MATEUS BRANDÃO MARQUES

DETERMINAÇÃO DE METAIS E METALOIDES EM TUBARÕES LIXA *Ginglymostoma cirratum* CAPTURADOS NO COMPLEXO ESTUARINO DE SÃO MARCOS, E SEUS EFEITOS CITOTÓXICOS E GENOTÓXICOS

SÃO LUÍS, MA

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Dissertação apresentada ao Programa de Pós-Graduação em CIÊNCIA & TECNOLOGIA AMBIENTAL da Universidade Federal do Maranhão como requisito à obtenção do título de MESTRE.

Orientador: Prof. Dr. Ricardo Luvizotto-Santos

Coorientadora: Prof[a]. Dr[a]. Rachel Ann Hauser-Davis

Linha de pesquisa: Biotecnologia e Tecnologias Aplicadas ao Meio Ambiente

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Aprovada em ___/____/____

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SÃO LUÍS, MA
2025

Peça a Deus que abençoe os seus planos, e eles darão certo.
Pv 16:3, NTLH

Quando o orientando escreve sem parar,
Com mil ideias que parecem brilhar,
Os orientadores, sábios, entram em cena,
Guiando o rumo, trazendo mais pena.

O efeito antagônico logo aparece,
O que empolga, eles logo esclarecem.

Corta excessos, ajustam o sentido,
Mostram que o caminho precisa ser medido.
Enquanto o orientando deseja voar,
Eles o ensinam primeiro a caminhar.

O que parece controle, é só direção,
Para transformar o rascunho em dissertação.
No final, o equilíbrio vai florescer,
Entre a pressa e a calma de quem quer aprender.

E o efeito antagônico, tão bem entendido,
Mostra-se o guia para o sucesso garantido.

(Mateus Brandão)

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RESUMO

Populações de elasmobrânquios têm sofrido declínios significativos nas últimas décadas devido a atividades antropogênicas, sendo a contaminação química uma das cinco principais ameaças a esse grupo. Apesar do uso de alguns biomarcadores bioquímicos para avaliar a saúde desses organismos, principalmente no que diz respeito à contaminação por metais e metaloides, as associações com biomarcadores genotóxicos ainda são escassas, especialmente para tubarões da costa amazônica brasileira. Neste contexto, foi realizado um estudo com tubarões lixa (*Ginglymostoma cirratum*) no Complexo Estuarino de São Marcos, Maranhão, onde foram determinados os níveis de metais/metaloides e diversos biomarcadores de estresse oxidativo, como glutationa reduzida (GSH), glutationa S-transferase (GST), e metalotioneína (MT), além de anomalias nucleares (micronúcleos, brotamento nuclear e células bilobadas). Diferenças significativas na acumulação de metais e metaloides entre machos e fêmeas foram observadas, mas não refletidas nas respostas antioxidantes. Concentrações mais altas de GSH e atividades de GST foram observadas nas brânquias e no fígado, enquanto as concentrações de MT foram maiores no músculo. Foi verificada uma baixa frequência de danos genotóxicos, atribuída a certas características do hábito de vida dos tubarões lixa, como um estilo de vida sedentário e um sistema eficiente de reparo de DNA. Foram detectadas diversas correlações moderadas a fortes entre metais/metaloides e respostas bioquímicas e genotóxicas, especialmente em fêmeas, destacando a função protetora da GST contra danos ao DNA. O efeito protetor foi observado apenas no fígado, onde o Se demonstrou sua ação antagônica contra o Hg tanto em fêmeas quanto em machos. Complementando esses achados, uma revisão narrativa destacou a necessidade de avaliar a integridade do DNA em elasmobrânquios para identificar ameaças ambientais e apoiar a conservação. A sensibilidade desses organismos aos poluentes exige o uso de biomarcadores genotóxicos como ferramentas-chave para monitorar impactos e mitigar riscos em ecossistemas marinhos. Os resultados reforçam a relevância de pesquisas integradas para proteger elasmobrânquios de ambientes sob impactos antrópicos e promover sua conservação global.

ABSTRACT

Elasmobranch populations have experienced significant declines in recent decades due to anthropogenic activities, with chemical contamination comprising one of the five main threats to this group. Although some biochemical biomarkers are used to assess the health of these organisms, particularly concerning contamination by metals and metalloids, associations with genotoxic biomarkers remain scarce, especially for sharks along the Brazilian Amazon coast. In this context, this study assessed the nurse shark (*Ginglymostoma cirratum*) in the São Marcos Estuarine Complex, Maranhão, determining metal/metalloid and oxidative stress biomarker levels, such as reduced glutathione (GSH), glutathione S-transferase (GST), and metallothionein (MT), as well as nuclear anomalies (micronucleus, nuclear buds, and bilobed cells). Significant differences in metal and metalloid accumulation between males and females were observed but were not reflected in antioxidant responses. Higher concentrations of GSH and GST activity were found in the gills and liver, while MT concentrations were higher in the muscle. A low frequency of genotoxic damage was detected, attributed to certain life habits of nurse sharks, such as a sedentary lifestyle and an efficient DNA repair system. Several moderate to strong correlations between metals/metalloids and biochemical and genotoxic responses were observed, especially in females, highlighting the protective role of GST against DNA damage. The protective effect was observed only in the liver, where Se demonstrated its antagonistic action against Hg in both females and males. Complementing these findings, a narrative review emphasized the need to evaluate DNA integrity in elasmobranchs to identify environmental threats and support conservation efforts. The sensitivity of these organisms to pollutants highlights the importance of genotoxic biomarkers as key tools for monitoring impacts and mitigating risks in marine ecosystems. These results reinforce the relevance of integrated research to protect elasmobranchs in environments under anthropogenic impact and to promote their global conservation.

LISTA DE SIGLAS E ABREVIATURAS

BL - Núcleo bilobado

BN - Brotamento nuclear

CESM - Complexo Estuarino de São Marcos

ETR - Elementos Terras Raras

GSH - Glutationa reduzida

GST - Glutationa S-Transferase

IUCN - União Internacional para a Conservação da Natureza (do inglês, *International Union for Conservation of Nature*)

MN - Micronúcleo

MT - Metalotíoneina

SUMÁRIO

1. INTRODUÇÃO

Os elasmobrânquios são um grupo de peixes cartilaginosos representados pelos tubarões e raias, organismos de suma importância para a manutenção do equilíbrio ecológico dos ecossistemas aquáticos que habitam (Rosa et al., 2006; Dulvy et al., 2017). Entretanto, em torno de 37,5% de todos os elasmobrânquios estão atualmente categorizados como ameaçados de extinção pela União Internacional para a Conservação da Natureza (em inglês, *International Union for Conservation of Nature*, IUCN) (IUCN, 2021) e 12,9% destes são classificados como "dados insuficientes", indicando ausência de informações mínimas para serem corretamente avaliados (Mace et al., 2008; Dulvy et al., 2021). No Brasil, 40% das 151 espécies nativas estão ameaçadas, sendo 18% criticamente em perigo, 5% em perigo, 16% vulnerável e 1% considerada regionalmente extinta (ICMBio, 2021).

A espécie *Ginglymostoma cirratum*, popularmente conhecida como tubarão lixa, pertence à classe Chondrichthyes, subclasse Elasmobranchii, ordem Orectolobiformes e família Ginglymostomatidae (Figueiredo, 1977). Como características morfológicas, o tubarão lixa apresenta boca terminal com barbilhões nasais moderadamente longos, suas nadadeiras são arredondadas e coloração do corpo variando de marrom escuro a claro na região dorsal, e região ventral amarelada (Figueiredo, 1977; Castro, 1993). A distribuição desta espécie é ampla em todo Atlântico ocidental (Figueiredo, 1997). Porém, no Brasil, o tubarão lixa tem apresentado declínio acentuado das populações ao longo da costa devido a pesca artesanal e a caça submarina (Brito, 2019), e, em razão de suas características biológicas como crescimento lento e maturação sexual tardia, figura como uma espécie em situação de vulnerabilidade em diversos estados (Alagoas, Bahia, Ceará, Paraíba, Pernambuco, Rio de Janeiro, Rio Grande do Norte e São Paulo) (Brito, 2019; ICMBIO, 2023). Além disso, é considerada uma espécie vulnerável (VU) pela Instrução Normativa 354/2023 e pela Portaria 148/2022 do MMA (Carlson et al., 2023).

A contaminação ambiental representa uma das principais ameaças para os elasmobrânquios (Alves et al., 2022). Diversos compostos potencialmente perigosos, como fármacos, pesticidas, hidrocarbonetos e metais, são transportados diariamente aos ambientes aquáticos costeiros (Dezorzi, 2021; Alvez et al., 2022; Boldrocchi et al., 2022),

podendo levar a efeitos nocivos e/ou acumular-se na biota, com o agravante da possibilidade de biomagnificação em mesopredadores, como os tubarões lixa (Wosnick et al., 2021).

Dentre os contaminantes mais estudados, os metais têm demonstrado grande capacidade de acumulação e de causar danos aos organismos aquáticos (Wang et al., 2022). O estresse provocado pela contaminação de metais, os quais possuem alta capacidade de assimilação em função de sua natureza não degradável e tóxica a depender do elemento e sua concentração, pode levar à manifestação de diversas disfunções fisiológicas, alterações estruturais em órgãos e tecidos e modificações comportamentais que afetam tanto o crescimento quanto a reprodução das espécies marinhas expostas (Da Fonseca, 2014; Delgado, 2019; Freitas et al., 2020). Metais e metaloides como arsênio (As), selênio (Se), mercúrio (Hg), titânio (Ti), rubídio (Rb) e Elementos Terras Raras (ETR) são exemplos destes tipos de contaminantes, alguns dos quais já foram estudados em elasmobrânquios e levam a efeitos adversos em indivíduos expostos, como, por exemplo, perturbação das vias metabólicas, imunossupressão e genotoxicidade, incluindo o aumento na frequência de anomalias nucleares (Trevizani, 2019; Dezorzi, 2021; Alves et al., 2022).

Para avaliação dos efeitos desses contaminantes na saúde de diversos organismos, incluindo os elasmobrânquios, são utilizados biomarcadores, que consistem em respostas biológicas a estressores (Alves et al., 2022). Essas respostas são evidenciadas através de alterações bioquímicas, celulares, histológicas, fisiológicas ou comportamentais indicativas da exposição e/ou efeito de agentes xenobióticos ou estressores ambientais (Jesus; Carvalho, 2008; Coppo et al., 2018). Entre os biomarcadores, destacam-se algumas proteínas como a metalotioneína (MT) e glutationa s-transferase (GST) e tripeptídeos como glutationa reduzida (GSH), que atuam como defesas antioxidantes essenciais na proteção celular contra os danos causados por espécies reativas induzidas por contaminantes (Alves et al., 2022; Rodrigues et al., 2022; Hauser-Davis et al., 2022; Alves et al., 2023). Além destes, outros biomarcadores, como danos genotóxicos, são igualmente importantes na avaliação da saúde de organismos expostos a contaminantes ambientais. Estes incluem anormalidades nucleares como

micronúcleo (MN), brotamento nuclear (BN) e núcleo bilobado (BL) que podem fornecer informações sobre danos ao material genético (Almeida et al., 2022; Norris et al., 2021).

Considerando as rotas de chegada de metais e metaloides na zona costeira, bem como seus efeitos na biota e a expectativa de que esse cenário não se altere no futuro próximo, os tubarões estarão cada vez mais expostos e consequentemente afetados por contaminantes persistentes por diferentes vias de assimilação, sobretudo pela sua dieta (Boldrocchi et al., 2022). Diante desse panorama, torna-se imprescindível a avaliação da saúde do tubarão-lixa por meio do uso de biomarcadores, visando analisar os possíveis efeitos da acumulação de metais como As, Se, Hg, Ti, Rb e ETR em seus tecidos. Essas informações são essenciais para a conservação dessa espécie vulnerável.

2. FUNDAMENTAÇÃO TEÓRICA

2.1 Regiões costeiras e estuários

As regiões costeiras são definidas como a interface ou áreas de transição entre o continente e a zona nerítica, recebendo influência de processos oceanográficos (ventos, correntes, ondas, marés e outros fatores físicos e biológicos) e abrigando diversos tipos de ecossistemas e habitats, como estuários, mangues, recifes, costões e florestas que favorecem a sobrevivência de várias espécies (Lopes et al., 2017). Por fornecerem diversos serviços ecossistêmicos, as regiões costeiras têm sido densamente ocupadas ao longo do tempo, apresentando uma elevada urbanização (Coutinho et al., 2016). Estima-se que atualmente em torno de 26,6% da população brasileira habita regiões costeiras, correspondendo a cerca de 50,7 milhões de pessoas que vivem de atividades relacionadas (direta ou indiretamente) à costa (Silva et al., 2019).

No entanto, a urbanização desordenada nas regiões costeiras, resulta na intensificação de impactos como erosão e intemperismo das rochas que liberam constituintes metálicos da crosta para o ambiente aquático (He et al., 2016). Além disso, impactos antrópicos como sobrepesca, lançamento de efluentes doméstico e industrial,

uso de pesticidas e intensas atividades portuárias, introduzem diversos constituintes químicos no ambiente (He et al., 2016).

Conectados às regiões costeiras, os estuários são ambientes com alta importância ecológica, social e econômica que também sofrem os efeitos da poluição ambiental (Do Nascimento et al., 2020). Os estuários são ambientes aquáticos costeiros, semi fechados com ligação direta ao mar aberto, apresentando a mistura entre a água doce, proveniente da drenagem continental, e a água marinha (Duarte; Vieira, 1997). Estes ambientes recebem e concentram material das bacias hidrográficas favorecendo a ciclagem de nutrientes e a produção primária (Duarte; Vieira, 1997; Jesus et al., 2020). Além disso, servem como habitat para diversas espécies que completam seu ciclo de vida, ou parte dele, neste ambiente (Jesus et al., 2020).

Grande parte da população humana fica localizada em regiões adjacentes aos estuários, devido à facilidade de acesso aos recursos naturais desses ambientes aliada às atividades econômicas como a portuária e a aquicultura (Silva et al., 2019; Fraga et al., 2021). Isto leva ao consequente lançamento de efluentes domésticos e industriais, muitos dos quais sem tratamento e em volumes elevados, impactando ecossistemas locais e gerando efeitos adversos aos organismos aquáticos residentes (Jesus et al., 2020).

Dentre os contaminantes mais comuns encontrados nos estuários estão os nutrientes (nitrogênio e fósforo), provenientes principalmente de atividades agrícolas, efluentes doméstico e industrial; metais (como Cd, Hg, Pb e Zn, dentre outros) que podem ser liberados por indústrias, minerações ou ainda pela ressuspensão por atividades de dragagem; hidrocarbonetos; produtos farmacêuticos e de cuidados pessoal e químicos como detergentes e produtos de limpeza (Santana et al., 2015; De Sousa et al., 2016).

2.1.1 Complexo Estuarino de São Marcos

O Complexo Estuarino de São Marcos (CESM) está localizado na porção da Amazônia Legal do estado do Maranhão, Brasil, apresentando uma transição climática entre o semiárido nordestino e a floresta amazônica ao norte do país (NUGEO, 2016). Os principais afluentes no CESM são provenientes da bacia do rio Mearim, como os rios

Pindaré, Grajaú e Mearim (Lima et al., 2021). Além disso, o complexo estuarino recebe também contribuições de cinco pequenas bacias hidrográficas localizadas nas margens leste, compreendendo os rios Cachorros, Bacanga e Anil, e oeste, abrangendo os rios Aurá e Salgado (Lima et al., 2021).

O CESM é a maior região estuarina do litoral norte brasileiro. Além disso, o complexo também desempenha um papel importante na economia do país, devido ao intenso fluxo de cargas para importação e exportação através do complexo portuário de São Luís (Montes et al., 2023). Este ambiente tem sido afetado principalmente pela contaminação de metais provenientes das atividades industriais e portuárias (Pinheiro-Sousa et al., 2021) que acabam prejudicando a biodiversidade marinha local, como algas, moluscos, crustáceos, peixes ósseos e cartilaginosos (Carvalho Neta et al., 2014; Santos et al., 2019; de Oliveira et al., 2019; Pinheiro-Sousa et al., 2021; Wosnick et al., 2021; Silva et al., 2023; Corrêa et al., 2023).

2.2 Contaminação e poluição por metais e metaloides

Os metais e metaloides são encontrados de forma natural na crosta terrestre cuja concentração varia espacialmente dependente do tipo de rocha da região e das condições geofísicas (Yu et al., 2015; Gontijo et al., 2017). Estes elementos são liberados naturalmente no ambiente por meio do intemperismo e erosão, ou ainda de pela atividade vulcânica (Figura 1) (Gontijo; Monteiro; Rosa, 2017). Entretanto, as principais fontes desses contaminantes atualmente compreendem atividades humanas, incluindo a mineração, fabricação de produtos metálicos, disposição de resíduos sólidos, combustão de combustíveis fósseis e efluentes municipais e industriais (Hill, 2010; Gontijo; Monteiro; Rosa, 2017). Com o aumento das atividades relacionadas à urbanização e industrialização próximas às zonas costeiras, as concentrações de metais e metaloides nestes ambientes têm aumentado significativamente nos últimos anos.

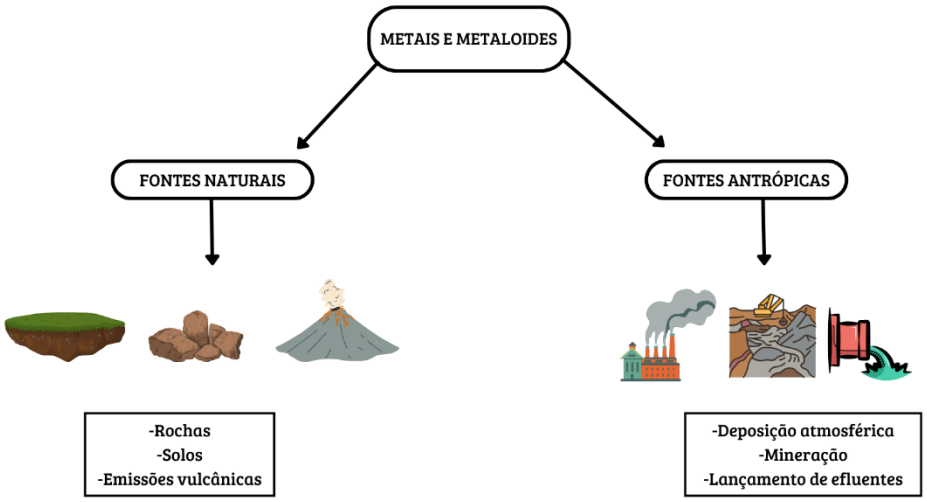


Figura 1 - Principais fontes de metais e metaloides em águas naturais. Fonte: O autor (2024).

Assim que atingem os ambientes aquáticos, os metais e metaloides passam por diversas transformações, mobilização e distribuição em diferentes formas físicas e químicas (Gontijo; Monteiro; Rosa, 2017). Esses elementos podem gerar danos aos organismos a depender da mobilidade, toxicidade e disponibilidade no ambiente (Gontijo; Monteiro; Rosa, 2017)

Alguns desses elementos são considerados essenciais para o crescimento e metabolismo de organismos (Hill, 2010). Porém, quando biodisponíveis em elevadas concentrações, podem ser tóxicos (Gontijo; Monteiro; Rosa, 2017). Além disso, existem também os metais não-essenciais, sem atividade biológica conhecida os quais podem ser tóxicos, até mesmo em baixas concentrações. De modo geral, os metais e metaloides apresentam alta capacidade de assimilação em função de sua natureza não degradável e por participarem de diversas interações químicas e bioquímicas como cátions, podendo levar à manifestação de disfunções fisiológicas, formação de danos nucleares, alterações estruturais em órgãos e tecidos alvo e modificações comportamentais que afetam o

crescimento e reprodução de organismos expostos, inclusive animais marinhos (Da Fonseca, 2014; Delgado, 2019; Freitas et al., 2020; Hauser-Davis et al., 2022).

O As, por exemplo, é um metaloide amplamente distribuído na crosta terrestre e pode ser encontrado na forma orgânica (sem efeito tóxico) e inorgânica (altamente tóxica) no ambiente aquático (Andrade; Rocha, 2016). Esse elemento é conhecido por ser capaz de inativar cerca de 200 enzimas, em particular, aquelas envolvidas na produção de energia celular e as relacionadas à síntese e reparo do DNA (Akter et al., 2005; Andrade; Rocha, 2016; Maciel et al., 2021).

Diferente do As, o selênio (Se), um metaloide que também possui uma ampla distribuição, geralmente encontrado junto às atividades antropogênicas (Selvaraj et al., 2013), possui ação antioxidante, com função no sítio ativo de algumas enzimas agindo na redução da toxicidade do As por meio da eliminação de espécies reativas de oxigênio (EROs) (Selvaraj et al., 2013; Wang et al., 2023).

O Ti, por sua vez, é o nono metal mais abundante na crosta terrestre, estando presente na maioria das rochas ígneas (Krebs, 2006). Esse elemento não apresenta função biológica conhecida e, embora ainda haja lacunas sobre seus efeitos em organismos aquáticos, algumas pesquisas indicam que este elemento pode afetar a reprodução em organismos aquáticos (Lee et al., 1990). No entanto, quando em formato de nanopartícula (dióxido de titânio) são tóxicas aos organismos aquáticos (Smii et al., 2021), oriundas tanto de fontes naturais (poeira vulcânica e compostos minerais) e antropogênica (produtos de higiene pessoal, processos de combustão e outros), cujo destino final geralmente são os ambientes aquáticos (Musial et al., 2012).

O Rb é um metal pouco abundante na crosta terrestre e raramente encontrado em concentrações altas o suficiente para extração comercial (Naidu et al., 2018; Xing et al., 2018). Esse elemento é conhecido pela sua capacidade de bioacumulação em tecidos tanto de peixes ósseos, como por exemplo, na espécie *Pangasianodon hypophthalmus*, quanto de em elasmobrânquios, como no tubarão da espécie *Galeocerdo cuvier* (Yamaguchi et al., 2007; Wosnick et al., 2024). Quando bioacumulado, o Rb pode causar efeitos tóxicos no organismo, como a inibição da espermatogênese, necrose celular e efeitos

neurológicos (Lieberman; Meltzer, 1970; Ringer, 1883), cujos danos são causados pela sua capacidade de substituir o potássio em reações bioquímicas celulares (Nielsen, 2012).

O Hg é um metal tóxico encontrado na crosta terrestre principalmente como mercúrio elementar ou como sulfeto (Bernhoft, 2012). Esse elemento é bioacumulável com alta capacidade de biomagnificação ao longo das teias tróficas, podendo gerar efeitos neurotóxicos, danos motores, sensoriais e reprodutivos (Bernhoft, 2012; Amorim-Lopes, 2020).

Ultimamente, além dos metais e metaloides já citados, tem surgido grande interesse pela ocorrência de elementos terras raras (ETRs) (Stefania et al., 2022). Os ETRs constituem um grupo de 17 elementos, incluindo 15 lantanídeos mais escândio e ítrio (Malhotra et al., 2020). Tais elementos podem ser ingeridos juntamente com água e alimentos e concentrados em diferentes níveis nos tecidos dos peixes podendo apresentar efeitos cumulativos e genotóxicos para organismos marinhos (Malhotra et al., 2020; Hanana; Kleinert; Gagné, 2021; Stefania et al., 2022).

2.3 Biomarcadores de contaminação ambiental

Biomarcador é uma ferramenta amplamente utilizada no monitoramento ambiental, tendo por finalidade avaliar os efeitos de xenobióticos, a sensibilidade individual das espécies e elucidar a relação causa efeito e dose-resposta na avaliação de risco à saúde (Amorim, 2003).

De acordo com Amorim (2003) e van der Oost, Beyer e Vermeulen (2003), os biomarcadores podem ser divididos em três tipos distintos, os quais fornecem informações sobre diferentes aspectos da exposição. O primeiro tipo compreende os biomarcadores de exposição, que indicam respostas a substâncias químicas presentes no organismo, sem, no entanto, dar informação quanto aos efeitos dessa exposição. O segundo tipo são os biomarcadores de efeito, que consistem em alterações bioquímicas, fisiológicas e outras que podem ser associadas ao comprometimento da saúde de organismos expostos. Por fim, o terceiro tipo compreende os biomarcadores de suscetibilidade, que indicam resposta de organismos à exposição a determinados

xenobiótico, levando em consideração fatores genéticos e alterações nos receptores que podem afetar a sensibilidade dos organismos expostos a essa substância, podendo indicar mecanismos (desenvolvimento) de resistência aos xenobióticos.

2.3.1 Metalotioneína

As metalotioneínas (MTs) são proteínas ricas em cisteínas encontradas no fígado, rim, brânquias, músculo, urina, plasma e sangue, compreendendo um potencial biomarcador de contaminação por metais, já que estão envolvidas na homeostase e detoxificação de metais e metaloides (Azevedo et al., 2021; Nordberg; Nordberg, 2022).

É sugerido por alguns estudos que as MTs atuam no transporte de íons metálicos para outras proteínas para controle de elementos livres como Zn e Cu. Além disso, possuem papel protetor como varredoras de radicais e espécies reativas de oxigênio, cujos níveis podem ser alterados conforme a sazonalidade, organismo, tamanho e sexo (Petering; Spieler, 1990; Melancon, 2008; Martins; Bianchini, 2009).

As MTs são ativadas pela entrada de metais no organismo, sejam eles essenciais ou não. Normalmente, as MTs são encontradas nas células na sua forma inativa, ligadas aos íons de zinco (Zn). Entretanto, quando íons metálicos entram na célula, as MTs se desligam do Zn para ligar-se aos metais através dos radicais -SH (sulfidrila). Essa ligação impede que os íons metálicos participem de reações (indevidas) dentro da célula (Andrews, 2000; Haq; Mahoney; Koropatnick, 2003).

O estresse oxidativo ocorre quando há um desequilíbrio entre a produção de espécies reativas de oxigênio (ERO) e a capacidade do organismo em neutralizá-las, levando a danos celulares. Essas EROs também induzem a expressão de metalotioneínas (MT), pois aumentam indiretamente os níveis de zinco (Zn). O Zn, ao se dissociar das MTs, ativa o fator de transcrição MTF-1, responsável pela expressão do gene da tioneína. Esse processo resulta em um aumento significativo na concentração de glutationa reduzida (GSH) e/ou de MTs para combater o estresse oxidativo causado por metais. Vale destacar que a GSH é o principal antioxidante celular, desempenhando um papel essencial na proteção contra danos oxidativos (Haq; Mahoney; Koropatnick, 2003). A MT tem sido

muito utilizada como um biomarcador específico de exposição aos metais. Sua atuação na biodisponibilidade de Zn, que interage com diversos fatores envolvidos no processo de apoptose e proliferação celular, torna-a um biomarcador de grande relevância em quadros neoplásicos. Além disso, as MTs têm sido aplicadas na avaliação de estresse oxidativo, cuja importância vem crescendo nos últimos anos, uma vez que é associada a vários elementos como As, Cu, Hg, Ti e outros, graças à nucleofilicidade de seus grupos sulfidrilas (Viarengo et al., 2000; Pedreiro et al., 2020; Nordberg; Nordberg, 2022).

Estudos indicam que a MT pode ser considerada um bom biomarcador de exposição a metais para elasmobrânquios. No entanto, existe uma variação na afinidade dessa biomolécula pelos metais, dependendo do indivíduo, seus hábitos e do órgão analisado. Um exemplo disso é o tubarão martelo *Sphyna tiburo*, que apresentou uma relação negativa entre a MT no músculo e o Hg (Walker et al., 2014). Outros estudos, como o de Cho et al. (2005), revelaram que em indivíduos como o tubarão gato (*Scyliorhinus torazame*), os níveis de MT aumentam com a exposição ao Cd, Cu e Zn no fígado e rins. Além disso, espécies de elasmobrânquios hidrotermais, como o tubarão de nariz longo *Deania hytricosa* e tubarão lanterna *Etsnopturus princeps*, mostraram sensibilidade aos metais, apresentando uma relação positiva da MT com Ag, Cu e Li no fígado (Company et al., 2010).

Um estudo mais recente realizado por Hauser-Davis et al. (2021) avaliou as MTs no fígado da espécie tubarão azul *Prionace glauca*, sugerindo-a como um biomarcador eficiente no mecanismo de desintoxicação de As, césio (Cs) e Ti. Da mesma forma, Martins et al. (2023) conduziram um estudo em raias-viola *Pseudobatos horkelii*, quando observaram que os metais exercem influência nos níveis de MT na espécie. A exposição a metais como Cd, Cr e Pb resultou em concentrações aumentadas de MT, indicando que esta proteína é induzida em resposta à presença de metais e metaloides. Isso sugere que as MTs desempenham um papel crucial na homeostase e desintoxicação de metais em *P. horkelii*.

2.3.2 Glutationa reduzida

A glutationa é um tripeptídeo essencial na biotransformação, eliminação e na

defesa antioxidante das células de organismos aeróbicos (Vašková et al., 2023), sendo encontrada na forma reduzida (GSH) e oxidada (GSSG). Sua ação contra as EROs é facilitada pela ação de enzimas como a glutationa peroxidase (GPx) e glutationa redutase (GR) (Adeoye et al., 2018).

A GSH age nas células dos organismos com uma variedade de funções como síntese de proteínas e DNA, sequestro de radicais livres, melhora da imunidade da célula e também na defesa antioxidante contra as EROs induzidas pelas atividades metabólicas de rotina e de contaminantes (Deponte et al., 2013; Ming et al., 2015). Nos espécimes aquáticos a GSH tende a melhorar o desenvolvimento de organismos (crescimento), aumentando sua imunidade e auxiliando na sua resiliência, uma vez que fortalece a resistência ao estresse ambiental (Ming et al., 2015).

A ação antioxidante da GSH nas células ocorre com o intuito de evitar o efeito de radicais tóxicos de oxigênio induzidos pela formação do superóxido (0_2^{-}.) e peróxido de hidrogênio (H_2O_2) (Vašková et al., 2023). O processo antioxidante se inicia com a GSH reduzindo o H_2O_2 na presença da GPx (usando como cofator o Se). Nesse processo a GSH é oxidada, tornando-se GSSG e em seguida, retorna à sua forma reduzida (GSH) pela GR que usa o NADPH como cofator de redução (Lu, 2009). A decomposição dos peróxidos resulta na produção de H_2O ou em um álcool correspondente (HOR).

O uso da GSH como biomarcador é comum em elasmobrânquios, uma vez que seus níveis têm sido associados a diversos estudos sobre estresse oxidativo, mostrando ser um potencial antioxidante frente à contaminação ambiental (Alves et al., 2022). A utilização da GSH em elasmobrânquios proporciona uma reserva funcional aumentada em determinadas fases da vida, ou seja, uma maior defesa antioxidante, como observado em fêmeas maduras da espécie *Carcharhinus falciformis* (Cruz-Ramírez et al., 2017). Hauser-Davis et al. (2021) observaram o potencial antioxidante desse biomarcador na contaminação por metais no fígado de *P. glauca*, uma vez que foi demonstrada uma relação entre a exposição ao Co e ao Zn. A GSH também foi associada à contaminação por ferro e ao estresse oxidativo em *P. horkelii* (Martins et al., 2023). Esses estudos indicam o potencial desse biomarcador na avaliação da saúde dos elasmobrânquios expostos à contaminação ambiental.

2.3.3 Glutationa S-Transferase

A glutationa S-Transferase (GST) é uma enzima essencial para a detoxificação intracelular de compostos xenobióticos. Sua principal função é catalisar a conjugação de compostos exógenos com o tripeptídeo glutationa (GSH). Dessa forma, a GST desempenha um papel crucial na proteção do organismo contra contaminantes (Huber; Almeida; Fátima, 2008).As GSTs são encontradas no meio biológico como homo- ou heterodímeros, apresentando dois sítios ativos por dímero cujas atividades são independentes. Cada sítio ativo consiste em pelo menos duas regiões de ligação, uma específica para a GSH e outra com menor especificidade para os substratos (eletrófilos). Os eletrófilos mais comuns das GSTs incluem haletos de alquila, epóxidos, compostos α, β-insaturados (como quinonas, iminoquinonas, aldeídos, cetonas, lactonas e ésteres), haletos de arila e nitroaromáticos (Huber; Almeida; Fátima, 2008).

As GSTs agem na biotransformação de xenobióticos na Fase II (ou de seus metabólitos oriundos da Fase I). Nessa etapa, a GSH atua como eliminador de EROS enquanto as GSTs metabolizam os subprodutos do estresse oxidativo (Dasari; Ganjayi; Meriga, 2018). A função da GST na célula é regular os níveis de EROS, prevenindo o acúmulo e protegendo as células contra a apoptose (Carvalho-Neta; Abreu-Silva, 2013).

A atividade da GST é frequentemente empregada como biomarcador para avaliar a exposição e efeitos de contaminantes em peixes, incluindo elasmobrânquios. Sua avaliação nesses animais não é recente e tem sido reconhecida como um biomarcador eficaz (Bodine; Luer; Ganjee, 1985; Barrera-Garcia et al., 2013; Rodrigues et al., 2022). Este biomarcador, por exemplo, demonstrou sensibilidade no tubarão lixa, sendo considerado crucial na resistência à carcinogênese química (Bodine; Luer; Ganjee, 1985). Em relação ao *P. glauca*, a GST mostrou sensibilidade à contaminação por As no fígado, apresentando resultados similares a outras espécies de peixes (Barrera-Garcia et al., 2013). Na espécie *Etmopterus perryi* (tubarão lanterna pigmeu), a GST forneceu informações sobre o estresse oxidativo induzido pelo Hg no tecido muscular, com sua atividade aumentada e correlacionada à catalase, um outro biomarcador de estresse

oxidativo (Rodrigues et al., 2022).

2.3.4 Danos genotóxicos

Diversos contaminantes ambientais, presentes em diferentes compartimentos, incluindo o ambiente aquático, podem causar alterações no material genético e são facilmente encontrados em organismos que habitam esses meios (ANVISA, 2018). Ao interagirem com o DNA, muitos desses contaminantes podem provocar danos genotóxicos ou até mesmo mutagênicos.

A genotoxicidade é caracterizada por qualquer tipo de dano que possa ser causado diretamente/indiretamente ao DNA, decorrente da capacidade de um agente interagir com o material genético e/ou com o aparato celular, incluindo os fusos mitóticos e topoisomerases (Santos et al., 2017; ANVISA, 2018). Já a mutagenicidade é responsável por alterações permanentes e hereditárias (propagado para células filhas) na estrutura do DNA, que podem afetar um único gene ou um conjunto deles (ANVISA, 2018).

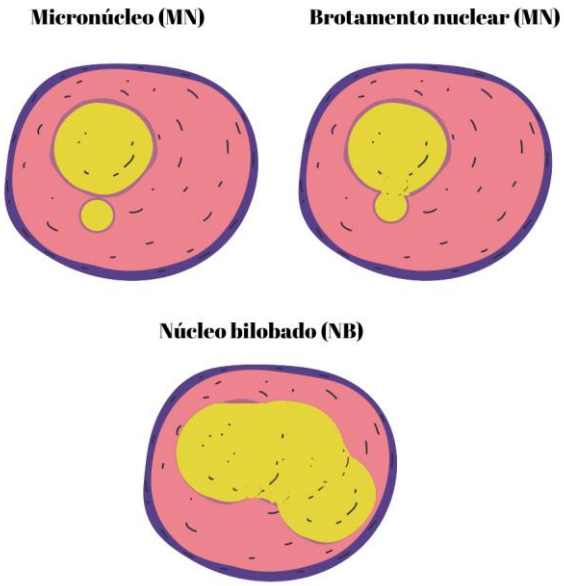
Como exemplo de danos ao material genético, tem-se as anomalias nucleares que podem fornecer informações quanto à genotoxicidade e mutagenicidade de compostos químicos, como o micronúcleo (MN), brotamento nuclear (BN) e núcleo bilobado (NB) (Figura 2) (Almeida et al., 2022), amplamente utilizados em estudos com organismos aquáticos (Pegado et al., 2019; Norris et al., 2021; Araújo et al., 2023; de Araújo et al., 2024).

O MN é um tipo de dano genotóxico caracterizado pela presença de um pequeno núcleo próximo ao núcleo original. O MN é formado durante a divisão celular, onde uma parte do material genético é expulso do núcleo original para o citoplasma, assumindo a forma de um pequeno núcleo durante a intérfase seguinte. O BN é uma pequena saliência de cromatina do núcleo principal, enquanto o NB é caracterizado pela presença de um núcleo com dois lobos no mesmo citoplasma celular (Toneline et al., 2014; Almeida et al., 2022).

Diversos estudos têm aplicado o Teste do Micronúcleo para avaliação da genotoxicidade e mutagenicidade. Esse método é amplamente utilizado devido ao seu

baixo custo e capacidade de detectar efeitos no nível cromossômico (Benetez-Trindade et al., 2014). Neste sentido, diversos estudos têm associados a contaminação por metais em moluscos e peixes a efeitos genotóxicos e mutagênicos por meio do estresse oxidativo (Benetez-Trindade et al., 2014; Do Amaral et al., 2019; Khan et al., 2019; Turan et al., 2020; Naik; Shyama; D'Costa; 2020). Esses danos genotóxicos também têm sido observados em elasmobrânquios, como o tubarão tigre,o tubarão limão *Negaprion brevirostris* e o tubarão lixa (Araújo et al., 2023), sendo relacionados tanto a fatores ambientais quanto à contaminação por metais.

Figura 2 - Representação das anomalias nucleares. Fonte: O autor (2024).



2.4 Tubarão lixa como bioindicador de contaminação ambiental

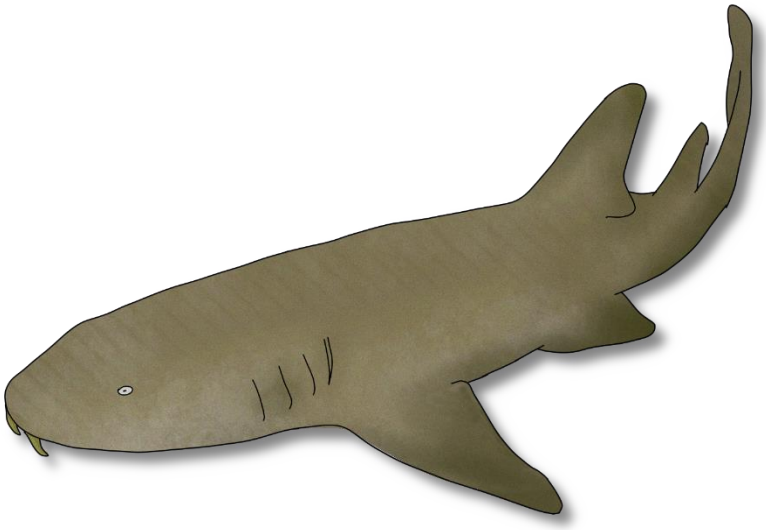
Os bioindicadores são organismos ou grupo de organismos usados para avaliações ambientais (Coppo et al., 2018). Estes devem ter relevância em seus respectivos ecossistemas, estarem adequadamente distribuídos, serem capazes de acumular e concentrar contaminantes e ainda possuírem capacidade de resposta frente às variações ambientais (Alves et al., 2022). A utilização de bioindicadores apresenta vantagens em

esforços de monitoramento ambiental, pois permite avaliar impactos cumulativos ao longo do tempo. (Holt et al., 2010; Alvez et al., 2022).

Os elasmobrânquios são um grupo de peixes que tem sido muito utilizado como bioindicadores de contaminação. De acordo com Alves et al. (2022), os elasmobrânquios englobam mais de 1.000 espécies conhecidas, uma grande parcela das quais age como predadora, sendo que qualquer variação ambiental pode acarretar impactos no ecossistema marinho, devido ao efeito de cascata ecológica, conferindo-lhes uma relevância ecológica ímpar. Além disso, esses indivíduos possuem importância no âmbito social, uma vez que constituem fonte de sustento e alimento para diversas populações humanas ao redor do mundo (Weigmann et al., 2016). A vasta distribuição dos elasmobrânquios é notável, tendo sido detectados em distintos ambientes, desde as águas salinas dos oceanos Pacífico, Atlântico equatorial e Índico até ambientes de água salobra, como estuários, e, em alguns casos, até mesmo em ecossistemas de água doce (Alves et al., 2022). Por fim, um atributo fundamental que eleva sua utilidade como bioindicador é a capacidade de bioacumulação, uma vez que ocupam posições geralmente elevadas nas teias alimentares. Devido à sua longa expectativa de vida, o nível trófico que ocupam está consistentemente associado às concentrações de substâncias, como os metais (Alves et al., 2022).

O tubarão lixa é um peixe de hábito bentônico, encontrado principalmente em águas litorâneas, mornas e calmas, alimentando-se de invertebrados de fundo, com distribuição no oceano Atlântico tropical e subtropical (Figura 3) (Gomes et al., 2019). Uma característica distintiva da espécie é a presença de barbilhões nasais moderadamente longos que chegam até a boca. Além disso, seus olhos ficam localizados posteriores aos cantos da boca e espiráculos diminutos presentes no mesmo nível dos olhos. Às quartas e quintas fendas branquiais são muito próximas entre si e quase sobrepostas. Suas nadadeiras possuem ápices arredondados, sendo a segunda nadadeira dorsal menor que a primeira (Gomes et al., 2019).

Figura 3 – Imagem representativa de tubarão lixa.



Fonte: Ilustração original Silva (2024)

Essa espécie é apresentada como residente (Wosnick *et al*., 2021), favorecendo seu uso como bioindicador pois pode atuar como fonte de informação sobre poluição localizada (Alves et al., 2022). O tubarão lixa sofre significativamente os impactos da pesca artesanal para comercialização da sua carne e apresenta alta vulnerabilidade aos poluentes por estar exposto cronicamente ao ambiente contaminado das águas costeiras. Isso é particularmente evidente em relação aos metais, como indicado pelas concentrações alarmantes de Cd, Hg e Pb já detectados na espécie (Lacerda et al., 2016; Hammerschlag et al., 2016; Pereira, 2019; Wosnick et al., 2021). Sendo assim, este organismo tende a apresentar respostas fisiológicas e comportamentais, que favorecem seu uso como bioindicador para avaliação de estressores ambientais e antropogênicos (Meneses; Pereira; Santos, 2011; Hammerschlag et al., 2016; Wosnick et al., 2021).

3. JUSTIFICATIVA

O crescimento populacional e a atividade portuária em ambientes costeiros, como as margens dos estuários e lagunas, são responsáveis pelo lançamento de volumes exorbitantes de efluentes residenciais, industriais e portuários provocando a degradação ambiental (Delgado, 2019). Assim, contaminantes como os metais, apesar de serem

encontrados naturalmente no ambiente, podem apresentar concentrações extremamente elevadas em ambientes aquáticos por meio de tais fontes, tornando-os motivo de preocupação quanto à poluição destas áreas, já que se tornam (bio)disponíveis e tóxicos principalmente aos organismos residentes (Delgado, 2019).

O tubarão lixa é um elasmobrânquio residente de águas costeiras, que se encontra na lista vermelha das espécies ameaçadas pela IUCN, devido às ameaças envolvendo a pesca para comercialização da carne, sendo, portanto, protegida em todo o território brasileiro pela legislação federal (IUCN, 2023; ICMBIO, 2021). Além disso, a contaminação ambiental tem se tornado outro fator de risco preocupante para esta espécie, principalmente no complexo estuarino de São Marcos, onde foram detectadas concentrações alarmantes de metais (Cd, Hg e Pb) nesta espécie, representando uma ameaça real para a conservação da espécie (Wosnick et al., 2021; Wosnick et al., 2024). Entretanto, estudos acerca da dinâmica de contaminantes nesta espécie ainda são escassos, e nenhum estudo acerca de elementos considerados de preocupação emergente, como Ti e elementos terras-rara, bem como a associação deles com danos genotóxicos estão disponíveis até o momento, tão pouco estudos associando o efeito protetivo do Se contra As e Hg.

4. HIPÓTESE

Tubarões lixa apresentam um elevado grau de contaminação por elementos traço e ETR, gerando sobre estes indivíduos respostas antioxidantes e alterações genotóxicas como resposta fisiológica à poluição do ambiente onde ocorrem.

5. OBJETIVOS

5.1 Geral

Avaliar a contaminação pelos elementos As, Se, Hg, Ti, Rb e ETR em tubarões lixa da região costeira do estado do Maranhão e relacionar as respostas bioquímicas e genotóxicas.

5.2 Específicos

- Determinar As, Se, Hg, Ti, Rb e ETR totais em diferentes tecidos (brânquias, fígado e músculo) de *G. cirratum*;
- Avaliar os níveis de metalotioneína (MT) nos tecidos branquiais, musculares e hepático para avaliar a via de detoxificação metal-MT
- Avaliar os níveis de glutationa reduzida (GSH) nos tecidos branquiais, musculares e hepático;
- Analisar a atividade da enzima de biotransformação glutationa–S-transferase (GST) das brânquias, fígado e músculo;
- Validar metodologia para análise de micronúcleo (MN) e anomalias nucleares (brotamento e núcleo bilobado) nas brânquias, fígado e músculo;
- Avaliar a mutagenicidade da contaminação por metais sobre a espécie através das alterações nucleares.

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7. ARTIGO I

GENETIC DAMAGE IN ELASMOBRANCHS: A REVIEW

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Objetivos Específicos da Dissertação atrelados ao artigo: Validar metodologia para análise de micronúcleo (MN) e anomalias nucleares (brotamento e núcleo bilobado) nos tecidos brânquias, fígado e músculo.

Abstract

DNA integrity is crucial for organismal health, and assessing DNA damage in aquatic organisms is essential for identifying environmental threats and informing conservation efforts. Pollutants such as metals, hydrocarbons, agrochemicals, pharmaceuticals, and climate change are linked to genetic damage, oxidative stress, and mutagenesis in several species, such as elasmobranchs (sharks and rays). Most studies focus on bivalves, crustaceans, and bony fish, with fewer assessments being carried out in cartilaginous fish. Concerning elasmobranchs, studies employing the micronucleus test and nuclear anomaly assays have aided in

understanding how this group responds to contamination by organic and inorganic pollutants. Notably, each species deals differently with these contaminants, presenting varied DNA damage levels, including low levels of response, probably associated to feeding habits, trophic position, maturation stage, sex and metabolism. Further investigations should be conducted in elasmobranchs to elucidate these variations and better understand DNA damage in this important ecological group.

1. Introduction

Elasmobranchs, a group that includes cartilaginous fish such as sharks and rays, are noteworthy for their ecological relevance in several aquatic ecosystems. These animals play a crucial role in the balance between species abundance and biodiversity, presenting unique evolutionary adaptations (Alves, 2022, Chynel et al., 2021, Collin, 2012). Some species are widely recognized as environmental contamination bioindicators, due to their wide distribution, ecological relevance, long-life expectancies, high food-chain positions, and chemical contamination bioaccumulation capacity, as well as comprising a significant source of food and income to human populations worldwide (Alves, 2022). Because of these features, however, these animals are particularly vulnerable to aquatic pollution and their effects, including genetic material damage (Alves, 2022, Alves et al., 2023, Araújo et al., 2023).

In fact, according to the International Union for Conservation of Nature (IUCN) classification criteria, over a third of elasmobranch species are threatened with extinction globally (Dulvy et al., 2021), especially those presenting coastal, sedentary habits or directly associated with the substrate, the ultimate sink for several environmental contaminants (Pimiento et al., 2023). The reasons for these alarming numbers are mainly related to excessive fishing practices and environmental contamination (Alves, 2022, Costa et al., 2022).

In this sense, studies on the effects of environmental contamination have recently been identified as priorities for conservation efforts for this group, highlighting the need for further elasmobranch assessments, which are still scarce (Alves, 2022, Consales and Marsili, 2021 Cooke, 2021). Several assessments concerning chemical contamination effects in elasmobranchs focus on the accumulation of metals and organic pollutants, known to cause antioxidant response alterations and DNA damage (Alves, 2022, Chynel et al., 2021). In

addition, environmental changes, such as increased temperature, variations in salinity and ocean acidification, also directly impact the physiology of these organisms, enhancing the effects of many contaminants (Alava et al., 2017, Alves, 2022). Despite this, research concerning genetic damage caused by pollutants and environmental changes have been largely directed at bony fishes (Wirgin and Waldman, 1998, Simoniello et al., 2009, Canedo et al., 2021), and a significant knowledge gap regarding the genetic effects of chemical contamination in elasmobranchs is observed, as most studies concerning this group focus only on population and ecological aspects (Batista et al., 2024, Ferragut-Perello et al., 2024). This is of significant concern, considering elasmobranch susceptibility to contamination, as well as their ecological and social importance (Araújo et al., 2023, Alves et al., 2023).

In this context, understanding the specific factors associated with genetic damage in this group is paramount to direct conservation efforts, allowing for in-depth understanding on how elasmobranchs respond to environmental changes, such as variations in temperature, salinity and pollution and assess their vulnerability to environmental stressors concerning DNA damage (Alves et al., 2016, Alava et al., 2017, Llorente, 2019).

2. Genetic damage and environmental chemical contamination

The integrity of DNA represents a crucial factor for organismal health, and can affect entire biological systems, depending on the extent of the genetic damage and the individual's capacity for repair (Youselfzadeh et al., 2021). In general, DNA damage resulting from metabolic activities, termed spontaneous damage, is readily repaired. However, DNA can be susceptible to several environmental stressors, including chemical and physical contamination (radiation), which can affect spontaneous damage processes (Carr and Hoekstra, 1995; De Bont and Van, 2004). These agents can induce a series of changes, such as genomic instability, replication errors, signal transduction pathway induction, transcription induction and cell cycle arrest (Valko, 2007; Chaudhary et al., 2023, Valko, 2007), which can, in turn, result in mutations or cell death.

DNA damage assessments play an important role in identifying threats and, consequently, in the conservation of aquatic organisms (Liyan et al., 2005, Alves et al., 2023). Damaged genetic material is often used as a chemical contamination biomarker, comprising a

biological indicator used to detect exposure to environmental stressors, and particularly useful in ecotoxicological studies to assess species sensitivity, environmental risks and, mainly, population health (Liyan et al., 2005, Nikitaki et al., 2015, Bae et al., 2020, Alves et al., 2023).

Several environmental pollutants, such as metals, polycyclic aromatic hydrocarbons (PAHs), agrochemicals and pharmaceuticals, among others, have been directly and indirectly associated with genetic damage in aquatic organisms (Alves, 2022, D'Costa et al., 2017 Akcha et al., 2021; Alves et al., 2023; Russo et al., 2023, Shahjahan, 2022). In this sense, exposed organisms become susceptible to the development of oxidative stress caused by the induction of Reactive Oxygen Species (ROS) (Alves et al., 2023), the modification of the expression of proteins, enzymes, and hormones important for the immune system (Shahjahan, 2022), and, consequently, to mutagenesis processes, resulting in direct genetic material damage and compromised genomic stability (Baines et al., 2021, Alves et al., 2023, Russo et al., 2023).

Concerning chemical contamination, PAHs are particularly known for their deleterious DNA effects. These compounds can be found in the environment due to forest fires, volcanic eruptions, humus compound reactions under the influence of soil microorganisms leading to the formation of coal or oil, and biosynthesis carried out by bacteria, algae and plants (Ewa and Danuta, 2017). However, the main PAH sources comprise anthropogenic activities associated with oil and coal burning and engine exhaust gasses, especially diesel and tobacco smoke (Ewa and Danuta, 2017). Structurally, PAHs are carbon compounds with two or more aromatic rings, known for their toxicity, with cytotoxic (displaying the ability to kill or damage cells), genotoxic, mutagenic and carcinogenic (displaying the ability to induce cancer or increase its incidence) effects (Chatterjee and Walker, 2017). Due to their low solubility, these compounds bind to lipid membranes and are transported by lipoproteins in the blood (Xue and Warshawsky, 2005; Ewa and Danuta, 2017).

Metals also have significant DNA effects, including strand breaking, repair pathway alterations and even epigenetic regulation influence (Morales et al., 2016). The effects of these elements on DNA occurs mainly through oxidative stress, which increases ROS production catalytically via redox cycling (Wright and Baccarelli, 2007, Jadoon and Malik, 2017). This affects cell membranes, disrupting lipid barriers, damaging proteins,

and ultimately harming DNA, through the removal of hydrogen atoms from deoxyribose, degradation of purines and pyrimidines, and the induction of single and double strand breaks (Flora et al., 2008; Jadoon and Malik, 2017), as well as deoxyribose oxidation and DNA strand breaks (Henle, 1996; Chattopadhyaya, 2014), producing 8-oxo-7,8-dihydroguanine (8-oxoG), a mutagenic lesion derived from guanine oxidation, often leading to G→T transversions (Linn, 1999, Chattopadhyaya, 2014).

Finally, organisms in general are exposed daily to various environmental stresses, such as climate change effects, including thermal stress, hypoxia and ocean acidification (Chatterjee and Walker, 2017, Lopes et al., 2021). These and many other environmental stressors induce mutations in trinucleotide repeats, resulting in neurodegenerative diseases through alt-non-homologous end joining (NHEJ) repairs (Chatterjee et al., 2015). Ocean acidification and increased temperature, in particular, are responsible for inducing DNA damage in many organisms, especially aquatic animals (Lopes et al., 2018), both through ROS and Reactive Nitrogen Species (RNS) overproduction (Lopes et al., 2018) and DNA methylation (Yue et al., 2023).

3. Genetic damage in elasmobranchs

Several elasmobranch species have been identified as environmental contamination bioindicators, due to certain characteristics such as widespread distribution, ecological importance, and high bioaccumulation capacity (Alves, 2022). Most studies on contamination in this group are related to the accumulation of metals and POPs, both of which can be transferred from mothers to their developing embryos (Alves, 2022, Chynel et al., 2021). It should also be noted that environmental changes, including increased temperature, variations in salinity and ocean acidification, tend to directly affect the physiology of these organisms, potentially intensifying the action of chemical contaminants (Alava et al., 2017, Alves, 2022).

Genetic damage in fish, however, has been most extensively studied in teleosts, with an outstanding understanding of the effects of environmental contamination in this group (Glei et al., 2009, Wirgin and Waldman, 1998 Simoniello et al., 2009; Canedo et al., 2021). Knowledge in this context concerning elasmobranchs, however, is still limited, despite their

susceptibility to chemical contamination and ecological and social importance (Araújo et al., 2023, Alves et al., 2023).

Exposure to environmental pollutants and climate change effects such as ocean warming and acidification, as well as specific species characteristics are preponderant factors concerning the genetic integrity of elasmobranchs (Lopes et al., 2018, Alves et al., 2023). Contamination by chemical compounds, such as surfactants, persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), and metals, comprise the main factors responsible for genetic damage in elasmobranchs, considering the accumulation of these pollutants in target tissues, whether by direct contact with sediment or through water or food, through bioaccumulation and biomagnification processes (Alves et al., 2016, Alves et al., 2023, Araújo et al., 2023). The former is the process by which organisms absorb and store contaminants from their environment (e.g., water, sediment, or food) over time, leading to higher concentrations within their bodies than in the surrounding environment, while the latter refers to the increasing concentration of contaminants as they move up the food chain, with meso and top predators, such as elasmobranchs, accumulating the highest levels due to consuming contaminated prey. The accumulation of these contaminants, although not directly resulting in DNA damage, can trigger ROS induction (Tchounwou et al., 2012, Alves et al., 2016), which if not neutralized by antioxidant defenses, can lead to cellular processes such as apoptosis, cellular necrosis, mutation and malformations (Alves et al., 2023, Menor, 2006). Deformities, for example, were observed in a ray individual, *Atlantoraja castelnaui*, which exhibited incomplete fusion of the right pectoral fin with the head and discontinuity of the muscle bundle near the rostrum (Hauser-Davis et al., 2020). The authors postulated that this morphological damage could be associated to the teratogenic properties of the metals (As, Hg and Cd) found in high concentrations in the specimen, a newborn probably exposed to maternal contaminant transfer.

Ocean acidification has also been identified as a problem for some elasmobranch species (Lopes et al., 2018, Pegado et al., 2019). As the pH of seawater decreases, some organisms tend to present an acid-base imbalance in body fluids, such as blood (Melzner et al., 2009, Pörtner, 2008). Although some species can compensate for these changes, other effects resulting from acidification, such as altered behavior and physiology, including decreased

antioxidant responses, become common (Esbaugh et al., 2016, Lopes et al., 2018). In this sense, the Lesser spotted dogfish *(Scyliorhinus canicula*) has demonstrated resistance to these changes, not presenting genotoxic effects even under high CO2 levels (Pegado et al., 2019), unlike the whitespotted bamboo shark (*Chiloscyllium plagiosum*), which demonstrated increasing DNA damage in several organs and a reduction in some antioxidant enzymes under the same conditions (Lopes et al., 2018). Ocean acidification generally results in an increase in H+ ions in the environment, and, alongside CO2 reactions with RND, result in a high production of ROS, leading to DNA damage (Feder and Hofmann, 1999, Sampaio et al., 2018). Furthermore, cellular acidosis can release metals such as Fe, which, in turn, produce hydroxyl radicals (Fenton reaction), causing cell membrane, protein and DNA damage (Lopes et al., 2018).

It is important to note that the effects of pollutants and ocean acidification on elasmobranch DNA also vary considerably according to species and specific characteristics, such as feeding habit, trophic position, maturation stage, sex and metabolism (Cruz-Ramírez et al., 2017, Lopes et al., 2018, Araújo et al., 2023). These particularities can significantly influence species' resilience in the face of climate and environmental change scenarios. For example, nurse sharks *(Ginglymostoma cirratum*), a resident organism, even in highly contaminated environments, presents a low damage frequency due to certain characteristics such as sedentary lifestyle and low metabolic activity (Araújo et al., 2023). In addition, the commonly applied elasmobranch capture technique to obtain samples has been known to influence the acid-base balance of sharks and rays (Giareta et al., 2023). Studies concerning longline fishing in sharks of the *Carcharhinidae genus*, for example, revealed significant changes in the acid-base balance of the blood of these individuals (Mandelman and Skomal, 2009). Despite the scarcity of studies relating capture stress to DNA damage, Cruz-Ramírez et al. (2017) verified damage to DNA, RNA and proteins in blood samples of silky sharks (*Carcharhinus falciformis*) captured in Mexico, indicating that sex and life stage can significantly influence capture damage.

Responses at a biological level, such as biochemical, histological and even morphological changes (appearance, pigmentation and deformations) that indicate the presence and/or action of stressors, are termed biomarkers (Depledge et al., 1995). The most commonly

applied biomarkers in genetic damage assessments are detected through the comet assay (CA), DNA strand break analysis and micronucleus test (MNT), widely used to detect genetic damage and evaluate the extent of the effects of environmental stressors (Bolognesi et al., 2014; Castro et al., 2021). These assays allow for the detection of specific effects, such as DNA disruption, by physical and chemical agents, through the diagnosis of genome integrity, as well as the identification of probable hereditary mutations (Kohn, 1983, Carr and Hoekstra, 1995). Although these methods have been routinely applied to different aquatic organisms as biomonitoring tools, most studies are focused only on bony fish, and DNA damage assessments in elasmobranchs employing these methods are still sorely lacking.

The micronucleus assay, in particular, has gained prominence in recent elasmobranchs studies, especially sharks, directly associated with the genotoxic effects of environmental pollutants, although research in this sense is still in its infancy (Araújo et al., 2023). Micronuclei (MN) formation results from chromosomal fragments, consisting of acentric chromatids or entire chromosomes that are not incorporated into daughter nuclei during cell division, generally induced by stress situations caused by physical, chemical or biological agents (Canedo et al., 2021, Krupina et al., 2021). This test is based on an anomaly score, and in most studies is carried out on slide using simple Giemsa staining with observation under an optical microscope (Luzhna et al., 2013). This is a sensitive and cheap test for detecting chromosomal damage, making it useful in environmental monitoring efforts (Michalová et al., 2020). Morphological mutagenicity patterns (Fig. 1) are mainly defined as (1) micronucleus (MN): the presence of a small nucleus (1/16–1/3) similar to the main nucleus, indicative of chromosomal fragmentation; (2) binucleate cells: where cells contain two similar nuclei, indicating failure in the cytokinesis process during cell division; (3) nuclear budding: where the nucleus presents a "bud" (chromatin displaced from the main nucleus), considered a process that precedes MN development, probably related to DNA amplification or the repair process by extrusion of the amplified DNA (Baršienė, 2006; Bolognesi et al., 2013). A fourth pattern, (4) bilobed nuclei, has also been defined, comprising a two-lobed shape associated with changes in cellular metabolism (Harabawy and Mosleh, 2014).

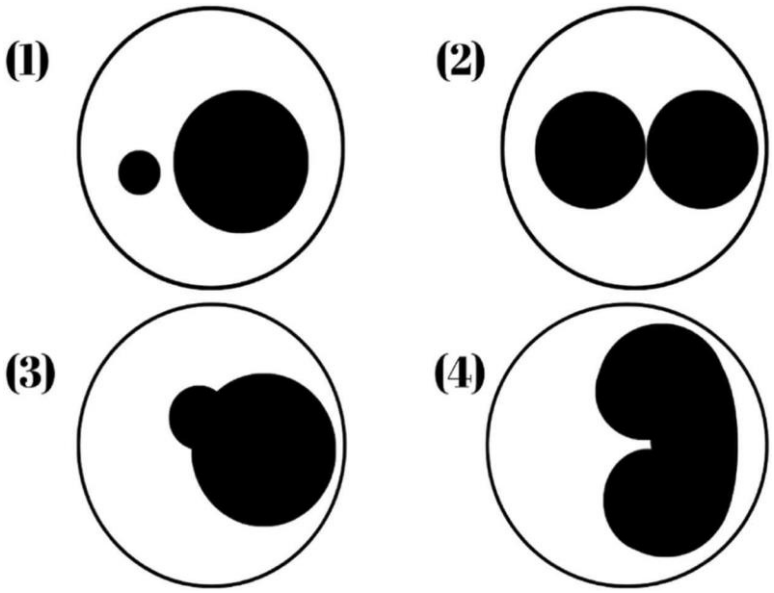

Fig. 1. Main nuclear anomalies. (1) micronucleus; (2) binucleate cells; (3) nuclear budding and (4) bilobed nucleus.

This technique has recently been applied to some elasmobranch families, such as Scyliorhinidae, Carcharhinidae and Ginglymostomatidae, providing an understanding of the sensitivity and adaptation of these species to environmental contaminants (Pegado et al., 2019, Araújo et al., 2023). For example, a study carried out in Portugal with the blood of 36 juvenile Lesser spotted dogfish discusses the importance of compensation mechanisms in protecting against DNA damage (nuclear anomalies) when exposed to high CO2 levels ($pCO2 \sim 900$ µatm, $\Delta -0.3$ pH units) (Pegado et al., 2019). The authors suggest that this species displays effective mechanisms to deal with these conditions, such as the ability to compensate for pH imbalances by increasing HCO3 levels without altering other biological parameters and the action of hemoglobin as a buffering agent (Pegado et al., 2019). Such mechanisms may directly influence nuclear anomalies, since no significant differences were found in micronuclei, segmented nuclei and bleeding nuclei induction in the different treatments (Pegado et al., 2019).

Other shark species, such as lemon sharks (*Negaprion brevirostris*), tiger sharks (*Galeocerdo cuvier*), and nurse sharks, demonstrate several responses to environmental contamination, with the first two showing greater sensitivity to genotoxic damage. This

sensitivity is detected through an increased frequency of certain genetic anomalies, such as micronuclei, bleeding nuclei, lobed nuclei, wedged nuclei, and binucleated cells (Araújo et al., 2023). A study carried out with the blood samples of 18 adult individuals of these species (males and females) from the Fernando de Noronha Archipelago, in Brazil, for example, discusses that some species such as nurse sharks display certain features, such as sedentary lifestyles, slow swimming, low metabolic activity and resident behavior, that aid in repairing DNA damage, resulting in decreased genetic damage. This was confirmed through a negative correlation between body length (associated with lifespan) and genotoxic damage, indicating that older organisms of this species tend to present less damage, probably due to kinetic differences in lymphocytes between old and young individuals (Araújo et al., 2023). However, species with active behavior, such as lemon sharks and tiger sharks, presented an opposite result, suggesting that lifestyle habits such as trophic position, diet, mobility, metabolic response and resistance influence the biomagnification of chemical compounds, resulting in direct DNA effects (Araújo et al., 2023).

Another genetic damage analysis applied to elasmobranch comprises the detection of the presence of damaged DNA, recognized as a sensitive biomarker in blue sharks (*Prionace glauca)* from Portugal (Alves et al., 2016). That study established the relationship between certain POPs (polychlorodibenzo-p-dioxins, polychlorodibenzofurans, polychlorinated biphenyls, polybrominated diphenyl ethers, polybrominated biphenyls and hexabromocyclododecane), metals and metalloids (Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Pb and Hg) in liver, muscle and brain samples from 20 juvenile blue shark individuals. A strong relationship between these contaminants and DNA damage levels was observed in muscle and liver, associated to ROS induction by the detected contaminants, such as hydroxyl radicals (•OH) and superoxide (O2•), as well as non-radical species like hydrogen peroxide (H2O2) (Harris, 1992, Mates, 2000). When present in excess, ROS can interact with DNA, leading to various forms of damage, including cross-links (DNA-protein), base modifications, intra-strand and inter-strand cross-links, and, ultimately, single and double-strand breaks (Jena, 2012; Hoseinifar et al., 2020).

In another study, whitespotted bamboo sharks from the Philippines, were investigated concerning the effects of ocean acidification on DNA in muscle, gill and liver

samples from 10 newly hatched sharks following exposure to high levels of CO2 (Lopes et al., 2018). High sensitivity, evidenced by increased damage to genetic material in the form of 8-Hydroxydeoxyguanosine) in the muscle and gills, induced by ROS, was observed. The detection of 8-OH-dG, an RNA nucleoside, is an important stress biomarker, formed due to ROS, first reported by Kasai et al. (1984). This biomarker can be applied both in vitro and in vivo, and is strongly associated with mutagenicity, in addition to being a sensitive biomarker, as it is capable of indicating low-level DNA damage (Dizdaroglu et al., 2001, Maclouf et al., 1987 Graille et al., 2020). Its formation comes from the action of hydroxyl radicals on the deoxyguanosine (dG) base of DNA, resulting in single-strand breaks (Ghorbanihaghjo et al., 2013, Pryor, 1997). High 8-OH-dG levels in cells indicate damage that can lead to genetic mutations, cell death and other deleterious effects (Kasai, 1997, Shen et al., 2007). The authors, however, highlight low liver damage levels, associated to an effective repair system in this organ.

Another DNA damage assessment was carried out in the gills and livers of 60 adult blue sharks from the North Atlantic, aiming to correlate this biomarker with both organic and inorganic contaminants (Alves et al., 2023). The results revealed the sensitivity of juvenile individuals to contamination by As and polychlorinated biphenyls, evidenced by organ damage and, as in the previously mentioned study on this same species, the breakdown of DNA material was associated with oxidative stress.

The CA, also termed microgel electrophoresis, has been applied in studies with elasmobranchs, although still rare. This method is based on the analysis of the length of the DNA "tail", or “comet”, visualized under a microscope after specific cell treatment, which extends from the cell nucleus, expressed as a percentage, thus indicating the amount of DNA damage (breakage) (Fig. 2) (Jiang et al., 2023). The results are classified into four categories, namely type 0 (no DNA damage), type 1 and 2 (small DNA damage), type 3 (extensive damage) and type 4 (complete DNA destruction) (Jiang et al., 2023).

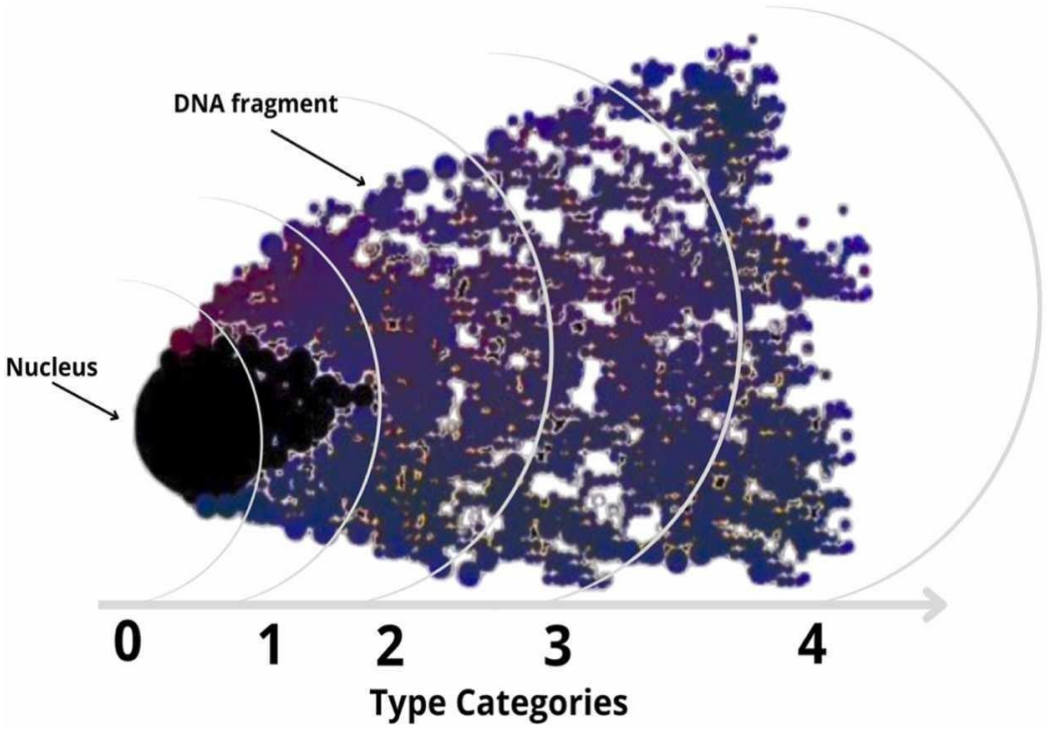

Fig. 2. Categories of DNA damage from 0 to 4 according to comet appearance. Type 0: no DNA damage; types 1 and 2: small DNA damage; type 3: extensive damage and type 4: complete DNA destruction.

This method has been applied in studies with elasmobranchs, although still rare. One study performed this assay on 10 individuals of *Scyliorhinus stellaris* (nursehound) in the northeastern Mediterranean Sea (Ergenler and Turan, 2024). The findings established a relationship between Cr, Cd, Fe, Ni, Pb and Zn and the extent of DNA damage in the gills and liver. The authors reported a significant increase in DNA damage in the gills compared to the liver, attributing this finding to the greater sensitivity of the gills, which are constantly exposed to water and have a high blood supply, which facilitates the absorption of toxic substances. In addition, the authors highlighted that the incidence of DNA damage in both organs is related to the formation of reactive oxygen species (ROS) induced by the presence of metals.

Although they are not commonly directly related to DNA damage in elasmobranchs, some studies also establish a relationship between enzymatic (superoxide dismutase, SOD, catalase, CAT, glutathione peroxidase, GPx, and glutathione S-transferase, GST) and non-enzymatic (reduced glutathione, GSH, and metallothionein, MT) enzymes in

protection against genetic damage caused by ROS (Ighodaro and Akinloye, 2018; Hoseinifar et al., 2020). A notable example is a study carried out with nurse sharks, where monooxygenases were used to assess aflatoxin B1 effects (Bodine et al., 1985). The authors indicate that this contaminant was able to reduce the activity of these enzymes, which normally play a protective role against electrophilic attacks on cellular DNA. In a subsequent investigation, involving the same species and contaminant, decreases in monooxygenase activity indicated a lower metabolizing capacity, resulting in potential DNA damage (Bodine et al., 1989). Another enzyme explored in this context is GPx, assessed in liver, muscle and brain samples of blue sharks captured in Portugal. A correlation was identified in muscle samples between GPx, DNA damage and high levels of contaminants (POPs and metals) (Alves et al., 2016). The researchers highlight that GPx acts mainly in preventing oxidative damage to DNA, thus indicating the importance of this enzyme in the antioxidant response and genetic protection in elasmobranchs.

Unlike the enzymes mentioned above, telomerase (TL), although not directly part of the cellular antioxidant system, also plays a vital role in DNA protection, acting directly on DNA and on telomere maintenance (Hori, 2022). The action of this enzyme was observed in blood samples from 16 elasmobranch species [(whale sharks (*Rhincodon typus*), Brownbanded bamboo sharks, (*Chiloscyllium punctatum*), Coral catsharks *(Atelomycterus marmoratus*), Zebra sharks (*Stegostoma fasciatum*), Pitted stingrays (*Dasyatis matsubarai*), Reticulate whipray (*Himantura uarnak*), bowmouth guitarfish (*Rhina ancylostoma*), Japanese bullhead shark (*Heterodontus japonicus*), Lesser spotted dogfish, Cloudy catshark (*Scyliorhinus torazame*), Pyjama shark (*Poroderma africanum*), Blotchy swellshark (*Cephaloscyllium umbratile*), Japanese wobbegong (*Orectolobus japonicus*), Bluespotted stingray (*Neotrygon kuhlii*), Giant devil ray (*Mobula mobular*), Round ribbontail ray (*Taeniurops meyeni*), Australian Ghost shark (*Callorhinchus milii*)] from Osaka Aquarium Kaiyukan from Japan (Hori et al., 2022). The authors highlight the presence of megatelomeres and low levels of oxidative stress in these species. Having a megatelomere may imply in greater protection against telomere shortening by compensating for the shortening that occurs during DNA replication, which is associated with aging and cell death that can be induced by oxidative stress (Barnes et al., 2019, Hori, 2022).

One study on nurse sharks and yellow stingrays (*Urobatis jamaicensis*) was conducted with the aim of investigating gene expression associated to DNA defense (Llorente, 2019). Several repair mechanisms were identified, namely mismatch repair (MMR), homologous recombination (HR) and non-homologous end joining (NHEJ). The first, mismatch repair (MMR), corrects base mismatches and small insertions or deletions (Llorente, 2019). Homologous recombination (HR) and non-homologous end joining (NHEJ) both repair double-stranded DNA breaks, with HR using sequence homology and NHEJ acting more quickly without a homologous template (Cai et al., 2019, Al-Jarf et al., 2023, Yu et al., 2020). According to the author, they play significant roles in DNA correction and maintenance in response to radiation and environmental pollutants. Therefore, an efficient repair system in these individuals would contribute to the development of an effective metabolic system (Llorente, 2019, Dey et al., 2023).

4. Conclusion

Advances in genetic damage assessments in elasmobranchs are crucial for understanding threats and conservation strategies. The use of genetic damage biomarkers is still incipient, and the MNT and damaged DNA assessments are the main techniques applied so far, providing the identification of direct responses related to the effects of contaminants. Although some elasmobranch species present defense and compensatory mechanisms, the resilience of some species is not a general pattern for this group, varying according to the biological characteristics of each species. This diversity in biological response highlights the need for specific approaches to the conservation of these animals, considering their particularities and local characteristics.

Future perspectives in this realm include studying the effects of persistent organic pollutants and the impact of rising sea temperatures and decreasing pH levels due to climate change on DNA integrity, along with the effects of increased UV radiation and radioactive materials from nuclear activities. Furthermore, understanding the genetic diversity within populations and its role in resilience to DNA damage and environmental stressors is also important. Comparative studies between different species of elasmobranchs can provide insights into species-specific responses to DNA damage, and applying techniques such as the

Comet assay, qPCR, and next-generation sequencing can also enhance the understanding of DNA damage and repair mechanisms in these species.

CRediT authorship contribution statement

Rachel Ann Hauser-Davis: Writing – review & editing, Writing – original draft, Visualization, Supervision, Investigation, Conceptualization. Ricardo Luvizotto-Santos: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Formal analysis, Conceptualization. Mateus Marques: Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization.

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9. ARTIGO II

Associations between metals and metalloids, oxidative stress and genotoxicity in nurse sharks (*Ginglymostoma cirratum*) from the Brazilian Amazon Coast

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Objetivos específicos da dissertação atrelados ao artigo:

- Determinar As, Se, Hg, Ti, Rb e ETR totais em diferentes tecidos (brânquias, fígado e músculo) de *G. cirratum*;
- Determinar os níveis de metalotioneína (MT) nos tecidos branquiais, muscular e hepático para avaliar a via de detoxificação metal-MT
- Avaliar os níveis de glutationa reduzida (GSH) nos tecidos branquiais, muscular e hepático;
- Analisar a atividade da enzima de biotransformação glutationa–S-transferase (GST) das brânquias, fígado e músculo;
- Validar metodologia para análise de micronúcleo (MN) e anomalias nucleares (brotamento e núcleo bilobado) nos tecidos brânquias, fígado e músculo;
- Avaliar a mutagenicidade da contaminação por metais sobre a espécie através das alterações nucleares.

Abstract

Elasmobranch populations have significantly declined in recent decades due to anthropogenic activities, with chemical contamination comprising one of the main threats to this group. Although some biochemical biomarkers have been utilized to assess elasmobranch health, especially concerning metal and metalloid contamination, associations with genotoxic biomarkers are still scarce and non-existent for Brazilian Amazon coast sharks. In this context, Nurse sharks (*Ginglymostoma cirratum*) from the São Marcos Estuarine Complex, Maranhão state, were analyzed for metals, metalloids, reduced glutathione (GSH), glutathione S-transferase (GST), metallothionein (MT), and nuclear anomalies (micronucleus, nuclear buds, and bilobed cells) in gills, liver, and muscle tissues. Females exhibited significantly higher arsenic (As) concentrations in muscle (23.14 ± 13.98 µg/g) and gills (4.53 ± 2.10 µg g^{-1}) compared to males (3.98 ± 2.61 µg g^{-1} and 1.51 ± 0.41 µg g^{-1}, respectively) ($p < 0.05$). Males showed higher selenium (Se) concentrations in muscle (0.52 ± 0.02 µg g^{-1}) compared to females (0.32 ± 0.09 µg g^{-1}) ($p < 0.05$), while rubidium (Rb) levels were higher in male liver (0.28 ± 0.05 µg g^{-1}) than in females (0.18 ± 0.04 µg g^{-1}) ($p = 0.001$). No significant differences

were observed for mercury (Hg), titanium (Ti), or rare earth elements (Ce and La) between sexes ($p > 0.05$). Higher GSH concentrations and GST activities were noted in gills and liver, while MT concentrations were higher in muscle. A low frequency of genotoxic damage was observed, likely due to the species' sedentary lifestyle and efficient DNA repair system. Moderate to strong correlations between metals/metalloids and biochemical/genotoxic responses were detected, particularly in females, highlighting the protective role of GST against DNA damage. Protective effects of Se against Hg toxicity were observed in the liver. Although metal concentrations did not exceed regulatory limits, the bioaccumulation patterns and physiological responses suggest that Nurse sharks are exposed to environmental contamination, with As and Se showing notable accumulation trends.

Keywords: Antioxidant; Biomarkers; Elasmobranchii; Nuclear anomalies.

Introduction

Earth's oceans and seas play a crucial role in sustaining animal and human life, covering approximately 70% of the globe's surface (Wang et al., 2020). These ecosystems contribute significantly to human well-being, encompassing primary and secondary production, biodiversity, global material and energy cycles, and resources such as fisheries, oil, gas, and minerals (Fleming et al., 2019; Gou et al., 2019). However, despite their vital importance, these ecosystems are being increasingly and negatively affected by environmental pollution, directly compromising the achievement of UN Goal 14, "Life Below Water" (United Nations, 2017; Häder et al., 2020), which aims to reduce marine pollution and preserve ecosystems essential for global sustainability (United Nations, 2017). The effects of environmental contamination have significantly intensified in certain areas in recent decades, including the Brazilian Amazon coast, mainly due to illegal mining activities near the coastal region, maritime transport, and vessel accidents (Fontes et al., 2023). This has led to serious biodiversity and marine ecosystem health implications (Fontes et al., 2023).

Exposure to metal and metalloids can cause significant organismal damage, *i.e.*, altered metabolic pathways, immunosuppression, and genotoxicity (Tchounwou et al., 2012; Alves et al., 2022). Elements such as arsenic (As), mercury (Hg), rubidium (Rb), titanium (Ti), selenium (Se), and rare earth elements (REE), for example, can significantly harm aquatic organisms

(Amorim-Lopes, 2020; Ghosh; Ghosh; Bhadury, 2021; Wang et al., 2023). Arsenic, for instance, inactivates enzymes involved in the DNA repair system and affects the central nervous system (Ghosh; Ghosh; Bhadury, 2021; Maciel et al., 2021), while Hg causes neurological effects (Bernhoft, 2012; Bolun et al., 2024). Titanium can affect reproduction aspects (Lee et al., 1990), while Rb is associated with cell necrosis and neurological effects (Lieberman; Meltzer, 1970; Ringer, 1883), and many REE are known to be genotoxic (Hanana; Kleinert; Gagné, 2021). Selenium, on the other hand, is an essential element that plays an antioxidant role and is known to reduce the toxic effects of certain elements such as As and Hg (Selvaraj et al., 2013; Wang et al., 2023), although it can also be harmful, when present over toxicity thresholds.

Cellular defense mechanisms are present in most living organisms to counteract the deleterious effects of chemical contamination. In this sense, several protein and enzyme biomarkers, such as reduced glutathione (GSH), glutathione S-transferase (GST), and metallothionein (MT), have been employed to assess oxidative stress and metal/metalloid exposure in exposed organisms. The glutathione-system members GSH and GST, both participate in the neutralization and excretion process of toxic compounds (Dasari et al., 2017; Averill-Bates, 2023), while MT regulates metal homeostasis, also playing against direct oxidative damage by scavenging reactive oxygen species (ROS) (Ruttkay-Nedecky et al., 2013; Yang et al., 2024). Nuclear anomalies, such as micronuclei and nuclear buds, among others, can be used to assess contaminant exposure effects, comprising genotoxicity indicators and revealing direct DNA damage caused by chemicals such as metals (Odetti et al., 2024).

Elasmobranchs, encompassing sharks and rays, comprising over 1,000 known species, are both directly and indirectly affected by pollution (Alves et al., 2022), mostly due to their relatively long lifespans and sensitivity to chemical contamination (Alves et al., 2016; Weigmann, 2016). These organisms are crucial for both ecological balance and human societies, serving as a vital source of income and food worldwide. However, research on the physiological impacts of metal and metalloid contamination in this group, including oxidative stress and genotoxicity, is still limited. In this sense, biochemical biomarkers are routinely employed to assess aquatic organismal health following exposure to chemical contaminants,

such as metals and metalloids (Alves et al., 2022), before damage at higher ecological levels, such as communities or ecosystems (Depledge; Aagaard; Gyorkost, 1995; Lemos et al., 2010). Although some oxidative stress biomarkers have been utilized to assess elasmobranch health, especially concerning metal and metalloid contamination, associations with genotoxic biomarkers are still sorely lacking for this group, and none are available for species from the Brazilian Amazon Coast, an elasmobranch hotspot (Noris et al., 2021; Araújo et al., 2023).

The Nurse shark *Ginglymostoma cirratum*, a mesopredator species with benthic habits that feed on small fish and bottom invertebrates, exhibits resident behavior (Wosnick et al., 2021) and is therefore, more vulnerable to localized chemical contamination, especially coastal, than other elasmobranchs species. It is classified as "vulnerable" on a global scale (Carlson et al., 2021) due to the adverse impacts of intensive fishing and marine pollution, particularly from metals and metalloids (Wosnick et al., 2021). In this context, Nurse sharks exhibit a high degree of bioaccumulation of metals and metalloids, which leads to the activation of antioxidant defense mechanisms and genotoxic alterations as a physiological response to the contamination of the environment in which they live.

This study aimed to determine metal and metalloid concentrations in Nurse sharks caught off the Brazilian Amazon coast, in Maranhão state, and associate these contaminants with enzymatic and protein oxidative stress and genotoxic biomarkers. The potential protective action of Se against the toxic effects of As and Hg was also evaluated.

Methodology

Study area

The São Marcos estuarine complex, in the state of Maranhão, Northeastern Brazil, is a highly metal-polluted environment due to intense port activity (Carvalho-Neta et al., 2013; Nunes et al., 2020; Almeida et al., 2021; Corrêa et al., 2023; Cutrim et al., 2025). . Nurse sharks were incidentally captured by artisanal fishers using bottom longlines in the main canal of the port complex (Figure 1) and subsequently taken to the Raposa Fishing Pier for processing and marketing

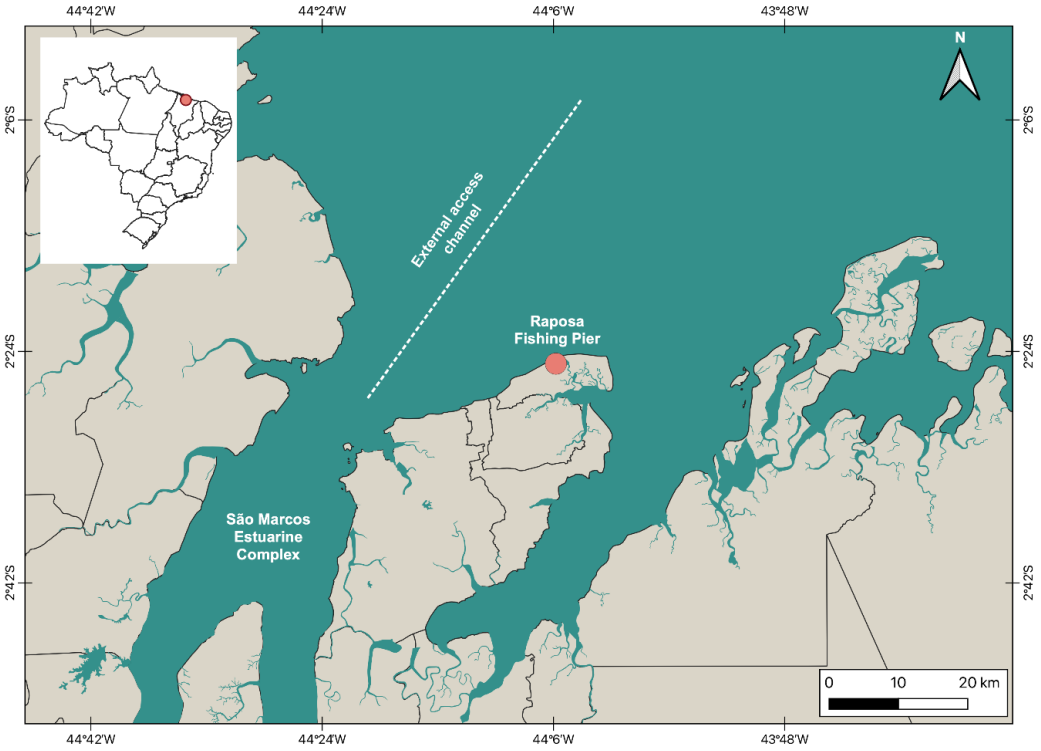

Figure 1. Nurse shark (*Ginglymostoma cirratum*) sampling area in the São Marcos Estuarine Complex, Maranhão, Brazilian Amazon Coast.

Nurse shark samplings and sample processing

The nurse sharks were measured and assessed for maturity according to Castro (2000). Four males (190 to 238 cm), two of which were mature, and ten females (180 to 237 cm), all mature, eight of which contained developing oocytes (Rêgo et al., 2019), were donated by local fishers. All were acquired fresh and whole between June and September 2023 (SISBIO-IBMA license no. 86594-1).

Total lengths (TL, cm) were determined using a tape measure. Gills, liver and muscle samples were removed from the carcasses and immediately placed in liquid nitrogen for transport to the laboratory and stored in an ultrafreezer (-80 ^{o}C) until further analyses. Tissue samples were stored in a phosphate-buffered saline solution (126.6 mmol L^{-1} NaCl, 4.8 mmol L^{-1} KCL, 1.5 mmol L^{-1} $CaCl_2$; 3.7 mmol L^{-1} $NaHCO_3$; 8.9 mmol L^{-1} Na_2HPO_4; 2.9 mmol L^{-1} NaH_2PO_4) as described by Cavalcante et al. (2008) for the genotoxicity analyses.

Metal and metalloid analyses

Muscle, gill, and liver samples (approximately 100 mg) were weighed and placed in sterile 15-mL polypropylene tubes for metal and metalloid analyses. Sample decomposition was performed by mixing 1 mL of distilled HNO_3 (67% v/v) with each sample and leaving the samples to react for 12 h, followed by decomposition on the following day conducted at 100 °C for about 5 h. After cooling to room temperature, the volumes of each sample were made up to 10 mL with ultrapure water.

The metal and metalloid determinations were performed by inductively coupled plasma mass spectrometry (ICP-MS), employing a NexIon 300X spectrometer (Perkin Elmer-Sciex, Norwalk, CT, USA). External calibration curves were used, all analyses were performed in quintuplicate and all analytical curves' determination coefficients were higher than 0.995. The concentrations of technologically critical elements—arsenic (As), mercury (Hg), selenium (Se), titanium (Ti), and rubidium (Rb)—as well as the rare earth elements cerium (Ce), dysprosium (Dy), erbium (Er), europium (Eu), gadolinium (Gd), holmium (Ho), lanthanum (La), lutetium (Lu), neodymium (Nd), praseodymium (Pr), samarium (Sm), terbium (Tb), thulium (Tm), ytterbium (Yb), and yttrium (Y) were evaluated.

Methodology accuracy was verified by the analysis of certified reference materials (DORM-5, fish muscle, NRC, Canada; NIST 2976, muscle tissue, National Institute of Standards and Technology; and BCR 668, muscle tissue, European Commission.

Oxidative stress biomarker analyses

Metallothionein determinations

Metallothionein determinations were carried out in triplicate according to Erk et al., (2002) through a thermal extraction procedure and quantified according to Ellman (1959). Briefly, samples (150 mg) were thawed and homogenized on ice employing an automatic microhomogenizer for 3 min in 2 mL polypropylene microtubes containing 500 mL of a Tris-HCl buffer (20 mmol L^{-1} pH 8.6) mixed with phenylmethyl sulfonyl fluoride (PMSF) (0.5 mmol L^{-1}) and β-mercaptoethanol (0.01%). The samples were then centrifuged for 60 min at 20,000 x g 4°C, and the supernatants were transferred to new sterile microtubes and heated at

70°C for 10 min, to denature non-thermoresistant proteins. Following another centrifugation in the same conditions for 30 min, the supernatants were mixed with a 1 mol L^{-1} HCl and 4 mmol L^{-1} EDTA solution, followed by the addition of 2 mol L^{-1} NaCl containing 0.43 mmol L^{-1} 5,5-dithiobis-2-nitrobenzoic acid buffered with 0.2 mol L^{-1} sodium phosphate, pH 8.0. Following an incubation period of 30 min, sample absorbances were determined at 412 nm on a Kasuaki DR-200BN-NM-BI microplate reader (Brazil) and MT levels were expressed as nmol g^{-1} wet weight (w.w.) calculated using reduced glutathione (GSH) as an external standard, due to a known stoichiometric ratio of 1 mol MT = 20 mol GSH (Kägi, 1991).

Reduced glutathione determinations

Reduced Glutathione (GSH) determinations were performed in triplicate according to Wilhelm-Filho et al. (2005). Briefly, 150 mg of the samples were homogenized utilizing an automatic microhomogenizer on ice in a 0.1 mol L^{-1} pH 6.5 sodium phosphate buffer containing 0.25 mol L^{-1} sucrose and subsequently centrifuged for 30 minutes at 11,000 x g at 4 °C. The supernatants were then separated from the precipitates and mixed with a 0.1 mol L^{-1} DTNB solution and 0.1 mol L^{-1} sodium phosphate buffer (pH 8.0), incubated for 15 min, and analyzed on a Kasuaki DR-200BN-NM-BI microplate reader (Brazil) at 412 nm. Concentrations were calculated using GSH as an external standard and expressed as µmol g^{-1} w.w.

Glutathione-S-transferase determinations

Glutathione-S-transferase (GST) activities were determined in triplicate according to Keen, Habig and Jakoby (1976), in which the conjugation of reduced glutathione (GSH) with CDNB, catalyzed by GST, produces a compound (GS-DNB) detectable at 340 nm. The samples (~150 mg) were homogenized employing an automatic microhomogenizer on ice at a 1:10 (w:v) ratio in a 0.1 mol L^{-1}, pH 7.2 potassium phosphate buffer containing 100 mmol L^{-1} PMSF as a protease inhibitor followed by centrifugation at 7.500 x g at 4 °C for 10 min. The supernatants containing the cytosolic fraction were then separated and mixed with a 0.1 mol L^{-1} pH 7.2 potassium phosphate buffer, 1-chloro-2,4-dinitrobenzene (CDNB) as substrate (35 mmol L^{-1}) and GSH (35 mmol L^{-1}). Reaction kinetics were monitored at 340 nm for 10 min at 15 s intervals at 25°C employing a BioTek Synergy H1 Hybrid Multidetection Microplate

Reader. Activities were expressed as μmol min^{-1} mg $protein^{-1}$ following total protein determinations by the biuret method.

Genotoxicity analyses

For the genotoxicity analyses, cell dissociation was carried out according to Kilemade et al. (2004) and Cavalcante et al. (2008), with modifications, for subsequent micronucleus (MN) test and nuclear anomaly (nuclear buds - NB and bilobed nuclei - BN) assessments. The muscle, gill, and liver samples (200 mg) were first rinsed with a phosphate-buffered saline (0.9%) and fragmented using sterilized surgical scissors. Then, 200 μL of trypsin-EDTA (0.25%) were added at 37 °C for cell dissociation, followed by incubation at room temperature (3 min for gills and liver and 6 min for muscle) and homogenization by manual inversion. To stop enzymatic digestion, 200 μL of bovine serum was added and the samples were left to stand for 10 min at room temperature. Larger tissue fragments were removed and discarded using an automatic pipette and 100 μL of a 0.9% saline solution were added to the remaining digested samples. The samples were then centrifuged at 1,000 rpm for 10 min, the supernatants discarded, and the precipitates resuspended in bovine serum mixed with an acetic acid and ethanol (3:1) solution. Then, 100 μL aliquots were removed and smears were prepared on glass slides and treated by rapid panoptic staining. The slides were then observed under an optical microscope (1,000x) and 1,000 cells per individual were counted. Micronuclei were classified according to Hooftman and Raat (1982), while other nuclear anomalies were classified according to Barsiené et al. (2006).

Statistical analyses

The metal and metalloid, genotoxicity, and biochemical data normality and homogeneity were verified by the Shapiro-Wilk and Levene tests, respectively. As the data were non-normally distributed, the Mann-Whitney test ($p<0.05$) was applied to compare elements and biomarker concentrations and activities between nurse shark males and females, and potential organ differences were evaluated by the Kruskal-Wallis test followed by the Dunnett *posthoc* test ($p<0.05$). Statistical correlations between the determined metals and biomarkers in the same organ for males and females were evaluated by the Spearman correlation test, according to the strength categorization proposed by Bryman and Cramer

(2011), as very weak when 0.00 < r < 0.19; weak when 0.20 < r < 0.39; moderate when 0.40 < r < 0.69; strong when 0.70 < r < 0.89; and very strong when 0.90 < r < 1.00. To verify the potential protective effects of Se against As and Hg, significant associations ($p<0.05$) were further evaluated by utilizing molar ratio calculations. All analyses were performed and graphs were constructed using the R v. 4.1.1 software (R Core Team, 2024).

RESULTS AND DISCUSSION

Metal and metalloid determinations

The Limits of Detection (LOD) and Limits of Quantification (LOQ) (Table 1), as well as the recoveries (Table 2), were calculated following the guidelines established by the Brazilian National Institute of Metrology and Industrial Quality (INMETRO, 2016). Among the investigated rare earth elements (REEs), only cerium (Ce) and lanthanum (La) were found above the LOQ. The remaining elements (As, Hg, Se, Ti, Rb) were detected in the Nurse shark tissues, with males and females analyzed separately. (Supplementary Table 1). Data are displayed in Figure 2 expressed as mg kg-1 w.w.

Females presented significantly higher As concentrations in muscle (23.14 ± 13.98 mg kg^{-1}) and gills (4.53 ± 2.10 mg kg^{-1}) when compared to males (3.98 ± 2.61 and 1.51 ± 0.41 mg kg^{-1}, respectively) ($p = 0.03$). No significant differences, however, were observed between males and females for Hg ($p = 0.07$). Regarding Se, males exhibited significantly higher concentrations in muscle tissue (0.52 ± 0.02 mg kg^{-1}) when compared to females (0.32 ± 0.09 mg kg^{-1}) ($p<0.02$. Regarding Rb, differences were observed only in the liver, with males exhibiting higher concentrations (0.28 ± 0.05 mg kg^{-1}) compared to females (0.18 ± 0.04 mg kg^{-1}) ($p = 0.001$). No significant difference was observed for gills, liver, or muscle for either Ti or Ce and La ($p > 0.07$).

Table 1. Limits of Detection (LD) and Limits of Quantification (LQ). Data are expressed as mg kg^{-1} wet weight.

Elements	As	Ce	Dy	Er	Eu	Gd	Hg	Ho	La	Lu	Nd	Pr	Rb	Se	Sm	Tb	Y
LOD	0.0038	0.00059	0.00006	0.00002	0.00002	0.00001	0.0012	0.00004	0.00007	0.0025	0.00008	0.00006	0.0041	0.21	0.00015	0.0043	0.00002
LOQ	0.013	0.002	0.0002	0.00006	0.00006	0.00002	0.0039	0.00014	0.00022	0.0084	0.00026	0.0002	0.014	0.7	0.00051	0.014	0.00006

Table 2. Observed and certified values for the analyzed reference materials and their recoveries (%). Data are expressed as mg kg^{-1} wet weight.

Element	**DORM-5**			**BCR 668**			**NIST 2976**		
	Observed values	**Certified values**	**Recovery (%)**	**Observed values**	**Certified values**	**Recovery (%)**	**Observed values**	**Certified values**	**Recovery (%)**
As	15.00 ± 1.30	13.30 ± 0.70	113	6.91 ± 0.46	7.10 ± 0.50	97	15.4 ± 1.0	13.3 ± 1.8	115
Ce	0.025 ± 0.005	-	-	0.082 ± 0.014	0.089 ± 0.007	92	0.080 ± 0.010	0.109 ± 0.008	74
Dy	0.0032 ± 0.0004	-	-	0.007 ± 0.001	0.0089 ± 0.0006	88	0.006 ± 0.001	-	-
Er	0.0016 ± 0.0002	-	-	0.004 ± 0.0001	0.0045 ± 0.0005	91	0.0030 ± 0.0004	-	-
Eu	0.0007 ± 0.0001	-	-	0.0026 ± 0.0003	-	-	0.001 ± 0.0001	0.002 ± 0.0003	81
Gd	0.0036 ± 0.0004	-		0.012 ± 0.002	0.013 ± 0.0006	96	0.010 ± 0.001	-	-

Hg	0.20 ± 0.02	0.30 ± 0.01	81	0.089 ± 0.010	-	-	0.051 ± 0.005	0.061 ± 0.003	85
Ho	0.0006 ± 0.0001	-		0.0010 ± 0.0001	0.0018 ± 0.0006	84	0.0010 ± 0.0001		
La	0.010 ± 0.002	-	-	0.079 ± 0.016	0.08 ± 0.006	99	0.046 ± 0.008	-	-
Lu	0.0002±0.0001	-	-	0.0003 ± 0.0001	0.0003 ± 0.00002	95	0.0003 ± 0.00002	-	-
Nd	0.020 ± 0.003	-	-	0.045 ± 0.014	0.054 ± 0.004	84	0.565 ± 0.015	-	-
Pr	0.003 ± 0.0005	-	-	0.010 ± 0.001	0.0123 ± 0.0011	88	0.008 ± 0.0008	-	-
Rb	2.20 ± 0.20	2.70 ± 0.10	82	2.60 ± 0.40	-	-	3.97 ± 0.58	4.14 ± 0.09	95
Se	2.40 ± 0.20	2.40 ± 0.11	102	1.48 ± 0.31	-	-	1.94 ± 0.28	1.8 ± 0.15	107
Sm	0.0032 ± 0.0002	-	-	0.010 ± 0.001	0.0112 ± 0.0008	96	0.008 ± 0.001	-	-
Tb	0.0005 ± 0.0001	-	-	0.0010 ± 0.0001	0.0016 ± 0.0001	90	0.0010 ± 0.0001	-	-
Y	0.015 ± 0.002	-	-	0.046 ± 0.006	0.059 ± 0.005	79	0.054 ± 0.009	-	-

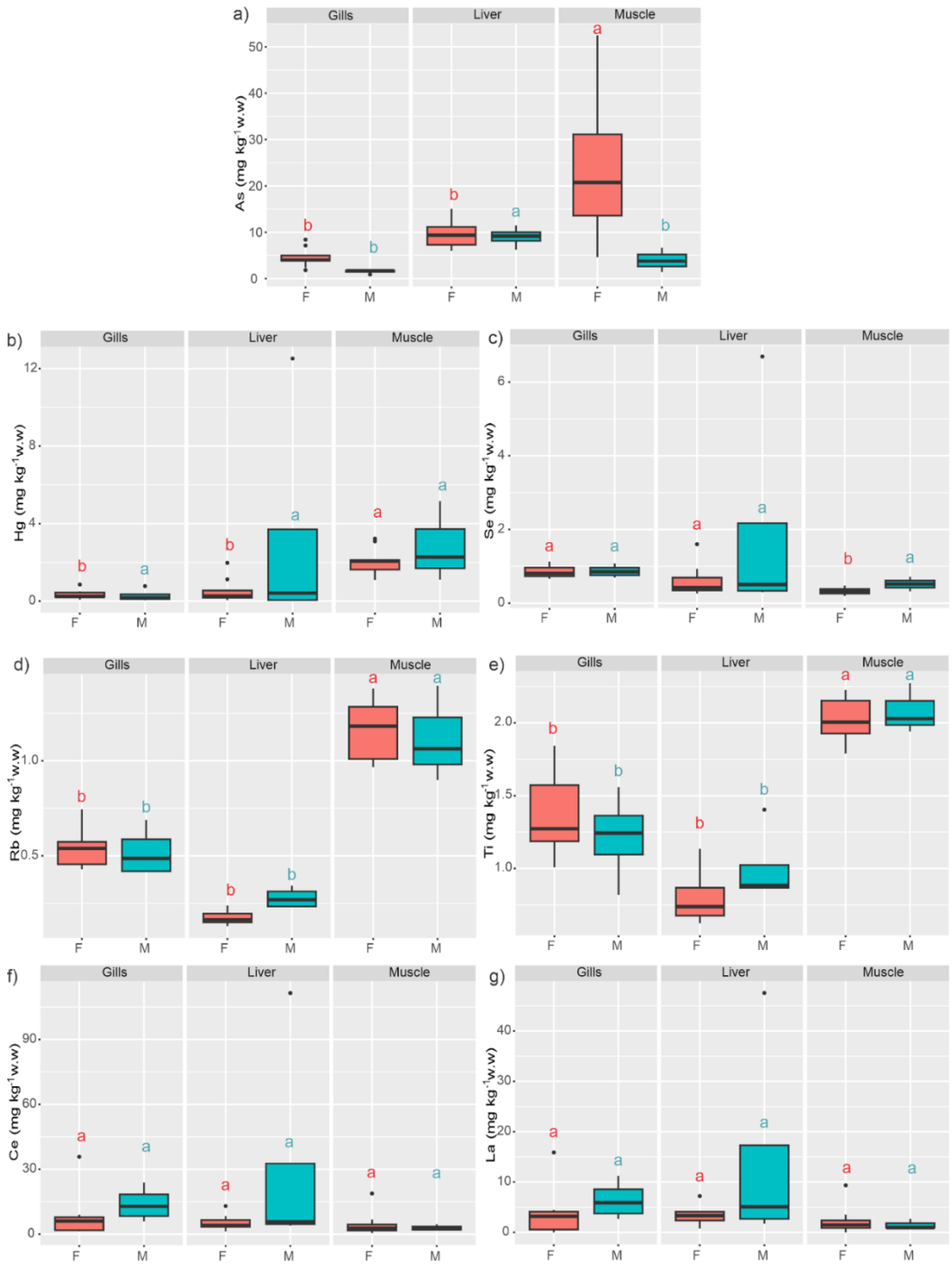

Figure 2. Metals and metalloids determined in the muscle, gill, and liver of *Ginglymostoma cirratum* from the Brazilian Amazon Coast. a) As, b) Hg, c) Se, d) Rb, e) Ti, f) Ce and g) La, Whiskers indicate maximum and minimum ranges, with vertical lines extending from the 10th to the 90th percentile and dots representing outliers. Different letters indicate significant

differences between organs (red for females and blue for males) according to the Kruskal-Wallis test followed by the Dunnett test ($p < 0.05$). F: females, M: males. Data are expressed as mg kg^{-1} w.w..

Concerning accumulation between tissues, females presented significantly higher As concentrations in muscle compared to the liver and gills ($p < 0.001$). In contrast, males presented the highest As in the liver, while the muscle and gills presented lower levels ($p < 0.01$). No differences were observed for Hg in males ($p > 0.05$), although females exhibited greater Hg accumulation in muscle, with lower concentrations in the liver and gills ($p < 0.05$). Similarly, Se was not significantly different between tissues in males ($p > 0.05$), while in females, this element was consistently higher in the gills and liver compared to muscle ($p < 0.001$). On the other hand, Rb and Ti were higher in muscle, and lower in the gills and liver ($p < 0.01$) in males. In females, on the other hand, both elements were higher in muscle, followed by gills and liver ($p < 0.0001$). Finally, no significant differences were noted for REE between organs ($p > 0.05$).

The accumulation of metals and metalloids in aquatic organisms is attributed to several biological and ecological factors, which vary according to sex, species, maturation, potential egg/embryo transfer, and habitat (Torres; Da Cunha; Dos Santos et al., 2017; Kim et al., 2019; Maciel et al., 2021).

In general, lower metal accumulation in females is often reported in elasmobranchs, attributed to female detoxification mechanisms, such as maternal transfer to eggs or embryos (Pantoja-Echevarría et al., 2021). In the specific case of Se, higher concentrations in females may be due to its active role in metabolic activities, as a protein and enzyme constituent (Pantoja-Echevarría et al., 2021), although further assessments are required to confirm this hypothesis.

As observed for Se, high As levels were also observed in females compared to males, probably due to the reproductive cycle and metabolism during oogenesis, which can influence the accumulation of several metals (Varol; Kaçar, 2023). The opposite was, however, noted in a study carried out with Caribbean Sharpnose sharks *Rhizoprionodon porosus* sampled in southeastern Brazil, where males presented significantly higher As concentrations (8.24 ± 1.20

mg kg^{-1} w.w.) compared to females (6.59 ± 1.87 mg kg^{-1} w.w.) in the liver (Maciel et al., 2021). This metalloid's availability is mainly related to accumulation via diet which, in the case of female Nurse sharks, is predominantly crustacean-based (Pratt; Carrier, 2001; Storelli; Marcotrigiano, 2004).

Rubidium and Se, in contrast, were higher in males than females. Lower metal and metalloid accumulation in males are not frequently reported for elasmobranchs, with most assessments indicating higher levels in females, *i.e.*, in the liver of Pacific Sleeper sharks *Somniosus pacificus*, where the concentrations of Rb were higher in females compared to males (0.44 ± 0.11 and 0.38 ± 0.07 mg kg^{-1} w.w., respectively) (McMeans et al., 2007). Some studies, however, indicate no significant differences between sexes, as demonstrated on another shark species, the Brown Smooth-hound *Mustelus henley*, where Se concentrations in muscle (0.17 ± 0.099 mg kg^{-1} w.w. for females and 0.17 ± 0.12 mg kg^{-1} w.w. for males) and liver (0.33 ± 0.29 mg kg^{-1} w.w. for females and 0.65 ± 0.65 mg kg^{-1} w.w.) were not significantly different between males and females (Pantoja-Echevarría et al., 2021).

The absence of significant differences in the accumulation of Hg, Ti, and the two REEs detected in the present study suggests that factors other than diet and habitat may be influencing the retention of these elements in both sexes. Possible explanations include similarities in metabolic rates, excretion mechanisms, or the strong influence of environmental factors on the bioavailability of these contaminants (Torres et al., 2017). Furthermore, the concentrations of these elements may be more influenced by environmental and behavioral factors than biological characteristics specific to each sex (Ju et al., 2021). The Nurse shark population in the São Marcos estuarine complex is chronically exposed to elevated Hg levels due to port activities, industrial operations, and coastal urbanization. Combined with their benthic habits, this exposure poses a considerable threat to this population (Wosnick et al., 2021). High levels of Ti and REEs, such as La and Ce, have been found in various organs of Tiger sharks in Southern Brazil, indicating systemic transport of these elements through multiple tissues (Wosnick et al., 2024). However, the harmful effects of Ti and REEs on marine organisms remain unknown.

Regarding toxicokinetics, most elements were detected in both muscle and liver. No significant differences were, however, observed between As concentrations in these tissues. This differs from another study also conducted in Brazil in the Caribbean Sharpnose shark *Rhizoprionodon porosus*, which As concentrations of 6.32 ± 2.41 mg kg^{-1} w.w. in female muscle and 7.21 ± 2.10 mg kg^{-1} w.w. in males, and 6.59 ± 1.87 mg kg^{-1} w.w. in female liver and 8.24 ± 1.20 mg kg^{-1} w.w. in males. In that study, muscle concentrations were significantly (25% higher) compared to liver, indicating potential bioaccumulation of this element. On the other hand, in Pacific Sleeper sharks, high As accumulation was detected in the liver (0.44 ± 0.11 mg kg^{-1} w.w.) (McMeans et al., 2007). This is expected, considering that the liver is the main metabolization organ in vertebrates, including sharks (Vélez-Alavez et al., 2013). Specifically, As is metabolized more efficiently in the liver, through the methylation of inorganic As, which results in a faster excretion of organic forms of As, avoiding bioaccumulation in less metabolically active tissues, such as muscle (Vélez-Alavez et al., 2013; Maciel et al., 2021).

Regarding Hg, high concentrations in muscle compared to other organs may be related to factors such as size, diet, habitat, and low efficiency of excretion mechanisms (Vélez-Alavez et al., 2013). Here, Hg concentrations in the muscle were significantly higher than in the gills and liver, indicating bioaccumulation. High Hg bioaccumulation in muscle (0.49 ± 0.08 mg kg^{-1} w.w.) compared to liver (0.08 ± 0.06 mg kg^{-1} w.w.) has also been reported in female Shortfin Mako sharks *Isurus oxyrinchus* caught off the coast of Baja California Sur (Vélez-Alavez et al., 2013). A similar pattern was observed in Blue sharks *Carcharhinus glaucus* from southwestern Portugal, where concentrations in muscle (0.00136 ± 0.0008 mg kg^{-1} w.w.) were higher than those detected in the liver (0.00028 ± 0.00034 mg kg^{-1} w.w.) (Alves et al., 2016). When organisms are exposed chronically to Hg, this element tends to accumulate in muscle with age increasing, due to its high bioaccumulation potential, especially in long-lived species such as sharks (Rodrigues et al., 2022). Furthermore, slower detoxification rates are noted for this element., and its accumulation in muscle is associated with its high affinity for amino acids present in this tissue (Vélez et al., 2021).

On the other hand, Se showed higher accumulations in the gills and liver compared to the muscle. Similar to what was observed herein, Se concentrations in female Brown Smooth-hounds *Mustelus henlei* captured off the coast of Southern California were also higher in liver (0.00033 ± 0.00029 mg kg^{-1} w.w.) compared to muscle (0.00017 ± 0.00009 mg kg^{-1} w.w.) (Pantoja-Echvarría et al., 2021), as well as those captured in the northern Mexican Pacific Ocean, (0.00014 ± 0.00007 mg kg^{-1} w.w. in liver and 0.00003 ± 0.00001 mg kg^{-1} w.w. in muscle) (Medina-Morales et al., 2020). The high levels of Se in biotransformation organs, such as gills and liver, are probably due to selenoproteins, such as glutathione peroxidase, which contribute to Hg demethylation, detoxification, and excretion into the bile in the form of cysteine-Hg (Medina-Morales et al., 2020; Pantoja-Echvarría et al., 2021).

Regarding Rb and Ti, significantly higher bioaccumulation was observed in the muscle than in the other organs assessed in this study. Studies in elasmobranchs generally do not focus on the toxicokinetics of these elements, with most focusing on the accumulation in only a single organ (McMeans et al., 2007; Wosnick et al., 2021; Ju et al., 2021; Suratno et al., 2022). In one study where inter-organ comparisons were conducted in Tiger sharks, *Galeocerdo cuvier* captured off the coast of Paraná, Brazil, Rb concentrations were also higher in muscle (0.78 ± 0.32 mg kg^{-1} w.w.) compared to gills (0.54 ± 0.18 mg kg^{-1} w.w.) and liver (0.31 ± 0.19 mg kg^{-1} w.w.) (Wosnick et al., 2024). The bioaccumulation of this element in Nurse sharks has been evaluated in only one study, also in Maranhão state, which reported muscle concentrations of 9.52 ± 4.55 mg kg^{-1} w.w. These high levels were associated with a space fuel leak resulting from the explosion of the VLS-1 V03 rocket in 2003, at the Brazilian Air Force Rocket Launch Center in Alcântara (Wosnick et al., 2021), probably due to this element's persistent characteristic and tendency to sink. Although Rb tends to bioaccumulate less than mercury (McMeans et al., 2007), it is also easily biomagnified (Wosnick et al., 2024) and may affect processes such as fish spermatogenesis and endocrine pathways (Yamaguchi et al., 2007). Its affinity with the muscle of aquatic organisms is related to its physicochemical behavior, similar to that of potassium (K), which causes it to be absorbed similarly (Sanders; Kirschner, 1983; Wosnick et al., 2024). Furthermore, the bioaccumulation of Rb in muscle results from slower diffusion, due to the lower permeability of the cell membrane to Rb compared to K (Sanders; Kirschner, 1983).

Concerning Ti, the same study in Tiger sharks revealed higher concentrations in muscle (3.48 ± 2.36 mg kg^{-1} w.w.) compared to other organs, such as gills (2.81 ± 1.52 mg kg^{-1} w.w.) and liver (1.35 ± 0.47 mg kg^{-1} w.w.) (Wosnick et al., 2024). The presence of this element in the aquatic environment is largely attributed to its increasing use in the form of nanoparticles in personal hygiene products, cosmetics, and the food industry (Wosnick et al., 2024). Its effects vary according to the exposure dose, ranging from changes in metabolic systems, to genomic damage and tissue lesions (Federici; Shaw; Handy, 2007; Shi et al., 2022). Some studies indicate that Ti in elasmobranchs has a low affinity for the liver, compared to tissues with lower metabolic activity, such as muscle (Hauser-Davis et al., 2021; Wosnick et al., 2024). In bony fish, the bioaccumulation of this element is considered low, with approximately 1 % from environmental exposure to the surrounding water column water, resulting in Ti concentrations through surface adsorption (Federici; Shaw; Handy, 2007). Other studies show lower accumulation in muscle via the dietary route compared to gills and liver (Suárez-Oubiña et al., 2023). As these studies were laboratory-based ranging from 14 to 90 days of exposure, exposure time may lead to greater bioaccumulation (Federici; Shaw; Handy, 2007; Suárez-Oubiña et al., 2023). In the case of elasmobranchs, such as the Nurse shark, which are long-lived and can be exposed to contamination through different matrices (water, sediment and diet), a probable trend of bioaccumulation in muscle, an organ with lower metabolic activity, is noted (Saratno et al., 2022; Wosnick et al., 2024).

Finally, the lack of difference in the accumulation of REE in the present study (Ce and La) also resembles those observed in *Galeocerdo cuvier* (Wosnick et al., 2024). In another species, such as the Nursehound *Scyliorhinus stellaris*, higher concentrations of these elements were recorded in the liver (0.00080 ± 0.00005mg kg^{-1} w.w.) and below the limit of detection in the muscle (Squadrone et al., 2022). Although REE are called "rare", they are relatively abundant in the Earth's crust but widely dispersed (Malhotra et al., 2020). Typically found in phosphates, carbonates, fluorides, and silicates, these elements are widely used in the electronic technology industries (Malhotra et al., 2020). However, studies on the toxicokinetics of these elements and their effects on aquatic organisms are scarce. Some studies indicate that REEs are more accumulated by organisms that feed on benthic organisms (Mayfield; Fairbrother, 2015), probably because sediments are the final environmental sinks for metals and metalloids

(Ferreira et al., 2021). When ingested, these elements may accumulate in greater quantities in the viscera and bones compared to muscle tissues (Mayfield; Fairbrother, 2015). When ingested, these elements may accumulate in greater quantities in the viscera and bones as in muscle tissues (Mayfield; Fairbrother, 2015). Once accumulated, elements like lanthanum (La), for example, can cause deformations in tissues such as gills and the liver, as observed in the cyprinid *Gobiocypris rarus* (Hua et al., 2017). In other species, such as Rainbow trout *Oncorhynchus mykiss*, exposure to Ce and La has been shown to affect the gene expressions of GST and MT, induce oxidative stress, and cause genotoxic effects (Hanana, Kleinert, Gagné, 2021).

Oxidative stress biomarkers

No significant differences in MT, GSH, or GST levels were observed between males and females ($p > 0.05$), although organ distribution varied for each (Figure 3). Concerning MT, variations were noted among organs for both males and females, significantly higher in muscle compared to gills (in males and females) and liver (only in females) ($p < 0.05$). Regarding GSH, higher levels were observed in the gills and liver in both males and females compared to muscle ($p < 0.05$). The same pattern was observed for GST activities, with higher levels in the liver, the main biotransformation organ ($p < 0.05$).

Metabolic responses, especially antioxidant defenses, are expected to increase during oogenesis to support energy expenditure and protect oocytes from oxidative stress (Barreira-Garcia et al., 2013; Ladisa; Ma; Habibi, 2022). However, in the present study, even with mature females, many with oocytes in formation and some already formed, no differences were observed in their antioxidant defenses and genotoxic damage compared to males. This may be related to the metabolic adaptations of females throughout their reproductive period, which may contribute to the maintenance of reproductive fitness and the protection of oocytes against oxidative stress of endogenous and exogenous origin (Ladisa; Ma; Habibi, 2022). In addition, it is important to highlight a possible similarity in the expression of antioxidant defenses between both sexes, which suggests the same capacity for a similar response to oxidative stress and contamination as already observed for Zebrafish *Danio rerio* (Glisic et al., 2015), Mummichog *Fundulus heteroclitus* (van Cleef et al., 2000) and North African catfish *Clarias*

gariepinus (M'Kandawire et al., 2017). Similar antioxidant responses in males and females can lead to comparable levels of MN and nuclear anomalies, as these defenses are crucial for neutralizing damage from free radicals that can cause genomic instability leading to genotoxic damage (Sharma et al., 2021).

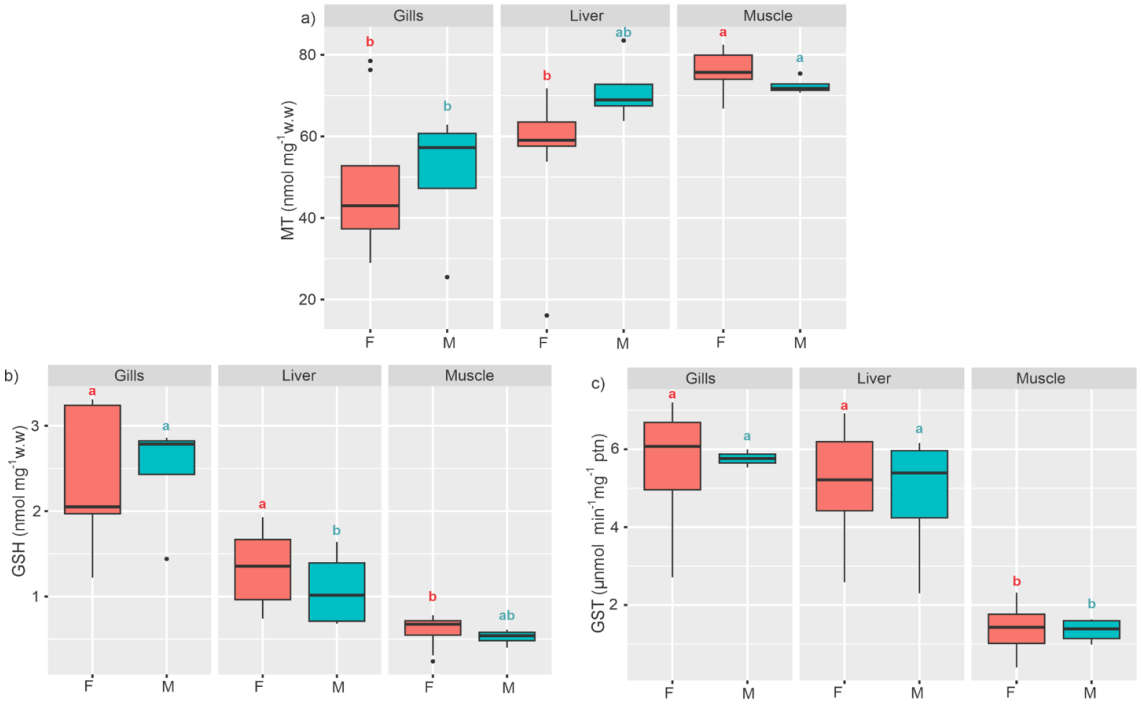

Figure 3. Metallothionein (a, nmol mg^{-1} w.w.) and GSH levels (b, nmol mg^{-1} w.w.) and GST activities (c, µmol min^{-1} mg $protein^{-1}$) in the gills, liver, and muscle of *Ginglymostoma cirratum* from the Brazilian Amazon Coast. Whiskers indicate maximum and minimum ranges, with vertical lines extending from the 10^{th} to the 90^{th} percentile and dots representing outliers. Different letters indicate significant differences between organs (red for females and blue for males) according to the Kruskal-Wallis test followed by the Dunnett test ($p < 0.05$). F: females, M: males.

Interorgan variations were observed concerning the determined biomarkers. In the case of MT, our results were higher than those reported for Blue sharks from the western North Atlantic Ocean, a nektonic species, ranging from 20 to 30 nmol mg^{-1} w.w. in its liver tissue and

50 to 60 nmol mg^{-1} w.w. in the muscle tissue (Hauser-Davis et al., 2021). It is important to highlight that pelagic organisms tend to accumulate metals, generally via water and diet, while demersal species, such as the Nurse shark, may also be exposed via sediment (Bervoets et al., 2013; Sow et al., 2019). These may lead to different accumulation of contaminants and, consequently, MT level variations (Bervoets et al., 2013; Sow et al., 2019). Higher MT levels were observed in the muscle compared to the gills. A similar pattern was recorded in deep-sea elasmobranchs (*Deania hystricosa*, *Etmopterus princeps*, and *Hydrolagus pallidus*), with high levels of muscle MT and low levels in the gills (Company et al., 2010). In Cloudy Catsharks *Scyliorhinus torazame*, on the other hand, the highest MT levels were detected in the liver, indicating its role in metal and metalloid detoxification (Cho et al., 2005). Although muscle is not as metabolically active as other organs (Hauser-Davis et al., 2022), an increase in MT levels may indicate a higher expression of the MT gene, induced by metal bioaccumulation (van Heerden et al., 2006). Unlike muscle, which is usually the last organ affected, the gills are considered the first target organ of metal toxicity, often presenting MT induction (Wang et al., 2014). However, in some fish species, gill MT presents species-specific variation, with some species showing low sensitivity to metal exposure (Cho et al., 2008; Wang et al., 2014). Studies indicate that variations in MT levels in the gills may be linked to the presence of different cell types, such as pavement cells and chloride cells (Cho et al., 2008). In particular, chloride cells regulate ionic balance and may influence MT levels. Thus, alterations in the amounts or function of these cells (induced by processes such as cell renewal, hyperplasia, necrosis, or apoptosis) may modify MT levels in gills, affecting an organism's defense capacity against exposure to metals and metalloids (Cho et al., 2008).

Regarding GSH levels, concentrations are similar to those detected in the muscle of Blue sharks from the western North Atlantic Ocean, ranging from 0.4 to 1.6 μmol g^{-1} w.w. (Hauser-Davis et al., 2021). These similarities can be attributed to the antioxidant function of GSH, since both demersal and pelagic species can be subject to the same levels of oxidative stress of endogenous and exogenous origin (Gabriel et al., 2020).

On the other hand, the GST enzyme activity in the present study showed higher concentrations than those observed in *Prionace glauca*, which had a mean value of 0.11 ± 0.03

(µmol min⁻¹ mg of protein⁻¹) in muscle (Alves et al., 2016). However, these values were lower than those observed in *Scyliorhinus canicula*, a demersal species from the Tyrrhenian Sea, where concentrations reached approximately 300 (µmol min⁻¹ mg of protein⁻¹) in liver tissue (Gorbi et al., 2004). Similarly, Great Lanternshark *Etmopterus princeps*, which inhabit areas surrounding hydrothermal vents, presented high activity in gills (33.2 ± 7.2 µmol min^{-1} mg ptn^{-1}), liver (61.3 ± 7.4 µmol min^{-1} mg ptn^{-1}), and muscle (52.8 ± 2.5 µmol min^{-1} mg ptn^{-1}) (Company et al., 2010). As noted for MT concentrations, GST activities tend to vary between species, depending on ecological characteristics, such as feeding habits and different exposure routes, with demersal species exhibiting more accumulation routes due to greater interaction with the sediment and swimming mobility (Barreira-García et al., 2012; Ozcelebi et al., 2021).

In general, Nurse sharks presented higher GSH concentrations in gills, while GST displayed higher activities in the gills and liver compared to muscle. Similar findings have been reported for Shortfin Mako sharks, where higher metabolic activity was found in the liver and lower in the muscle (Vélez-Alavez et al., 2013). It is known that the gills and liver play essential roles in elasmobranch metabolism. The gills are the first defense organ, involved in respiration, osmotic regulation, and ammonia excretion (Wegner, 2015), while the liver acts mainly in detoxification, energy storage, nutrient conversion, hormonal balance, and coagulation (Melo et al., 2019). These functions increase oxidative stress in these organs, requiring high metabolic responses (Rivera-Ingraham, 2017). However, it is important to note that elevated levels of these antioxidants in the gills and liver may also be related to capture stress caused by capture, inducing a wide range of physiological changes related to extreme physical effort, including hypoxia and hemorrhage (Cruz-Ramírez et al., 2017; Tao, 2024). These factors are largely responsible for affecting the acid-base balance in the blood, causing metabolic and respiratory acidosis, as noted in Carcharhinidae sharks caught by longline fisheries (Mendoza; Aguilera; Carreón Martínez, 2002; Tao, 2024). In addition, sharks with greater swimming activity tend to exhibit more intense metabolic responses, due to the greater consumption of O_2 during physical activity, which generates an overproduction of ROS (López-Cruz et al., 2010).

Genotoxicity assessment

The genotoxic damage (MN, NB, and BC) were not significantly different between males and females ($p > 0.05$) (Figure 4), with frequencies lower than 5%. However, when comparing organs, MN frequencies in males were higher in the gills and lower in muscle ($p < 0.05$). Similarly, BR frequencies were different only in males, higher in the liver compared to muscle ($p < 0.05$). Finally, BL frequencies were significantly different among organs only for females, higher in the liver and gills and lower in muscle ($p < 0.01$).

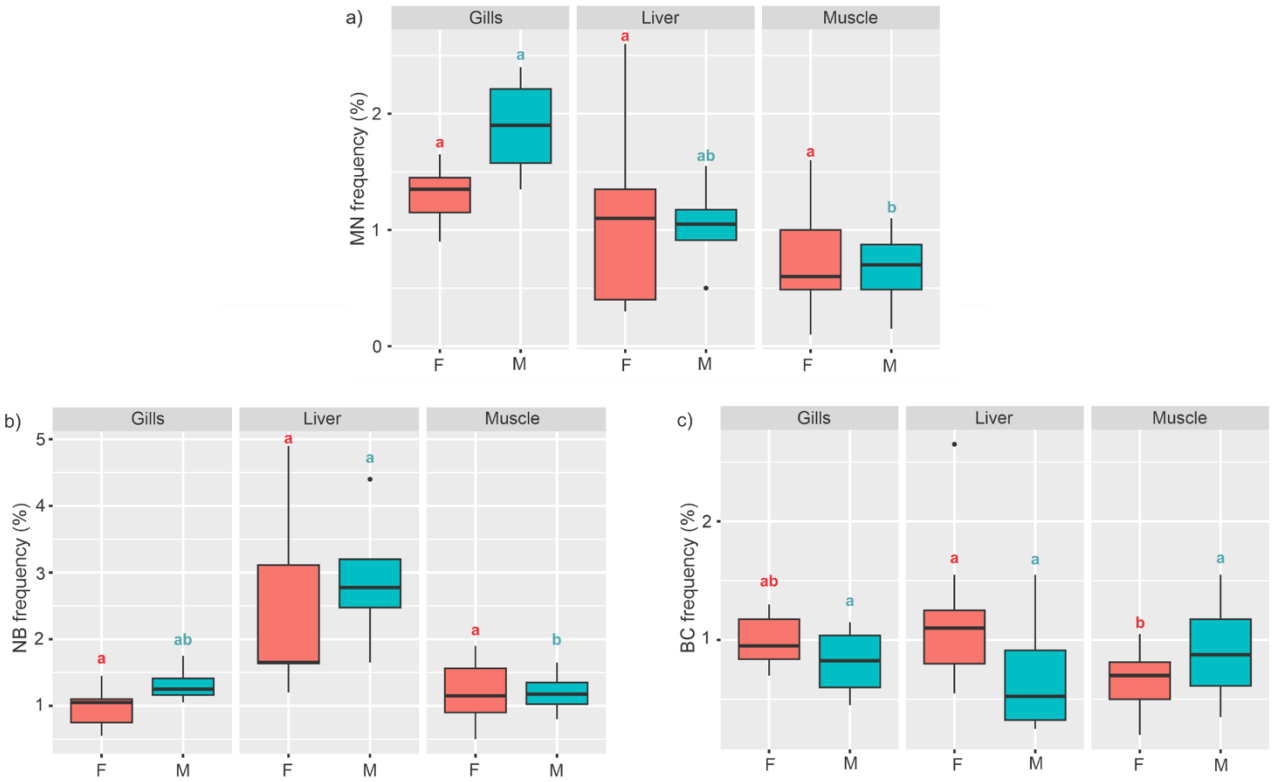

Figure 4. Frequency of genotoxic damage in the gills, liver, and muscle of *Ginglymostoma cirratum* from the Brazilian Amazon Coast. a) MN (%), b) NB (%), and c) BC (%). Whiskers indicate maximum and minimum ranges, with vertical lines extending from the 10th to the 90th percentile and dots representing outliers. Different letters indicate significant differences between organs (red for females and blue for males) according to the Kruskal-Wallis test followed by the Dunnett test ($p < 0.05$). F: females, M: males.

The genotoxic damage in the present study was similar to that found in blood samples from sharks in the Fernando de Noronha region, an isolated island off the coast of the state of Pernambuco, Northeastern Brazil (Araújo et al., 2024). The frequency of MN and BC in males and females for Nurse sharks from Fernando de Noronha was less than 2% (Araújo et al., 2024). However, other species, such as Tiger sharks and Lemon sharks (Negaprion brevirostris), presented a higher damage frequency compared to Nurse sharks in the same region (Araújo et al., 2024). Blood samples tend to have less damage as cell renewal rates are higher than liver and gill cells (Stopper et al., 2020; Kisnanto; Lusiyanti; Erawati, 2021). In contrast, these organs are more susceptible to genotoxic damage (Arslan; Boyacioglu, 2021), due to greater cellular variety (Datta Munshi, 1964; Sardet; Pisam; Maetz, 1979; Arslan; Boyacioglu, 2021). In the case of Nurse sharks, the low damage frequency may be associated with trophic position as a mesopredator and its sedentary behavior, which results in a low metabolic rate and, possibly, a low mitotic index (Araújo et al., 2024). In addition, it seems to present highly active DNA repair systems, which play significant roles in maintaining the integrity of the genome of this species and contributing to its longevity (Llorente, 2019).

Finally, genotoxic damage frequencies were higher in the gills and liver, which are more metabolically active and the target of several pollutants, including metals and pesticides which induce the formation of free radicals, increasing genotoxic damage (Bolognesi et al., 2011; Arslan et al., 2015; Raza et al., 2022). Due to the constant oxidative stress caused by free radicals induced by contaminants and metabolic activities, these organs may exhibit overloaded repair systems, making them less efficient when compared to muscle (Shyama et al., 2017), since muscle metabolic activity is generally associated with the production of ROS during swimming (López-Cruz et al., 2010; Roman et al., 2021). The sensitivity of these organs to DNA damage has also been observed in Nursehound sharks (*S. stellaris*) from the northeastern Mediterranean Sea, with greater sensitivity observed in the gills due to their high blood flow and constant exposure to salt water (Ergenler and Turan, 2024).

Statistical correlations

Associations between the biomarkers and metals and metalloids were observed in the muscle, gills, and liver of both, male and female nurse sharks (Supplementary Figures 1, 2 and

3). Positive correlations were observed between BC and NB in muscle for both males ($r = 1.0$, $p < 0.05$) and females ($r = 0.59$, $p < 0.05$), while in gills these correlations were evident only for females ($r = 0.80$, $p < 0.05$). Nuclear anomalies also exhibited correlations ranging from moderate to strong with the biochemical biomarkers exclusively in the gills and liver of females. In gills, a negative correlation between MN and GST ($r = -0.70$, $p < 0.01$), and positive correlations between NB and MT ($r = 0.61$, $p < 0.05$), and between BC and MT ($r = 0.71$, $p < 0.01$) were observed. In the liver, a very strong negative correlation was detected between NB and GST ($r = -0.95$, $p < 0.0001$).

All metals and metalloids were correlated with the determined genotoxic biomarkers. In muscle, correlations were observed only for females, both moderate, between Ti and NB ($r = -0.63$, $p < 0.01$) and between As and NB ($r = -0.68$, $p < 0.01$). In female gills, associations ranged from moderate to strong, between Ti and MN ($r = 0.78$, $p < 0.001$), Se and NB ($r = 0.73$, $p < 0.01$), Se and BL ($r = 0.66$, $p<0.05$), La and NB ($r=0.81$, $p<0.01$), and La and BC ($r = 0.71$, $p < 0.05$). In the liver, Se was correlated with NB for both females ($r = 0.73$, $p < 0.01$) and males ($r = -1.0$, $p <0.05$). In this same organ, Hg was negatively correlated with NB for budding ($r =- 1$, $p <0.05$).

The positive correlations between NB and BC observed in the muscle and gills (both male and female) in this study may be related to the shared mechanisms underlying these types of genotoxic damage. Both NB and BC are involved in abnormal cell division (Fenech et al., 2011) and frequently co-occur (Torres-Bugarín et al., 2014). These nuclear anomalies indicate damaged and amplified genetic material (Harabawy et al., 2014). Prior studies on Nurse sharks have reported similar correlations and significant associations between these anomalies (Araújo et al., 2024), suggesting a tendency for their co-occurrence. Correlations were observed between the biochemical biomarkers and genotoxicity in gills and liver, specifically between GST and MT. The association between nuclear anomalies and GST suggests a possible role for this biotransformation enzyme in reducing ROS levels in these tissues, thereby mitigating DNA damage and reinforcing its protective function (Alves et al., 2016; Tierbach et al., 2018). In Blue sharks, DNA damage was also negatively correlated with glutathione peroxidase activity, indicating that the induction or inhibition of this enzyme may result in

minor or major DNA damaging effects, respectively (Alves et al., 2016). The positive association between nuclear anomalies and MT reflects the crucial role of this metalloprotein in mitigating metal-induced oxidative DNA damage (Chiaverini & De Ley, 2010). However, the observed correlation may suggest that the metal-binding capacity of MT is overwhelmed in this assessment, resulting in increased DNA damage even with high levels of this protein (Qu & Waalkes, 2015).

Correlations between these elements were also observed for antioxidant biomarkers and other metals and metalloids. In female muscle, a strong positive correlation was observed between Se and MT ($r = 0.74$, $p < 0.01$), a moderate positive correlation between Se and Hg ($r = 0.60$, $p < 0.05$), a strong negative correlation between Rb and GST ($r = -0.74$, $p < 0.01$), and a moderate positive correlation between Rb and Ti ($r = 0.64$, $p < 0.01$) were also noted. Gills presented strong and very strong positive correlations between As and GSH in females ($r = 0.78$, $p < 0.01$) and As and MT in males ($r = 1.0$, $p < 0.05$). Furthermore, very strong positive associations were detected in males between Se and GSH ($r=1.0$, $p<0.05$), Se and MT ($r = 1.0$, $p < 0.05$), and Se and As ($r = 1.0$, $p < 0.05$). Concerning Hg, correlations were observed only in males between GST, MT, As, and Se, all positive and very strong ($r = 1$, $p < 0.05$). Finally, in the liver, Se was correlated with GST ($r = -0.75$, $p < 0.01$), Ti ($r = 0.60$, $p < 0.01$), and La ($r = 0.62$, $p < 0.05$) only in females, while Se was correlated with Ce in both females ($r = 0.72$, $p < 0.01$) and males ($r = 0.80$, $p < 0.05$). Regarding Hg, this element correlated strongly and very strongly with GST ($r = -0.79$, $p < 0.01$) and Se ($r = 0.73$, $p < 0.01$) in female liver, and with Se in males, ($r = 1.0$, $p < 0.05$). Similarly to muscle, Rb was positively and moderately correlated with Ti in both males and females ($r = 0.55$, $p <0.05$).

The observed associations between genotoxic damage and metals and metalloids, such as Ti, As, Se, Hg, and La, are mainly attributed to the accumulation of these elements in the analyzed organisms, as well as their ability to induce breaks in the genetic material, resulting in nuclear anomalies (Bolognesi; Hayashi, 2011; Qiu et al., 2020; Araújo et al., 2024). In Nurse sharks from the Fernando de Noronha Archipelago, these correlations were identified for both essential and non-essential metals, such as Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn (Araújo et al., 2024). Furthermore, REEs, such as lanthanum (La), have been noted as

associated with nuclear anomalies in bony fish, and can cause DNA damage even at low concentrations (Qiu et al., 2020). In contrast, Hg was not associated with this type of damage in Blacktip shark *Carcharhinus limbatus*. This lack of association may be due to the specific physiology of this elasmobranch species, potentially making it less susceptible to Hg toxicity. This may result in certain biomarkers not responding to Hg toxic effects (Noris et al., 2021). In general, organisms activate defense mechanisms, such as metabolic changes to reduce ROS and DNA repair systems, in response to contaminant accumulation (YadaV; Trivedi, 2009; Melo et al., 2013). However, apoptosis or senescence processes may be triggered in affected cells, reducing the formation of nuclear anomalies (Melo et al., 2013).

The correlations between metals and metalloids observed in this study were predominantly positive, as in the case of Se, MT, and Hg. Selenium, although an antioxidant, can also cause damage to cell membranes and proteins through ROS formation when present in high concentrations (Siscar et al., 2014; Hauser-Davis et al., 2022). It has been shown that Se influences the levels of oxidative stress biomarkers, such as MT, GSH, and GST (Siscar et al., 2014; Hauser-Davis et al., 2016; Hauser-Davis et al., 2022). For example, in *Danio rerio*, Se increased the levels of MT and GSH as a compensatory response to ROS (Hauser-Davis et al., 2016). However, Se can induce metal dyshomeostasis by substituting sulfur in proteins (Abbas and Authman, 2009).

Metallothionein plays a crucial role in the detoxification of Se and other metals and metalloids, as observed in Blue sharks (Hauser-Davis et al., 2021) and Brazilian electric ray *Narcine brasiliensis* (Hauser-Davis et al., 2022). In the present study, positive correlations were observed between Se and the determined biomarkers, as well as between Se and metals. However, these correlations were not identified in previous studies, suggesting possible detoxification deficiencies in Nurse shark samples from the same area (Wosnick et al., 2021). Selenium also exhibits antioxidant properties that can mitigate the detrimental effects of metals, depending on circulating concentration. In addition, it plays a key role alongside antioxidant proteins, such as glutathione (GSH). The selenite anion, for example, reacts with GSH to form GSSeSG, a mixed disulfide essential for metal detoxification, contributing to GSH regeneration and cellular redox balance maintenance (Gaxiola-Robles et al., 2014). Several

studies suggest that the interaction of Se with other toxic elements exerts a protective effect, as observed for other elasmobranchs, such as Silky sharks *Carcharhinus falciformis* (Wang et al., 2023), Blue sharks (Hauser-Davis et al., 2021), Scalloped Hammerhead sharks *Sphyrna lewini* (Borgés-Tiznado et al., 2015), as well as in the Pearl Cichlid *Geophagus brasiliensis*, a bony species (Land et al., 2018).

The negative correlation observed between Rb and GST in muscle may be associated with the toxic effects of Rb (Cheng et al., 1984). Although Rb has no defined metabolic function, it can replace K due to physicochemical similarities (Wosnick et al., 2021). When bioaccumulated, Rb has been noted as interfering with GSH-mediated antioxidant responses, and GSH deficiency compromises detoxification processes, affecting metal absorption and metabolism (Cheng et al., 1984).

The positive correlations between Hg, GST, and MT indicate possible physiological responses to mitigate oxidative stress caused by Hg (Choi et al., 2023). GSH is assisted by enzymes such as GST, which conjugates and detoxifies most contaminants, including Hg. Mercury has a high affinity for glutathione, forming the excretable compound GS-HgCH (Rodrigues et al., 2022). A model proposed by Bhattacharya et al. (1997) suggests that GST, GSH, and Hg in its oxidized form (Hg^{2+}) play a crucial role in MT induction for xenobiotic detoxification. In this system, GST facilitates Hg^{2+} cell entry, allowing the formation of the Hg-GSH complex. This complex enters the cell nucleus, where Hg^{2+} binds to the metal response element (MRE) in the MT gene, inducing transcription of the MT gene, responsible for the production of MT, which aids in metal and metalloid detoxification and regulation (Gundacker et al., 2007).

Positive correlations between MT, GST, and Hg are rarely reported in elasmobranch assessments (Barreira-García et al., 2012; Walker et al., 2014; Wosnick et al., 2021; Hauser-Davis et al., 2021; Rodrigues et al., 2022; Martins et al., 2023). This was, however, observed herein, suggesting that GST and gill MT may be useful as Hg exposure biomarkers in Nurse shark. GST facilitates Hg excretion by conjugating it with GSH, while MT binds to Hg to mitigate its toxicity (Gundacker et al., 2007; Farina; Aschner, 2019).

Regarding As, positive correlations between this element and antioxidant defense biomarkers, such as GSH and MT, were observed herein. In Mako sharks, As causes the production of ROS, such as $O_2^{\bullet -}$, $H^{\bullet}$, and H_2O_2, leading to changes in GSH levels and other antioxidant responses (Vélez-Alavez et al., 2013). Metallothionein, in addition to reducing ROS, contributes to cell viability maintenance by mitigating As-induced cell cycle arrest, and restoring energy and lipid metabolism (Qi et al., 2021).

The positive correlation observed between Rb and Ti and Hg and As points to similar environmental sources in the study area (Acquavita; Floreani; Covelli, 2021; Wosnick et al., 2021), a highly metal-polluted environment, presenting high Hg, As, Al, Pb, Fe, Cr, and Zn levels due to intense port activity (Carvalho-Neta et al., 2013; Nunes et al., 2020; Almeida et al., 2021; Corrêa et al., 2023). Both Hg and As are associated with various metabolic and cellular alterations, including changes in GST, catalase (CAT), and ChE levels, as well as histological and genetic malformations in local organisms such as the Pemecou sea catfishes *Sciades herzbergii* (Carvalho-Neta et al., 2013; Ribeiro et al., 2023). However, in other fish groups, the correlation between Hg and As is often negative (Ponton et al., 2022), suggesting that Hg methylation favors the formation of organic forms of As with lower biological absorption, requiring further assessments.

Potentially protective Se effects

Significant correlations between Se and As, and Se and Hg are described in Table 3. Potentially protective effects of Se were observed in both sexes and all organs analyzed in the present study through significant correlations. It is noteworthy that significant associations between Se and As were observed only in the gills of males; however, in this same tissue, no significant correlation between Se and Hg was found. In females, no significant correlations between Se and As were observed.

Table 3. Selenium molar ratios between As and Hg with significant correlations in Nurse sharks *Ginglymostoma cirratum* from the Brazilian Amazon Coast.

Nurse shark sex	Tissue	Elements	Molar ratio	Probable effect
Female	Muscle	Se x Hg	0.40:1	None

	Liver	Se x Hg	2.94:1	Protection
Male	Gills	Se x As	0.55:1	None
	Liver	Se x Hg	1.52:1	Protection

Selenium is an essential element for the nervous system in various organisms (Wang et al., 2023). Although potentially toxic at high concentrations, selenium can exert an antagonistic effect against metals with no known biological function when present at adequate species-specific concentrations (Bergés-Tiznado et al., 2015; Hauser-Davis et al., 2022). An optimal protective effect is generally observed at a 1:1 molar ratio (or greater) between Se and the toxic element (Escobar-Sánchez et al., 2011). The observed correlations indicated a protective effect only in the liver of both sexes for Hg, with a ratio (Se:Hg) of 2.94:1 in females and 1.52:1 in males. However, no protective effect against As was observed in male gills, nor against Hg in female muscle tissue.

The protective effect of Se has been studied in several elasmobranch species (Escobar-Sánchez et al., 2011; McMeans et al., 2007; Selvaraj et al., 2012; Bergés-Tiznado et al., 2015; Hauser-Davis et al., 2021; Wang et al., 2023). A study carried out by McMeans et al. (2007) with two species, Greenland shark *S. microcephalus*, and Pacific Sleeper shark *S. pacificus*, highlighted an average protective effect of Se against Hg, with molar ratios of 12.36:1 *(S. microcephalus*) and 3.26:1 (*S. pacificus*) in muscle samples. In *S. lewini*, protection against Hg was also observed, with excess Se noted in muscle (5.3:1), liver (154.0:1), kidney (136.5:1), and brain (34.5:1) (Bergés-Tiznado et al., 2015). More recent studies point to a protective effect in Blue sharks, with Se molar ratios of 12:1 in the liver and 1.5:1 in muscle (Hauser-Davis et al., 2021). Similarly, a protective effect was also observed in Silky sharks, with a molar ratio of 1.83:1 in muscle (Wang et al., 2023).

The excess of Se relative to Hg in the liver (Table 3) seemingly indicates that Se is bound to proteins in selenocysteine and selenomethionine forms (Bergés-Tiznado et al., 2015). This excess ensures sufficient Se availability to replace that sequestered by Hg, allowing for the normal synthesis of selenoproteins (Escobar-Sánchez et al., 2011; Torres et al., 2014). Furthermore, studies show that Se antagonism in tissues, together with its association with

antioxidants (Torres et al., 2014; Zwolak, 2020), tends to significantly reduce DNA damage induced by metals and metalloids, as well as apoptosis-mediated cell death (Selvaraj et al., 2012; Zwolak, 2020).

Thus, we believe Se action may occur in the Nurse shark analyzed in the present study, considering the low frequency of genotoxic damage and the correlation between Se and antioxidant defenses in organs with a protective effect, as discussed in the previous section. On the other hand, although a protective effect was observed only in the liver, this protection did not manifest in the gills and muscles of both females and males. In *P. glauca*, for example, the absence of a protective effect was also noted in muscle, where the molar ratio of Se to Hg was 0.2:1 (Escobar-Sánchez et al., 2011). It is worth noting that differences in protective effects tend to vary according to sex, age, and geographic distribution of specimens (Hauser-Davis et al., 2021). The absence of a protective effect against As in the gills of males may be related to the chemical form of selenium present in this tissue. Se in the form of selenite (SeO_3^{2-}) can increase arsenite retention and toxicity, which could explain the lack of Se protection against As in this organ (Kaur et al., 2020).

Conclusions

Differences in the accumulation of several metals and metalloids between organs and sex of Nurse sharks from the Brazilian Amazon coast were observed. In contrast, the absence of significant differences in the REE determined in the present study suggests a possible similarity in the bioaccumulation of Ce and La between the sexes analyzed.

Genotoxic damage was low, probably due to its sedentary lifestyle, trophic position, and highly active DNA repair system. The slightly elevated frequencies of anomalies observed in the gills and liver indicate the susceptibility of these organs to genotoxicity. Biochemical biomarkers were similar between sexes, but different among organs, with higher levels and activities in the gills and liver compared to the muscle.

Moderate to strong correlations between several metals/metalloids, biomarkers, and genotoxic responses were observed in the gills and liver, especially in females. Glutathione-S-

transferase was negatively correlated with genotoxic biomarkers, suggesting a protective role against DNA damage. MT positively correlates with nuclear anomalies, evidencing its role against oxidative stress, suggesting a compromise of the antioxidant system. Se, Hg, and Rb exhibited strong correlations in the gills and liver. The association of As and Hg with genotoxic damage appears to be linked to the bioaccumulation of these metals and their ability to cause DNA breaks. The protective effect was observed only in the liver, where Se demonstrated its antagonistic action against Hg in both females and males. However, this effect was not observed for As, possibly due to the chemical form of selenium present in the gills.

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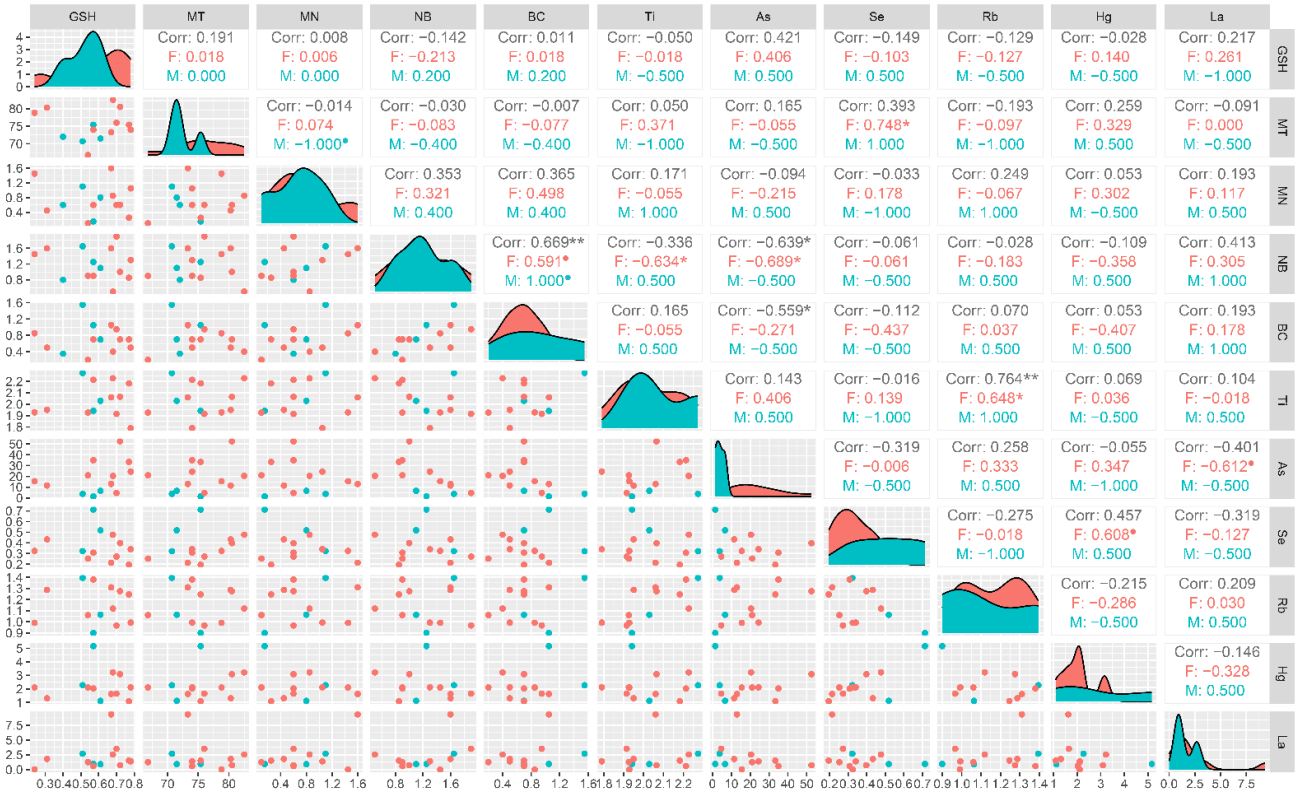
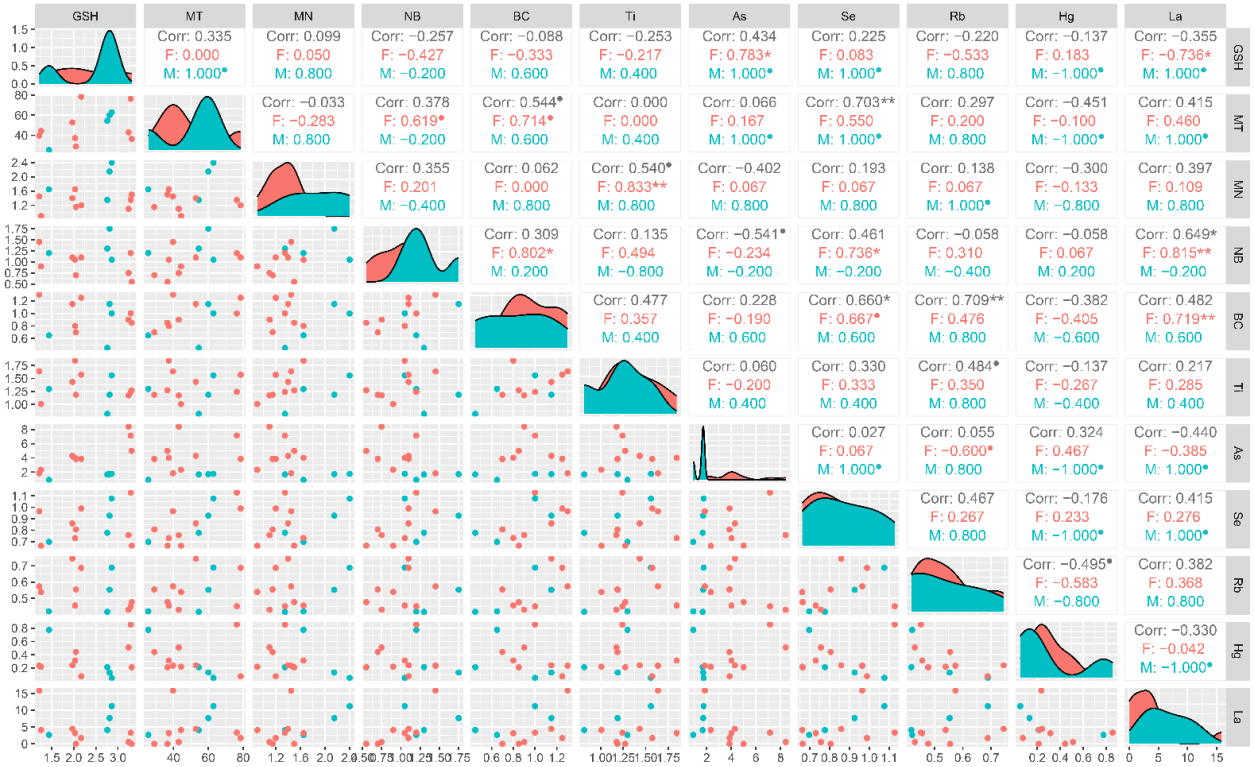
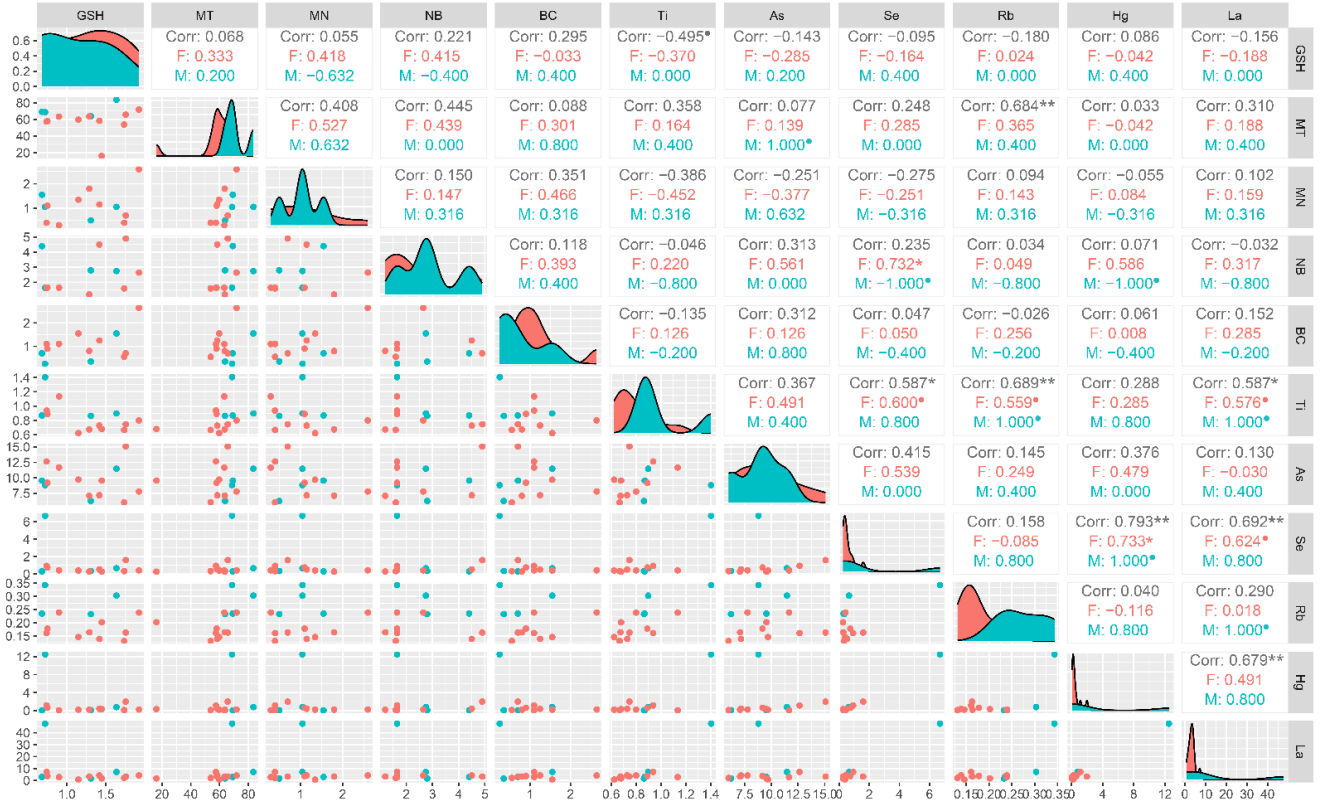
Supplementary Figure 1. Spearman correlation matrix for metals, genotoxicity biomarkers, and biochemical biomarkers in muscle samples of Nurse sharks. F: females, M: males. Significant correlations are marked with " . " ($p < 0.05$), " * " ($p < 0.01$), and " ** " ($p < 0.001$). Negative values indicate negative correlations between variables, while positive values indicate positive correlations.



Supplementary Figure 2**.** Spearman correlation matrix for metals, genotoxicity biomarkers, and biochemical biomarkers in gill samples of Nurse sharks. F: females, M: males. Significant correlations are marked with " . " ($p < 0.05$), " * " ($p < 0.01$), and " ** " ($p < 0.001$). Negative values indicate negative correlations between variables, while positive values indicate positive correlations



Supplementary Figure 3. Spearman correlation matrix for metals, genotoxicity biomarkers, and biochemical biomarkers in liver samples of Nurse sharks. F: females, M: males. Significant correlations are indicated as " . " ($p < 0.05$), " * " ($p < 0.01$) and " ** " ($p < 0.001$). Negative values indicate negative correlations between variables, while positive values indicate positive correlations.



Supplementary Table 1. Concentration of total metals and metalloids in Nurse shark across different tissues and sexes. Mean ± SD (min - max).

Tissue	Sex	Metals and metalloids (mg kg^{-1} w.w.)						
		As	Ce	Hg	La	Rb	Se	Ti
Gills	F	4.53 ± 2.10 (1.85 - 8.41)	8.11 ± 10.77 (1.46 - 35.8)	0.35 ± 0.23 (0.08 - 0.86)	4.74 ± 5.09 (0.54 - 15.9)	0.55 ± 0.11 (0.43 - 0.75	0.84 ± 0.16 (0.66 - 1.13)	1.38 ± 0.27 (1.01 - 1.84)
	M	1.51 ± 0.41 (0.91 - 1.76)	13.87 ± 8.00 (5.92 - 23.9)	0.29 ± 0.33 (0.05 - 0.78)	6.41 ± 3.83 (2.66 - 11.2)	0.52 ± 0.13 (0.42 - 0.69)	0.87 ± 0.17 (0.70 - 1.08)	1.22 ± 0.31 (0.82 - 1.56)
Liver	F	9.59 ± 2.83 (6.01 - 15.1)	5.11 ± 3.53 (1.31 - 13.0)	0.51 ± 0.61 (0.06 - 1.98)	3.37 ± 1.74 (0.75 - 7.20)	0.18 ± 0.04 (0.13 - 0.24)	0.59 ± 0.41 (0.26 - 1.60)	0.79 ± 0.16 (0.62 - 1.14)
	M	9.01 ± 2.17 (6.23 - 11.5)	31.7 ± 53.2 (3.91 - 111)	3.35 ± 6.12 (0.04 - 12.5)	14.9 ± 21.9 (1.72 - 47.6)	0.28 ± 0.50 (0.23 - 0.34)	2.00 ± 3.13 (0.30 - 6.69)	1.01 ± 0.26 (0.86 - 1.40)
Muscle	F	23.14 ± 13.98 (4.65 - 52.5)	4.46 ± 5.34 (0.53 - 18.4)	2.03 ± 0.69 (1.09 - 3.22)	2.52 ± 2.71 (0.58 - 9.34)	1.16 ± 0.15 (0.97 - 1.38)	0.32 ± 0.09 (0.20 - 0.48)	2.03 ± 0.15 (1.79 - 2.23)
	M	3.98 ± 2.61 (1.46 - 6.66)	2.89 ± 1.54 (1.66 - 4.62)	2.85 ± 2.08 (1.12 - 5.16)	1.51 ± 1.01 (0.91 - 2.68)	1.12 ± 0.25 (0.90 - 1.40)	0.52 ± 0.20 (0.32 - 0.71)	2.08 ± 0.17 (1.94 - 2.27)

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We advise you to read the Sex and Gender Equity in Research (SAGER) guidelines and the SAGER checklist (PDF) on the EASE website, which offer systematic approaches to the use of sex and gender information in study design, data analysis, outcome reporting and research interpretation.

For further information we suggest reading the rationale behind and recommended use of the SAGER guidelines.

Definitions of sex and/or gender

We ask authors to define how sex and gender have been used in their research and publication. Some guidance:

- Sex generally refers to a set of biological attributes that are associated with physical and physiological features such as chromosomal genotype, hormonal levels, internal and external anatomy. A binary sex categorization (male/female) is usually designated at birth ("sex assigned at birth") and is in most cases based solely on the visible external anatomy of a newborn. In reality, sex categorizations include people who are intersex/have differences of sex development (DSD).
- Gender generally refers to socially constructed roles, behaviors and identities of women, men and gender-diverse people that occur in a historical and cultural context and may vary across societies and over time. Gender influences how people view themselves and each other, how they behave and interact and how

power is distributed in society.

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- Institutional affiliations: Authors should use either the full, standard title of their institution or the standard abbreviation of the institutional name so that the institutional name can be independently verified for research integrity purposes.

Studies in humans and animals

Authors must follow ethical guidelines for studies carried out in humans and animals.

Studies in humans

Work which involves the use of human subjects should be carried out in accordance with the World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects.

Manuscripts should follow the International Committee of Medical Journal Editors (ICMJE) recommendations for the conduct, reporting, editing and publication of scholarly work in medical journals and aim to be representative of human populations in terms of sex, age and ethnicity. Sex and gender terms should be used correctly, as outlined by WHO (World Health Organization).

Manuscripts must include a statement that all procedures were performed in compliance

with relevant laws and institutional guidelines and have been approved by the appropriate institutional committee(s). The statement should contain the date and reference number of the ethical approval(s) obtained.

Manuscripts must also include a statement that the privacy rights of human subjects have been observed and that informed consent was obtained for experimentation with human subjects.

This journal will not accept manuscripts that contain data derived from unethically sourced organs or tissue, including from executed prisoners or prisoners of conscience, consistent with recommendations by Global Rights Compliance on Mitigating Human Rights Risks in Transplantation Medicine. For all studies that use human organs or tissues, sufficient evidence must be provided that these were procured in line with WHO Guiding Principles on Human Cell, Tissue and Organ Transplantation. The source of the organs or tissues used in clinical research must be transparent and traceable. If your manuscript describes organ transplantation you must additionally declare within the manuscript that:

- autonomous consent free from coercion was obtained from the donor(s) or their next of kin.
- organs and/or tissues were not sourced from executed prisoners or prisoners of conscience.

Studies in animals

All animal experiments should comply with ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

Studies should be carried out in accordance with Guidance on the operation of the Animals (Scientific Procedures) Act 1986 and associated guidelines, EU Directive 2010/63 for the protection of animals used for scientific purposes or the NIH (National Research Council) Guide for the Care and Use of Laboratory Animals (PDF) or those of an equivalent internationally recognized body.

The sex of animals, and where appropriate, the influence (or association) of sex on the results of the study must be indicated and a statement included in your manuscript that such guidelines as listed above have been followed.

Writing and formatting

File format

We ask you to provide editable source files for your entire submission (including figures, tables and text graphics). Some guidelines:

- Save files in an editable format, using the extension .doc/.docx for Word files and .tex for LaTeX files. A PDF is not an acceptable source file.
- Lay out text in a single-column format.
- Remove any strikethrough and underlined text from your manuscript, unless it has scientific significance related to your article.
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We advise you to read our Step-by-step guide to publishing with Elsevier.

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You are required to include the following details in the title page information:

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Abstract

You are required to provide a concise and factual abstract which does not exceed 250 words. The abstract should briefly state the purpose of your research, principal results and major conclusions. Some guidelines:

- Abstracts must be able to stand alone as abstracts are often presented separately from the article.
- Avoid references. If any are essential to include, ensure that you cite the author(s) and year(s).
- Avoid non-standard or uncommon abbreviations. If any are essential to include, ensure they are defined within your abstract at first mention.

Keywords

You are required to provide 1 to 7 keywords for indexing purposes. Keywords should be written in English. Please try to avoid keywords consisting of multiple words (using "and" or "of").

We recommend that you only use abbreviations in keywords if they are firmly established in the field.

Highlights

You are required to provide article highlights at submission.

Highlights are a short collection of bullet points that should capture the novel results of your research as well as any new methods used during your study. Highlights will help increase the discoverability of your article via search engines. Some guidelines:

- Submit highlights as a separate editable file in the online submission system with the word "highlights" included in the file name.
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including spaces.

We encourage you to view example article highlights and read about the benefits of their inclusion.

Graphical abstract

You are required to provide a graphical abstract at submission.

The graphical abstract should summarize the contents of your article in a concise, pictorial form which is designed to capture the attention of a wide readership. A graphical abstract will help draw more attention to your online article and support readers in digesting your research. Some guidelines:

- Submit your graphical abstract as a separate file in the online submission system.
- Ensure the image is a minimum of 531 x 1328 pixels (h x w) or proportionally more and is readable at a size of 5 x 13 cm using a regular screen resolution of 96 dpi.
- Our preferred file types for graphical abstracts are TIFF, EPS, PDF or MS Office files.

We encourage you to view example graphical abstracts and read about the benefits of including them.

Tables

Tables must be submitted as editable text, not as images. Some guidelines:

- Place tables next to the relevant text or on a separate page(s) at the end of your article.
- Cite all tables in the manuscript text.
- Number tables consecutively according to their appearance in the text.
- Please provide captions along with the tables.
- Place any table notes below the table body.
- Avoid vertical rules and shading within table cells.

We recommend that you use tables sparingly, ensuring that any data presented in tables is not duplicating results described elsewhere in the article.

Figures, images and artwork

Figures, images, artwork, diagrams and other graphical media must be supplied as

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When submitting artwork:

- Cite all images in the manuscript text.
- Number images according to the sequence they appear within your article.
- Submit each image as a separate file using a logical naming convention for your files (for example, Figure_1, Figure_2 etc).
- Please provide captions for all figures, images, and artwork.
- Text graphics may be embedded in the text at the appropriate position. If you are working with LaTeX, text graphics may also be embedded in the file.

Artwork formats

When your artwork is finalized, "save as" or convert your electronic artwork to the formats listed below taking into account the given resolution requirements for line drawings, halftones, and line/halftone combinations:

- Vector drawings: Save as EPS or PDF files embedding the font or saving the text as "graphics."
- Color or grayscale photographs (halftones): Save as TIFF, JPG or PNG files using a minimum of 300 dpi (for single column: min. 1063 pixels, full page width: 2244 pixels).
- Bitmapped line drawings: Save as TIFF, JPG or PNG files using a minimum of 1000 dpi (for single column: min. 3543 pixels, full page width: 7480 pixels).
- Combinations bitmapped line/halftones (color or grayscale): Save as TIFF, JPG or PNG files using a minimum of 500 dpi (for single column: min. 1772 pixels, full page width: 3740 pixels).

Please do not submit:

- files that are too low in resolution (for example, files optimized for screen use such as GIF, BMP, PICT or WPG files).
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All images must have a caption. A caption should consist of a brief title (not displayed on the figure itself) and a description of the image. We advise you to keep the amount of text in any image to a minimum, though any symbols and abbreviations used should be explained.

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If you submit usable color figures with your accepted article, we will ensure that they appear in color online.

Please ensure that color images are accessible to all, including those with impaired color vision. Learn more about color and web accessibility.

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We encourage the use of supplementary materials such as applications, images and sound clips to enhance research. Some guidelines:

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- Include a concise, descriptive caption for each supplementary file describing its content.
- Provide updated files if at any stage of the publication process you wish to make changes to submitted supplementary materials.
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We publish all video and animation files supplied in the electronic version of your article. For more detailed instructions, we recommend that you read our guidelines on submitting video content to be included in the body of an article.

Research data

We are committed to supporting the storage of, access to and discovery of research data, and our research data policy sets out the principles guiding how we work with the research community to support a more efficient and transparent research process.

Research data refers to the results of observations or experimentation that validate research findings, which may also include software, code, models, algorithms, protocols, methods and other useful materials related to the project.

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For this journal, the following instructions from our research data guidelines apply.

Option C: Research data deposit, citation and linking

You are **required** to:

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- Cite and link to this dataset in your article.
- If this is not possible, make a statement explaining why research data cannot be shared.

Data statement

To foster transparency, you are required to state the availability of any data at submission. Ensuring data is available may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you can state the reason why (e.g., your research data includes sensitive or confidential information such as patient data) during the submission process. This statement will appear with your published article on

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Linking to the data underlying your work increases your exposure and may lead to new collaborations. It also provides readers with a better understanding of the described research.

If your research data has been made available in a data repository there are a number of ways your article can be linked directly to the dataset:

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- You can also link relevant data or entities within the text of your article through the use of identifiers. Use the following format: Database: 12345 (e.g. TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

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Data in Brief and MethodsX: co-submission

You are encouraged to publish research **data**, **methods** or **protocols** related to your manuscript as a co-submission article in *Data in Brief* or *MethodsX*. By publishing a co-submission, you further advance research reproducibility, interoperability, and open science. In case both your original research article and your co-submission article(s) get accepted for publication, they will be linked together on ScienceDirect.

When submitting your original research article, please follow the ***co-submission process*** active for this journal:

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    - Methods article template (*MethodsX*)
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    - When you upload the files for your original research article, in the ‘Attach Files’ step in the Editorial Manager submission process, please also upload the file(s) for your co-submission.
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- Divide your article into clearly defined and numbered sections. Number subsections 1.1 (then 1.1.1, 1.1.2, ...), then 1.2, etc.
- Use the numbering format when cross-referencing within your article. Do not just refer to "the text."
- You may give subsections a brief heading. Headings should appear on a separate line.
- Do not include the article abstract within section numbering.

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The theory section should lay the foundation for further work by extending the background you provided in the introduction to your article. The calculation section should represent a practical development from a theoretical basis.

Glossary

Please provide definitions of field-specific terms used in your article, in a separate list.

Acknowledgements

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We ask you to use the following format for appendices:

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- Give separate numbering to formulae and equations within appendices using formats such as Eq. (A.1), Eq. (A.2), etc. and in subsequent appendices, Eq. (B.1), Eq. (B. 2) etc. In a similar way, give separate numbering to tables and figures using formats such as Table A.1; Fig. A.1, etc.

References

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Any references cited within your article should also be present in your reference list and vice versa. Some guidelines:

- References cited in your abstract must be given in full.
- We recommend that you do not include unpublished results and personal communications in your reference list, though you may mention them in the text of your article.
- Any unpublished results and personal communications included in your reference list must follow the standard reference style of the journal. In substitution of the publication date add "unpublished results" or "personal communication."
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Linking to cited sources will increase the discoverability of your research.

Before submission, check that all data provided in your reference list are correct, including any references which have been copied. Providing correct reference data allows us to link to abstracting and indexing services such as Scopus, Crossref and PubMed. Any incorrect surnames, journal or book titles, publication years or pagination within your references may prevent link creation.

We encourage the use of Digital Object Identifiers (DOIs) as reference links as they provide a permanent link to the electronic article referenced.

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This journal does not set strict requirements on reference formatting at submission. Some guidelines:

- References can be in any style or format as long as the style is consistent.
- Author names, journal or book titles, chapter or article titles, year of publication, volume numbers, article numbers or pagination must be included, where applicable.
- Use of DOIs is recommended.

Our journal reference style will be applied to your article after acceptance, at proof stage. If required, at this stage we will ask you to correct or supply any missing reference data.

Reference style

All citations in the text should refer to:

- Single author: the author's name (without initials, unless there is ambiguity) and the year of publication.
- Two authors: both authors' names and the year of publication.
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Citations can be made directly (or parenthetically). Groups of references can be listed either first alphabetically, then chronologically, or vice versa. Examples: "as demonstrated (Allan, 2020a, 2020b; Allan and Jones, 2019)" or "as demonstrated (Jones, 2019; Allan, 2020). Kramer et al. (2023) have recently shown".

The list of references should be arranged alphabetically and then chronologically if necessary. More than one reference from the same author(s) in the same year must be

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Abbreviate journal names according to the List of Title Word Abbreviations (LTWA).

Examples:

Reference to a journal publication:

Van der Geer, J., Handgraaf, T., Lupton, R.A., 2020. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.sc.2020.00372.

Reference to a journal publication with an article number:

Van der Geer, J., Handgraaf, T., Lupton, R.A., 2022. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2022.e00205.

Reference to a book:

Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.

Reference to a chapter in a book:

Mettam, G.R., Adams, L.B., 2023. How to prepare an electronic version of your article, in: Jones, B.S., Smith, R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Reference to a website:

Cancer Research UK, 2023. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2023).

Reference to a dataset:

Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions [dataset]. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.

Reference to software:

Coon, E., Berndt, M., Jan, A., Svyatsky, D., Atchley, A., Kikinzon, E., Harp, D., Manzini, G., Shelef, E., Lipnikov, K., Garimella, R., Xu, C., Moulton, D., Karra, S., Painter, S., Jafarov, E., & Molins, S., 2020. Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88) [software]. Zenodo. https://doi.org/10.5281/zenodo.3727209.

Web references

When listing web references, as a minimum you should provide the full URL and the date

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You can list web references separately under a new heading directly after your reference list or include them in your reference list.

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We encourage you to cite underlying or relevant datasets within article text and to list data references in the reference list.

When citing data references, you should include:

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Add [dataset] immediately before your reference. This will help us to properly identify the dataset. The [dataset] identifier will not appear in your published article.

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If a template is not available for this journal, follow the format given in examples in the reference style section of this Guide for Authors.

Submitting your manuscript

Submission checklist

Before completing the submission of your manuscript, we advise you to read our submission checklist:

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- Spelling and grammar checks have been carried out.
- All references in the article text are cited in the reference list and vice versa.
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11. CONTRIBUIÇÕES ATRELADAS À DISSERTAÇÃO

11.1 Importância Social

O presente estudo destaca a relevância social de monitorar os impactos da contaminação ambiental em espécies de grande importância ecológica, como os tubarões lixa (*Ginglymostoma cirratum*). Esses organismos desempenham papel fundamental nos ecossistemas marinhos e também têm valor cultural e social em comunidades costeiras que dependem da pesca. Com a crescente preocupação global em relação à saúde dos oceanos, este trabalho fornece dados que podem sensibilizar a população sobre a necessidade de ações para a preservação dessas espécies, fomentando a educação ambiental. Além disso, ao explorar a saúde dos tubarões lixa na costa amazônica brasileira, o estudo contribui para a conscientização sobre os riscos da contaminação ambiental para a biodiversidade marinha e, consequentemente, para o bem-estar humano, dado que populações humanas também dependem dos recursos marinhos para subsistência.

11.2 Importância Econômica

Do ponto de vista econômico, o estudo evidencia a necessidade de proteger espécies marinhas como os tubarões lixa, que possuem grande potencial para as atividades de pesca artesanal e renda. A conservação dessa espécie pode gerar benefícios econômicos para comunidades locais que dependem de recursos marinhos, como a pesca sustentável e o turismo voltado para a observação de tubarões em ambientes naturais. Além disso, a presente pesquisa ressalta os impactos econômicos negativos associados à poluição por metais e metaloides, que comprometem a saúde dos ecossistemas e, consequentemente, a produtividade pesqueira. Dados robustos sobre a contaminação química também podem auxiliar na criação de políticas públicas voltadas para a gestão de recursos marinhos, promovendo um uso mais eficiente e sustentável, com reflexos positivos na economia regional e nacional, especialmente em áreas como o Maranhão, fortemente dependentes da pesca e da atividade portuária.

11.3 Importância Ambiental

O presente estudo contribui para a compreensão dos impactos da contaminação por metais e metaloides em ecossistemas marinhos. A pesquisa revela como espécies vulneráveis, como os tubarões lixa, respondem a essas pressões ambientais, utilizando biomarcadores para monitorar o estado de saúde dos organismos e dos ecossistemas. Este trabalho reforça a necessidade de ações de mitigação contra a poluição marinha, especialmente em áreas sensíveis como a costa amazônica brasileira. Além disso, o estudo apresenta evidências do papel protetor do selênio contra os efeitos tóxicos do mercúrio, um método que pode orientar estratégias de manejo ambiental. A conservação dos tubarões lixa não só protege a biodiversidade marinha, mas também mantém o equilíbrio ecológico, essencial para o funcionamento saudável dos ecossistemas oceânicos e costeiro


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