Squared: x2=0.0049; p=0.999). However, the total areas of larvae developing in the waters of the IMP river were significantly smaller (Median = 7.65; Interquartile Interval = 5.61 – 10.45 mm^2) compared to the larvae developing in the REF river (9,97mm^2; 8.64 – 11.19) (MW: W=1042; p=0.0099, Fig. 2).

Energetic metabolism

The Hexokinase (HK) enzyme activity had a significant difference in the time (F=38.32; p<0.0001) and the interaction between the streams and time of exposure (F=10.3; p<0.00005). At 12 hours, after fertilization (Period 1), the HK activity among individuals developing in IMP water was significantly higher than the individuals developing in REF water (p<0.05). At 24 hours, period already inherent to organogenesis (Period 1), there was no significant difference observed (F=2.6; p>0.05), but at 48 and 72 hours (Period 2 – morphogenesis phase), there was a decrease in enzymatic activity, notably marked in IMP compared to REF (F=6.55, p<0.05; F=11.84, p<0.05, respectively, fig. 3A).

The analysis of phosphofructokinase (PFK) presented statistical differences between rivers (F=42.01, p<0.0001), between times (F=188.74, p<0.0001), and in the interaction of rivers and times (F=25.03, p<0.0001). In period 1 of development, at 12 h and 24 h, the enzymatic activity was significantly higher for the IMP river than the REF river (F=88.13, p<0.0001; F=27.53, p<0.0001, respectively). At other times there were no significant differences between rivers (p>0.05), but a decrease in enzyme activities was observed in both locations (Fig. 3B).

There were significant differences for lactate dehydrogenase (LDH) enzyme between rivers (F= 17.657, p<0.0001), between times (F= 37.99, p<0.0001), and in the interaction of rivers and times (F=55.05, p<0.0001). In 12h of development (Period 1), the enzymatic activity of individuals from the IMP river was higher compared to the REF river (F=164.43, p<0.0001), with a subsequent reduction in morphogenesis phase at 72h (F=17.78, p<0 .0001, fig. 3D).

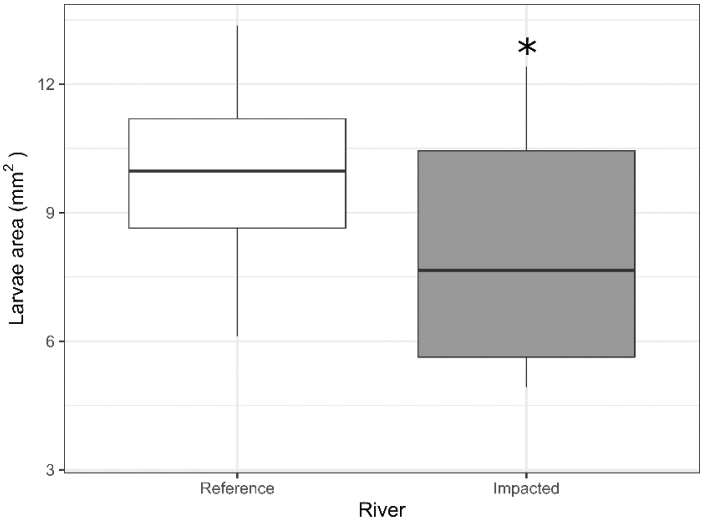

Figure 2. Median (bold line), interquatile (box) and interpercetile intervals (bar) of larvae area (72h) developing in the waters of the Manoel Gomes (REF) and Tormenta (IMP) streams. Asterisk indicates the statistical difference of the Impacted group in relation to the Reference group, by the Mann-Whitney-U test.

The citrate synthase (CS) activity was different between rivers (F=9.98, p<0.05), between times (F=33.91, p<0.0001) and between rivers and times (F=3.31, p< 0.05), and at 24h the enzymatic activity of the IMP river reduced when compared to the REF river (F=17.15, p<0.0001, fig. 3C).

Statistical differences were observed for malate dehydrogenase (MDH) between times (F=6.67, p<0.05), rivers (F=45.314, p<0.0001), and in the interaction between rivers and times (F=11.68, p<0.0001). In 48h, the morphogenesis phase, it was observed that in the IMP river the means were reduced compared to the REF river (F=39.95, p<0.00001, fig. 3E).

Antioxidant system

There were significant differences for catalase (CAT) activity between times (F=64.419, p<0.0001) and the interaction between rivers and time (F=8.44, p<0.0001). At 48h, during the morphogenesis phase, the animals exposed to IMP water had a significant lower average compared to REF (F=11.97, p<0.05), and at 72h, they showed higher averages compared to the REF (F=12.55, p<0.05, fig. 4A).

The glutathione peroxidase (GPx) enzyme showed statistical differences between rivers (F=7.65, p<0.05), times (F=24.17, p<0.0001), and in the interaction of rivers and times (F=33.56, p<0.0001). At Period 1, in 12h, individuals developing in IMP

showed higher activity concerning REF (F=27.24, p<0.0001), with an inversion of the mean values in 24h (F=9.62, p<0.05), and in 48h (F=70.87, p<0.0001, fig. 4B).

The enzyme glutathione reductase (GR) showed significant difference in activity only in relation to time (F=77.08, p<0.0001), and between rivers the means were considered equal (F=2.29, p=0.13). However, it is important to emphasize that, as the development period progressed, the activity averages gradually decreased, until in 72 hours the averages were close to zero (Fig. 4C).

There were significant differences of glutathione transferase (GST) activity in time (F=29.61; p<0.0001) and in the interaction of time and rivers (F=15.67, p<0.0001). At 12 hours (Period 1), GST activity among individuals developing in waters from the IMP river was significantly higher than that observed among individuals of the REF river, while in 48 hours (Period 2), there was an inversion of GST activity, being lower among individuals from IMP (p<0.05; Fig. 4D).

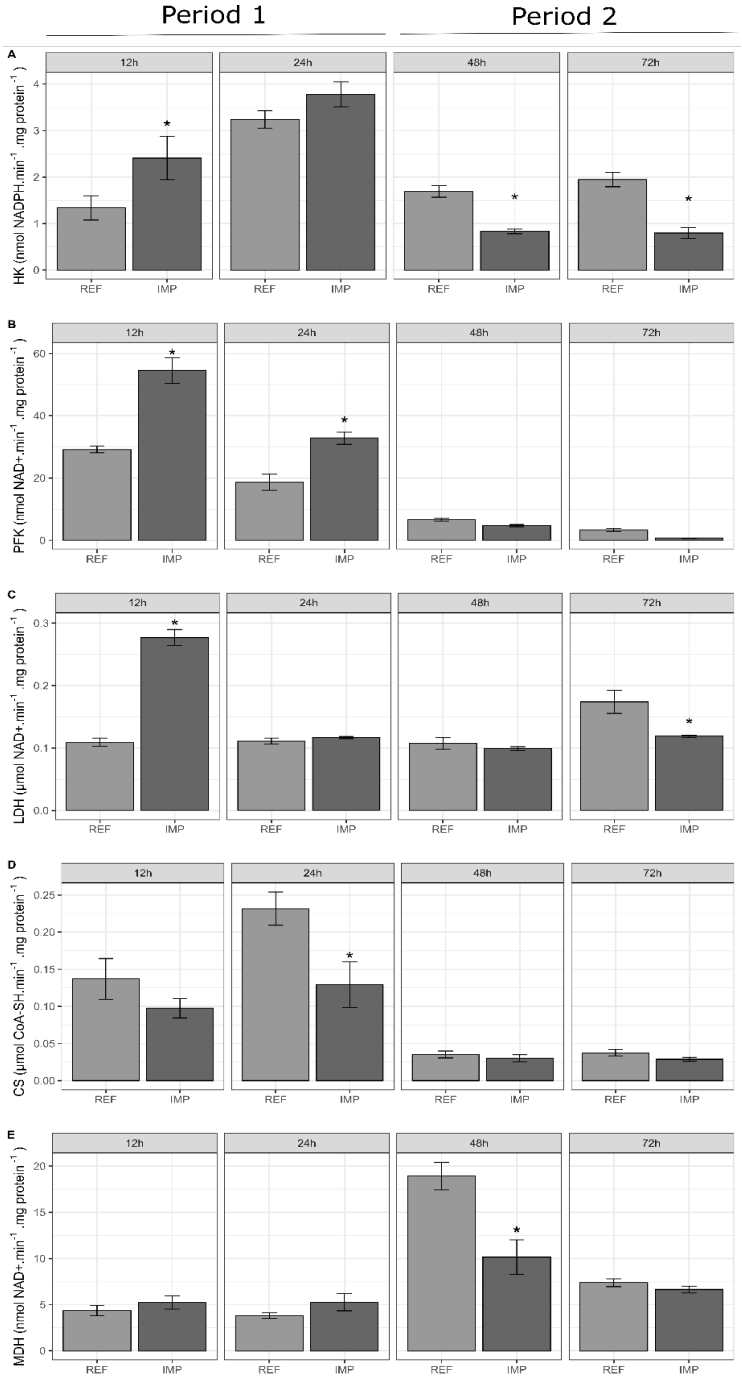

Figure 3. Means and standard errors of the activity of energy metabolism enzymes in individuals developing in reference (REF) and impacted (IMP) waters, during 72 hours of experimentation. A) hexokinase (HK); B) phosphofructokinase (PFK); C) lactate dehydrogenase (LDH); D) citrate synthase (CS). E) malate dehydrogease (MDH). Period 1 - fertilization to organogenesis stages; Period 2 - morphogenesis phase. (*) significant differences between streams in the same period of embryonic development.

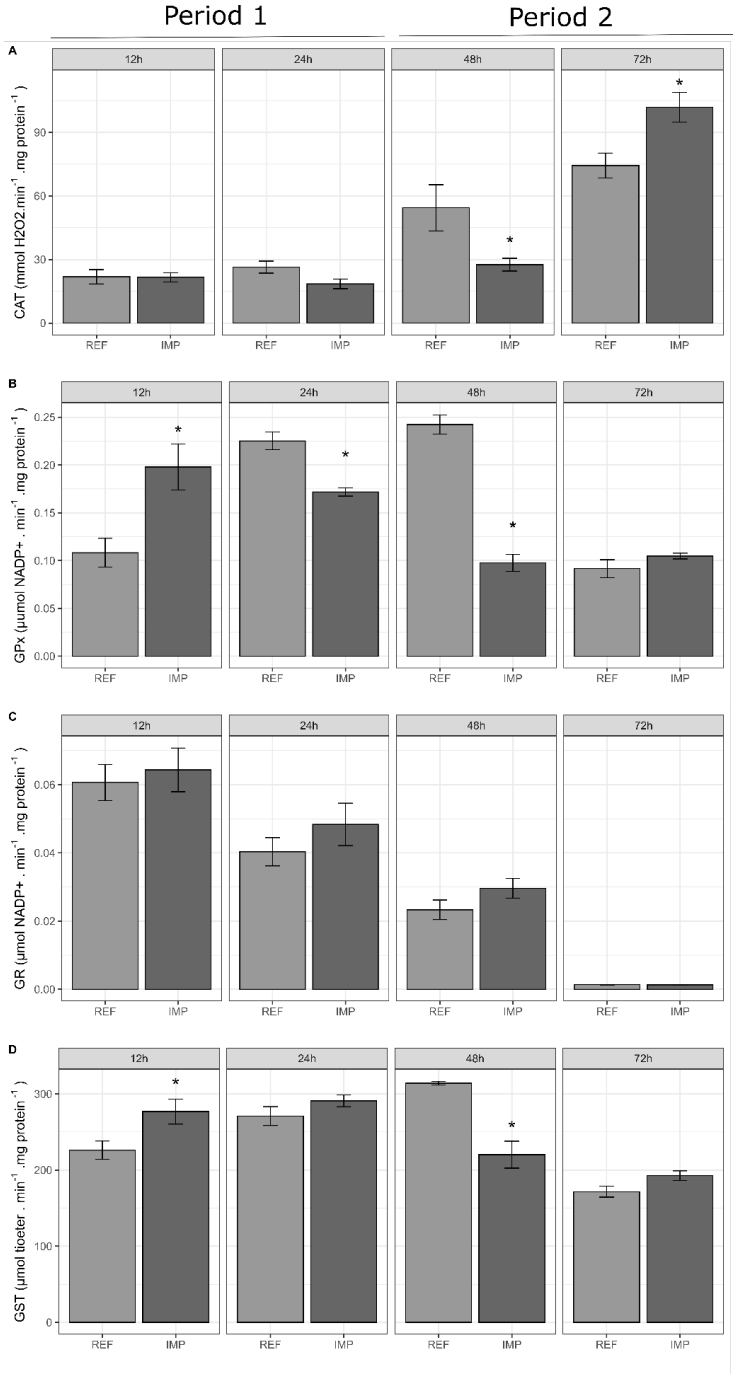

Figure 4. Means and standard errors of enzyme activities of the antioxidant system in individuals developing in reference (REF) and impacted (IMP) waters, during 72 hours of experimentation. A) catalase (CAT); B) glutathione peroxidase (GPx); C) glutathione reductase (GR); D) glutathione S-transferase (GST). Period 1 - fertilization to organogenesis stages; Period 2 - morphogenesis phase. (*) significant differences between streams in the same period of embryonic development.

Cell disorders

The lipid peroxidation (LPO) was significant different between rivers ($F=19.20$, $p<0.0001$) and times ($F=25.58$, $p<0.0001$), with higher averages being observed in IMP when compared to REF (fig. 5A).

Cholinesterase enzyme activity (ChE) presented significant differences between rivers ($F= 82.260$; $p<0.0001$), time ($F= 95.9$; $p<0.0001$) and in the interaction of rivers and time ($F= 30.2$; $p<0.0001$). In Period 1 (12 e 24h), the activity reduces according to the start of development, but without statistical difference between the groups. In morphogenesis phase at 48h (Period 2), lower values of the enzymatic activity of the IMP site were observed concerning the REF ($F=111.49$; $p<0.0001$), and this inhibition was maintained in 72h ($F=61.43$; $p<0.0001$; Fig. 5B).

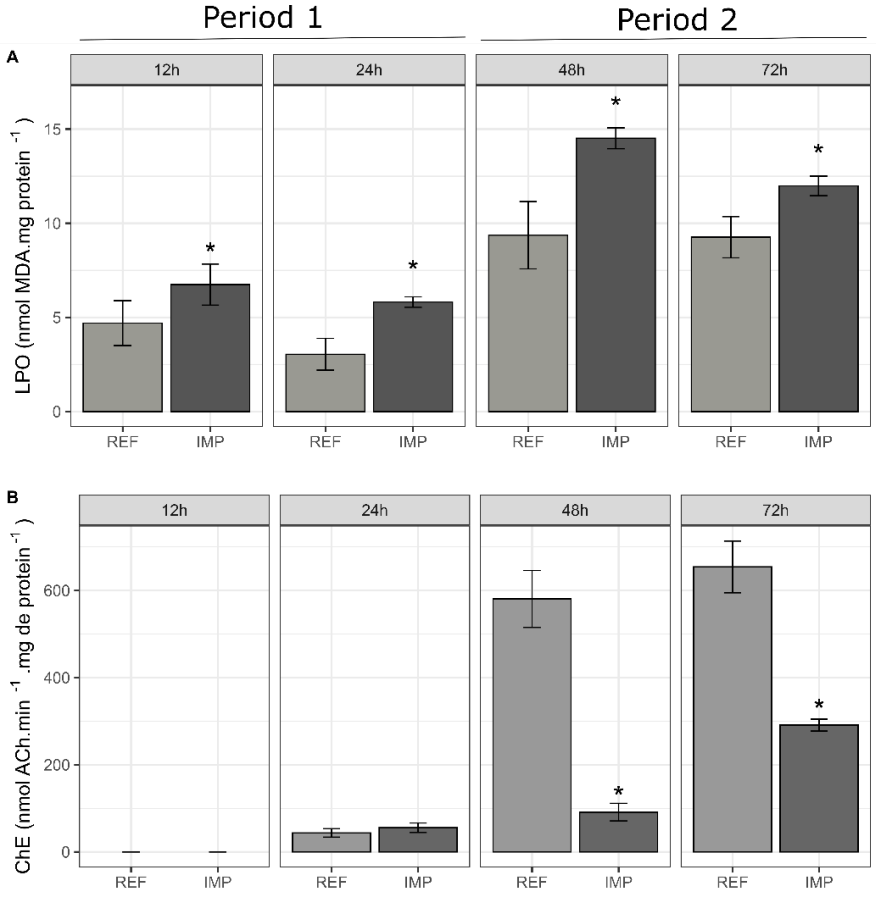

Figure 5. Means and standard errors of lipoperoxidation reaction and ChE activity in individuals developing in reference (REF) and impacted (IMP) waters, during 72 hours of experimentation. A) lipoperoxidation (LPO); B) cholinesterase enzyme activity (ChE). Period 1 - fertilization to organogenesis stages; Period 2 - morphogenesis phase. (*) significant differences between streams in the same period of embryonic development.

DISCUSSION

Effects of IMP waters exposure during the period of the initial development until eggs eclosion (Period 1)

The results will address the metabolic changes observed among individuals developing in the waters of the Tormenta (IMP) River during the first development periods, as summarized in Figure 6.

The animals exposed to IMP stream water manifested an induction in HK activity in 12h and a PFK induction activity in 12 and 24h compared to the animals exposed to REF water. These two enzymes are part of the glycolytic pathway, the HK converting the glucose to glucose-6-phosphate and PFK forming fructose-1,6-biphosphate from fructose-6-phosphate (Kuettner *et al.* 2010). The activity of CS is often used as an indicator of aerobic capacity, and it was reduced in the animals of the IMP group, possibly related to the negative effects of contaminants present in these waters on cellular energy production routes. Exposure of zebrafish larvae to 50 ug/L of the pyrethroid permethrin reduced CS activity when compared to the control (Nunes *et al.* 2019), as well as to *Clarias batrachus* exposed to organochlorine endosulfan for 21 days (Tripathi and Verma 2004) and in *Chinook salmon* exposed to contaminants of emerging concern (Yeh *et al.* 2017). The presence of contaminants may function as inhibitors of Krebs Cycle enzymes, accumulating metabolic intermediates and resulting in a reduction in the speed of the pathway and the production of ATP (Betarbet *et al.* 2000).

The LDH induction observed in 12h is possibly related to a search for an alternative way to maintain ATP production, which indicates that disturbances may have occurred in the energy production of these animals. The LDH activity may be a response to qualifying stressful environmental situations, such as low oxygen availability (Zakhartsev *et al.* 2004). As reported by Das *et al.* (2004), the elevation of nitrite increased LDH activity in juveniles of three different fish species, suggesting that in a situation of anoxia the body tries to compensate for the respiratory metabolism by the anaerobiosis. This higher activity may be associated with an inhibition of the respiratory chain by the water contaminants, which promotes a reduction in ATP production, since high levels of NADH in the cell inhibit the Krebs Cycle. Moreover, an increase in LDH activity was also observed in *Cyprinus carpio* exposed to glyphosate-based herbicides (Gholami-

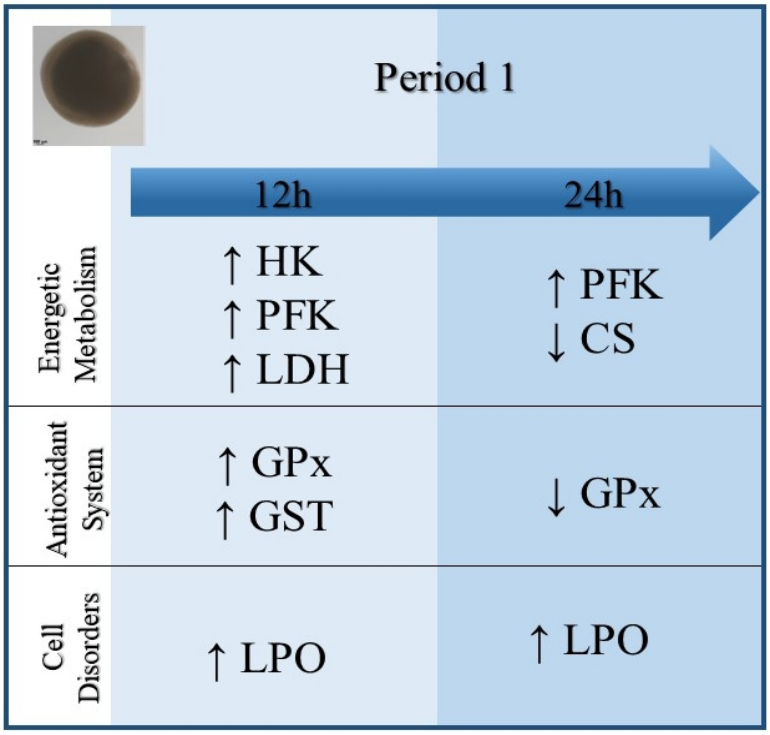

Figure 6. Summary of energetic metabolism, antioxidant system, and cell disorders results of Rhamdia quelen individuals developing in waters of Tormenta River (IMP), during the initial development until eggs eclosion (12 and 24 h, Period 1).

Seyedkolaei *et al.* 2013) and in *Catla catla* exposed to methyl parathion (Khare *et al.* 2019), as well as in zebrafish embryos exposed to antibiotics (Oliveira *et al.* 2020). Our findings are corroborated by these facts, suggesting that the action of contaminants interfere with metabolic function, requiring enzymatic adaptations, inducing the anaerobic pathway, enabling physiological regulations due to exposure to xenobiotics in water.

The biomarkers used to monitor the antioxidant defense were CAT, GPx, GR, and LPO. In 24h exposition, the animals in the IMP group exhibited a reduction in GPx activity compared to REF, suggesting that the initial increase (12h) of GPx and GST activities could lead to a GSH depletion due to its use by GPx associated with GST in a detoxification process. To reinforce this hypothesis, the GR activity was not influenced in the first 24 h period, which did not promote the proper recycling of GSSG during this period, which consequently led to lower availability of GSH in response to demand. The induction of GST activity accompanied by GPx inhibition was also observed in *Brycon cephalus* exposed to the organophosphate methyl parathion (Monteiro et al. 2006), as well as in *Cyprinus carpio* exposed to the organophosphate trichlorfon (Woo and Chung 2020), indicating that phase II biotransformation can deplete GSH levels, increasing the risk of oxidative stress (Kaur and Jindal 2017).

While GPx activity is stimulated in the first hours of development, CAT enzymatic activity is reduced in both rivers. Hypotheses for this behavior include the use of the GPx pathway for H_2O_2 degradation, as well as the low expression of the CAT enzyme in the early stages of development. According to Cong *et al.* (2020), the CAT activity reduces after 96 h of exposure to high concentrations of dimethyl phthalate in *Danio rerio*, with up or downregulation of the transcriptional expression of the respective gene.

By increasing the lipoperoxidation reaction of individuals exposed to IMP waters, it can be proposed that cellular damage occurred in the first hours of exposure. The increase in lipid peroxidation potentiates structural changes in DNA due to increased membrane permeability (Vieira *et al.* 2017). Such pattern was also observed in *Alburnus mossulensis* exposed to low concentrations (1.25 ug/L) of fenpropathrin, which demonstrates that certain pesticides can disrupt the cellular defense system (Banaee *et al.* 2014), as also reported by other researchers (Cong *et al.* 2020; Kaur and Jindal 2017).

Effects of IMP waters exposure during the larvae period (Period 2)

The results observed among individuals developing in the waters of the Tormenta (IMP) River during the second development period (Period 2) is summarized in Figure 7.

In period 2, considered as morphogenesis phase, *R. quelen* embryos are denominated larvae and have an open mouth and operculum (Rodrigues-Galdino *et al.* 2010), leading to an even higher contact with the water, which may justify the modulation of several enzymes from this stage of development.

During this period, a reduction in the HK and PFK activity was observed for both, REF and IMP groups compared

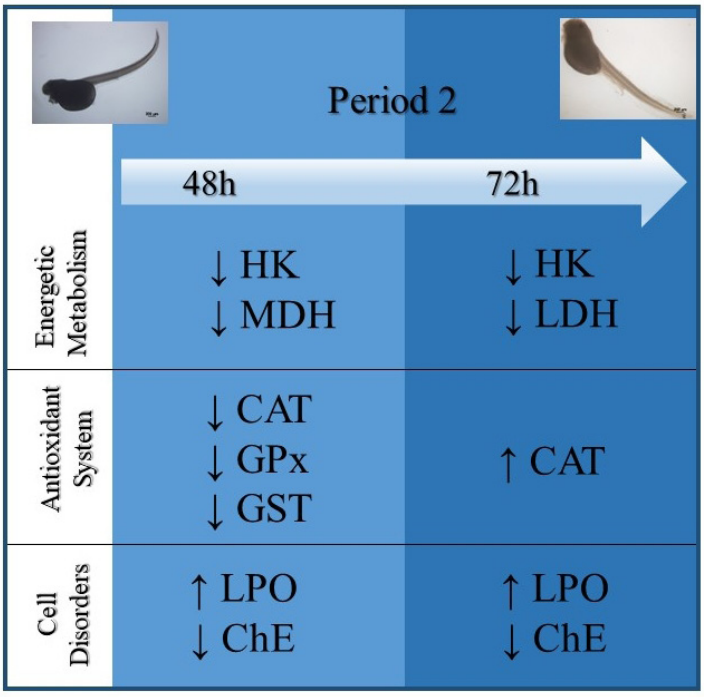

Figure 7. Summary of energetic metabolism, antioxidant system, and cell disorders results of Rhamdia quelen individuals developing in waters of Tormenta River (IMP), during the larval phase (48 and 72 h, Period 2).

to the first period, possibly related to the adaptation to the consumption of different nutrients from the yolk sac, thus modifying the substrate oxidation pathways for cellular energy production. Furthermore, in animals of the IMP group, it was possible to observe a significant reduction in HK compared to REF. Additionally, PFK activity was extremely low in this period, which may result in the accumulation of intermediates above it (such as glucose 6 phosphate), which also contributes to the inhibition of HK. This may be due to contaminants present in the water, as several authors have shown that there is an inhibition of this enzyme in the presence of aluminum (Exley *et al.* 1994), heavy metals (Fu and Xi 2019; Sabir *et al.* 2019; Moniruzzaman *et al.* 2020), as well as in the presence of atrazine (Wang *et al*. 2018), and the increase in ROS itself may result in the inhibition of this enzymatic activity (Anastasiou *et al.* 2011). In addition, the inhibition in MDH activity in 48h, may point to a reduction in the aerobic metabolism, as suggested by Mishra and Shukla (1997, 2003) for *Clarias batrachus* exposed to endosulfan. We emphasize that the reduction of CS activity in this period was even more intensified among animals exposed to IMP waters, which corroborates the proposal of inhibition of MDH activity. This change in enzymatic activity can lead to reduced energy availability, also inhibiting the regulation of growth and development of animals in the IMP group, as observed by Qiu *et al.* (2021).

Concerning the antioxidant system, in the larval phase, there was a GR activity reduction in both groups, which promotes a decrease in the GSH availability. When this substrate has its levels reduced, the activity of dependent enzymes also changes, as observed by the reduced activity of GPx and GST, especially among animals from the IMP group. Therefore, it is suggested that for the IMP group there was a reduction in the cell's antioxidant defense potential, especially due to the possible GSH reduction. In contrast to the GR and GPx activity reduction during morphogenesis, there was an increase in CAT activity, possibly explained by the similar function to GPx of oxidizing H_2O_2 into H_2O and O_2. However, it is noteworthy that this may also be a result like that found in *Brycon cephalus* exposed to sublethal concentrations of methyl parathion (Monteiro et al. 2006) or in *Cyprinus carpio* exposed to sublethal concentrations of chlorpyrifos (Stoyanova et al. 2020), in which a decrease in GPx and an increase in CAT activity were identified.

The entry of xenobiotic compounds into rivers through the leaching of chemical molecules existent in the soil causes activation of enzymes with a detoxification function, such as GSTs from non-target organisms. GSTs are part of a family of enzymes that catalyze the conjugation of reduced glutathione to a wide range of substrates, ensuring the formation of polar compounds that are more easily excreted (Mannervik and Jemth 2001). In 48h GST activity appears inhibited in IMP, possibly because of GSH depletion. We emphasize that this response may be a consequence of the opercular opening, causing the larvae to have greater exposure to inadequate quality water, thus increasing the action of stressors present in the water. Because of the reduced activity of the antioxidant system, an intense increase in the lipoperoxidation reaction was observed, which is therefore indicative of the installation of oxidative stress and cell damage. Sites contaminated with different xenobiotics can induce significant lipid peroxidation rises, as described by numerous researchers (Kaur and Jindal 2017; Shukla *et al.* 2017, Cong *et al.* 2020; Gonçalves *et al.* 2020).

After 48 h of fertilization, ChE activity became more effective among animals in both groups. However, when comparing the enzymatic activity of ChE between IMP and REF, a significant ChE activity inhibition was observed. This fact indicates the presence of organophosphate pesticides, as reported by Nimet *et al.* (2017). *Colossoma macropomum* fish exposed to trichlorfon organophosphate, used against acanthocephalic parasite infections, show decreased AChE activity (Duncan *et al.* 2019), the same as *R. quelen* when exposed to cadmium (Pretto *et al.* 2010). The present results support the indications of Sandoval-Herrera *et al.* (2019), who suggest that the inhibition of ChE induced by exposure to pesticides reduces the escape capacity of fish, reducing ecological fitness even when exposed to low concentrations of organophosphates.

CONCLUSION

In Period 1, encompassing the start of development up to the eggs eclosion, the animals exposed to the waters of the IMP stream initially have a more anaerobic metabolism with activation of the antioxidant system and higher oxidative stress (LPO). In Period 2, equivalent to the larval phase, after consumption of the yolk, and opercular opening, changes in carbohydrate metabolism are observed, lower activity of HK and LDH, higher activity of the antioxidant system, especially for peroxide inactivation (increased CAT activity), intense LPO, and inactivation of the cholinesterase enzyme. As a consequence of the amendments presented, the dimensions of the larvae (body area in mm^2) had a significant decrease in the area of animals in the IMP environment. All aspects evaluated may have resulted in smaller animals, which ecologically can lead to animals being more sensitive to environmental pressures.

The main finding of this study was the physiological alteration of eggs and larvae of *Rhamdia quelen* developing in waters of Tormenta (IMP), where the land use is remarkably agricultural, with smaller vegetation cover, higher temperature, less dissolved oxygen and higher water turbidity. The alterations of land use over the basin of the impacted river is a warning to preserve the *fitness* of native species in this region, constantly exposed to this impacting situation.

DECLARATIONS

Ethics Approval: The experiment was authorized by the Ethics Committee in Animal Experimentation of the Universidade Estadual do Oeste do Paraná (CEUA- n.33-17).

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

Availability of data and materials: Not applicable.

Competing Interests: The authors declare that they have no competing interests.

Funding: This work has been financially supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico- CNPq (422443/2016-6).

Author Contributions: CMTOP: Data curation, Visualization, Investigation, Writing-original draft. SR: Conceptualization, Data curation, Visualization, Writing-original draft. LHC: Visualization, Writing-original draft. CZN: Conceptualization. TMS: Writing-review & editing. MRD: Methodology, Writing-original draft. LD: Methodology. ATBG: Conceptualization, Data curation, Visualization, Writing-original draft, Methodology, Resource, Funding acquisition, Formal analysis, Project administration, Supervision. All authors read and approved the final manuscript.

Acknowlegement: Authors are grateful for the financial support of CNPq and the logistical support of UNIOESTE.

REFERENCES

Aebi, H. (1984). Catalase in Vitro. Methods Enzymol. 105, 121–126. doi:10.1016/S0076-6879(84)05016-3

Alabaster, J. S., Calamari, D., Dethlefsen, V., Konemann, H., Lloyd, R., & Solbé, J. F. (1988). Water quality criteria for European freshwater fish. Chemistry and ecology, 3(3), 165-253. https://doi.org/10.1080/02757548808070844

Amaral Júnior, Hilton, Silvano Garcia, Paulo Fernando Warmling, Bruno Correia da Silva, and Natália da Costa Marchiori (2015). **Assim Cultivamos o Jundiá Rhamdia Quelen No Estado de Santa Catarina**. EPAGRI/CNPq/MPA/FAPESC, 81 p.

Anastasiou, D., Poulogiannis, G., Asara, J. M., Boxer, M. B., Jiang, J. K., Shen, M., Bellinger, G., Sasaki, A.T., Locasale, J.W., Auld, D.S., Thomas, C.J., Heiden, M.G.V., Cantley, L. C. (2011) Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. Science, 334(6060):1278-1283. https://doi.org/10.1126/science.1211485

Andrews, J. W., Knight, L. H., & Murai, T. (1972). Temperature requirements for high density rearing of channel catfish from fingerling to market size. The Progressive Fish-Culturist, 34(4), 240-241. doi: 10.1577/1548-8659(1972)34[240:trfhdr]2.0.co;2

Azevedo-Linhares, M., Souza, A.T.C., Lenz, C.A., Leite, N.F., Brito, I.A., Folle, N.T., Garcia, J.E., Neto, F.F., Ribeiro, C. O. (2018) Microcystin and pyriproxyfen are toxic to early stages of development in *Rhamdia quelen*: An experimental and modelling study. Ecotoxicology and environmental safety. 166:311-319. https://doi.org/10.1016/j.ecoenv.2018.09.064.

Baldissera, M. D., Souza, C. F., Zanella, R., Prestes, O. D., Meinhart, A. D., Da Silva, A. S., & Baldisserotto, B. (2021). Behavioral impairment and neurotoxic responses of silver catfish Rhamdia quelen exposed to organophosphate pesticide trichlorfon: protective effects of diet containing rutin. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 239, 108871. https://doi.org/ 10.1016/j.cbpc.2020.108871

Baldwin, J., Elias, J.P., Wells, R.M.G., Donovan, D.A. (2007) Energy Metabolism in the Tropical Abalone, Haliotis Asinina Linné: Comparisons with Temperate Abalone Species. Journal of Experimental Marine Biology and Ecology 342 (2):213–25. https://doi.org/10.1016/j.jembe.2006.09.005.

Banaee, M., Sureda, A., Zohiery, F., Hagi, B.N., Garanzini, D.S. (2014) Alterations in Biochemical Parameters of the Freshwater Fish, Alburnus Mossulensis, Exposed to Sub-Lethal Concentrations of Fenpropathrin. International Journal of Aquatic Biology 2 (2):58–68. https://doi.org/10.22034/ijab.v2i2.32.

Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V., Greenamyre, J.T. (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nature Neuroscience, 3: 1301–1306. https://doi.org/10.1038/81834.

Bradford, M.M. (1976) A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal. Biochem. 72:248–254. https://doi.org/ 10.1006/abio.1976.9999

BRASIL(2005). Ministério do Meio Ambiente. Resolução CONAMA nº 357, de 15 de junho de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. Website: http://pnqa.ana.gov.br/Publicacao/RESOLUCAO_CONAMA_n_357.pdf> . Acessed 05 May 2021

Buege, J.A., Aust, S.D. (1978) Microsomal Lipid Peroxidation. Methods Enzymol. 52:302–310. https://doi.org/ 10.1016/s0076-6879(78)52032-6

Carneiro, P.C.F., Bendhack, F., Mikos, J.D., Shorer, M., Oliveira Filho, P.R.C., Baldisserotto, B., Golombieski, J., Silva, I.V.F., Miron, D., Esquivel, B.M.; Garcia, J.R.E. (2002) Jundiá: 2002. Um grande peixe para a região sul. Panorama da Aqüicultura.12 (69):41-46.

Childress, J.J., Somero, G.N. (1979) Depth-Related Enzymic Activities in Muscle, Brain and Heart of Deep-Living Pelagic Marine Teleosts. Marine Biology 52 (3):273–83. https://doi.org/10.1007/BF00398141.

Chippari-Gomes, A. R., Gomes, L. C., & Baldisserotto, B. (1999). Lethal temperatures for silver catfish, Rhamdia quelen, fingerlings. Journal of Applied Aquaculture, 9(4), 11-21. https://doi.org/10.1590/S0103-84782000000600026

Cong, B., Liu, C., Wang, L., Chai, Y. (2020) The Impact on Antioxidant Enzyme Activity and Related Gene Expression Following Adult Zebrafish (*Danio rerio*) Exposure to Dimethyl Phthalate. Animals (Basel). 10 (4):1–12. https://doi.org/10.3390/ani10040717.

Das, P. C., Ayyappan, S., Das, B. K., Jena, J. K. (2004) Nitrite toxicity in Indian major carps: sublethal effect on selected enzymes in fingerlings of Catla catla, Labeo rohita and Cirrhinus mrigala. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 138(1):3-10. https://doi.org/10.1016/j.cca.2004.03.010.

De Castro Marcato, A.C., de Souza, C.P., Fontanetti, C.S. (2017) Herbicide 2,4-D: a review of toxicity on non-target organisms. Water Air. Soil. Pollut. 228 (3):120. https://doi.org/10.1007/s11270-017-3301-0.

Duncan, Wallice Paxiúba, Jhonatan Junior Silva Idalino, André Gentil da Silva, Rebeca Fontenele Moda, Hallana Cristina Menezes da Silva, Daniele Aparecida Matoso, and Ana Lúcia Silva Gomes. (2019) Acute Toxicity of the Pesticide Trichlorfon and Inhibition of Acetylcholinesterase in Colossoma Macropomum (Characiformes: Serrasalmidae). Aquaculture International. https://doi.org/10.1007/s10499-019-00497-w.

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88–95. https://doi.org/10.1016/0006-2952(61)90145-9.

Exley, C., Birchall, J. D., Price, N. C. (1994) Aluminum inhibition of hexokinase activity in vitro: a study in biological availability. Journal of inorganic biochemistry. 54 (4):297-304.https://doi.org/10.1016/0162-0134(94)80035-9.

Ferreira, E.B., Cavalcanti, P.P., Nogueira, D.A. (2013) ExpDes.pt: Experimental Designs package (Portuguese).

Flohé, L., Günzler, W.A. (1984) Assays of Glutathione Peroxidase. Methods in Enzymology 105, 114–21. https://doi.org/10.1016/s0076-6879(84)05015-1.

Fu, Z., & Xi, S. (2019) The effects of heavy metals on human metabolism. Toxicology mechanisms and methods. 30 (3):167-176. https://doi.org/10.1080/15376516.2019.1701594.

Galdino, Alana Marielle Rodrigues. (2009) "Estadiamento Dos Embriões e Larvas e Avaliação Da Qualidade Das Larvas e Pós Larvas Do Peixe Neotropical Jundiá Rhamdia Quelen (Siluriformes, Heptapteridae) Produzidas Sob Diferentes Temperaturas de Incubação." Universidade Federal do Paraná.

Ghisi, N.C., Larentis, C., de Oliveira, E. C., Neves, M. P., Zavaski, A. G., de Almeida Roque, A., ... & Delariva, R. L. (2020). Environmental assessment of Neotropical streams using fish as bioindicators: a multibiomarker and integrated approach. Hydrobiologia, 1-18. https://doi.org/10.1007/s10750-020-04460-2

Gholami-Seyedkolaei, S. J., Mirvaghefi, A., Farahmand, H., Kosari, A. A. (2013) Effect of a glyphosate-based herbicide in Cyprinus carpio: assessment of acetylcholinesterase activity, hematological responses and serum biochemical parameters. Ecotoxicology and environmental safety, 98:135-141. https://doi.org/10.1016/j.ecoenv.2013.09.011.

Global Forest Watch. (2021) Website: <https://www.globalforestwatch.org/>. Acessed 22.09.21.

Gomes, L.C., Golombieski, J.I., Gomes, A.R.C., Baldisserotto, B. (2000) Biologia Do Jundiá *Rhamdia quelen* (Teleostei, Pimelodidae). Ciência Rural 30 (1):179–85.

Gomes, M.F., de Paula, V.D.C.S., Martins, L.R.R., Garcia, J.R.E., Yamamoto, F.Y., de Freitas, A.M. (2021) Sublethal effects of triclosan and triclocarban at environmental concentrations in silver catfish (*Rhamdia quelen*) embryos. Chemosphere, 263,:127985. https://doi.org/10.1016/j.chemosphere.2020.127985.

Gonçalves, C., Marins, A.T., Amaral, A.M.B., Nunes, M.E.M., Müller, T.E., Severo, E., Feijó, A., Rodrigues, C.C.R., Zanella, R., Prestes, O.D., Clasen, B., Loro, V.L. (2020) Ecological Impacts of Pesticides on Astyanax Jacuhiensis (Characiformes: Characidae) from the Uruguay River, Brazil. Ecotoxicology and Environmental Safety. 205 (205):1–8. https://doi.org/10.1016/j.ecoenv.2020.111314.

Graeff, A., Segalin, C.A., Pruner, E.N., Junior, H.A. (2008) Produção de alevinos de Jundiá (*Rhamdia quelen*) (140). Florianópolis.

Grivennikova, V.G., Vinogradov, A.D. (2006) Generation of Superoxide by the Mitochondrial Complex I. Biochimica et Biophysica Acta - Bioenergetics 1757 (5–6):553–61. https://doi.org/10.1016/j.bbabio.2006.03.013.

Kaur, M., Jindal, R. (2017) Oxidative Stress Response in Liver, Kidney and Gills of Ctenopharyngodon Idellus (Cuvier & Valenciennes) Exposed to Chlorpyrifos. MOJ Biology and Medicine. 1 (4):103–12. https://doi.org/10.15406/mojbm.2017.01.00021.

Keen, H., Jakoby, B. (1976) Mechanism for the Several Activities of the Glutathione. 251:(20).

Khare, A., Chhawani, N., Kumari, K. (2019) Glutathione reductase and catalase as potential biomarkers for synergistic intoxication of pesticides in fish. Biomarkers, 24(7):666-676. https://doi.org/10.1080/1354750X.2019.1651902.

Kuettner, E. B., Kettner, K., Keim, A., Svergun, D. I., Volke, D., Singer, D., Hoffmann, R., Müller, E. C., Otto, A., Kriegel, T.M., Sträter, N. (2010) Crystal structure of hexokinase KlHxk1 of Kluyveromyces lactis: a molecular basis for understanding the control of yeast hexokinase functions via covalent modification and oligomerization. Journal of Biological Chemistry. 285 (52):41019-41033. https://doi.org/10.1074/jbc.M110.185850.

Malafaia, G., de Souza, A.M., Pereira, A.C., Goncalves, S., da Costa Araujo, A.P., Ribeiro, R.X., Rocha, T.L. (2020) Developmental toxicity in zebrafish exposed to polyethylene microplastics under static and semi-static aquatic systems. Science of The Total Environment. 700:134867. https://doi.org/10.1016/j.scitotenv.2019.134867.

Mannervik, Bengt, and Per Jemth. (2001) Measurement of Glutathione Transferases. Curr Protoc Toxicol 6:6–10. https://doi.org/10.1002/0471140856.tx0604s01

MapBiomas – Coleção da Série Anual de Mapas de Cobertura e Uso de Solo do Brasil. (2021) Website: https://mapbiomas.org/. Acessed 28.09.21

Marins, A.T., Cerezer, C., Leitemperger, J.W., Severo, E.S., Costa, M.D., Fontoura, D.O., Nunes, M.E.M., Ribeiro, L.C., Zanella, R., Loro, V.L. (2021) A mixture of pesticides at environmental concentrations induces oxidative stress and cholinergic effects in the neotropical fish Rhamdia quelen. Ecotoxicology. 30 (1):164-174. https://doi.org/10.1007/s10646-020-02300-6.

Mishra, R., Shukla, S. P. (1997) Impact of endosulfan on cytoplasmic and mitochondrial liver malate dehydrogenase from the freshwater catfish (*Clarias batrachus*). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology. 117 (1):7-18. https://doi.org/10.1016/s0742-8413(96)00197-1

Mishra, R., Shukla, S. P. (2003) Endosulfan effects on muscle malate dehydrogenase of the freshwater catfish *Clarias batrachus*. Ecotoxicology and Environmental Safety. 56 (3):425-433. https://doi.org/10.1016/S0147-6513(03)00006-X

Modesto, V., Ilarri, M., Souza, A. T., Lopes-Lima, M., Douda, K., Clavero, M., & Sousa, R. (2018). Fish and mussels: importance of fish for freshwater mussel conservation. Fish and Fisheries, 19(2), 244-259. https://doi.org/10.1111/faf.12252

Moniruzzaman, M., Kumar, S., Das, D., Sarbajna, A., Chakraborty, S. B. (2020) Enzymatic, non enzymatic antioxidants and glucose metabolism enzymes response differently against metal stress in muscles of three fish species depending on different feeding niche. Ecotoxicology and Environmental Safety. 202:110954. https://doi.org/10.1016/j.ecoenv.2020.110954.

Monteiro, D.A., de Almeida, J.A., Rantin, F.T., Kalinin, A.L. (2006) Oxidative stress biomarkers in the freshwater characid fish, Brycon cephalus, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 143:141–149. https://doi.org/10.1016/j.cbpc.2006.01.004.

Nimet, J., Guimarães, A. T. B., & Delariva, R. L. (2017) Use of muscular cholinesterase of *Astyanax bifasciatus* (Teleostei, Characidae) as a biomarker in biomonitoring of rural streams. Bulletin of environmental contamination and toxicology. 99:232-238. https://doi.org/10.1007/s00128-017-2111-9.

Nunes, M. E. M., Schimith, L. E., da Costa-Silva, D. G., Lopes, A. R., Leandro, L. P., Martins, I. K., Mello, R. S., Hartmann, D. D., Carvalho, N. R., Rosa, P. C., Trevisan, R., Di Giulio, R. T., Posser, T.and Franco, J. L. (2019) Acute exposure to permethrin modulates behavioral functions, redox, and bioenergetics parameters and induces DNA damage and cell death in larval zebrafish. Oxidative medicine and cellular longevity, 2019. https://

doi.org/10.1155/2019/9149203.

Oliveira, R. C. S., Oliveira, R., Rodrigues, M. A. C., de Farias, N. O., Sousa-Moura, D., Nunes, N. A., Andrade, T.S., Grisolia, C. K. (2020) Lethal and sub-lethal effects of nitrofurantoin on zebrafish early-life stages. Water, Air, & Soil Pollution, 231(2):1-14.https://doi.org/10.1007/s11270-020-4414-4

Pereira, C.R., Barcellos, L.J.G., Kreutz, L.C., Quevedo, R.M., Ritter, F., Silva, L.B. (2006) Embryonic and Larval Development of Jundiá (Rhamdia Quelen, Quoy and Gaimard, 1824, Pisces, TeleosteI), a South American Catfish. Brazilian Journal of Biology- Revista Brasleira de Biologia 66 (4):1057–63. https://doi.org/10.1590/S1519-69842006000600013.

Pimpão, C. T., Moura, E., Fredianelli, A. C., Galeb, L. G., Rocha, R. M. V. M., & Montanha, F. P. (2012). Evaluation of toxicity in silver catfish. New Advances and Contributions to Fish Biology. IntechOpen. https://doi.org/10.5772/53899

Pretto, A., Loro, V.L., Morsch, V.M., Moraes, B.S., Menezes, C., Clasen, B., Hoehne, L., Dressler, V. (2010) Acetylcholinesterase activity, lipid peroxidation, and bioaccumulation in silver catfish (*Rhamdia quelen*) exposed to cadmium. Arch. Environ. Contam. Toxicol. 58:1008–1014. https://doi.org/10.1007/s00244-009-9419-3

Qiu, Q., Zou, H., Zou, H., Jing, T., Li, X. P., Yan, G., Geng, N., Zhang, B., Zhang, Z., Zhang, S., Yoa, B., Zhang, G. Zou, C. (2021) 3-Bromopyruvate-induced glycolysis inhibition impacts larval growth and development and carbohydrate homeostasis in fall webworm, Hyphantria cunea Drury. Pesticide Biochemistry and Physiology, 104961.https://doi.org/10.1016/j.pestbp.2021.104961.

R Core Team (2019) R: A language and environment for statistical computing.

Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018.

Reis, J.A.T. dos, Mendonça, A.S.F., 2009. Análise técnica dos novos padrões brasileiros para amônia em efluentes e corpos d'água. Eng. Sanit. e Ambient. 14, 353–362. https://doi.org/10.1590/S1413-41522009000300009

Rodrigues-Galdino, A.M., Maiolino, C.V., Forgati, M., Donatti, L., Mikos, J.D., Carneiro, P.C.F., Rios, F.S.A. (2010) Development of the Neotropical Catfish Rhamdia Quelen (Siluriformes, Heptapteridae) Incubated in Different Temperature Regimes. Zygote 18 (2):131–44. https://doi.org/10.1017/S096719940999013X.

Sabir, S., Akash, M. S. H., Fiayyaz, F., Saleem, U., Mehmood, M. H., Rehman, K. (2019) Role of cadmium and arsenic as endocrine disruptors in the metabolism of carbohydrates: inserting the association into perspectives. Biomedicine & Pharmacotherapy. 114:108802. https://doi.org/10.1016/j.biopha.2019.108802.

Saborowski, R., Buchholz, F. (2002) Metabolic Properties of Northern Krill, Meganyctiphanes Norvegica, from Different Climatic Zones. II. Enzyme Characteristics and Activities. Marine Biology 140 (3):557–65. https://doi.org/10.1007/s00227-001-0734-0.

Sandoval-Herrera, Natalia, Freylan Mena, Mario Espinoza, and Adarli Romero. (2019) Neurotoxicity of Organophosphate Pesticides Could Reduce the Ability of Fish to Escape Predation under Low Doses of Exposure."Scientific Reports. 9 (1):1–11. https://doi.org/10.1038/s41598-019-46804-6.

SEAB- Secretaria da Agricultura e do Abastecimento. (2021) Website: <"https://www.agricultura.pr.gov.br/deral/ProducaoAnual"> Acessed 05.04.21.

Shukla, S., Jhamtani, R.C., Dahiya, M.S., Agarwal, R. (2017) Oxidative Injury Caused by Individual and Combined Exposure of Neonicotinoid, Organophosphate and Herbicide in Zebrafish. Toxicology Reports. 4:240–44. https://doi.org/10.1016/j.toxrep.2017.05.002.

Sies, H., Koch, O.R., Martino, E., Boveris, A. (1979) Increased biliary Glutathione Disulfide release in chronically ethanol-treated rats. FEBS Lett. 103:287–290. https://doi.org/10.1016/0014-5793(79)81346-0.

Silva de Assis, H.C. (1998). Der einsatz von biomarkern zur summarischen erfassung vom gewässerverschmutzungen. (Ph.D. thesis). University of Berlin, Berlim.

Souza, C.M., Jr., Shimbo, J.Z., Rosa, M.R., Parente, L.L., Alencar, A.A., Rudorff, B.F.T., Hasenack, H., Matsumoto, M., Ferreira, L.G., Souza-Filho, P.W.M., de Oliveira, S.W., Rocha, W.F., Fonseca, A.V., Marques, C.B., Diniz, C.G., Costa, D., Monteiro, D., Rosa, E.R., Vélez-Martin, E., Weber, E.J., Lenti, F.E.B., Paternost, F.F., Pareyn, F.G.C., Siqueira, J.V., Viera, J.L., Neto, L.C.F., Saraiva, M.M., Sales, M.H., Salgado, M.P.G., Vasconcelos, R., Galano, S., Mesquita, V.V., Azevedo, T. (2020) Reconstructing Three Decades of Land Use and Land Cover Changes in Brazilian Biomes with Landsat Archive and Earth Engine. *Remote Sens. 12:*2735. https://doi.org/10.3390/rs12172735.

Stoyanova, S., Georgieva, E., Velcheva, I., Iliev, I., Vasileva, T., Bivolarski, V., Tomov, S., Nyeste, K., Antal, L., Yancheva, V. (2020) Multi-Biomarker Assessment in Common Carp (*Cyprinus Carpio*, Linnaeus 1758) Liver after Acute Chlorpyrifos Exposure. Water (Switzerland) 12 (6):1–19. https://doi.org/10.3390/w12061837.

Thuesen, Erik V., Kelly D. McCullough, and James J. Childress. (2005) "Metabolic Enzyme Activities in Swimming Muscle of Medusae: Is the Scaling of Glycolytic Activity Related to Oxygen Availability?" Journal of the Marine Biological Association of the United Kingdom 85 (3):603–11. https://doi.org/10.1017/S0025315405011537.

Tripathi, G., Verma, P. (2004) Endosulfan-mediated biochemical changes in the freshwater fish Clarias batrachus. Biomed Environ Sci, 17(1):47-56.

Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M., Mazur, M. (2006) Free Radicals, Metals and Antioxidants in Oxidative Stress-Induced Cancer. Chemico-Biological Interactions 160 (1):1–40. https://doi.org/10.1016/j.cbi.2005.12.009.

Vieira, C.E.D., Costa, P.G., Cabrera, L.C., Primel, E.G., Fillmann, G., Bianchini, A., Martinez, C.B.R. (2017) A Comparative Approach Using Biomarkers in Feral and Caged Neotropical Fish: Implications for Biomonitoring Freshwater Ecosystems in Agricultural Areas.Science of the Total Environment 586:598–609. https://doi.org/10.1016/j.scitotenv.2017.02.026.

Wang, S., Zheng, S., Zhang, Q., Yang, Z., Yin, K., Xu, S. (2018) Atrazine hinders PMA-induced neutrophil extracellular traps in carp via the promotion of apoptosis and inhibition of ROS burst, autophagy and glycolysis. Environmental pollution, 243:282-291. https://doi.org/10.1016/j.envpol.2018.08.070.

Wickham, H., (2009) ggplot2: elegant graphics for data analysis. Springer New York.

Woo, S. J., Chung, J. K. (2020) Effects of trichlorfon on oxidative stress, neurotoxicity, and cortisol levels in common carp, Cyprinus carpio L., at different temperatures. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 229:108698. https://doi.org/10.1016/j.cbpc.2019.108698

Woynarovich, E. (1983) A Propagação Artificial de Peixes de Aguas Tropicais : Manual de Extensão.

Wu, Y., Liu, J., Shen, R., Fu, B. (2017) Mitigation of nonpoint source pollution in rural areas: from control to synergies of multi ecosystem services. Science of the Total Environment. 607:1376-1380. http://dx.doi.org/10.1016/j.scitotenv.2017.07.105.

Yeh, A., Marcinek, D. J., Meador, J. P., Gallagher, E. P. (2017) Effect of contaminants of emerging concern on liver mitochondrial function in Chinook salmon. Aquatic toxicology, 190:21-31. https://doi.org/10.1016/j.aquatox.2017.06.011.

Zakhartsev, M, T Johansen, H O Portner, and R Blust. (2004) Effects of Temperature Acclimation on Lactate Dehydrogenase of Cod (Gadus Morhua): Genetic, Kinetic and Thermodynamic Aspects. The Journal of Experimental Biology 207:95–112. https://doi.org/10.1242/jeb.00708.

Zaniboni-Filho, E. (2004) Piscicultura das espécies nativas de água doce. Aquicultura: experiências brasileiras. Florianópolis: Multitarefa, 1:337-368.

Zhuo, D., Liu, L., Yu, H., Yuan, C. (2018) A national assessment of the effect of intensive agro-land use practices on nonpoint source pollution using emission scenarios and geo-spatial data. Environmental Science and Pollution Research. 25:1683-1705. https://doi.org/10.1007/s11356-017-0118-8.