

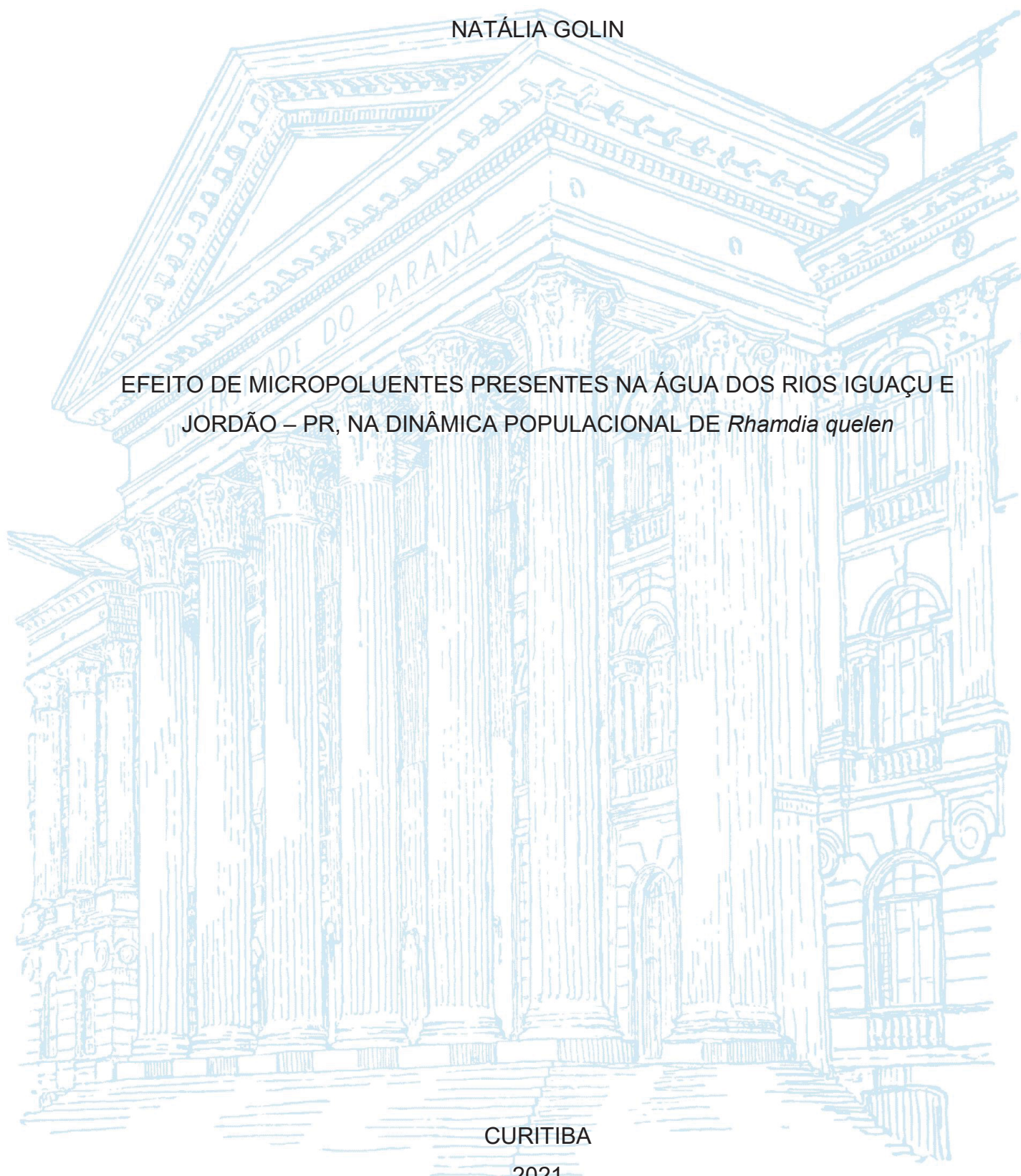
UNIVERSIDADE FEDERAL DO PARANÁ

NATÁLIA GOLIN

EFEITO DE MICROPOLUENTES PRESENTES NA ÁGUA DOS RIOS IGUAÇU E  
JORDÃO – PR, NA DINÂMICA POPULACIONAL DE *Rhamdia quelen*

CURITIBA

2021



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Dissertação apresentada ao curso de Pós-Graduação em Ecologia e Conservação, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Ecologia e Conservação.

Orientador: Prof. Dr. Ciro Alberto de Oliveira

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## ATA DE SESSÃO PÚBLICA DE DEFESA DE MESTRADO PARA A OBTENÇÃO DO GRAU DE MESTRE EM ECOLOGIA E CONSERVAÇÃO

No dia trinta de julho de dois mil e vinte e um às 08:30 horas, na sala [https://meet.google.com/rms\\_eqtx-xff](https://meet.google.com/rms_eqtx-xff), MODALIDADE PARECER, foram instaladas as atividades pertinentes ao rito de defesa de dissertação da mestranda **NATÁLIA GOLIN**, intitulada: **EFEITO DE MICROPOLUENTES PRESENTES NA ÁGUA DOS RIOS IGUAÇU E JORDÃO ? PR, NA DINÂMICA POPULACIONAL DE Rhamdia quelen**, sob orientação do Prof. Dr. CIRO ALBERTO DE OLIVEIRA RIBEIRO. A Banca Examinadora, designada pelo Colegiado do Programa de Pós-Graduação em ECOLOGIA E CONSERVAÇÃO da Universidade Federal do Paraná, foi constituída pelos seguintes Membros: CIRO ALBERTO DE OLIVEIRA RIBEIRO (UNIVERSIDADE FEDERAL DO PARANÁ), IZABELLA DE ANDRADE BRITO (SECRETARIA DO DESENVOLVIMENTO SUSTENTÁVEL E DO TURISMO), ELTON CELTON DE OLIVEIRA (UNIVERSIDADE TECNOLÓGICA FEDERAL DO PARANÁ). A presidência iniciou os ritos definidos pelo Colegiado do Programa e, após exarados os pareceres dos membros do comitê examinador e da respectiva contra argumentação, ocorreu a leitura do parecer final da banca examinadora, que decidiu pela APROVAÇÃO. Este resultado deverá ser homologado pelo Colegiado do programa, mediante o atendimento de todas as indicações e correções solicitadas pela banca dentro dos prazos regimentais definidos pelo programa. A outorga de título de mestre está condicionada ao atendimento de todos os requisitos e prazos determinados no regimento do Programa de Pós-Graduação. Nada mais havendo a tratar a presidência deu por encerrada a sessão, da qual eu, CIRO ALBERTO DE OLIVEIRA RIBEIRO, lavrei a presente ata, que vai assinada por mim e pelos demais membros da Comissão Examinadora.

CURITIBA, 30 de Julho de 2021.

Assinatura Eletrônica

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Presidente da Banca Examinadora

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## TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ECOLOGIA E CONSERVAÇÃO da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **NATÁLIA GOLIN** intitulada: **EFEITO DE MICROPOLUENTES PRESENTES NA ÁGUA DOS RIOS IGUAÇU E JORDÃO ? PR, NA DINÂMICA POPULACIONAL DE *Rhamdia quelen*** , sob orientação do Prof. Dr. CIRO ALBERTO DE OLIVEIRA RIBEIRO, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa. A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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## RESUMO

A bacia do rio Iguaçu possui grande importância ecológica para os ecossistemas aquáticos no sul do Brasil devido à sua ictiofauna altamente endêmica, no entanto, a poluição química tem ameaçado sua biodiversidade. Um importante afluente do rio Iguaçu, o rio Jordão, contribui para essa poluição, pois drena grandes áreas agrícolas além de receber efluentes domésticos e industriais antes de desembocar no rio Iguaçu. O objetivo deste estudo foi avaliar os efeitos de micropoluentes encontrados na água dos rios Iguaçu, Jordão e a água de suas confluências na fase embrio-larval de *R. quelen*, por meio de análises de sobrevivência, deformidade e biomarcadores bioquímicos. Além disso, foi investigada as possíveis consequências a nível populacional por meio de modelagem matemática. Ovos fertilizados de *R. quelen*, transcorridos 8 horas pós-fertilização (8hpf) foram expostos até 96 hpf à amostras de água do rio Iguaçu a montante (RI), rio Jordão (RJ) e encontro dos rios (ER), nas quais foram também realizadas análises químicas para detecção de metais tóxicos e poluentes orgânicos. A taxa de sobrevivência após a exposição não apresentou diferenças comparativamente com o grupo controle, enquanto os índices de deformidade foram maiores para os três pontos, principalmente para RJ, que também teve o maior número de contaminantes detectados. A atividade da superóxido dismutase foi aumentada em ER, enquanto os níveis de tiol não proteico foram reduzidos em ER e RJ. O Índice Integrado de Respostas de Biomarcadores (IBR) sugere maior resposta ao estresse oxidativo para larvas tratadas com ER, enquanto as larvas RJ podem estar enfrentando danos oxidativos. A modelagem matemática sugeriu que a água do RJ levaria à maior redução populacional (46,19%), seguida da RI (40,48%) e da ER (33,33%). Embora todas as amostras de água tenham induzido alguma forma de toxicidade, os embriões e larvas expostos a RJ tiveram os resultados mais proeminentes. Os resultados demonstram que as espécies de peixes encontradas na bacia do Rio Iguaçu podem estar sendo afetadas pela presença de micropoluentes na água, colocando em risco a dinâmica entre as espécies. O presente estudo revela a importância em avaliar o impacto das atividades antrópicas ao longo da bacia do rio Iguaçu, com atenção especial para a microbacia do rio Jordão, a qual ainda não havia sido estudada.

Palavras-chave: Ecotoxicologia, Modelagem, Biomarcadores, Embriões, Larvas.

## ABSTRACT

The Iguaçu basin have high ecological importance for aquatic ecosystems in southern Brazil due to it expressive endemic ichthyofauna rate, but chemical pollution may threaten this biodiversity. Jordão river is one of the main tributaries of Iguaçu river and contribute to this pollution, since it drains large agricultural areas and receive domestic or industrial effluents before failing into the Iguaçu river. The objective of the current study was to evaluate the toxic effects of micropollutants detected in the Iguaçu and Jordão rivers after exposure to embryo-larval phase of *R. quelen*. The survival rate, deformities and biochemical biomarker analyses will be used to investigate the consequences at the population level by means of mathematical modelling. *R. quelen* fertilized eggs after 8 hours post-fertilization (8 hpf) were exposed to 96 hpf to water samples from Iguaçu River upstream (IR), Jordão River (JR), and downstream of both rivers (MR), where inorganic and organic chemicals analysis were carried out. Survival rate was not a sensitive endpoint, while the deformity indices were higher in individuals exposed to water from the three studied sites, especially JR, which also had the higher number of contaminants detected. Superoxide dismutase activity was increased in MR, while non-protein thiol levels were reduced in MR and JR. The Integrated Biomarker Response suggests higher oxidative stress response for MR-treated larvae, while JR-larvae could be facing oxidative damage. Mathematical modelling suggested that JR would lead to the greater population reduction (46.19%), followed by IR (40.48%), and MR (33.33%). Although all studied sites induced some toxicity, JR-exposed embryos and larvae showed the most prominent results than other studied sites. It appears that JR site is impacted by micropollutants in order to affect the fish population, but probably due to the dilution with Iguaçu river, does not influence the toxic potential of the Iguaçu River, at least not in an additive or synergistic way.

Keywords: Ecotoxicology, Modelling, Biomarkers, Embryos, Larvae.

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## 1 INTRODUÇÃO GERAL

A água é um recurso fundamental para a vida, porém, atividades como lançamento de esgotos doméstico e industrial, bem como o intenso uso de agrotóxicos, vem alterando a qualidade desse importante recurso natural em rios e mananciais do Brasil. Essas fontes de poluição, além de tornarem a água imprópria para o consumo, também comprometem os ecossistemas aquáticos, afetando direta ou indiretamente os organismos que vivem nestes ambientes (PARANÁ, 2008; PEREIRA, 2004).

A bacia do rio Iguaçu possui elevada importância econômica na geração de energia hidroelétrica devido ao seu relevo e às relações com suas características geomorfológicas. Além disso, as altas taxas de endemismo observadas em espécies de peixes chega a uma taxa de 70% das espécies nativas, que não são encontradas em outro local, e, portanto, considerada como uma das áreas prioritárias para conservação e uso sustentável da biodiversidade brasileira (BRASIL, 2007). No entanto, a bacia do rio Iguaçu tem sido intensamente modificada por atividades antrópicas, como a construção de barragens ao longo do rio, além de ser afetada pelos impactos da agropecuária e grandes aglomerados urbanos e industriais. Essas atividades em conjunto, tem colocado sob risco as populações de espécies da fauna aquática e semiaquática da bacia, gerando um quadro bastante preocupante de degradação ambiental com reflexos negativos para a biodiversidade local (BAUMGARTNER et. al, 2012; BRASIL, 2018). O rio Jordão é o principal afluente da margem direita do rio Iguaçu. Em relação a sua ictiofauna, a microbacia possui o mesmo padrão geral encontrado no rio Iguaçu, contendo espécies com elevado grau de endemismo. As fontes potencialmente poluidoras da sua bacia são agricultura, pecuária, áreas urbanas, indústrias, aterros sanitários, lixões e estações de tratamento de esgoto (PARANÁ, 2008; 2013). Parte das águas do rio Jordão desaguam no reservatório de Segredo através de um desvio construído para abastecer o mesmo, ou diretamente à jusante da barragem.

No Brasil, a implementação da Política Nacional de Recursos Hídricos, Lei nº 9.433 (BRASIL, 1997), tem como objetivo garantir à atual e às futuras gerações a disponibilidade de água em padrões de qualidade adequados aos seus respectivos usos. Para preservar adequadamente os recursos hídricos, como os rios, é necessário um monitoramento adequado destas águas, por meio de métodos

capazes de identificar o real impacto antrópico nesses ambientes aquáticos. Neste contexto, o Brasil como outros países, tem utilizado principalmente a determinação de parâmetros físicos e químicos previstos na legislação no diagnóstico da qualidade da água nos reservatórios, rios e mananciais (DOS SANTOS *et al.*, 2016). O uso dos testes ecotoxicológicos está previsto na Resolução CONAMA nº 357/2005, porém a realização não é obrigatória. De acordo com dos Santos *et al.* (2016) os resultados apenas das análises obrigatoriamente previstas na legislação não retratam o real impacto ambiental causado pelos micropoluentes encontrados na água, e portanto, os potenciais efeitos sobre os sistemas biológicos. Deste modo, é indicado que as análises físico-químicas sejam realizadas em conjunto com os ensaios que demonstrem uma interação entre os organismos vivos e a exposição aos micropoluentes (CAIRNS JUNIOR, 2002; PALMA *et al.*, 2010). Além disso, os efeitos da poluição são majoritariamente o resultado da exposição a uma mistura complexa de poluentes, como observado na junção de dois rios afetados por fontes difusas, por exemplo. Portanto, medições baseadas em efeitos biológicos em vários níveis de organização (por exemplo, molecular, celular, organismo e população), são as mais vantajosas e integrativas para avaliação do impacto ambiental em ambientes aquáticos (KLUMPP *et al.*, 2002).

Alguns países têm adotado, além dos testes padrões previstos na legislação, ensaios ecotoxicológicos como uma ferramenta complementar às análises físico-químicas no diagnóstico de qualidade de água (EMBRY *et al.*, 2010; CONNON *et al.*, 2012). Os testes de ecotoxicidade fora da esfera regulamentar, permitem avaliar a contaminação ambiental por diversas fontes de poluição, como efluentes domésticos, industriais, agrícolas, além de investigarem efeitos sinérgicos e antagônicos presentes em misturas (BEYER *et al.*, 2014). A ISO-15088 (ISO, 2007) recomenda o uso de ovos de *Danio rerio* nos testes ecotoxicológicos, mas não prevê o uso de espécies nativas para esse tipo de avaliação. O Jundiá, *Rhamdia quelen* (Quoy e Gaimard, 1824) é uma espécie de peixe nativo da América do Sul, amplamente utilizado em pisciculturas e facilmente adaptado às condições experimentais de laboratório (BALDISSEROTO *et al.*, 2020). Por ser muito utilizado em cultivo, os estudos sobre seu processo reprodutivo já são bastante conhecidos. Além disso, a espécie possui similaridades filogenéticas com diversas outras espécies endêmicas da Bacia do Rio Iguaçu incluindo o Surubim do Iguaçu sob risco de extinção (BAUMGARTNER *et al.*, 2012). Deste modo, as avaliações de risco

através da ecotoxicologia podem ser facilitadas com o uso dessa espécie nos estudos do impacto de micropoluentes encontrados na bacia do rio Iguaçu, ou em outros corpos hídricos do Brasil também impactados pela atividade antrópica (BRITO *et al.*, 2017).

Além dos parâmetros de desenvolvimento, como taxa de sobrevivência e deformidades em embriões e larvas de peixes avaliados nos testes de embriotoxicidade, os biomarcadores também devem ser investigados. O uso de biomarcadores auxilia na interpretação dos efeitos observados nos níveis iniciais de desenvolvimento, o que favorece uma melhor avaliação dos efeitos dos micropoluentes, complementando os testes ecotoxicológicos (TRIEBSKORN, 1997). Os biomarcadores geralmente mostram os primeiros sinais detectáveis de resposta ao estresse subletal em um organismo exposto a um determinado xenobiótico, e, portanto, tem um papel preditivo neste tipo de avaliação (VALAVANIDIS *et al.*, 2006). Alguns mecanismos são ativados frente a ação de xenobióticos nos organismos, como o processo de detoxicação e a atividade do sistema de defesa antioxidante, os quais são essenciais para manutenção da homeostase corporal (LEE *et al.*, 2006). Alguns contaminantes, devido a sua capacidade pró-oxidante, promovem o aumento da formação de espécies reativas de oxigênio (ROS) e outros radicais livres no organismo, levando a um processo de estresse oxidativo e prejudicando características fisiológicas. Devido a isso, enzimas antioxidantes como a Superóxido Dismutase (SOD) e a Catalase (CAT) são ativadas na tentativa de mitigar possíveis danos oxidativos (AJIMA *et al.*, 2021). A Glutathione S-Transferase (GST) é uma enzima de biotransformação de fase II, a qual pertence a um grupo de enzimas multifuncionais que participam dos processos antioxidantes e de desintoxicação de compostos tóxicos (Vojoudi *et al.* 2017). Outro biomarcador bastante importante na avaliação da qualidade do ambiente aquático é a Acetilcolinesterase (AChE), visto que distúrbios na sua atividade causados pela exposição a poluentes, pode levar a efeitos neurológicos importantes do ponto de vista toxicológico (THANOMSIT *et al.*, 2021). A AChE é responsável pela remoção do neurotransmissor acetilcolina (ACh) da fenda sináptica. No entanto, quando há inativação ou uma diminuição da sua atividade, ocorre um acúmulo de ACh na fenda sináptica, interferindo no funcionamento normal do sistema nervoso pela impossibilidade de formação de novos impulsos (FULTON e KEY, 2009).

Mesmo que o conceito de ecotoxicologia seja amplo, podendo ser aplicado em níveis mais complexos de organização biológica como populações, comunidades e o próprio ecossistema, os testes que têm sido utilizados em ecotoxicologia avaliam apenas a perturbação a nível individual sem um enfoque populacional. Isso gera incertezas de como estes resultados ou efeitos experimentais podem ser traduzidos para níveis mais complexos de organização biológica. Os efeitos como sobrevivência em ambiente natural, taxa de desenvolvimento e reprodução não são considerados nesses testes, negligenciando, portanto, impactos em larga escala como população e comunidades (FORBES *et al.*, 2001; HALBACH *et al.* 1983; STARK, 2005). Com o objetivo de aplicar os resultados a partir de testes individuais para níveis populacionais, Brito *et al.* (2017) desenvolveram modelos matemáticos preditivos a partir de estudos nos estágios iniciais de desenvolvimento, os quais vem sendo utilizados recentemente na avaliação de efeitos de poluentes e misturas complexas em ambientes aquáticos impactados pela atividade antrópica (BRITO *et al.*, 2018; AZEVEDO-LINHARES, *et al.*, 2018; BARRETO *et al.*, 2020; FOLLE *et al.*, 2021; NAGAMATSU *et al.*, 2021). Com base nos dados de toxicidade encontrados nas análises laboratoriais o modelo simula os efeitos tóxicos dos poluentes em escala populacional nos ambientes naturais ao longo do tempo (BRITO *et al.*, 2017).

O modelo desenvolvido para o Jundiá (*Rhamdia quelen*) (BRITO *et al.*, 2017), tem sido aplicado também para outras espécies nativas importantes do ponto de vista de conservação como o Dourado (*Salminus brasiliensis*) e o Curimatá (*Prochilodus lineatus*), expostos a águas afetadas por despejos provenientes de Estações de Tratamento de Esgoto (ETE) (BARRETO *et al.*, 2020). Por meio da modelagem matemática foram avaliadas previsões sobre o impacto em populações dessas espécies, a partir das análises obtidas com os estágios iniciais de desenvolvimento. Através do modelo, os resultados demonstraram que em condições similares de exposição natural, essas populações poderiam ser drasticamente reduzidas e em alguns casos levadas à extinção, caso permanecesse a condição de estresse testada. Portanto, esse tipo de abordagem atende a dois desafios impostos hoje nos estudos de ecotoxicologia: (i) compreender o efeito dos poluentes a nível populacional e, (ii) possibilitar a avaliação dos efeitos de misturas complexas sobre a biota de determinado ambiente aquático. Acreditamos, portanto, que esse tipo de estudo contribui significativamente para a conservação das espécies de peixes, bem como a manutenção de recursos hídricos, monitoramento

de qualidade da água, sobretudo àquela destinada ao abastecimento público, funcionando como uma importante ferramenta para a definição de políticas públicas de conservação.

## 1.1 OBJETIVOS

### 1.1.1 Objetivo Geral

Avaliar os efeitos tóxicos de micropoluentes detectados na água dos rios Iguaçu e Jordão nos estágios iniciais de *R. quelen* a partir de testes ecotoxicológicos e modelagem matemática.

### 1.1.2 Objetivos específicos

1. Avaliar os efeitos dos micropoluentes encontrados nas águas dos rios Iguaçu e Jordão na sobrevivência e deformidades dos embriões e larvas de *R. quelen* por meio de testes ecotoxicológicos;
2. Avaliar através de biomarcadores bioquímicos os efeitos dos micropoluentes encontrados nas águas dos rios Iguaçu e Jordão no estresse oxidativo e neurotoxicidade em larvas de *R. quelen*;
3. Utilizar a modelagem matemática para avaliar os efeitos encontrados nos níveis iniciais de desenvolvimento na dinâmica populacional de indivíduos adultos da espécie *R. quelen*;
4. Discutir a utilização desse tipo de abordagem com uma ferramenta para a definição de políticas de conservação.

## 2 ARTIGO CIENTÍFICO

### **Toxicological responses in embryo and larvae of *Rhamdia quelen* exposed to micropollutants in Jordao River Southern Brazil**

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#### **Abstract**

The Iguazu basin present high ecological importance due to it expressive endemic ichthyofauna rate, but chemical pollution may threaten this biodiversity. Jordão river is one of the main tributaries of Iguazu River and contribute to this pollution, since it drains large agricultural areas and receive domestic or industrial effluents before failing into the Iguazu river. The objective of the current study was to evaluate the toxic effects of micropollutants detected in the Iguazu and Jordão rivers after exposure to embryo-larval phase of *R. quelen*. The survival rate, deformities and biochemical biomarker analyses were used to investigate the consequences at the population level by means of mathematical modelling. *R. quelen* fertilized eggs (8hpf) were exposed for 96 hours to water samples from Iguazu River upstream (IR), Jordão River (JR), and downstream of both rivers (MR), where inorganic and organic chemicals analysis were carried out. Survival rate was not a sensitive endpoint, while the deformity indices were higher in individuals exposed to water from the three studied sites, especially JR, which also had the higher number of contaminants detected. Superoxide dismutase activity was increased in MR, while non-protein thiol levels were reduced in MR and JR. The Integrated Biomarker Response suggests higher oxidative stress response for

MR-treated larvae, while JR-larvae could be facing oxidative damage. Mathematical modelling suggested that JR would lead to the greater population reduction (46.19%), followed by IR (40.48%), and MR (33.33%). Although all studied sites induced some toxicity, JR-exposed embryos and larvae showed the most prominent results than other studied sites. It appears that JR site is impacted by micropollutants in order to affect the fish population, but probably due to the dilution with Iguaçu river, does not influence the toxic potential of the Iguaçu River, at least not in an additive or synergistic way.

**Keywords:** Ecotoxicology, modeling, embryos, Iguaçu River, Jordão River.

## Introduction

Water is a fundamental resource for life, but the discharge of domestic and industrial effluents and the intense use of pesticides have been altering the water quality of rivers (Srivastava et al., 2020). Among the main contaminants entering aquatic ecosystems through human activities, metals and organic pollutants, such as persistent toxic organic pollutants (POPs) and polycyclic aromatic hydrocarbons (HAPs), present high toxicity, potential for bioaccumulation, resistance to biodegradation, and can be transported over long distances and to different compartments in the environment (Jones and Voogt, 1999; Yao et al., 2014). Therefore, in addition to making water unfit for consumption, chemical pollution is also a major cause of aquatic biodiversity loss, compromising these ecosystems (Vardhan et al., 2019; Vörösmarty et al., 2010).

The Iguaçu River basin, southern Brazil, presents high economic and ecological importance, with more than 70% of endemic fish species, being considered one of the priority areas for conservation and sustainable use of Brazilian biodiversity (Baumgartner et. al, 2012; MMA - Ministry of the Environment, 2007). In addition to the construction of numerous dams along its course, the Iguaçu River has been heavily modified due to intensive agriculture and large urban agglomerations on its drainage area. This intense environmental degradation has threatened the diversity of the local ichthyofauna (Nardelli et al., 2016).

The Jordão River sub-basin has a drainage area of 4,730.60 km<sup>2</sup>, with an extension of 168 km to its mouth in the Iguaçu River (Paraná, 2008). It is the main tributary of the right bank of the Iguaçu River, and, regarding its ichthyofauna, it has a remarkably high degree of endemism, even when compared to the Iguaçu River itself (Baumgartner et. al, 2012). The potentially polluting sources in this basin are agriculture, livestock, urban areas, industries,

landfills, dumps, and sewage treatment plants (Paraná, 2013). The land use in the Jordão River basin is composed of 45.9% agriculture, 40% fields and pastures, and 1.1% urban areas, where the highest load of point source pollution is domestic wastewater, while the diffuse one was related to agriculture (Paraná, 2008). The basin comprises seven cities, where three of them do not have sewage system (IBGE, 2017). Moreover, the pulp and paper industry are very representative in this basin (Paraná, 2013).

In Brazil, the implementation of the National Water Resources Policy, Law No. 9,433 (Brasil, 1997), aims to guarantee current and future generations the availability of water in quality standards appropriate for their respective uses. To preserve water resources such as rivers, it is necessary to monitor these waters properly, using techniques that can really assess the quality of these aquatic environments (Cairns Jr., 2002). Foreseen by legislation, Brazil has employed the general determination of physical and chemical parameters of the water (CONAMA - Brazilian National Environmental Council, 2005). However, physicochemical analysis by itself does not portray the environmental impact caused by pollutants and does not demonstrate the effects on biological systems. Thus, for a better assessment of water quality, physicochemical analysis should be complemented with the use of organisms (Palma et al., 2010). Moreover, environmental contaminants rarely occur in isolation and the aquatic biota is often exposed to a mixture of chemicals with multiple, and sometimes unknown, effects on organisms and their populations. Therefore, analyses based on biological endpoints at different levels of organization are the most appropriate and integrative for environmental impact assessment and biomonitoring (Klumpp et al., 2002).

Ecotoxicity tests can elucidate additive, synergistic, and antagonistic effects of chemical mixtures from different pollution sources directly on the biota (Beyer et al., 2014). Moreover, some xenobiotics present at low concentrations in water, even under legal limits, can be toxic to aquatic organisms if combined with other substances (Silva et al., 2002). Therefore, understanding the sensitivity of fish to pollutants is essential for the environmental risk assessment process (Belanger et al., 2010). There are national and international standard protocols for testing acute toxicity on fish (USEPA - United States Environmental Protection Agency, 1996a, USEPA- United States Environmental Protection Agency, 1996b, OECD - Organisation for Economic Co-operation and Development, 2019). The Fish Embryo Test (FET) was proposed as an alternative to testing on adult fish. In the embryo-larval phase, fish are highly sensitive to chemical pollutants present in water (Kendall et al., 2001). Therefore, acute toxicity tests at this stage represent an important tool that has been used to identify the potential toxic effects of xenobiotics and their synergistic effects on aquatic life (Braunbeck et

al., 2015). Toxicity can be verified through physiological changes, malformations, and mortality of embryos and larvae. In addition, the toxic effects observed on this phase can also be extrapolated to other phases of the life cycle (Belanger et al., 2013; Hallare et al., 2005).

Even though ISO-15088, International Organization for Standardization (ISO, 2007) recommends the use of *Danio rerio* eggs in ecotoxicological tests, *Rhamdia quelen* (Quoy and Gaimard, 1824), being a South American native fish, represents a good study model for conservational purposes on the Iguaçú River. It is widely used in fish farms and easily adapted to laboratory conditions, having a well-known reproductive process. In addition, the species has similarities with several endemic species from the Iguaçú River (Baumgartner et al., 2012). The catfish *R. quelen* shows sensitivity to contaminants and well-characterized responses to stress. Thus, ecotoxicological risk assessments performed with this species can be more meaningful to Brazilian water bodies (Gomes et al, 2021; Marins et al, 2021).

Ecotoxicological tests usually assess effects at the individual level, with few studies investigating how these effects can be translated to the populational level and what, in fact, the ecological implications would be (Forbes et al., 2001; Gledhill and Van Kirk, 2011). Factors such as reproductive parameters and natural rates of survival and development are not commonly related to the effects observed experimentally on the individual, which can have major impacts on the population (Halbach et al. 1983; Stark, 2005). Predictive mathematical models have been used to link test results at the individual level to impacts at the population level (Brito et al., 2017; Nagamatsu et al, 2021). Based on the toxicity data from bioassays, these models can simulate toxic effects of pollutants on a population scale in natural environments (Barreto et al., 2020).

In addition to survival and deformity rates, biochemical biomarkers can also be analyzed in developing embryos, providing more complete results and a better understanding of pollutant toxicity mechanisms on fish (Triebkorn, 1997). The analysis of biomarkers allows the early detection of toxicity, representing the first signs of stress response in organisms exposed to xenobiotics and, therefore, useful sublethal indicators of environmental contamination (Valavanidis, et al., 2006).

Thus, seeking to examine the influence of the Jordão River on the pollution of the Iguaçú River and its effects on the biota, the objective of this study was to evaluate the toxic effects of the Iguaçú, Jordão and the combination of their waters to the embryo-larval phase of *R. quelen*, through survival, deformity, and biochemical biomarker analyses, besides the investigation of possible consequences at the population level by means of mathematical modelling.

## Materials and Methods

### *Study area*

The Iguaçu River basin is the largest and most important in the state of Paraná, stretching over 72,000 km<sup>2</sup>, with a drainage area of 186,321 km<sup>2</sup>, comprising about 101 municipalities along southern Brazil and northern Argentina (Baumgartner et al, 2012). This basin is considered a World Ecoregion of freshwater fish distribution due to its high rate of endemism (Abell et al., 2008). The Iguaçu River originates in the Metropolitan Region of Curitiba (MRC) and runs 1,320 km until it flows into the Iguaçu Falls, where it joins the Paraná River. This river receives large amounts of urban and industrial waste, being classified as the second most polluted river in Brazil (IBGE, 2010). The fish biodiversity has been reduced over the years, mainly due to anthropic actions (Baumgartner et. al, 2012). The Jordão River drains an extensive agricultural region and flows into the Iguaçu River (Paraná, 2008; 2013) and no studies have been developed until now to evaluate the chemical impact to biota. Therefore, it is expected that this will be a great source of discharge of pollutants into the Iguaçu River.

### *Water sampling*

In order to investigate the effects of micropollutants detected in Jordão and Iguaçu Rivers to fish population, three sites were studied where water samples were collected in January 2020. The first site named Iguaçu River (IR) located 5 km upstream the Jordão River mouth; the second site named Jordão River (JR) is located about 1 km before it flows into the Iguaçu River; Then, the third studied site called meeting of the rivers (MR) was in the Iguaçu River, 5 km downstream of the Jordão River mouth (Figure 1).

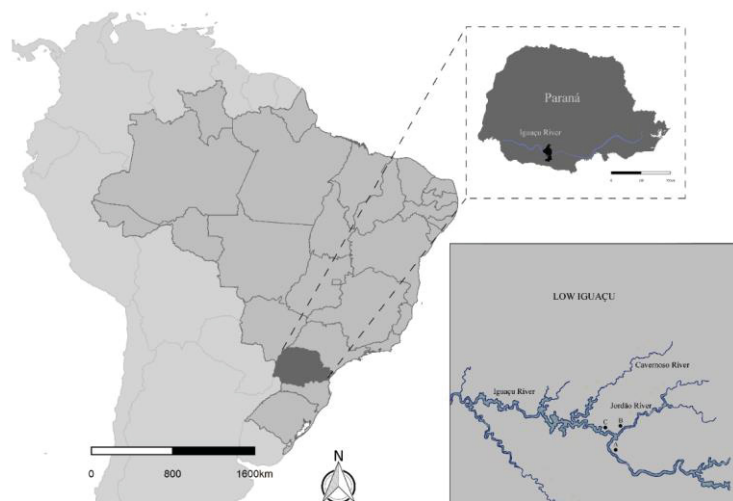


Figure 1. Map showing the studied sites location in the Lower Iguacu River Basin, Paraná State Southern Brazil. A) Iguacu River (IR): upstream the Jordão River mouth ( $25^{\circ} 47'10.43'' \text{S } 52^{\circ} 8'7.61'' \text{W}$ ); B) Jordão River (JR): before it joins the Iguacu River ( $25^{\circ} 46'18.80'' \text{S } 52^{\circ} 6'54.69'' \text{W}$ ); C) Meeting of the Rivers (MR): Iguacu River downstream of the Jordão River mouth ( $25^{\circ} 45'54.85'' \text{S } 52^{\circ} 8'49.50'' \text{W}$ ). The dark spot corresponds to the study area.

The water samples were collected 30 cm from the water surface and transported refrigerated to the Cellular Toxicology Laboratory at Federal University of Paraná. In the laboratory, the samples were filtered on paper discs ( $12 \mu\text{m}$  porosity) to remove suspended materials and then preserved at  $4^{\circ}\text{C}$  for 24 hours until the experiments were carried out. Water samples collected for organic chemical analysis were stored in amber glass bottles and kept refrigerated until analysis, while for metal analysis were acidified with nitric acid ( $\text{pH} < 2$ ).

### *Chemical analysis*

The following organic contaminants were analyzed in the water samples: polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), and polychlorinated biphenyls (PCBs). The extraction of organic contaminants followed the protocol EPA 3510C (Separatory Funnel Liquid-Liquid Extraction) (EPA - US Environmental Protection Agency, 1996), as described by Barreto et al. (2020). For toxic metal analysis were considered Cadmium (Cd), Lead (Pb), and Arsenic (As) due to the historical of recent analyses (Yamamoto et al., 2018). For the quantification of the metal contents, the technique of inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used, according to the protocol 6010C US EPA (EPA, 2007), using the ES-710 Varian ICP-OES equipment.

### *Model species*

The silver catfish *R. quelen* has a geographical distribution from southern Mexico to central Argentina, is an omnivorous species, with a tendency to piscivore and benthic habit (Gomes et al. 2000; Gomiero et al., 2007). This species is functionally and phylogenetically like several native endemic fish species of the Iguaçu River basin, such as the siluriforms *Rhamdia branneri* (Haseman, 1911), *Rhamdia voulezi* (Haseman, 1911), *Pimelodus ortmanni* (Haseman, 1911), and *Pimelodus britskii* (Garavello & Shibatta, 2007) (Baumgartner et al., 2012). The habit similarities found with other species from the Iguaçu River basin, allow a more reliable transposition of the results to the species found in the rivers under study. Also, *R. quelen* is widely used in fish farms from Southern Brazil, so the knowledge about reproduction and experimental facilities are easily available, including the eggs obtaining (Baldisseroto et al, 2020).

The fertilized eggs of *R. quelen* were supplied by the Pisciculture Station Panamá ([www.pisciculturapanama.com.br](http://www.pisciculturapanama.com.br)) through induced reproduction. Gametes were collected from a single pair of progenitors to reduce the genetic variability of individuals. The fertilized eggs were transported to the laboratory in oxygenated plastic bags in polystyrene boxes ( $24^{\circ}\text{C}\pm 1$ ). The viable eggs were selected 8 hours post fertilization (hpf) using a Leica Stereomicroscope (0.7-4.5x), according to Rodrigues-Galdino et al. (2010).

#### *Fish embryo-larval toxicity tests*

The embryos were exposed to 4 treatments: water from the Jordão River (JR), Iguaçu River upstream (IR) and downstream from the mouth of the Jordão River, called Meeting of the Rivers (MR) and reconstituted water (Control group) ( $6.5\text{ mgL}^{-1}\text{ CaCl}_2$ ,  $133.5\text{ mgL}^{-1}\text{ MgSO}_4$ ,  $0.4\text{ mgL}^{-1}\text{ KCl}$ ,  $10.5\text{ mgL}^{-1}\text{ NaHCO}_3$ ). The exposure started at 8 hpf in 24-well culture plates (Corning®), where one embryo was added per well containing 2 ml of test water. A total of 960 embryos were distributed in 40 plates, 10 for each experimental time evaluated (24, 48, 72, and 96 hpf). An integrated treatment technique was used, where each plate contained all treatments, one in each row, ensuring that all groups were exposed to the same conditions, including those variables on a microscale. The row position of each treatment within the plates was randomized, so all treatments occupied all possible row positions. The plates were kept in a dark environment, with controlled temperature at  $24^{\circ}\text{C}\pm 1$ . Dissolved oxygen in all test waters was maintained between 6 and  $8\text{ mgL}^{-1}$  by means of aeration.

The plates were analyzed at 24, 48, 72, and 96 hpf, with half of the test water in each well renewed every 24 h. The survival rate was registered daily, where mortality was determined by coagulation of embryos, lack of somite formation, or lack of heartbeat, according to the OECD protocol (OECD - Organisation for Economic Co-operation and Development, 2013). At each exposure period, the larvae were collected, fixed in Karnovsky fixative solution (2.5% glutaraldehyde, 4% paraformaldehyde in 0.1 mol L<sup>-1</sup> sodium phosphate buffer, pH 7.3) for 24 h, washed with 0.1 mol L<sup>-1</sup> sodium phosphate buffer, and stored at 4 °C until the deformity analysis.

The deformity analysis was carried out on Zeiss Stereomicroscopic (SteREO Discovery.V12) and classified using the methodology adapted from Powers et al. (2011), being divided into three types: 1) spine (stunted skeletal growth and torsion in the tail), 2) cranial (absence or atrophy of the eyes and/or barbels), 3) thorax (distension and pericardial/yolk sac edema). A score of 1 to 3 was assigned to everyone with malformation for each type of deformity presented, where 1 = low severity, 2 = moderate severity, and 3 = high severity, according to Barreto et al., (2021). The scores of the types of deformities presented by the individual were added, thus obtaining a total deformity score. Finally, the individual scores on each row/plate were added and then divided by the number of surviving organisms on that treatment row, resulting in a Deformity Index.

#### *Biochemical biomarkers*

To assess whether the micropollutants detected in the river waters are bioavailable and may induce oxidative stress or neurotoxicity in *R. quelen* embryo-larvae until 96hpf, another experimental design was carried out. A total of 20 glass beakers were used, 5 for treatment. A total of 120 embryo-larvae was added to each beaker with 200ml of water from their respective treatments. Every 24 hours, 100 ml of test water was renewed, and dead individuals were removed. At the end of the experimental period, the surviving larvae were placed in 2 mL microtubes and stored at -80 °C. Subsequently, the individuals were homogenized in 500 µL of Tris-HCl buffer (20 mM, 1.0 mM EDTA, pH 7.6) and centrifuged at 12,000 xg for 10 min at 4 °C. Aliquots of 200 µL were separated and stored at -80 °C for the analysis of biochemical biomarkers. All biomarker determinations were performed in a microplate reader (Varioskan Lux, Thermo Scientific).

The following biomarkers were evaluated according to the methodologies presented: (i) Superoxide Dismutase (SOD) activity (Kono, 1978; Crouch et al., 1981); (ii) Acetylcholinesterase (AChE) activity (Ellman et al., 1961); (iii) Catalase activity (CAT)

(Aebi, 1984); (iv) Glutathione S-transferase (GST) activity (Keen et al., 1976); (v) Glucose-6-phosphate dehydrogenase (G6PDH) activity (Glock and Mclean, 1953); (vi) Lipid peroxidation (LPO) - FOX method (Jiang et al., 1991, 1992), (vii) Concentration of non-protein thiols (NPT) (Sedlak and Lindsay, 1968) and (viii) Protein carbonylation (PCO) (Levine et al., 1994)

The results obtained from biochemical biomarkers were used to calculate the Integrated Biomarker Response (IBR), as described by Beliaeff and Burgeot (2002) modified by Sanchez et al. (2013). Firstly, the results of the parameters of each treatment were divided by the results of the corresponding control. To reduce the variance, logarithmic transformations ( $Y_i$ ) were performed. The mean ( $\mu$ ) and standard deviation (SD) of the results were calculated for all analyzes and the formula  $Z_i = (Y_i - \mu)/SD$  was applied for each treatment. The difference between treatments and control ( $Z_i - Z_0$ ) was used to obtain an integrated value for each biomarker analyzed. After performing the calculations, the results were plotted on a radar chart and added to obtain the IBR index.

#### *Data analysis*

Statistical analyzes were performed using the RStudio statistical program (R Core Team, 2018) with a significance level of 5%. The assumptions of normality (Shapiro-Wilk) and homoscedasticity (Levene's test) of the data on survival, deformities, and for each biomarker were checked. The data were transformed with  $\ln P$  and then ANOVA was applied together with the Tukey test. Multiple paired comparisons were performed to verify the differences between treatments and control, also considering possible differences between exposure times within the same treatment for survival.

#### *Mathematical modelling*

The survival rates and deformity indices obtained in the acute ecotoxicity tests in the embryo-larval stage were used to calculate an impact value that was applied on a stochastic model based on the individual (Brito et al., 2017). The propagation of the effects observed on embryos and larvae to the population dynamics of *R. quelen* was evaluated, considering that the pollutants contained in the water samples tested affect all stages of development of the species (embryonic-larval, juvenile, and adult).

To obtain the impact value of each treatment, the steps described below were followed.

- (I) First, the number of alive and normal larvae after 96hpf was registered for each treatment. Equation (1) was performed to obtain the rate of individuals that were not impacted by the pollutants in the water samples.

$$\begin{aligned} & \textbf{Non – impacted individuals (\%)} \\ & = \frac{\textbf{Number of individuals alive and without deformities}}{\textbf{Total number of individuals}} \times 100 \end{aligned} \quad (1)$$

- (II) Subsequently, the impact value of each treatment was obtained using equation (2).

$$\textbf{Impact Value} = \frac{\textbf{Non – impacted individuals in the treatment (\%)}}{\textbf{Non – impacted individuals in the Control (\%)}} \quad (2)$$

The effect of treatments on individuals was modeled by multiplying the maximum survival probability of each life phase (embryo-larval, juvenile, and adult) by their respective impact value, as presented by Brito et al. (2017). As a result of the population modelling simulations, the number of *R. quelen* adults over the years was obtained for each treatment. For the sake of interpretation of the results, they were transformed into relative population density using equation (3). The control population represents the undisturbed scenario, with no impact on the maximum probabilities of survival. Therefore, the average population density of the control treatment is 1.0, but it varies for the other treatments according to their impact values. The first 100 years on the simulation were based on the maximum probability of survival of all life stages (without impact), so that the population would reach asymptotic behavior. In the year 101, the impact values from each treatment were applied, considering that all life stages would be equally affected (embryonic-larval, juvenile, and adult).

$$\begin{aligned} & \textbf{Relative population density (\%)} \\ & = \frac{\textbf{Adult population size of each year}}{\textbf{Mean population size in a non – perturbative scenario}} \times 100 \end{aligned} \quad (3)$$

## Results and discussion

### *Chemical analysis*

The chemical analysis of micropollutants detected in tested water showed that JR presented the most complex mixture followed by IR, while MR showed the lower number of micropollutants detected. All contaminants analyzed were found in concentrations below the limits established by Brazilian legislation for use class 2 (CONAMA, 2005), in which Iguaçu is classified (Table 1). There are more restrictive limits for arsenic ( $0.14 \mu\text{g L}^{-1}$ ), hexachlorobenzene ( $0.00029 \mu\text{g L}^{-1}$ ), and PCBs ( $0.000064 \mu\text{g L}^{-1}$ ) that are applied when potential fishing or fish farming occurs, in which case, arsenic and PCBs would have been present in concentrations above the limits (Table 1).

Fluoranthene and pyrene were detected only in JR site. These two PAHs are usually the most abundant compounds associated with pyrogenic sources, considered important persistent contaminants because they are largely unaffected by degradation or removal processes other than sedimentation (Page et al., 1999; Burgess et al., 2003). As these chemicals are indicative of human activities such as fossil fuel combustion, industrial discharge or urban runoff (Jordan et al., 2021). In fact, the JR runs through seven municipalities, including one of the state's biggest city, Guarapuava (Peyerl et al., 2018). Also, the pulp and paper industry, known as an important PAH source, is the main industrial branch in the region (Peres, 2009; Gupta et al., 2018). Recently ECHA (2018a, 2018b) described the carcinogenic, mutagenic, toxic for reproduction, persistent, bioaccumulative, and toxic properties of PAHs, highlighting the serious risk of exposure to human health and the environment.

The low or undetectable levels of OCPs can be explained by no longer production nor used in large scale due to their toxicological properties and restrictions, but irregular use or deposit may still be a potential source (Rissato et al., 2006). Paraná is the second larger pesticide consumer in Brazil, and, considering the well-developed and intensive agriculture activities in the region around the Jordão river, these ecosystems are prone to contamination by agricultural runoff (IPARDES, 2020). PCBs are persistent organic pollutants related with reproductive, immunological and carcinogenicity to aquatic organisms even under trace concentrations. As these compounds are lipophilic and biomagnificated in the food chain, the chronic exposure is a constant risk to biota, and the presence of these molecules in both Iguaçu and Jordão rivers are strong evidence of industrial activity impact. Specifically in Iguaçu river the presence of these compounds revealed that the larger industrial park in the state of Paraná located in Curitiba city, even more than 200 km upstream, is the most important source of pollutants to Iguaçu basin. The presence of Cd, Pb, and As in the water of Iguaçu river reinforce the mentioned above but also revealed the urban waste discharge,

mining and manufacturing and fertilizer or pesticide use (Zhou et al., 2020), also including fossil fuel uses (Allen et al., 2012) or leachates from preservative-treated wood (Hingston et al., 2001). All these activities are found along both studied rivers. These toxic metals have the effects well described by USEPA (2017), and even in low concentrations they are considered priority pollutants to environmental studies (USEPA, 2017). As in the current study the water was filtered removing organic particles, the concentrations of micropollutants detect could be underestimated considering that the concentration of organic or inorganic pollutants in particulate matter, increase the bioavailability to biota.

Table 1. Organic pollutants and metals detected in water samples from Jordão River (JR), Iguaçú River downstream (ME) and upstream from the mouth of Jordão River (IR). The last column shows the limits established by the Brazilian legislation CONAMA n° 357/2005 (CONAMA, 2005) for water of Class 2. LoD = limit of detection. PAH LoD = 0.010  $\mu\text{g L}^{-1}$ , OCP, PCB, and PBDE LoD = 0.062  $\text{ng L}^{-1}$ , As LOD = 0.68  $\mu\text{g L}^{-1}$ , Cd LOD = 0.01  $\mu\text{g L}^{-1}$ , Pb LOD = 0.13  $\mu\text{g L}^{-1}$ .

	Water Sample			
	Meeting of the rivers (ME)	Iguaçu River (IR)	Jordão River (JR)	Limit ( $\mu\text{g L}^{-1}$ )
<b>Organic pollutants</b>	$\mu\text{g L}^{-1}$			
<i>Polycyclic aromatic hydrocarbons (PAHs)</i>				
Fluoranthene	< LoD	< LoD	0.032	-
Pyrene	< LoD	< LoD	0.055	-
Total PAHs	< LoD	< LoD	0.09	-
<i>Organochlorine pesticides (OCPs)</i>				
Hexachlorobenzene (HCB)	< LoD	0.000102	< LoD	0.0065
o,p'-DDT	< LoD	< LoD	0.000084	0.002
Total OCPs	< LoD	< LoD	0.00008	-
<i>Polychlorinated biphenyls (PCBs)</i>	< LoD	0.00027	0.00021	0.001
<i>Polybrominated diphenyl ethers (PBDEs)</i>	< LoD	< LoD	< LoD	-
<b>Toxic Metals</b>	$\mu\text{g L}^{-1}$			
Cadmium (Cd)	< LoD	0.06	< LoD	1
Lead (Pb)	< LoD	2.06	0.16	10
Arsenic (As)	0.27	3.52	< LoD	10

### *Fish embryo-larval toxicity tests*

*Survival rates:* The embryos (24 hpf) of *R. quelen* exposed to MR presented the lowest survival rate (76.67%), different from the control and IR (both 93.33%), but no differences in larvae survival for the other tested periods of exposure and treatments was observed (Figure 2). According to Brito et al. (2018) the exposure of *R. quelen* embryos and larvae to environmental samples from Upper Iguaçú River similar results were found, where the survival did not vary greatly among the treatments. In addition, Gomes et al. (2021) after

exposure of embryos from the same species to environmentally relevant concentrations of triclosan, triclocarban, and their binary mixture, observed high survival rates for all treatment groups, ranging from 92.8% to 98%. In fact, regarding survival rates and compared to other native species, *R. quelen* embryos shown to be more resistant, probably explained by the egg structure and lower permeability of the embryonic membranes (Barreto et al., 2020). Moreover, *Danio rerio* embryos exposed to water from river high impacted by pesticides in southern Brazil also did not show differences in survival rates across treatments (Severo et al., 2020). The results described above revealed that survival rate is not the most sensitive endpoint, but posterior effects due to malformations for example could be more expressive to understand the impact on population. It is well described that chronic exposure initiating in the early stages of development and during the live cycle can lead to irreversible damages do populations and community of fish, even under low concentration (Folle et al., 2021). According to Gemuse et al. (2021), juveniles of *Oreochromis niloticus* chronically exposed to upper Iguaçú water threat the fish community of Iguaçú river.

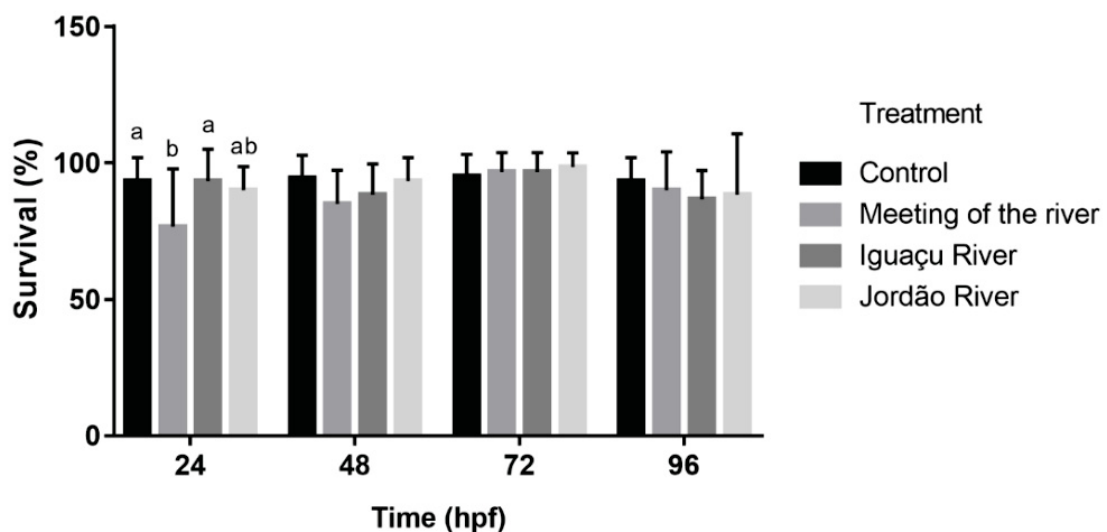
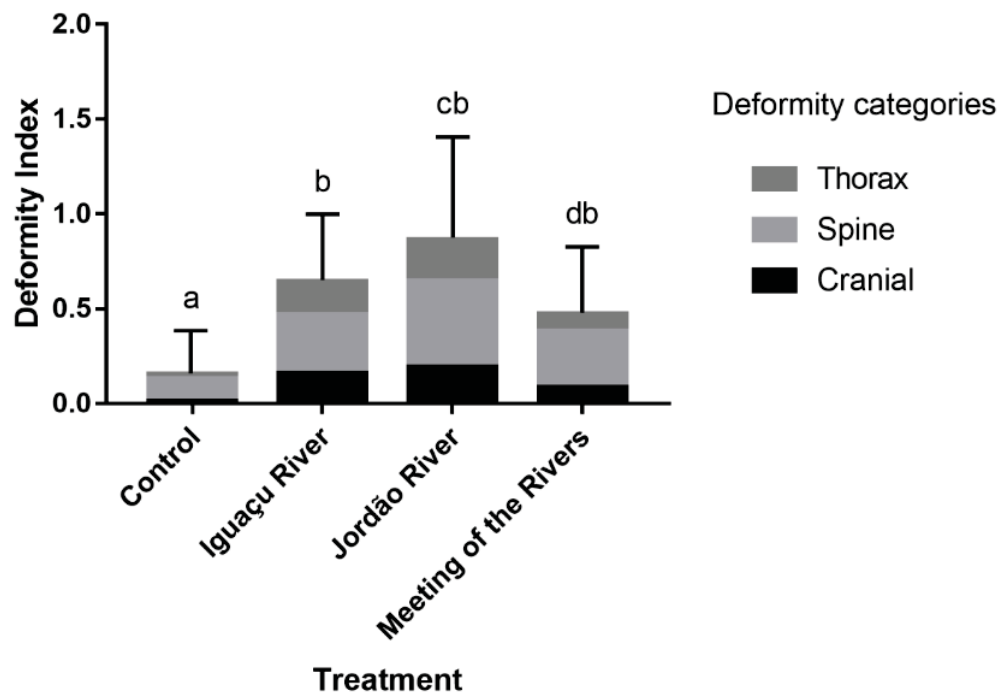


Figure 2. Survival rate of *R. quelen* embryo exposed to water from Iguaçú and Jordão rivers. Different letters indicate significant differences between the treatments ( $p < 0.05$ ).

Still, the chemical composition of MR could reach the developing embryo and cause a higher mortality than IR before 24 hpf. Li et al. (2009) and Sun et al. (2016) described that arsenic, for example, can cross the egg chorion, delaying hatch and inducing mortality in fish embryos. Moreover, according to Azevedo-Linhares, et al. (2018) the mixture of pyriproxyfen pesticide and microcystin, or toxic metals (Nagamatsu et al., 2021) presented high toxicity to

*R. quelen* under initial stages of development. A complete chemical analysis is too hard, and so it is impossible to describe the complex mixture of contaminants and pollutants present in water impacted by an extremely high diversity of chemicals. On this way, the events discussed here do not consider other elements or molecules that certainly are present in the studied sites, where many pathways of increased toxicity due to the joint action of other pollutants may have occurring (Cheng et al., 2021).

*Deformity indices:* The deformity indices present high levels larvae exposed to the three studied sites comparatively with the control group (0.152) (Figure 3) highlighting here that individuals exposed to MR (0.471) showed a tendency to be less affected than those exposed to water from IR (0.644) and JR (0.865). This finding suggests that the high concentration of chemicals in JR are directly affecting the deformity index. Spine deformities were the most frequent and severe morphological damages in larvae from all treatments, while cranial and thorax deformities did not differ among the groups (Figure 4). This result is in line with the results of the chemical analysis, where samples with a higher number of contaminants detected, also generated higher deformity indices.



**Figure 3.** General deformity index, with scores of spine, cranial, and thorax larvae malformations. Different letters indicate significant differences between the treatments ( $p < 0.05$ ).

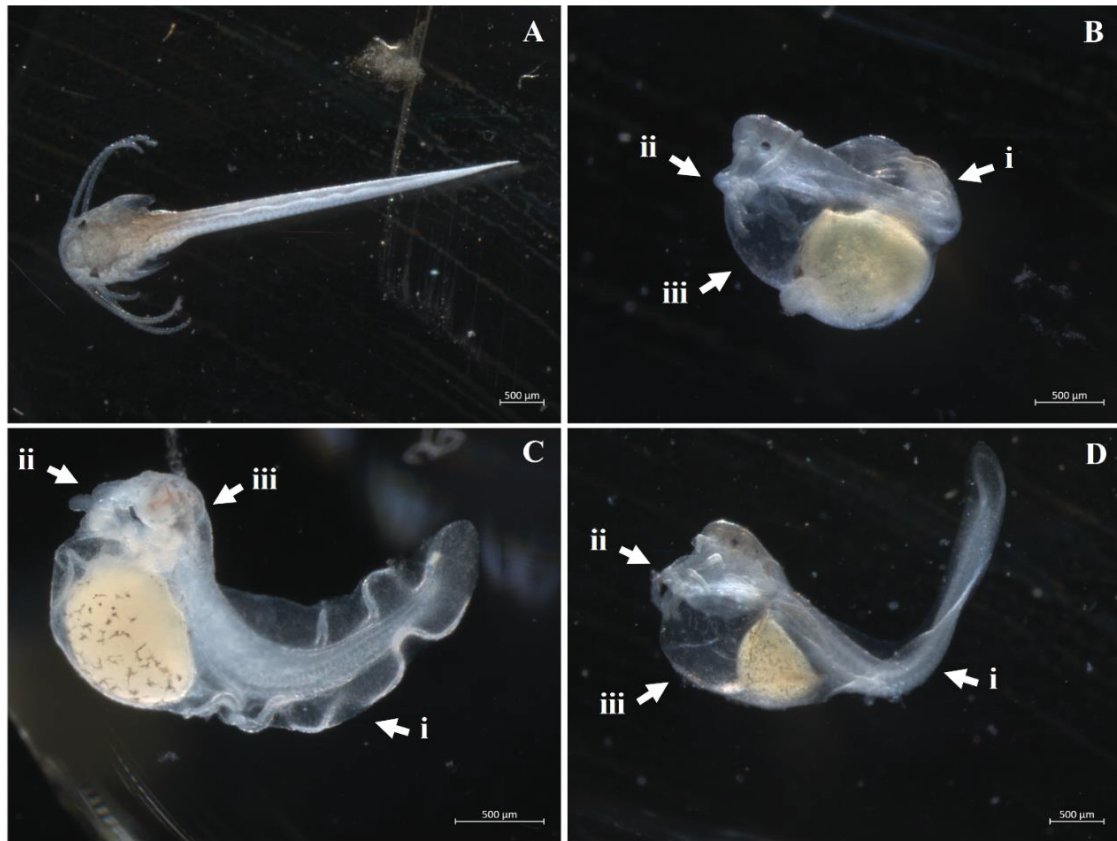


Figure 4. Deformities observed in *R. quelen* larvae after exposure to 72 hpf. (A) Normal larvae from control group. (B) Malformed larvae exposed to the Iguaçú River water (IR): (i) spinal torsion and atrophy, (ii) stunted barbels, and (iii) pericardial edema. (C) Malformed larvae exposed to the Jordão River water (JR): (i) spinal torsion, (ii) stunted barbels, and (iii) absence of eyes. (D) Malformed larvae exposed to the water from meeting of the rivers (MR): (i) spinal torsion, (ii) stunted barbels, and (iii) pericardial edema.

Deformities can represent major ecophysiological challenges for individuals and a devastating effect on fish populations, as they affect individual fitness, decreasing the probability of survival or the ability to reproduce (Corrales et al., 2014; Sfakianakis et al., 2015). A vast array of morphological aberrations on fish have been associated to pollutant exposure (Eissa et al., 2021). For example, lead-exposed embryos of the siluriform *Clarias gariepinus* developed gross morphological malformations, including different kinds of notochordal curvature, such as lordosis, scoliosis, kyphosis, and C-shaped body (Osman et al., 2007). Pyrene exposure increased the deformity rates of *Sebastiscus marmoratus* embryos, impairing skeleton development by disrupting the proliferation of chondrocytes (cartilage cells) (Shi et al., 2012). 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a by-product of numerous industrial processes such as pesticide production and paper bleaching, can also induce deformities, especially spine and tail ones, on the early life stages of several fish species (Elonen et al., 1998; Giesy et al., 2002). Moreover, Kessabi et al. (2013) found a high incidence of spinal deformities on fish from polluted sites, suggesting a correlation between

environmental exposure to a mixture of pollutants, such as metals, PAHs, and estrogenic compounds, and spinal deformities.

Although none of the contaminants analyzed here were present in concentrations above the limits established by Brazilian legislation, water from the three studied sites induced severe malformations in *R. quelen* larvae, reinforcing the importance of bioassays, since physicochemical analysis alone would not reflect the real conditions of water quality. Therefore, even at low or undetected concentrations, pollutants may still promote deleterious effects on the biota (Hu et al., 2009; DeCourten et al., 2020; Gemuse et al., 2021). Considering that the mouth of the Jordão River is located 2 km downstream from Governador Ney Aminthas de Barros Braga (Segredo) Hydroelectric Power Plant, it is also important to consider the factors influencing water quality downstream of reservoir systems. Some studies have showed the self-purification properties of reservoirs, with decreased water flow velocity, increased adsorption to suspended solids, and sedimentation of contaminants (Zhao et al., 2012; Gao et al., 2016). On this way, including the high dilution presented in the reservoir (Muller et al., 2018), was expected that individuals exposed to the Iguaçu water could presented better health condition with mitigant effects on the impact of micropollutants, but this was not observed. These findings revealed that despite of all advantages described above to reservoirs in terms of chemical impact, the high incidence of deformities in individuals exposed to Iguaçu water indicate the bioavailability of them to biota, inputting relevant risk to biodiversity.

#### *Biochemical biomarkers*

Comparatively with the control group the SOD activity was higher in larvae exposed to the water from MR, while the concentration of NPT decrease in larvae exposed to water from MR and JR. There were no significant differences among treatments for the other considered biochemical biomarkers (Figure 5).

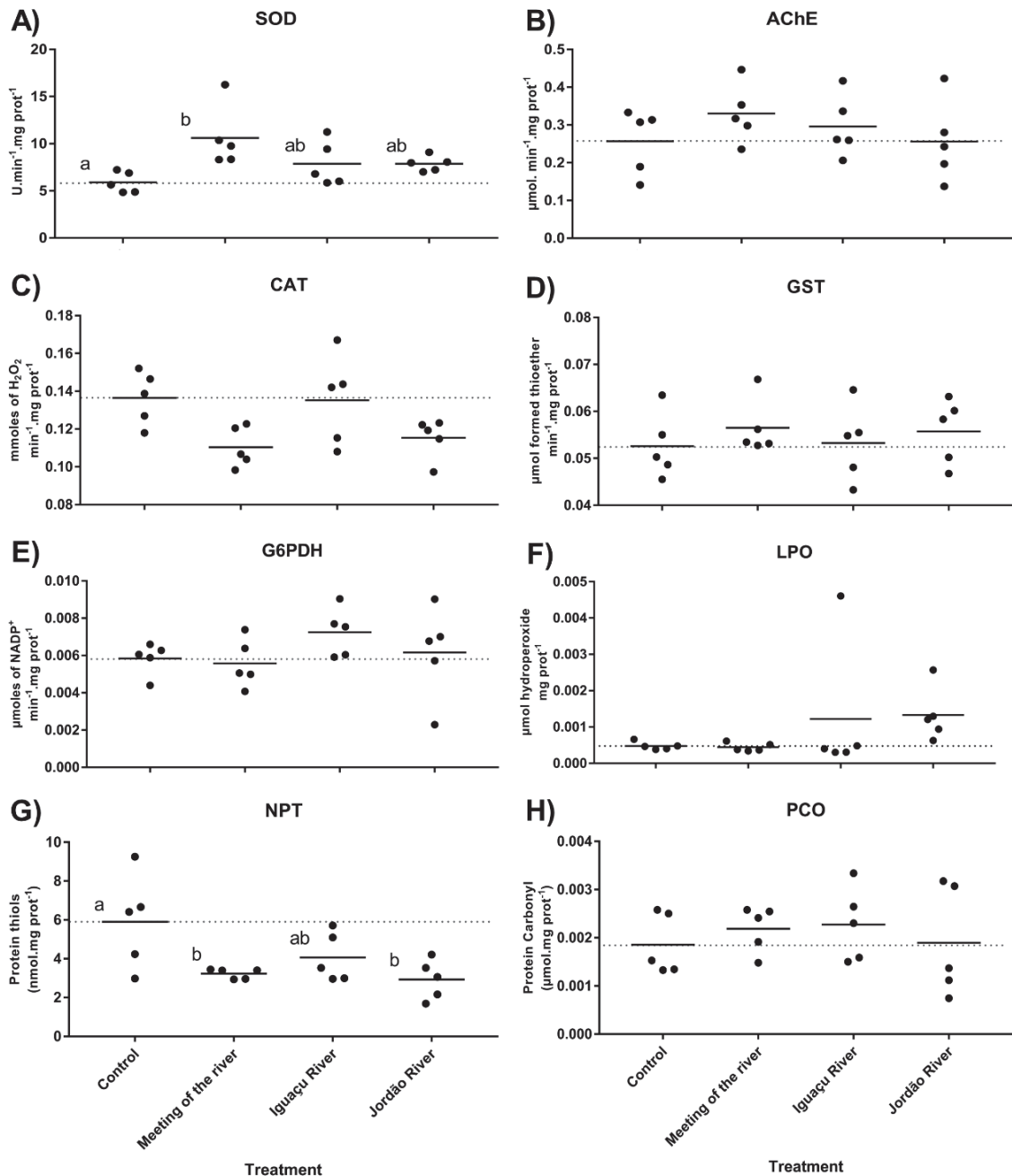


Figure 5. Biochemical biomarkers on *R. quelen* larvae after 96 hpf to water from Iguacu and Jordão rivers. A) Superoxide dismutase (SOD) activity. B) Acetylcholinesterase (AChE) activity. C) Catalase (CAT) activity. D) Glutathione S-transferase (GST) activity. E) Glucose-6-phosphate dehydrogenase (G6PDH) activity. F) Lipid peroxidation (LPO). G) Concentration of non-protein thiols (NPT). H) Protein carbonylation (PCO). Different letters indicate significant differences between the treatments ( $p < 0.05$ ).

Pollutants can exert their toxicity through the reactive oxygen species (ROS) production, such as superoxide radicals ( $O_2^{\cdot-}$ ), a common by-product of xenobiotic redox cycling or because of biotransformation (Fortuño et al., 2009). The  $O_2^{\cdot-}$  is the main precursor of the hydroxyl radical ( $\cdot OH$ ), the most reactive among ROS. The antioxidant enzyme SOD constitutes the first line of defense from antioxidant mechanism in cells, catalyzing the

dismutation of  $O_2^{\cdot-}$  into hydrogen peroxide ( $H_2O_2$ ), further metabolized by other enzymes and so preventing  $\bullet OH$  formation and oxidative damage (Ighodaro and Akinloye, 2018; Bevilaqua et al., 2020). Organisms react to xenobiotic exposure by upregulating the biosynthesis of this antioxidant protein and multiple studies have shown that SOD can be induced in fish by the presence of xenobiotics and increased ROS production (Winston and Di Giulio, 1991; Klotz and Steinbrenner, 2017). Wang et al. (2020) exposed *D. rerio* embryos to pesticides and their mixtures and found increased ROS levels and SOD activity (Wang et al., 2020). Therefore, the increase in SOD activity suggests MR-treated larvae were exposed to xenobiotics and prone to oxidative stress through  $O_2^{\cdot-}$ .

NPT consists mostly of glutathione (GSH), which is an efficient  $\bullet OH$  scavenger (Yadav and Mishra, 2013). In addition, GST uses GSH for xenobiotic conjugation and excretion, depleting the cellular GSH pool, leading to  $\bullet OH$  accumulation and suppressed capacity of GSH regeneration due to oxidative stress (Lackner, 1998). In fact, although it was not statistically significant, an increase in GST could also be observed on MR larvae, which would be in line with the reduced NPT levels found. Wu et al. (2011) exposed *D. rerio* embryos to endocrine-disrupting chemicals and their mixture and found reduced GSH levels. Different pesticides can also lead to reduced levels of GSH in this same species (Li et al., 2018). Thus, a reduction of NPT levels indicates that MR and JR impaired the redox balance and antioxidant response of *R. quelen* larvae, thereby increasing their susceptibility to oxidative stress damage.

The increase of SOD activity in individuals exposed to water from Iguaçú and Jordão rivers and the decrease of NPT levels also in the same group revealed again that the micropollutants found in these ecosystems are bioavailable and induce the antioxidant mechanism. The absence of effects in macromolecules showed that the mechanisms it is efficient to annulate the cell damages. Despite of that, it is known that the high incidence of antioxidant mechanism activation leads to a systemic toxicology event that is sometime responsible for chronic diseases in animals (Stobbe et al., 2011). The presence of low concentrations of a diversity of micropollutants found in water from Iguaçú and Jordão rivers can induces the oxidative stress mechanisms in larvae of *R. quelen*, that associated with other disturbs described in the current study, show a potent chemical impact to biota from Iguaçú basin.

The IBR is represented in Figure 6, where scores above or below zero (control) indicate the stimulus or inhibition of a given biomarker. The micropollutants detected in water from MR lead to the highest IBR score, followed by the IR and the JR with similar scores.

Differently from observed to the isolated event, the IBR permit to demonstrate that LPO (JR) and PCO (IR) play an important information, where although these parameters were not statistically significant were enough to demonstrate the value of biochemical biomarkers in the diagnostic of water quality. The same can be observed to AChE activity found in individuals exposed to water from Iguaçú and Jordão rivers. Although the chemical analysis revealed the lower diversity of micropollutants in water from MR including the lowest deformity index among the three studied sites, curiously the global response of biomarkers in the present study showed the worst conditions of the Iguaçú and Jordão water. One explanation could be relationated to the high turbulence of the river observed in this site, that could agitate the sediment and so increasing the bioavailability of other chemicals not detected int the current study

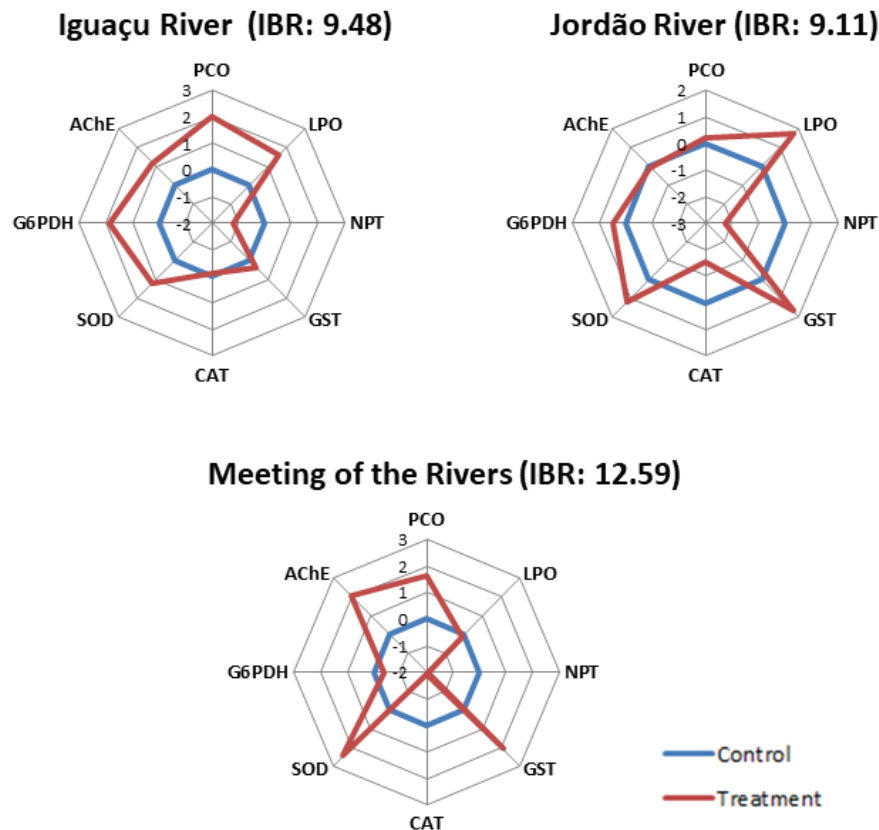


Figure 6. Integrated biomarker response (IBR) of biochemical biomarkers in larvae of *R. quelen* exposed 96 hpf to water from Iguaçú and Jordão rivers. The radar charts show the oscillation of biomarker responses of the treated groups (red line) in relation to the control group (blue baseline). The area below zero indicates a reduction in the parameter, while values above zero indicate an increase in the parameter.

It could be suggested that MR-exposed embryos and larvae were at an early stage of oxidative stress response, in which SOD activity was activated to annulate ROS effects.

Meanwhile, JR-exposed larvae, which had a higher deformity index, were already experiencing oxidative damage, which can be seen by the LPO stimulus in the IBR chart. Studies have shown the relationship between oxidative stress, lipid peroxidation, and major skeletal disorders, such as scoliosis and lordosis, in fish (Lewis-McCrea and Lall, 2007, 2010; Boglione et al., 2013). Fish skeletal tissues contain a significant amount of lipids with critical roles in regulating bone metabolism, being highly vulnerable to lipid peroxidation and skeletal anomalies (Lall and Lewis-McCrea, 2007), which could explain JR higher deformity index, especially spine malformations.

### *Mathematical modelling*

Based on the larvae survival observed experimentally at the end of 96 hpf, the impact value of each treatment was calculated (Table 2) and imposed on the survival of all life stages of *R. quelen* to investigate the impact that the water from Iguaçu and Jordão rivers on the population of the species. The temporal evolution of the relative population density is shown in Figure 7.

Table 2. Impact values used in each scenario simulated by the mathematical model. The survival probabilities of the three life stages assumed for the control are the maximal survival probabilities described for *R. quelen* in the literature. Non-impacted larvae correspond to alive larvae without deformities at 96 hpf.

Treatment	Non-impacted larvae	Impact Value	Survival probability		
			Larvae	Juvenile	Adult
Control	91.67	1.00	0.60	0.80	0.65
Meeting of the rivers (MR)	86.67	0.95	0.57	0.76	0.62
Iguaçu River (IR)	80.00	0.87	0.52	0.70	0.57
Jordão River (JR)	78.33	0.85	0.51	0.68	0.55

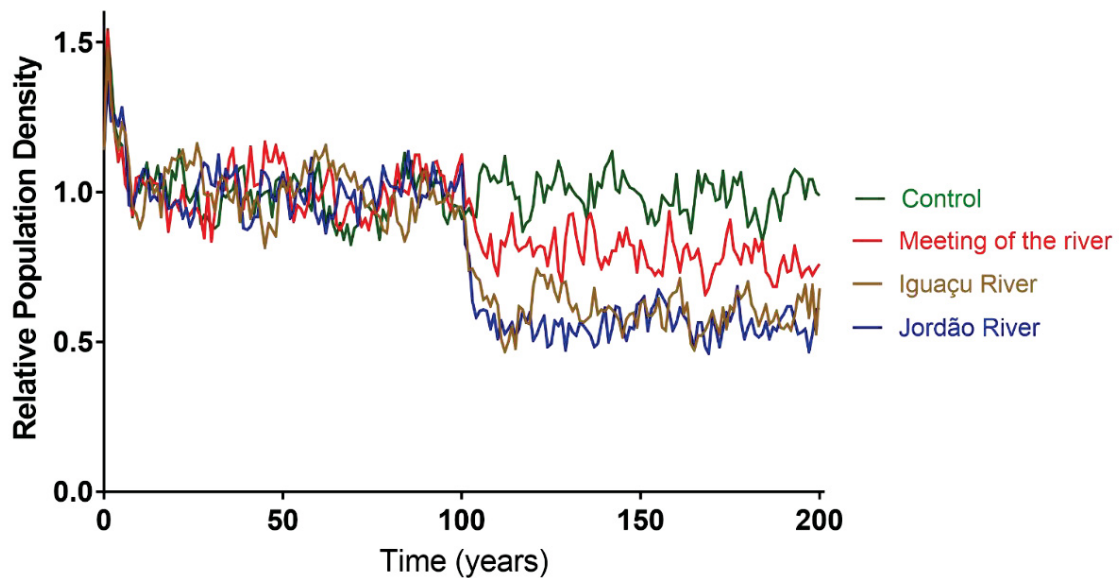


Figure 7. Temporal evolution of relative population size of *R. quelen* considering different impact values calculated from embryo-larvae exposition to control and water samples from rivers.

In the long run, according to the model simulations, *R. quelen* populations inhabiting the Jordan River, and being exposed to the same physicochemical characteristics as the water sample tested here, would have the greatest population reduction (46.19 %). The Iguazu River upstream (IR) and downstream (MR) of the Jordão River would lead to the second and third greatest population reduction (40.48 % and 33.33 %, respectively), compared to a 13.33 % reduction in the control treatment. This finding suggests a greater risk of exposure of fish species inhabiting JR than the other two points, suggesting that JR would not exert greater impact on IR fish populations due to the influx of its polluted waters, at least not in an additive or synergistic way. Clearly, it is important to consider that the tests carried out here used a single sample from these points, showing a punctual profile of the toxic potential of these waters. Given seasonal climate and water flow changes, the results could be variable. In any way, the results are concerning for the ichthyofauna of the Iguazu River basin, especially considering the high endemic rates of this basin.

The siluriforms are the most representative order in the Iguazu River basin, with the highest species richness (Larentis et al., 2016). Most of JR endemic species also belong to this group, such as *Trichomycterus crassicaudatus*, *Trichomycterus igobi*, *Trichomycterus plumbeus*, and *Ancistrus agostinhoi* (Baumgartner et al., 2012). Moreover, the siluriform *Steindachneridion melanodermatum*, which used to occur along the studied stretches and is known as the biggest native predator in the Iguazu, is an endangered species which population is severely fragmented and in continued decline due to decreased habitat quality (ICMBio,

2018). There is a reproduction program for this species, aiming to repopulate the river and restore its population levels, but the species habitat quality requirements have received little attention. Thus, considering the results shown to *R. quelen* populations and the risk of exposure suggested to JR endemic fauna, local environmental agencies must focus on the protection of this river biodiversity by means of better water management practices to achieve better water quality parameters.

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### 3 CONSIDERAÇÕES FINAIS

O Rio Jordão (JR) apresentou a mistura mais complexa de micropoluentes presentes na água, seguido do Rio Iguaçu (IR), enquanto no encontro dos Rios (MR) apresentou a menor quantidade de micropoluentes avaliados neste estudo;

A taxa de sobrevivência não se apresentou como um parâmetro sensível aos micropoluentes avaliados neste período do estudo, apenas os indivíduos de *R. quelen* expostos às águas do MR em 24hpf tiveram suas taxas de sobrevivência reduzidas quando comparadas ao grupo Controle;

A água dos três locais estudados induziu malformações em larvas de *R. quelen*, sugerindo uma correlação entre malformação e mistura de poluentes. Os indivíduos de *R. quelen* expostos à água do Rio Jordão apresentaram um maior índice de deformidade, seguido pelos do Rio Iguaçu e Encontro dos Rios;

O aumento na atividade da SOD e a redução dos níveis de NPT indicam que a água dos rios Iguaçu e Jordão apresentam moléculas que afetam o equilíbrio redox nas larvas de *R. quelen*, aumentando sua suscetibilidade aos danos do estresse oxidativo.

A aplicação de modelagem matemática demonstrou que em condições naturais similares às avaliadas neste estudo, a dinâmica populacional de *R. quelen* seria afetada, havendo uma redução populacional de 46,19% no Rio Jordão, 40,48% e 33,33%, respectivamente no Rio Iguaçu a montante (IR) e a jusante (MR) do Rio Jordão.

O estudo mostra que as espécies de peixes encontradas na bacia do Rio Iguaçu podem estar sendo afetadas pela presença de micropoluentes na água, colocando em risco a dinâmica entre as espécies.

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