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A multibiomarker approach to investigate paracetamol effects in the reproduction regulatory axis of a male Neotropical catfish *Rhamdia quelen*

Uma abordagem de multibiomarcadores para investigar os efeitos do paracetamol no eixo de regulação da reprodução de machos do bagre Neotropical *Rhamdia quelen*

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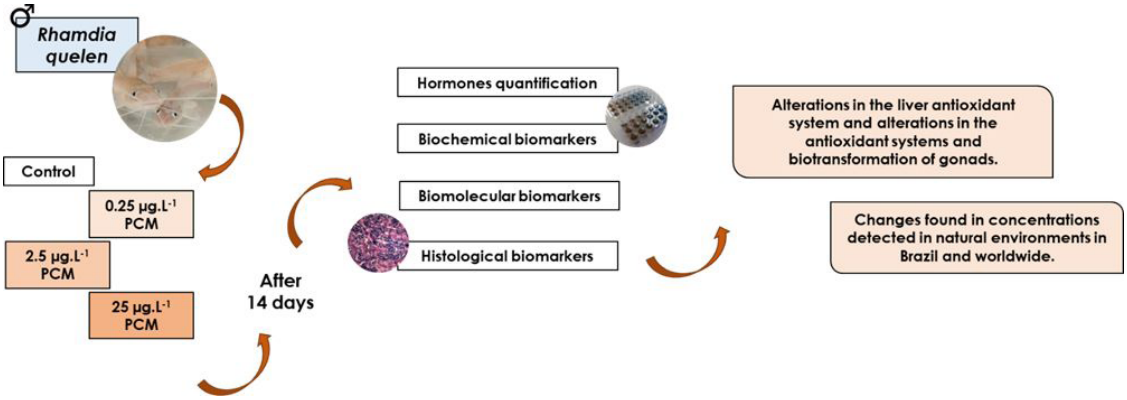
Abstract: Aim: Paracetamol (PCM), or acetaminophen, is one of the most used drugs for human treatment of pain and fever. Since it has often been found in the aquatic environment, the aim of this study was to investigate the effects of PCM on the reproductive axis of male *Rhamdia quelen* catfish. **Methods:** Different biomarkers were evaluated in hypothalamus, liver and gonads, as well as the plasma sexual hormone quantification. The fish were exposed to three PCM concentrations: 0.25, 2.5 and 25 $\mu g.L^{-1}$ and to a control group (solvent acetone 0.0003%). After 14 days of exposure, fish were anesthetized, for blood sampling and biometrics, and after euthanasia, the tissues were sampled. Plasma was used for 11- keto testosterone and 17β - estradiol quantification. The hypothalamus was collected for brain aromatase (*cyp19a1b*) gene expression; the liver for the vitellogenin (*vtg*) gene expression and biochemical biomarkers; and gonad for the biochemical and histological biomarkers analyses. **Results:** No alterations were observed in the hormone's levels, sexual maturation level or in *cyp19a1b* and *vtg* gene expression. In the liver the non-protein thiols concentration increased at 2.5 $\mu g.L^{-1}$ of PCM, and the superoxide dismutase (SOD) activity was reduced at 0.25 $\mu g.L^{-1}$ of PCM. In gonads, glutathione S-transferase (GST) activity decreased and SOD activity increased at 25 $\mu g.L^{-1}$ of PCM, while the glutathione peroxidase (GPx) activity reduced after exposure to both PCM concentrations. **Conclusion:** The results showed that environmental concentrations of PCM can cause alterations in the antioxidant system, mainly in the gonads of *R. quelen* males. However, without significant change in the hormones levels or in the expression of genes related to the reproduction axis. These alterations occurred at concentrations already found in aquatic environment, including in Brazil.

Keywords: acetaminophen; endocrine disruptor; emerging contaminant; fish.

Resumo: Objetivo: O paracetamol (PCM), ou acetaminofeno, é um dos medicamentos mais utilizados no tratamento humano da dor e da febre. Por ser frequentemente encontrado no ambiente aquático, o objetivo deste estudo foi investigar os efeitos do PCM no eixo reprodutivo de machos do bagre *Rhamdia quelen*. **Métodos:** Diferentes biomarcadores foram avaliados no hipotálamo, fígado e gônadas, bem como a quantificação de hormônios sexuais. Os peixes foram expostos a três concentrações de PCM: 0,25, 2,5 e 25 µg.L^{-1} e ao grupo controle (solvente acetona 0,0003%) e após 14 dias foram anestesiados para coleta de sangue e biometria, e a eutanásia foi feita para coleta de tecidos. Do sangue foi obtido plasma para quantificação de 11- keto testosterona e 17β - estradiol. O hipotálamo foi coletado para a expressão do gene da aromatase cerebral (*cyp19a1b*); o fígado para a expressão do gene da vitelogenina (*vtg*) e biomarcadores bioquímicos; e gônadas para os biomarcadores bioquímicos e histológicos. **Resultados:** Não foram observadas alterações nos níveis hormonais, no nível de maturação sexual ou na expressão dos genes *cyp19a1b* e *vtg*. No fígado, a concentração de tióis não protéicos aumentou em 2,5 µg.L^{-1} de PCM, e a atividade da superóxido dismutase (SOD) foi reduzida em 0,25 µg.L^{-1} de PCM. Nas gônadas, a atividade da glutationa S-transferase (GST) diminuiu e a atividade da SOD aumentou em 25 µg.L^{-1} de PCM, enquanto a atividade da glutationa peroxidase (GPx) diminuiu após a exposição a ambas as concentrações de PCM. **Conclusões:** Esses resultados mostraram que concentrações ambientais de PCM podem causar alterações no sistema antioxidante, principalmente nas gônadas de machos de *R. quelen*, porém sem alterar significativamente os níveis hormonais ou a expressão de genes relacionados ao eixo reprodutivo. Essas alterações podem ocorrer em concentrações já encontradas em ambientes aquáticos, inclusive no Brasil.

Palavras-chave: acetaminofeno; desregulador endócrino; contaminante emergente; peixe.

Graphical Abstract



1. Introduction

The population growth leads to the increased use of pharmaceutical products to improve life quality. They are emerging contaminants due to the presence in water in low concentrations and not included in legislation related to water quality. Therefore, they are an environmental issue once they can reach the water bodies, by the sewage containing human and animal excreta, industrial waste or by incorrect disposal (Ebele et al., 2017).

The analgesic and antipyretic drug, such as paracetamol (PCM), also called acetaminophen is one of the most therapeutic molecules used, and can consequently contaminate aquatic ecosystems (Parolini, 2020). In Brazil, for example, it has already been considered one of the drugs with the highest prevalence in use by children (Pizzol et al., 2016). However, it is a drug responsible for the poisoning of many adults in the country, whether intentional or not (Okuyama et al., 2022). PCM is being found in water bodies worldwide (Phong Vo et al., 2019; Wilkinson et al., 2022). The global average detected in surface waters is around 0.161 µg.L^{-1}, with a maximum of 230 µg.L^{-1}. In the Latin America and Caribbean region, the average is 0.74 µg.L^{-1} with a maximum of 37 µg.L^{-1} (Aus der Beek et al., 2016). In Brazil, concentrations of 3 and up to 42 µg.L^{-1} were found (Veras et al., 2019).

Adverse effects have already been found in aquatic biota, either in invertebrates and/or in vertebrates, such as oxidative stress in the microcrustacean *Daphnia magna* Straus, 1960 (Liu et al., 2019), neurotoxicity in the estuarine polychaeta *Hediste diversicolor* O.F. Müller, 1776 (Barbosa et al., 2020),

nephrotoxicity in the catfish *Rhamdia quelen* Quoy & Gaimard, 1824 (Perussolo et al., 2019) and alterations in embryonic development in the fish *Cyprinus carpio* Linnaeus, 1758 (Gutiérrez-Noya et al., 2021). Besides these effects, there is a concern related to its potential action as an endocrine disruptor (Ebele et al., 2017). It has been demonstrated that PCM is able to alter sex hormones levels (Cohen et al., 2018) and estrogen/androgen receptor (Kar et al., 2021). In a 21-day subchronic water exposure, for example, 2.5 $\mu g.L^{-1}$ of PCM was able to reduce testosterone and increase estradiol levels in catfish *Rhamdia quelen*, in addition to reducing spermatogenesis (Guiloski et al., 2017).

To evaluate the endocrine disrupting action of contaminants in fish, in addition to hormones and their receptors quantification, other parameters can be measured, including the expression of genes related to reproductive cycle. In most studies and in OECD guidelines, vitellogenin is used as a biomarker of endocrine disruption in fish. However, impairment of the liver, for example, the organ that produces this protein, can also lead to changes in its expression or production in some cases (Baumann et al., 2020). Thus, other organs and parameters that may result in indirect changes in these deregulation parameters must be evaluated. In this way, hypothalamus, pituitary, liver and gonad are the targeted tissues for composing the HPLG axis also known as the reproductive axis (Hachfi et al., 2012).

The native Neotropical species *Rhamdia quelen* can be used to evaluate the endocrine effects of drugs (Guiloski et al., 2017; Vicentini et al., 2022). This catfish is one of the favorites of local aquaculture due to its commercial value and its growing conditions (Montanha et al., 2011). It has distribution from the south of South America to the south of Mexico, being distributed in the regions of Brazil (Gomes et al., 2000). There are reports of these animals in the state of Goiás (Araújo & Tejerina-Garro, 2007), Rio de Janeiro (Venancio et al., 2010), Minas Gerais (Casatti & Castro, 2006), São Paulo (Negrelli et al., 2019), Paraná (Carvalho et al., 2022), Santa Catarina (Guerini et al., 2014) and Rio Grande do Sul (Corrêa et al., 2010), for example. It is an omnivorous species, whose sexual maturity is reached in the first year of life and has two reproductive peaks (spring and summer) (Montanha et al., 2011).

The present study aimed to investigate the PCM potential effect as an endocrine disruptor in males of the Neotropical fish *Rhamdia quelen*, assessing biochemical and molecular biomarkers such as vitellogenin and brain aromatase in the HPLG axis.

2. Material and Methods

2.1. Animal acclimation and experimental design

The adult males of *Rhamdia quelen* fish (13.89 ± 2.10 cm, 24.73 ± 1.85 g) obtained in the pisciculture of the Universidade Estadual do Oeste do Paraná (UNIOESTE) were acclimatized for 30 days in laboratory, under controlled conditions (constant aeration, temperature of 25 ± 2 °C, photoperiod of 12h and daily feed). After this period, the animals were separated into four different groups of exposure: three test groups (0.25, 2.5 and 25 $\mu g.L^{-1}$ of PCM) and one control (acetone 0.0003%), in three replicates per group. During the experiment, 50% of water was daily exchanged, containing 10% of the initial paracetamol/acetone concentration, according to degradation data found by Perussolo et al. (2019). The experimental conditions were the same as the acclimation period (constant aeration, temperature of 25 ± 2 °C, photoperiod of 12h and daily feed). After 14 days of exposure, each fish was individually exposed immersed for 1-3 minutes in a glass container with benzocaine in a concentration of 0.1 $\mu g.L^{-1}$. The blood was taken from the caudal vein and centrifuged (2000×g for 5 minutes). The plasma obtained was used for hormonal quantification. The fish was immersed again in benzocaine bath and euthanasia was performed by medullary section. After this, hypothalamus, liver and gonad were taken and frozen at –80 °C for molecular and biochemical biomarkers. A portion of each gonad was prepared for the histological analysis. This experiment was approved by the Ethics Committee for the Use of Animals of the Universidade Federal do Paraná (UFPR) under the number 995. The PCM concentration during the exposure and water physico-chemical parameters are described in Perussolo et al. (2019).

This study was carried out following the rules and approved by the Ethics Committee on Animal Use of the Universidade Federal do Paraná (UFPR) under number 995.

2.2. Gonado and hepatosomatic index

The gonadosomatic (GSI) and hepatosomatic (HSI) indexes were calculated by the following Equation 1:

$$GSI \text{ or } HSI = (gonad \text{ or } liver \text{ } weight / body \text{ } weight) \times 100 \quad (1)$$

2.3. Hormone quantification

To determine 17β- estradiol and 11- keto testosterone levels, 25 µL and 50 µL of plasma were used, respectively. The method applied was enzyme-linked Immunosorbent assay (ELISA) by the use of commercially available kits (17β- Estradiol from IBL International and 11- Keto Testosterone from Cayman Chemical). Analyzes were performed as described by the manufacturers and concentrations were expressed in $pg.mL^{-1}$.

2.4. Molecular biomarkers

Hypothalamus and liver were used to *cyp19a1b* and *vtg* expression, respectively. The total RNA was extracted with the RNeasy minikit (QIAGEN), following the manufacturer's instructions. After extraction, 1 µg of RNA from hypothalamus and 2 µg from the liver was treated with DNAse I (Invitrogen). With Reverse Transcription System (Promega), the treated RNA was retrotranscribed into complementary DNA (cDNA). This material was submitted to a quantitative PCR (qPCR).

For *cyp19a1b*, 80 ng of cDNA, 12.75 µL of Master Mix (Promega), 0.8 µM of each primer (*cyp19a1b*Fw: 5′-GCAGAAGTTACCGTTGATGGA-3′ and *cyp19a1b*Rv: 5′-TTG GCTTTAGGGAAGAAC-3′, Silva de Assis et al., 2018) and RNAse free water to a final volume of 25 µL were added at each reaction. For *vtg*, 40 ng of cDNA, 10.2 µL of Master Mix (Promega), 1.2 µM of each primers (*vtg*Fw: 5'- CATCATTGCTCGTGCTGTCA- 3'; *vtg*Rv: 5'- AGAGGCAACCACAACCTGTA-3', Fernandes et al. (2021) and RNAse free water for a final volume of 20 µL were used. The beta actin gene was applied as reference gene for the both *cyp19a1b* and *vtg* genes expression analysis (βacFw: 5′-CACTGGTATTGTGATGGACTC-3′ and βacRv: 5′-TCATGAGGTAGTCAGTCAGGTC-3′, Silva de Assis et al., 2018). For each treatment was used RNA of all the group samples and for each plate was used a control of reaction. The qPCR was carried in two replicates for each sample in 96-well plates (MicroAmp®) in a StepOnePlus Real Time PCR System device. For *cyp19a1b* and beta actin, the cycling parameters were 2 minutes at 95 °C and 40 cycles of 15 seconds at 95 °C, 15 seconds at 59 °C and 30 seconds at 72 °C. For *vtg*, the parameters were 2 minutes at 95 °C and 40 cycles of 15 seconds at 95 °C, 15 seconds at 59 °C, 40 seconds at 72 °C. For all these genes, controls of no reverse transcription of samples and control of the plate reaction was carried out to ensure that no genomic DNA was present. The relative expression of *cyp19a1b* and *vtg* was calculated following Livak & Schmittgen (2001).

2.5. Biochemical biomarkers

Liver and gonad were homogenized in potassium phosphate buffer 0.1 M pH 7, in proportion 1:10 (weight/volume). The samples were centrifuged at 15.000 x *g* for 30 minutes at 4 °C. Supernatant aliquots for all analyzes were kept at -80 °C.

The total proteins were quantified following the method of Bradford (1976). Ethoxyresorufin-*O*-deethylase (EROD) activity was measured based on Burke & Mayer (1974) using 2.6 µM of nicotinamide adenine dinucleotide phosphate (NADPH) and 2.6 µM of 7-ethoxyresorufin solution. The activity of the glutathione *S*-transferase (GST) enzyme was measured using 3 mM of 1-chloro-2,4-nitrobenzene (CDNB) and L-Glutathione reduced, based on Keen et al. (1976). Superoxide dismutase (SOD) activity was measured according to Gao et al. (1998), using 15 mM of pyrogallol. The activity of catalase (CAT) was based on Aebi (1984), in which the consumption of the 20 mM hydrogen peroxide reaction is measured. Glutathione peroxidase (GPx) activity was measured by the method of Hafeman et al. (1974), in which the consumption of the 1.5 mM hydrogen peroxide present in a solution is measured. The non-protein thiols concentration (GSH) was measured based on Sedlak & Lindsay (1968) and for lipoperoxidation (LPO) level measurement was used the FOX method (Ferrous Oxidation), proposed by Jiang et al. (1992).

2.6. Histological biomarker

Gonadal fragments were fixed in ALFAC solution (alcohol 80%, formaldehyde 40% and acetic acid 100%) for 20 h. After this, they were dehydrated in alcoholic series (70%, 80%, 90% and 100%), diaphanized in xylol and included in Paraplast®. Seven µm thick sections were stained with hematoxylin-eosin (HE) and the slides were analyzed under light microscopy for evaluation of the gonadal stage.

2.7. Data analysis

The biomarkers results were submitted to generalized linear model (GLM) analysis, suggested for ecotoxicological data (Szöcs & Schäfer, 2015), after an outlier analysis. The models were adjusted through Gamma, Gamma (identity), Gaussian, Inverse Gaussian and quasipoisson error distributions.

One-way analysis of variance (ANOVA) was performed, with lsmeans contrasts ($p \leq 0.05$). Non-metric multidimensional scaling (nMDS) was performed for the integrated comparison of all biomarkers, using MASS and vegan libraries (Oksanen et al., 2015). The statistical analyses were made in R (3.2.2. version).

3. Results

3.1. Gonado and hepatosomatic index

The gonadosomatic (GSI) and hepatosomatic (HSI) indexes did not present significant alterations compared to control (Figure 1). However, fish exposed to 25 $\mu g.L^{-1}$ of PCM have presented higher HSI than those exposed to 0.25 $\mu g.L^{-1}$ ($p = 0.031$; Figure 1B).

3.2. Hormone quantification

Plasma 17β-estradiol and 11- keto testosterone levels did not present significant alterations in fish exposed to PCM, but fish exposed to 2.5 and 25 $\mu g.L^{-1}$ of PCM showed a reduction of approximately 66% in 17β-estradiol level comparing to control group (Figure 2).

3.3. Molecular biomarkers

The hypothalamic *cyp19a1b* and the hepatic *vtg* expression did not change significatively by PCM exposure (Figure 3).

3.4. Biochemical biomarkers

In the liver, EROD and GST activities, enzymes related to the biotransformation process, were not significantly altered in the PCM presence. Although, GSH concentration ($p < 0.001$) presented a significant increase in fish at 2.5 $\mu g.L^{-1}$ group compared to the fish from control and 0.25 $\mu g.L^{-1}$ groups. Regarding the antioxidant system, CAT and GPx enzymes activities were not significantly affected in fish PCM exposed groups. However, SOD activity was lower in fish at 0.25 $\mu g.L^{-1}$ group when compared to control and 25 $\mu g.L^{-1}$ groups ($p < 0.001$).

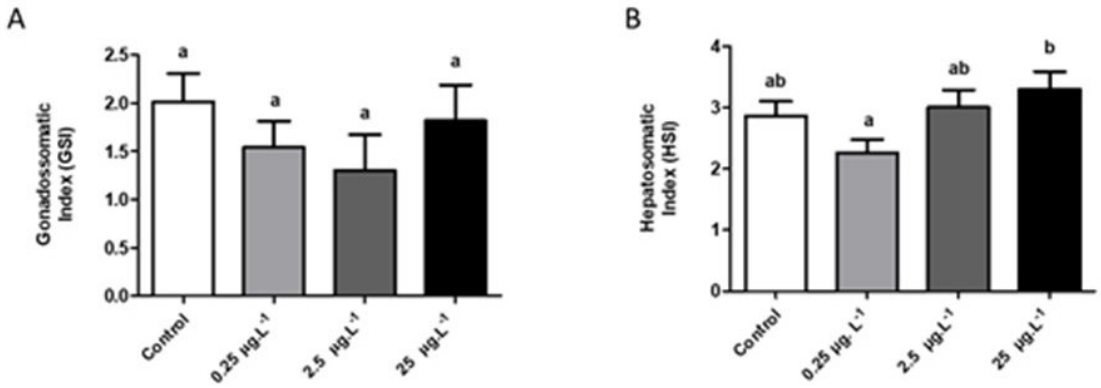


Figure 1. Gonado (A) and hepatosomatic index (B) of *Rhamdia quelen* males exposed to paracetamol. The results are expressed as mean ± standard error. Different letters indicate significant differences ($p \leq 0.05$).

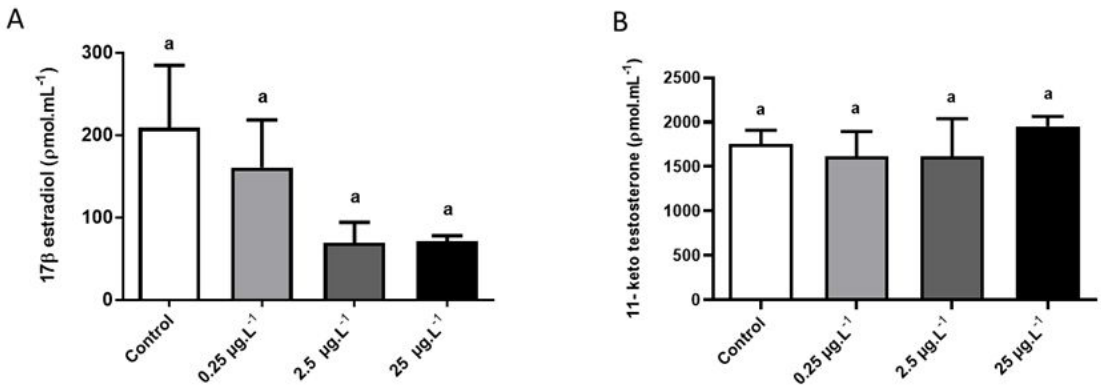


Figure 2. 17β- estradiol (A) and 11- keto testosterone (B) of *Rhamdia quelen* males exposed to paracetamol. The results are expressed as mean ± standard error. Different letters indicate significant differences ($p \leq 0.05$).

The fish at 0.25 µg.L^{-1} group presented lower LPO levels than those observed in the fish from other groups, including control ($p < 0.001$; (Figure 4).

In gonads, GST activity decreased in fish from 25 µg.L^{-1} group compared to control ($p = 0.029$). SOD had its activity increased by 75% in fish from 0.25 µg.L^{-1} group compared to those from 25 µg.L^{-1} ($p < 0.01$). Fish from groups exposed to PCM showed a reduction in GPx activity when compared to control ($p < 0.001$).

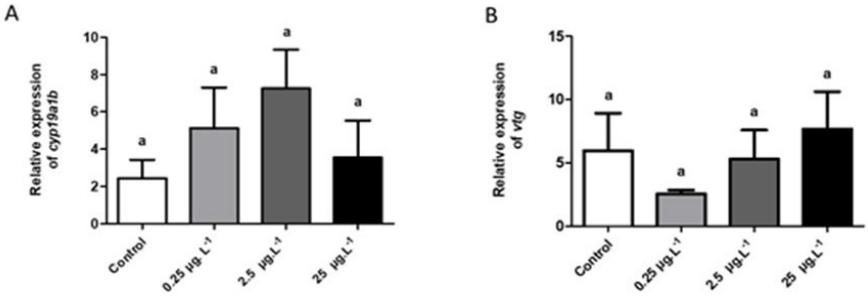


Figure 3. Hypothalamic *cyp19a1b* and hepatic *vtg* expression in *Rhamdia quelen* males exposed to paracetamol. The results are expressed as mean ± standard error. Different letters indicate significant differences ($p \leq 0.05$).

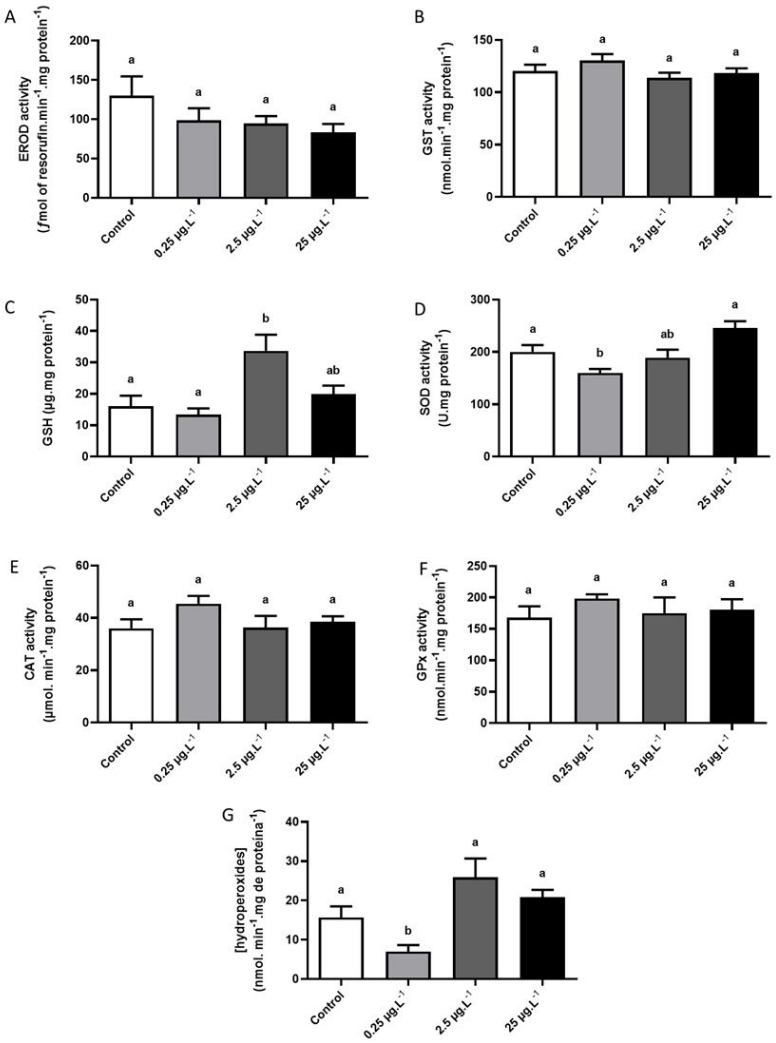


Figure 4. Biochemical biomarkers in the liver of *Rhamdia quelen* males exposed to paracetamol. (A) EROD; (B) GST; (C) GSH; (D) SOD; (E) CAT; (F) GPx and (G) LPO. The results are expressed as mean ± standard error. Different letters indicate significant differences ($p \leq 0.05$).

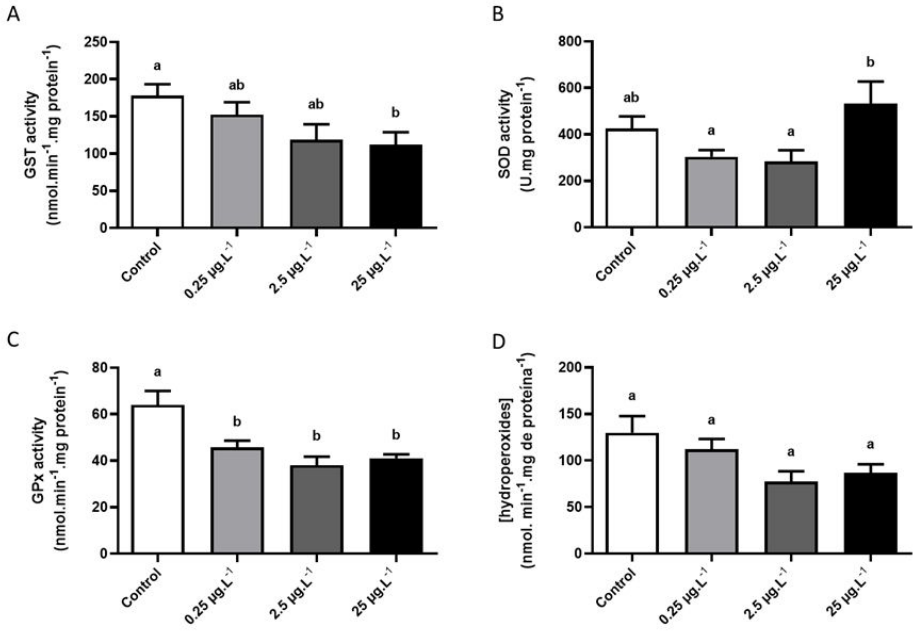


Figure 5. Biochemical biomarkers in the gonad of *Rhamdia quelen* males exposed to paracetamol. (A) GST; (B) SOD; (C) GPx and (D) LPO. The results are expressed as mean ± standard error. Different letters indicate significant differences ($p \leq 0.05$).

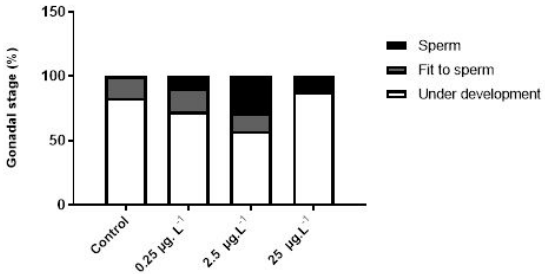


Figure 6. Frequency of different gonadal maturation stage in *Rhamdia quelen* males exposed to paracetamol.

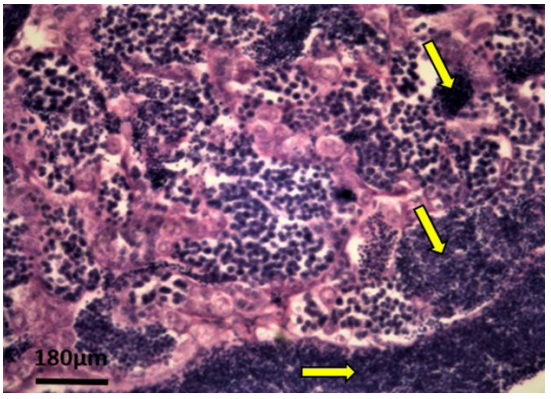


Figure 7. Developing testis of *Rhamdia quelen* male, few seminiferous tubules containing sperm (yellow arrow). H/E.

LPO levels showed no difference between treatments (Figure 5).

3.5. Histological biomarker

Most of the gonads were in development stage, with no sperm formation (Figure 6). Some animals, both in control and PCM treatments groups, were either able to sperm (seminiferous tubules full of sperm, Figure 7) or had already sperm (seminiferous tubules were not so full of sperm, especially in the center of the gonad).

3.6. Multivariate analysis

The nMDS presented a stress of 0.2248. The PCM groups that were most spatially distant from the control group, focusing on the right quadrants (Figure 8).

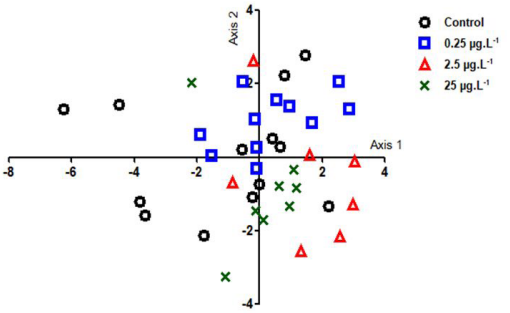


Figure 8. Multivariate analysis (nMDS) of *Rhamdia quelen* males exposed to paracetamol, with the biomarkers (except histological biomarker) represented in two axis.

3. Discussion

In our study, paracetamol was able to change biochemical biomarkers in tissues related to reproduction. During the PCM metabolism, the cell produces N-acetyl-p-benzoquinoneamine (NAPQI), a toxic bioactive metabolite that must be excreted for not causing oxidative stress (Santos et al., 2020). One way for this neutralization is through conjugation with glutathione that significantly increased in fish exposed to 2.5 $\mu g.L^{-1}$ PCM after 14 days. In the presence of high concentrations of PCM, cofactors such as sulfate, glucuronic acid and glutathione are eventually depleted and the metabolite can accumulate, causing damage for the cells (Masteling et al., 2016). However, at the highest tested concentration an increase in GSH was not observed as well as lipid-type cell damage. The GSH induction occurred at the median concentration. The increase in GSH indicates the reduction of paracetamol-induced liver damage (drug-induced liver damage) in these conditions. This prevents damage caused by NAPQI, such as oxidative stress, nuclear DNA fragmentation and mitochondrial dysfunction (Saide et al., 2019). Acting on the electron transport chain in the mitochondria, NAPQI can influence the body's energy metabolism and lead to tissue necrosis that is being avoided, in this case, by neutralizing the paracetamol metabolite (Ramachandran & Jaeschke, 2019).

Unlike the hepatic tissue, the gonadal tissue showed alterations of the enzyme activity of the biotransformation system (GST) and the antioxidant system (SOD and GPx) after 14 days of exposure. These results are differently than expected, since the enzyme activities changes in this tissue are more difficult to detect than in the liver (Hamed et al., 2016). In this case, there was a reduction in GST and GPx activities at 25 $\mu g.L^{-1}$. This could mean a lack of the substrate such as GSH, a cofactor for these enzymes. Although it was not measured in this tissue, it can be induced to prevent the effects of NAPQI, the bioactive metabolite. As PCM was able to alter the biotransformation and antioxidant system in gonads after 14 days of exposure, it was sufficient to prevent damage to macromolecules such as lipids. Therefore, all the alterations can alter the gonads homeostasis and cause some effect in the reproduction such as the disruption of gonad development and sex differentiation, affecting the number of germ cells, leading to episodes of sterility, mainly at a long-term exposure (Delbes et al., 2022).

About sex hormone levels of *Rhamdia quelen* males, 17β-estradiol and 11-keto testosterone did not show significant changes. Despite estrogen showing a reduction in the highest concentrations tested, this difference was not significant. No alterations for estradiol and 11-keto testosterone levels was observed in *Danio rerio* Hamilton, 1822 exposed to 0.5 and 10 $\mu g\ L^{-1}$ of PCM for 6 weeks (Galus et al., 2013). However, for the same species used in this study, *Rhamdia quelen*, exposed to 0.25 $\mu g.L^{-1}$ and 2.5 $\mu g.L^{-1}$ of PCM for 21 days, a reduction in 11- keto testosterone levels and an increase in estradiol levels were observed (Guiloski et al., 2017). Comparing the results it seems that exposure time plays a role in the potential of PCM as endocrine disruptor, at least in male fish. These results may influence other parameters, such as the expression of some genes related to reproduction.

In this study, important genes for the fish reproductive process in the hypothalamus and liver were evaluated. Based on the literature we expected a change in the hypothalamic *cyp19a1b* and the hepatic *vtg* expression, as it has already been observed in other fish species (Dang, 2014; Lin et al., 2018). It has been reported that exposure to PCM for 90 days resulted in concentration-dependent increase of the vitellogenin protein concentration in liver of male medaka fish (*Oryzias latipes* Temminck & Schlegel, 1846), from 0.095 to 950 $\mu g.L^{-1}$ (Kim et al., 2012). Therefore, these gene expressions were not significantly affected by PCM after 14 days of exposure. Generally, the alterations are observed in some fish species after longer-term exposure. The tested conditions in this study such as exposure time and PCM concentration were not able to induce vitellogenin and brain aromatase expression. The *vtg* induction is expected, since this protein is not normally expressed in males, only in cases of estrogen induction (Dang et al., 2011; Dang, 2014). The same was expected for the *cyp19a1b*, responsible for converting estradiol into testosterone, with estradiol being the signaling hormone for the production of vitellogenin (Silva de Assis et al., 2018).

Vitellogenin induction can also be indirectly visualized by histology, through the yolk present in reproductive cells in females (Juin et al., 2017). However, if vitellogenin is somehow being induced in males, other changes should be seen, such as a reduction in male gametes, developmental delay or the presence of female germ cells (Forner-Piquer et al., 2020; Mushirobira et al., 2021).

Therefore, in the present study no alterations were observed in the histology of the gonads, being possible to visualize all stages of gametogenesis. This results is are the same observed for males of *Danio rerio* after 6 weeks of PCM exposure at 0.5 and 10 $\mu g.L^{-1}$, that showed all spermatogenesis stages, including spermatozoa, with no observable effect on testis morphology (Galus et al., 2013). For the same species of this study, *Rhamdia quelen*, exposed for 21 days at 0.25 $\mu g.L^{-1}$, males showed similar conditions with cells at different stages of development. However, at exposure to 2.5 $\mu g.L^{-1}$, spermatogenesis inhibition was observed, emphasizing that, PCM can become estrogenic in a longer-term exposure (Guiloski et al., 2017). In the present study, the non-visualization of alterations in the gonads can means that the biotransformation and antioxidant system altered were not able to cause histological damage.

Vitellogenin and gonadal histology have been showing sensitive endpoints for fathead minnow, medaka and zebrafish in 21-day assays for most endocrine disruptors (Dang et al., 2011). These endpoints can be different for different organisms. For crustaceans, for example, the young number per adult or per brood were used as disruption biomarker. For *Moina macrocopa* Straus, 1820, after 7 days of exposure to PCM, adults had no change in the offspring number, but between concentrations of 950 and 8580 $\mu g.L^{-1}$ (Kim et al., 2012). For fish, concentration and exposure time were important for the biochemical responses of detoxification and the antioxidant system in the liver of *Oncorhynchus mykiss* Walbaum, 1792, an important reproductive organ for producing vitellogenin (Ramos et al., 2014). For *Rhamdia quelen*, vitellogenin and gonadal histology were not sensitive for PCM exposure for 14 days exposure. The present study demonstrated that parameters normally indicated for endocrine disruption studies may not always be used in a general way. These parameters related to reproductive axis may be affected in the long-term exposure, making the exposure time an important factor to be evaluated.

Many factors can affect an endocrine disruptor effect. Some modulating factors must be considered, such as temperature, photoperiod, water quality, feeding rate, feed composition, population density, population sex ratio and individual genetics (Wang, 2018). For example, differences of 48 h EC50 results from PCM to *Daphnia magna* have already been described. One of the possible justification for such results is the nutrition. This can affect glutathione metabolism, which is a metabolic pathway used for PCM metabolism and excretion, and feed composition is something that may vary among studies (Nunes et al., 2014). For PCM, other factors can also be considered, such as size and exposure time, are important factors for PCM toxicity. For example, in the study of Guiloski et al. (2017) males' fish of *R. quelen* heavier and bigger than the fish of this study, were exposed to PCM for 21 days. They presented a reduction in the hepatic activity of enzymes in the biotransformation system and an increase in enzymes in the antioxidant system, unlike what was found in the present study. This information is important for studies for studies related to endocrine disruption and for the evaluation of tissues linked to reproduction.

In this study, by the univariate and multivariate analyses, the most biochemical changes were observed at 2.5 and 25 $\mu g.L^{-1}$. These concentrations have already been found in surface waters in Brazil. Concentrations of 18 $\mu g.L^{-1}$ of PCM were found in human environments close to the Amazon Basin (Rico et al., 2021). In Pernambuco, concentrations of 3 to 42 $\mu g.L^{-1}$ were found (Veras et al., 2019). Concentrations close to those tested in this study, from 3.4 to 30.4 $\mu g.L^{-1}$, were detected in rivers in São Paulo, a region where *Rhamdia quelen* has already been found (Starling et al., 2019). In Curitiba, in the state of Paraná, concentrations of up to 0.37 $\mu g.L^{-1}$ were found, with 0.26 $\mu g.L^{-1}$ in one of the most polluted rivers in the city (Kyamer et al., 2015). In the same region, in a public water supply reservoir, 0.69 $\mu g.L^{-1}$ of PCM was found (Calado et al., 2019). The concentrations used in this study are environmentally relevant, making studies with endocrine physiology of *Rhamdia quelen* exposed to emerging contaminants of great importance, mainly due to its commercial value and its presence in different aquatic environment of the country.

4. Conclusion

Paracetamol can cause sublethal effects to males of *Rhamdia quelen* exposed to low concentrations for 14 days. Effects were found mainly in relation to biochemical biomarkers in the gonads. However, these exposure conditions are not sufficient to cause changes in hormones levels or genes related to the reproductive axis expression. The results compared with the literature, showed that factors such as fish weight and size, gonad maturation and exposure time are important to assess the effects of an endocrine disruptor such as paracetamol.

The present study demonstrated that paracetamol concentrations already found in aquatic environments, including Brazil, can cause sublethal effects in tissues related to the reproductive system. At a long-term exposure can lead reproductive changes due to the alteration of homeostasis, such as biochemical, genetic and histological changes of this important axis in an important specie for South America aquiculture and biodiversity.

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References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121-126. PMid:6727660. http://dx.doi.org/10.1016/S0076-6879(84)05016-3.

Araújo, N.B., & Tejerina-Garro, F.L., 2007. Fish diversity and composition in Cerrado streams, Ribeirão Ouvidor basin, upper Paraná River basin, Goiás, Brazil. Rev. Bras. Zool. 24(4), 981-990. http://dx.doi.org/10.1590/S0101-81752007000400014.

Aus der Beek, T., Weber, F., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., & Küster, A., 2016. Pharmaceuticals in the environment: global occurrences and perspectives. Environ. Toxicol. Chem. 35(4), 823-835. PMid:26666847. http://dx.doi.org/10.1002/etc.3339.

Barbosa, I., Pizarro, I., Freitas, R., & Nunes, B., 2020. Antioxidative and neurotoxicity effects of acute and chronic exposure of the estuarine polychaete *Hediste diversicolor* to paracetamol. Environ. Toxicol. Pharmacol. 77, 103377. PMid:32251999. http://dx.doi.org/10.1016/j.etap.2020.103377.

Baumann, L., Holbech, H., Schmidt-Posthaus, H., Moissl, A.P., Hennies, M., Tiedmann, J., Weltje, L., Segner, H., & Braunbeck, T., 2020. Does hepatotoxicity interfere with endocrine activity in zebrafish (*Danio rerio*)? Chemosphere 238, 124589. PMid:31437630. http://dx.doi.org/10.1016/j.chemosphere.2019.124589.

Bradford, M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72(1-2), 248-254. PMid:942051. http://dx.doi.org/10.1016/0003-2697(76)90527-3.

Burke, M.D., & Mayer, R.T., 1974. Ethoxyresorufin: direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metab. Dispos. 2(6), 583-588. PMid:4155680.

Calado, S.L.M., Esterhuizen-Londt, M., Assis, H.C.S., & Pflugmacher, S., 2019. Phytoremediation: green technology for the removal of mixed contaminants of a water supply reservoir. Int. J. Phytoremediation 21(4), 372-379. PMid:30656959. http://dx.doi.org/10.1080/15226514.2018.1524843.

Carvalho, B.M., Faria, L., Miiller, N.O.R., Spach, H.L., & Vitule, J.R.S., 2022. Length-weight relationships of native and non-native fishes in a subtropical coastal river of the Atlantic Rain Forest. Acta Limnol. Bras. 34, e5. http://dx.doi.org/10.1590/s2179-975x2821.

Casatti, L., & Castro, R.M.C., 2006. Testing the ecomorphological hypothesis in a headwater riffles fish assemblage of the rio São Francisco, southeastern Brazil. Neotrop. Ichthyol. 4(2), 203-214. http://dx.doi.org/10.1590/S1679-62252006000200006.

Cohen, I.V., Cirulli, E.T., Mitchell, M.W., Jonsson, T.J., Yu, J., Shah, N., Spector, T.D., Guo, L., Venter, J.C., & Telenti, A., 2018. Acetaminophen (paracetamol) use modifies the sulfation of sex hormones. EBioMedicine 28, 316-323. PMid:29398597. http://dx.doi.org/10.1016/j.ebiom.2018.01.033.

Corrêa, F., Claudino, M.C., & Garcia, A.M., 2010. Guia fotográfico e aspectos da biologia dos principais peixes de água doce do Parque Nacional da Lagoa do Peixe, RS. R. Eletr. Cad. Ecol. Aquat. 5, 28-43.

Dang, Z., 2014. Fish biomarkers for regulatory identification of endocrine disrupting chemicals. Environ. Pollut. 185, 266-270. PMid:24316064. http://dx.doi.org/10.1016/j.envpol.2013.11.006.

Dang, Z., Li, K., Yin, H., Hakkert, B., & Vermeire, T., 2011. Endpoint sensitivity in fish endocrine disruption assays: regulatory implications. Toxicol. Lett. 202(1), 36-46. PMid:21295121. http://dx.doi.org/10.1016/j.toxlet.2011.01.016.

Delbes, G., Blázquez, M., Fernandino, J.I., Grigorova, P., Hales, B.F., Metcalfe, C., Navarro-Martín, L., Parent, L., Robaire, B., Rwigemera, A., van Der Kraak, G., Wade, M., & Marlatt, V., 2022. Effects of endocrine disrupting chemicals on gonad development: mechanistic insights from fish and mammals. Environ. Res. 204(Pt B), 112040. PMid:34509487. http://dx.doi.org/10.1016/j.envres.2021.112040.

Ebele, A.J., Abdallah, M.A., & Harrad, S., 2017. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerg. Contam. 3(1), 1-16. http://dx.doi.org/10.1016/j.emcon.2016.12.004.

Fernandes, L.P.S., Mathias, F.T., Richardi, V.S., & Silva de Assis, H.C., 2021. Cloning, partial sequencing and 17β-estradiol modulation of hepatic vitellogenin gene of the Neotropical catfish *Rhamdia quelen.* J. Appl. Ichthyology 37(4), 545. http://dx.doi.org/10.1111/jai.14203.

Forner-Piquer, I., Beato, S., Piscitelli, F., Santangeli, S., Di Marzo, V., Habibi, H.R., Maradonna, F., & Carnevali, O., 2020. Effects of BPA on zebrafish gonads: focus on the endocannabinoid system. Environ. Pollut. 264, 114710. PMid:32417572. http://dx.doi.org/10.1016/j.envpol.2020.114710.

Galus, M., Kirischian, N., Higgins, S., Purdy, J., Chow, J., Rangaranjan, S., Li, H., Metcalfe, C., & Wilson, J.Y., 2013. Chronic, low concentration exposure to pharmaceuticals impacts multiple organ systems in zebrafish. Aquat. Toxicol. 132-133, 200-211. PMid:23375851. http://dx.doi.org/10.1016/j.aquatox.2012.12.021.

Gao, R., Yuan, Z., Zhao, Z., & Gao, X., 1998. Mechanism of pyrogallol autoxidation and determination of superoxide dismutase enzyme activity. Bioelectrochem. Bioenerg. 45(1), 41-45. http://dx.doi.org/10.1016/S0302-4598(98)00072-5.

Gomes, L.C., Golombieski, J.I., Gomes, A.R.C., & Baldisserotto, B., 2000. Biologia do jundiá *Rhamdia quelen* (Teleostei, Pimelodidae). Cienc. Rural 30(1), 179-185. http://dx.doi.org/10.1590/S0103-84782000000100029.

Guerini, S., Prado, G.P., & Passos, M.G., 2014. Hábito alimentar de *Rhamdia quelen* (Siluriformes: Pimelodidae) em um trecho do Rio Bonito no município de São Domingos, Santa Catarina. Uningá Rev. 18, 10-15.

Guiloski, I.C., Ribas, J.L.C., Piancini, L.D.S., Dagostim, A.C., Cirio, S.M., Fávaro, L.F., Boschen, S.L., Cestari, M.M., Cunha, C., & Assis, H.C.S., 2017. Paracetamol causes endocrine disruption and hepatotoxicity in male fish *Rhamdia quelen* after subchronic exposure. Environ. Toxicol. Pharmacol. 53, 111-120. PMid:28545014. http://dx.doi.org/10.1016/j.etap.2017.05.005.

Gutiérrez-Noya, V.M., Gómez-Oliván, L.M., Ramírez-Montero, M.C., Islas-Flores, H., Galar-Martínez, M., & García-Medina, S., 2021. Survival and malformations rates, oxidative status in early life stages of *Cyprinus carpio* due to exposure to environmentally realistic concentrations of paracetamol. Sci. Total Environ. 768, 144585. PMid:33454465. http://dx.doi.org/10.1016/j.scitotenv.2020.144585.

Hachfi, L., Couvray, S., Simide, R., Tarnowska, K., Pierre, S., Gaillard, S., Richard, S., Coupé, S., Grillasca, J., & Prévot-D'Alvise, N., 2012. Impact of Endocrine Disrupting Chemicals [EDCs] on Hypothalamic-Pituitary-Gonad-Liver [HPGL] axis in fish. World J. Fish Mar. Sci. 4, 14-30.

Hafeman, D.G., Sunde, R.A., & Hoekstra, W.C., 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J. Nutr. 104(5), 580-587. PMid:4823943. http://dx.doi.org/10.1093/jn/104.5.580.

Hamed, R.R., Saleh, N.S.M., Shokeer, A., Guneidy, R.A., & Abdel-Ghany, S.S., 2016. Glutathione and its related enzymes in the gonad of Nile Tilapia (*Oreochromis niloticus*). Fish Physiol. Biochem. 42(1), 353-364. PMid:26476660. http://dx.doi.org/10.1007/s10695-015-0143-9.

Jiang, Z.Y., Hunt, J.V., & Wolff, S.P., 1992. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal. Biochem. 202(2), 384-389. PMid:1519766. http://dx.doi.org/10.1016/0003-2697(92)90122-N.

Juin, S.K., Sarkar, S., Maitra, S., & Nath, P., 2017. Effect of fish vitellogenin on the growth of juvenile catfish, *Clarias gariepinus* (Burchell,1822). Aquacult. Rep. 7, 16-26. http://dx.doi.org/10.1016/j.aqrep.2017.05.001.

Kar, S., Sangem, P., Anusha, N., & Senthilkumaran, B., 2021. Endocrine disruptors in teleosts: evaluating environmental risks and biomarkers. Aquac. Fish. 6(1), 1-26. http://dx.doi.org/10.1016/j.aaf.2020.07.013.

Keen, J.H., Habig, W.H., & Jakoby, W.B., 1976. Mechanism for the several activities of the glutathione S-transferases. J. Biol. Chem. 251(20), 6183-6188. PMid:977564. http://dx.doi.org/10.1016/S0021-9258(20)81842-0.

Kim, P., Park, Y., Ji, K., Seo, J., Lee, S., Choi, K., Kho, Y., Park, J., & Choi, K., 2012. Effect of chronic exposure to acetaminophen and lincomycin on Japanese medaka (*Oryzias latipes*) and freshwater cladocerans *Daphnia magna* and *Moina macrocopa*, and potential mechanisms of endocrine disruption. Chemosphere 89(1), 10-18. PMid:22560975. http://dx.doi.org/10.1016/j.chemosphere.2012.04.006.

Kyamer, R.D., Mizukawa, A., Ide, A.H., Marcante, L.O., Santos, M.M., & Azevedo, J.C.R., 2015. Determination of anti-inflammatory in water and sediment and its relations with water quality in Alto Iguaçu watershed, Curitiba-PR. R. Bras. Recur. Hidr. 20, 657-667.

Lin, W., Guo, H., Li, Y., Wang, L., Zhang, D., Hou, J., Wu, X., Li, L., Li, D., & Zhang, X., 2018. Single and combined exposure of microcystin-LR and nitrite results in reproductive endocrine disruption via hypothalamic-pituitary-gonadal-liver axis. Chemosphere 211, 1137-1146. PMid:30223329. http://dx.doi.org/10.1016/j.chemosphere.2018.08.049.

Liu, S., Ding, R., & Nie, X., 2019. Assessment of oxidative stress of paracetamol to *Daphnia magna* via determination of Nrf1 and genes related to antioxidant system. Aquat. Toxicol. 211, 73-80. PMid:30954018. http://dx.doi.org/10.1016/j.aquatox.2019.03.014.

Livak, K.J., & Schmittgen, T.D., 2001. Analysis of relative gene expression data using real time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25(4), 402-408. PMid:11846609. http://dx.doi.org/10.1006/meth.2001.1262.

Masteling, R.P., Castro, B.B., Antunes, S.C., & Nunes, B., 2016. Whole-organism and biomarker endpoints in *Daphnia magna* show uncoupling of oxidative stress and endocrine disruption in phenolic derivatives. Ecotoxicol. Environ. Saf. 134, 64-71. http://dx.doi.org/10.1016/j.ecoenv.2016.08.012.

Montanha, F. P., Nagashima, J. C., & Kirnew, M. D., 2011. The physiological characteristics and reproductive of *Rhamdia quelen*. R. Ci. Eletr. Med. Vet. 9(17), 1-8.

Mushirobira, Y., Kamegai, K., Amagai, T., Murata, R., Nagae, M., & Soyano, K., 2021. Expression profiles of hepatic vitellogenin and gonadal zona pellucida subtypes in gray mullet (*Mugil cephalus*) with 17α-ethinylestradiol-induced gonadal abnormality. Aquat. Toxicol. 237, 105863. PMid:34082271. http://dx.doi.org/10.1016/j.aquatox.2021.105863.

Negrelli, D.C., Vieira, D.H.M.D., Tagliavini, V.P., Abdallah, V.D., & Azevedo, R.K., 2019. Molecular and morphological analysis of *Henneguya jundiai* n. sp. (Cnidaria: Myxosporea), a new parasite of the gills of *Rhamdia quelen* in Brazil. Acta Trop. 197, 105053. PMid:31173737. http://dx.doi.org/10.1016/j.actatropica.2019.105053.

Nunes, B., Antunes, S.C., Santos, J., Martins, L., & Castro, B.B., 2014. Toxic potential of paracetamol to freshwater organisms: a headache to environmental regulators? Ecotoxicol. Environ. Saf. 107, 178-185. PMid:24949899. http://dx.doi.org/10.1016/j.ecoenv.2014.05.027.

Oksanen, J.F., Blanchet, G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., & Wagner, H., 2015. Vegan: Community Ecology Package. R Package, version 2.3-0. Vienna: R Foundation for Statistical Computing.

Okuyama, J., Galvão, T., & Silva, M., 2022. Estimates of paracetamol poisoning in Brazil: analysis of official records from 1990s to 2020. Front. Pharmacol. 13, 829547. PMid:35350767. http://dx.doi.org/10.3389/fphar.2022.829547.

Parolini, M., 2020. Toxicity of the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) acetylsalicylic acid, paracetamol, diclofenac, ibuprofen and naproxen towards freshwater invertebrates: a review. Sci. Total Environ. 740, 140043. PMid:32559537. http://dx.doi.org/10.1016/j.scitotenv.2020.140043.

Perussolo, M.C., Guiloski, I.C., Lirola, J.R., Fockink, D.H., Corso, C.R., Bozza, D.C., Prodocimo, V., Mela, M., Ramos, L.P., Cestari, M.M., Acco, A., & Assis, H.C.S., 2019. Integrated biomarker response index to assess toxic effects of environmentally relevant concentrations of paracetamol in a neotropical catfish (*Rhamdia quelen*). Ecotoxicol. Environ. Saf. 182, 109438. PMid:31310901. http://dx.doi.org/10.1016/j.ecoenv.2019.109438.

Phong Vo, H.N., Le, G.K., Hong Nguyen, T.M., Bui, X., Nguyen, K.H., Rene, E.R., Vo, T.D.H., Thanh Cao, N.D., & Mohan, R., 2019. Acetaminophen micropollutant: historical and current occurrences, toxicity, removal strategies and transformation pathways in different environments. Chemosphere 236, 124391. PMid:31545194. http://dx.doi.org/10.1016/j.chemosphere.2019.124391.

Pizzol, T.S.D., Tavares, N.U.L., Bertoldi, A.D., Farias, M.R., Arrais, P.S.D., Oliveira, M.A., Luiza, V.L., & Mengue, S.S., 2016. Use of medicines and other products for therapeutic purposes among children in Brazil. Rev. Saude Publica 50(Suppl. 2), 12s. http://dx.doi.org/10.1590/s1518-8787.2016050006115.

Ramachandran, A., & Jaeschke, H., 2019. Acetaminophen hepatotoxicity: a mitochondrial perspective. Adv. Pharmacol. 85, 195-219. PMid:31307587. http://dx.doi.org/10.1016/bs.apha.2019.01.007.

Ramos, A.S., Correia, A.T., Antunes, S.C., Gonçalves, F., & Nunes, B., 2014. Effect of acetaminophen exposure in *Oncorhynchus mykiss* gills and liver: detoxification mechanisms, oxidative defense system and peroxidative damage. Environ. Toxicol. Pharmacol. 37(3), 1221-1228. PMid:24816177. http://dx.doi.org/10.1016/j.etap.2014.04.005.

Rico, A., Oliveira, R., Nunes, G.S.S., Rizzi, C., Villa, S., López-Heras, I., Vighi, M., & Waichman, A.V., 2021. Pharmaceuticals and other urban contaminants threaten Amazonian freshwater ecosystems. Environ. Int. 155, 106702. PMid:34139589. http://dx.doi.org/10.1016/j.envint.2021.106702.

Saide, K., Sherwood, V., & Wheeler, G.N., 2019. Paracetamol-induced liver injury modelled in *Xenopus laevis* embryos. Toxicol. Lett. 302, 83-91. PMid:30282005. http://dx.doi.org/10.1016/j.toxlet.2018.09.016.

Santos, P.R.O., Costa, M.J., Santos, A.C.A., Silva-Zacarín, E.C.M., & Nunes, B., 2020. Neurotoxic and respiratory effects of human use drugs on a Neotropical fish species, *Phalloceros harpagos.* Comp. Biochem. Physiol. C Toxicol. Pharmacol. 230, 108683. PMid:31874287. http://dx.doi.org/10.1016/j.cbpc.2019.108683.

Sedlak, J., & Lindsay, R.H., 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25(1), 192-205. PMid:4973948. http://dx.doi.org/10.1016/0003-2697(68)90092-4.

Silva de Assis, H.C., Navarro-Martín, L., Fernandes, L.S.P., Cardoso, C.C., Pavoni, D.P., & Trudeau, V.L., 2018. Cloning, partial sequencing and expression analysis of the neural form of P450 aromatase (*cyp19a1b*) in the South America catfish *Rhamdia quelen.* Comp. Biochem. Physiol. B Biochem. Mol. Biol. 221–222, 11-17. PMid:29655871. http://dx.doi.org/10.1016/j.cbpb.2018.04.001.

Starling, M.C.V.M., Amorim, C.C., & Leão, M.M.D., 2019. Occurrence, control and fate of contaminants of emerging concern in environmental compartments In Brazil. J. Hazard. Mater. 372, 17-36. PMid:29728279. http://dx.doi.org/10.1016/j.jhazmat.2018.04.043.

Szöcs, E., & Schäfer, R.B., 2015. Ecotoxicology is not normal: a comparison of statistical approaches for analysis of count and proportion data in ecotoxicology. Environ. Sci. Pollut. Res. Int. 22(18), 13990-13999. PMid:25953608. http://dx.doi.org/10.1007/s11356-015-4579-3.

Venancio, A.C.P., Aguiar, G.R., Lopes, P.S., & Alves, D.R., 2010. Metazoan parasites of Mandi-amarelo *Pimelodus maculatus* and of Jundiá *Rhamdia quelen* (Osteichthyes: Siluriformes) of Paraíba do Sul River, Volta Redonda, Rio de Janeiro. Rev. Bras. Parasitol. Vet. 19(3), 157-163. http://dx.doi.org/10.1590/S1984-29612010000300006.

Veras, T.B., Paiva, A.L.R., Duarte, M.M.M.B., Napoleão, D.C., & Cabral, J.J.S.P., 2019. Analysis of the presence of anti-inflammatories drugs in surface water: a case study in Beberibe river - PE, Brazil. Chemosphere 222, 961-969. http://dx.doi.org/10.1016/j.chemosphere.2019.01.167.

Vicentini, M., Fernandes, L.S.P., Marques, A.E.M.L., Osório, F.H.T., Baika, L.M., Risso, W.E., Martinez, C.B.R., Grassi, M.T., Fávaro, L.F., Mela, M., Cestari, M.M., & Silva de Assis, H.C., 2022. Effects of cadmium on the female reproductive axis of a Neotropical fish. Chemosphere 286(Pt 1), 131639. PMid:34346330. http://dx.doi.org/10.1016/j.chemosphere.2021.131639.

Wang, N., 2018. Increasing the reliability and reproducibility of aquatic ecotoxicology: learn lessons from aquaculture research. Ecotoxicol. Environ. Saf. 161, 785-794. PMid:29960649. http://dx.doi.org/10.1016/j.ecoenv.2018.06.044.

Wilkinson, J.L., Boxall, A.B.A., Kolpin, D.W., Leung, K.M.Y., Lai, R.W.S., Galbán-Malagón, C., Adell, A.D., Mondon, J., Metian, M., Marchant, R.A., Bouzas-Monroy, A., Cuni-Sanchez, A., Coors, A., Rojo, P.C.M., Gordon, C., Cara, M., Moermond, M., Luarte, T., Petrosyan, V., Perikhanyan, Y., Mahon, C.S., McGurk, C.J., Hofmann, T., Kormoker, T., Iniguez, V., Guzman-Otazo, J., Tavares, J.L., Figueiredo, F.G., Razzolini, M.T.P., Dougnon, V., Gbaguidi, G., Traoré, O., Blais, J.M., Kimpe, L.E., Wong, M., Wong, D., Ntchantcho, R., Pizarro, J., Ying, G., Chen, C., Páez, M., Martínez-Lara, J., Otamonga, J., Poté, J., Ifo, S.A., Wilson, P., Echeverría-Sáenz, S., Udikovic-Kolic, N., Milakovic, M., Fatta-Kassinos, D., Ioannou-Ttofa, L., Belušová, V., Vymazal, J., Cárdenas-Bustamante, M., Kassa, B.A., Garric, J., Chaumot, A., Gibba, P., Kunchulia, I., Seidensticker, S., Lyberatos, G., Halldórsson, H.P., Melling, M., Shashidhar, T., Lamba, M., Nastiti, A., Supriatin, A., Pourang, N., Abedini, A., Abdullah, O., Gharbia, S.S., Pilla, F., Chefetz, B., Topaz, T., Marcellin, K.Y., Aubakirova, B., Beisenova, R., Olaka, L., Mulu, J.K., Chatanga, P., Ntuli, V., Blama, N.T., Sherif, S., Aris, A.Z., Looi, L.J., Niang, M., Traore, S.T., Oldenkamp, R., Ogunbanwo, O., Ashfaq, M., Iqbal, M., Abdeen, Z., O'Dea, A., Morales-Saldaña, J.M., Custodio, M., Cruz, H., Navarrete, I., Carvalho, F., Gogra, A.B., Koroma, B.M., Cerkvenik-Flajs, V., Gombač, M., Thwala, M., Choi, K., Kang, H., Ladu, J.L.C., Rico, A., Amerasinghe, P., Sobek, A., Horlitz, G., Zenker, A.K., King, A.C., Jiang, J., Kariuki, R., Tumbo, M., Tezel, U., Onay, T.T., Lejju, J.B., Vystavna, Y., Vergeles, Y., Heinzen, H., Pérez-Parada, A., Sims, D.B., Figy, M., Good, D., & Teta, C., 2022. Pharmaceutical pollution of the world's rivers. Proc. Natl. Acad. Sci. USA 119(8), e2113947119. PMid:35165193. http://dx.doi.org/10.1073/pnas.2113947119.

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