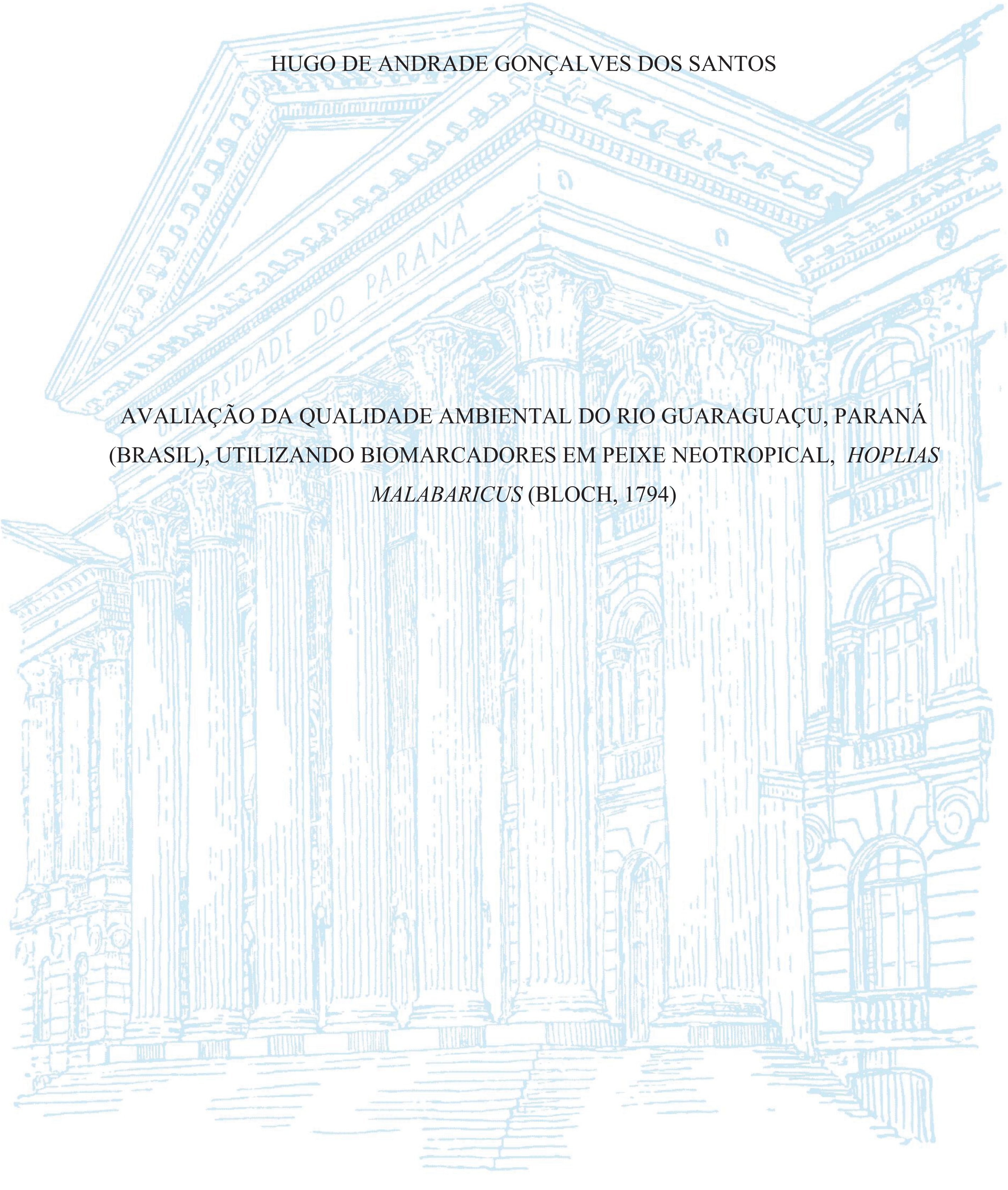
UNIVERSIDADE FEDERAL DO PARANÁ

HUGO DE ANDRADE GONÇALVES DOS SANTOS



AVALIAÇÃO DA QUALIDADE AMBIENTAL DO RIO GUARAGUAÇU, PARANÁ (BRASIL), UTILIZANDO BIOMARCADORES EM PEIXE NEOTROPICAL, *HOPLIAS MALABARICUS* (BLOCH, 1794)

CURITIBA
2023

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

Orientadora: Profa. Dra. Helena Cristina da Silva de Assis

Coorientador: Prof. Dr. Andre Andrian Padial

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação ECOLOGIA E CONSERVAÇÃO da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **HUGO DE ANDRADE GONÇALVES DOS SANTOS** intitulada: **AVALIAÇÃO DA QUALIDADE AMBIENTAL DO RIO GUARAGUAÇU, PARANÁ (BRASIL), UTILIZANDO BIOMARCADORES EM PEIXE NEOTROPICAL, HOPLIAS MALABARICUS (BLOCH, 1794)** , que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.
A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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Dedico este trabalho para minha mãe Denise e meu pai Carlos, que sempre apoiaram minhas decisões e me incentivaram a seguir o caminho que escolhesse. Quero também dedicar às pessoas especiais que não se encontram mais entre nós, mas guardo em minha memória todos os momentos bons que passei com elas e os seus ensinamentos, em memória dos meus avôs Afrânio e Armênio, minhas avós Eliza e Rosa, minha tia Maria, meu tio João, meu primo Pedro e meu tio avô Otheres. Dedico por último ao meu irmão de quatro patas que se foi durante o período do mestrado, meu companheiro de dezesseis anos, o gato "mais gato de todos os gatos", Félix III, que esteve comigo desde meus oito anos. Sinto saudades de todos.

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“AQUELE QUE AJUDA OS OUTROS SIMPLESMENTE PORQUE ISSO DEVE OU PRECISA SER FEITO, E PORQUE É A COISA CERTA A FAZER, É SEM DÚVIDA, UM VERDADEIRO SUPER-HERÓI. ” (STAN LEE)

RESUMO

A água é um recurso limitado e essencial para a vida que sofre contaminação por diversos xenobióticos oriundos de atividades antrópicas. Dentre os principais contaminantes encontrados em corpos hídricos, pode-se destacar os agrotóxicos e os elementos traço, que são caracterizados por possuírem alta persistência ambiental e capacidade de bioacumulação em organismos aquáticos, o que podem acarretar diversos problemas a saúde. O rio Guaraguaçu encontrado no bioma da Mata Atlântica, é o principal rio do litoral paranaense por sua biodiversidade única, importância econômica e abastecimento de água para os municípios de Matinhos, Pontal do Paraná e Paranaguá. No entanto, esse rio vem sofrendo ao longo dos anos com a contaminação de suas águas devido ao crescimento urbano e o lançamento de esgoto não tratado. Além disso, sofre influência de um aterro sanitário localizado próximo a sua região intermediária em um de seus afluentes, o rio Pery. Deste modo, o objetivo deste estudo foi avaliar a influência da ação antrópica na qualidade da água do Rio Guaraguaçu a partir das análises químicas da água e de biomarcadores em peixes da espécie *Hoplias malabaricus*. Os peixes foram coletados em três setores com gradientes ecológicos distintos ao longo do rio, sendo: S1- prístino; S2- impactado; S3- menos impactado. Amostras de água foram coletadas para análise da presença de elementos traço e agrotóxicos. Nos peixes, foram analisados biomarcadores bioquímicos, histopatológicos e de genotoxicidade. Músculo de peixe foi coletado para análise de bioacumulação de elementos traço. A partir da avaliação da qualidade ambiental do rio Guaraguaçu, os resultados encontrados mostraram que a atividade da acetilcolinesterase cerebral, diminuiu no setor 2 comparado aos setores 1 e 3, indicando a presença de compostos anticolinesterásicos na água. Brânquia e fígado foram os tecidos que mais apresentaram alterações, principalmente no setor 2, com aumento da atividade do sistema antioxidante. A lipoperoxidação aumentou tanto no setor 2 como no 3, caracterizando um dano celular. Biomarcadores histopatológicos mostraram diferentes lesões no fígado e brânquias dos peixes do setor 2. Para os biomarcadores de genotoxicidade, a presença de micronúcleo ocorreu em pelo menos um organismo de cada setor, indicando presença de substâncias mutagênicas na água. O presente trabalho buscou evidenciar a problemática atual da qualidade da água do Rio Guaraguaçu para criar um banco de dados de suma importância para o conhecimento tanto dos residentes com grau de vulnerabilidade social que necessitam do rio para sobrevivência e subsistência quanto para futuras tomadas de decisões por parte dos governantes.

Palavras-chaves: Ecotoxicologia; Sistema antioxidante; Modelo biológico; Genotoxicidade.

ABSTRACT

Water is a limited and essential resource for life that suffers contamination from various xenobiotics originating from anthropogenic activities. Among the main contaminants found in water bodies, pesticides and trace elements can be highlighted, which are characterized by their high environmental persistence and bioaccumulation capacity in aquatic organisms, leading to various health problems. The Guaraguaçu River, located in the Atlantic Forest biome, is the main river in the coastal region of Paraná, Brazil, due to its unique biodiversity, economic importance, and water supply to the municipalities of Matinhos, Pontal do Paraná, and Paranaguá. However, this river has been experiencing water contamination over the years due to urban growth and the discharge of untreated sewage. Additionally, it is influenced by a landfill located near its intermediate region in one of its tributaries, the Pery River. Therefore, the objective of this study was to evaluate the influence of anthropogenic activities on the water quality of the Guaraguaçu River through chemical analysis of water and biomarkers in *Hoplias malabaricus* fish. The fish were collected in three sectors with distinct ecological gradients along the river: Sector 1 - pristine; Sector 2 - impacted; Sector 3 - less impacted. Water samples were collected for analysis of trace elements and pesticides. Biochemical, histopathological, and genotoxicity biomarkers were analyzed in the fish. Fish muscle samples were collected for analysis of trace element bioaccumulation. The results of the Guaraguaçu River environmental quality assessment showed that the activity of cerebral acetylcholinesterase decreased in Sector 2 compared to Sectors 1 and 3, indicating the presence of anticholinesterase compounds in the water. Gill and liver tissues showed the most alterations, especially in Sector 2, with an increase in the activity of antioxidant system. Lipoperoxidation increased in both Sector 2 and 3, indicating cellular damage. Histopathological biomarkers revealed different lesions in the liver and gills of fish from Sector 2. For genotoxicity biomarkers, the presence of micronuclei occurred in at least one organism from each sector, indicating the presence of mutagenic substances in the water. The present study aimed to highlight the current issues regarding the water quality of the Guaraguaçu River, to create a highly important database for the knowledge of both residents with social vulnerability who rely on the river for survival and sustenance, as well as for future decision-making by policymakers.

Keywords: Ecotoxicology; Antioxidant system; Biological model; Genotoxicity

LISTA DE ABREVIATURAS E SIGLAS

ACh - Acetilcolina

AChE - Acetilcolinesterase

Al – Alumínio

ANOVA – Análise de Variância

APA – Área de Proteção Ambiental

As – Arsênio

ATP – Adenosina Trifosfato

BL – Blebbed

BN – Binucleus

CAT - Catalase

Cd - Cádmio

CDNB – 1-Cloro-2,4-Dinitrobenzeno

Cfa - Clima subtropical, com verão quente

CONAMA - Conselho Nacional do Meio Ambiente

Cr - Cromo

CTAF – Centro de Tecnologias Avançadas em Fluorescência UFPR

Cu - Cobre

CYP450 - Citocromo P450

DNA - Ácido Desoxirribonucleico

EEG – Estação Ecológica do Guaraguaçu

EC – Commission Regulation of the European Community (Regulamento da Comissão da Comunidade Europeia)

EROS - Espécies Reativas de Oxigênio

FAO – Food and Agriculture Organization (Organização das Nações Unidas para Alimentação e Agricultura)

Fe – Ferro

FOX – Ferrous Oxidation in Xylenol orange (Oxidação Ferrosa do laranja de Xilenol)

GPx – Glutationa Peroxidase

GR - Glutationa Redutase

GSH – Glutationa Reduzida

GSSG - Glutationa oxidada ou Glutationa dissulfeto

GST - Glutationa-S-Transferase

HCl – Ácido Clorídrico

H_2O - Água

H_2O_2 - Peróxido de Hidrogênio

Hg – Mercúrio

HNO_3 – Ácido Nítrico

IAP – Instituo Ambiental do Paraná

ICMBio – Instituto Chico Mendes de Conservação da Biodiversidade

ICP-OES – Espectrometria de Emissão Ótica com Plasma Indutivamente Acoplado

KNO_3 – Nitrato de Potássio

LAA – Laboratório de Análises Ambientais

LAMAQ – Laboratório Multiusuário de Análises Químicas

LASB – Laboraório de Análise e Síntese em Biodiversidade

LB – Lobed

LEC – Laboratório de Ecologia e Conservação

LPO - Lipoperoxidação

LQ – Limite de Quantificação

LTA – Laboratório de Toxicologia Ambiental

MC – Células Mucosas

MET – Metalotioneína

MG – Minas Gerais

Mn - Manganês

N – Focos Necróticos

$NaBH_4$ – Borohidreto de Sódio

NADPH - Nicotinamida Adenina Dinucleotídeo Fosfato

NaOH – Hidróxido de Sódio

ND – Não Detectado

Ni – Níquel

NT – Notched

O_2 – Oxigênio

O^{2-} - Radical Superóxido

OH^- - Radical Hidroxila

PAHs - Polycyclic Aromatic Hydrocarbons (Hidrocarbonetos Policíclicos Aromáticos -HPAs)

Pb - Chumbo

PCA1 – Eixo x

PCA2 – Eixo y

PCoA – Análises de Coordenadas Principais

PCoA1 – Eixo 1 (x)

PCoA2 – Eixo 2 (y)

PERMANOVA – Análise Multivariada de Permutação de Variância

pH – Potencial Hidrogeniônico

PL – Lamela Primária

PNRH - Política Nacional de Recursos Hídricos

POPs – Poluentes Orgânicos Persistentes

RDC – Resolução da Diretoria Colegiada

S1 – Setor ou Seção 1

S2 – Setor ou Seção 2

S3 – Setor ou Seção 3

SEM – Scanning Electron Microscope (Microscópio Eletrônico de Varredura -MEV)

SINGREH - Sistema Nacional de Gerenciamento de Recursos Hídricos

SisBio – Sistema de Autorização e Informação em Biodiversidade

SL – Lamela Secundária

SOD - Superóxido Dismutase

TECLAB – Tecnologia em Análises Laboratoriais

UFPR – Universidade Federal do Paraná

U.S. EPA – United States Environmental Protection Agency (Agência de Proteção Ambiental dos Estados Unidos)

U.S. Geological Survey - United States Geological Survey (Serviço Geológico dos Estados Unidos)

UTFPR – Universidade Tecnológica Federal do Paraná

V – Vaso Sanguíneo

VC – Vacuolated

Zn - Zinco

SUMÁRIO

1 APRESENTAÇÃO DA DISSERTAÇÃO

O presente trabalho contempla o trabalho de pesquisa do discente Hugo de Andrade Gonçalves dos Santos do Programa de Pós-Graduação em Ecologia e Conservação da Universidade Federal do Paraná (UFPR). Inicialmente, apresenta-se uma introdução geral afim de ingressar no assunto do trabalho e construir o cenário em que se encontra a presente pesquisa, as hipóteses a serem testadas, seguindo pelos objetivos geral e específicos que o nortearam. O presente trabalho apresentou resultados significativos que irá gerar uma publicação científica no formato de artigo, que será enviado para a revista Science of The Total Environment (STOTEN).

Esta dissertação está apresentada no formato de artigo, seguindo o modelo em língua inglesa, apresentando resumo, introdução, metodologia, resultados e discussão, além da conclusão. O trabalho intitulado como: “Biomonitoring Top Fish Predators using Biomarkers Unveil Human Impacts in a Coastal River from a World Heritage Site”, contempla a análise de biomarcadores bioquímicos, histopatológicos e de genotoxicidade em traíras (*H. malabaricus*), além da avaliação dos parâmetros físico-químicos da água, e análises químicas de agrotóxicos e elementos traço em água e em músculo de peixe, para se avaliar a qualidade da água do principal rio da costa paranaense, o Rio Guaraguaçu.

Por fim, apresenta-se as considerações finais abordando os resultados do trabalho de forma simplificada e unificada, com algumas recomendações para futuros projetos e trabalhos.

2. INTRODUÇÃO GERAL

Do total da superfície terrestre mais de 70% é recoberta por água, sendo aproximadamente 97% de água salgada, encontrada em oceanos e mares (Esteves, 1998). Dentre as reservas de água doce, mais de 2% são encontrados em geleiras, estabelecendo uma porcentagem menor que 1% de água disponível para consumo em rios, lagos e aquíferos (Esteves, 2011; Häder et al, 2020), demostrando a necessidade de se cuidar de um recurso tão limitado. Do total de água doce disponível no mundo, aproximadamente 12% se encontram em território brasileiro (Silva; Pompêo; Paiva, 2015; Elste et al., 2019). A importância da água, pode ser ainda evidenciada quando 60% do corpo humano adulto é composto por esse recurso e em alguns organismos essa composição pode alcançar 90%, sendo essencial para todos os organismos vivos (U.S. Geological Survey, 2019).

Logo, o desenvolvimento da população humana junto do crescimento exacerbado da população mundial, da expansão urbana e do modelo consumista/capitalista de viver, estão ligados diretamente ao uso da água de forma direta ou indireta como recurso essencial para diversos processos, entre as quais se destaca o abastecimento, geração de energia, irrigação na agricultura, produção, navegação e aquicultura (Moraes; Jordão, 2002; Tundisi; Tundisi, 2011). Contudo, desde o início do século XX, esse recurso tem enfrentado grande pressão e competição devido a conflitos de interesses entre os seres humanos, resultando em uma redução em sua qualidade para consumo e ameaçando a existência de organismos nos ecossistemas aquáticos. Isso é principalmente causado pelo lançamento de esgoto, contaminação por agrotóxicos e elementos traço, superexploração, invasão de espécies exóticas, mudanças climáticas, regulação de fluxo (como a construção de reservatórios) e mudanças no uso da terra, que levam à erosão do solo e ao assoreamento dos corpos hídricos (Silva; Pompêo; Paiva, 2015; Dudgeon, 2019).

Dentre os principais contaminantes dos corpos hídricos, pode-se destacar os agrotóxicos e elementos traço. A degradação da água por agrotóxicos é um dos principais causadores da contaminação dos recursos hídricos, junto com a contaminação por esgotos domésticos, já que são encontrados em águas superficiais e subterrâneos do mundo todo, em função do amplo uso em áreas agrícolas e urbanas (Armas et al., 2007; Klaassen; Watkins III, 2012; Sharma et al., 2019; Rathi; Kumar; Vo, 2021). Com seus altos riscos ambientais e à saúde pública, a legislação brasileira possui a lei nº 7.802/1989 voltada sobretudo para o ciclo de vida dos agrotóxicos, desde a produção até o descarte ecologicamente correto dos resíduos, além do registro, classificação, controle, inspeção e fiscalização desses produtos (Brasil, 1989). A preocupação com os agrotóxicos se deve a variedade de classes e composições que podem possuir. Os agrotóxicos podem variar de acordo com a espécie alvo a ser exterminada, desde fungicidas, rodencidas, inseticidas até herbicidas. No entanto, muitas vezes não só as espécies alvo são afetadas assim como todo o meio ambiente circundante. A composição dessas substâncias também pode variar bastante desde organoclorados, organofosforados, carbamatos e piretróides, tornando-se uma das principais causas de contaminação ambiental (Sharma et al., 2019; Souza et al., 2020).

A concentração exacerbada dos elementos traço no meio ambiente, está relacionado principalmente a atividades do ser humano, como no uso em tintas anti-incrustantes utilizadas em embarcações, despejo de resíduos pelas indústrias, refinarias de petróleo, processos de clareamento de metais, fabricação de plásticos e carvão ativado, encontrados em agrotóxicos, na queima de combustíveis, mineração, descarte incorreto de resíduos sólidos, utilizados em baterias e até em aditivos alimentares como é o caso do manganês (Pereira, 2004; Esteves, 2011). Considerados na sua maioria elementos com grande poder carcinogênico, mutagênico, teratogênico, nefrotóxico, imunotóxico, neurotóxico, com capacidade de desestruturar proteínas enzimáticas (Al-Sabti et al., 1994; Moraes; Jordão, 2002; Klaassen; Liu; Diwan, 2009; Häder et al., 2020; Nordberg; Nordberg, 2022).

Por isso, o Brasil possui em sua legislação normas, resoluções, regulamentações e leis voltadas para avalição da qualidade da água, controle dos efluentes liberados nos corpos hídricos, controle das atividades potencialmente poluidoras e criação de instituições voltadas para segurança hídrica, que mesmo sendo deficientes e necessitem de aperfeiçoamentos, são capazes de determinar os níveis de poluição aceitáveis de determinados sistemas, onde o uso de suas águas são indispensáveis para atividades humanas (Pereira, 2004; Häder et al., 2020). A resolução CONAMA nº 357/2005 tem por finalidade classificar os corpos de água e instaurar diretrizes ambientais para o seu enquadramento, como também estabelecer condições e padrões de lançamento de efluentes. O artigo 2º desta resolução, nos parágrafos XXI e XXII, estabelece que ensaios ecotoxicológicos são determinantes para análise do efeito deletério de agentes físicos ou químicos a diversos organismos aquáticos com intuito de avaliar o potencial de risco à saúde humana (CONAMA, 2005).

Para complementar e alterar a resolução 357/05 foi criada a resolução CONAMA 430/2011, que dispõe sobre as condições, parâmetros, padrões e diretrizes para controle do lançamento de efluentes em corpos hídricos (CONAMA, 2011). Além das resoluções CONAMA, em 1997 foi criado a lei nº 9433 que institui a Política Nacional de Recursos Hídricos (PNRH), junto da criação do Sistema Nacional de Gerenciamento de Recursos

Hídricos (SINGREH). No artigo 1º dessa lei nos parágrafos I e II, a importância e a preocupação com a água foram evidenciadas, ao caracterizar esse recurso como um bem de domínio público, limitado e dotado de valor econômico (Brasil, 1997).

No entanto, no Brasil a legislação voltada para a ecotoxicidade de lançamento de fontes poluidoras é pouco exigente (Magalhães; Ferrão Filho, 2008), mesmo tendo estudos na área de ecotoxicologia que já avaliam os efeitos que determinados contaminantes podem causar em organismos vivos em diferentes níveis de organização (Castro et al., 2014; Cavalcanti et al., 2016; Dalzochio et al., 2016; Pereira et al., 2020). Com a falta de legislações mais abrangentes, diversos rios brasileiros importantes para abastecimento, lazer e subsistência correm o risco de degradação, como o rio Guaraguaçu, importante rio da planície litorânea do Paraná devido a sua rica biodiversidade e seus serviços ambientais (Contente; Stefanoni; Spach, 2011; Elste et al. 2019; Cavallini; Reis; Tiepolo, 2020).

2.1. RIO GUARAGUAÇU

O litoral do Paraná (Brasil) que ocupa uma área de cerca de 6.058 km^2, é caracterizado pela presença do bioma da Mata Atlântica considerado *hotspot* da biodiversidade. As características morfológicas e de relevo da região é determinada pela presença da Serra do Mar e a planície litorânea (Torres, 2019), logo a presença de chuvas orográficas na região litorânea é comum (IAT, 2006). O clima da região é subtropical úmido com uma precipitação média anual de 2.500 mm com um padrão claro de chuva sazonal (Lana et al. 2001; Abreu-Mota et al., 2014), invernos secos com precipitações de até 60 mm e verão chuvoso podendo ultrapassar 1000 mm de precipitação (Silva, 2008; Vitule, 2008).

No litoral paranaense existem duas bacias hidrográficas, a bacia da baía de Guaratuba e da baía de Paranaguá, que são subdivididas em várias sub-bacias e fazem parte da bacia do Atlântico Sul (Staszczak; Rocha, 2018). Um dos principais rios tributários da bacia hidrográfica da baía de Paranaguá encontrado em sua região sul, é o rio Guaraguaçu (25º45’W e 48º35’S”) que possui padrão meandrante característico de rios de baixa energia, percorrendo a planície litorânea e envolto por floresta ombrófila densa de terras baixas, abrangendo assim os municípios do Pontal de Paraná, Paranaguá e Matinhos (IAT, 2006; Cavallini, 2018; Elste et al., 2019; Torres, 2019; Cavallini; Reis; Tiepolo, 2020). Caracteriza-se por ser um rio com grande influência também de marés, tendo seu vazamento em direção ao mar durante a maré baixa, com um certo período de estagnação e sofrendo posteriormente refluxo durante a enchente da maré (IAT, 2006).

O Rio Guaraguaçu possui importância ambiental, além de possuir relevância para os municípios do litoral do Paraná, provendo o abastecimento de água para os municípios de Pontal do Paraná, Matinhos e Paranaguá (Silva, 2008; Elste et al, 2019), assim como uma área utilizada para prática de atividades e lazer, como a pesca que pode ser esportiva ou de subsistência para os moradores da região (Reis et al., 2015). Entretanto, o aumento das contaminações e degradações ao longo dos trechos do rio Guaraguaçu ameaçam os usos múltiplos desse rio (Fig. 1).

Fig. 1. Possíveis fontes de contaminação dos corpos hídricos, como por exemplo o Rio Guaraguaçu.

Nessa região, há pouca proteção das margens e há presença de atividades de agricultura. Mesmo sendo considerada em sua maioria de origem familiar/subsistência, possuindo como principais cultivos a banana, mandioca, o feijão, o milho e o arroz (ZEE, 2016), existe a possibilidade da utilização de agrotóxicos causando riscos de contaminantes nos afluentes do rio Guaraguaçu, e do maior escoamento de contaminantes por águas pluviais. Além disso, a presença da mineradora Nova Prata na rodovia Alexandra Matinhos (PR-508) voltada para materiais relacionados a construção civil, como areia, e da presença constante de embarcações no rio e tráfego de carros pela rodovia PR-407, pode aumentar a contaminação por combustíveis, óleos, graxas e outros fluidos (IAT, 2006; Reis et al., 2015; ZEE, 2016; Cavallini, 2018; Araújo; Vitule; Padial, 2021).

No entanto, a contaminação ocorre principalmente devido ao lançamento de efluentes doméstico e o escoamento de contaminantes para um de seus principais afluentes (cujo canal foi retificado para escoamento), o rio Pery, a partir de um aterro sanitário encontrado no município do Pontal do Paraná (Singo; Araújo-Ramos; Rocha, 2020). Tal fenômeno é principalmente observado após o verão, quando a região recebe milhares de turistas, sobrecarregando os sistemas de drenagem e tratamento de esgoto, caracterizando a contaminação ao longo do rio como heterogênea (Elste et al, 2019). O gradiente de impacto antrópico do rio Guaraguaçu, desde sua nascente mais preservada, passando por uma área a jusante de maior ação antrópica onde se encontra o rio Pery, até sua foz caracterizada pela transição para um ambiente estuarino, assim como sua variação sazonal, já foi descrito anteriormente e tem relação com a diversidade de plantas aquáticas, ocorrência de espécies invasoras e impactos ecológicos como homogeneização biótica (Araújo; Vitule; Padial, 2021; Sato; Costa; Padial, 2021; Galvanese et al, 2022). Logo, a utilização do biomonitoramento se torna uma ferramenta essencial ao avaliar a qualidade ambiental através da análise dos possíveis efeitos de contaminantes na saúde dos organismos, especificamente peixes ao se tratar do presente trabalho.

2.2. AVALIAÇÃO DE QUALIDADE AMBIENTAL/MODELO BIOLÓGICO

A escolha do modelo biológico na avaliação da qualidade ambiental depende de inúmeras características inerentes aos organismos. Dentre os principais modelos biológicos utilizados, destacam-se: as microalgas, crustáceos, peixes, invertebrados bentônicos, bivalves, poliquetas (López-Doval; Barata; Díez, 2015) e até girinos de determinadas espécies de sapo (Fernandes et al., 2021). Os organismos devem seguir pelo menos alguns dos requisitos seguintes para serem utilizados no biomonitoramento: abundantes e com ampla distribuição; possuírem relevante representação ecológica; conhecimento prévio de sua biologia, fisiologia e hábitos alimentares; possuir importância comercial; fácil cultivo e serem nativos (Magalhães; Ferrão Filho, 2008). Apesar de todas as possibilidades possíveis para uso no monitoramento ambiental, a escolha do organismo será dependente da área de estudo e dos objetivos do trabalho a ser realizado (Resh, 2008).

Os peixes são organismos essenciais para os ambientes aquáticos circundantes para avaliar a condição e funcionamento desses ecossistemas, entretanto, a presença de xenobióticos na água pode causar efeitos adversos no metabolismo desses organismos (Burkina; Zlabek; Zamaratskaia, 2015; Ballesteros et al., 2017). No Brasil, algumas espécies de peixes utilizadas em trabalhos para biomonitoramento, são o *Geophagus brasilienses*

(Oliveira et al., 2019), *Hoplias malabaricus* (Pantaleão et al., 2006) e *Rhamdia quelen* (Souza-Bastos et al., 2017).

O *H. malabaricus* (Bloch, 1794) (Fig. 2), popularmente conhecido como traíra, é caracterizado como predador de topo de cadeia, sendo comumente utilizado em trabalhos para análise de biomagnificação de certos contaminantes de rios e reservatórios por sua capacidade de indicar respostas de efeitos crônicos e de bioacumulação (Castro et al., 2014; Mela et al., 2014). A espécie é carnívora com uma estratégia de predação por emboscada ("senta e espera") não sendo considerada uma boa nadadora (Chu-Koo; Pérez, 2007), com a sua fase adulta principalmente relacionada na maioria das vezes a ambientes limnéticos (lênticos), em águas rasas e perto de vegetações marginais ou submersas (Carvalho; Fernandes; Moreira, 2002; Reis et al., 2017; Paula; Risso; Martinez, 2021; Leite et al., 2021).

Fig. 2. *Hoplias malabaricus*.

Apresenta boca com dentes adaptados para segurar e engolir presas inteiras, possuindo hábitos alimentares generalistas que são dependentes de sua fase de vida, considerada estritamente insetívora durante sua fase juvenil e na fase adulta preferencialmente piscívora (Moraes; Barbola, 1995; Silva, 2008; Pessoa et al., 2013; Montenegro et al., 2013). Conserva grande amplitude ecológica ao mostrar resistência a diferentes perturbações, possuindo uma das maiores tolerâncias à privação alimentar registradas ao conseguir manter suas taxas metabólicas mesmo após um longo período de jejum, aguentam altas temperaturas da água, sobrevivem em ambientes com baixos níveis de oxigênio dissolvido (Barbieri, 1989; Costa et al., 2007; Cruz-Esquivel; Marrugo-Negrete, 2022), além de conseguirem viver em ambientes aquáticos com pH ácido, como encontrado em riachos no Peru (Chu-Koo; Pérez, 2007).

O ciclo reprodutivo da traíra ocorre tanto em ambientes de água limpa quanto em ambientes degradados e acontece quase que exclusivamente nos períodos de primavera (setembro-outubro) e verão, apesar de possuir um desenvolvimento gonodal assíncrono caracterizado pelo parcelamento da desova, considerado como uma adaptação da espécie para evitar a competição pela busca de alimento e local de desova (Barbieri, 1989; Gomes et al., 2015; Melo et al., 2017). O tamanho médio para maturação sexual se encontra por volta dos 16 cm de comprimento (Moraes; Barbola, 1995), entretanto sabe-se que a partir dos 23 cm 100 % das fêmeas já se tornam aptas à reprodução (Barbieri, 1989), conhecido por ser um peixe capaz de ultrapassar os 40 cm de comprimento (Balboni; Colautti; Baigún, 2011) chegando até no máximo 65 cm (Chu-Koo; Pérez, 2007) e pesar mais de 1 kg (Carvalho et al., 2022). Destaca-se que apesar de ser uma espécie com hábito alimentar voraz (Monteiro; Rantin; Kalinin, 2013), possui comportamento de cuidado parental com suas crias (Gomes et al., 2015; Melo et al., 2017).

Os estudos toxicológicos com traíra no geral, possuem como objetivo principal analisar os impactos de contaminantes aos ambientes aquáticos in situ (Lozano et al., 2013; Carvalho; Fernandes; Moreira, 2002), ou são controladas em laboratório para análise das respostas a determinadas concentrações de contaminantes já que a mesma possui habilidade de se adaptar a condições experimentais (Costa et al., 2007; Silva de Assis et al., 2013; Paula; Risso; Martinez, 2021). A biologia reprodutiva, comportamental, morfológica e fisiológica de *H. malabaricus* também já foi foco de diversos estudos a fim de detalhar tal espécie (Barbieri, 1989; Domanico; Delfino; Freyre, 1993; Marques; Gurgel; Lucena, 2001; Carvalho; Fernandes; Moreira, 2002; Chu-Koo; Pérez, 2007; Balboni; Colautti; Baigún, 2011; Lima et al., 2017), assim como já foi evidenciado a relevância da espécie para a alimentação humana (Castro et al., 2014).

Logo, possuindo a maioria das características para ser utilizado como modelo biológico a traíra se tornou uma ótima espécie para avaliação da qualidade ambiental de corpos hídricos. No Rio Guaraguaçu uma das espécies nativas de peixe com ampla distribuição durante toda sua extensão é o *H. malabaricus* (Silva, 2008; Occhi, 2020; Carvalho et al., 2022), mostrando a possibilidade e relevância de analisar biomarcadores nessa espécie.

2.3. BIOMARCADORES DE CONTAMINAÇÃO AMBIENTAL

O uso de biomarcadores pode ser instrumento eficaz em várias etapas do biomonitoramento da qualidade ambiental de ecossistemas aquáticos (Van der Oost; Beyer; Vermeulen, 2003). Existem diversos biomarcadores que se destacam em bioensaios e refletem a saúde dos organismos e podem indicar a presença, o efeito e em alguns casos até o grau de contaminação do ambiente, como: os biomarcadores bioquímicos, de genotoxicidade, hematológicos, fisiológicos e histopatológicos. Dentre os biomarcadores mais utilizados no monitoramento ambiental, estão os biomarcadores bioquímicos. O uso de biomarcadores bioquímicos podem corroborar para detecção de alterações ambientais de forma precoce. Esses biomarcadores permitem a identificação de efeitos no organismo em níveis mais basais de organização (celular, bioquímico, molecular), reforçando a compreensão de repostas mais rápidas, antes que ocorra a morte do indivíduo (Van der Oost; Beyer; Vermeulen, 2003; Friberg et al., 2011; López-Doval; Barata; Díez, 2015).

Tratando-se de biomarcadores bioquímicos para avaliação de neurotoxicidade, destaca-se a análise da atividade enzimática da acetilcolinesterase (AChE). Os organofosforados e carbamatos são relatados como inibidores eficazes da AChE (Cavalcanti et al., 2016), se ligando ao sítio ativo da enzima e inibindo sua ação. Logo, a função de hidrolisar o neurotransmissor acetilcolina (ACh) em colina e ácido acético é interrompida, fazendo que ocorra acumulação da acetilcolina na fenda sináptica, atuando assim sobre o sistema nervoso parassimpático, ocasionando a hiperexcitação colinérgica, danos irreversíveis e morte do indivíduo (Van der Oost; Beyer; Vermeulen, 2003; Cavalcanti et al., 2016). A diminuição da atividade da acetilcolinesterase pode afetar a coordenação motora dos organismos (Burkina; Zlabek; Zamaratskaia, 2015), podendo interferir em comportamentos, locomoção e na fisiologia dos organismos.

Enzimas dos sistemas de biotransformação podem corroborar para a desintoxicação/transformação de xenobióticos e seus metabólitos, e o sistema antioxidante pode atuar no combate de radicais livres para evitar que ocorram danos oxidativos nos diferentes tecidos analisados. Como a metabolização de xenobióticos no organismo ocorre principalmente no fígado, é um dos órgãos mais utilizados para análises destes biomarcadores de biotransformação.

O sistema de biotransformação nos organismos, como nos peixes, ocorre em duas fases. As reações da fase I estão relacionadas com a adição de um grupo funcional polar (–OH, –NH2, –SH ou –COOH) em compostos lipofílicos, incluindo reações de oxidação, redução e hidrólises, caracterizando a fase inicial de desintoxicação e excreção (Kroon; Streten; Harries, 2017; Klaassen; Watkins III, 2012). Os principais grupos de enzimas envolvidas nas reações da fase de biotransformação são as pertencentes ao grupo Citocromo P450 (CYP450). Na fase II da biotransformação ocorre a conjugação do xenobiótico ou seus metabólitos com um ligante endógeno. A glutationa-S-transferase (GST), enzima da fase II, catalisa a conjugação de metabólitos da fase I com a forma reduzida de glutationa (GSH), facilitando assim a excreção de produtos químicos ao adicionar mais grupos polares tornando-os mais hidrofílicos (Van der Oost; Beyer; Vermeulen, 2003; Burkina; Zlabek; Zamaratskaia, 2015; Kroon; Streten; Harries, 2017) intensificando a excreção de xenobióticos.

Assim como o sistema de biotransformação, o sistema antioxidante também é muito importante, pois ajuda para a redução de estresses oxidativos ocasionados por contaminantes. O estresse oxidativo caracteriza-se por ser um desequilíbrio entre a geração de compostos oxidantes, como o radical superóxido (O^{2-}), peróxido de hidrogênio (H_2O_2) e o radical hidroxila (OH^-) que são espécies reativas de oxigênio (EROS) e a atuação do sistema antioxidante, o que favorece a ocorrência de lesões oxidativas em macromoléculas e estruturas celulares, podendo resultar em morte celular (Winston; Di Giulio, 1991; Gutteridge, 1993; Barbosa et al., 2010). Com a presença de contaminantes no ambiente, os organismos aumentam a produção de espécies reativas de oxigênio, sendo a mitocôndria uma das principais fontes endógenas geradoras de EROS (Rover Júnior et al., 2001).

Desta forma, enzimas como a superóxido dismutase (SOD), catalase (CAT), encontrada em todas as células aeróbicas e em altos níveis no fígado e rim dos organismos, e glutationa peroxidase (GPx) são ativadas e ajudam na defesa antioxidante (Fig. 3) no organismo (Barbosa et al., 2010; Fernandes et al., 2021). A SOD, uma metaloenzima que pode ser encontrada no citosol, sendo dependente de cobre (Cu^{2+}) e zinco (Zn^{2+}) (SOD-Cu/Zn), e na mitocôndria necessitando do manganês como cofator (SOD-Mn) (Meister; Anderson, 1983; Yadav; Trivedi, 2009; Barbosa et al., 2010), catalisa o radical superóxido em peróxido de hidrogênio (Mccords; Fridovich, 1969) que posteriormente será degradado pela CAT em água (H_2O) e oxigênio (Pastorino et al., 2021). A GPx pode atuar na catalização de peróxido de hidrogênio, corroborando com a atividade da CAT, além de contribuir na redução de outros peróxidos em seus álcoois correspondentes (Mills, 1957; Mannervick, 1985). A GPx durante o processo de catalização de peróxidos, emprega a glutationa reduzida (GSH) como cofator, gerando glutationa oxidada ou glutationa dissulfeto (GSSG) como produto, sendo necessário a ação da glutationa redutase (GR) para a regeneração de GSH, a partir de GSSG, na presença de nicotinamida adenina dinucleotídeo fosfato (NADPH) (Meister;

Anderson, 1983; Huber; Almeida, 2008). Logo, tais enzimas, são importantes para evitar que danos oxidativos ocorram, sendo especialmente importante na proteção da membrana celular contra a lipoperoxidação (LPO) (Dalzochio et al., 2016; Kroon; Streten; Harries, 2017).

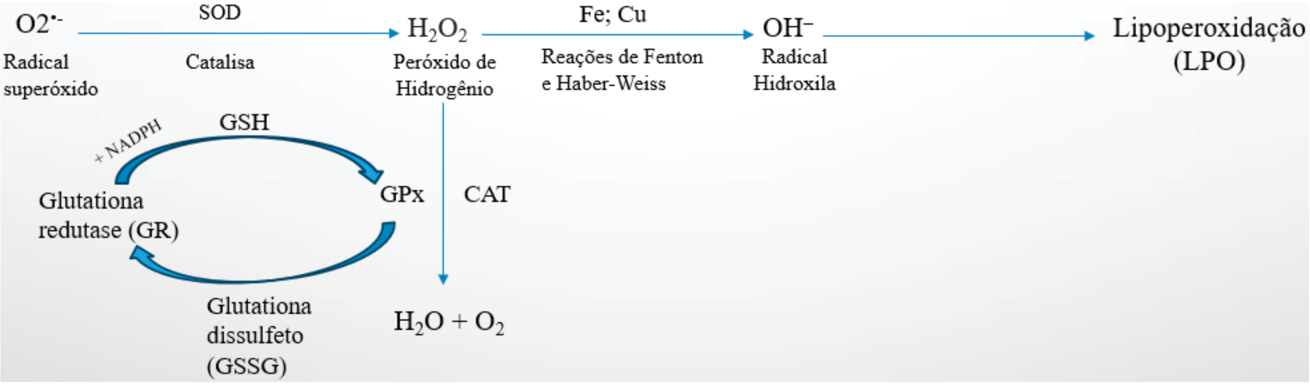

Fig. 3. Sistema antioxidante. Caso a GPx e CAT não catalisem o peróxido de hidrogênio, a partir da presença de ferro e cobre, pode ocorrer Reações de Fenton e Haber-Weiss, produzindo radical hidroxila, o qual, não possui enzima conhecida capaz de catalisá-lo, logo ocorre lipoperoxidação devido ao acúmulo de radical hidroxila.

Além do uso de atividades enzimáticas como biomarcadores bioquímicos, também é comumente utilizada a metalotioneína (MET) como biomarcador, principalmente para controle da concentração de elementos traço no organismo da maioria dos grupos de animais (El-Khayat et al., 2020). A metalotioneína é uma proteína não enzimática que possui baixo peso molecular, alto teor de cisteína e ausente de aminoácidos aromáticos, sintetizada primariamente no fígado e rins, sua produção é dependente da disponibilidade de minerais como zinco e selênio no organismo (Thirumoorthy et al., 2011). Por apresentar grupos tiol (–SH) dos resíduos de cisteína permitindo que a MET se ligue a metais pesados específicos, essa proteína possui propriedades de desintoxicação de elementos traço não essenciais como mercúrio e cádmio, além de trabalhar na homeostase de elementos essenciais como cobre e manganês. Ademais, a metalotioneína atua como antioxidante contra espécies reativas de oxigênio, protegendo contra danos no DNA e estresse oxidativo (Amiard et al., 2006; Atli; Canli, 2008; Fabrin et al., 2018; Nordberg; Nordberg, 2022).

Outros biomarcadores são comumente utilizados para complementar as respostas adquiridas com a avaliação dos biomarcadores bioquímicos e assim ilustrar os inúmeros danos causados pelos xenobióticos em diferentes níveis de organização no organismo, como os biomarcadores de genotoxicidade, através da análise de Micronúcleo Písceo por exemplo, e os biomarcadores histopatológicos.

Dentre os órgãos mais utilizados em análises histopatológicas, assim como na análise bioquímica, pode-se destacar o fígado, principal órgão para biotransformação de xenobióticos, e a brânquia. A análise de brânquia em peixes é amplamente utilizada por se tratar de um órgão com várias funções, incluindo troca gasosa, regulação iônica e excreção de metabólitos (Hedayati, 2018).

Biomarcadores de genotoxicidade são essenciais para verificação de danos/modificações no DNA, do potencial carcinogênico de contaminantes e assim acrescentar informações para alcançar um melhor direcionamento dos estressores existentes em determinados ambientes aquáticos (Palhares; Grisolia, 2002; Yadav; Trivedi, 2009). Dentre os testes e métodos para avaliação genotóxica em organismos, pode-se destacar o ensaio de Micronúcleo Písceo, bastante utilizado com peixes para avaliação na ocorrência de mutagênese e consequentemente o surgimento de micronúcleos nas células desses organismos (Al-Sabti; Metcalfe, 1995; Obiakor; Okonkwo; Ezeonyejiaku, 2012; Oliveira; Valdes, 2019).

Os micronúcleos presentes nos eritrócitos dos peixes, são corpos contendo cromatina citoplasmática formados quando fragmentos cromossômicos sem centrômero ou cromossomos se retardam durante a anáfase e não se incorporam aos núcleos das células filhas durante a divisão celular (Al-Sabti; Metcalfe, 1995; Pantaleão et al., 2006). Os micronúcleos são identificados facilmente como resultado de atividade de quebra cromossômica (clastogênica) pelos contaminantes (Palhares; Grisolia, 2002; Ali; El-Shehawi; Seehy, 2008; Martins; Paz; Brentano, 2010).

Logo, utilizar inúmeros biomarcadores com diferentes objetivos para análise, gera uma ampla perspectiva sobre a qualidade ambiental ao fornecer dados baseados nos efeitos integrados de variados estressores ambientais na saúde dos organismos, populações, comunidades e ecossistema como um todo, tornando essa estratégia amplamente utilizada em pesquisas avançadas e planos de gestão ambiental eficazes (Georgieva et al., 2021). Essa prática de uso de multibiomarcadores, é considerada vantajosa por permitir ações corretivas em áreas impactadas, por permitir ações preventivas para a conservação ambiental e criar informações capazes de alertar toda a população humana que pode estar sendo afetada por diversos contaminantes naquele ambiente (Burkina; Zlabek; Zamaratskaia, 2015; Kroon; Streten; Harries, 2017; Salgado et al., 2021).

Portanto, essa dissertação busca contribuir com o melhor entendimento da qualidade da água do rio Guaraguaçu e assim criar uma base de dados capaz de fomentar a criação de novas leis e tomadas de decisões por parte dos governantes, além de evidenciar a problemática do rio para a população ribeirinha que usa o rio para subsistência.

2.4. HIPÓTESES

As hipóteses preditivas seriam que o setor que sofre com uma ação antrópica maior, sofrerá mais alterações dos biomarcadores e que o peixe *Hoplias malabaricus* seria um modelo biológico ideal para análise de forma complementar de todos os biomarcadores, conseguindo diferenciar a qualidade ambiental de cada setor.

2.5. OBJETIVOS

2.5.1. GERAL

Avaliar a influência da ação antrópica e ambiental na qualidade da água utilizando análises químicas e biomarcadores de contaminação ambiental em peixe predador de topo de cadeia trófica *H. malabaricus*.

2.5.2. ESPECÍFICOS

Avaliar os parâmetros físico-químicos da água para melhor representar e interpretar as possíveis respostas dos biomarcadores;

Quantificar a concentração de diferentes tipos de agrotóxicos na água e determinados elementos traço na água e biota, como alumínio (Al), arsênio (As), cádmio (Cd), cobre (Cu), cromo (Cr), chumbo (Pb), ferro (Fe), manganês (Mn), níquel (Ni), mercúrio (Hg) e zinco (Zn) para compreender as interferências antrópicas existentes no rio Guaraguaçu e como isso pode interferir na vida dos peixes e consequentemente na vida da população que depende do rio para sobrevivência e subsistência;

Evidenciar a influência do rio Pery, único afluente da margem direita, a partir da análise dos biomarcadores e dos parâmetros da água, sobre o rio Guaraguaçu para avaliar a possibilidade da formação de uma divisão de setores com diferentes gradientes ecológicos.

3. Biomonitoring Top Fish Predators using Biomarkers Unveil Human Impacts in a Coastal River from a World Heritage Site’

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3.1. HIGHLIGHTS:

- Fe, Al and Mn were detected in water.

- Presence of blood genotoxicity in impacted and non-impacted sectors.

- Biochemical and histopathological biomarkers alterations in impacted sectors.

- Essential trace elements were detected in fish tissue.

3.2. ABSTRACT

The use of biomarkers in fish for biomonitoring is a valuable approach to reveal effects of human impacts on biota health. Top predator fish are effective models for monitoring human activities' impacts on aquatic ecosystems. The Guaraguaçu River, the largest river-system on coastal region of South Brazil and a World Heritage site, is ecologically and economically important for nearby municipalities with tourism potential. The river receives contaminants from disorderly urban growth, including discharges of domestic sewage and small fishery boats mainly, particularly during the tourist season. Our study aimed to assess impact of anthropogenic activities on water quality in the Guaraguaçu River by analyzing environmental contamination biomarkers in the top fish predator *Hoplias malabaricus*. Fish were collected using a fyke net trap across sectors representing a gradient of anthropic impact: sector 1 - pristine; sector 2 – impacted; and sector 3 - less impacted. Water samples were collected to analyze the presence of trace elements and pesticide. Biomarkers of the antioxidant system, histopathology, genotoxicity, neurotoxicity, and concentration of trace elements were analyzed in fish tissues. In water samples Al, Fe and Mn were detected, but no pesticides were found. In fish muscle, zinc and iron were detected. Brain acetylcholinesterase activity decreased in impacted sectors, indicating neurotoxic effects. The antioxidant system increased activity in gills and liver, and damage from lipoperoxidation was observed, particularly in sector 2 when compared to sector 1, suggesting oxidative stress. Histopathological biomarkers revealed lesions in the liver and gills of fish in impacted sectors. Micronuclei, a genotoxicity biomarker, were observed in organisms from all sectors. Our results demonstrate the detrimental effects of poor water quality on biota health, even when contaminants are not detected in water. This information is important for the social-vulnerable residents who rely on the river for survival, as well as for decisions-makers.

Keywords: Ecotoxicology; Environmental health; Biomonitor; Water contaminants; Guaraguaçu River; social-vulnerable residents.

3.3. INTRODUCTION

Human activities significantly influence water quality, disrupting the natural, and causing imbalances in aquatic ecosystems (Chaudhry; Malik, 2017; Malik et al., 2020). Pollution from sewage, pesticides, mining, intense navigation, and improper waste disposal are primary environmental problems resulting from human actions (Häder et al., 2020; Malik et al., 2020). These issues characterize the Anthropocene era, a geological period defined by the profound influence of human activities on Earth. Unfortunately, these actions have consequences for humans, leading to controversies and illustrating the concept of the "tragedy of the commons" (Dudgeon, 2019). In this sense, ecotoxicological studies have becoming increasingly relevant, particularly in freshwater ecosystems. Indeed, pesticides and trace elements are among the main contaminants found in water bodies (Khoshnood, 2017). These substances are prevalent in surface water and groundwater globally, primarily due to their extensive use in agricultural and urban settings. Pesticides exhibit varying compositions and properties, leading to differences in environmental persistence and toxicity levels. They can have detrimental effects on non-target organisms, including humans, such as carcinogenic, mutagenic, teratogenic, or endocrine-disrupting effects (Hashimi; R. Hashimi; Ryan, 2020; Souza et al., 2020).

Trace elements or trace metals (Jarapala; Kandlakunta; Thingnganing, 2014), previously referred to as heavy metals (Duffus, 2002), and are naturally occurring chemical elements found in small concentrations, typically below 0.1% by volume. Some trace elements, such as iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu), are essential for the proper functioning of living organisms, playing critical roles in various physiological processes (Sfakianakis et al., 2015). However, other trace elements such as mercury (Hg), lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni) lack recognized biological functions and are generally toxic to a wide range of organisms. It is worth noting that even essential elements can become highly toxic to plants and animals at high concentrations, such as copper (Mela et al., 2013; Tesser; Rocha and Castro, 2021).

Trace elements and pesticides pollution present a significant problem due to their bioaccumulation tendency in living organisms, particularly in the liver, muscle, and kidney, even in the absence of detectable concentrations in water (Mela et al., 2014; Ali et al., 2020). In addition to their bioaccumulative nature, trace elements can generate free radicals and reactive oxygen species through oxidative mechanisms, causing DNA damage and impairing crucial enzymes essential for bodily functions (Häder et al., 2020; Nordberg; Nordberg, 2022).

Biomonitoring has emerged as a method to assess changes in environmental quality using living organisms, allowing for the monitoring of stressors' effects on biological system across various regions worldwide, including Africa (Barhoumi et al., 2012; Saad Abdelkarim, 2020), Asia (Saleh; Marie, 2016; Kumar et al., 2021), Europe (Pastorino et al., 2021) and South America (Santana et al., 2018; Montes et al., 2020). The use of biomonitors and biomarkers are crucial in biomonitoring for evaluation of water quality management and conservation (Santana et al., 2018). Among the biomonitors, fish are an excellent biological model for studying aquatic ecosystems due to their constant exposure to environmental conditions. They inhabit diverse aquatic environments, occupy various trophic levels (Calado et al., 2019) and play a significant ecological role in influencing trophic structure, nutrient cycling, and energy flow within the food chain (Kroon; Streten; Harries, 2017). This study used the freshwater fish species *H. malabaricus* (Bloch, 1794, order Characiformes, family Erythrinidae), commonly known as trahira, that is widely distributed in the Neotropical region extends across South America and Central America, including Mexico, in rivers, reservoirs, and lakes, exhibiting ecological plasticity (Chu-Koo; Pérez, 2007; Grassi et al., 2017; Leite et al., 2021; Cruz-Esquivel; Marrugo-Negrete, 2022), and normally consumed by humans (Lozano et al., 2013). As a top predator in the food chain, it is commonly used in toxicological studies as a biomonitoring species (Mela et al., 2014; Paulino et al., 2020; Escalante-Rojas et al., 2021; Leite et al., 2021; Paula; Risso and Martinez, 2021; Cruz-Esquivel; Marrugo-Negrete, 2022). In the Guaraguaçu River of Paraná (Fig. 1), Brazil, this native species holds ecological significance as a potent top predator (Gazola-Silva et al., 2007).

Biomarkers provide insights into the effects on organisms at cellular, biochemical, and molecular levels, allowing for the understanding of early responses prior to individual mortality (Van der Oost; Beyer and Vermeulen, 2003). Analysis of biotransformation system, neurotoxicity, genotoxicity and antioxidant system are commonly used biomarkers in biomonitoring studies. Histopathological biomarkers are also employed to assess integrated tissue and organ injuries as they reveal morphological changes, based on the duration and intensity of exposure to the xenobiotic, as well as the adaptive capacity of organisms in cases of chronic exposure where the toxic agent causes cellular injury without resulting in death. (Georgieva et al., 2021; Leão-Buchir et al., 2023).

Tropical and sub-tropical freshwater ecosystems from the global south are still understudied compared to those in the Northern hemisphere. One of the still understudied ecosystems is the Guaraguaçu River, a Coastal River in South Brazil, located within a set of estuaries, called 'Lagamar mosaic', with high ecological and economical importance considered by UNESCO as a World Heritage Site (https://whc.unesco.org/en/list/). Although Lagamar mosaic is one of the most well-preserved remnants of one of the world's biodiversity hotspots (i.e., the Atlantic Forest, Myers et al. 2000), the Guaraguaçu River have been suffering from intense degradation due to disorderly urban growth, including discharges of domestic sewage and small fishery boats mainly, particularly during the tourist season (Elste et al. 2019). It was expected that more pronounced biomarker responses would be observed in the river sector considered to be more affected by human activities. The aim of this study was to evaluate the influence of the environment and anthropogenic activities on water quality by analyzing chemical analysis and biomarkers of environmental contamination in the top predator fish species *H. malabaricus*. It is important to emphasize that this is the first study using biomarkers in fish from Guaraguaçu River.

3.4. MATERIALS AND METHODS

3.4.1. Study Area

The Guaraguaçu River (25º45'W and 48º35'S) is part of the fourth largest sub-basin of the Paraná coastal plain, known as the Guaraguaçu River Basin, with a drainage area of 395.5 km². It originates in the Serra da Prata within the Saint-Hilaire/Lange National Park, a well-preserved area in the state of Paraná (Contente; Stefanoni and Spach, 2011), and flows into the Paranaguá Bay through the Cotinga Channel (Cavallini; Reis and Tiepolo, 2020). Spanning 60 km across the coastal plain, the river experiences a subtropical climate characterized by hot, rainy, and humid summers (Cfa). It is renowned for its biodiversity and environmental services, making it the largest and most significant river on the Paraná coast. The municipalities of Pontal do Paraná, Paranaguá, and Matinhos rely on it for their water supply (Vitule; Umbria and Aranha, 2006; Elste et al., 2019).

Despite the presence of conservation units such as the Guaraguaçu Ecological Station, Palmito and Rio da Onça State Parks, and the Guaratuba Environmental Protection Area (APA), as well as two indigenous lands inhabited by the Guarani ethnic group (Elste et al., 2019), the Guaraguaçu river area faces constant transformation and degradation. This can be attributed to several environmental impacts, including irregular agricultural areas along the Paraná coast, port activities at the Port of Paranaguá (Contente; Stefanoni and Spach, 2011), mining operations, urban expansion, aquaculture tanks, and the discharge of domestic effluents (Elste et al., 2019). Furthermore, a landfill located in the municipality of Pontal do Paraná is releasing contaminants into one of the river's main tributaries, the Pery River, which has been modified for drainage purposes. These factors contribute to the ongoing environmental deterioration of the area (Singo; Araújo-Ramos and Rocha, 2020).

3.4.2. Fish and water sampling

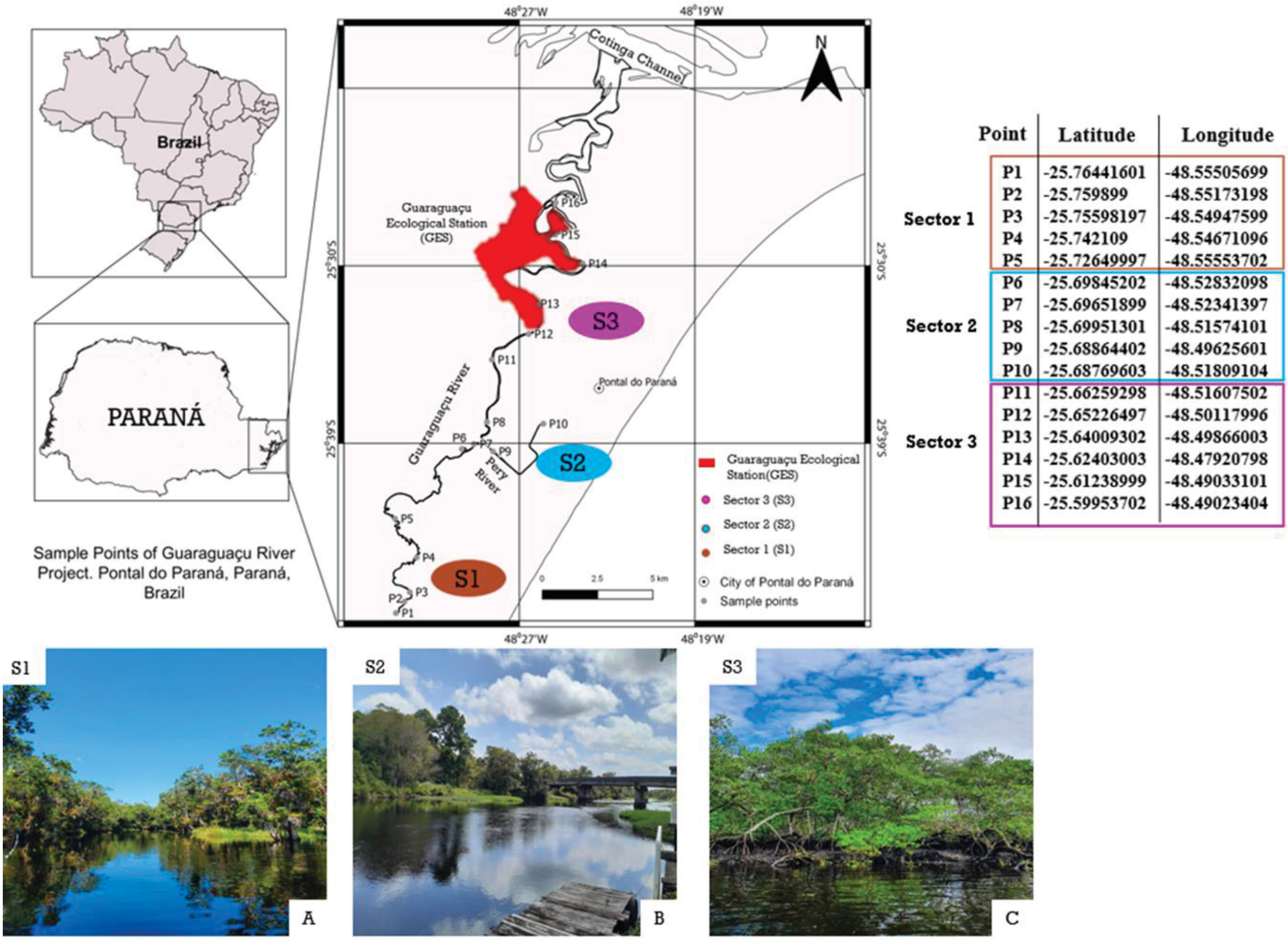
The fish species *H. malabaricus* was sampled in collaboration with the Laboratory of Analysis and Synthesis in Biodiversity (LASB) and Laboratory of Ecology and Conservation (LEC-DEA) from the Federal University of Paraná (UFPR). The LASB and LEC are responsible for the "Guaraguaçu Project," a long-term project for monitoring the biodiversity of the Guaraguaçu River (see https://lasbufprbio.wixsite.com/home).

The fish sampling was conducted at 16 specific locations that were characterized and georeferenced by the Guaraguaçu Project. These sampling points were divided into three sectors along the Guaraguaçu River, each representing distinct ecological gradients (Fig. 4). *H. malabaricus* is characterized as an opportunistic sedentary predator, being normally present in the vegetation of the margins of lentic environments, having territorial characteristics due to its reproductive and parental care (Chu-Koo; Pérez, 2007; Montenegro et al., 2013; Gomes et al., 2015). The classification of the river into different sectors was primarily based on the influence of tides, which is one of the main characteristics considered in this division, together with urbanization and human occupation:

Sector 1 (S1, preserved) (Fig. 4A): More pristine; located upstream of the river near the springs at the base of the mountains; characterized by a well-preserved area with little or no anthropogenic interference. This sector exhibits higher fish abundance in all trophic levels and is not directly influenced by tides. It is notable the presence of a large and dense “caixetal” (pioneer vegetal formation influenced by river).

Sector 2 (S2, contaminated) (Fig. 4B): Located downstream; characterized by higher levels of anthropogenic activity. In this area, there are fishermen, ranches, landfill, marinas and irregular housing settlements. The presence of the mining company also contributes to the anthropogenic impact. This sector is more susceptible to pollution due to sewage discharge and presence of landfill, and it may be considered visually the most polluted compared to other sectors of the river. However, the tidal influence is relatively low.

Sector 3 (S3, less impacted) (Fig. 4C): The mouth of Paranaguá Bay marks the estuarine transition (brackish water), mangrove region (pioneer vegetal formation influenced by river and sea). The presence of Palmito State Park (Guaraguaçu Ecological Station- EEG), contributed to the conservation efforts. The tidal effects are more pronounced in this sector, resulting in higher salinity levels compared to upstream areas. Fishing activities are more prevalent in this sector, reflecting the importance of the estuarine environment for fishery resources. Additionally, this sector of the river tends to have a wider channel due to the proximity to the sea (IAT, 2006; Cavallini et al., 2018; Araújo; Vitule and Padial, 2021).



	Point	Latitude	Longitude
Sector 1	P1	-25.76441601	-48.55505699
	P2	-25.759899	-48.55173198
	P3	-25.75598197	-48.54947599
	P4	-25.742109	-48.54671096
	P5	-25.72649997	-48.55553702
Sector 2	P6	-25.69845202	-48.52832098
	P7	-25.69651899	-48.52341397
	P8	-25.69951301	-48.51574101
	P9	-25.68864402	-48.49625601
	P10	-25.68769603	-48.51809104
Sector 3	P11	-25.66259298	-48.51607502
	P12	-25.65226497	-48.50117996
	P13	-25.64009302	-48.49866003
	P14	-25.62403003	-48.47920798
	P15	-25.61238999	-48.49033101
	P16	-25.59953702	-48.49023404

Fig. 4. Guaraguaçu River and river sectors. A- Sector 1; B- Sector 2; C- Sector 3.

Sampling fish of sector 1 was carried out between points 1 and 3 (approximately 600 meters between the points). In sector 2, between points 6 and 8 (approximately 985 meters between the points), while in sector 3, organisms were collected between points 15 and 16 (approximately 1,400 meters between the points).

The fish were collected using fyke net traps with a mesh size of 5 mm and a net of 10 x 1 m, as well as wicker fish traps. Twenty-seven specimens were collected, with eleven individuals in sectors 1 and 2, and five in sector 3. There were slight variations among individuals in terms of size, life stage, and male/female ratio (Supplementary Material 1). The sampling was conducted under the authorization of the Brazilian Institute of Biodiversity Conservation (ICMBio), in accordance with the Permanent License for Collection of Zoological Material (SisBio) No. 24779-1 (Authentication Code: 26744745). This license ensures compliance with animal welfare and ethical standards according to international guidelines. Additionally, the species *H. malabaricus* (Bloch, 1794) from the Guaraguaçu River was registered and assigned the number MHNCI 6190 at the Capão da Imbuia Natural History Museum - Curitiba, Paraná, Brazil.

The sampling was conducted during March/April, which correspond to the end of summer, and a period with a higher likelihood of high tide influence. This period of year is also characterized by increase rainfall, which can contribute to surface runoff and percolation of waste into the river (Contente; Stefanoni and Spach, 2011). Anthropogenic activities, such as tourism and recreation, are more prevalent during this period, potentially leading to water contamination.

During the sampling, water physicochemical parameters including salinity, pH, temperature, conductivity, and transparency were measured. These parameters provide insights into the overall water quality and can help identify potential sources of contamination. The water pH was measured using a portable pH meter PG1400 (GEHAKA). Conductivity was quantified using a CG1400 conductivity meter (GEHAKA). Temperature was determined by calculating the average of temperatures measured by the pH meter and conductivity meter. Transparency was measured using a Secchi Disk. Although salinity was not directly measured with a salinometer due to the logistical limitations, it is present only in sector 3 (transition with the mangrove) (Galvanese et al., 2022) and it was calculated from conductivity by a mathematical conversion.

The composite samples of surface water were collected using amber bottles (1L per sector) for pesticide analysis and plastic bottles with the addition of 1.5 ml of ultrapure nitric acid (1L per sector) for trace element analysis. The samples were maintained in ice during the transportation and subsequent laboratory analysis.

3.4.3. Samples for biomarkers and chemical analysis

The fish were anesthetized using benzocaine and their size and weight were measured. Blood samples were taken from the caudal vein using a heparinized syringe for the micronucleus test. Subsequently, euthanasia was performed by spinal cord sectioning. A fragment of the liver and gills from each specimen was collected for histopathological analysis and fixed in ALFAC solution (80% alcohol, formaldehyde, and glacial acetic acid) for 16 hours.

A fragment of muscle, liver, brain, posterior kidney, and gills was sampled for biochemical biomarkers and stored in liquid nitrogen for transport. In the Environmental Toxicology Laboratory (LTA) at the Federal University of Paraná, the samples were stored at -80°C until analysis.

Muscle tissue samples from *H. malabaricus* were collected in the field, with five separates muscle samples being obtained per sector of the Guaraguaçu River. These samples were then stored in a freezer for subsequent analysis of trace elements to assess bioaccumulation levels.

3.4.4. Analysis of pesticides in water

After storing the samples in amber bottles in the refrigerator, they were sent to the Technology in Laboratory Analysis (TECLAB). The "United States Environmental Protection Agency" (U.S. EPA, 1996a) Continuous Liquid-Liquid Extraction (EPA 3520 C) method was used for sample preparation and extraction. This method describes a procedure for isolating organic compounds from samples, including appropriate concentration techniques to prepare the extract for the specific analysis. After extraction, analysis was performed using a GCMS 240 adv functionality gas chromatograph (Agilent GC) based on the U.S. EPA (2014) Gas Chromatography/Mass Spectrometry (GC-MS) method for Semivolatile Organic Compounds (EPA 8270E).

3.4.5. Analysis of trace elements in water

Cadmium (Cd), lead (Pb), copper (Cu), chromium (Cr), manganese (Mn), and nickel (Ni) were quantitatively analyzed using an Atomic Absorption Spectrophotometer, GBC - Avanta model, at the Multiuser Laboratory of Chemical Analysis (LAMAQ) at the Federal Technological University of Paraná (UTFPR). The analysis method involved a digestion procedure utilizing concentrated nitric acid. Specifically, 100 mL of sample, along with standards, and blank solutions, were heated in Erlenmeyer flasks on a heating plate. Triplicate samples were prepared, with each sample subjected to 2 mL of nitric acid. Subsequently, 100 mL of the digested sample were evaporated at a time until reaching 500 mL of evaporated sample for each sector. Once cooled, the residue was transferred to a 100 mL volumetric flask, and 1 mL of a 13% potassium nitrate (KNO_3) solution was added. The final volume of 100 mL was achieved by dilution with distilled water.

For the preparation of the calibration curve, standards of 0.5 mg/L, 1 mg/L, 1.5 mg/L, and 2 mg/L were used. For the preparation of the blank, 100 mL of distilled water was subjected to the same analytical process as the samples. The standards and the blank were also transferred to 100 mL volumetric flasks and supplemented with a 13% KNO_3 solution, but in a volume of 2 mL. Both the samples and the standards, as well as the blank, were analyzed using the atomic absorption spectrophotometer.

The analysis of aluminum (Al), arsenic (As), iron (Fe), and zinc (Zn) followed practically the same method used for the other trace elements, with only some alterations regarding the sample quantity and the equipment used. At the Plant Nutrition Laboratory of UFPR, triplicates of the samples were prepared with a volume of 80 mL for each analyzed sector in Erlenmeyer flasks, under digestion with 1.6 mL of concentrated nitric acid and a constant temperature of 80 ºC on a heating plate. Triplicate blanks were prepared with 80 mL of MilliQ water plus 1.6 mL of concentrated nitric acid in each sample. After evaporating the samples to 25 mL, an Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES) instrument, specifically a Varian 720-ES, was used to analyze the trace elements. The results were expressed in mg/L.

For mercury (Hg) analysis, an Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES) by Thermo Scientific, model iCAP 6500, with axial view was used. This instrument is located at the Environmental Analysis Laboratory (LAA) of the Department of Chemistry at the UFPR. The analytical curve was constructed using a 100 mg/L standard solution of mercury (Hg) from AccuStard (New Haven, USA), which is a mono-element standard solution. The determination of Hg was performed through the chemical vapor generation technique within a concentration range of 0.2 to 5.0 µg/L in a 1% (v/v) nitric acid (HNO_3) medium. The analytical curve exhibited a correlation coefficient above 0.999. For the preparation of solutions for chemical vapor generation, 1 % m/v solid sodium borohydride ($NaBH_4$) of analytical grade from Reatec (Brazil), 0.4% (m/V) sodium hydroxide (NaOH) of analytical grade from Synth (Brazil), 6 M/L concentrated hydrochloric acid (HCl) of analytical grade from Reatec (Brazil) previously distilled, and 65% HNO_3 from Merck (Germany) were used.

3.4.6. Analysis of trace elements in muscle of *H. malabaricus*

The methodology was based on and adapted from the United States Environmental Protection Agency (U.S. EPA, 1996b) method 3052, which involves microwave-assisted acid digestion of siliceous and organic-based matrices, for the analysis of Cd, Cr, Pb, Ni, Mn, Cu, As, Zn, Fe and Al in the samples.

In the Plant Nutrition Laboratory at UFPR, the samples were placed in an oven at a constant temperature of 40°C for drying. After 24 hours, the dried samples were weighed and reached a constant weight of approximately 0.1 g. The samples were then transferred to Teflon tubes for microwave digestion. To initiate the digestion, 3 mL of HNO_3 was added to each sample and allowed to react for another 24 hours. Subsequently, an additional 1 mL of nitric acid and 1 mL of hydrogen peroxide (H_2O_2) 35% were added, and the sample was left to rest for another day for the reaction of HNO_3 and H_2O_2. Before placing the samples in the microwave, 3 mL of Milli-Q water (ultrapure) were added to each Teflon tube. During the digestion process, triplicate blank solutions were also prepared in a similar manner to the samples. The digestion process was carried out using a MARS 6 microwave digestion system.

The heating program in the microwave consisted of a pre-digestion stage with two steps. The first step involved a 5-minute heating ramp up to 80°C, followed by another 5-minute ramp up to 130°C. Finally, there was a 15-minute cooling period. For the digestion process, two stages were used with a 5-minute heating ramp up, varying power from 1030-1800 W, temperature ranging from 125-180°C, each stage lasting 10 minutes. After digestion, Milli-Q water was added to the samples to reach a volume of 20 mL. An Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) instrument, specifically a Varian 720-ES, was used to analyze the trace elements. The results were expressed in mg/kg (= μg/g).

3.4.7. Biochemical biomarkers

The muscle and brain were homogenized in potassium phosphate buffer (0.1 M, pH 7.5) at a ratio of 1:10 (w/v) using a micro homogenizer, and centrifuged for 20 minutes at 12,000 xg, 4 °C. For the muscle and brain, the supernatant was used for acetylcholinesterase (AChE) activity. The analysis of AChE activity was based on the method of Elmann et al. (1961), modified for microplate by Silva de Assis (1998). The absorbance was read at a wavelength of 405 nm.

The liver was homogenized in potassium phosphate buffer (0.1 M, pH 7.0) at a ratio of 1:10 (w/v), while the kidney at a ratio of 1:5 (w/v), and then centrifuged for 30 minutes at 15,000 xg, 4 °C. Aliquots of the supernatant were taken for measurement of glutathione S-transferase (GST) activity, superoxide dismutase (SOD) activity, catalase (CAT) activity, glutathione peroxidase (GPx) activity, concentration of non-protein thiols (GSH), and lipoperoxidation (LPO). The gills were homogenized in potassium phosphate buffer (0.1 M, pH 7.0) at a ratio of 1:5 (w/v), followed by centrifugation for 30 minutes at 15,000 xg, 4 °C. Aliquots of the supernatant were taken for measurement of GST activity, SOD activity, CAT activity, GPx activity, GSH concentration, and LPO.

The analysis of Glutathione S-transferase (GST) activity followed the method proposed by Keen et al. (1976) based on the catalysis of the reaction between the substrate 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) by GST. The measurement was performed at a wavelength of 340 nm.

The activity of SOD was evaluated using the method proposed by Gao et al. (1998), which is based on the ability of superoxide dismutase to inhibit the auto-reduction of pyrogallol. The absorbance was measured at a wavelength of 440 nm.

The method for CAT analysis was based on Aebi (1984). The principle of the method is to evaluate the decrease in absorbance at 240 nm due to the consumption of H_2O_2 by catalase, resulting in the production of oxygen (O_2) and water (H_2O). The absorbance was measured at 240 nm.

The analysis of GPx activity was performed using the method described by Hafeman et al. (1974). Based on the measurement of the decrease in absorbance at 340 nm, caused by the reduction of oxidized glutathione (GSSG), which is catalyzed by glutathione reductase (GR) in the presence of nicotinamide adenine dinucleotide phosphate (NADPH).

The concentration of GSH was analyzed using the method developed by Sedlak and Lindsay (1968). The absorbance was measured at 405 nm. The concentration of GSH was determined by comparing it to a standard curve of GSH.

The analysis of LPO was performed using the FOX assay (Ferrous Oxidation in Xylenol Orange), proposed by Jiang et al. (1992). This method is based on the rapid oxidation of iron (Fe^{+2}) mediated by peroxides under acidic conditions, followed by the formation of the Fe^{+3}-xylene orange complex in the presence of the stabilizer butylated hydroxytoluene. The absorbance was measured at 570 nm.

A fragment of liver was used for metallothionein (MET) analysis. The liver was homogenized in a solution consisting of Tris HCl buffer (20 mM, pH 8.6), 1.71 g of sucrose, 50 μL of phenylmethylsulfonyl fluoride (PMSF), and 1 μL of β-mercaptoethanol, at a ratio of 1:5 (w/v). The homogenate was then centrifuged for 30 minutes at 15,000 xg, 4 °C. From the supernatant, two aliquots were taken: 300 μL for metallothionein activity analysis and 10 μL for protein analysis. The method was proposed by Viarengo et al. (1997) and the absorbance was measured at a wavelength of 405 nm. A negative control was performed, as well as a standard curve using GSH.

The Bradford method (1976) was used for protein quantification in the samples, with a standard curve prepared using bovine serum albumin.

3.4.8. Histopathological biomarkers

In the laboratory, the ALFAC solution was replaced with 70% alcohol and kept for 24 hours, followed by another change to 70% alcohol and kept until the samples were embedded in Paraplast®. The liver and gill samples were cut using a microtome, with a thickness set at 5 µm, and stained with hematoxylin/eosin. Images were captured using an Olympus BX51 microscope at the Center for Advanced Fluorescence Technologies (CTAF-UFPR). The Lesion Index was calculated based on histopathological findings, according to Bernet et al. (1999) and modified by Mela et al. (2013). All identified lesions and tissue alterations were classified into categories based on their biological significance: 1 - minimal lesion, easily reversible; 2 - moderate lesion, reversible in most cases; and 3 - severe lesion, generally irreversible. Scores ranging from 0 to 6 were assigned to establish the severity of the lesions. The lesion index for each group of liver or gill lesions was calculated using the following formula:

$$Iorg = \sum rp \sum alt\ (a \times w)$$

, where: org represents the organ (constant), rp represents the reaction pattern, alt represents the alteration, a represents the score value and w represents the importance factor of the lesion.

For scanning electron microscopy analysis of gills, the samples were fixed and stored in 3% glutaraldehyde for a minimum period of 24 hours. They then underwent a gradual dehydration process in ethanol (from 50% to 100% in 10-minute intervals), followed by critical point drying using a Bal-tec CPD 030 instrument. Once the samples were completely dehydrated, they were gold-coated using a Balzers SCD 050 device. Finally, readings and images of the tissue lesions were obtained using a JEOL JSM 6360-LV scanning electron microscope (SEM) at the Electron Microscopy Center of UFPR.

3.4.9. Biomarkers of genotoxicity

To assess the frequency of micronuclei, the technique described by Hooftman and Raat (1982) was used. In the field, a drop of blood was placed on a microscope slide, and using a 45-degree angle, a coverslip was slid to perform a smear, resulting in one slide per fish. The slides were left to air dry and then fixed in absolute ethanol for 15 minutes. Once dry, the slides were stained with 10% Giemsa diluted in 90 mL of phosphate/sorensen buffer (pH 6.8) and left for 12 minutes. For each slide, 2000 cells/erythrocytes were analyzed under a 100x magnification using an optical microscope. The frequency of the following nuclear morphological alterations, proposed by Carrasco et al. (1990), was also determined in the blood samples: Blebbed (BL), Lobed (LB), Vacuolated (VC), Notched (NT), and Binucleus (BN).

3.4.10. Statistical analysis

The analysis of the data was performed by examining the variation in biomarker responses (response variable) among the three sampling sectors (predictor variable).

The Levene and Shapiro-Wilk tests were used to assess the assumptions of homogeneity of variances and normality, respectively. The assumptions were met in most of the analyses, allowing the use of one-way ANOVA followed by Tukey's test. In cases where the assumptions were not met, a permutation ANOVA was performed or a data log transformation was made. The results were expressed as mean ± standard error with a significance level of $p < 0.05$. For an integrated analysis of the biochemical biomarker responses in the gills, liver, and kidney, multivariate statistics using Principal Coordinates Analysis (PCoA) were employed, considering only the first two axes to describe differences between sectors and similarities among biomarkers. The analysis was conducted using RStudio software (R CORE TEAM, 2017), and the PCoA graphs were generated using STATISTICA software.

For the analysis of trace elements in the muscle of *H. malabaricus*, a PERMANOVA (Permutation Multivariate Analysis of Variance) with Euclidean distance matrix was performed using RStudio. This analysis was conducted to compare the influence of trace elements among the sampled sectors. The PERMANOVA allows for the assessment of differences among groups while considering multivariate data.

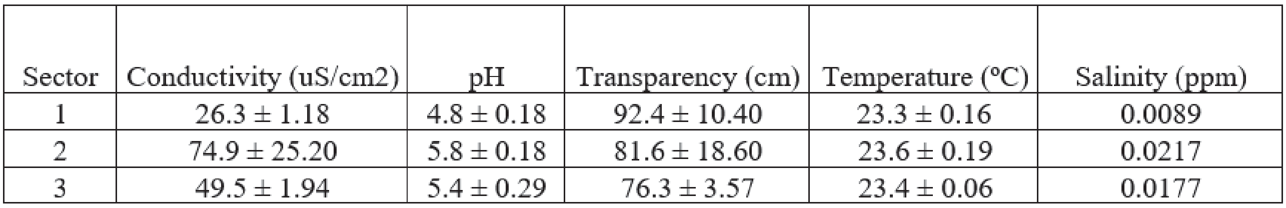
The physicochemical parameters did not undergo statistical analyses. This is mainly due to the nature of quantifying and obtaining data related to a "snapshot" period of the parameters, as the Guaraguaçu River is influenced by tides that occur twice a day, during the flood tide and ebb tide. In addition, water features were estimated in only one sampling point per sector, so there were no real replicates to compare sites. As a result, it becomes impractical to conduct statistical analysis for comparing the sectors in terms of physicochemical parameters, as they do not represent concrete daily patterns or even have independent replicates. However, the findings were able to describe trends in the river, as well supported by previous studies (IAT, 2006; Vitule; Umbria and Aranha, 2006; Elste et al., 2019).

3.5. RESULTS

3.5.1. Physicochemical parameters

Although with only one snapshot sampling, analysis of the water's physicochemical parameters (Table 1) suggests an apparent difference into the three sectors, particularly regarding conductivity and transparency. The conductivity in sector 2 was higher than in sector 3, which is directly influenced by tides. When analyzing transparency, there was a scale from greater to lesser transparency.

Table 1. Mean values and standard errors (Mean ± SE) of the physical-chemical parameters of the sixteen collection points of the “Guaraguaçu Project” divided to characterize the three sectors of the Guaraguaçu River.



Sector	Conductivity (uS/cm2)	pH	Transparency (cm)	Temperature (°C)	Salinity (ppm)
1	26.3 ± 1.18	4.8 ± 0.18	92.4 ± 10.40	23.3 ± 0.16	0.0089
2	74.9 ± 25.20	5.8 ± 0.18	81.6 ± 18.60	23.6 ± 0.19	0.0217
3	49.5 ± 1.94	5.4 ± 0.29	76.3 ± 3.57	23.4 ± 0.06	0.0177

Legend: Points 1-5 = Sector 1; Points 6-10 = Sector 2; Points 11-16 = Sector 3. Points 8 and 9 (Pery rectified channel) of the guaraguaçu project belonging to sector 2 had extremely high conductivity: 154.4 and 110 uS/cm respectively. Salinity is expressed only as a mean value.

3.5.2. Chemical Analysis

3.5.2.1. Pesticides in water

The intermediate region of the Guaraguaçu River can be considered the most impacted by anthropogenic stressors. However, the results of the analysis of pesticides in water (Supplementary Material 2) showed that these contaminants are not present in significant quantities in any of the three sectors. All sectors had concentrations below the limit of quantification (LQ) and, therefore, when compared to CONAMA 357/05, almost all are below the maximum concentration allowed by the legislation, except the pesticides Aldrin + Dieldrin, Pentachlorophenol, Chlordane, Endrin and Lindane. Because as some are below the quantification limit, there is no way to know the exact value, besides that there are some pesticides that do not have a limit by legislation (see all data in Supplementary Material 2).

3.5.2.2. Trace elements in water

The Guaraguaçu River and its tributaries are classified for the most part, according to CONAMA 357/05 as class 2, water that can be used for human consumption after conventional treatment, protection of aquatic communities, recreation, irrigation of vegetables, aquaculture, and fishing activities (ZEE, 2016).

The results showed that the Guaraguaçu River region does not exhibit significant contamination by no essential trace elements, at least when analyzed in water (Supplementary Materials 3, 4 and 5). Manganese (Mn), an essential element, was quantified, but its concentration in all three sectors were below the maximum limit allowed by legislation (total manganese - maximum of 0.1 mg/L) according to CONAMA 357/05. Fe and Al were quantified and are above the legal limits (Dissolved Aluminum - maximum of 0.1 mg/L; Dissolved Iron - maximum of 0.3 mg/L) according to CONAMA 357.

3.5.2.3. Trace elements in the muscle of *H. malabaricus*

In the water samples, manganese, iron and aluminum were quantified. However, in the analysis of trace elements in fish muscle tissues, several elements were quantified, including chromium (Cr), copper (Cu), manganese (Mn), iron (Fe), zinc (Zn), aluminum (Al), and arsenic (As), which were included due to their biological importance in the metabolism and physiological processes of aquatic organisms (Supplementary Material 5). Brazilian legislation attempts to establish limits for trace elements in food by Resolution of the Collegiate Board - RDC (2013). It establishes the MERCOSUR Technical Regulation on maximum limits for inorganic contaminants in food (Brazil, 2013) and Decree No. 55.871 (1965), which, although repealed in 2019, remains the only document that establishes maximum limits for nickel, zinc, copper, and chromium in food (BRAZIL, 1965). Comparing Brazilian legislation is largely in line with international regulations as Food and Agriculture Organization (FAO, 1983) and Commission Regulation of the European Community (EC, 2006).

Among the trace elements analyzed, lead (Pb), cadmium (Cd), and nickel (Ni) were not quantified in the muscle tissue of *H. malabaricus* in any of the three sectors, similar to water samples. Sector 3 was the only sector where at least one organism had concentrations of As and Cu above the limit of quantification of the equipment (Arsenic = 0.02 mg/L; Copper = 0.01 mg/L). However, the copper concentration remained below the limit determined by legislation. Al, Fe and Mn do not have a maximum limit of concentration in food according to the legislation. Aluminum was quantified in all organisms from sector 3 of the Rio Guaraguaçu. The arsenic value found in a single organism from sector 3 (5.569 mg/kg) exceeded the concentration limit stipulated by the legislation (As = 1 mg/kg). Chromium was found in two organisms from sector 2 (3.469 and 2.281 mg/kg) and one organism from sector 3 (5.551 mg/kg), exceeding the limit (Cr = 0.1 mg/kg). When analyzing the concentration of zinc, only five organisms out of fifteen had concentrations below the limit stipulated by Decree No. 55,871 of 1965 (50 mg/kg). Iron and zinc were the only elements quantified in all organisms analyzed in all sectors.

The correlation of each original variable (trace elements) with the x and y axes (PCA1 and PCA2, respectively), revealed that two elements stood out in explaining nearly 99% of the influence on the sectors of the Guaraguaçu River (Supplementary Material 7). The x-axis (PCA1) explained approximately 82% of the variation and, according to the loadings, was positively associated with the concentration of Zn. On the other hand, the y-axis (PCA2) explained approximately 17% of the variation and was negatively associated with Fe. By analyzing the ordination graph (Fig. 5), it can be observed that Fe is more correlated with sector 3, while Zn is more correlated with S1. However, from a statistical point of view, there is no significant difference among the three sectors regarding trace element concentrations (p-value = 0.19 > 0.05) (Supplementary Material 6).

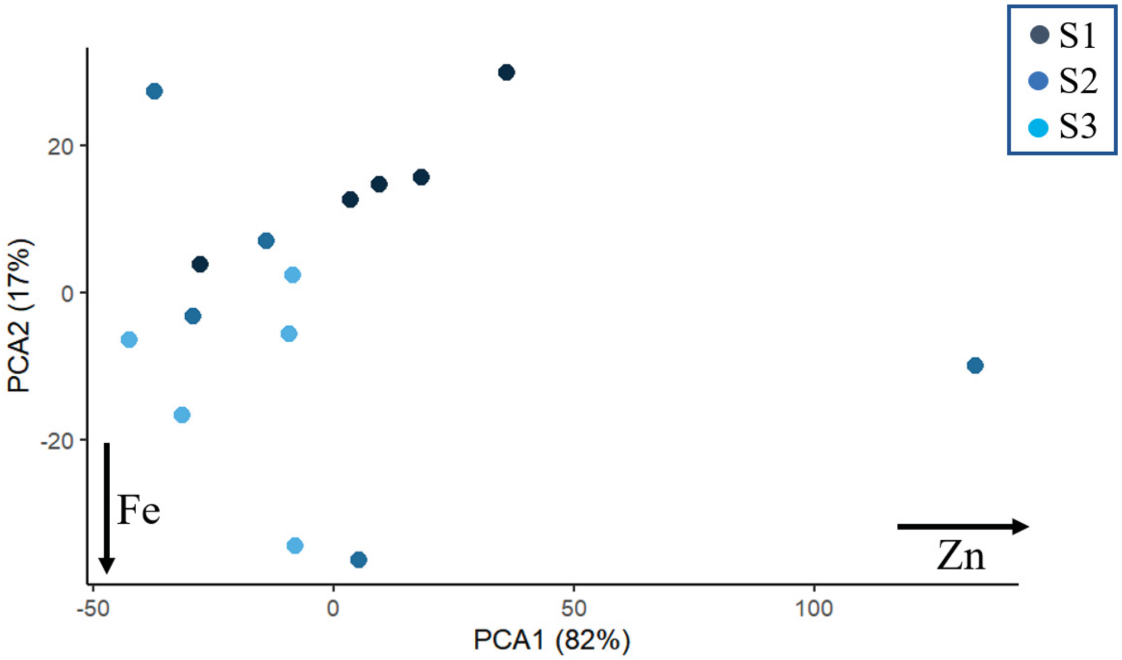


Fig. 5. Principal Coordinate Analysis ordination, showing the first two axes (PCA1 and PCA2) that represent most of the data variation (percentages in the graph). The Fe and Zn elements are the two that are mostly related to the axes, with high Fe values mainly in sector 3(S3), and high Zn values in sector 1 (S1).

3.5.3. Biochemical biomarkers

The Guaraguaçu River showed significant differences among the three sectors analyzed based on biochemical biomarker analysis in the brain, muscle, gills, liver, and kidney (Supplementary Material 8). In the brain, there was a significant difference in AChE activity (F=10.17; p=0.0008) (Fig. 6A), with a low activity observed in S2, which is considered more impacted by human activities.

In muscle tissue, a significant difference was observed (F= 7.95; p = 0.002) among the sectors, with a low acetylcholinesterase activity in S3, which is less impacted, compared to S2, which is more influenced by human activities (Fig. 6B). However, there was no significant difference between S1 (control sector and considered pristine) and the other two sectors.

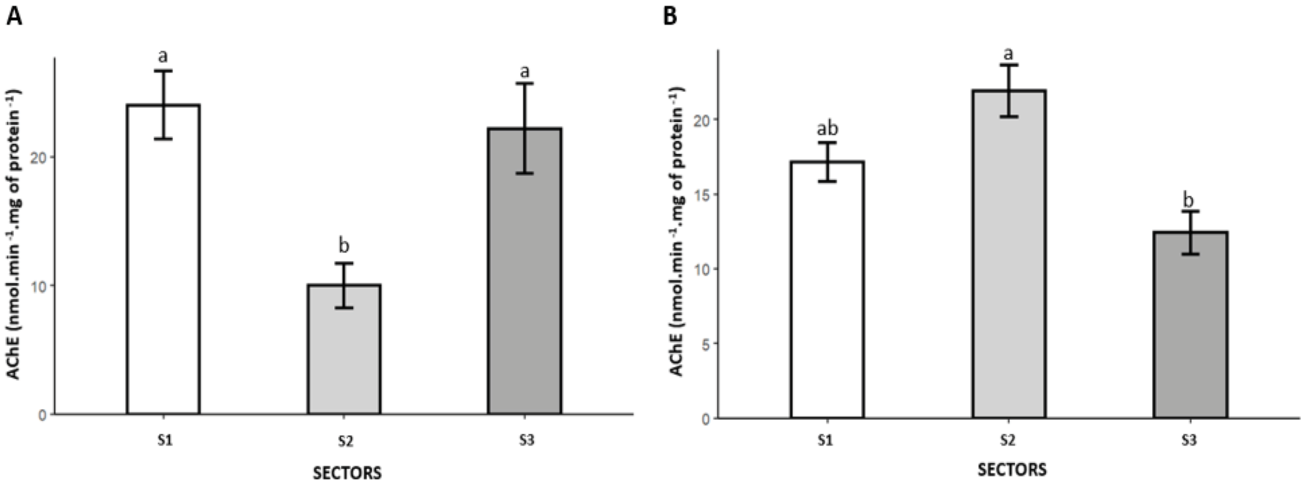


Fig. 6. Acetylcholinesterase (AChE) activity (Mean ± Standard Error) in the brain (A) and muscle (B) of *Hoplias malabaricus* in three sectors characterized by different ecological gradients. Different letters indicate significant difference ($p<0.05$) by Tukey's test.

In the comparison among the three sectors regarding the effects of biomarker activities in the fish gills, a higher activity of GST was observed in S3 (F= 7.96; p= 0.003). For GSH, there was no significant difference among the three sectors (F= 1.55; p = 0.244), and the activity of CAT decreased in S2 and S3 (F= 8.46; p = 0.002). However, there was an increase in GPx activity (F=8.48; p = 0.003), LPO (F= 17.33; $p < 0.01$), and SOD activity (F= 11.24; p = 0.0006) in S2 and S3. Analyzing the Principal Coordinate Analysis (PCoA) (Fig. 7A), created to interpret the percentage of explanation that the biomarkers exert on the sectors of the Guaraguaçu River, it is considered that, except for the higher CAT activity in S1, all other biomarkers showed a higher correlation and activity in S2 and S3, in addition to an increase in LPO. In fact, there is a visual separation among the sectors considering the set of biomarkers, where S1 is the one that stands apart from the others.

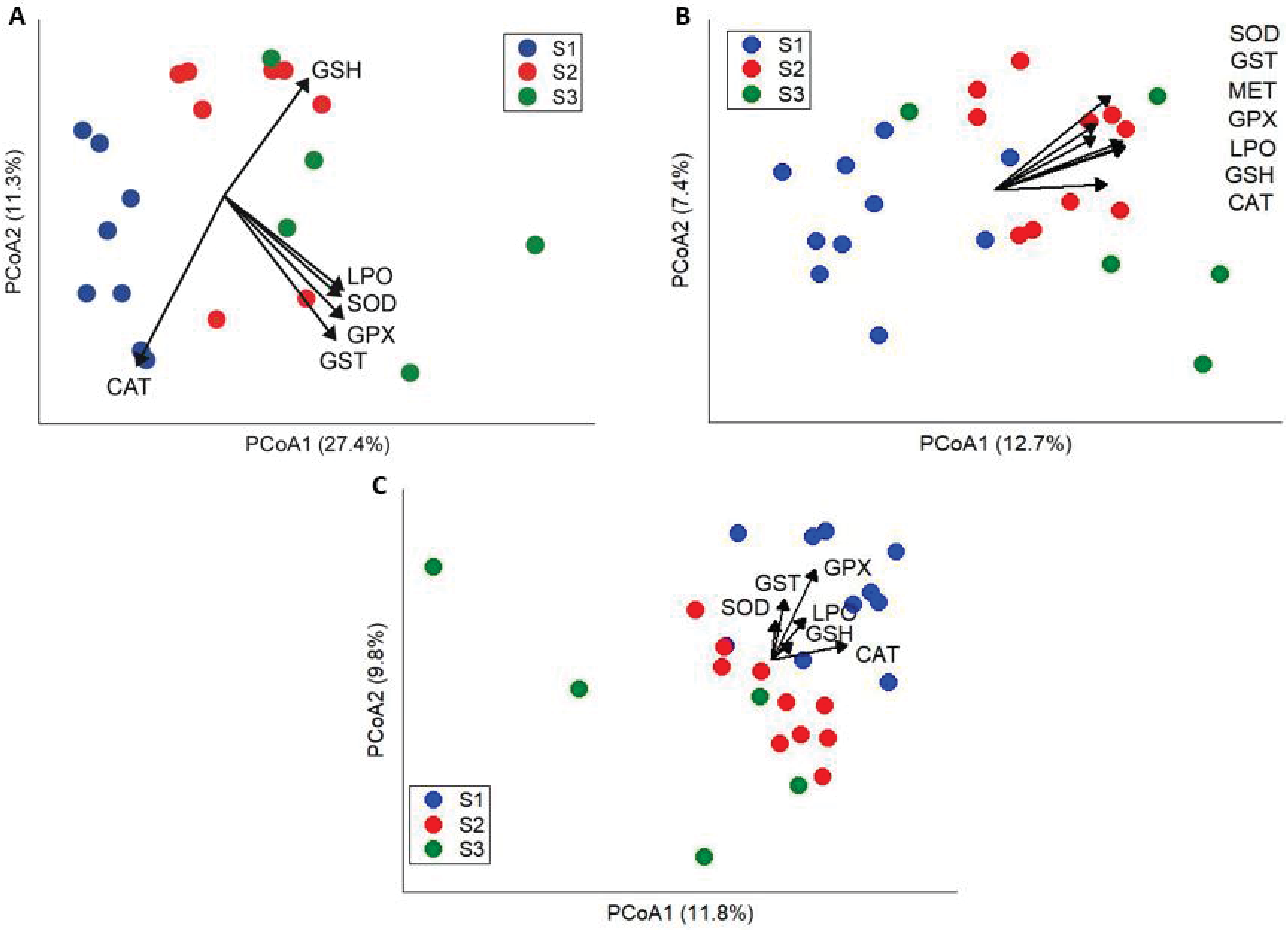


Fig. 7. Principal Coordinate Analysis ordination of biochemical biomarkers in gills (A): Axis 1 (PCoA1) explains 27.4% of the variation, while Axis 2 (PCoA2) explains 11.3% of the variation; PCoA of biochemical biomarkers in liver (B): Axis 1 (PCoA1) explains 12.7% of the variation, while Axis 2 (PCoA2) explains 7.4% of the variation. The order of the arrows is directly related to the order of the biomarkers in the upper right corner of the graph; and PCoA of biochemical biomarkers in kidney (C): Axis 1 (PCoA1) explains 11.8% of the variation, while Axis 2 (PCoA2) explains 9.8% of the variation.

In terms of the effects of biomarker activities found in the liver, it can be noted that there was an increase in the GST activity (F= 3.68; p= 0.04) and GPx activity (F= 5.97; p= 0.01) in S2 compared to S1, but there was no significant difference between S3 and the other two sectors in the activity of both enzymes. However, for GSH (F= 25.79; p< 0.01), SOD activity (F= 16.29; p<0.01), and LPO (F= 17.39; p<0.01), there was an increase in S2 and S3 when compared to S1. CAT activity (F= 1.33; p= 0.28) and MET (F= 0.71; p = 0.05079) did not show significant differences among the three sectors. The PCoA used to illustrate the collective responses of the liver biomarkers (Fig. 7B) shows that the biomarkers, for the most part, exhibited higher responses in Sectors 2 and 3, with S2 showing higher activity than S1 in almost all biomarkers. As in the gill biomarkers, there is a clear separation among sectors for the combination of liver biomarkers, highlighting the regionalization of health impacts on the fish in the Guaraguaçu River.

The posterior kidney is known to have the primary function of excreting contaminants and their metabolites from organisms, making it extremely important for biomarker analysis. In the case of the posterior kidney, it was found that the only biomarkers that showed significant differences among the three sectors were GST (F= 18.31; $p<0.01$) and GPx (F= 14.82; p = 0.0001), with increased activities in sectors 2 and 3 (Fig. 7C). Similar to the previous results, there is a distinct difference among the sectors when considering the set of biomarkers in the kidney.

3.5.4. Histopathological biomarkers

The histopathological biomarkers demonstrated a spatial difference in the Guaraguaçu River among the three sampling sectors, when analyzed using optical microscopy in gills (Fig.8 A-F) and liver (Fig. 9 A-D) and scanning electron microscopy in gills (Fig. 8 G-L), indicating the likely presence of xenobiotics such as trace elements. More pronounced responses of the biomarkers, consequently leading to greater tissue lesions and alterations, were observed in the fish collected in sector 2, considered to be more affected by anthropogenic activities. When analyzing the mean Bernet index for each sector in each tissue relative to the number of individuals collected in each sector, a significant difference was found among the three sectors both for gills ($p<0.05$; F-value = 98.9) (Fig. 8M) and for liver ($p<0.05$; F-value = 25.6) (Fig. 9E), with sector 2 showing the highest indexes (25.27 and 24.18, respectively for gills and liver), followed by sector 3 (with 3.2 and 7.6), and lastly sector 1 (with 0.73 and 2.91).

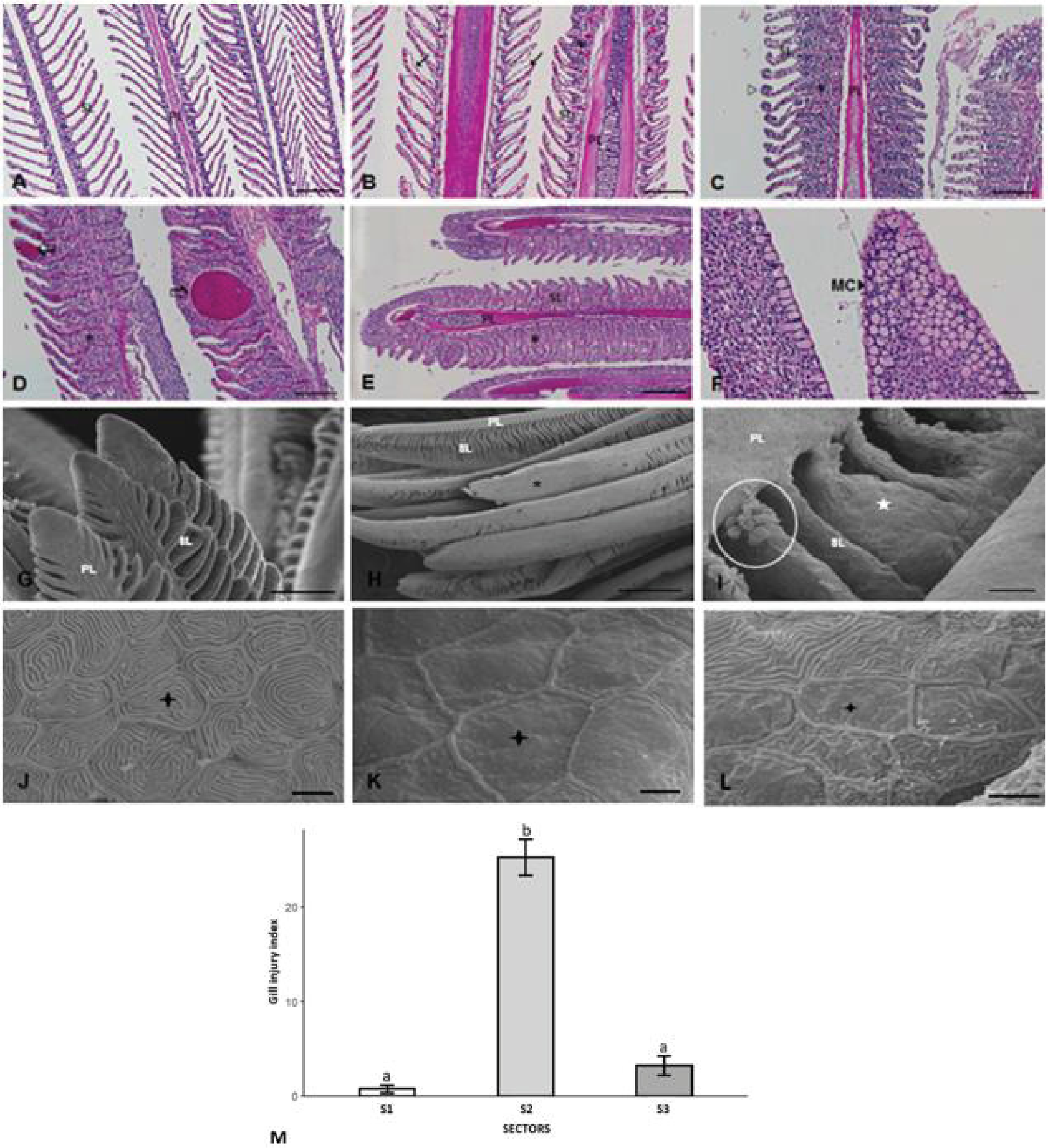


Fig. 8. Histological gill sectors of *H. malabaricus* counterstained with hematoxylin/eosin using optical microscopy (**A-F**), and images using scanning electron microscopy (**G-L**). **(A)** Gill of sector 1: Primary lamella (PL) and secondary lamella (SL); **(B)** Gill sector 3: Primary lamella (PL), secondary lamella (SL), epithelial lifting (↙) and partial hyperplasia (*); **(C)** Gill sector 2: Primary lamella (PL), partial hyperplasia (*) in secondary lamella (SL) and rolling of secondary lamella (▷); **(D)** Gill sector 2: Aneurysm (⇨) and partial hyperplasia (*) in secondary lamella (SL); **(E)** Gill sector 2: Primary lamella (PL) and total hyperplasia of secondary lamella (SL); **(F)** Gill sector 2: Total hyperplasia of secondary lamella (SL) and increase of mucus cells (MC). Scale bar: (**A-E**) 100 µm, **(F)** 50 µm. **(G)** Gill of sector 1: General view of gill filaments and lamellae showing normal morphological features. Primary lamellae (PL) and secondary lamellae (SL). Scale bar: 50 µm; **(H)** Gill of sector 2: Primary lamellae (PL) and secondary lamellae (SL). It is possible to observe Hyperplasia in secondary lamella (SL) with lamellae fusion (*). Scale bar: 100 µm; **(I)** Gill of sector 2: Primary lamellae (PL) and secondary lamellae (SL), partial hyperplasia (in circle) and aneurysm (★). Scale bar: 20 µm; **(J)** Gill of sector 1: Note well-organized pavement cells and organized microridges (✦). Scale

bar: 5 µm; **(K)** Gill of sector 2: We can see alteration in the branchial epithelium with significant reduction of microridges (*) in pavement cells. Scale bar: 5 µm; **(L)** Gill of sector 3: Branchial epithelium with reduction of microridges (*) in pavement cells. Scale bar: 5 µm.; **(M)** Graph of the histopathological lesion index in the gill tissue. Results are expressed as mean ± standard error for normal data. Analysis of variances ANOVA ($p < 0.05$; F-value = 98.9). Different letters mean statistically significant differences between sectors.

The changes found in the gills, particularly in S2, included aneurysm and hyperplasia with partial fusion of the secondary lamellae (Fig. 8D), folding of the tips of the secondary lamellae (Fig. 8C), hyperplasia with total fusion of the secondary lamellae and an increase in mucous cells (Fig. 8F), with a higher percentage of occurrence in sector 2 related to increase of mucous cells (27.17%) and hyperplasia with total fusion of the secondary lamellae (23.20%), classified as level 1 (minimal lesion) and level 2 (moderate lesion), respectively, in the biological importance grading of the Bernet index (Bernet et al., 1999). In S3, the observed alterations were epithelial lifting and hyperplasia with partial fusion (Fig. 8B). When analyzing the images captured by SEM, alterations in the gill epithelium with reduced microridges were observed in gills of fish from S2 and S3 (Fig. 8K and 8L), with major alterations found in S2. In S1, intact gill structures without tissue alterations were observed (Fig. 8A and Fig. 8G).

In the liver, most of the alterations also occurred in fish tissues from sector 2 of the Guaraguaçu River. Among the alterations found in fish from sector 2, the presence of melanomacrophage centers (Fig. 9B), blood congestion in sinusoids, hepatocyte vacuolization (Fig. 9D), and necrosis can be mentioned. The alterations related to hepatocyte vacuolization (30%) and necrosis (26%) had a higher percentage of occurrence in sector 2, indicating two irreversible changes. In fish from sector 3, which is considered to have less anthropogenic influence, the presence of cell death or necrosis (Fig.9C) was still observed.

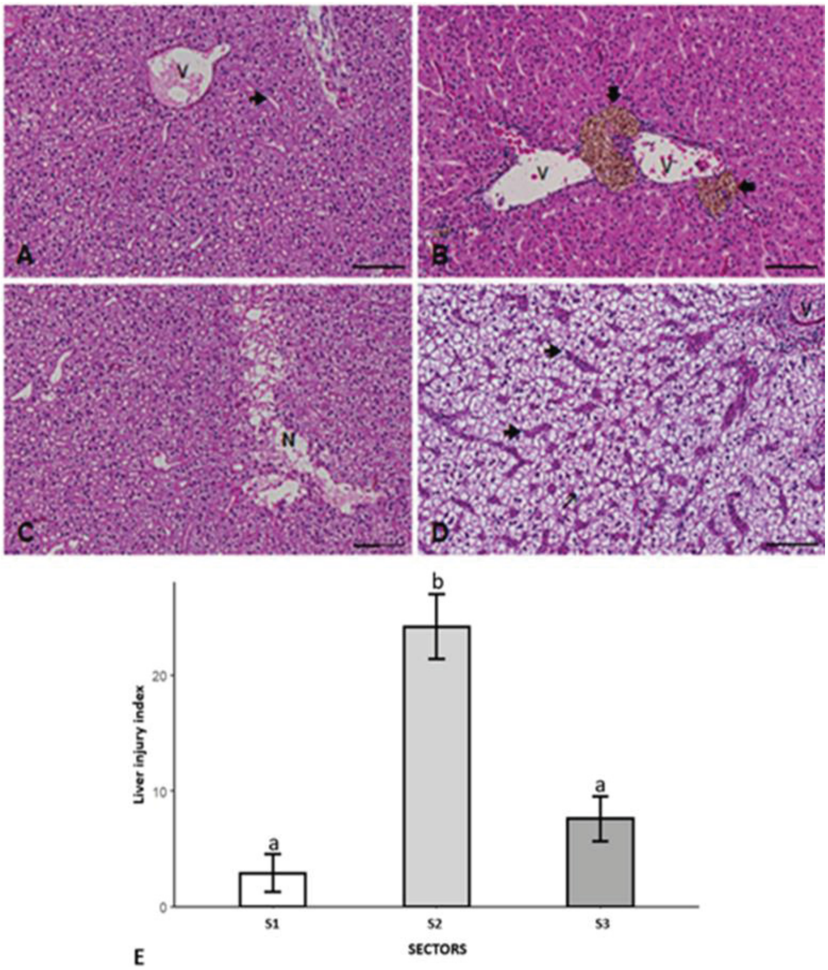


Fig. 9. Histopathological liver of *H. malabaricus* counterstained with hematoxylin/eosin. **(A)** Sector 1: Blood vessel (V) and sinusoids (➔); **(B)** Sector 2: Melanomaceophages centers (⬇) surrounding the blood vessels; **(C)** Sector 3: Necrotic foci (N); **(D)** Liver sector 2: Blood vessel (V), blood congestion in sinusoids (➔) and vacuolation of hepatocytes (↗). Scale bars = 100 µm; **(E)** Graph of the histopathological lesion index in the liver tissue. Results are expressed as mean ± standard error. Analysis of variances ANOVA ($p < 0.05$; F-value = 25.6). Different letters mean statistically significant differences.

3.5.5. Biomarker of genotoxicity

The results showed that there was no statistically significant difference in the presence of micronuclei or other nuclear morphological alterations (blebbed, notched, lobed, binucleus, and vacuolated) among the three analyzed sectors of the Guaraguaçu River (Table 2). From the analysis of 2000 erythrocytes, the presence of micronuclei occurred in all three sectors, with three micronuclei found in S1, two in S2, and two in S3, totaling seven micronuclei for all the blood samples analyzed.

Table 2. Frequency of Micronucleus and nuclear morphological alterations in erythrocytes of *H. malabaricus* in the three sectors of Guaraguaçu River. (Median; 1st quartile; 3rd quartile).

		Nuclear morphological alterations					
Section	Statistical	Micronucleus	Blebbed	Notched	Lobed	Binucleus	Vacuolated
	F-value	0.285	0.386	3.381	2.303	0.277	1.635
	P-value	0.754	0.683	0.050	0.121	0.760	0.215
S1		3 (0; 0; 0.5) a	17 (1; 0; 2) a	50 (4; 2.5; 6.5) a	17 (1; 0; 2) a	5 (0; 0; 1) a	98 (10; 5.5; 12) a
S2		2 (0; 0; 0) a	14 (1; 0; 3) a	25 (2; 1; 3) a	26 (1; 1; 4) a	3 (0; 0; 0.5) a	70 (3; 1; 8) a
S3		2 (0; 0; 0) a	4 (1;0; 1) a	27 (5; 3; 6) a	2 (0; 0; 1) a	2 (0; 0; 1) a	64 (11; 11; 12) a

Statistics were performed by ANOVA test followed by Tukey's post hoc test. When normality assumptions were not found, an ANOVA with permutations was performed. Same letter means no significant difference (p>0.05 or p=0.05).

3.6. DISCUSSION

The Guaraguaçu River have a great social and economic importance in providing water supply and subsistence fishing (Reis et al., 2015) mostly to social-vulnerable residents of Pontal do Paraná, Paranaguá and Matinhos municipalities (Elste et al., 2019). In addition, this river plays an important seasonal role for sport fishing tourism; and is central to aquatic biodiversity conservation (Vitule; Umbria and Aranha, 2006). In spite of that, our results clearly demonstrated that human impacts are reflecting in environmental health of the river.

This is in line with the recent ecological studies carried out in Guaraguaçu River. Eutrophication process and mass-development of invasive aquatic macrophytes, particularly the African tanner-grass *Urochloa arrecta* (Hack. ex T. Durand & Schinz) Morrone & Zuloaga occurs mainly in the intermediate region of the river (Elste et al., 2019; Araújo; Vitule; Padial, 2021). Recent studies have reported the biotic homogenization impacts related to the gradient of anthropogenic impact found in the Guaraguaçu River (Sato; Costa; Padial, 2021; Galvanese et al., 2022). Here, we add to this knowledge by investigating how the health of a top predator fish is affected by the human impact gradient of the Guaraguaçu River.

3.6.1. Physicochemical parameters

The results are in line with the well-known increase in contamination and degradation along the intermediate stretches of the Guaraguaçu River (Elste et al. 2019), posing a threat to its multiple uses. The lower pH in Sector 1, which is considered pristine is influenced almost exclusively by the Serra do Mar (Abreu-Mota et al., 2014). The biogenic material imported from upstream rivers settles in this region due to its almost stagnant water character, resulting from low water flow. Because of organic matter decomposition, the environment becomes more acidic, giving the water a brownish coloration. Additionally, the absence of a constant flow of vessels leads to minimal particulate matter, which explains the higher water transparency in this sector.

It can be observed that the conductivity in sector 2 is higher than in sector 3, which is influenced by tidal effects due to its proximity to the mouth of the Guaraguaçu River. Consequently, sector 3 would be expected to have the highest conductivity and salinity in the river (Galvanese et al., 2022). This can be mainly explained by highlighting that Points 8 and 9 of the "Guaraguaçu Project," with conductivities of 154.4 and 110 µS/cm, respectively, are located within the straightened channel of the Pery River, near the landfill in the municipality of Pontal do Paraná, and in the presence of domestic sewage discharge. These points possible be influenced by increased conductivity due to excessive concentrations of elements such as phosphorus and nitrogen, and their ions, which are naturally limiting factors for biotic growth (Souza et al., 2019; Singo; Araújo-Ramos and Rocha, 2020). This finding confirms and strengthens the results of other studies conducted in different water bodies worldwide, where anthropogenic nutrient inputs significantly alter physicochemical parameters such as conductivity (Arif; Kumar and Parveen, 2020; Wu et al., 2020). In fact, high salinity has been previously observed in sector 3 (Bora; Thomaz and Padial, 2020), however, a profile of the salt wedge with measurements considering tides and seasonal variations is still necessary to establish the salinity value in this sector.

3.6.2 Chemical Analysis

3.6.2.1 Pesticides and trace elements in water

The results may indicate that agriculture around of the Guaraguaçu River, as shown and stated by the Ecological Economic Zoning of Paraná (2016), have poor impact in the water quality. Indeed, it is historically mostly of family/subsistence origin, with the main crops being banana, cassava, beans, corn, and rice. However, it is worth noting that even though no pesticides were detected using the method employed, this does not necessarily indicate the absence of pesticides in the region.

Analyzing the presence of trace elements on water, manganese, iron and aluminum were found. Two of them, Fe and Al, exceeding the legal limits and the intrinsic characteristics of the geomorphology and pedology of the studied region should take into account. The influence of the rainy collection period can contribute to the increase of these essential trace elements due to weathering of rocks and leaching of the surrounding soils into the river. However, it is worth noting that previous works have shown that even in the sediment matrix there is no significant presence of trace elements in the region of the Guaraguaçu River. In Angeli et al. (2020), when analyzing 135 sediment sample surfaces in a wide region of the Paranaguá Bay estuarine complex, where the Guaraguaçu River flows, it was also shown that there is no strong influence of trace elements. Samples collected at the mouth of the Guaraguaçu River did not demonstrate significant contamination by elements such as Cr, Cu, Ni, and Pb.

Despite that, in Cavallini et al. (2018), when analyzing fecal samples of *Lontra longicaudis* (OLFERS, 1818), a top predator in the Guaraguaçu River, lead concentrations around 1 mg.L^{-1} were found, indicating abnormalities. This reinforces the idea of the possibility of trophic biomagnification of trace elements in the biota. Concentrations of cadmium, manganese, and lead were also determined in Neotropical otter feces (Cavallini; Reis and Tiepolo, 2020), which are considered potentially toxic and bioaccumulative metal ions. This contamination may be related primarily to the proximity of the Guaraguaçu River mouth to the port of Paranaguá, as well as the historical and rapid development of the Paraná coast.

Therefore, even if trace elements have not been quantified or have low concentration values in the water matrix, it does not necessarily indicate that the region is not influenced by these elements. Species such as *H. malabaricus*, as well as the Neotropical otter used in some studies, are top predator species that can undergo biomagnification processes, leading to harmful effects on their health. This is particularly concerning as these animals are commonly consumed by humans, as demonstrated in the study by Leite et al. (2021), which investigated the bioconcentration of trace elements in the muscle tissue in two Neotropical rivers in Brazil, providing evidence of biomagnification along the food chain.

3.6.2.2. Trace elements in the muscle of *H. malabaricus*

Trace elements, especially non-essential ones, are mostly considered elements with significant carcinogenic, mutagenic, teratogenic, nephrotoxic, immunotoxic, and neurotoxic potential, capable of disrupting enzymatic proteins in the bodies of living organisms, such as fish (Häder et al., 2020; Nordberg; Nordberg, 2022). Therefore, the results suggested that the Guaraguaçu River is not experiencing concerning levels of pollution when considering contamination by non-essential elements such as Pb, Cd, and Ni, as they were not quantified in the fish muscle tissue. However, the non-essential element Cr was found in three individuals (two in sector 2 and one in sector 3) with concentrations above the legal limits. Chromium can be naturally found in rocks, soil, and living organisms, but its concentration tends to increase in aquatic environments due to certain human activities such as industrial processes and wastewater discharge, causing serious health problems in organisms (Jarapala; Kandlakunta; Thingnganing, 2014), including oxidative stress, DNA damage, cell apoptosis, and alterations in gene expression (Leite et al., 2021).

In addition to non-essential trace elements, there are those considered essential for life, which are major components of enzymes, hormones, and animal body cells (Qu et al., 2014). However, if there is an excessive concentration of these essential elements such as Zn, Fe, Cu, and Mn in the water body, sediment, or other matrices, they can cause serious health problems in organisms.

Manganese plays an important role as a cofactor of various enzymes, being an essential element found in various sources such as food, soil, and water (Li; Yang, 2018). Zinc is associated with the maintenance of the immune system, body growth, cell division, DNA synthesis, cellular metabolism, reproduction, and participation in protein and cell membrane structure (Garai et al., 2021). It is also considered to have a protective effect against the toxicity of cadmium and lead (Kumar et al., 2021). Similarly, iron (Fe) plays an important role in the growth and development of organisms, being essential for cellular metabolism. However, excessive iron can cause tissue damage (Ayhan; Yaman, 2022), while extremely high concentrations of zinc can decrease immune function (Javed; Usmani, 2016). Zinc toxicity is species-specific and varies with different stages of fish development, environmental factors, and the concentration of the element in the environment. It can result in gill tissue destruction, alterations in swimming behavior, respiratory problems leading to cardiac failure, and fish mortality (Skidmore, 1964).

In Kumar et al. (2021), a study conducted in three fishing locations in Mumbai, India, which are influenced by boat traffic and domestic and industrial effluents, bioaccumulation of metals was observed in thirty fish species. Similar to the current study, zinc (Zn) was the most found element in fish. However, it is noted that the concentration of Zn in the present study is much higher than the concentrations reported in Kumar et al. (2021), which consequently found chromium (Cr) concentrations above the legal limits set by Brazilian regulations in almost all analyzed species.

Therefore, it can be observed that the concentration of a specific trace element in organisms depends on the species being studied, the nature and feeding habits of the fish, the studied environment, external influences on that environment, such as potential sources of contamination, and the sampling period (Tesser; Rocha and Castro, 2021; Leite et al., 2021). Among the sources of trace elements, natural sources can be highlighted, mainly related to geological leaching of rocks and soil erosion caused by water flow, as well as anthropogenic sources, which can include the discharge of domestic and industrial effluents into water bodies, mining activities, and agriculture (Voigt et al., 2015).

The intrinsic characteristics of each fish species are strong factors in the accumulation of trace elements. Voigt et al. (2015) observed that high concentrations of Al, Zn, Fe, and Mn in all analyzed tissues can possibly be related to sediment contamination and the life history and interaction of the *Geophagus brasiliensis* species. Similarly, due to *H. malabaricus* being a carnivorous species with an ambush predation strategy, primarily inhabiting lentic environments such as shallow waters near marginal or submerged vegetation during its adult phase (Reis et al., 2017; Paula; Risso and Martinez, 2021; Leite et al., 2021), the influence of sediment can be a factor in the possibility of bioaccumulation of trace elements.

The higher concentration of iron observed in the fish from S3, as demonstrated by the ordination (Fig. 2), can be related to the geology and geomorphology of the area. This sector is characterized by the presence of marine terraces, which represent ancient marine levels that have varied over the past six thousand years. These terraces have an erosive surface, characterized by dark brown coloration caused by the enrichment of organic matter and iron hydroxides. This, in turn, influences the coloration of the Guaraguaçu River waters, which are predominantly transparent but have a black-reddish hue due to both soil erosion and material originating from the forest (IAT, 2006).

Among the main soils found in the coastal plain of Paraná, Espodossols and Organossols can be highlighted. Organossols are hydromorphic soils, poorly evolved, and mainly derived from organic matter in different stages of decomposition under permanent water saturation conditions (IAT, 2006). On the other hand, Espodossols are characterized by their sandy texture with the accumulation of organic matter and/or iron oxides. They are also moderately to extremely acidic soils and can have high levels of extractable aluminum (Zaroni; Santos, 2021).

Espodossols mainly occur in flat terrain and are therefore found only in the coastal plain of Paraná. Due to the large amount of sand and high permeability, these soils are highly unsuitable for agricultural use (Silva et al., 2013), which also explains the low agricultural influence in the region, with a focus on family farming. Thus, the concentration of organic matter, iron oxides, and the acidic pH found in the waters of sector 1 of the Guaraguaçu River, possibly related to the zinc concentration, may be associated with the intrinsic characteristics of the soils in the region.

Another factor that could explain the concentration of Mn in almost all analyzed individuals, as well as Fe and Zn in all organisms is the fact that these trace elements are essential. The presence of these elements in a larger number of organisms and in considerable concentrations could be another explanation for the possibility of bioaccumulation, as shown in various studies with different fish species in environments experiencing similar anthropogenic interference to the Guaraguaçu River (Kamaruzzaman et al., 2011; Jesus et al., 2014; Jarapala; Kandlakunta;Thingnganing, 2014; Javed; Usmani, 2016). The sampling period can also affect the bioavailability of these elements in the environment. For example, there may be a higher concentration of trace elements, such as aluminum, during rainy periods (Leite et al., 2021), due to the increased input of sediments and effluents carried by rainfall.

The presence of aluminum (Al) occurring almost exclusively in the muscle tissues of fish from S1 and S3, characterized as pristine and minimally impacted, respectively, suggests that the concentration be derived from natural sources. Aluminum in natural waters originates from weathering of rocks and minerals, and there are no specific regulatory limits for its concentration in fish. However, it is important to investigate the influence of aluminum on the health of fish in the Guaraguaçu River, as this element can be neurotoxic and can cause respiratory and reproductive diseases to animals (Gemsemer et al., 2018).

3.6.3. Biochemical biomarkers

The decrease in acetylcholinesterase activity in S2 may be related to the presence of a neurotoxic substance with anticholinesterase effects, potentially impairing motor coordination and locomotion of organisms (Oliveira et al., 2019). The inhibition of AChE activity is widely known as an exposure biomarker for organophosphate and carbamate pesticides (Fajardo and Ocampo, 2018). However, diverse contaminants have also been classified as inhibitors of AChE activity, including metals and other classes of organic environmental pollutants (Fu et al., 2018).

The gills are the main and often the first route of contact with xenobiotics in addition to being a crucial organ for respiration and osmoregulation in fish (Kumar et al., 2017). GST is one of the enzymes involved in the biotransformation of xenobiotics into more hydrophilic metabolites for subsequent excretion (Kroon; Streten; Harries, 2017), which also plays a role in biological stress control due to abiotic factors. The increase in GST activity in S3 can be directly related to the presence of salinity, as this sector is known to be influenced by tides. This change in water salinity can cause osmotic stress in aquatic animals such as fish (Evans; Kultz, 2020). The presence of salinity may also explain the decrease in catalase activity in the gills of S3. Mozanzadeh et al. (2021) demonstrated in their study that an increase in salinity could reduce catalase activity in certain fish tissues, such as the liver. This suggests that the oxidative stress response of fish to changes in water salinity are related to the specificity of each species, as well as the developmental stage of the organism and the concentration of salt in the water.

Both xenobiotics and environmental factors such as salinity, temperature, and pH can cause oxidative stress by producing reactive oxygen species (ROS), which can be neutralized by antioxidant system (SOD, CAT, GPx, and GST) as well as GSH (Chowdhury; Saikia, 2020; García-Caparrós et al., 2021). The increase in SOD and GPx activity may be related to the presence of elevated concentrations of ROS, especially in S2, which is heavily influenced by anthropogenic activities. Despite the increased activity of antioxidant enzymes, the lipoperoxidation also increased, which can indicate the presence of concerning concentrations of certain xenobiotics.

Liver is commonly used for biomarker analysis because it is one of the main organs responsible for the metabolism and detoxification of xenobiotics. The cofactor reduced glutathione plays a role in binding to GST and GPx enzymes to detoxify the body from xenobiotics and combat reactive oxygen species, respectively (Burkina; Zlabek and Zamaratskaia, 2015; Kroon; Streten; Harries, 2017). The increased of GST, GPx and SOD activities, and concentration of GSH and LPO demonstrate that Sector 2 indeed be the most impacted by anthropogenic actions, leading to changes in enzymatic activities and increased cases of lipoperoxidation, which are damages to the lipid membrane, indicating tissue damage in the fish.

Metallothionein plays a crucial role in immune response by participating in the detoxification and transport of metals within the bodies of various animal groups, including vertebrates and invertebrates. El-Khayat et al. (2020) observed a strong positive correlation between metallothionein activity and the presence of metals such as cadmium, lead, and copper in various fish tissues, with the liver showing the highest correlation, highlighting the potential of metallothionein as a biomarker in toxicological studies and for assessing environmental stress. However, in the case of the Guaraguaçu River, no significant difference in metallothionein concentration was observed among the three sectors of the river.

3.6.4. Histophatological biomarkers

The use of histopathological analysis provides additional information when combined with various other biomarkers, allowing for a more comprehensive and integrated study in biomonitoring. Among the lesions found in the gills of sector 2, only hyperplasia with partial or total fusion indicating moderate alterations with potential reversibility and are related to an increase in the number of cells, consequently reducing the space between the gill filaments. The presence of hyperplasia, as well as an increase in mucous cells, represents tissue protection mechanisms against pathogens and contaminants from the external environment, however it hinders gas exchange by the organisms, decreasing blood oxygenation (Mallatt, 1985; Marinović et al., 2021).

In previous studies focused on aquatic environments contaminated by sewage discharge (Liebel; Tomotake and Oliveira-Ribeiro, 2013; Pereira et al., 2020), the presence of trace elements (Salgado et al., 2021; Oliveira et al., 2022), and pesticides (Oliveira et al., 2019), organisms exhibited similar responses and lesions to those found in the present study. These include epithelial lifting of the secondary lamella found in sector 2, that can happen due to infiltration of fluid between epithelium and basement membrane that ultimately increase diffusion distance for gas exchange; aneurysms, found principally in gills of sector 2, are related to blood accumulation and dilation of the branchial artery (Flores-Lopes; Thomaz, 2011); and loss of microridges in pavement cells related to the gills of sector 2 and 3, where this structure which primarily serve to increase the surface area of cells in contact with the external environment and also act as structures capable of retaining mucus to protect the entire gill structure (Mela et al., 2013). Blood congestion, can leads to the disruption of pillar cells due to the intense blood flow to the secondary lamellae, can cause aneurysms (Hassaninezhad et al., 2014).

Freitas et al. (2022) studied histopathological biomarkers in *H. malabaricus* in the Mearim River, located in the Brazilian Amazon, which is affected by contamination from anthropogenic activities. The fish in this region also exhibited biological responses to contaminants, including congestion, aneurysms, hyperplasia with partial or total fusion of lamellae, mucus proliferation, and epithelial lifting, as well as the fish captured in sector 2 of the present work. All these alterations indicate a strong influence of anthropogenic actions and water degradation due to the presence of contaminants in the study area, suggesting that gill alterations do not have a specific relationship with a particular xenobiotic, as similar responses can be observed for different contaminants. According to Oliveira et al., 2022, the gill lesions reflect a generalized stress response.

Another important aspect to highlight is the influence of seasonality on the responses of biomarkers to xenobiotics (Salgado et al., 2019; Marinović et al., 2021). The concentration of water contaminants can vary with the water flow and the influence of a wetter or drier period. Salgado et al. (2021) found higher responses of histopathological biomarkers in fish collected during the cold-dry period, which may be related to lower precipitation and, consequently, lower water flow, facilitating higher concentrations of contaminants in the water or even in sediments. However, Pereira et al. (2020), when evaluating environmental contamination of a river in northeastern Brazil through the analysis of histopathological biomarkers in the gills of *Psectrogaster amazônica*, found that the indexes of histological alterations were higher during the rainy season, which can be explained by the greater input of contaminants through surface runoff. Therefore, it is crucial to conduct a comprehensive study on the influence of seasonality on organism responses to contaminants, as the results can vary from region to region and, primarily, due to the biology of the individuals.

Among the liver alterations and severe lesions, necrosis or cell death, and hepatocyte vacuolization can be highlighted in this study. Vacuolation, found in liver fish of sector 2, indicate decrease of stored energy in the form of glycogen or represent a degenerative change in which there is fluid distension of organelles such as endoplasmic reticulum and Golgi apparatus (Mela et al., 2007). Increased hepatocyte vacuolation, is often cited as a toxicological response in fish, although the exact composition of the vacuolation and mechanism of formation are frequently not elucidated. According to Gonzales et al. (1993), necrosis found in the fish liver are usually related to contaminants found in water or sediment. The principle of hepatic necrosis results from the presence of chemicals within cells causing disturbs on biochemical process as enzyme inhibition, failure on protein synthesis, carbohydrate metabolism, reactive oxidative species production, damages in cell membrane and failure of ATP synthesis (Mela et al., 2013; Kumar et al., 2017). These two irreversible lesions, found in fish's liver of sectors 2 and 3, demonstrate and indicate the presence of contaminants capable of altering the health of organisms.

Melanomacrophage centers is a collection of macrophages that contain hemosiderin, lipofuscin, and seroids as well as the melanin pigment caused by inflammation of most teleost (Rabitto et al., 2005). These structures increase in size or frequency in conditions of environmental stress and have been suggested as reliable biomarkers for water quality in terms of both deoxygenation and anthropogenic chemical pollution (Mela et al., 2007, 2013). The presence of melanomacrophage centers, can be associated with regions with history of contamination (Viana et al., 2021), influenced for example by trace elements as copper (Mela et al., 2013). These trace elements can affect the normal functioning of the liver and be responsible for hepatic insufficiency (Savassi et al., 2020). Blood congestion is a liver dysfunction due to venous congestion, usually as a result of dysfunction of the heart, which is also known as congestive heart failure (Cotran et al., 2005). The fish liver is especially liable to chemical products due to the slow blood flow in relation to the cardiac output. Liver and gills alterations, such as those observed in this study, could result in severe physiological problems and provide reliable information on stress to a broad range of environmental pollutants.

3.6.5. Biomarker of genotoxicity

The frequencies of micronuclei generally vary according to the season of the year, the type of contaminant involved, and the fish species under evaluation (Ali; El-Shehawi; Seehy, 2008; Obiakor; Okonkwo; Ezeonyejiaku, 2012). It is important to highlight the presence of micronuclei in at least one fish from each sector, which emphasizes the possibility of water contamination in this river, particularly related to anthropogenic activities (Canedo et al., 2021) such as domestic sewage discharge and the presence of trace elements (Hussain et al., 2018; Ali et al., 2020). The presence of these impacts increasingly plausible since previous studies (Francisco et al., 2019; Lehun et al., 2021) have found micronuclei in organisms living in regions impacted by practically the same probably causes found in the Guaraguaçu River. These alterations would rarely occur naturally, as they are considered mutagenic characteristics, as the increase in frequency is usually influenced by exposure to clastogenic or aneugenic substances that are closely linked to environmental disturbances (Hayashi, 2016; Ali et al., 2020).

Salgado et al., 2019 reported the lack of micronuclei or vacuolated alterations, but blebbed, notched, and lobed nuclei were found. However, Oliveira et al., 2019 observed all the alterations described in the present study, indicating that the variation in the presence or absence of nuclear morphological alterations also depends on the contamination history of the study site and the biomonitor species used, as each organism has different biology. This was demonstrated in Rodriguez-Cea et al. (2003) using different fish species, where only brown trout showed potential as a biomonitor species by demonstrating higher sensitivity to genotoxic compounds such as the trace element cadmium when comparing contaminated and non-contaminated regions. Indeed, in addition to the characteristics of the study area and the biological model, one of the main reasons that makes it challenging to use nuclear alterations in erythrocytes as biomarkers to interpret genotoxic damage is the distinct origins of micronucleus formation and other nuclear abnormalities (Krupina; Goginashvili; Cleveland, 2021).

3.7. CONCLUSION

In the water samples, pesticides were not detected, just manganese, iron and aluminum were quantified, indicating a low concentration of trace elements. However, in the muscles of the fish some trace elements were detected. This can evidence that the study with a top predator is interesting, because it can analyze the accumulation of xenobiotics over time, since these animals can consume other living organisms. The use of biomarkers may be more sensitive than chemical techniques, because, although the presence of pesticides and some metals in water was not evidenced, prominent responses of biomarkers were presented. Severe damage was analyzed mainly in sector 2 with a certain level of human impact, such as neurotoxicity, lipoperoxidation, histopathological damage in the liver and gills and blood mutagenicity. These results support the hypothesis proposed in this study and create a valuables dataset that can inform the problems for the social-vulnerable residents who use the river for survival, as well as for decision-making by policymakers.

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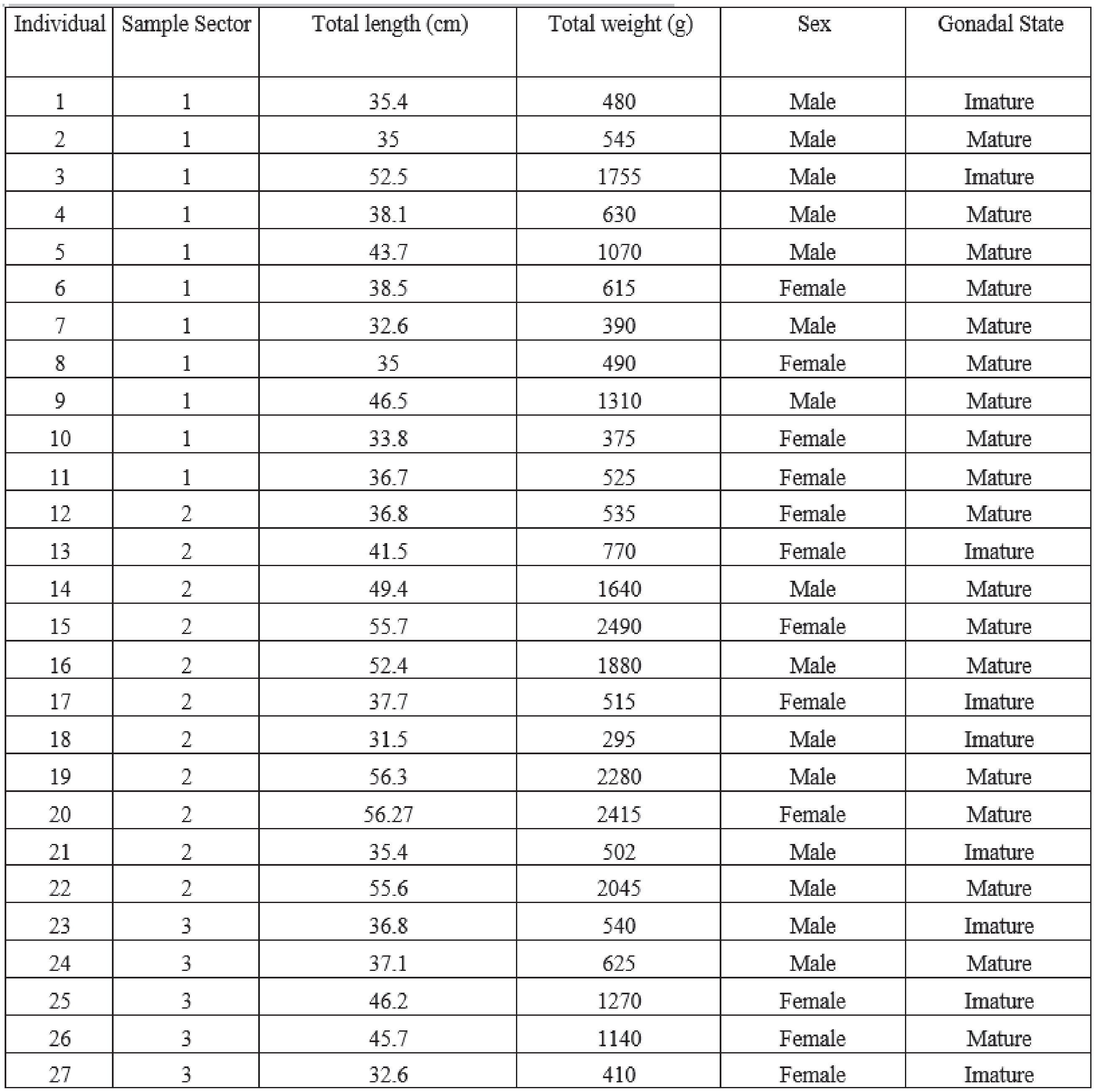
3.10. SUPPLEMENTARY MATERIAL

Supplementary material 1. Measurements of length and weight, in addition to the sex and gonadal state of the twenty-seven fish captured along the Guaraguaçu River divided into three sectors.



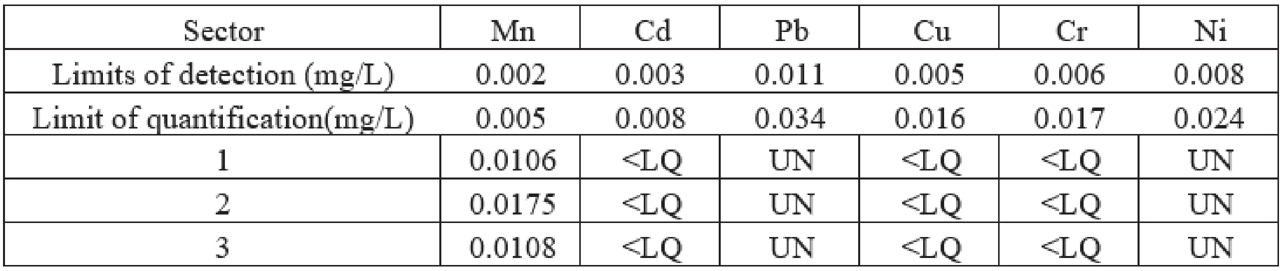
Individual	Sample Sector	Total length (cm)	Total weight (g)	Sex	Gonadal State
1	1	35.4	480	Male	Imature
2	1	35	545	Male	Mature
3	1	52.5	1755	Male	Imature
4	1	38.1	630	Male	Mature
5	1	43.7	1070	Male	Mature
6	1	38.5	615	Female	Mature
7	1	32.6	390	Male	Mature
8	1	35	490	Female	Mature
9	1	46.5	1310	Male	Mature
10	1	33.8	375	Female	Mature
11	1	36.7	525	Female	Mature
12	2	36.8	535	Female	Mature
13	2	41.5	770	Female	Imature
14	2	49.4	1640	Male	Mature
15	2	55.7	2490	Female	Mature
16	2	52.4	1880	Male	Mature
17	2	37.7	515	Female	Imature
18	2	31.5	295	Male	Imature
19	2	56.3	2280	Male	Mature
20	2	56.27	2415	Female	Mature
21	2	35.4	502	Male	Imature
22	2	55.6	2045	Male	Mature
23	3	36.8	540	Male	Imature
24	3	37.1	625	Male	Mature
25	3	46.2	1270	Female	Imature
26	3	45.7	1140	Female	Mature
27	3	32.6	410	Female	Imature

1627 Supplementary material 2. Pesticides analyzed in water in the three sectors.

Analysis	Sector 1	Sector 2	Sector 3	LQ	Reference	Date analysis
Alachlor	< 0,05 µg/L	< 0,05 µg/L	< 0,05 µg/L	0,05	EPA 3510C:1996, 8270D:2014	04/04/2022
Aldrin + Dieldrin	< 0,03 µg/L	< 0,03 µg/L	< 0,03 µg/L	0,03	EPA 3510C:1996, 8270D:2014	04/04/2022
Atrazine	< 0,05 µg/L	< 0,05 µg/L	< 0,05 µg/L	0,05	EPA 3510C:1996, 8270D:2014	04/04/2022
Bentazone	< 100,0 µg/L	< 100,0 µg/L	< 100,0 µg/L	100,0	EPA 3510C:1996, 8270D:2014	04/04/2022
Chlordane	< 0,05 µg/L	< 0,05 µg/L	< 0,05 µg/L	0,05	EPA 3510C:1996, 8270D:2014	04/04/2022
Endosulfan	< 0,05 µg/L	< 0,05 µg/L	< 0,05 µg/L	0,05	EPA 3510C:1996, 8270D:2014	04/04/2022
Endrin	< 0,03 µg/L	< 0,03 µg/L	< 0,03 µg/L	0,03	EPA 3510C:1996, 8270D:2014	04/04/2022
Heptachlor + Heptachlor epoxide	< 0,01 µg/L	< 0,01 µg/L	< 0,01 µg/L	0,01	EPA 3510C:1996, 8270D:2014	04/04/2022
Hexachlorobenzene	< 0,005 µg/L	< 0,005 µg/L	< 0,005 µg/L	0,005	EPA 3510C:1996, 8270D:2014	04/04/2022
Lindane	< 0,05 µg/L	< 0,05 µg/L	< 0,05 µg/L	0,05	EPA 3510C:1996, 8270D:2014	04/04/2022
Metolachlor	< 0,05 µg/L	< 0,05 µg/L	< 0,05 µg/L	0,05	EPA 3510C:1996, 8270D:2014	04/04/2022
Methoxychlor	< 0,03 µg/L	< 0,03 µg/L	< 0,03 µg/L	0,03	EPA 3510C:1996, 8270D:2014	04/04/2022
Pendimethalin	< 0,05 µg/L	< 0,05 µg/L	< 0,05 µg/L	0,05	EPA 3510C:1996, 8270D:2014	04/04/2022
Pentachlorophenol	< 0,05 µg/L	< 0,05 µg/L	< 0,05 µg/L	0,05	EPA 3510C:1996, 8270D:2014	04/04/2022
Propanyl	< 0,1 µg/L	< 0,1 µg/L	< 0,1 µg/L	0,1	EPA 3510C:1996, 8270D:2014	04/04/2022
Simazine	< 0,05 µg/L	< 0,05 µg/L	< 0,05 µg/L	0,05	EPA 3510C:1996, 8270D:2014	04/04/2022
Trifluralin	< 0,05 µg/L	< 0,05 µg/L	< 0,05 µg/L	0,05	EPA 3510C:1996, 8270D:2014	04/04/2022
Malathion	< 0,05 µg/L	< 0,05 µg/L	< 0,05 µg/L	0,05	EPA 3510C:1996, 8270D:2014	04/04/2022
Parathion	< 0,04 µg/L	< 0,04 µg/L	< 0,04 µg/L	0,04	EPA 3510C:1996, 8270D:2014	04/04/2022
Permethrin	< 0,05 µg/L	< 0,05 µg/L	< 0,05 µg/L	0,05	EPA 3510C:1996, 8270D:2014	04/04/2022
Azinphos	< 0,01000	< 0,01000	< 0,01000	0,01000	EPA 3510C:1996, 8270D:2014	04/04/2022

1628 Legend: LQ- Limit of Quantification; EPA- Environmental Protection Agency

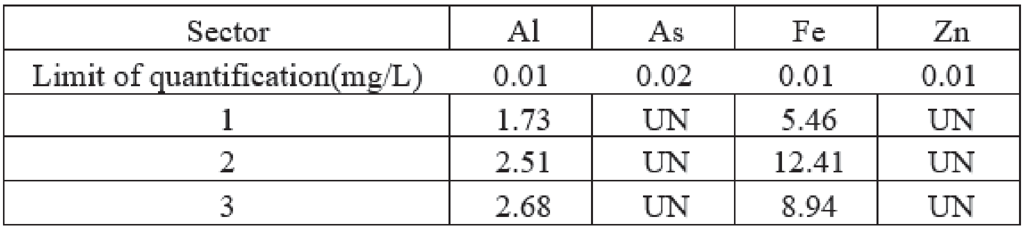
Supplementary material 3. Analysis of trace elements in water using a Flame Atomic Absorption Spectrophotometer (FAAS)



Sector	Mn	Cd	Pb	Cu	Cr	Ni
Limits of detection (mg/L)	0.002	0.003	0.011	0.005	0.006	0.008
Limit of quantification(mg/L)	0.005	0.008	0.034	0.016	0.017	0.024
1	0.0106	<LQ	UN	<LQ	<LQ	UN
2	0.0175	<LQ	UN	<LQ	<LQ	UN
3	0.0108	<LQ	UN	<LQ	<LQ	UN

Legend: LQ- Limit of Quantification; UN- Undetectable

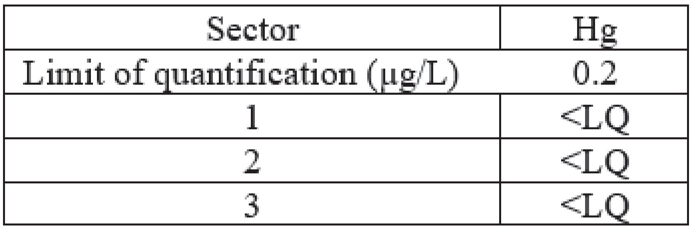
Supplementary material 4. Analysis of trace elements in water using Inductively Coupled Plasma Optical Emission Spectrometry (ICP - OES)



Sector	Al	As	Fe	Zn
Limit of quantification(mg/L)	0.01	0.02	0.01	0.01
1	1.73	UN	5.46	UN
2	2.51	UN	12.41	UN
3	2.68	UN	8.94	UN

Legend: UN- Undetectable

Supplementary material 5. Analysis of mercury in water using Inductively Coupled Plasma Optical Emission Spectrometry (ICP - OES)



Sector	Hg
Limit of quantification (μg/L)	0.2
1	<LQ
2	<LQ
3	<LQ

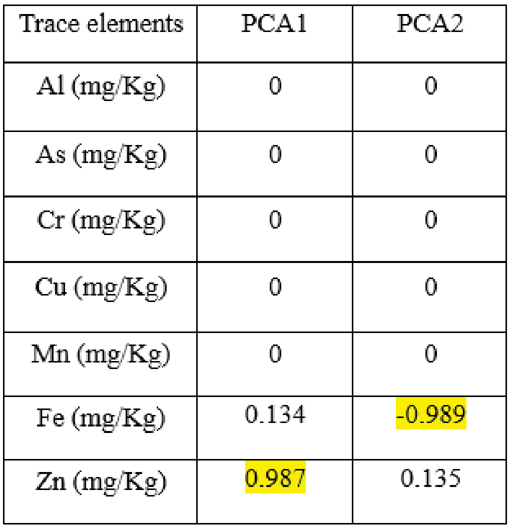
Legend: LQ- Limit of Quantification

Supplementary material 6. Table of concentration (mg/Kg) of trace elements in *Hoplias malabaricus* muscle in the three sampling sectors.

	Trace elements									
	Al	As	Cr	Cu	Mn	Fe	Zn	Cd	Ni	Pb
LQ (mg/L) *	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
Brazilian Legislation (mg/Kg) **	-	1	0.1	30	-	-	50	0.05	5	0.3
Statistical***	p-value = 0.19 (> 0.05)									
Trace elements (mg/Kg)										
Sector	Al	As	Cr	Cu	Mn	Fe	Zn	Cd	Ni	Pb
S1	3.871	ND	ND	ND	4.608	34.585	83.939	ND	ND	ND
S1	14.675	ND	ND	ND	8.830	35.904	68.525	ND	ND	ND
S1	8.159	ND	ND	ND	ND	34.666	75.676	ND	ND	ND
S1	ND	ND	ND	ND	6.646	22.852	103.558	ND	ND	ND
S1	ND	ND	ND	ND	5.349	39.987	36.713	ND	ND	ND
S2	ND	ND	ND	ND	ND	15.502	30.947	ND	ND	ND
S2	ND	ND	ND	ND	ND	38.911	51.339	ND	ND	ND
S2	2.014	ND	3.469	ND	14.572	75.367	194.459	ND	ND	ND
S2	ND	ND	2.281	ND	ND	46.845	34.749	ND	ND	ND
S2	ND	ND	ND	ND	5.164	84.382	64.138	ND	ND	ND
S3	2.727	ND	5.551	2.671	2.417	80.452	51.458	ND	ND	ND
S3	3.592	ND	ND	2.415	ND	44.252	56.152	ND	ND	ND
S3	5.362	ND	ND	ND	ND	60.098	30.586	ND	ND	ND
S3	2.432	5.569	ND	ND	ND	48.370	21.206	ND	ND	ND
S3	4.482	ND	ND	3.435	ND	52.089	54.259	ND	ND	ND

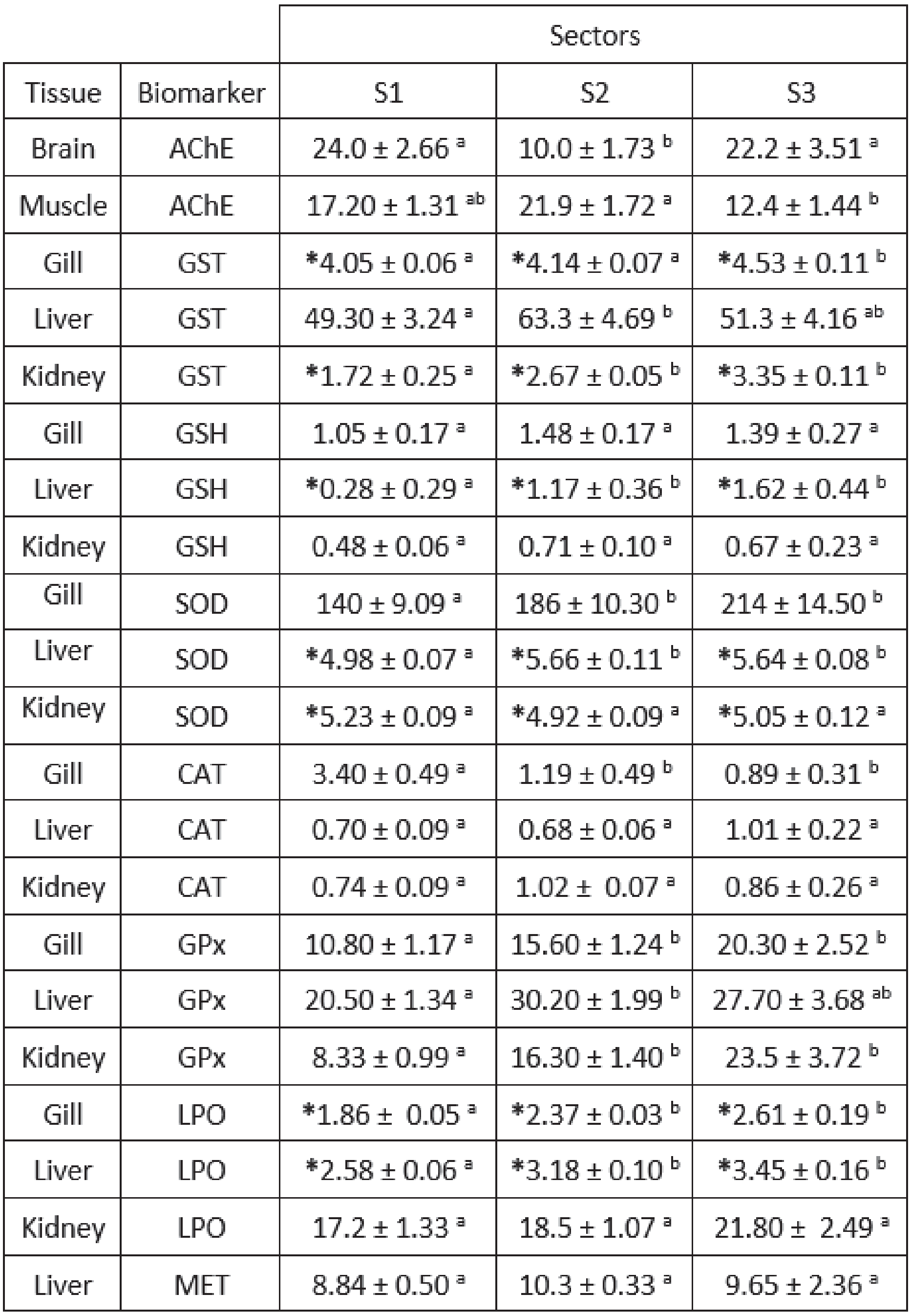
Legend: * LQ: ICP-OES Limit of Quantification ; ** DECREE 55.871 of 03/26/1965, Modifies DECREE No. 50.040, 01/24/1961, referring to regulatory norms for the use of food additives / ANVISA: RDC No. 42, 08/29/2013, Provides for the Technical Regulation MERCOSUR on Maximum Limits of Inorganic Contaminants in Food; ***p-value > 0.05 means no significant difference between sectors; ND - Not Determined.

Supplementary material 7. Loadings table representing the correlation of each original variable (trace elements) with the x (PCA1) and y (PCA2) axes. Zn and Fe are the two elements that have the greatest relationship with the PCA1 and PCA2 axes, respectively.



Trace elements	PCA1	PCA2
Al (mg/Kg)	0	0
As (mg/Kg)	0	0
Cr (mg/Kg)	0	0
Cu (mg/Kg)	0	0
Mn (mg/Kg)	0	0
Fe (mg/Kg)	0.134	-0.989
Zn (mg/Kg)	0.987	0.135

Supplementary Material 8. Mean values and standard errors (Mean ± SE) of the biochemical biomarkers responses.



		Sectors		
Tissue	Biomarker	S1	S2	S3
Brain	AChE	24.0 ± 2.66 ^{a}	10.0 ± 1.73 ^{b}	22.2 ± 3.51 ^{a}
Muscle	AChE	17.20 ± 1.31 ^{ab}	21.9 ± 1.72 ^{a}	12.4 ± 1.44 ^{b}
Gill	GST	*4.05 ± 0.06 ^{a}	*4.14 ± 0.07 ^{a}	*4.53 ± 0.11 ^{b}
Liver	GST	49.30 ± 3.24 ^{a}	63.3 ± 4.69 ^{b}	51.3 ± 4.16 ^{ab}
Kidney	GST	*1.72 ± 0.25 ^{a}	*2.67 ± 0.05 ^{b}	*3.35 ± 0.11 ^{b}
Gill	GSH	1.05 ± 0.17 ^{a}	1.48 ± 0.17 ^{a}	1.39 ± 0.27 ^{a}
Liver	GSH	*0.28 ± 0.29 ^{a}	*1.17 ± 0.36 ^{b}	*1.62 ± 0.44 ^{b}
Kidney	GSH	0.48 ± 0.06 ^{a}	0.71 ± 0.10 ^{a}	0.67 ± 0.23 ^{a}
Gill	SOD	140 ± 9.09 ^{a}	186 ± 10.30 ^{b}	214 ± 14.50 ^{b}
Liver	SOD	*4.98 ± 0.07 ^{a}	*5.66 ± 0.11 ^{b}	*5.64 ± 0.08 ^{b}
Kidney	SOD	*5.23 ± 0.09 ^{a}	*4.92 ± 0.09 ^{a}	*5.05 ± 0.12 ^{a}
Gill	CAT	3.40 ± 0.49 ^{a}	1.19 ± 0.49 ^{b}	0.89 ± 0.31 ^{b}
Liver	CAT	0.70 ± 0.09 ^{a}	0.68 ± 0.06 ^{a}	1.01 ± 0.22 ^{a}
Kidney	CAT	0.74 ± 0.09 ^{a}	1.02 ± 0.07 ^{a}	0.86 ± 0.26 ^{a}
Gill	GPx	10.80 ± 1.17 ^{a}	15.60 ± 1.24 ^{b}	20.30 ± 2.52 ^{b}
Liver	GPx	20.50 ± 1.34 ^{a}	30.20 ± 1.99 ^{b}	27.70 ± 3.68 ^{ab}
Kidney	GPx	8.33 ± 0.99 ^{a}	16.30 ± 1.40 ^{b}	23.5 ± 3.72 ^{b}
Gill	LPO	*1.86 ± 0.05 ^{a}	*2.37 ± 0.03 ^{b}	*2.61 ± 0.19 ^{b}
Liver	LPO	*2.58 ± 0.06 ^{a}	*3.18 ± 0.10 ^{b}	*3.45 ± 0.16 ^{b}
Kidney	LPO	17.2 ± 1.33 ^{a}	18.5 ± 1.07 ^{a}	21.80 ± 2.49 ^{a}
Liver	MET	8.84 ± 0.50 ^{a}	10.3 ± 0.33 ^{a}	9.65 ± 2.36 ^{a}

Legend: **AChE**- acetylcholinesterase; **GST**- Glutathione S-transferase; **GSH**- Reduced Glutathione; **SOD**- Superoxide dismutase; **CAT**- Catalase; **GPx**-Glutathione peroxidase; **LPO**- Lipoperoxidation; **MET**- Metallothionein; **S1**- Sector 1; **S2**- Sector 2; **S3**- Sector 3; (*) Show the use of log transformation to achieve the assumptions of homogeneity and normality of the data; Different letters mean difference between sectors.

4. CONSIDERAÇÕES FINAIS

Apesar do Rio Guaraguaçu possuir uma importância social provendo o abastecimento de água para os municípios de Pontal do Paraná, Matinhos e Paranaguá (Elste et al., 2019), econômica voltada para a pesca de subsistência (Reis et al., 2015) e ecossistêmica, a partir da análise dos parâmetros físico-químicos da água foi demonstrado uma clara e aparente diferença quando separado entre os três setores descritos, principalmente ao observar a condutividade e transparência dos setores. Observa-se que a condutividade no setor 2 se encontra maior que no setor 3, setor que sofre com a influência de marés por estar mais próximo da foz do rio, e consequentemente seria o setor com maior condutividade e salinidade do Rio Guaraguaçu (Staszczak; Rocha, 2018). Tal fato, pode ser explicado principalmente pela influência do canal retificado do rio Pery, que possui um aterro sanitário além da presença de lançamento de esgoto doméstico, podendo estar influenciando e apresentando um aumento da condutividade pelo excesso da concentração de elementos, como fósforo e nitrogênio, e seus íons considerados naturalmente limitantes do crescimento biótico (Reis et al., 2015; Souza et al., 2019; Singo; Araújo-Ramos; Rocha, 2020).

Nas análises químicas da água, não foi encontrado uma contaminação por agrotóxicos no Rio Guaraguaçu, pela metodologia utilizada. O elemento traço manganês foi quantificado, mas abaixo do limite estipulado por lei. Ferro e alumínio, dois elementos essenciais, apresentaram concentrações maiores do que o estipulado por lei e sua presença pode estar relacionada às características geomorfológicas e pedológicas da região. Entretanto, assim como os agrotóxicos, os elementos traço podem bioacumular no tecido animal e ocasionar sérios problemas para a saúde do organismo. A ausência de agrotóxicos em concentrações significativas pode estar relacionada com a agricultura que historicamente em sua maioria é de origem familiar/subsistência, possuindo como principais cultivos a banana, mandioca, o feijão, o milho e o arroz (ZEE, 2016).

Apesar da maioria dos elementos traços não estarem presentes na água de acordo com os métodos utilizados, no músculo das traíras, o elemento Cr foi encontrado em dois organismos do setor 2 e um do setor 3, os setores caracterizados por possuírem diferentes graus de impacto, o que pode indicar a contaminação da região por determinadas substâncias. No geral, os únicos elementos que apareceram em todos os organismos de todos os setores foram Fe que não possui limite estabelecido, e Zn que possuía limite, mas a legislação que o estabelecia foi revogada. A presença de tais elementos em todas as amostras pode estar relacionada ao tipo de solo presente (IAT, 2006) ou sedimento na região do rio Guaraguaçu, já que estatisticamente não há diferença significativa entre os três setores para todos os elementos traço analisados. No entanto, vale destacar a importância de uma atualização na legislação brasileira ao estipular limites de mais elementos traço em alimentos, água e sedimento devido principalmente o risco de em concentrações exacerbadas, mesmo elementos essenciais, causarem problemas e riscos à saúde dos peixes e consequentemente dos seres humanos.

Ao analisar as respostas das atividades de biomarcadores bioquímicos, verificamos que existe uma diferença entre os setores amostrados. Evidencia-se assim, que principalmente no setor 2, que possui extrema influência antrópica, possui um aumento do sistema antioxidante e de biotransformação, além de aumento de lipoperoxidação comparado com o setor 1 caracterizado por ser conservado. O setor 2 também apresentou uma inibição de acetilcolinesterase cerebral, comparado aos outros dois setores. A inibição da atividade de AChE é amplamente conhecida como biomarcador de exposição para pesticidas organofosforados e carbamatos (Fajardo; Ocampo, 2018). No entanto, diversos contaminante também tem sido classificados como inibidores da atividade de AChE, incluindo metais e outras classes de poluentes orgânicos ambientais (Fu et al., 2018). Esses resultados corroboraram com a hipótese estipulada pelo presente trabalho. Com isso, verificou-se também a importância da espécie *H. malabaricus* como excelente modelo biológico para utilização em biomonitoramento, já que foi capaz de demonstrar diferentes resultados para diferentes biomarcadores.

Os resultados de histopatologia, reforçaram o encontrado na análise dos biomarcadores bioquímicos. Lesões teciduais da brânquia e fígado, foram mais evidentes em peixes do setor 2, considerado mais antropizado, além de peixes do setor 3 que mesmo com menos impactos, ainda apresentou danos teciduais nos organismos. Dentre as lesões encontradas em brânquias podem se destacar principalmente no setor 2 a presença de aneurisma e hiperplasia com fusão parcial da lamela secundária, dobras das extremidades das lamelas secundárias, hiperplasia com fusão total das lamelas secundárias e aumento das células de muco. No setor 3, as alterações mais comumente encontradas foram lifting epitelial e hiperplasia com fusão parcial. Ao analisar o fígado, entre as alterações encontradas em peixes do setor 2, pode-se citar a presença de centro de melanomacrófagos, congestão sanguínea nos sinusóides, vacuolização dos hepatócitos e necrose. Já para o setor 3, destaca-se a presença de morte celular, ou necrose.

Ao se analisar a presença de micronúcleo, todos os três setores indicaram mutagenicidade nos organismos. Além disso, alterações nucleares também foram observadas como “blebbed”, “notched”, “lobed”, “binucleus” e “vacuolated”, entretanto não houve diferença estatística significativa entre os três setores analisados do Rio Guaraguaçu. Os produtos oriundos e utilizados pelas atividades antrópicas são as principais causas de alterações morfológicas no núcleo de células de peixes (Canedo et al., 2021), como agrotóxicos (Oliveira et al., 2019), lançamento de esgoto (Lehun et al.,2021), elementos traço (Francisco et al., 2019; ALI et al., 2020) e hidrocarbonetos policíclicos aromáticos (HPAs) (Benincá et al., 2011).

Logo, vale salientar a importância do presente trabalho, ao destacar seu pioneirismo sendo um dos primeiros, se não o primeiro, trabalho na análise da qualidade da água do rio Guaraguaçu que utiliza o biomonitoramento com peixes nativos para análise de biomarcadores. A utilização do biomonitoramento junto do monitoramento tradicional se torna uma ferramenta eficaz, ao conseguir evidenciar os problemas encontrados em um dos bens e recursos mais preciosos de qualquer ecossistema, os seus organismos. Com os resultados aqui alcançados, busca-se incentivar futuros trabalhos no importante rio Guaraguaçu criando um alerta para a necessidade de uma melhora na qualidade de vida de todos os residentes com grau de vulnerabilidade social que precisam desse rio para subsistência, abastecimento e sofrem com as consequências da contaminação desse rio, além de criar um direcionamento para criação de ações governamentais capazes de controlar, diminuir e até mesmo encerrar com atividades antrópicas capazes de interferir na qualidade da água desse importante rio da costa paranaense.

Entretanto, salienta-se que sejam necessários outros trabalhos para análise de possíveis contaminantes em sedimentos. Uma análise mais profunda da possibilidade da presença de outros xenobióticos como HPAs e poluentes orgânicos persistentes (POPs) também é de extrema importância, além da expansão das análises para mais de uma espécie de peixe ou até mesmo para mais de uma espécie de animal em diferentes níveis tróficos, com a possibilidade de análise em períodos distintos do ano ao avaliar os efeitos da sazonalidade no Rio Guaraguaçu, para que assim se possa ter um direcionamento maior para quais xenobióticos podem estar influenciando a saúde dos organismos da região do Guaraguaçu.

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