UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCIÊNCIAS - CÂMPUS DE BOTUCATU
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (ZOOLOGIA)

# SISTEMAS PARASITO-HOSPEDEIRO COMO SENTINELAS DE POLUIÇÃO AQUÁTICA NA BACIA HIDROGRÁFICA DO TIETÊ-JACARÉ, SUDESTE DO BRASIL

**Lucas Aparecido Rosa Leite**

**Botucatu, SP**
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**Orientadora:** Vanessa Doro Abdallah Kozlowiski, Dra.

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Palavras-chave: Bioindicação de acumulação; Bioindicação de efeito; Parasitologia ambiental; Parasitologia de peixes; Poluição aquática.

Dedico esta tese a todos os meus professores, do primário à pós-graduação. Sem vocês
eu não seria nada do que sou hoje e muito menos teria chegado até aqui.

*“Quando eu deixar este mundo, não deixarei arrependimentos*
*Deixarei algo para ser lembrado, e eles não se esquecerão”.*
Beyoncé.

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# *Sumário*

## SUMÁRIO

# Resumo

# RESUMO

O avanço da urbanização e do desenvolvimento da industrialização e do agronegócio são uma constante ameaça à conservação da biodiversidade, especialmente em ambientes aquáticos. Nesses ambientes, bioindicadores podem ser muito úteis na detecção e prevenção de impactos ambientais que colocariam em risco a estruturação do ecossistema. Dentre os organismos com potencial bioindicador, encontra-se um grupo bastante peculiar e comumente esquecido em estudos de biodiversidade: os parasitos. Os estudos envolvendo parasitos e seus possíveis potenciais como bioindicadores da saúde do ambiente aumentaram muito nas últimas décadas, entretanto, na região da América do Sul ainda é um campo de pesquisa pouco explorado. No presente estudo, avaliou-se o potencial bioindicador de parasitos em duas vertentes: a bioindicação de efeito e a bioindicação de acumulação de metais-traço, em dois rios do Estado de São Paulo: o rio Jacaré-Pepira e o Jacaré-Guaçú. No estudo com bioindicação de efeito, foi testada a variação nos índices de prevalência, intensidade e abundância de cinco espécies da classe Monogenea (*Gussevia astronoti, Gussevia asota, Anacanthorus serrasalmi, Rhinoxenus piranhus* e *Amphithecium speirocamarotum*) em relação à sazonalidade (período seco e chuvoso), às variáveis químicas orgânicas e inorgânicas da água dos rios e em relação ao fator de condição de seus hospedeiros *Serrasalmus maculatus* e *Astronotus crassipinnis*. Os resultados mostraram variações significativas dos índices de prevalência, abundância e intensidade de algumas das espécies de monogenéticos testadas, com *G. asota* mostrando interações tanto com os parâmetros químicos da água quanto com os fatores de condição dos hospedeiros, se mostrando a espécie mais sensível das cinco analisadas. Já no estudo com bioindicação de acumulação, avaliou-se o potencial acumulativo para metais-traço de parasitos pertencentes à três taxa: Cestoda (*Proteocephalus macrophalus*), Nematoda (*Hysterothylacium* sp.) e Digenea (*Phyllodistomum* sp.) parasitos de *Cichla kelberi* e de *Hoplias malabaricus*. As três espécies demonstraram uma alta capacidade de acumulação, especialmente cestoides e nematoides. Além disso, verificou-se que peixes infectados tendem a concentrar menos metais em seus tecidos que hospedeiros não-infectados. Em geral os estudos mostram que os parasitos de peixes dos rios Jacaré-Pepira e Jacaré-Guaçú possuem potencial bioindicador e podem ser usados em estudos de diagnóstico e monitoramento ambiental. Além disso, expande-se ainda mais o conhecimento relacionado ao parasitismo e bioindicação na região neotropical. Por fim, realizou-se um estudo de avaliação de risco de contaminação por consumo humano e de bioacumulação de metais-traço na musculatura de *H. malabaricus*, também dos rios Jacaré-Pepira e Jacaré-Guaçú, onde observou-se que os peixes do rio Jacaré-Guaçú possuem concentrações significativamente maiores que os do Jacaré-Pepira. Além disso, nos peixes do rio Jacaré-Guaçú, o elemento Al teve o quociente de risco (HQ) acima de 1, que indica possível risco de contaminação caso seja consumido por humanos. Outros elementos que merecem destaque são o Cr e o Cd. O Cr estava em concentrações muito acima do permitido na legislação brasileira e o Cd teve um fator de bioconcentração alto, que pode indicar um processo de biomagnificação.

**Palavras-chave:** Parasitologia ambiental; bioindicação de efeito; bioindicação de acumulação; poluição aquática; parasitologia de peixes.

# *Absctract*

# ABSTRACT

The advance of urbanization and the development of industrialization and agribusiness are a constant threat to the conservation of biodiversity, especially in aquatic environments. In these environments, bioindicators can be very useful in detecting and preventing environmental impacts that would jeopardize the structuring of the ecosystem. Among the organisms with potential for bioindication, there is a very peculiar and commonly overlooked group in biodiversity studies: parasites. Studies involving parasites and their potential as bioindicators of the environmental health have increased in recent decades, however, in the South American region it is still a little explored field of research. In the present study, the potential of parasites as bioindicators was assessed in two ways: the effect bioindication, and the bioindication of trace metals accumulation in two rivers in the state of São Paulo: the Jacaré-Pepira River and the Jacaré-Guaçú River. In the study with effect bioindication, the variation in the rates of prevalence, abundance, and intensity of five species of the Monogenea class (*Gussevia astronoti*, *Gussevia asota*, *Anacanthorus serrasalmi, Rhinoxenus piranhus* e *Amphithecium speirocamarotum*) was tested compared to the seasonality (dry and wet season), to the organic and inorganic chemical variables of the rivers waters, and in relation to the condition factors of its fish hosts *Serrasalmus maculatus* and *Astronotus crassipinis*. The results showed significant variations in the prevalence, abundance, and intensity rates of some of the monogenetic species tested, with *G. asota* showing interactions both with the chemical parameters of the water and with de condition factors of the hosts, proving to be the most sensitive species of the five analyzed. In the study with accumulation bioindication, the potential for trace metal accumulation of parasites belonging to three different taxa was evaluated: Cestoda (*Proteocephalus macrophalus*), Nematoda (*Hysterothylacium* sp.) and Digenea (*Phyllodistomum* sp.) parasites of *Cichla kelberi* and *Hoplias malabaricus*. The three species showed a high capacity for accumulation, specially cestodes and nematodes. In addition, it has been found that infected fish tend to concentrate less amounts of metals in their tissues than non-infected hosts. In general, our studies show that fish parasites in the Jacaré-Pepira and Jacaré-Guaçú rivers have potential for bioindication and can be used in diagnostic and environmental monitoring studies. In addition, the knowledge related to parasitism and bioindication in the neotropical region further expands. Finally, a study was carried out to assess the risk of contamination by human consumption and bioaccumulation of trace metals in the musculature of *H. malabaricus*, also from the Jacaré-Pepira and Jacaré-Guaçú rivers, where fish from the Jacaré-Guaçú River have trace metals concentrations significantly higher than those from the Jacaré-Pepira. In addition, in fish from the Jacaré-Guaçú River, the element Al had a hazard quotient (HQ) above 1, which indicates a possible risk of contamination if consumed by humans. Other elements that are worth mentioning are Cr and Cd. Cr was in concentrations much higher than the allowed by the Brazilian legislation, and Cd had a high bioconcentration factor, which may indicate a biomagnification process.

**Keywords:** Environmental parasitology; effect bioindication; accumulation bioindication; water pollution; fish parasitology.

# *Introdução geral*

# 1 INTRODUÇÃO GERAL

## 1.1 Parasitos de peixes como bioindicadores da saúde do ambiente

O parasitismo é um dos mais bem sucedidos meios de vida apresentados pelos seres vivos (Poulin e Morand, 2000), e é uma relação tão antiga que desempenhou um papel crucial no surgimento da vida na terra, através daquilo que se chama de parasitismo molecular (Araújo et al., 2003; Nee e Smith, 1990). O termo em si vive à sombra de dualidades, pois parasitos são intrinsecamente ligados à geração de diversidade, ao passo que podem causar a extinção de espécies, podem causar problemas na reprodução dos hospedeiros, mas aumentar a taxa de crescimento da população, e podem estimular uma resposta imunológica, ao mesmo tempo que estimulam uma infecção crônica secundária (Hudson, 2005). O termo parasitismo em si é comumente associado a patogenicidade quando na verdade esse nem sempre é o produto final da relação, sendo que muitas vezes os parasitos são inofensivos à saúde do hospedeiro e de extrema importância para sua sobrevivência (Araújo et al., 2003).

Historicamente, o papel dos parasitos no funcionamento do ecossistema tem sido considerado de pouca importância, no entanto, há evidências crescentes de que os efeitos mediados pelos parasitos podem ser extremamente significativos na modelagem de funções ecossistêmicas e na estruturação das cadeias alimentares, sendo considerados como importantes condutores da biodiversidade (Hudson et al., 2006). Em contrapartida à inegável importância desses organismos na manutenção dos ecossistemas, os parasitos enfrentam alguns paradigmas, especialmente ligados à sua conservação, pois sofrem com o estigma de serem causadores de impactos negativos aos hospedeiros e são constantemente visados em estratégias de erradicação (Dougherty et al., 2015). Sendo assim, entender o parasitismo como um componente crucial na biodiversidade, com a inclusão desses organismos em estratégias de conservação é de extrema importância para se garantir a manutenção dos ecossistemas e da sobrevivência das espécies hospedeiras.

Os impactos ambientais são uma constante ameaça à biodiversidade global, e no que se diz respeito a biodiversidade de parasitos, podem causar nas próximas décadas a extinção de 5 a 10% das espécies dos principais clados conhecidos, algumas destas espécies ainda nem descritas pela taxonomia (Carlson et al., 2017; Carlson et al., 2020). Estes organismos, respondem aos impactos de diferentes formas dependendo do seu grupo taxonômico, do seu hospedeiro e do hábitat que ocupa (Lafferty, 1997), podendo nos ajudar a detectar, identificar e até mesmo a prever tais impactos no ambiente (Lafferty, 1997; Vidal-Martínez et al., 2009).

Nas últimas décadas, os estudos que avaliam a força dos impactos ambientais sobre os parasitos e/ou parasitismo tiveram um aumento significativo (Sures et al., 2017), o que levou ao estabelecimento do termo “Parasitologia Ambiental” (Goater et al., 2013; Nachev e Sures, 2016), que torna-se uma disciplina aceita dentro da parasitologia e que engloba todos os estudos que relacionam parasitos e o meio ambiente, especialmente sobre o impacto antropogênico sobre eles e do papel dos parasitos como bioindicadores da qualidade ambiental. Atualmente, as duas áreas mais estudadas dentro desse espectro são 1) os parasitos como bioindicadores de acumulação e 2) os parasitos como bioindicadores de efeito.

Na bioindicação de acumulação, avalia-se o potencial dos parasitos em acumular um determinado poluente, geralmente em comparação aos tecidos do seu hospedeiro. Os metais-traço (também designados como elementos-traço ou metais pesados) são os compostos mais avaliados em estudos com bioacumulação (Sures et al., 2017), porém alguns poluentes orgânicos como bifenilos policlorados (PCBs), pesticidas organoclorados (OCPs), éteres de difenila polibromados (PBDEs), hidrocarbonetos aromáticos policíclicos (PAHs), ftalados, inseticidas, piretróides, N,N-dietil-meta-toluamidas (DEET) (Molbert et al., 2020) e até mesmo fragrâncias e filtros-UV (protetores solares) (Mille et al., 2020) também já foram estudados. Muitos desses estudos mostram que os parasitos conseguem acumular os compostos em quantidades muito superiores à de seus hospedeiros ou em relação a outros bioindicadores de vida-livre, como, por exemplo, os mexilhões (Sures et al., 1997; Sures et al., 1999a; Sures et al., 1999b). Os grupos de parasitos mais utilizados para este fim são os acantocéfalos, cestoides e nematoides (Sures et., 2017), pois são os que geralmente preenchem os principais requisitos necessários para a utilização em estudos desse tipo, que são: facilidade de coleta e identificação, serem grandes em tamanho para que se tenha tecido suficiente para as análises das concentrações dos compostos e com vasta quantidade de informações sobre sua biologia disponível (Sures, 2003; Sures, 2004).

Na bioindicação de efeito, estuda-se as mudanças fisiológicas, de composição química, comportamental ou numérica dos parasitos frente à um agente estressor (Vidal-Martínez et al, 2009). Estes estudos podem ser realizados tanto ao nível de espécie quanto ao nível de comunidade e as métricas utilizadas para avaliar as respostas em relação aos impactos ambientais podem ser vários, dependendo da área de estudo, do tipo de poluente ou qualquer que seja a variável interferente, química ou física. As comumente utilizadas são, ao nível de espécie: alterações nas taxas de prevalência, intensidade e abundância dos parasitos de acordo com as mudanças no meio, e ao nível de comunidade: composição, riqueza e diversidade das espécies de acordo com as áreas de estudo ou concentração dos poluentes ou variáveis químicas e físicas coletadas do ambiente (Blanar et al., 2009; Falkenberg et al., 2019; Gilbert e Avennant-Oldweage, 2017; Igeh et al., 2020; Lacerda et al., 2017; Poulin, 2020).

O avanço da parasitologia ambiental só reforça ainda mais o quão importantes e extremamente úteis estes pequenos, peculiares e frequentemente esquecidos organismos podem ser, não só para a manutenção da biodiversidade, agindo nas cadeias alimentares, mas também ao auxiliar na detecção e monitoramento dos impactos ambientais causados pelo ser humano. Os esforços de pesquisa, com os mais variados grupos de parasitos nas diferentes regiões do globo tornam-se de grande importância para criação de metodologias que incluam estes organismos de maneira aplicada em programas de monitoramento ambiental e conservação da biodiversidade (incluindo a de parasitos).

## 1.2 Os hospedeiros analisados no estudo

### 1.2.1 Hoplias malabaricus *(Bloch, 1794)*

*Hoplias malabaricus* (Bloch, 1794) (Characiformes: Erythrinidae) (Figura 1), popularmente conhecida no Brasil como traíra, é uma espécie de peixe que possui ampla distribuição por toda América do Sul e Central. Trata-se de um peixe carnívoro predador, se alimentando de pequenos peixes e crustáceos, adaptado à ambientes com baixa integridade, preferencialmente em ambientes lênticos, mas também podendo ser encontrado em ambientes lóticos (Gião et al., 2020; Leite et al., 2021; Guimarães et al. 2021).

Por possuir uma carne de alta qualidade, com índices baixos de gordura e muito saborosa, é muito visada na pesca e utilizada na alimentação, principalmente da população ribeirinha. Estima-se que até 9 toneladas deste peixe sejam capturadas anualmente no Brasil (Secretaria de Monitoramento e Controle do Ministério da Pesca e Aquicultura – MPA, 2011).

**Figura 1**: *Hoplias malabaricus* (Bloch, 1794) (Characiformes: Erythrinidae), coletada do rio Jacaré-Pepira, sub-bacia do Tietê-Jacaré, Estado de São Paulo, Brasil. Foto: L.A.R. Leite.

A fauna parasitológica de *H. malabaricus* já foi bastante estudada em diversas bacias da América do Sul. No Brasil, existem estudos nas bacias do rio Paraná, Amazônica, Uruguai, Atlântico Nordeste Ocidental e Oriental, São Francisco, Tocantins-Araguaia, Rio Paraguai e Atlântico Sudeste, com aproximadamente 50 espécies de parasitos já registrados, sendo Monogenea e Nematoda os grupos com mais espécies registradas (Gião et al. 2020), este último com registros de espécies de potencial zoonótico como as do gênero *Contracaecum* Railliet & Henry, 1912, *Eustrongylides* Jägersk, 1909 e *Hysterothylacium* Ward & Magath, 1917 (Gião et al., 2020; Leite et al., 2021).

### 1.2.2 Cichla kelberi *Kullander & Ferreira, 2006*

*Cichla kelberi* Kullander & Ferreira, 2006 (Cichliformes: Cichlidae), conhecido popularmente como tucunaré, é um peixe nativo da bacia dos rios Amazonas e do Tocantins-Araguaia no norte do Brasil (Kullander e Ferreira, 2006), mas amplamente introduzido em diversas bacias brasileiras (Espínola et al., 2010), inclusive na bacia do alto rio Paraná, através do escape de pesqueiros recreacionais (Ota et al., 2018), onde é muito apreciado devido ao seu apreço na pesca esportiva (Winemiller, 2001). Trata-se de um predador voraz cuja dieta se baseia em crustáceos, pequenos peixes, incluindo casos de canibalismo, insetos e plantas (Mendonza et al., 2018), podendo ser encontrado preferencialmente em ambientes lênticos e de águas transparentes (Espínola et al., 2010).

Em áreas onde a espécie foi introduzida, a fauna parasitária de *C. kelberi* tende a possuir uma baixa riqueza de espécies, e é constituída basicamente de espécies generalistas (Yamada e Takemoto, 2013), com exceção de algumas que possuem especificidade parasito-hospedeiro, como *Proteocephalus macrophallus* (Diesing, 1850) (Scholz et al., 1996). Em ambientes introduzidos, os principais taxa registrados de parasitos são Monogenea, Digenea, Nematoda, Cestoda e, em alguns casos, Crustacea (Kohn et al., 2011; Santos- Clapp and Brasil-Sato, 2014; Yamada e Takemoto, 2013). Na bacia do Tietê-Jacaré, no rio Jacaré-Pepira, foram registradas 9 espécies, na maioria monogéticos do gênero *Gussevia* Kohn & Paperna, 1964 (Januário et al., 2019).

**Figura 2**: *Cichla kelberi* Kullander & Ferreira, 2006 (Cichliformes: Cichlidae). Foto: A.S. Leão.

### 1.2.3 Serrasalmus maculatus *Kner, 1856*

*Serrasalmus maculatus* Kner, 1856 (Characiformes: Serrasalmidae), popularmente conhecida como pirambeba, é a única espécie desse gênero endêmica da bacia do alto rio Paraná, (Ota et al., 2018) e que era anteriormente identificada como *Serrasalmus spilopleura* Kner, 1858 (Graça e Pavanelli, 2007). É um predador mutilante, com alimentação preferencialmente piscívora, mas com tendência generalista, englobando itens alimentares como insetos, crustáceos, moluscos e até plantas (Villares Junior et al., 2008) e que possui boa capacidade de adaptação à ambientes lênticos artificiais, onde se tornou muito abundante (Behr e Signor, 2008).

Na bacia do alto rio Paraná, a fauna parasitária de *S. maculatus* é composta por Monogenea, Digenea, Nematoda, Cestoda Acanthocephala e Copepoda, com pelo menos 17 espécies de parasitos registrados, a maioria pertencente ao filo Nematoda, com aproximadamente 7 espécies registradas (Takemoto et al., 2009; Lehun et al., 2020).

**Figura 3**: *Serrasalmus maculatus* Kner, 1856 (Characiformes: Serrasalmidae), coletada do rio Jacaré-Pepira, sub-bacia do Tietê-Jacaré, Estado de São Paulo, Brasil. Foto: F.F. Januário.

### 1.2.4 Astronotus crassipinnis *(Heckel, 1840)*

*Astronotus crassipinnis* (Heckel, 1840) (Cichliformes: Cichlidae), popularmente conhecido como oscar, é um peixe de hábitos diurnos, sedentário (Sánchez-Botero e Araujo-Lima, 2001), endêmico da bacia amazônica e introduzido na bacia do alto rio Paraná através do seu intenso uso no aquarismo (Ota et al., 2018). Na bacia do alto rio Paraná, essa espécie é comumente identificada erroneamente como *Astronotus ocellatus* (Agassiz, 1831) (Júlio Júnior et al., 2009). Trata-se de uma espécie com dieta onívora, com tendência à carnivoria, alimentando-se de pequenos peixes, crustáceos, insetos e plantas, e habitando preferencialmente locais rasos entre galhos e plantas aquáticas nas margens dos rios (Santos et al., 2018).

A fauna parasitária de *A. crassipinnis* é relativamente pouco conhecida. Na Amazônia, foram registradas ao menos 15 espécies de metazoários parasitando peixes dessa espécie (Atroch, 2018; Santos et al., 2018). Na bacia do Tietê-Jacaré, um levantamento realizado nos rios Jacaré-Pepira e Jacaré-Guaçú por Januário et al. (2018) encontrou 15 espécies de metazoários, divididas em Monogenea, Digenea, Nematoda, Crustacea, Oligoqueta e Myxozoa.

**Figura 4**: *Astronotus crassipinnis* (Heckel, 1840) (Cichliformes: Cichlidae), coletada do rio Jacaré-Pepira, sub-bacia do Tietê-Jacaré, Estado de São Paulo, Brasil. Foto: F.F. Januário.

### 1.3 As áreas de estudo

A Bacia Hidrográfica do Tietê-Jacaré (BH-TJ) (Figura 5) está localizada na região central do Estado de São Paulo e abrange 34 municípios, fazendo divisa com as bacias do Piracicaba/Capivari/Jundiaí, do Mogi-Guaçu, do Tietê/Sorocaba, Tietê-Batalha e Médio Paranapanema (Estado de São Paulo, 2015). O clima na região da BH-TJ, segundo a classificação de Köeppen-Geiser, é o Cwa, isto é, clima subtropical úmido com inverno seco e verão quente e chuvoso (Peel et al., 2007), com precipitação média anual de 1.500 a 2.000 mm e temperaturas médias superiores a 22°C no mês mais quente e menores que 18°C no mês mais frio (Instituto de Pesquisas Meteorológicas, 2017).

A BH-TJ está dividida em seis sub-bacias (Tabela 1) de acordo com a área de drenagem dos principais rios, que são: O rio Tietê e os rios Jacaré-Guaçu e Jacaré-Pepira, ambos desaguando no rio Tietê.

As principais atividades econômicas na BH-TJ estão ligadas principalmente à agroindústria, com destaque para indústria sucroalcooleira que é responsável por aproximadamente 13% de toda a produção de Estado de São Paulo, ocupando quase que 50% (5.810,192 Km$^2$) da área total da bacia (Estado de São Paulo, 2016). Outro setor importante na região é a produção e processamento de cítricos, principalmente laranja, representando 11% de toda a produção nacional (Estado de São Paulo, 2016).

A região apresenta 1.106 Km$^2$ de vegetação natural remanescente que ocupa, aproximadamente 9% da área total da bacia, onde as fitofisionomias predominantes são compostas pela Floresta Estacional Semidecídua e Cerradão. A região ainda possui três Estações Ecológicas (EE) (EE de Bauru, EE de Itapirina e EE de São Carlos) e oito Unidades de Conservação de Uso Sustentável (APA Corumbataí-Tejupa, APA Ibitinga, APA Piracicaba-Juqueri Mirim e APA rio Batalha; Floresta Estadual Pederneiras; RPPN Floresta das Águas Perenes, Olavo Egydio Setúbal e Amadeu Botelho) (Estado de São Paulo, 2015).

**Tabela 1:** Caracterização espacial das sub-bacias da Bacia Hidrográfica do Tietê-Jacaré, Estado de São Paulo, Brasil (Fonte: adaptado de Estado de São Paulo, 2015).

| | **Sub-bacia** | **Área** | |
|---|---|---|---|
| | | **Km$^2$** | **%** |
| 1 | Sub-bacia do rio Jacaré-Guaçú e afluentes do rio Tietê. | 4.183,47 | 35,4 |
| 2 | Sub-bacia do rio Jacaré-Pepira e afluentes diretos do rio Tietê. | 2.670,28 | 22,6 |
| 3 | Sub-bacia do rio Jaú, ribeirão da Ave Maria, Ribeirão do Sapé e afluentes diretos do rio Tietê. | 1.527,61 | 12,9 |
| 4 | Sub-bacia do rio Lençóis, ribeirão dos Patos e afluentes diretos do rio Tietê. | 1.436,61 | 12,2 |
| 5 | Sub-bacia do rio Bauru, ribeirão Grande, ribeirão Pederneiras e afluentes diretos do rio Tietê. | 826,8 | 7,0 |
| 6 | Sub-bacia do rio Claro, ribeirão Bonito, ribeirão de Veado, ribeirão Água Limpa e afluentes diretos do rio Tietê. | 1.159,1 | 9,8 |

**Figura 5:** Bacia hidrográfica do Tietê-Jacaré, Estado de São Paulo, Brasil. Fonte: SigRH.

A BH-TJ possui apenas 26,5% de vegetação das Áreas de Preservação Permanente (APP) preservadas. Das seis sub-bacias que compõem a BH-TJ, as dos rios Jacaré-Guaçú e Jacaré-Pepira possuem os menores índices de degradação, correspondendo a 66,41% e 64,83% de APP degradada, respectivamente. As duas sub-bacias também apresentam os maiores percentuais de fragmentos de vegetação remanescente de toda BH-TJ, que corresponde a 37% do total de vegetação remanescente da bacia (Estado de São Paulo, 2015).

A região possui três tipos de ecossistemas aquáticos bem definidos: lóticos – caracterizados por redes hídricas correntes que transportam materiais físicos, químicos e biológicos (12.747 Km$^2$); lênticos – caracterizado por ambientes aquáticos de água represada sem movimento corrente (172.126 Km$^2$); e áreas úmidas – caracterizadas por ambientes onde o solo está saturado com água ou inundadas em partes do ano (408.519 Km$^2$) (Estado de São Paulo, 2016).

Com relação à qualidade das águas superficiais, a sub-bacia do rio Jacaré-Guaçú apresenta Índice de Qualidade das Águas (IQA) considerado bom, já com relação ao Índice de Qualidade das Águas para Proteção da Vida Aquática (IVA), este é classificado como regular. Já a sub-bacia do Jacaré-Pepira apresenta IQA e IVA classificados como bom. Com relação ao Índice do Estado Trófico (IET), a sub-bacia do rio Jacaré-Guaçú apresenta um IET majoritariamente mesotrófico, isto é, situação intermediaria entre o baixo teor de nutrientes e alto teor de nutrientes, enquanto a sub-bacia do rio Jacaré-Pepira possui um IET classificado como oligotrófico, ou seja, possuidor de grande enriquecimento em nutrientes (Estado de São Paulo, 2016).

Tanto a sub-bacia do rio Jacaré-Gaçú, quanto do rio Jacaré-Pepira são utilizados no abastecimento rural de diversos municípios do Estado de São Paulo, dentre eles Araraquara, Ibitinga, São Carlos, Brotas e Torrinha. Além disso, ambas as sub-bacias possuem potencial risco à contaminação, já que se encontram em áreas de consumo de agrotóxicos, maior produção agrícola, mineração e inundação (Estado de São Paulo, 2016).

### 1.3.1 Rio Jacaré-Pepira

O rio Jacaré-Pepira (Figura 6), Bacia Hidrográfica do Tietê-Jacaré, localizado na porção central do Estado de São Paulo, é um rio de sexta ordem com área total de 2.612 Km$^2$. Sua nascente fica localizada a 900 m de altitude na Serra de Itaqueri, município de São Pedro (22º32’55”S e 47º54’50”O) e sua foz a 400 m de altitude, no rio Tietê no município de Ibitinga (21º45’28”S e 48º49’44”O) (Barrella, 1989; Almeida et al., 1999; Metzger et al., 1998). O rio é considerado um dos mais conservados de toda a bacia, sendo popularmente conhecido como “Pantaninho Paulista” devido à presença de áreas alagadas em sua várzea, tornando-o mantenedor de grande biodiversidade na região, estando inclusive inserido em uma Área de Proteção Ambiental (APA), a APA Ibitinga, que tem como objetivo proteger as várzeas formadas pelo rio onde ocorrem importantes remanescentes de vegetação em estagio avançado de regeneração e a fauna a ela associada (Estado de São Paulo, 2017).

### 1.3.2 Rio Jacaré-Guaçú

O rio Jacaré-Guaçu (Figura 6), Bacia Hidrográfica do Tietê-Jacaré, também localizado na porção central do Estado de São Paulo, é um rio de quarta ordem e um importante tributário da margem direita do rio Tietê, possuindo uma extensão aproximada de 1.100 Km$^2$. O rio é formado pela junção do ribeirão do Feijão que nasce na Serra do Cruzeiro e do ribeirão Lobo, que tem sua nascente localizada na Serra de Itaqueri, e sua foz ocorre no rio Tietê, no município de Ibitinga (Esguícero e Arcifa, 2011; Rodríguez, 2001). Este rio recebe uma grande quantidade de esgoto doméstico e industrial, principalmente através de seus tributários. Além disso, suas águas são caracterizadas pela alta concentração de coliformes fecais, pesticidas e metais (Esguícero e Arcifa, 2011; Rodríguez, 2001).

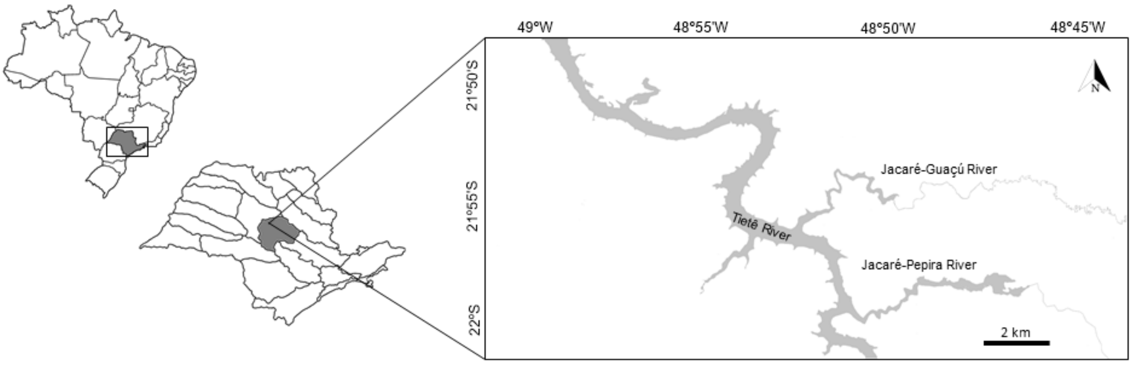

**Figura 6:** Mapa dos dois rios estudados, Jacaré-Pepira e Jacaré-Guaçú, localizados dentro da sub-bacia do Tietê-Jacaré, estado de São Paulo, Brasil. Fonte: Google Earth.

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# *Objetivos*

# 2 OBJETIVOS

## 2.1 Geral

Avaliar o potencial para bioindicação de efeito e de acumulação de poluentes em metazoários parasitos de quatro espécies de peixes coletados de dois rios com diferentes graus de antropização, o rio Jacaré-Pepira e o Jacaré-Guaçú, sub-bacia do Tietê-Jacaré, Estado de São Paulo, Brasil e avaliar se existe risco de contaminação por metais através do consumo humano da musculatura dos peixes nos dois rios.

## 2.2 Específicos

- Avaliar o potencial para a acumulação de metais-traço em três espécies de endohelmintos parasitos em relação aos seus peixes hospedeiros: *Proteocephalus macrophallus* (Cestoda), parasito de *Cichla kelberi* e *Hysterothylacium* sp. (Nematoda) e *Phyllodistomum* sp. (Digenea) parasitos de *Hoplias malabaricus;*
- Verificar se as concentrações dos metais recuperados dos parasitos são mais altas que as recuperadas dos hospedeiros;
- Comparar as concentrações recuperadas dos tecidos (músculo, intestino e fígado) de peixes infectados em comparação à peixes não-infectados;
- Analisar as possíveis influências do tamanho das infrapopulações parasitárias e do tipo de infecção (infecção simples e infecção combinada) nas concentrações de metais-traço recuperadas dos tecidos dos hospedeiros infectados;
- Avaliar o potencial para bioindicação de efeito de cincos espécies de parasitos da classe Monogenea*:* *Anacanthorus serrasalmi*, *Amphithecium speirocamarotum* e *Rhinoxenus piranhus*, parasitos de *Serrasalmus maculatus* e *Gussevia asota* e *Gussevia astronoti*, parasitos de *Astronotus crassipinnis*;
- Analisar os padrões de distribuição (prevalência, abundância e intensidade médias) dos monogenéticos de acordo com os períodos seco e chuvoso;
- Analisar se a variância nos parâmetros químicos da água dos dois rios influencia na abundância dos parasitos;
- Analisar as possíveis relações entre o fator de condição dos peixes com as variáveis químicas da água dos rios e com a abundância de parasitos.

# *Artigo 1*

## ARTIGO 1.

# *Proteocephalus macrophallus* (Cestoda: Proteocephalidae) infecting *Cichla kelberi* (Cichliformes: Cichlidae) as a bioindicator for trace metal accumulation in a neotropical river from southeastern Brazil

## ABSTRACT

Here, we evaluate the potential for trace metal accumulation of nine elements (Al, Cr, Mn, Fe, Ni, Cu, As, Cd and Pb) in the cestodes *Proteocephalus macrophallus* parasitizing the tucunaré, *Cichla kelberi*, in the Jacaré-Guaçú River, southeastern Brazil. For metal quantification in the tissues of hosts and parasites an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) was used. All recovered trace metals were in higher amounts in cestodes than in the tissues (muscle, intestine and liver) of their hosts. The best accumulated element was lead, with concentrations up to 10,000 times higher in parasites than in the liver of the fish hosts. Other well-accumulated elements were cadmium, with concentrations up to 238 time higher in parasites, and aluminum and nickel, with concentrations ranging from 140 to 128 times higher in parasites. In addition, non-infected fish had higher concentrations of cadmium and lead in their tissues than infected fish. In infected fish, the size of the cestodes infrapopulations influenced in the concentrations of arsenic, cadmium, and lead in the hosts tissues. In general, specimens of *P. macrophallus* had a good capacity for metal accumulation for all the analyzed elements, with emphasis on Al, Ni, Cd and Pb, and therefore could be a useful tool in trace metal bioaccumulation indication.

**Keywords:** Accumulation indicators; trace-metal accumulation; heavy metal pollution; environmental parasitology; fish parasitology.

## 1 INTRODUCTION

The impact of pollutants on the aquatic biota has been gaining considerable attention in recent decades due to the rapid urban growth and industrial development (Effendi, 2016; Poulin, 1992; Reza & Singh, 2010). Among the most problematic pollutants are the trace metals, which despite having a natural origin in the environment, also have anthropogenic origin, mainly from activities such as mining, use of fertilizers and pesticides in agriculture and trough domestic sewage (Erasmus et al., 2020; Merian et al., 2004; Reza & Singh, 2010). In fish, metal pollution is associated with body deformities in larvae and in adults, compromising their development and reproduction, and even leading to animal's death (Sfakianakis et al., 2015; Zeitoun & Mehana, 2014). In addition, the bioaccumulation and biomagnification of toxic metals such as lead, and mercury can have implications in human health through the consumption of contaminated fish meat (Castro-González & Méndez-Armenta, 2006).

Parasites are an important part of the biodiversity playing very important roles in the ecosystem and, although they are most often associated with public health problems, only a small portion of these organisms are in fact of medical or veterinary importance (Poulin & Morand, 2004). In fish, parasites play a strong role in the host's biology, controlling individual characteristics such as behavior, migratory and reproductive habits that will lead to changes in populations and in the structuring of fish communities (Luque & Poulin, 2008). As their hosts, parasites are also affected by pollution, and due to their high sensitiveness to negative changes in the ecosystem, they can respond to pollution even at its lowest levels and even faster than their hosts, and therefore can be used as bioindicators of environmental health (Lafferty, 1997; Sures, 2004; Vidal-Martínez et al., 2009). Regarding trace metal pollution in the aquatic environment, in the host-parasite relationship parasites act as bioaccumulators, which means that they can accumulate amounts of metals at scales much larger than their fish hosts (Sures et al., 1997; Sures et al., 1999; Sures, 2001).

Despite being a research field in development for at least two decades (Sures et al., 1997), in the neotropical region, studies involving parasitism and the bioaccumulation of trace metals are considerably recent, and the first study of this type was in Chile, conducted by Woelfl et al. (2009), who evaluated the accumulative potential of the tapeworm *Diphyllobothrium latum* (Linnaeus, 1758) parasitizing *Oncorhynchus mykiss* (Salmoniformes: Salmonidae). Since then, there have been some studies in Brazil involving acanthocephalans (Reis et al., 2017; Duarte et al., 2020), nematodes (Leite et al., 2017; Leite et al., 2020), cestodes (Leite et al., 2019), and digeneans (Leite et al., 2021). Still, there are some gaps regarding the accumulation dynamics exerted by fish parasites, and their potential roles not only as bioaccumulators, but also in other types of responses of both host and their parasites to metal pollution.

Therefore, our objective is to evaluate the potential for trace metal accumulation of the species *Proteocephalus macrophallus* Diesing, 1850 (Cestoda: Proteocephalidae), compared to its fish host, the tucunaré *Cichla kelberi* Kullander & Ferreira, 2006 (Cichliformes: Cichlidae) collected from and anthropized neotropical aquatic environment, the Jacaré-Guaçú River, located in southeastern Brazil, in order to verify if the parasites have a better capacity to accumulate trace metals compared to their hosts, and also if the size of the parasitic infrapopulations influences the hosts' accumulation capacity.

## 2 MATERIAL AND METHODS

### 2.1 Study area

The study was conducted in the Jacaré-Guaçú River, a fourth-order river, and an important tributary of the Tietê River, in the upper Paraná River basin, one of the most important Brazilian basins, an important source of the local biodiversity, with approximately 230 species of fish and 300 species of fish parasites (Langeani et al., 2007; Lehun et al., 2020). This river has the greatest surface water availability of the hydrographic sub-basin to which it belongs, but it is an extremely problematic area. Land use around the river is heavily agricultural, with 70% of the land being used for this purpose, however, industrial use is also significant. In addition, the river waters present a strong risk of contamination, as they receive large amounts of industrial sewage and because they are in an area of high consumption of pesticides. Some metals such as aluminum are in concentrations above the limit allowed by legislation, offering risks to the integrity of the aquatic biota, as well as to the riverside population that consumes fish from this river (Estado de São Paulo, 2016; Rodríguez, 2001; Esguícero and Arcifa, 2011).

### 2.2 Fish, parasites, and tissues sampling

*Cichla kelberi* specimens were collected between May 2017 and September 2019, totaling 29 fish collected. For fish collection, waiting nets with meshes of different sizes were used. After being collected, fish were anesthetized with a solution based on eugenol, and the euthanasia performed by spinal section. After that, fish were individualized in plastic bags and frozen until the necropsy.

The collections were made under the authorization of the Chico Mendes Institute for Biodiversity Conservation (SISBIO - authorization nº 40998-3). The euthanasia methodology was authorized by the Ethics Committee on the Use of Animals (CEUA - authorization nº 3353050417) of the Universidade do Sagrado Coração (USC) and follows the guidelines suggested by the National Council for the Control of Animal Experimentation (CONCEA).

From the hosts, infected or not, individual samples of muscle tissue, liver, and intestine (approximately 600 mg) were taken. Removed samples were washed with deionized water and frozen at -20ºC until the moment of analysis for metal recovery.

Samples of *Proteocephalus macrophallus* were also taken from those hosts that tested positive for infection. In cases were the individual infrapopulation does not reached the mass necessary for the analysis of metal recovery (approximately 150 mg), a pool was made with those collected from other fish, if it meets specific criteria, such as: being of the same parasite species, be collected from the same species of fish, and from the same collection period. The collection and processing of the samples follow the same methodology used for fish hosts tissues and are according to Leite et al. (2021).

## 2.3 Trace metal analysis

For metal recovery in fish and parasites tissues, the samples were first digested with a solution of 3 mL $HNO_3$ and 2 mL $H_2O_2$ in a microwave oven MARS 5 (CEM GmbH, Kamp-Lintfort, Germany), with the following programming: 400W at 180ºC for 6 minutes, 600W at 180ºC for 8 minutes and 800W at 180ºC for 12 minutes. To the digested samples, deionized water was added to a volume of 10 mL. Then, using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (7700e, Agilent, USA), the concentrations of the elements Al, Cr, Mn, Fe, Ni, Cu, As, Cd, and Pb were obtained. The ICP-MS was calibrated using the Certified Reference Material (CRM) DORM-4 (Fish Protein Certified Reference Material for Trace Metals and other Constituents) (National Research Council of Canada, NRC – CNRC) (Leite et al., 2021).

## 2.4 Statistical analysis

The quantitative descriptors of parasitism of prevalence, and mean intensity of infection were calculated according to Bush et al. (1997).

The Shapiro Wilk test was applied to verify data distribution. The Mann-Whitney test was applied to verify the differences between metal concentrations in infected and non-infected fish. The Kruskal-Wallis test was applied to verify the variance between the metal concentrations in the tissues (muscle, intestine, and liver) of *C. kelberi* and the concentrations in the tissues of *P. macrophallus*. All tests were considered statistically significant when $p \leq 0.05$.

Principal Component Analysis (PCA) in conjunction with Spearman's Rank Correlation Coefficient was used to establish the possible relationships between the size of parasitic infrapopulations and the concentrations of metals in host tissues.

Bioconcentration Factors (BCF) was calculated using the ratios $C_{[parasite]}/C_{[host\ tissue]}$, where C is the metal concentration obtained in the respective tissue (Sures et al., 1999).

## 3 RESULTS

All the tapeworm parasites used in the study were adults, with an infection prevalence of 76%, and a mean infection intensity of 39 ± 10 parasites per fish. The 29 specimens of *C. kelberi* collected from the Jacaré-Guaçú River were 10 females and 19 males, with mean standard length of 22.7 ± 3 cm and mean total weight of 335 ± 144.8 g. In total, 22 fish were infected with specimens of *P. macrophallus*, and seven were not infected.

The trace metal concentrations recovered from specimens of *P. macrophallus* and from the tissues (muscle, intestine and liver) of the specimens infected and non-infected of *C. kelberi* are in Figure 1 and 2. Although Al, Mn and Cr had numerically higher concentrations in the livers of non-infected fish hosts, only Cd and Pb statistically differed between infected and non-infected fish with infected fish having statistically lower concentrations (Mann-Whitney test, $p \leq 0.05$) of these metals in the intestinal and liver tissues (Figure 2).

The tissue samples of *P. macrophallus* had statistically higher concentrations (Kruskal-Wallis test, $p \leq 0.05$) in almost all trace metals compared to the tissues of *C. kelberi* except for Fe and Cu in the liver, which had no statistically significant difference. The bioconcentration factors showed that tapeworms have a higher affinity for the accumulation of Al, Ni, Cd and particularly Pb (Table 1). In comparison to the host's musculature, the concentrations of Al in the parasites were up to 140 times higher, while for Ni it was up to 128 times higher and Cd up to 238 times higher. Pb was the element with the highest BCF, with concentrations in the parasites 10,000 times higher than the host muscle. The least accumulated elements were Fe and Cu, with an average BCF of 1 in comparison to the host liver. For the muscle, the order of BCF was (in decreasing order): Pb, Cd, Al, Ni, Mn, Cr, Fe, Cu and, As; intestine: Pb, Al, Ni, Cr, Cd, Fe, As, Mn and, Cu; liver: Pb, Al, Ni, Cr, As, Mn, Cd, Fe and, Cu.

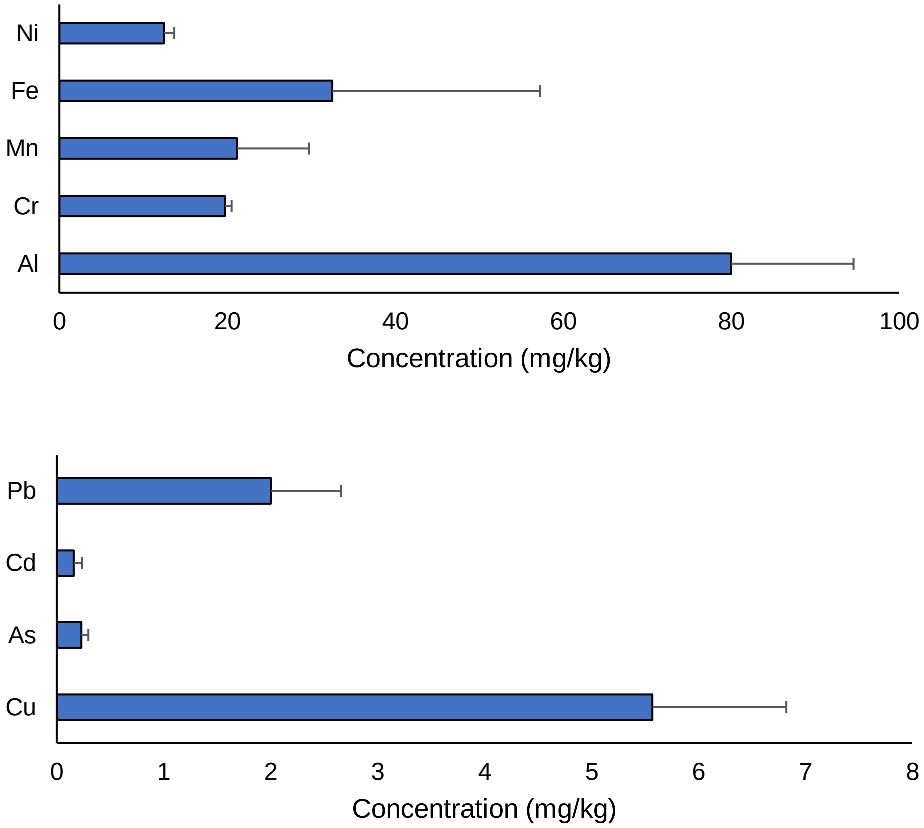

**Figure 1:** Trace metal concentrations (mg/kg) in the tissues of *Proteocephalus macrophallus* (Cestoda: Proteocephalidae) infecting *Cichla kelberi* (Cichliformes: Cichlidae), from the Jacaré-Guaçú River, Tietê-Jacaré sub-basin, state of São Paulo, Brazil.

In the Principal Component Analysis (PCA) that correlates the sizes of the *P. macrophallus* infrapopulations with the trace metal concentrations in the tissues (muscle, intestine and liver) of *C. kelberi*, significant negative correlations were found ($p \leq 0.05$) for As in the intestine (rs= -0,382) and liver (rs= -0,417), and for Cd in the muscle (rs= -0,593) and intestine (rs= -0,524) (Figure 3, Table 2), indicating that as the infection rates of *P. macrophallus* in the host was increased, the concentrations of As and Cd were decreased.

**Table 1**: Bioconcentration Factors (BCF) (± SD) of trace metal in *Proteocephalus macrophallus* (Cestoda: Proteocephalidae) compared to the tissues (muscle, intestine, and liver) of its fish host *Cichla kelberi* (Cichliformes: Cichlidae) from the Jacaré-Guaçú River, state of São Paulo, Brazil.

| **Element** | **Tapeworm/Muscle** | **Tapeworm/Intestine** | **Tapeworm/Liver** |
|---|---|---|---|
| **Al** | 140 ± 30 | 98 ± 29 | 105 ± 12 |
| **Cr** | 77 ± 20 | 64 ± 13 | 69 ± 11 |
| **Mn** | 87 ± 54 | 13 ± 12 | 17 ± 9 |
| **Fe** | 68 ± 35 | 23 ± 11 | 1 ± 0.9 |
| **Ni** | 128 ± 26 | 95 ± 15 | 99 ± 12 |
| **Cu** | 40 ± 24 | 10 ± 5 | 1 ± 0.3 |
| **As** | 31 ± 20 | 19 ± 16 | 18 ± 9 |
| **Cd** | 238 ± 86 | 48 ± 31 | 15 ± 8 |
| **Pb** | 325 ± 160 | 264 ± 70 | 10637 ± 12815 |

**Table 2:** Spearman's Rank Correlation Coefficient that correlates the infrapopulations sizes of *Proteocephalus macrophallus* (Cestoda: Proteocephalidae) and the trace metal concentrations in the muscle (A), intestine (B), and liver (C) of its fish hosts *Cichla kelberi* (Cichliformes: Cichlidae) from the Jacaré-Guaçú River, Tietê-Jacaré sub-basin, state of São Paulo, Brazil.

| **Element** | **Muscle** | **Intestine** | **Liver** |
|---|---|---|---|
| Al | -0.028 | -0.124 | -0.141 |
| Cr | -0.133 | -0.301 | -0.216 |
| Mn | -0.066 | -0.285 | -0.358 |
| Fe | -0.034 | -0.209 | -0.049 |
| Ni | -0.191 | -0.218 | -0.140 |
| Cu | -0.096 | -0.100 | -0.175 |
| As | -0.296 | **-0.382** | **-0.417** |
| Cd | **-0.592** | **-0.524** | 0.003 |
| Pb | 0.085 | -0.234 | -0.271 |

Bold values indicate statistically significant correlation ($p \leq 0.05$).

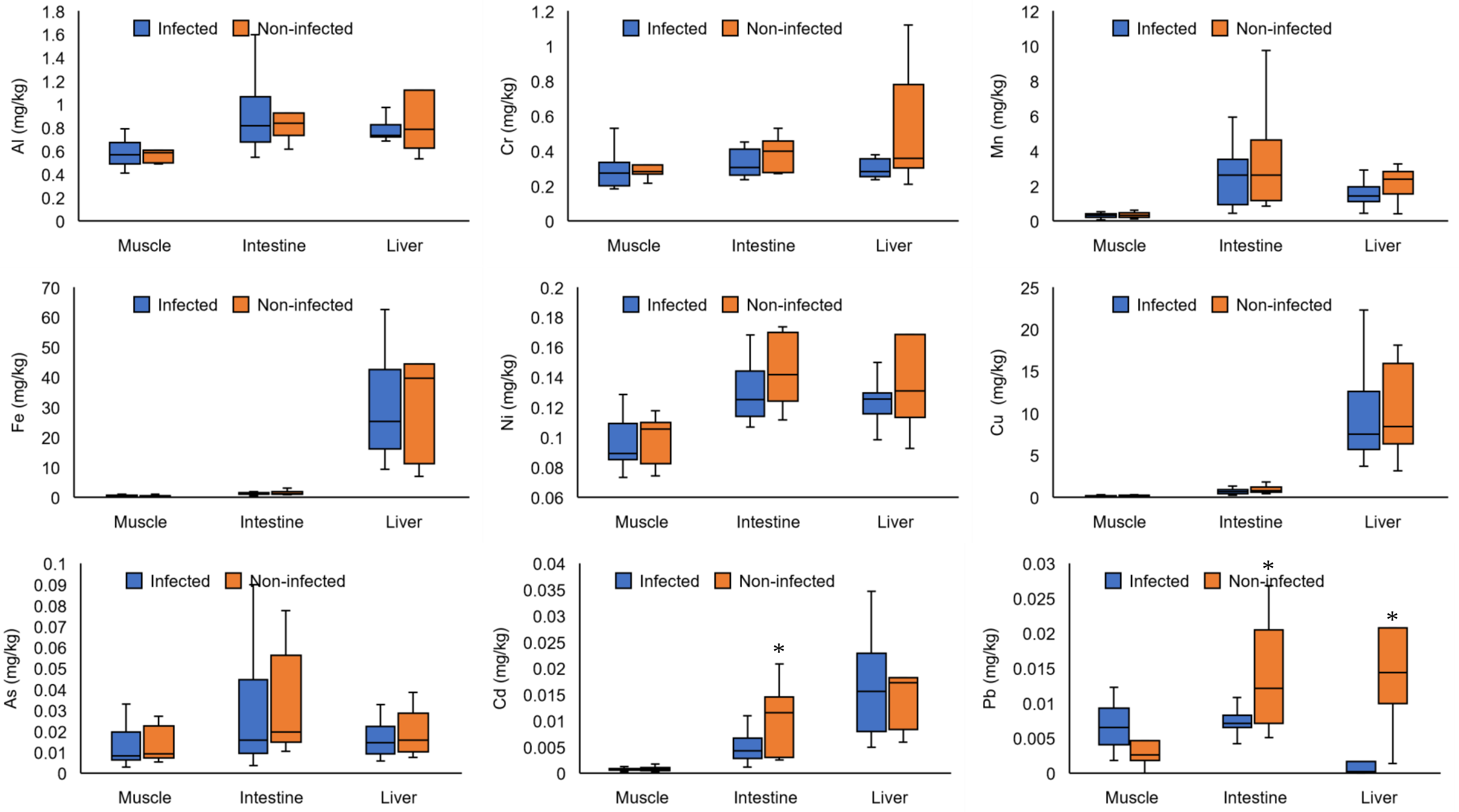

**Figure 2**: Trace metal concentrations (mg/kg) in the tissues (muscle, intestine, and liver) of infected and non-infected specimens of *Cichla kelberi* (Cichliformes: Cichlidae), from the Jacaré-Guaçú River, state of São Paulo, Brazil. *Statistically different (Mann-Whitney test, $p < 0.05$).

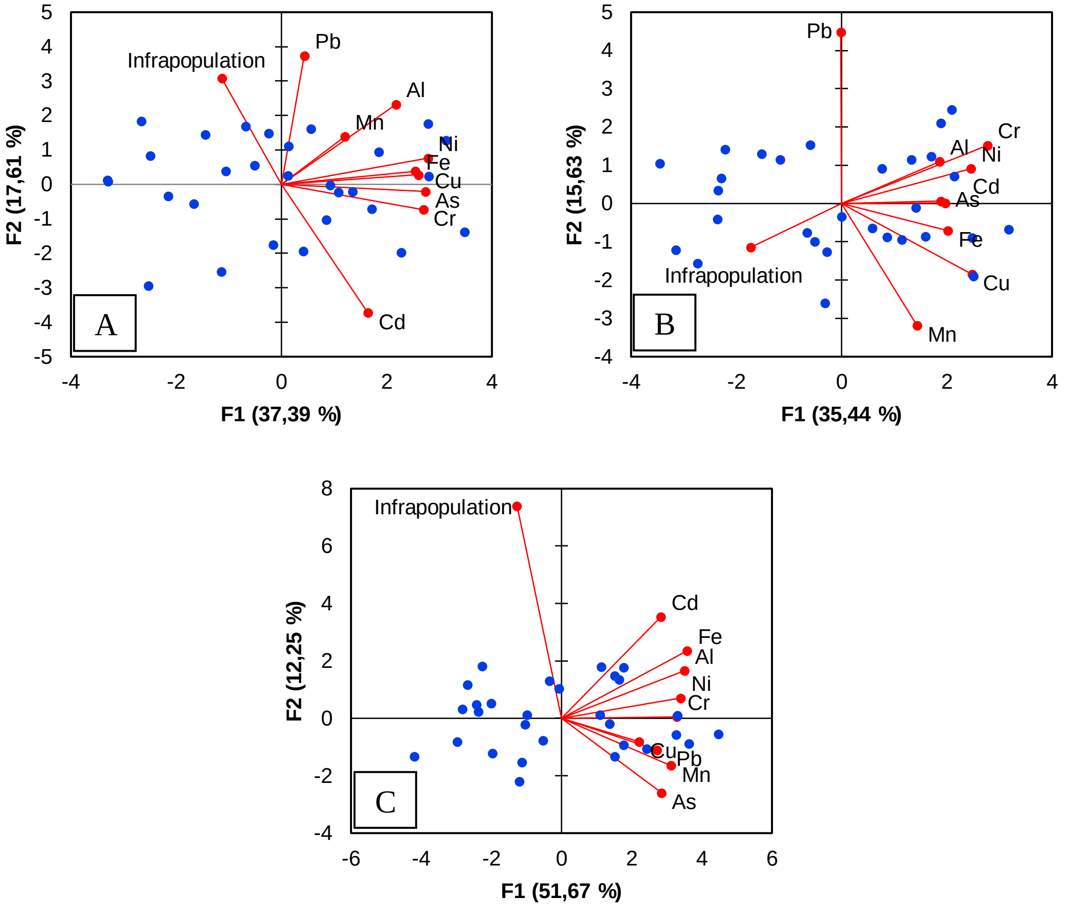

**Figure 3:** Principal Component Analysis (PCA) that correlates the infrapopulation sizes of *Proteocephalus macrophallus* (Cestoda: Proteocephalidae) and the trace metal concentrations in the muscle (A), intestine (B), and liver (C) of its fish hosts *Cichla kelberi* (Cichliformes: Cichlidae) from the Jacaré-Guaçú River, Tietê-Jacaré sub-basin, state of São Paulo, Brazil.

## 4 DISCUSSION

Some of the requirements for a parasitic organism to be considered a good indicator of trace metal accumulation are the high distribution of the species and the easy identification of both parasite species and its fish host (Sures, 2003). The genus *Proteocephalus* is very prevalent in fish of the genus *Cichla* (Januário et al., 2019; Lehun et al., 2020; Santos et al., 2011; Yamada & Takemoto, 2013), which facilitates the application of studies with metal bioaccumulation using theses parasites, in addition, the genus *Cichla* which despite being endemic to the Amazon basin (Kulander & Ferreira, 2006), has become an invasive species and consequently very common in several other Brazilian basins, including the upper Paraná River basin (Yamada & Takemoto, 2013), were the Jacaré-Guaçú River is included, which facilitates their capture and use in these types of studies without causing damage to the aquatic ecosystem.

This is not the first time that a species of the genus *Proteocephalus* has been used as a possible indicator of trace metal accumulation. Eira et al. (2009) tested the accumulative capacity of *Proteocephalus macrocephalus* (Creplin, 1825) parasitizing an eel (*Anguilla anguilla*) in a contaminated river in Portugal, and found higher concentrations of Cr, Ni, Pb, and Zn in the parasites compared to the tissues (liver, kidney, and muscle) of its fish host. In another study, Brázová et al. (2012, 2015) evaluated the accumulation of nine metals in the tissues (muscle, liver, kidney, brain, and hard roe) of the perch (*Perca fluviatilis*) and in its tapeworm parasites *Proteocephalus percae* (Müller, 1780), and found significantly higher amounts of As, Cd, Cr, Cu, Mn, Ni, Pb, and Zn in the parasites compared to their fish hosts. More recently, in a river very close to the Jacaré-Guaçú River, the Jacaré-Pepira River, but with lower levels of degradation, Leite et al. (2019) evaluated the accumulative potential of *P. macrophallus* also infecting *C. kelberi* (misidentified in the study as *Cichla ocellaris*) and found higher levels of 13 metals (Al, As, Ba, Cd, Cr, Fe, Hg, Mg, Mn, Ni, Pb, Ti, and Zn) in the tapeworms compared to the tissues (muscle, intestine and liver) of their hosts.

Based on the previously published studies and the results obtained by the present study, it is possible to observe that species of the genus *Proteocephalus* that have already been assessed as possible accumulation bioindicators, have a good affinity for the accumulation of the elements Cd, Pb, Al, and Cr. This pattern, however, is not clearly established, with several variations, especially related to the bioconcentration factors from study to study. For example, in the studies by Brazová et al. (2012 and 2015) the accumulation of Pb in the liver tissues of the hosts was the highest (BCF = 65.9 and 67.4) compared to the other elements, whereas in the study by Eira et al. (2009), the accumulation of Pb in the tissues of the parasites compared to the liver of the host was below 1. While in the present study, the rates of Pb accumulation of *P. macrophallus* in relation to the liver of them fish host reached up to 10,000. Sures et al. (2017) listed all studies so far with bioaccumulation of trace elements with several groups of parasites, and in general, regarding to the Cestoda class, Pb and Cd are well accumulated elements in both freshwater and marine environments.

In the case of parasitic tapeworms, two main factors can influence the parasite's accumulation capacities: the life cycle, and the parasite body part used for the trace metals quantification (Baruš et al., 2011; Leite et al., 2019; Scholz, 1999; Sures et al. 1997). According to Baruš et al. (2011), plerocercoids have a longer exposure time to metals when compared to adult individuals, so larval stages are expected to have greater accumulation capacity. Regarding the body part of the tapeworm that was used, Sures et al. (1997) found higher amounts of metals in pregnant proglottids of *Monobothrium wageneri* Nybelin, 1922 and *Bothriocephalus scorpii* (Müller, 1776) compared to the anterior parts (scolex, neck, and proglottids without eggs). As in previous studies with the same species (Leite et al., 2019) we did not differentiate the body parts in the analysis, but the amount of tissue analyzed was largely composed of pregnant proglottids, which function as metal deposits and consequently could explain the high concentrations recovered in these tissues. In addition, all parasite specimens used were adults.

There is a theory that endoparasites can act as true filters, sequestering compounds from parasitized hots and consequently reducing its metal concentrations, which could help in an increasing in the survival rates in polluted or low integrity environments. This "good side" of parasitism has already been observed in studies with other endoparasites, such as Acanthocephala (Brázová et al., 2015; Filipović et al., 2003; Paller et al., 2016; Sures et al., 1999; Sures et al., 2003) Nematoda (Bergey et al., 2002; Hursky et al., 2015; Leite et al., 2021), and Digenea (Leite et al., 2021). In the present study, we found statistically lower concentrations of cadmium and lead in the intestines and livers of non-infected hosts, and the same was also observed by Torres et al. (2015) who found smaller amounts of Cd and Pb in uninfected specimens of the European hake (*Merluccius merluccius*) than in fish infected by the cestode *Clestobothrium crassiceps* (Rudolphi, 1819), and by Tučerková et al. (2002) that also found Cd concentrations in non-infected perch (*P. fluviatilis*) compared to infected ones by *P. percae*. An explanation for this phenomenon is the biodilution theory (Soler-Jiménez et al., 2020), which attributes the differences in the concentrations of the compounds to the amount of biomass found, that is, a parasitized host had higher biomass than non-parasitized hosts, which causes the accumulated pollutants to be more diluted in the tissues, which include parasites, and consequently have their concentrations reduced. And that leads to another important point to be discussed: the role of the size of the parasitic infrapopulations (which is directly related to the amount of biomass found) in the concentrations of metals in the tissues of the parasitized hots.

In the present study it was observed that arsenic and cadmium concentrations were in lower amounts in fish with larger infrapopulations of *P. macrophallus*. There are still few studies that consider the sizes of the parasitic infrapopulations, and the concentration of metals recovered from the hosts tissues. However, some studies show the existence of a negative relationship between the sizes of the infrapopulations and metal concentrations in the tissues (Al-Hasawi et al., 2019; Brázová et al., 2015; Leite et al., 2021), indicating that fish with smaller infrapopulations tend to have higher amounts of metals than fish with larger ones. Here again, as well in situations of comparison between parasitized and non-parasitized fish, the biodilution theory can be applied. Fish with smaller infrapopulations, have less biomass than larger infrapopulations, so as the increase in infrapopulations and consequently biomass occurs, trace metal concentrations tend to decrease. These results are promising, and future studies should focus not only highlighting the already indisputable capacity of parasites to accumulate metals, but also on other mechanisms in the host-parasite relationship that involve the accumulation of pollutants, so that they can be useful not only as a tool for pollution detection, but also in other aspects such as this possible benefit to the hots. The remaining question is whether these possible reductions in metal concentrations in the host's tissues are significant to warrant an increase in the survival rates of these organisms in situations where the hosts is exposed to situations of low integrity in the habitat.

## 5 CONCLUSIONS

In general, specimens of *P. macrophallus* had a good capacity for metal accumulation for all the elements analyzed, with emphasis on Al, Ni, Cd, and Pb. Furthermore, such elements as lead and cadmium, in addition to having been well accumulated by the tapeworms, had their concentrations also significantly corelated to the presence or absence of parasitism and also to the sizes of parasitic infrapopulations.

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# *Artigo 2*

## ARTIGO 2.

# Seasonal patterns of infestation by monogenean parasites of fish and their relationship with water parameters in two rivers with different disturbance gradients in southeastern Brazil

## ABSTRACT

Here, we evaluate the relationships between the infestation rates of five monogenean parasites species with the dry and wet seasons, with the organic and inorganic parameters of the water of two rivers: the Jacaré-Pepira and Jacaré-Guaçú, and with the condition factors of its fish hosts: *Serrasalmus maculatus* and *Astronotus crassipinnis*, in the state of São Paulo, southeastern Brazil. Fish were collected between January and December 2017. *Anacanthorus serrasalmi*, *Amphithecium speirocamarotum*, and *Gussevia asota* had higher abundance rates (Student's *t* test, $p \leq 0.05$) in the wet season. *Gussevia asota* had its abundance negatively correlated to nitrate in the Jacaré-Pepira River and with total nitrogen and potassium in the Jacaré-Guaçú River. Regarding the fish hosts condition factors, was observed a positive correlation with the abundances of *G. asota* in the Jacaré-Guaçú River, and with *A. serrasalmi* in the Jacaré-Pepira River. In general, wet season favored an increasing in the infestation rates of the monogeneans parasites in their host species, mainly in the river considered as the most polluted, the Jacaré-Guaçú River. Of the five parasites species analyzed in this study, only *Gussevia astronoti* and *Rhinoxenus piranhus* had no interaction with seasonality, river water variables, or fish host condition factors. On the other hand, *G. asota* had interactions both with water parameters (nitrate and total nitrogen) and with the hosts condition factors, which reflected in the abundance and intensity rates, showing itself as a species sensitive to changes in the environment and, therefore, that can be considered as a bioindicator organism.

**Keywords**: Environmental parasitology; effect bioindication; Monogenea; Fish parasitology; Host-parasite relationship.

## 1 INTRODUCTION

Parasites play crucial roles in the environment and are considered as true puppeteers in the ecosystems structuring (Dougherty et al., 2015), being responsible, in some cases, for up to 78% of the interactions in the food chains (Lafferty et al., 2006), whose biomass can scale together with the host's body biomass (Poulin and George-Nascimento, 2007), and may even surpass the biomass of top chain predators (Kuris et al., 2008). Its eminent presence in the structuring of animal communities means that there is also an intrinsic relationship with the physical environment, where negative changes in several characteristics of the habitat directly affect the occurrence and distribution of parasitic species, and consequently their hosts, which can bring losses at all levels of the food chain and to the functioning of the ecosystem (Lafferty et al., 2006; Silva-Souza et al., 2006).

Environmental parasitology, which covers the use of parasites as indicators of environmental health (Sures et al., 2017), is a field that has been gaining strength in recent decades and demonstrates the different types of response of parasites due to disturbances in the environment, in addition to the high effectiveness of these organisms in detecting pollution, organic and inorganic, and other negative impacts, especially in aquatic ecosystems (freshwater and marine) (Carravieri et al., 2020; Lafferty, 2008; Marcogliese and Pietrock, 2011; Sures et al., 2017; Vidal-Martínez e Wunderlich, 2017).

Monogenea, Digenea, Nematoda, Acanthocephala, Cestoda, Hirudinea and Copepoda are the taxa commonly found parasitizing fish in Latin American waters (Lehun et al., 2020; Luque and Poulin, 2007; Luque et al., 2017). Among these groups, endoparasites, especially acanthocephalans, nematodes, and cestodes are potential sentinels and pollution indicators, especially for trace metals (Duarte et al., 2020; Leite et al., 2017; Leite et al., 2018; Leite et al., 2019; Leite et al., 2021; Nachev et al., 2013; Sures et al., 2017; Vidal-Martínez e Wunderlich, 2017), organic pollutants (Carravieri et al., 2020), and more recently for microplastics (Hernandez-Milian et al., 2019). Ectoparasites, on the other hand, especially monogeneans, may not be the ideal group for use in studies with accumulation bioindication (Sures, 2003; Sures, 2004), but they can be very useful in another type of approach: effect bioindication.

Effect bioindication considers the physiological, behavioral, or numerical responses of the parasites to a stressor (Sures, 2006; Vidal-Martínez et al., 2009). And in this case, parasites of the Monogenea class can be very useful as bioindicators, as they tend to respond numerically, increasing or decreasing their populations, according to the increase in chemical pollution in the environment (Sanchez-Ramírez et al., 2007; Gilbert and Avenant-Oldewage, 2017), being able to respond to eutrophication and organic pollution, trace metals pollution, and pollution caused by effluents discharge (Gilbert and Avenant-Oldewage, 2017).

In Brazil, habitat, and species losses due to human negative activities is as growing reality and occurs in all biomes in the country (Coelho et al., 2020; Ferreira et al., 2015; Lima et al., 2020; Rangel, 2012; Roque et al., 2016). Identify problems that interfere with the health of the environment, even on the smallest scales, is of crucial importance in diagnosing and mitigation of these problems. In this study we evaluated the variation in rates of prevalence, mean intensity, and mean abundance of five monogenean parasite species in relation to the organic and inorganic water parameters of two neotropical rivers, the Jacaré-Pepira and Jacaré-Guaçú, in southeastern Brazil, and also their relationship with the condition factors of the two fish hosts species analyzed: *Serrasalmus maculatus* Kner, 1858 (Characiformes: Serrasalmidae) and *Astronotus crassipinnis* (Heckel, 1840) (Cichliformes: Cichlidae).

## 2 MATERIAL AND METHODS

### 2.1 Study area

Two rivers with different degrees of disturbance were selected: the Jacaré-Pepira River and the Jacaré-Guaçú River.

Both are important tributaries of the Tietê River, one of the most important rivers in the state of São Paulo, with 1,100 km in length, and inserted in the upper Paraná River basin, one of the most important Brazilian basins and a major maintainer of local biodiversity with more than 230 fish species, and 300 species of fish parasites (Langeani et al., 2007; Lehun et al., 2020).

The Jacaré-Pepira River (-21.904128S, -48.842116W), is the river considered as the most conserved of the two, has characteristics very similar to those found in the Brazilian Pantanal, with wetlands and swampy areas in the rainy seasons, its role as a maintainer of the biodiversity makes this river inserted into an area of environmental protection.

The Jacaré-Guaçú River (-21.808657S, -48.871903W), as it is closer to an urban area, the municipality of Ibitinga, in the state of São Paulo, receives loads of domestic and industrial sewage and is also influenced by areas of agriculture and livestock. It is considered a more polluted river with high concentrations of pesticides, metals, and fecal coliforms (Rodríguez, 2001; Esguícero and Arcifa, 2011).

### 2.2 Fish and parasites collection

Fish collections were carried out in between January and December 2017, totaling 12 samplings of *S. maculatus* and *A. crassipinnis* (Table 1). Fish collections followed the guidelines of the scientific fishing license, under the authorization of the Instituto Chico Mendes de Biodiversidade (ICMBio), through the Biodiversity Authorization and Information System (SISBIO), represented by the number 55914-1. Fish captures was carried out with the help of local fishermen, who used simple waiting nets of different meshes sizes and placed at varying heights.

**Table 1**: Number of specimens (N), standard length (SL) and total weight (TW) of the two fish hosts species collected from the Jacaré-Pepira and Jacaré-Guaçú rivers, Tietê-Jacaré sub-basin, state of São Paulo, Brazil.

| **Fish species** | **Jacaré-Pepira** | | | **Jacaré-Guaçú** | | |
|---|---|---|---|---|---|---|
| | **N** | **SL (cm)** | **TW (g)** | **N** | **SL (cm)** | **TW (g)** |
| *S. maculatus* | 40 | 15.73 ± 0.44 | 198.36 ± 15.85 | 40 | 14.95 ± 1.97 | 169.99 ± 66.8 |
| *A. crassipinnis* | 22 | 19.11 ± 2.70 | 400.23 ± 143.97 | 40 | 18.75 ± 2.03 | 372.9 ± 98.3 |

After collected, fish were stored in individual plastic bags to avoid contamination in their parasitic fauna and then transported in a thermic box to the laboratory where they were kept refrigerated in a freezer for a maximum period of one month until parasite collection. At the time of analysis, the standard length (cm), sex, and total weigh (g) of the hosts were recorded.

For the monogenean parasites sampling, fish specimens had their body surfaces, gills and nostrils washed and the contents were sieved with 53 µm. After that, the filtered content was placed in petri dishes and looked at under a stereomicroscope. Collected parasites were stored in glass tubes with 70% alcohol.

For species identification, parasites were mounted using Gray and Wess for clarification of the sclerotized structures or stained with Gomori’s trichrome and mounted with Canada balsam.

### 2.3 River water analysis

Water samples were collected on the surface and bottom of the Jacaré-Pepira and Jacaré-Guaçú rivers and transported to the laboratory according to the Standard methods for the examination of water and wastewater (Apha, 2012). Samples were collected from January and December 2017, in monthly terms, totaling 12 samples from each analyzed river.

Chemical analysis of water samples was performed according to the methodology described in the standard methods for examination of water and wastewater (APHA, 2012). The analyzed parameters were Ammonia (N-$NH_4$ mg/$L^{-1}$), Nitrate (N-$NO_3$ mg/$L^{-1}$), Total phosphorus (TP) (mg/$L^{-1}$) and Potassium (mg/$L^{-1}$) by a Multiparameter Photometer for Nutrient Analysis (Hanna - HI 83225), Total Kjeldahl Nitrogen (TKN) by titration (4500-$N_{org}$B) and Nitrite (mg/$L^{-1}$) using a Spectrophotometer (DR 2500, ODYSSEY - HACH).

The classification of water samples was performed according to the values established by CONAMA Resolution 357/2005 for class 1 freshwater bodies (Brazil, 2005).

## 2.4 Statistical analysis

The quantitative descriptors of parasitism of prevalence, mean abundance and mean intensity of infestation were calculated according to Bush et al. (1997). The student's *t*-test was used to visualize statistical variation between the parasitic descriptors and seasonality (dry and wet seasons), and also to test the variation between the water parameters in the two different rivers and according to the season, results were considered statistically significant when $p \leq 0.05$.

The Principal Component Analysis (PCA) was used to correlate the river water parameters of both rivers according to the season, and to correlate parasitic abundances with the river water parameters and with the condition factors (KN) of the fish hosts. For correlation, was used the Spearman's Rank Correlation Coefficient.

For the calculation of the fish relative condition factors, a standard length/weight relationship was created, and through the coefficient angular equation, the expected weight (EW) of the fish individuals was estimated through the expression $EW = SL.y^x$, where SL represents the standard length of the individuals, and X and Y are in coefficient angular equation. The Condition Factors (KN) was obtained through $K = EW/W$, where EW is the expected weight and W the observed weight of the fish individuals (Nash et al., 2006).

## 3 RESULTS

Tables 2 and 3 show the quantitative descriptors of prevalence, mean intensity, and mean abundance of the five monogenean species in dry and wet seasons. Except for *Rhinoxenus piranhus* Kritsky, Boeger and Thatcher, 1988, which was collected from the host's nostrils, all species were collected from the fish gills. Numerically, in most species, are observed higher values for mean abundance and intensity in wet season compared to the dry season in both rivers. Student's *t*-test, however, show that in the Jacaré-Pepira River only *Anacanthorus serrasalmi* Van Every and Kritsky, 1992 had mean values of abundance and intensity statistically different ($p \leq 0.05$) between seasons, with higher infestation rates in the wet season. In the Jacaré-Guaçú River, *Gussevia asota* Kritsky, Thatcher and Boeger, 1989 and *Amphithecium speirocamarotum* Boeger and Jégu, 1997 had their abundances higher also in the wet season ($p \leq 0.05$). Remaining cases had no statistically significant differences ($p \geq 0.05$).

Figure 1 show the mean values of the concentrations (mg/L$^{-1}$) of the inorganic and organic variables of the waters sampled from both rivers in dry and wet seasons. In the PCA (Figure 2) it is possible to observe the variables well discriminated between the rivers (Figure 2A), and it also shows that of all variables, only nitrite was in higher concentrations in the samples from Jacaré-Pepira River (Figure 2B). The rest was in higher concentrations in the waters of the Jacaré-Guaçú River.

**Table 2:** Prevalence, mean intensity and mean abundance of the monogenean parasites of *Astronotus crassipinnis* and *Serrasalmus maculatus* in dry and wet seasons in the Jacaré-Pepira River, Tietê-Batalha river basin, state of São Paulo, Brazil.

| **Species** | | **Prevalence (%)** | | **Mean Intensity** | | **Mean Abundance** | |
|---|---|---|---|---|---|---|---|
| | | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** |
| *A. crassipinnis* | *G. astronoti* | 81 | 100 | 12 ± 3 | 13 ± 1 | 9 ± 3 | 13 ± 1 |
| | *G. asota* | 69 | 100 | 5 ± 1 | 4 ± 2 | 4 ± 1 | 4 ± 2 |
| *S. maculatus* | *A. serrassalmi* | 81 | 100 | 6 ± 1 | 10 ± 1 | 5 ± 1 | 10 ± 1 |
| | *R. piranhus* | 57 | 100 | 6 ± 1 | 6 ± 1 | 4 ± 1 | 6 ± 1 |

Analyzing the water variables of each river according to the season, it its observed that, except for potassium in the Jacaré-Guaçú waters, there was no variance between the concentrations of the parameters (Student's *t*-test $p \geq 0.05$). But when comparing the rivers, it is possible to observe a variation for all variables, indicating a statistically significant difference ($p \leq 0.05$) for ammonia, phosphorus, nitrate, nitrite, and total nitrogen. So, except for nitrite that was found in higher concentrations ($p \leq 0.05$) in the waters of the Jacaré-Pepira River, all other parameters were higher, regardless of the period, in the Jacaré-Guaçú River.

**Table 3**: Prevalence, mean intensity, and mean abundance of the monogenean parasites of *Astronotus crassipinnis* and *Serrasalmus maculatus* in dry and wet seasons in the Jacaré-Guaçú River, Tietê-Batalha river basin, state of São Paulo, Brazil.

| **Species** | | **Prevalence (%)** | | **Mean Intensity** | | **Mean Abundance** | |
|---|---|---|---|---|---|---|---|
| | | **Dry** | **Wet** | **Dry** | **Wet** | **Dry** | **Wet** |
| *A. crassipinnis* | *G. astronoti* | 90 | 95 | 12 ± 2 | 11 ± 1 | 10 ± 2 | 11 ± 1 |
| | *G. asota* | 85 | 75 | 4 ± 1 | 8 ± 2 | 3 ± 1 | 7 ± 2 |
| *S. maculatus* | *A. serrassalmi* | 100 | 100 | 10 ± 1 | 7 ± 1 | 10 ± 1 | 7 ± 1 |
| | *A. speirocamarotum* | 75 | 100 | 2 ± 1 | 5 ± 1 | 3 ± 1 | 5 ± 1 |

Analyzing the water variables of each river according to the season, it its observed that, except for potassium in the Jacaré-Guaçú waters, there was no variance between the concentrations of the parameters (Student's *t*-test $p \geq 0.05$). But when comparing the rivers, it is possible to observe a variation for all variables, indicating a statistically significant difference ($p \leq 0.05$) for ammonia, phosphorus, nitrate, nitrite, and total nitrogen. So, except for nitrite that was found in higher concentrations ($p \leq 0.05$) in the waters of the Jacaré-Pepira River, all other parameters were higher, regardless of the period, in the Jacaré-Guaçú River.

In the PCA that correlates the chemical variables of the water with the abundance of parasites, *G. asota* correlated negatively with nitrate in the Jacaré-Pepira River ($rs = -0.659$, $p \leq 0.05$) (Figure 3A) and also in the Jacaré-Guaçú River ($rs = -0.633$, $p \leq 0.05$) (Figure 3B), and with total nitrogen in Jacaré-Guaçú River ($rs = -0.428$, $p \leq 0.05$) (Figure 3B). *Amphithecium speirocamarotum* also had a negative correlation with potassium ($rs = -0{,}605$, $p \leq 0.05$) (Figure 3B) in the Jacaré-Guaçú River. Which means that, as the concentrations of nitrate, total nitrogen and potassium in the water increased, the abundance of *G. asota* and *A. speirocamarotum* tended to decrease.

There was no statistically significant variation between the condition factors (Figure 4 and 5) of *A. crassipinnis* and *S. maculatus* in the two rivers and in the different periods analyzed (Student's *t*-test $p \geq 0.05$). Regarding to the relationships between condition factors and parasitic species abundance, the PCA indicated a positive relationship between the abundance of *G. asota* in the Jacaré-Guaçú River (rs = 0.736, $p \leq 0.05$) (Figure 6B) and for *A. serrasalmi* in the Jacaré-Pepira River (rs = 0.350, $p \leq 0.05$) (Figure 7A), indicating that fish with higher condition factors are more likely to be parasitized by these monogenean species than fish with lower ones.

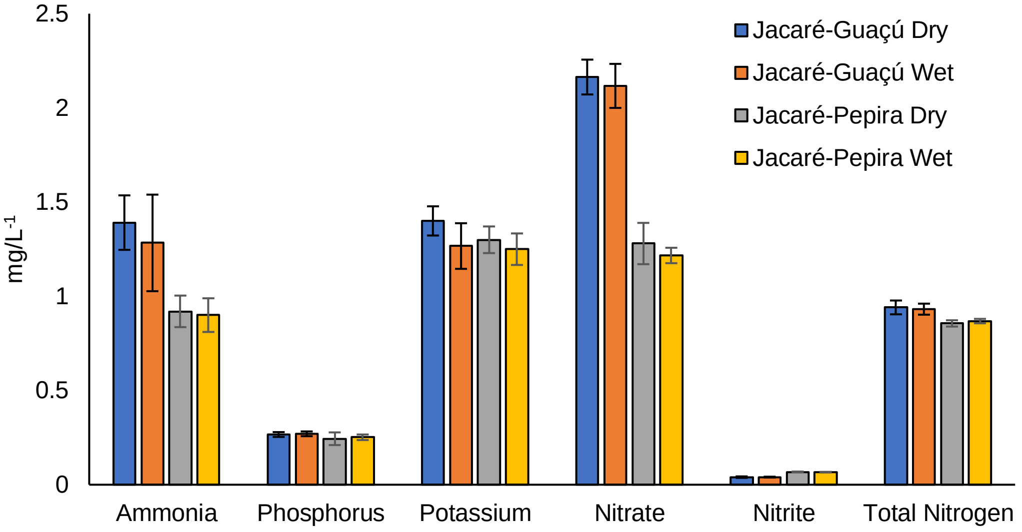


**Figure 1**: Concentrations ($mg/L^{-1}$) of the inorganic and organic variables analyzed from the waters of the Jacaré-Pepira and Jacaré-Guaçú rivers in dry and wet seasons.

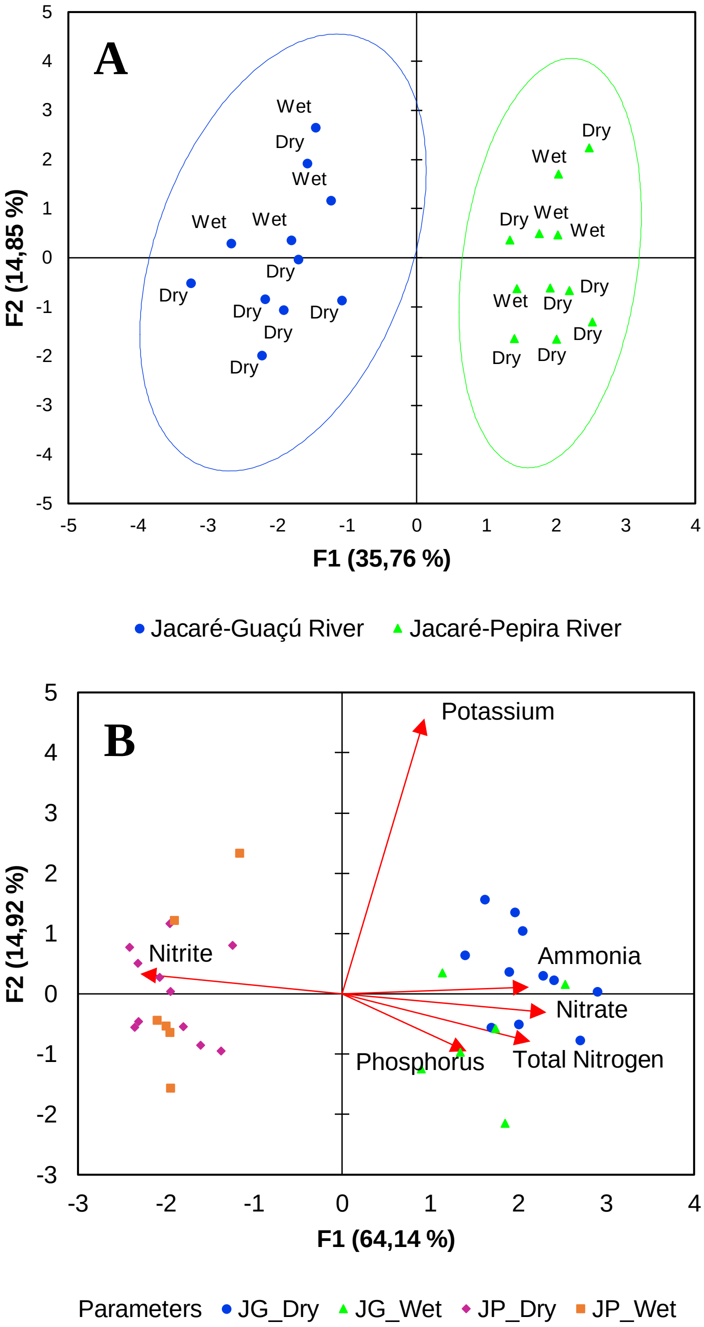


**Figure 2**: Principal Component Analysis of the inorganic and organic variables in the waters of the Jacaré-Pepira and Jacaré-Guaçú rivers in dry and wet seasons.

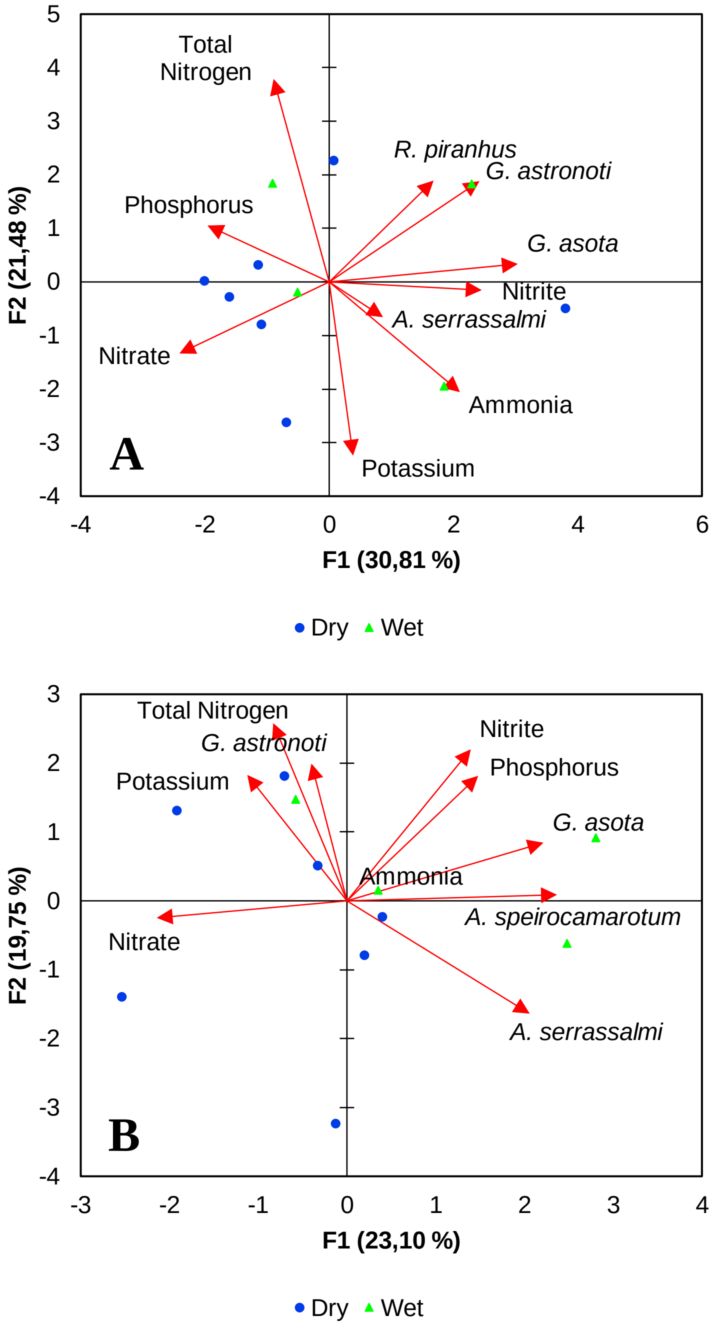
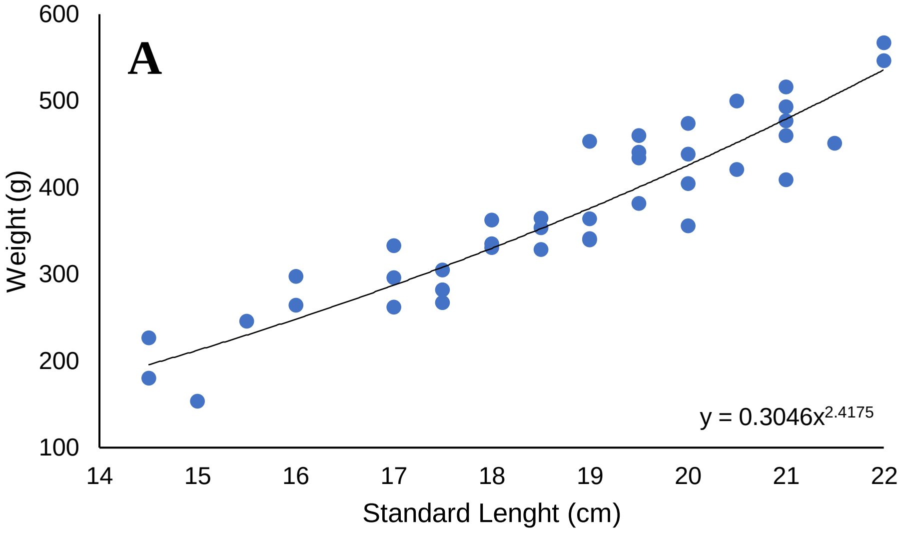


**Figure 3:** Principal Component Analysis that correlates the abundance of the monogenean parasites of *Astronotus crassipinis* and *Serrasalmus maculatus* with inorganic and organic variables analyzed from the waters, in dry and wet seasons, of the Jacaré-Pepira (A) and Jacaré-Guaçú (B) rivers, Tietê-Batalha River basin, southeastern Brazil.



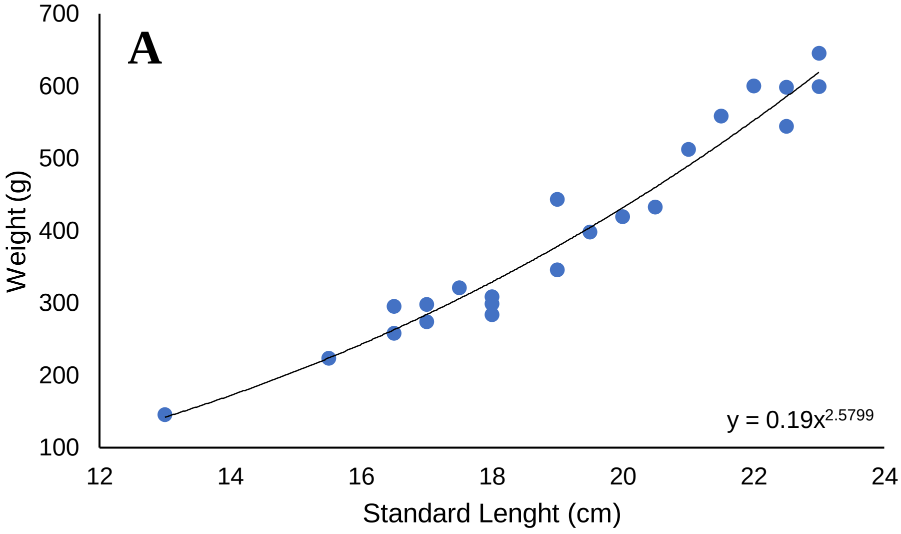


**Figure 4:** Condition factor of the specimens of *Astronotus crassipinnis* from the Jacaré-Pepira (A) and Jacaré-Guaçú (B) river, Tietê-Batalha River basin, southeastern Brazil.

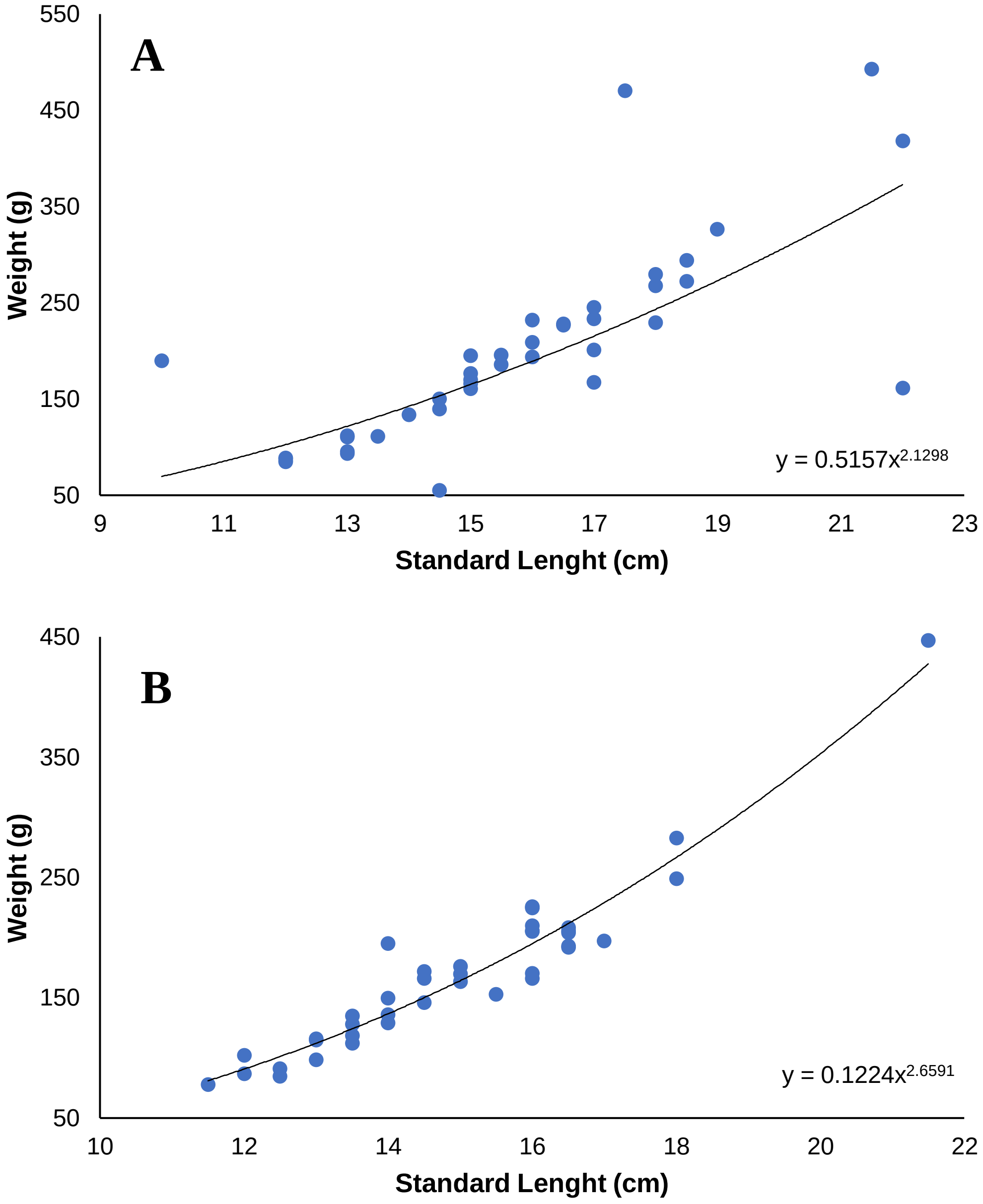


**Figure 5:** Condition factor of the specimens of *Serrasalmus maculatus* from the Jacaré-Pepira (A) and Jacaré-Guaçú (B) river, Tietê-Batalha River basin, southeastern Brazil.

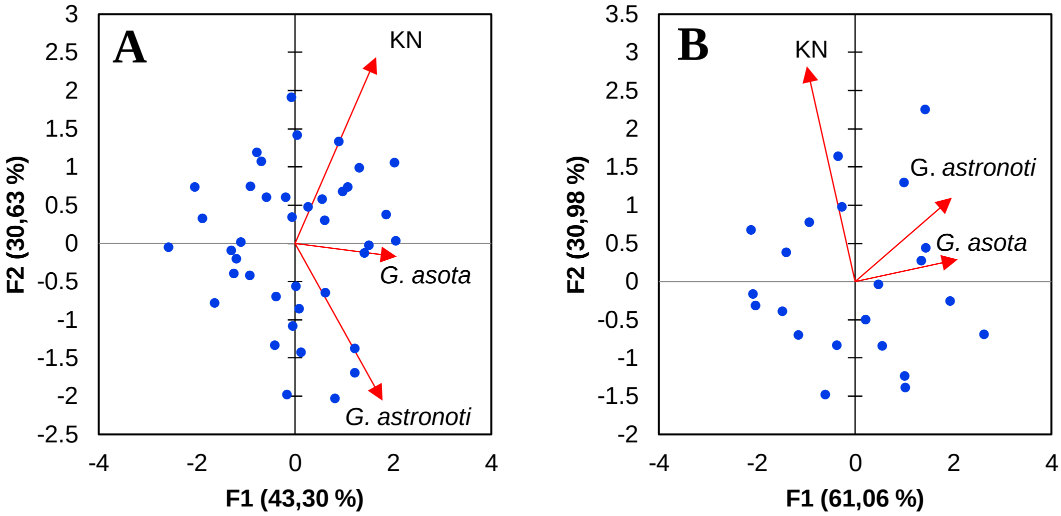


**Figure 6:** Principal Component Analysis that correlates the condition factors (KN) of *Astronotus crassipinnis* and the abundance of its monogenean parasites in the Jacaré-Pepira (A) and Jacaré-Guaçú (B) rivers, Tietê-Batalha River basin, southeastern Brazil.

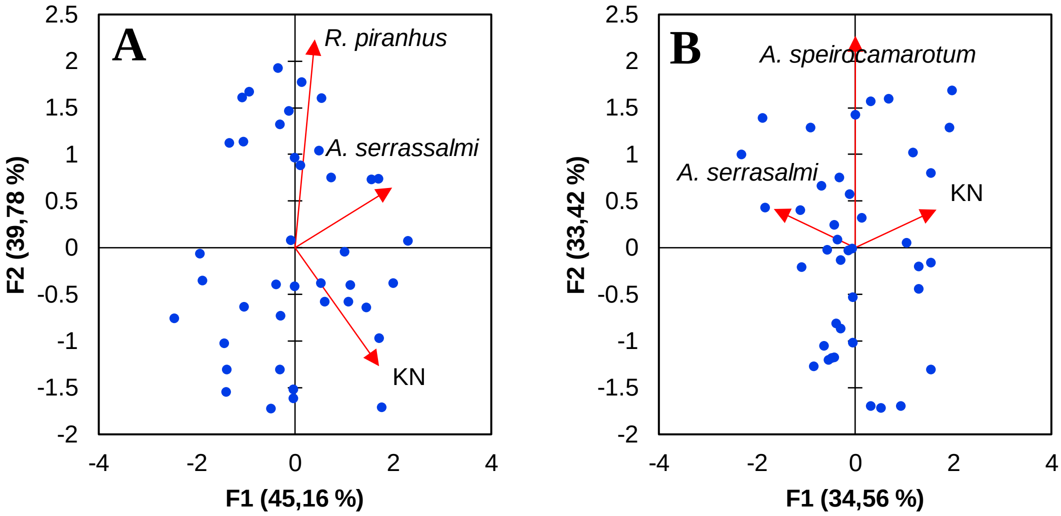


**Figure 7:** Principal Component Analysis that correlates the condition factors (KN) of *Serrasalmus maculatus* and the abundance of its monogenean parasites in the Jacaré-Pepira (A) and Jacaré-Guaçú (B) rivers, Tietê-Batalha River basin, southeastern Brazil.

## 4 DISCUSSION

In effect bioindication, among the several parasite's response mechanisms against an environmental stressor (in a general way), the complexity of the live cycle has always been a factor that apparently favored heteroxenous species (Dias et al., 2017) since their life cycles allow a broad view of the functioning of the ecosystem at different trophic levels. In Blanar et al. (2009) meta-analysis, however, was observed that monoxenous parasites had effect sizes higher than heteroxenous against several types of pollutants. One of the reasons for this is that fish ectoparasites are in direct contact with water and consequently with pollutants, which can directly affect their abundances, since the host's immune response can also be affected (Falkenberg et al., 2019; Madi and Ueta, 2009). In the present study, a significant response of monoxenous cycle parasites could also be visualized, since that some of the monogenean species of the two fish hosts analyzed had significant variation in their abundances and infestation rates according to the season, water variables and to the host's condition factor.

Recently, a meta-analysis conducted by Poulin (2020) about the seasonal dynamics in parasite infections in aquatic ecosystems shows, in a general way, that the wet season in tropical aquatic ecosystems peaks in parasitic abundance, but with a closer look at specific groups, freshwater monogeneans had no statistical difference between seasons, only coastal fish monogeneans had significant difference in the prevalence rates but peaking in the dry season. In the present study, was observed a different pattern, *A. serrasalmi*, *G. asota*, and *A. speirocamarotum* had significantly higher abundance rates in wet season and the same pattern was also observed for dactylogyrids in the Brazilian Amazon, were *Wittingtonocotyle caetei*, *W. jeju*, *Urucleidoides* sp., *U. eremitus* and *Anacanthorus* sp. parasitizing *Hoplerythrinus unitaeniatus* and *Hoplias malabaricus* (Characiformes: Erythrinidae) had higher rates of prevalence and abundance in the wet season (Gonçalves et al., 2016). In parasites of the family Dactylogyridae, structural characteristics of the habitat have no consequences on the prevalence and abundance of species, although abiotic factors are important to the occurrence and distribution of monogeneans, they alone are not determining factors in the season changes or patterns of occurrences. Water temperature still the major determinant, since these parasites have a high sensitivity to temperature changes, which in the species reproductive dynamics plays an important role in egg production (Buchmann, 1988; Chubb, 1977; Lacerda et al., 2018). In another study, Igeh et al. (2020) assessing the season variation along with water and sediment variables in the infestation patterns of *Cichlidogyrus philander* Douëllou, 1993 infecting the gills of *Pseudocrenilabrus philander* (Cichliformes: Cichlidae) in a dam in South Africa, found no relationship between the chemical variables of water and sediments and the monogenean infestation rates, observing only a seasonal influence, which had the highest peaks of infestation in wet season. Thus, despite the clear influence of the seasonality, especially related to the temperature, in the rates of abundance and intensity of infestation of the monogenean parasites, seasonality alone cannot be considered as the only cause in the infrapopulations variations (Igeh et al., 2020).

The chemical parameters in the waters of the two rivers did not differentiate between dry and wet seasons, but two of them were in concentrations above that allowed by the current Brazilian legislation (Brazil, 2005): ammonia, on the Jacaré-Pepira and Jacaré-Guaçú rivers, and phosphorus on the Jacaré-Pepira River. Ammonia is naturally present in surface waters and liquid effluents. Its concentration is generally low in groundwater, because of the adsorption of soil and clay particles and is not easily leached from soils. It is produced largely by deaminating organic compounds containing nitrogen and by hydrolysis of urea. The high concentrations of the ammonium ion found can have major ecological implications, such as: influencing the amount of oxygen dissolved in the water. Another form of action can be at basic pH, where this ion turns into ammonia gas (free, gaseous $NH_3$), which, depending on the concentration, can be toxic to fish. According to Esteves (2011), concentrations equal to or higher than 0.5 $mg/L^{-1}$ of ammonia are considered lethal to fish. In the case of phosphorus, despite values slightly above what is allowed, and due to its direct connection with eutrophication mechanisms, this value just fairly above will not affect the overgrowth of algae that could cause eutrophication (Apha, 2005; Sperling, 1996).

In addition to the parameters found in concentrations above the allowed by the legislation, others, such as nitrate, total nitrogen, and potassium, had an influence on the abundance of two of the five monogenean species analyzed: *G. asota* and *A. speirocamarotum*. Nitrate is a common nutrient in freshwater environments and usually comes from areas of agriculture or sewage disposal (Pacheco and Fernandes, 2016; Padilla et al., 2018), and can be toxic to the aquatic biota, including fish species (Camargo et al., 2005; Freitag et al., 2015). In the present study, it was observed that the abundance of *G. asota* decreased significantly as the nitrate concentrations in water increased. While high concentrations of nitrate can cause the death of the fish, non-lethal concentrations can alter their epidermal structure and ensure a significant reduction in infestation rates of monogenean species. Smallbone et al. (2016) found that nitrate concentrations ranging from 50 to 250 mg/L significantly reduced the gyrodactylid *Gyrodactylus turnbulli* Harris, 1986 infrapopulations in two populations of *Poecilia reticulata* (Cyprinodontiformes: Poeciliidae), suggesting that nitrate protects the host against infestations/infections. Although there is no study that directly links the effect of increasing or decreasing concentrations of total nitrogen and potassium on monogenetic parasites abundance rates, a negative relationship between the concentrations of these water parameters and the abundance of monogenean species is already commonly observed (Blanar et al., 2009; Lacerda et al., 2018). In addition, our study shows that even at not so high concentrations (from a toxicological point of view) changes in concentrations already reflect directly on the distribution of the parasites, showing how sensitive these organisms are to changes in the aquatic environment.

An intrinsic relationship between the fish condition factors and the organic and inorganic parameters of the water and also with the dry and wet seasons was not observed, even knowing that some of these variables can directly affect the fish development (Gonçalves et al., 2016; Lizama et al., 2006). On the other hand, it was observed that the abundances of *G. asota* and *A. serrasalmi* were positively correlated with the condition factors of their fish hosts. Falkenberg et al. (2017), assessing the relationships between fish gill parasites and environmental factors in two estuarine environments in Brazil also found a positive relationship between fish condition factors and the abundance of parasites, which was attributed to the fact that the higher the condition factor, the larger the available surface, which can provide an increase in infestation rates, therefore, larger fish can tolerate higher levels of parasitism (Igeh et al., 2020; Falkenger et al., 2017; Lizama et al., 2006). In addition, the age of the host causes changes in its biology that can directly influence in the trophic levels of the food web and consequently in its associated parasitic fauna (Dogiel et al., 1961).

## 5 CONCLUSIONS

In general, wet season favored an increasing in the infestation rates of the monogeneans parasites in their host species, mainly in the river considered as the most polluted, the Jacaré-Guaçú River. Of the five parasites species analyzed in this study, only *Gussevia astronoti* Kritsky, Thatcher and Boeger, 1989 and *R. piranhus* had no interaction with river water variables or fish host condition factors. On the other hand, *G. asota* had interactions both with water parameters (nitrate and total nitrogen) and with the hosts condition factors, which reflected in the abundance and intensity rates, showing itself a species sensitive to changes in the environment and, therefore, that can be considered as a bioindicator organism.

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# *Artigo 3*

## ARTIGO 3.

# Bioaccumulation and health risk assessment of trace metal contamination in the musculature of the trahira fish (*Hoplias malabaricus*) from two neotropical rivers in southeastern Brazil

## ABSTRACT

In the present study, we evaluated the bioaccumulation and health risk of trace metal contamination in the musculature of the trahira fish (*Hoplias malabaricus*), collected from the Jacaré-Pepira and Jacaré-Guaçú rivers, in southeastern Brazil. During the period from May 2017 to November 2019, 90 fish were collected, 45 from each river. River water samples were also taken during the same collection periods. From fish, muscle tissue samples were taken, and together with river water samples, analyzed for the recovery of trace metals (Al, Cr, Mn, Fe, Ni, Cu, As, Cd, and Pb) through the technique of Inductively Coupled Plasma Mass Spectrometry (ICP-MS). In general, fish as well as the waters of the Jacaré-Guaçú River had higher concentrations of metals. The elements Al, Cr and Cd stood out from the others analyzed metals for having a hazard index (HQ) above 1 (Al), for being up to 10 times above the concentrations allowed by Brazilian legislation (Cr) and for having a high bioconcentration factor (Cd), indicating a biomagnification process through the food chain. These elements should receive attention as they can offer risks to the health of fish, which can jeopardize the survival of their populations, and especially to humans who use these animals as a food source.

**KEYWORDS:** Hazard quotient; Trace metal contamination; River pollution; Heavy metal pollution; Bioaccumulation.

## 1 INTRODUCTION

Fish are an important source of nutrition for humans, as their meat contains high amounts of proteins, fatty acids, vitamins, and essential metals (Çamur et al., 2021). In Brazil, an average of 4.7 kg of fish is consumed *per capita*/year. Southeast Brazil is the region that consumes the least amount of fish meat in the country, with fish meat representing 6.1% of the population's diet, of which, 23% represents consumption from outside home. In addition, the rural areas consume 34.4% more fish than the population living in urban areas of the country (Instituto Brasileiro de Geografia e Estatística, 2020).

Brazil has an immense hydrographic structure, with the most extensive river network in the world, and having the greatest hydrological potential on the planet, which directly reflects in the complexity of structuring aquatic animal communities, where fish represent enormous diversity, with approximately 2,500 species (Peressin and Silva, 2015). Due to the great diversity of hydrographic basins and also of fish species, fishery (both industrial and artisanal) in Brazil is an important economic tool (Petrere Jr., 1986), which places the country in the fourth position in the ranking of the largest fishing countries in Latin America (Silva, 2014). Artisanal fishing plays a fundamental role in Brazil fish production, being responsible for more than 50% of landings (Cetra and Petrere Jr., 2001), and can be divided into three categories: 1) professional artisanal fishing, focusing on commercialization, 2) subsistence artisanal fishing, supplying food for the fishermen and their families, and 3) amateur/sport fishing, practiced only as tourism or leisure, and the fish cannot be commercialized (Castro et a., 2008).

Fishing activities develop over time, and there is also an increase in the anthropic pressure exerted on aquatic environments, both lotic and lentic. In Brazil, the unrestrained increase, diffusion, and little importance for environmental causes of other economic activities, such as the agricultural industry, for example, has caused irreparable damage, leading to the contamination of water bodies, including the organisms that lives in these environments (Findley, 1988; Gunkel et al., 2007; Silva et al., 2016).

Among the countless pollutants that reach water bodies, trace metals are extremely problematic because they can be very toxic even at lowest concentrations in the aqueous state (Sahu and Basti, 2021). Agricultural activities, and discharges of industrial and domestic sewage are the main sources of contamination in both lentic and lotic environments. In water, non-reactive or non-toxic forms of certain metals can be transformed into reactive or toxic forms, which in turn can directly affect life in water (Sahu and Basti, 2021), being bioaccumulated or biomagnified trough food web, including fish. Because they are at the top of the food chain, they can accumulate higher amounts of metals in their tissues, and this in turn becomes a public health problem, since humans can feed on contaminated tissue, leading to intoxication (Raknuzzaman et al., 2016).

*Hoplias malabaricus* (Bloch, 1794) (Characiformes: Erythrinidae) is a freshwater, carnivore, and top food chain predator. It occurs throughout South and Central America, especially in lentic environments. It is a fish well adapted to waters with high turbidity and low oxygen availability (Gião et al., 2020). This fish has a very tasty and high-quality meat, that makes the species highly sought after in fishing. It is estimated that up to 9 tonnes of fish of this genus are caught per year (Secretaria de Monitoramento e Controle do Ministério da Pesca e Aquicultura – MPA, 2011). In this study, we aimed to evaluate the concentrations of trace metals in the muscle tissue of *H. malabaricus* and in the water of two rivers where this species occurs, the Jacaré-Pepira and Jacaré-Guaçú rivers, in southeastern Brazil, and to analyze if there is a risk of contamination by consumption by humans through the hazard coefficient.

## 2 MATERIAL AND METHODS

### 2.1 Study area

This study was conducted in the Jacaré-Pepira and Jacaré-Guaçú rivers, in the Tietê-Batalha River basin, in the Midwest portion of the state of São Paulo, southeastern Brazil.

### 2.2 Fish and river water sampling

Fish and water sampling was conducted between May 2017 and November 2019. Fish specimens were collected using gill nets with the aid of local fishermen. Bottom and surface water samples were collected in the same point as the fish, using a Van Dorn water sampler. After collection, fish were stored in individual plastic bags, and water samples in sterile plastic bottles, and then stored in a freezer at -25ºC.

In the laboratory, fish were identified, measured, weighed, and then necropsied, with samples of internal lateral musculature removed and store individually in sterile plastic tubes.

### 2.3 Trace metal analysis

For metal recovery in the muscle tissues of fish, samples were first digested with a solution of 3 mL $HNO_3$ and 2 mL $H_2O_2$ in a microwave oven MARS 5 (CEM GmbH, Kamp-Lintfort, Germany), with the following programming: 400W at 180ºC for 6 minutes, 600W at 180ºC for 8 minutes and 800W at 180ºC for 12 minutes. To the digested samples, deionized water was added to a volume of 10 mL. Then, using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (7700e, Agilent, USA), the concentrations of the elements Al, Cr, Mn, Fe, Ni, Cu, As, Cd, and Pb was obtained. The ICP-MS was calibrated using the Certified Reference Material (CRM) DORM-4 (Fish Protein Certified Reference Material for Trace Metals and other Constituents) (National Research Council of Canada, NRC – CNRC).

For river water trace metal analysis, samples were analyzed in natura by the ICP-MS, and the methods was validated using the CRM NIST-1643f (Trace Elements in Natural Water) (National Institute of Standards & Technology, USA).

### 2.4 Statistical analysis

The significant differences between the metal concentrations in the musculature of fish and in river waters were obtained through the Mann-Whitney test, considering the result as significant when $p \leq 0.05$.

The Principal Component Analysis (PCA) was applied to verify the distribution of metal concentrations in fish musculature in the two different rivers and in river water according to seasonality. The Agglomerative Hierarchical Cluster Analysis (AHC) was applied to identify possible pollution sources.

Hazard Coefficient (HQ) was calculated according to Yang et al. (2013), using the following formula:

$$HQ = \frac{EDI}{RfD}$$

Where EDI is the estimated daily intake, and *RfD* is the reference dose (mg/kg), previously stablished by the Food and Agriculture Organization of the United Nations (FAO) (FAO, 2021). Estimated daily intake (EDI) was calculated using the fallowing formula:

$$\text{EDI} = \text{C}_{fish} \times \left[\frac{dc_{fish}}{bw}\right]$$

Were $C_{\text{fish}}$ is the metal concentration (mg/kg) obtained in fish musculature, $dc_{\text{fish}}$ is the daily fish consumption (g/day) *per capita* by the population of southeastern Brazil, and *bw* is the average body weight (kg) of the target population according to the Brazilian Institute of Geography and Statistics (IBGE) (IBGE, 2020).

The Bioconcentration Factors (BCF) was calculated using the following ratio:

$$BCF = \frac{C_{fish}}{C_{water}}$$

Were $C_{\text{fish}}$ is the metal concentration obtained from the musculature of *H. malabaricus* and $C_{\text{water}}$ is the metal concentration obtained from the river water of the Jacaré-Pepira and Jacaré-Guaçú rivers.

## 3 RESULTS

The accuracy, detection, and quantification limits of the ICP-MS analysis for metal quantification in the samples of muscle tissue of *Hoplias malabaricus* and in the waters from the Jacaré-Pepira and Jacaré-Guaçú rivers together with the concentration values obtained in the certified reference materials (CRM) are shown in tables 1 and 2. For the muscle tissues of *H. malabaricus*, the accuracy of the analysis varied from 92% (As) to 111% (Fe), while for the water samples of the two rivers, the accuracy varied from 88% (As) to 111% ( Cr), which indicates that the results obtained from the ICP-MS analysis are reliable.

**Table 1:** Trace metal concentrations in certified reference material (DORM-4) as well as accuracy, detection (DL), and quantification (QL) limits determined by the ICP-MS analysis.

| Element | DORM-4 (mg/kg) | DORM-4 analyzed (mg/kg) | Accuracy (%) | DL (µg/L) | QL (µg/L) |
|---|---|---|---|---|---|
| Al | 1280 ± 340 | 1382.4 ± 328.2 | 108 | 0.01 | 0.01 |
| Cr | 1.87 ± 0.18 | 2.03 ± 0.23 | 109 | 0.03 | 0.09 |
| Mn | 3.17 ± 0.26 | 3.26 ± 0.29 | 103 | 0.02 | 0.06 |
| Fe | 343 ± 20 | 380.7 ± 18.2 | 111 | 0.14 | 0.48 |
| Ni | 1.34 ± 0.14 | 1.31 ± 0.17 | 98 | 0.05 | 0.05 |
| Cu | 15.7 ± 0.46 | 15.3 ± 0.64 | 100 | 0.02 | 0.02 |
| As | 6.87 ± 0.44 | 6.32 ± 0.38 | 92 | 0.01 | 0.03 |
| Cd | 0.299 ± 0.018 | 0.290 ± 0.018 | 97 | 0.01 | 0.05 |
| Pb | 0.404 ± 0.062 | 0.412 ± 0.082 | 102 | 0.01 | 0.03 |

**Table 2:** Trace metal concentrations in certified reference material (NIST-1643f) as well as the accuracy of the ICP-MS analysis.

| Element | NIST-1643f (µg/L) | NIST-1643f analyzed (µg/L) | Accuracy (%) |
|---|---|---|---|
| Al | 132.5 ± 1.2 | 140.88 ± 1.4 | 106 |
| Cr | 18.32 ± 0.10 | 20.28 ± 0.4 | 111 |
| Mn | 36.77 ± 0.58 | 38.90 ± 0.2 | 106 |
| Fe | 92.51 ± 0.77 | 99.37 ± 1.1 | 107 |
| Ni | 59.2 ± 1.4 | 52.81 ± 0.1 | 89 |
| Cu | 21.44 ± 0.70 | 22.53 ± 0.4 | 105 |
| As | 56.85 ± 0.37 | 50.04 ± 0.1 | 88 |
| Cd | 5.83 ± 0.13 | 5.85 ± 0.1 | 100 |
| Pb | 18.30 ± 0.08 | 19.04 ± 0.4 | 104 |

In total, 90 fish specimens were collected, 45 from each river. Fish collected from the Jacaré-Pepira River had a mean standard length, and body mass of 28.1 ± 3.42 cm, 447 ± 133 g, and 1.07 ± 0.16, respectively. For the Jacaré-Guaçú, the mean standard length, and body mass were 30.2 ± 3.55 cm, 489 ± 150 g, respectively.

The trace metal concentrations recovered from the *H. malabaricus* muscle tissues in the Jacaré-Pepira, and Jacaré-Guaçú Rivers are shown in Figure 1. The elements distribution (from the highest to the lowest concentration) in the fish muscle was almost the same in both rivers, Fe and Al being the ones with the highest concentrations, followed by Ni, Mn, Cu, Cr, As, Cd, and Pb in the Jacaré-Pepira River, and Ni, Mn, Cu, Cr, Pb As, and Cd in the Jacaré-Guaçú River. Numerically, fish from the Jacaré-Guaçú River had higher mean concentrations for all elements compared with fish from the Jacaré-Pepira River, which can be clearly visualized in the Principal Component Analysis (PCA) (Figure 2), but the Mann-Whitney test, however, only showed as significantly different ($p \leq 0.05$) the concentrations of the elements Cr, Mn, Cu, and As, which were higher in the musculature of *H. malabaricus* from the Jacaré-Guaçú River.

Figure 3 shows the trace metal concentrations recovered from the water samples from the Jacaré-Pepira and Jacaré-Guaçú rivers in dry and wet seasons. The elements with the highest concentrations were Fe, Mn, and Al, which had numerically higher concentrations in wet season in at least one of the two analyzed rivers. In general, seasonality was more influential in the metal concentrations in the waters from the Jacaré-Pepira river (Table 3), with significant differences for the elements Mn, Ni, and As, which had higher concentrations in the wet season (Mann-Whitney test, $p \leq 0.05$) and for Fe and Cd with higher concentrations in the dry season, this pattern can be better visualized in Figure 4A, that shows the Principal Component Analysis (PCA) with variables indicating higher values in the wet season. In the Jacaré-Guaçú River, only Al was in statistically higher concentrations in the wet season. Comparing the two rivers, the waters of the Jacaré-Guaçú river had higher concentrations for Cr, Mn and As, while the Jacaré-Pepira river had higher concentrations of Cu (Table 3), this also can be easily visualized from the PCA analysis in Figure 4C.

The Bioconcentration Factors (BCF) of the metal concentrations obtained in the muscular tissues of *H. malabaricus* compared to those obtained in the waters of both rivers are shown in Table 4. The BCF values, equal to or higher than 1, which indicates bioaccumulation, were Cd, Al, Cr, and Ni in Jacaré-Pepira River, and Cd, Al, Ni, Cr, Pb and Fe in the Jacaré-Guaçú River, with emphasis on Cd that was in concentrations up to

10 times higher in the musculature of the fish from the Jacaré-Pepira River and 4 times higher in Jacaré-Guaçú River fish (Table 4).

Regarding the risks of metal contamination by consumption of fish musculature by humans, obtained through the hazard coefficient (HQ), only the metal Al from fish of the Jacaré-Guaçú River had HQ value above 1, which indicates a risk of contamination (Table 5).

The Agglomerative Hierarchical Cluster Analysis (HCA) dendrogram that analyzes the potential sources and similarities of metals in the tissues of *H. malabaricus* in both rivers are shown in Figure 5. Figure 5A shows that in the Jacaré-Pepira the HCA formed two main groups, group 1 composed of the elements Mn, Cr, Pb, As, Fe, and Ni, and group 2 composed of the elements Cd, Al, and Cu. This indicates that the source of group 1 is common, and the group 2 has another common source. In the Jacaré-Guaçú River (Figure 5B), three main groups were formed, group 1 formed by Ni, Fe, Cr, and Al, group 2 formed by Pb, As, and Mn, and group 3 formed by Cd and Cu, also indicating that the groups had different sources.

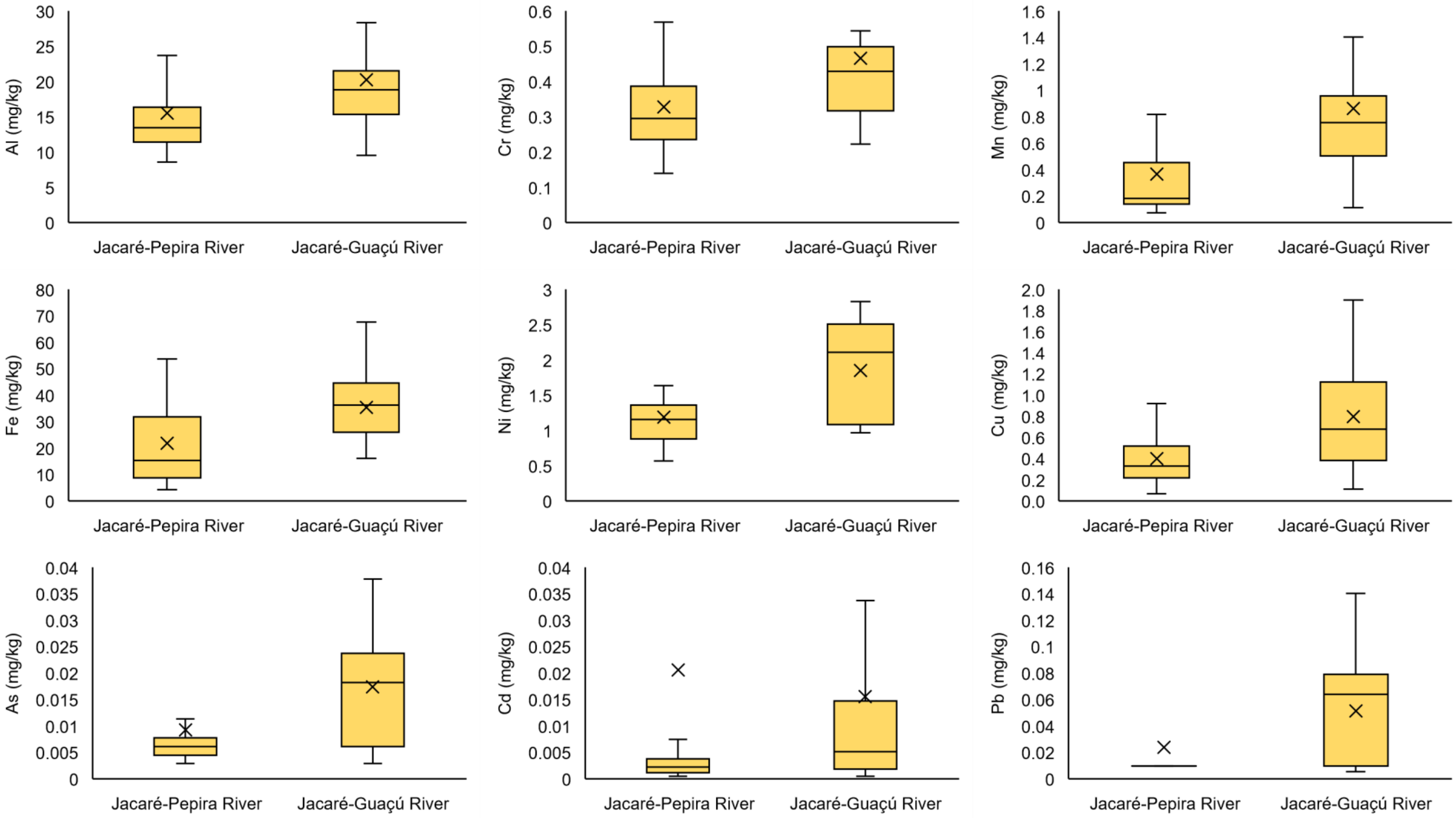

**Figure 1:** Trace metal concentrations in the musculature of *Hoplias malabaricus* (Bloch, 1794) collected from the rivers Jacaré-Pepira and Jacaré-Guaçú, southeastern Brazil.

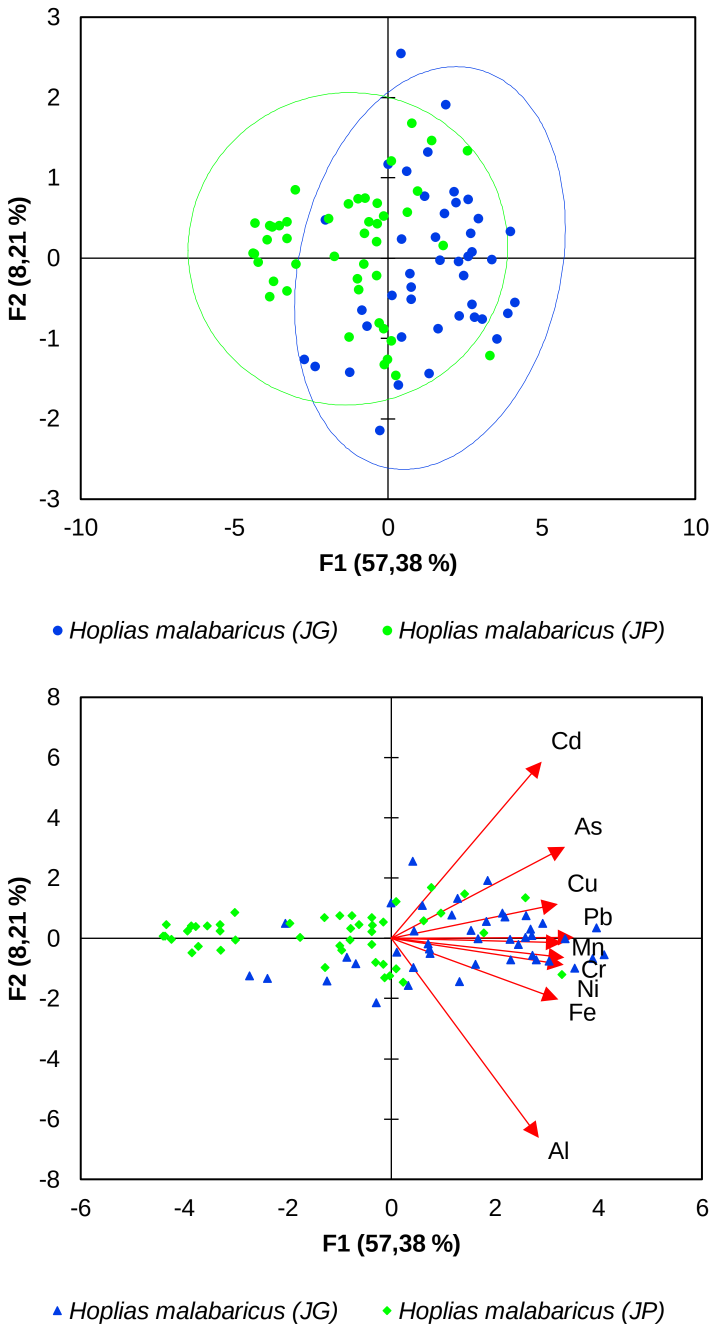

**Figure 2:** Principal Component Analysis (PCA) considering the trace metal concentrations distribution in the musculature samples of *Hoplias malabaricus* (Bloch, 1794) from the Jacaré-Pepira (JP) and Jacaré-Guaçú (JG) rivers, southeastern Brazil.

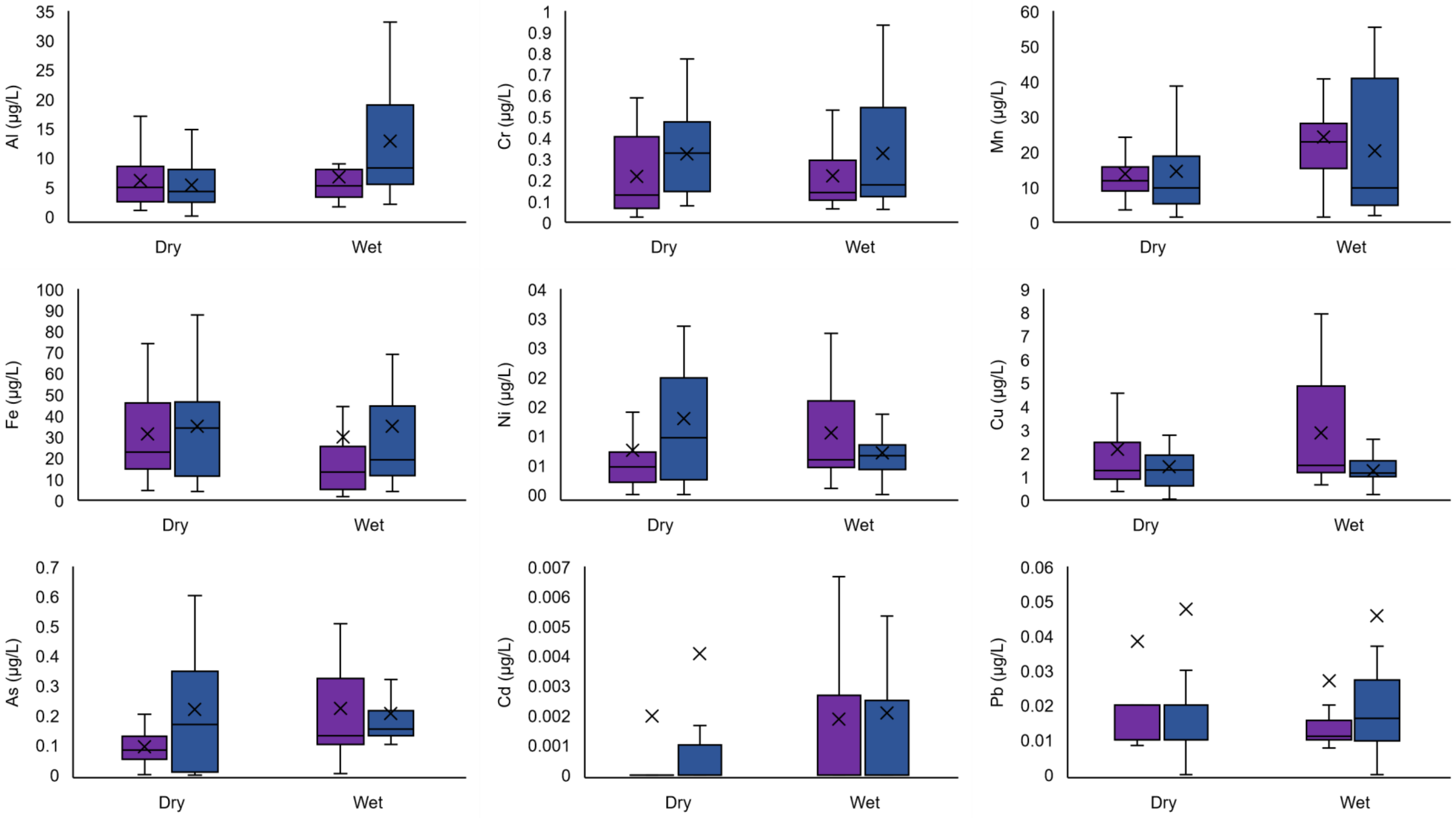

**Figure 3:** Trace metal concentrations in the water samples from the Jacaré-Pepira (purple) and Jacaré-Guaçú (blue) rivers, southeastern Brazil, during dry and wet seasons.

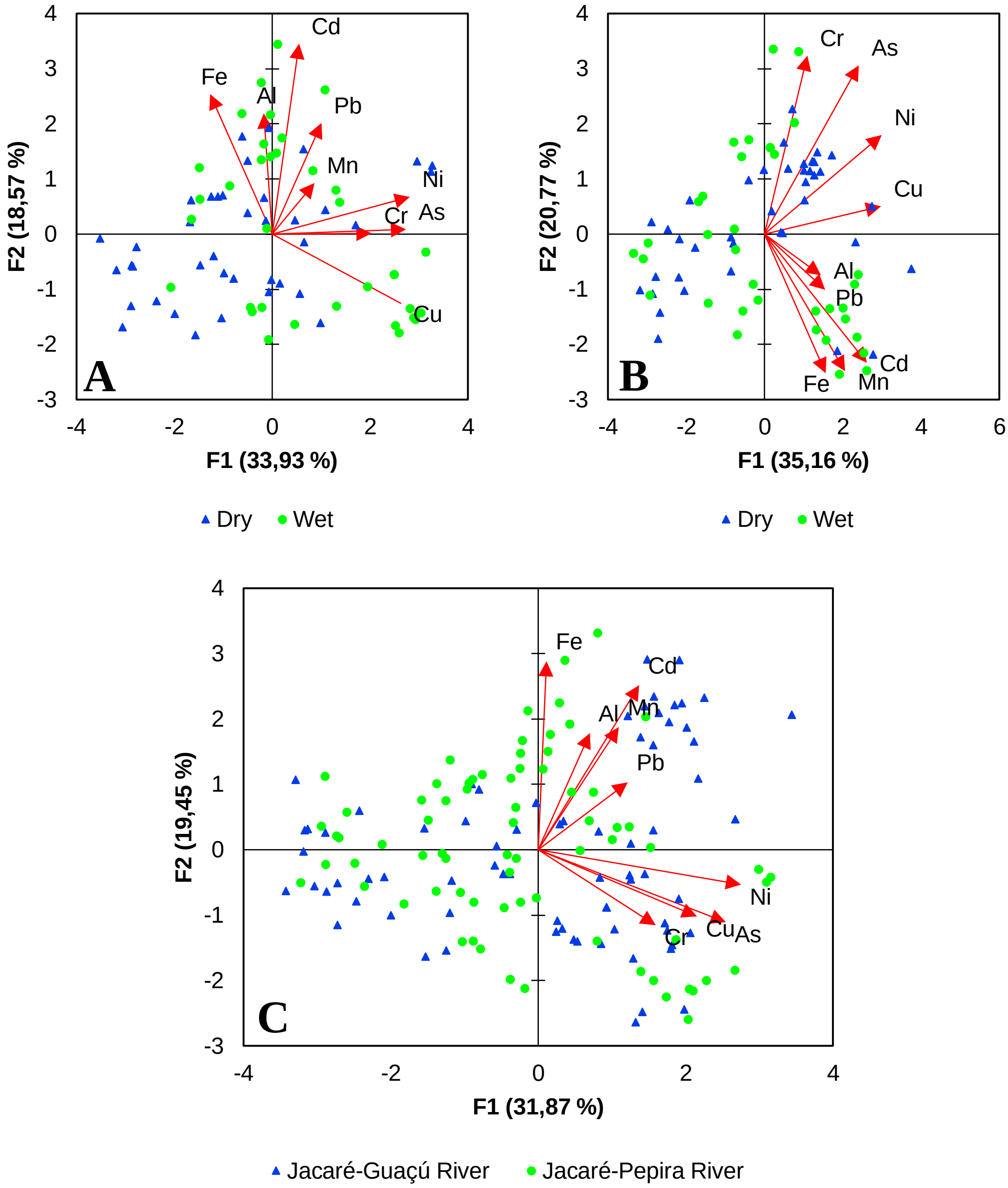

**Figure 4:** Principal Component Analysis (PCA) considering the trace metal concentrations distribution in water samples of the Jacaré-Pepira River (A), Jacaré-Guaçú River (B) during dry and wet seasons, and without considering the seasonality in both rivers (C).

**Table 3:** Differences in trace-metal concentrations in river water samples, considering wet and dry seasons, and between the two analyzed rivers, Jacaré-Pepira River and Jacaré-Guaçú River, southeastern Brazil.

| **Element** | **Dry/Wet Season** | | **Jacaré-Pepira/Jacaré-Guaçú** |
|---|---|---|---|
| | **Jacaré-Pepira** | **Jacaré-Guaçú** | |
| Al | n.s. | Wet | n.s. |
| Cr | n.s. | n.s. | Jacaré-Guaçú |
| Mn | Wet | n.s. | Jacaré-Guaçú |
| Fe | Dry | n.s. | n.s. |
| Ni | Wet | n.s. | n.s. |
| Cu | n.s. | n.s. | Jacaré-Pepira |
| As | Wet | n.s. | Jacaré-Guaçú |
| Cd | Dry | n.s. | n.s. |
| Pb | n.s. | n.s. | n.s. |

Mann-Whitney significance level: $p \leq 0.05$

n.s.: not statistically significant difference.

In case of significant difference, the samples exhibiting significantly higher metal concentrations is given in each case.

**Table 4**: Bioconcentration factors of the trace metals recovered in the musculature of *Hoplias malabaricus* (Bloch, 1794) compared to those recovered from the water samples of the Jacaré-Pepira and Jacaré-Guaçú rivers, southeastern Brazil.

| **Element** | **Fish musculature/River water** | |
|---|---|---|
| | **Jacaré-Pepira River** | **Jacaré-Guaçú River** |
| Al | 2.41 | 2.27 |
| Cr | 1.50 | 1.43 |
| Mn | 0.02 | 0.05 |
| Fe | 0.71 | 1.01 |
| Ni | 1.33 | 1.82 |
| Cu | 0.16 | 0.59 |
| As | 0.06 | 0.08 |
| Cd | 10.66 | 4.98 |
| Pb | 0.73 | 1.10 |

**Table 5:** Hazard Quotient (HQ) for trace-metal contamination in *Hoplias malabaricus* (Bloch, 1794) musculature from the Jacaré-Pepira and Jacaré-Guaçú rivers, southeastern Brazil.

| **Element** | **Hazard Quotient** | |
|---|---|---|
| | **Jacaré-Pepira River** | **Jacaré-Guaçú River** |
| Al | 0.856 | 1.116 |
| Mn | 0.021 | 0.049 |
| Cu | 0.044 | 0.088 |
| As | 0.034 | 0.064 |
| Cd | 0.045 | 0.034 |
| Pb | 0.053 | 0.112 |

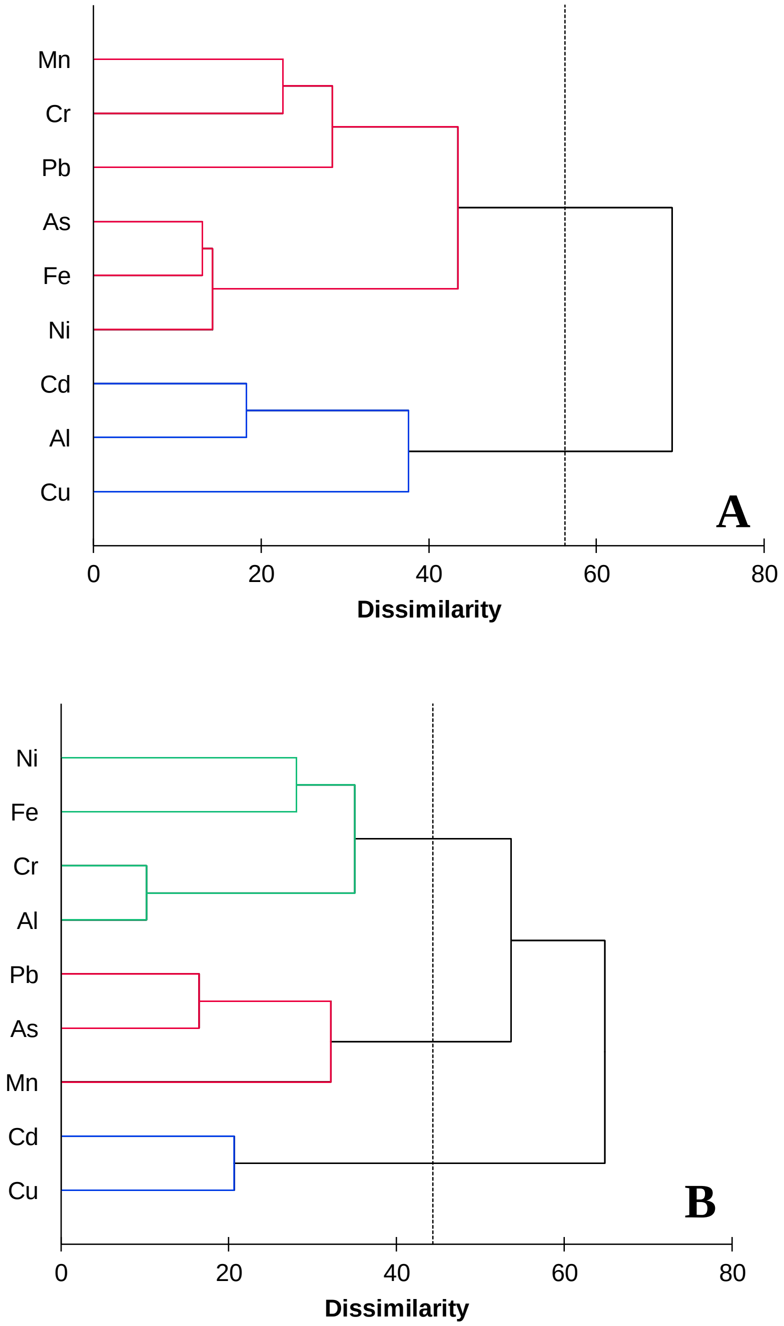

**Figure 5:** Agglomerative Hierarchical Cluster Analysis (AHC) of the trace metals in the musculature of *Hoplias malabaricus* (Bloch, 1794) from the Jacaré-Pepira (A) and Jacaré-Guaçú (B) rivers, southeastern Brazil.

## 4 DISCUSSION

Most metals tend to accumulate in non-edible fish parts such as the gills, liver, and intestines, which are key organs in the uptake and absorption routes of these compounds (Silva de Jesus et al., 2014; Sures and Siddall, 1999). In the muscle, which is one of the main edible organs, metals only exceed the maximum accepted limits for consumption when they have already exceeded in non-edible organs (Silva de Jesus et al., 2014). According to the maximum values for inorganic contaminants stipulated by the Brazilian National Health Surveillance Agency (ANVISA) for fish, chromium was the only element above limits, with concentrations up to 10 times higher than the accept by the current legislation, which is 0.1 mg/kg (Brasil, 2013). The aluminum, despite being also in high concentrations in the musculature of the analyzed specimens of *H. malabaricus*, does not have a minimum value stipulated for consumption by the Brazilian legislation.

Chromium is a transitional element found in several compounds in the Earth's crust, and it is essential for organisms to potentiate the action of insulin (Stoecker, 2004). In fish, the toxicity of Cr is high and depends on age, development, and other physiological variables (Aslam and Yousafzai, 2017) in addition to being pH dependent (Stoecker, 2004). Chromic acid is much more toxic to aquatic organisms than the basic one and is directly linked to damage to the external surface of the fish, behavioral changes, gill hypertrophy, swimming difficulties, and feeding difficulties, severely affecting the biochemistry and hematology of the fish (Ali et al., 2021; Aslam and Yousafzai, 2017; Stoecker, 2004). In humans, chromium toxicity varies according to the type of exposure, and it can cause oxidative stress, DNA damage, cell apoptosis, alterations in gene expression and even potentiate the onset of cancer (Stocker, 2004).

Regarding the Hazard Quotient, aluminum was the only element that obtained an index with a value above 1, and only for fish from the Jacaré-Guçú River, which indicates that there is a risk of contamination by human consumption of the musculature of *H. malabaricus* from this river. Aluminum is the third most abundant element in the earth's crust, is widely distributed in all environments, and is not considered essential for any organism (Yokel, 2004). Its source of natural origin can easily surpass sources of anthropogenic origin. In natural waters, for example, Al originates from the weathering of rocks and minerals. Its anthropogenic origin is primarily through mining and waste. Aluminum accumulation in fish is pH dependent and increases as the pH of the water decreases. Aluminum can be neurotoxic to animals and can also cause respiratory and reproduction problems for aquatic organisms (Gebara et al., 2021; Gemsemer, 2018; Yokel, 2004). In humans, although toxicity is rare, it can cause degenerative diseases of the nervous system, such as Alzheimer's disease (Linhart et al., 2020), and also of bones, such as osteomalacia (Yokel, 2004).

In the fish and waters samples from the Jacaré-Guaçú River, the concentrations of the elements Cr, Mn, Cu, and As were at significantly higher concentrations than in the waters and fish of the Jacaré-Pepira River. This result may reflect the gradient of the disturbance levels in both rivers. The Jacaré-Guaçú river is closer to an urban perimeter, receives domestic and industrial sewage, and its waters are already known to have high concentrations of metals (Rodríguez, 2001; Esguícero and Arcifa, 2011), while the Jacaré-Pepira river is farm from the urban perimeter, and is inserted within an area of environmental protection, which can guarantee a greater integrity of the environment. Regarding seasonality, metals as Al, Mn, Ni, and As had higher concentrations in the wet season. Carvalho et al. (2021), performing a spatio-temporal analysis of the concentration of trace metals in an urban supply river in northeastern Brazil, also found higher concentrations of metals in the wet season. The same pattern was also observed by Coimbra et al. (2018) who, when analyzing the metal concentrations in the musculature of *H. malabaricus* and in the water of a lake in Brazil, also found higher levels of metals in the wet season. This pattern possibly occurs because, as the water volume increases, pollutant compounds of anthropogenic origin are transported to the river where, together with those from natural origin, lead to an increase in their respective concentrations (Li and Zhang, 2010; Vega et al., 1998).

In the dynamics between metals in water-fish interactions, Cd is an element that drew attention for presenting the highest bioconcentration factor (BCF) value, with concentrations up to 10 times higher in fish than in water. Since *H. malabaricus* is a carnivorous fish, it may be indicative of cadmium biomagnification through the food chain, in addition to water uptake through the gills. Cadmium is normally accumulated in non-edible organs such as intestines, liver, kidneys, and gills, but in the case of larger fish, there may also be accumulation in the musculature (Ruangsonboon and Wongrat, 2006). The toxicity effects of cadmium on fish include nephrotoxic, induction of oxidative stress and immunotoxic problems (Kumar and Singh, 2010).

## 5 CONCLUSIONS

The results obtained in the present study provide a preliminary overview of the current status of trace metal contamination in the waters and *H. malabaricus* specimens analyzed from the Jacaré-Pepira and Jacaré-Guaçú rivers. In general, trace metal concentrations in the waters and fish of the Jacaré-Guaçú were higher than in the Jacaré-Pepira, which shows that the Jacaré-Guaçú is the one that suffers more anthropogenic action between the two rivers. In addition, some elements such as Al, Cr and Cd, due to its high concentrations, should receive some attention as they can offer risks to the health of fish, which can jeopardize the survival of their populations, and especially to humans who use these animals as a food source.

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# *Considerações Finais*

# 3 CONSIDERAÇÕES FINAIS

Os estudos apresentados mostram que os parasitos dos peixes, endêmicos ou introduzidos, da Bacia Hidrográfica do Tietê-Jacaré, nos rios Jacaré-Pepira e Jacaré-Guaçú, podem ser ferramentas úteis no diagnóstico da poluição aquática nesses rios, uma vez que responderam de maneira satisfatória às mudanças no ambiente, seja através da bioacumulação de metais-traço ou de respostas numéricas de abundância e prevalência frente a alterações no meio, como variáveis químicas da água e da sazonalidade.

Foi possível observar um gradiente de perturbação nos dois rios, onde o rio Jacaré-Pepira é o rio mais conservado, já que os níveis de metais em suas águas, seus peixes e parasitos são estatisticamente menores, e o rio Jacaré-Guaçú o mais poluído, possuindo níveis maiores de metais tanto no meio abiótico quanto no biótico.

Diante disso, salienta-se a importância do monitoramento ambiental dessas áreas, que são importantes mantenedoras da biodiversidade da região e oferecem aporte econômico através da pescaria artesanal, que por sua vez pode ser afetada caso os níveis de metais excedam os limites permitidos, trazendo prejuízos não só a biota aquática, mas especialmente a população ribeirinha, pescadores e moradores e a quem consome o pescado advindo dessas áreas.

# *Apêndice*

## ARTIGO 4.

# Patterns of distribution and accumulation of trace metals in *Hysterothylacium* sp. (Nematoda), *Phyllodistomum* sp. (Digenea) and in its fish host *Hoplias malabaricus,* from two neotropical rivers in southeastern Brazil*

Lucas A. R. Leite[a,*], Walter dos R. Pedreira-Filho[b], Rodney K. de Azevedo[c] e Vanessa D. Abdallah[c]

[a]Universidade Estadual Paulista (UNESP), Instituto de Biociências, Câmpus de Botucatu, Brazil.
[b]Fundação Jorge Duprat de Segurança e Medicina do Trabalho (FUNDACENTRO), Centro Técnico Nacional (CTN), Divisão de Agentes Químicos, São Paulo, Brazil.
[c]Centro universitário CESMAC, Programa de Pós-graduação em Análise de Sistemas Ambientais, Maceió, Brazil.

***Corresponding author:**
Lucas A. R. Leite
E-mail: leitearlucas@gmail.com

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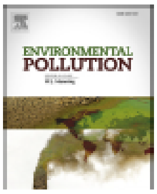

# Patterns of distribution and accumulation of trace metals in *Hysterothylacium* sp. (Nematoda), *Phyllodistomum* sp. (Digenea) and in its fish host *Hoplias malabaricus*, from two neotropical rivers in southeastern Brazil[☆]

Lucas Aparecido Rosa Leite [a, *], Walter dos Reis Pedreira Filho [b], Rodney Kozlowiski de Azevedo [c], Vanessa Doro Abdallah [c]

[a] *Universidade Estadual Paulista (UNESP), Instituto de Biociências, Câmpus de Botucatu, Brazil*
[b] *Fundação Jorge Duprat de Segurança e Medicina do Trabalho (FUNDACENTRO), Centro Técnico Nacional (CTN), Ministério da Economia (ME), São Paulo, Brazil*
[c] *Centro Universitário CESMAC, Programa de Pós-graduação em Análise de Sistemas Ambientais, Maceió, Brazil*

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ABSTRACT

Here we evaluated the potential for trace metal accumulation of two parasitic species, *Hysterothylacium* sp. (Nematoda) and *Phyllodistomum* sp. (Digenea), found parasitizing *Hoplias malabaricus*, a characiform fish also known as *trahira*, collected from two neotropical rivers, Jacaré-Pepira and Jacaré-Guaçú, in southeastern Brazil. Fish were collected between July 2017 and July 2019, totaling 90 fish specimens analyzed, 45 from each river. From fish, we take samples of three different tissues: muscle, intestine and liver. Along with the parasite samples taken from fish hosts, tissue samples were analyzed by an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for obtaining the trace metal (Al, Cr, Mn, Fe, Ni, Cu, As, Cd e Pb) concentrations. All elements were found in statistically higher concentrations in the parasites, both nematodes and digeneans, than in the host tissues, but in comparison, was observed that *Hysterothylacium* sp. had higher concentrations than those obtained in *Phyllodistomum* sp. We also found that uninfected fish had statistically higher concentrations of metals than infected ones. And in those who are infected, the size of the parasitic infrapopulations correlated negatively with the concentrations of trace metals obtained in the hosts tissues, that is, the concentrations in fish showed a tendency to decrease as the parasitic infrapopulations increased, or vice versa. In addition, our results show that the influence of the parasitic infrapopulations on metal concentrations in the fish host is not affected in cases of mono-infection or co-infection.

## 1. Introduction

Trace metals are natural components of the aquatic environments, but their levels have increased considerably in some areas due to domestic, industrial and agricultural activities, leading to the pollution of these environments (Merian et al., 2004; Otachi et al., 2014), compromising the water quality and endangering the biotic integrity of the ecosystem. Thus, several tools have been suggested in order to detect aquatic pollution in its different forms and at different levels, either through physicochemical analyzes of water, that primarily describe the concentrations of the respective pollutants, or through the use of bioindicators (Khan and Thulin, 1991; Lafferty and Kuris, 2005).

Bioindicators are organisms that reflect the biotic or abiotic state of an ecosystem and the impact produced on the environment through genetic, biochemical, physiological, morphological, ecological or behavioral changes (Parmar et al., 2016). Vidal-Martínez et al. (2010) divide bioindicators into two categories: 1) effect bioindicators, which are organisms used to detect environmental impacts through changes in their physiology, chemical, behavioral or numerical composition; and 2) accumulation bioindicators, which are organisms that efficiently absorb substances

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[☆] This paper has been recommended for acceptance by Prof. Wen-Xiong Wang.
[*] Corresponding author.
*E-mail address:* lar.leite@unesp.br (L.A. Rosa Leite).

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