UNIVERSIDADE FEDERAL DO PARÁ
NÚCLEO DE ECOLOGIA AQUÁTICA E PESCA DA AMAZÔNIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA AQUÁTICA E PESCA

Juliana De Souza Araujo Damasceno

Elementos traço em peixes marinhos da Amazônia: aspectos ecológicos e ecotoxicológicos

Tese de Doutorado

Belém – PA, 2020

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Elementos traço em peixes marinhos da Amazônia: aspectos ecológicos e ecotoxicológicos

Trace elements in marine fish in the Amazon: ecological and ecotoxicological aspects

Tese apresentada à Universidade Federal do Pará, como parte das exigências para a obtenção do título de *Doctor scientiae* no Programa de Pós-Graduação em Ecologia Aquática e Pesca.

Orientador: Dr. Tommaso Giarrizzo
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Co-Orientador: Dr. Marcelo de Oliveira Lima
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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ecologia Aquática e Pesca na área de concentração de Ecologia Aquática, Universidade Federal do Pará

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"Não fui eu que lhe ordenei? Seja forte e corajosa! Não se apavore, nem desanime, pois o Senhor, o seu Deus, estará com você por onde você andar".

Josué 1:9 NVI (adaptado)

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Resumo

Os trabalhos que integram esta tese tratam especialmente de temas associados à contaminação por elementos traço em peixes marinhos na Costa Amazônica, fazendo estimativas quanto ao risco de exposição à saúde pública pelo consumo destes peixes, e avaliando alguns aspectos ecológicos sobre os tubarões que são comercializados ao longo da costa. Foram analisados um total de 54 espécies de peixes marinhos. De modo geral, diferentes espécies de peixes coletadas nas águas costeiras da Amazônia acumulam doses de arsênio (As), mercúrio (Hg), chumbo (Pb) e cádmio (Cd). O As foi o elemento mais abundante em todos os peixes, especialmente nos de posição trófica baixa, estando inclusive acima dos limites máximos permitidos em guias internacionais. Os peixes associados aos ambientes recifais foram mais suscetíveis ao acúmulo de Hg. O arsênio inorgânico (iAs), Hg e Pb apresentaram individualmente potencial risco não carcinogênico à saúde pelo consumo de algumas espécies cartilaginosas. Espécies de tubarões classificadas na categoria de vulneráveis e ameaçadas foram encontradas sendo comercializadas nos mercados de peixe. Além das altas doses de elementos tóxicos presentes, as assinaturas de $\delta^{15}N$ indicam que os tubarões capturados na costa amazônica possuem menor posição trófica do que as mesmas espécies em outras partes do mundo, o que pode ser explicado pelo fato de estes indivíduos serem retirados do ambiente ainda juvenis. Por fim, encontramos que o tubarão *M. higmani* descarrega grande parte dos elementos traço, essenciais e não essenciais, para a prole durante a gestação e que a dinâmica do suprimento de nutrientes do embrião-mãe é um reflexo direto da dieta e dos habitats da mãe durante o período gestacional. O fígado acumula mais elementos não essenciais que o músculo e a maior parte dos elementos são biodiluídos pelo crescimento

dos embriões. Além disso, a proporção molar de Se: Hg sugere que o Se pode ter um papel protetor contra a toxicidade do Hg durante os estágios iniciais do desenvolvimento deste tubarão. Concluímos que, coletivamente, os elementos tóxicos foram encontrados o suficiente para serem considerados como um risco potencial à saúde humana, e que o consumo regular de carne de tubarão ao longo da costa norte do Brasil pode representar um risco para a saúde das populações humanas locais através da exposição a altos níveis de As e Hg. Por sua vez, a escala e os impactos da pesca de tubarões nesta região são desconhecidos; consequentemente, são necessários mais dados para avaliar se a pesca é sustentável.

Palavras-chave: Amazônia, Arsênio, Cádmio, Chumbo, Descarga Maternal, Elasmobrânquios, Isótopos estáveis, Mercúrio, Peixes Marinhos, Risco de Exposição.

Abstract

The studies that are part of this thesis deal especially with issues associated with contamination by trace elements in marine fish on the Amazon Coast, making estimates regarding the risk of exposure to public health through the consumption of these fish, and evaluating some ecological aspects about sharks that are marketed at along the coast. A total of 54 species of marine fish were analyzed. In general, different species of fish collected in the coastal waters of the Amazon accumulate doses of arsenic (As), mercury (Hg), lead (Pb) and cadmium (Cd). Arsenic was the most abundant element in all fish, especially those with low trophic position, even being above the maximum limits allowed in international guides. Fish associated with the reef were more susceptible to Hg accumulation. Inorganic arsenic (iAs), Hg and Pb individually presented a potential non-carcinogenic health risk due to the consumption of some cartilaginous species. Vulnerable and threatened species of shark have been found to be traded in fish markets. In addition to the high doses of toxic elements present, the $\delta^{15}N$ signatures indicate that sharks caught on the Amazon coast have a lower trophic position than the same species in other parts of the world, which can be explained by the fact that these individuals are removed from the environment still young. Finally, we find that the shark *M. higmani* discharges much of the trace elements, essential and non-essential, to the litter during pregnancy and that the dynamics of the nutrient supply of the mother embryo is a direct reflection of the diet and habitats of the mother during the gestational period. The liver accumulates more non-essential elements than muscle and most elements are biodiluted by the growth of embryos. In addition, the molar ratio of Se: Hg suggests that Se may have a protective role against Hg toxicity during the early stages of this shark's development.

We conclude that, collectively, the toxic elements were found enough to be considered as a potential risk to human health, and that regular consumption of shark meat along the northern coast of Brazil may pose a risk to the health of local human populations through exposure to high levels of As and Hg. In turn, the scale and impacts of shark removals in this region are unknown; consequently, more data is needed to assess whether fishing is sustainable.

Key-Worlds: Amazon, Arsenic, Cadmium, Elasmobranchs, Exposure Risk, Lead, Marine fish, Maternal offloading, Mercury, Stable Isotopes.

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Diretrizes

A presente Tese de Doutorado foi elaborada sob formato de compilação de artigos científicos, desenvolvidos sobre o tema "contaminação por elementos traço em peixes marinhos na Costa Amazônica", submetidos a periódicos avaliados de acordo com o critério Qualis-CAPES na área de Biodiversidade. As diretrizes dos artigos seguem Resolução Nº 4.782, de 24 de fevereiro de 2016, a qual aprovou o Regimento do Programa de Pós-Graduação em Ecologia Aquática e Pesca da Universidade Federal do Pará PPGEAP/UFPA. A Tese foi estruturada baseando-se nas seguintes diretrizes do Regimento do PPGEAP em destaque:

CAPÍTULO XXII:

" § 1o A elaboração da Tese por agregação de artigos científicos deverá ser constituída por um documento que incorpore artigos completos, publicados ou submetidos a revistas especializadas com corpo editorial, e um texto integrador.

§ 2o Para o que prevê o parágrafo anterior, serão considerados somente os artigos científicos elaborados após o ingresso do estudante no Curso de Doutorado e que sejam diretamente relacionados com o tema desenvolvido na Tese, devendo o estudante ser o primeiro autor de, no mínimo, 2 (dois) dos trabalhos incluídos."

Estrutura da tese

Os trabalhos a seguir tratam especialmente de temas associados à contaminação por elementos traço em peixes marinhos na Costa Amazônica, fazendo estimativas quanto ao risco de exposição à saúde pública pelo consumo destes peixes (capítulo 1), e avaliando alguns aspectos ecológicos sobre os tubarões que são comercializados ao longo da costa (capítulo 2 e capítulo 3). Levando em consideração as diretrizes, a Tese segue a seguinte estrutura:

Capítulo Introdutório, onde é feita uma breve abordagem sobre a vulnerabilidade do ambiente costeiro, especialmente o amazônico, como também algumas características dos quatro elementos traço abordados nos capítulos subsequentes. Além disso, é apresentada a problemática sobre os riscos ecológicos e de saúde pública mediante o consumo de carne de tubarão na Amazônia. São apresentados também os métodos que foram utilizados, um breve resumo sobre os resultados e as conclusões e considerações finais.

O capítulo 1, "Trace elements (As, Hg, Pb and Cd) in Amazon marine fish and their implications to human health", fornece as concentrações de 4 elementos traço em 47 espécies de peixes, analisando variação por habitat na acumulação, e estimando o risco à saúde pelo consumo de 27 espécies com importância comercial.

O capítulo 2, "As, Hg, Pb and Cd in 13 commercial shark species from Amazon Coastal waters", fornece as concentrações de quatro elementos traço e a assinatura isotópica de ^{15}N em 13 espécies de tubarões encontrados à venda em mercados de peixes ao longo da Costa Amazônica, além de observar a biomagnificação de Hg e biodiluição de As, como também o posicionamento trófico das espécies.

O capítulo 3, "Maternal offloading of trace elements and isotopic fractionation in the smalleye smooth-hound shark *Mustelus higmani* (Springer and Lowe, 1963)", fornece dados sobre a descarga materna de 16 elementos traço, e o fracionamento isotópico mãe-embrião no tecido muscular e hepático da espécie de tubarão placentotrófica *M. higmani*.

Apêndice, catálogo ilustrativo das espécies de peixes marinhos da Costa Norte do Brasil amostradas neste estudo.

Anexos, referências à produção científica em decorrência de pesquisas desenvolvidas durante o Doutorado (Anexos 1- 9).

Capítulo Integrador

1. Introdução Geral

1.1. A Zona Costeira como ambiente vulnerável à contaminantes

As regiões costeiras tropicais são um dos ecossistemas mais produtivos do mundo (Sigman & Hain, 2012), o que levou a uma concentração da população humana, indústria e projetos de desenvolvimento e agricultura. Mais de 50% da população mundial vive a menos de 50 km dos ambientes marinhos e estuarinos (Marques-Júnior *et al*., 2009), e como resultado, o ambiente aquático costeiro aparece como o destino final para diferentes fluxos de água doce terrestre, sedimentos, nutrientes e outros contaminantes de origem antropogênica (Kroon et al., 2016; Nkwoji et al., 2020).

Desta forma, as regiões estuarinas, por exemplo, além de funcionarem como berçário, área de forrageamento e abrigo, possuem papel fundamental na ciclagem de contaminantes inorgânicos como metais e metaloides (Singer *et al*., 2013; Li *et al.,* 2014). A exemplo desses, os elementos como mercúrio (Hg), arsênio (As), cádmio (Cd) e chumbo (Pb) estão entre os mais tóxicos para os organismos, bem como à saúde pública (ATSDR, 2017). Por se ligarem facilmente a macromoléculas e membranas, acumulam-se em quase todos os tecidos dos organismos aquáticos como os peixes (Santos *et al*., 2003).

1.2. A Costa Amazônica

Sustentando uma indústria pesqueira de US$ 610 milhões, a produtividade pesqueira na Costa Amazônica destaca-se como um dos principais componentes da economia nacional e regional, além também de fornecer recursos alimentares para as populações locais (FAO, 2014; Sea Around Us, 2017). As estimativas de produção primária apontam para um hábito oligotrófico e estratificado, cujas plumas da Amazônia e do Orinoco são a principal fonte de nutrientes (Hu et al., 2004).

Todavia, a bacia amazônica possui uma fonte natural e antropogênica substancial de elementos como Hg e As (Bundschuh et al., 2012; Arrifano et al., 2018; Siqueira et al., 2018; da Silva Júnior et al., 2019), que acabam no mar através da grande descarga de água e sedimentos do continente (Scarpelli, 2005; Isaac & Ferrari, 2017). Além disso, existe uma proposta de exploração de petróleo na foz do rio Amazonas. Se aprovado, o ecossistema e a abundância de contaminantes podem mudar completamente no futuro, uma vez que acidentes com derramamentos de hidrocarbonetos ocorrem com freqüência (NAP, 2003), e efeitos deletérios são esperados (Rodrigues et al., 2010; D'Costa et al., 2017).

1.3. Os elementos traço

As fontes naturais de Hg incluem intemperismo de rochas e emissões vulcânica, no entanto, as atividades antropogênicas como a produção de fungicidas orgânicos,

equipamentos elétricos, baterias, na medicina, no setor militar, queimadas e construção de barragens são responsáveis por um a dois terços do Hg presente na atmosfera e o ambiente aquático (Authman, 2015; Bosch et al., 2016). O Hg se caracteriza por sua capacidade de bioacumulação e biomagnificação na cadeia trófica (Hylander *et al*., 2003; Coelho *et al*., 2013; Souza-Araujo et al., 2016). Por sua elevada toxicidade, principalmente na forma de metilmercúrio (MeHg), pode induzir a uma série de danos, principalmente neurodegenrativos, à saúde humana e animal quando da ocorrência de exposição excessiva de longo prazo (Oliveira *et al*., 2010; Chan, 2011; Dorea *et al*., 2012).

O As é amplamente distribuído na natureza devido a fontes naturais e antropogênicas, através de atividades de fundição, fabricação de vidro, fabricação e uso de pesticidas arsênico, herbicidas, fungicidas e conservantes de madeira (ATSDR, 2007; Bosch et al., 2016). Nos organismos marinhos, pode ser encontrado em diversas formas químicas e estados de oxidação, sendo que em sua maioria, encontra-se presente em formas orgânicas não tóxicas como arsenobetaína (Fattorini *et al.,* 2006). Apenas 2-10% do arsênio total apresenta-se em sua forma de maior toxicidade (Fattorini *et al.,* 2006; ATSDR, 2007). Os primeiros sintomas da exposição ao As, em humanos, incluem dor abdominal, vômito, diarreia, fraqueza muscular e rubor da pele, enquanto a toxicidade crônica pelo As leva a doenças de pele e câncer.

O Pb é um elemento que ocorre naturalmente em rochas, solos e na hidrosfera, com uma abundância de 0,0016% na crosta terrestre (Davidson et al. 2014). Mas, sua crescente abundância no meio ambiente é oriunda de fontes antropogênicas como a

mineração de metais comuns, fabricação de baterias, tintas à base de Pb e gasolina com chumbo (Authman, 2015). Uma vez no ambiente marinho, o Pb é facilmente absorvido na corrente sanguínea do peixe e acumulado nos ossos, brânquias, rins, fígado e escamas do corpo. Desta forma, exposição tóxica ao Pb em humanos devido ao consumo de peixes pode resultar em problemas neurológicos, efeitos hematológicos, insuficiência renal, hipertensão e câncer (Bosch et al., 2016).

O Cd é um elemento amplamente encontrado na crosta terrestre, mas em concentrações bastante escassas, variando de 0,1 a 5 ppm (Morrow, 2001). Dentre as fontes antropogênicas de Cd, destacam-se a fundição de outros metais (principalmente o Zn), a queima de combustíveis fósseis, a incineração de resíduos como as baterias de níquel-cádmio (Ni-Cd) (ATSDR, 2012). O Cd é altamente tóxico para os seres humanos e possui uma meia-vida biológica longa, o que leva a uma eliminação lenta da carga corporal acumulada. Os efeitos na saúde humana incluem hipertensão e função cardiovascular, distúrbios neurológicos, fraqueza e defeitos esqueléticos (ATSDR, 2012; Bosch et al., 2016).

O uso de traçadores químicos como isótopos estáveis de $\delta^{15}N$ e $\delta^{13}C$ têm sido amplamente aplicados como ferramenta para estudos ecológicos com a abordagem de biomagnificação/biodiluição de elementos traço (Kehrig *et al*., 2013). Por serem transferidos e acumulados ao longo da cadeia trófica e representarem a verdadeira assimilação de recursos alimentares, a análise de isótopos estáveis tornou-se complementar para estudos de dieta e metodologias de habitats (Fry, 2005). Ademais,

possibilitam elucidar as relações tróficas dentro da rede alimentar e as relações entre acumulação de contaminantes e a posição trófica (Kehrig *et al*., 2013).

1.4. O comércio de carne de tubarão na Amazônia

Os tubarões são um componente importante do topo da cadeia alimentar em ecossistemas marinhos. As espécies ocorrem desde regiões temperadas com clima frio até águas tropicais, em profundidades que vão desde a superfície até 1000 m ou mais (Compagno, 2008). Como espécies chave, os tubarões desempenham papéis importantes na comunidade pelo efeito 'top-down' de controle populacional (Arreguín-Sánchez, 2011). Por outro lado, a pressão da pesca e a degradação do habitat são considerados os dois principais fatores responsáveis pelos declínios globais relatados nas populações de tubarões nos últimos 50 anos (Ferreti et al., 2010; Worm et al., 2013; Dulvy et al. 2014).

Em consonância com os declínios da população de tubarões associados 'finning' (Fowler & Séret, 2010; Heithaus et al., 2010), o uso crescente de carne de tubarão como alimento está impactando os estoques em todo o mundo (Borrel et al., 2011; Taylor et al., 2014; Ong & Gan, 2016; McKinney et al., 2016), incluindo o Brasil (Figura 1) (Barreto et al., 2017; Bornatowisk et al., 2018). Melo Palmeira et al, (2013), por exemplo, relataram espécimes de *Pristis perotteti*, uma espécie criticamente ameaçada, sendo vendidos em mercados de peixes na Costa Norte do Brasil, enquanto Feitosa et al (2018) usaram sequências de DNA para identificar 427 amostras de tubarões, popularmente conhecidos como 'cação', obtidas da pesca local, constatando que nove das 17 espécies identificadas foram listadas em alguma categoria de ameaça de extinção.

Além das implicações ecológicas da pesca de tubarões, remoção e declínio populacional associado, o consumo de carne de tubarão também pode expor os seres humanos à potencial contaminação por compostos organo-halogenados e oligoelementos (Pethybridge et al., 2010; Barrera-Garcia et al., 2012; Lopez et al., 2013; Rumbold et al., 2014; Weijs et al., 2015). Em um estudo realizado em peixes comercializados em mercados de peixe do município de Belém – PA, na Amazônia brasileira, Souza-Araujo et al. (2016) encontraram doses de Hg em cações ultrapassando até três vezes o limite de segurança estabelecido pela OMS.

Figura 1: Tubarões juvenis (cações) desfigurados postos a venda no mercado de peixes do município de Bragança – PA (A), e em redes de supermercados de Belém – PA (B).

Os efeitos da exposição a contaminantes, na região amazônica brasileira, se restringe basicamente ao Hg e em comunidades ribeirinhas (Grotto et al., 2010; Oliveira

et al., 2010, Crespo-Lopez et al., 2011; Dorea et al., 2012), onde o peixe é a principal fonte de proteína animal. No entanto, estudos sobre demais elementos traço, como também as implicações ecológicas e de saúde pública pelo consumo de predadores marinhos, como o tubarão, são escassos. Desta forma, torna-se essencial o desenvolvimento de pesquisas científicas que possam somar informações, e elucidar padrões de acumulação, variação espacial, biomagnificação de contaminantes, bem como avaliar o risco de exposição à saúde pública pelo consumo de produtos marinhos.

2. Objetivos

2.1. Geral

- Avaliar a contaminação por elementos traço em peixes marinhos de importância ecológica e comercial da Costa Amazônica.

2.2. Específicos

- Fornecer as primeiras informações sobre as concentrações dos elementos não essenciais As, Hg, Cd e Pb em peixes marinhos na Amazônia; (capítulo 1)

- Avaliar o risco de exposição à saúde pública ao As, Hg, Cd e Pb mediante o consumo de espécies de importância comercial; (capítulo 1)

- Investigar quais espécies de tubarões, e o status de conservação, são comercializados como cação nos mercados de peixe da costa Amazônica; (capítulo 2)

- Inferir sobre à exposição ao As, Hg, Cd e Pb mediante ao consumo de tubarões; (capítulo 2)

- Avaliar a biomagnificação dos elementos dentre os tubarões comercializados; (capítulo 2)

- Determinar a descarga materna de 16 elementos (essenciais e não essenciais) em tecido muscular e hepático na espécie de cação *M. higmani*; (capítulo 3)

- Avaliar a dinâmica nutricional mãe-embrião através do fracionamento isotópico de 13C e 15N em tecido muscular e hepático em *M. higmani*; (capítulo 3)

- Verificar o papel protetor do Se sobre a toxicidade do Hg em mães e embriões de *M. higmani*; (capítulo 3)

- Relacionar as concentrações dos elementos essenciais e não essenciais, e de isótopos estáveis em músculo e fígado com o comprimento total dos embriões. (capítulo 3)

3. Metodologia

3.1. Área de estudo

O estudo foi realizado na área de atuação da pesca artesanal e industrial na Plataforma Continental do Amazonas, em um trecho que compreende os estados do Amapá, Pará e parte do Maranhão, desde o cabo do Oiapoque – AP até a baía de São Marcos – MA (04ºS; 50ºW a 01ºS; 44ºW) (Figura 1).

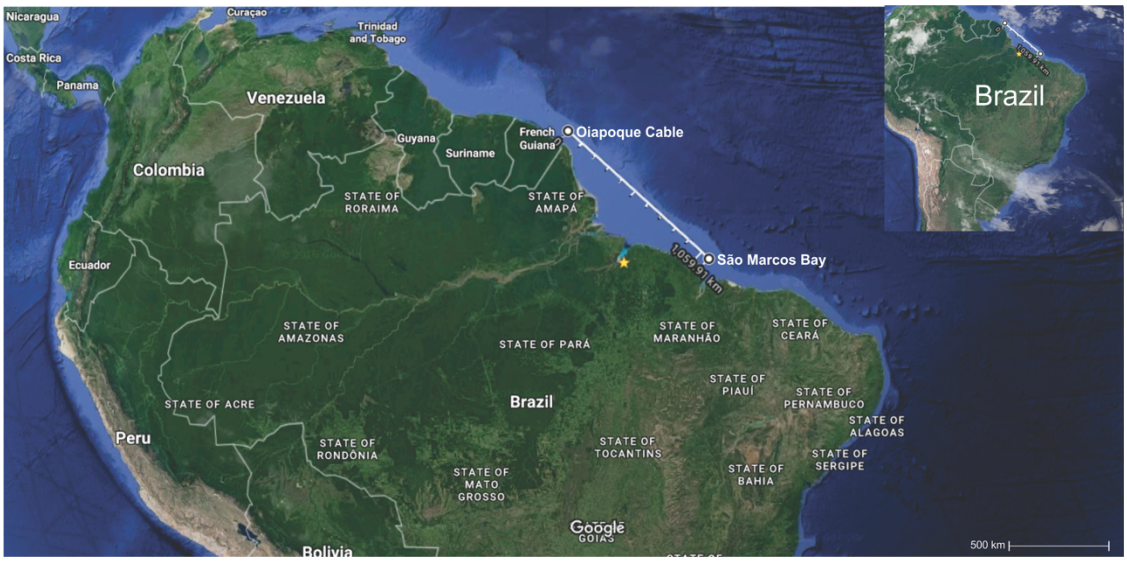

Figura 2: Área de abrangência dos embarques pesqueiros artesanais e industriais em um trecho que compreende desde o Cabo do Oiapoque – AP até a Baía de São Marcos – MA. Fonte: Google Earth.

A região em questão é formada pela descarga dos rios Amazonas ao norte e rio Tocantins ao sul da Ilha de Marajó (Sioli, 1966) e mistura de aproximadamente 6.300 km^3/ano de águas continentais e 9,3 x 10^8 t/ano de sedimentos com águas oceânicas (Meade *et al*., 1979). Juntamente com a grande deposição sedimentar causada pela ação

de erosão, o desenvolvimento de ilhas e planícies alagadas, favorece a manutenção de ecossistemas estuarinos e manguezais. A precipitação anual pode variar entre 2.300 a 3.500 mm (Fisch, 1998).

Ademais, a grande quantidade de nutrientes fornecidos por esses ecossistemas, bem como a influência da descarga de sedimentos pelos rios amazônicos contribuem para uma alta produtividade biológica, biomassa e complexa teia alimentar, influenciando positivamente a atividade pesqueira na região (Isaac, 2006).

3.2. Amostragem

As espécies amostradas para o capítulo 1 e 3 foram obtidas entre março de 2015 e julho de 2017, por amostragens da fauna acompanhante em embarcações da frota industrial do camarão-rosa, em parceria com o projeto "Biodiversidade e suas implicações na Amazônia Azul Brasileira" do Centro de Pesquisa e Gestão de Recursos Pesqueiros do Litoral Norte (CEPNOR/ICMBio), e o projeto "SHRIMP_NEN: Rede cooperativa multidisciplinar para subsidiar o manejo da pesca dos estoques de camarões da região Norte e Nordeste do Brasil com foco ecossistêmico". Para o capítulo 2, as amostras foram obtidas em agosto de 2017, em coletas nos mercados de peixe dos municípios de Belém, Vigia, Curuçá, Salinópolis e Bragança no estado do Pará.

Os indivíduos capturados foram classificados ao menor nível taxonômico possível, e foi realizada a biometria, mensurando o comprimento total (CT) e peso total (P) para. De cada indivíduo foi coletado uma amostra de aproximadamente 10 g de músculo da porção dorso-superior direita por o tecido muscular desta região possuir baixo

teor de lipídios e carbonato inorgânico. Além deste, foram também coletados tecidos de fígado da espécie *M. higmani*, sendo armazenados em sacos de polietileno e mantidos congelados até as análises de metais e isótopos estáveis. Parte do tecido muscular coletado será armazenado em eppendorf de 2 ml com Etanol 100% P.A. para posterior confirmação genética das espécies quando necessário.

3.3. Análise molecular

Para a identificação molecular das amostras, o DNA genômico total foi extraído do tecido muscular usando um Kit de Purificação de DNA Wizard Genomic (Promega Corporation, Madison, WI - EUA), seguindo o protocolo do fabricante. Um fragmento do gene do Citocromo C Oxidase I (COI), padronizado como DNA Barcoding, foi amplificado usando os iniciadores: COI 5′TCAACCAACCACAAAGACATTGGCC3 'e COI 5' TAGACTTCTGGGTGGCCAAAGAATCA 3 '(Ward et al. 2005). As amostras foram amplificadas em um volume final de 25 µL, contendo 4 µl de DNTP (1,25 mM), 2,5 µl de solução tampão 10X, 1 µl de MgCl2 (25 Mm), 0,25 µl de cada primer (200 ng / µl), 1–1,5 µl de DNA genômico (100 ng / µl), 1 U de Taq DNA polimerase (5 U / µl) e água purificada para completar o volume final da reação. As reações em cadeia da polimerase (PCRs) foram realizadas em um termociclador (Applied Biosystems) sob o seguinte protocolo térmico: desnaturação inicial a 93 ° C por 3 min; 35 ciclos de desnaturação a 94 ° C por 30 s, recozimento (a temperaturas de 50 a 60 ° C, dependendo da espécie) por 45 s e extensão a 72 ° C por 45 s, com uma extensão final de 5 minutos a 72 ° C. Todas as reações positivas foram sequenciadas em um sequenciador automático ABI 3500 (Applied Biosystems).

Após o sequenciamento de DNA barcoding, cada sequência foi comparada com sequências semelhantes nos seguintes bancos de dados públicos: GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) e BoldSystems V4 (http: // www.boldsystems. org). Apenas sequências 100% semelhantes com espécies válidas foram consideradas.

3.4. Análise de elementos traço

As concentrações dos elementos traço em amostras de tecido muscular e hepático foram determinadas por espectrometria de massa acoplada ao plasma induzida (ICP-MS). Cada amostra foi primeiramente homogeneizada com tesoura cirúrgica ou bastão de PTFE, e uma alíquota de 0,1 g (peso úmido) de tecido foi colocada em um frasco de PTFE com 1,5 ml de HNO_3 (65% PA) e 0,5 ml de H_2O_2 foi adicionado. As amostras foram aquecidas em forno de microondas (MarsXpress, CEM Corporation) ao longo de uma rampa de temperatura (1º passo: 800W, 180º C, 10 minutos; 2º passo: 1200W, 200º C, 5 minutos; 3º passo: 1000W, 100º C, 10 minutos) e depois esfriou por 20 minutos em banho frio. As soluções digeridas foram então transferidas para frascos de polietileno, que foram cheios até 10 ml com HNO_3 (1%), e armazenados a 4 ºC até a análise por ICP-MS. Para o controle de qualidade, amostras de material de referência certificado DORM-3 e Dolt-4 (0,05 g dw) (Conselho Nacional de Pesquisa, Canadá), triplicatas e brancos foram analisados simultaneamente com as amostras do estudo. A porcentagem de recuperação variou de 75,72% a 109,91% para todos os elementos do DORM-3 e de 75,32 a 113,39% para todos os elementos do DOLT-4.

3.5. Análise de isótopos estáveis

Amostras de tecido muscular e hepático foram secas em um forno de laboratório padrão a 60° C por 24 horas e depois homogeneizadas a um pó fino usando um almofariz e pilão de porcelana. Os lipídios do tecido muscular foram extraídos agitando por um minuto o tecido em pó em tubos criogênicos adicionados em 1,9 ml de solução de clorofórmio-metanol (1: 2). Os tubos criogênicos foram então colocados em banho-maria a 30 °C por 24 horas, em seguida foram centrifugados por 4-6 minutos e o solvente foi filtrado. Este processo foi repetido uma vez. Os resíduos resultantes foram secos em capela de exaustão por 24-48h para evaporar o solvente restante (Hussey et al. 2012). Para tecido hepático, o processo de extração lipídica foi repetido duas vezes, fornecendo altos níveis conhecidos de lipídios nesse tecido (Hussey et al. 2012).

Após a extração lipídica, a uréia foi extraída em ambos os tecidos dos peixes cartilagenosus agitando por um minuto o tecido em pó em tubos criogênicos adicionados de 1,9 ml de água deionizada. Os tubos criogênicos foram então colocados em banho-maria a 30 °C por 24 horas, em seguida foram centrifugados por 4-6 minutos e a água removida com uma seringa médica. O processo de lavagem com água foi repetido três vezes e as amostras foram secas em um liofilizador (Li et al. 2016).

Depois disso, uma alíquota de aproximadamente 400 - 600 μg de tecido para cada amostra foi pesada e comprimida em cápsulas de estanho de 5 mm x 3,5 mm de massa conhecida. Os valores de isótopos esáveis de $\delta^{15}N$ e $\delta^{13}C$ das amostras foram então determinados usando um Espectrômetro De Massa Com Razão Isotópica de fluxo contínuo (IR-MS, Finnigan MAT Deltaplus, Thermo Finnigan, San Jose, CA, EUA)

equipado com um analisador elementar (Costech, Valencia, CA, EUA). As assinaturas isotópicas são expressas na notação delta (δ) e são definidas como partes por mil (‰) em relação a um padrão conhecido, como segue:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$$

Onde R_{Sample} e $R_{Standard}$ correspondem aos isótopos estáveis ($^{13}C/^{12}C$ e $^{15}N/^{14}N$) no experimental e no padrão (controle), respectivamente. A precisão foi avaliada pelo desvio padrão das análises replicadas de quatro padrões (NIST1577c, padrão interno do laboratório (músculo tilápia), USGS 40 e uréia (n = 68 para todos), medidos ≤0,18 ‰ para $\delta^{15}N$ e ≤0,14 ‰ para $\delta^{13}C$ A precisão, com base nos valores certificados do USGS 40 (n = 68 para $\delta^{15}N$ e $\delta^{13}C$) analisados ao longo das leituras e não utilizados para normalizar as amostras, mostrou uma diferença de -0,05 para $\delta^{15}N$ e -0,07 ‰ para $\delta^{13}C$ do valor certificado A precisão da instrumentação foi verificada durante o período em que essas amostras foram analisadas foi baseada nos padrões NIST 8573, 8547 e 8574 para $\delta^{15}N$ e 8542, 8573 e 8574 para $\delta^{13}C$ (n = 20 para todos). Os valores certificados foram -0,17, -0,10, -0,14 ‰ para $\delta^{15}N$ e -0,10, -0,06 e 0,14 ‰ para $\delta^{13}C$, respectivamente.

3.6. Avaliação dos riscos de exposição

A avaliação do risco à saúde humana aos elementos As, iAs, Hg, Cd e Pb pelo consumo de peixe foi estimada usando as seguintes equações:

3.6.1 Consumo diário estimado (EDI)

$$EDI = (te \times DC) / PC$$

Onde, EDI ($\mu g.kg^{-1}_{bw}\ dia^{-1}$) é a ingestão diária estimada; te é a concentração média de elemento traço ($\mu g.g^{-1}ww$); DC é o consumo diário de peixes da população da Amazônia brasileira ($g.dia^{-1}$), conforme relatado por Mangas et al. (2016); PN é o peso corporal médio humano (70 kg para uma pessoa adulta). O valor diário de consumo per capita de produtos aquáticos no Brasil foi de 39,72g em 2011 (IBGE, 2011), mas na Amazônia brasileira estima-se 73,86g (Mangas et al., 2016), superior à média mundial de 54,19g por dia em 2011. 2014 (FAO, 2016). O As inorgânico (iAs) foi estimado (10% do total de As), uma vez que a USEPA (2000) sugere o uso de iAs para a avaliação do risco à saúde humana em vez da exposição de As total (USFDA, 1993).

3.6.2 Quociente de Perigo (HQ)

$$HQ = EDI / RfD$$

HQ é o quociente de risco e é a razão da potencial exposição a uma substância e o nível em que nenhum efeito adverso é esperado. RfD é a dose de referência oral ($\mu g\ .kg^{-1}bw\ dia^{-1}$) disponível para os elementos traço (Capítulo 1:Tabela 2), que é uma estimativa de uma dose diária de contaminantes que provavelmente não apresenta risco considerável de efeitos deletérios à saúde humana (IRIS, 2019; USEPA, 2013). Se o HQ obtido for <1, indica que não é provável que ocorram efeitos adversos e, portanto, pode ser considerado como tendo risco insignificante. Caso contrário, se >1, não há probabilidade estatística de ocorrência de danos. Trata-se de uma declaração de que a concentração de exposição excede a concentração de referência (RfD), e o consumo de

produtos aquáticos pode impor um risco à saúde dos consumidores, especialmente pessoas suscetíveis, como mulheres grávidas (Karaminasab et al., 2015).

3.6.3 Índice de Risco (HI)

$$HI = HQ\ (iAs) + HQ\ (Se) + HQ\ (Hg) + HQ\ (Pb) + HQ\ (Cd)$$

O índice de risco (HI) dos HQs é expresso como a soma dos quocientes de risco (USEPA, 2011). HI é o índice de risco, HQ (iAs) é o quociente de risco para a ingestão de As inorgânico e assim por diante.

3.6.4. Risco de Câncer (TR)

$$TR = EDI \times CPSo/1000$$

O risco de câncer (TR) é usado para indicar o risco carcinogênico. CPSo é a inclinação oral da potência cancerígena ($mg.kg^{-bw}\ dia^{-1}$). Como não há CPSo estabelecido para Hg e Cd, o valor de TR para ingestão apenas iAs e Pb foi calculado para mostrar o risco carcinogênico. O fator de inclinação oral (CPSo) de iAs é 1,5 $mg.kg^{bw-}dia^{-1}$ e de Pb é 0,0085 $mg.kg^{bw-}dia^{-1}$ (IRIS, 2019).

3.7. Análise de dados

Para o cálculo do nível trófico (Nt), utilizou-se a seguinte fórmula:

$$Nt= [(\delta^{15}N_{amostra} - \delta^{15}N_f)/ 2{,}54] + 2$$

Onde $\delta^{15}N_{amostra}$ é a média da assinatura isotópica de cada espécie; $\delta^{15}N_f$ é a média da assinatura isotópica do consumidor primário, que neste estudo foi a espécie *Rhinoptera bonasus*; 2,54 representa a média do fracionamento trófico do ecossistema e 1 representa o posicionamento do consumidor primário (Jackson *et al*., 2013).

Os dados foram transformados para atingir os requisitos de normalidade e homogeneidade quando necessário. Diferenças nas concentrações dos elementos traço entre espécies, habitats e tecidos foram testadas com PERMANOVA univariada em matrizes de distâncias Euclidianas com base em 9999 permutações (Anderson, 2001). Os cálculos e testes foram realizados usando o PERMANOVA+ para o software PRIMER-E (Anderson *et al*., 2008).

Correlações lineares de Pearson foram utilizadas para testar a relação entre $\delta^{15}N$ *vs*. concentrações dos elementos traço, $\delta^{13}C$ *vs*. concentrações dos elementos traço, concentrações dos elementos traço *vs.* comprimento total e a relação entre a concentração de Hg *vs.* razão molar Se:Hg.

O teste - T para uma amostra foi utilizado para testar a diferença na concentração de elementos traço entre mães e embriões no tecido muscular e hepático de *M. higmani*, sendo a concentração do tecido de cada mãe o valor teórico comparado à suas respectivas ninhadas. Estes cálculos foram realizados usando o Rstudio (Version 1.1.383).

4. Resultados

- Dentre os elementos tóxicos analisados, o arsênio é o mais abundante em todos os peixes, estando inclusive acima dos limites máximos permitidos em guias internacionais;

- iAs, Hg e Pb apresentaram individualmente potencial risco não carcinogênico à saúde pelo consumo de algumas espécies cartilaginosas;

- Dentre as 13 espécies de tubarões encontradas sendo comercializadas, duas espécies são listadas como ameaçadas de extinção (EN), duas são vulneráveis (VU), três estão quase ameaçadas (NT), quatro são menos preocupantes (LC) e duas são deficientes em dados, DD, de acordo com o RedList da IUCN;

- Altas doses de As (até 40x acima do limite de segurança) e Hg, foram encontradas nos tubarões que estavam sendo comercializados;

- As maiores concentrações de Hg foram encontradas em espécies com maiores assinaturas de $\delta^{15}N$ (biomagnificação), enquanto que as maiores doses de As foram nas espécies com menores $\delta^{15}N$ (biodiluição);

- As assinaturas de $\delta^{15}N$ nos tubarões capturados na costa amazônica são menores do que as mesmas espécies em outras partes do mundo;

- Os embriões da espécie de tubarão *M. higmani* possuem maiores concentrações dos elementos traço no músculo, tanto essenciais quanto não essenciais, do que suas respectivas mães;

- O fígado das mães acumula mais elementos não essenciais que o dos embriões;

- A dinâmica do suprimento de nutrientes do embrião-mãe é um reflexo direto da dieta e dos habitats da mãe durante o período gestacional;

- A razão molar de Se: Hg sugere que o Se exerce papel protetor contra a toxicidade do Hg durante os estágios iniciais do desenvolvimento de *M. higmani*.

- A maior parte dos elementos são biodiluídos pelo crescimento dos embriões;

5. Conclusão e considerações finais

Neste estudo concluímos que as espécies de peixes marinhos da Costa Amazônica apresentam, de modo geral, baixas concentrações de elementos traço, a exceção do As. O arsênio é o elemento mais abundante em todos os peixes, especialmente nos de posição trófica baixa. Isto pode estar diretamente relacionado à influência da pluma do Amazonas sobre a região costeira, onde o arsênio é descarregado juntamente com a grande quantidade de água e sedimentos (Scarpelli, 2005).

iAs, Hg e Pb apresentaram risco potencial à saúde não carcinogênico individualmente pelo consumo de algumas espécies cartilaginosas. Coletivamente, esses elementos foram encontrados o suficiente para serem considerados como um risco potencial à saúde humana. Dessa forma, as pessoas que consomem continuamente peixes cartilaginosos contaminados com elementos tóxicos, como as encontradas aqui, estão sob risco alvo de câncer a longo prazo. Complementarmente, os resultados do estudo sobre o consumo de tubarões especificamente (capítulo 2) confirmam que o consumo regular de carne de tubarão ao longo da costa norte do Brasil pode representar um risco para a saúde das populações humanas locais através da exposição a altos níveis de As e Hg.

Além da exposição à saúde humana, a captura e comercialização de tubarões pode impactar negativamente o ecossistema em uma escala global (Bird et al., 2018). A maioria dos espécimes capturados para este fim são juvenis ou sub-adultos e, consequentemente, a manutenção das populações futuras podem ser afetadas, principalmente daquelas ameaçadas ou vulneráveis. Além do fato de que as assinaturas de $\delta^{15}N$ destes juvenis demonstram que eles não possuem a mesma posição trófica que os adultos de outras

regiões e, portanto, podem possuir uma significância trófica no ecossistema marinho da Costa Amazônica que vem sendo negligenciada por todos estes anos.

Um outro fator importante a ser levantado é que por mais que as assinaturas isotópicas de $\delta^{15}N$ dos tubarões juvenis sejam similares às que foram encontradas em outros peixes ósseos predadores, os tubarões possuem maior acumulação de elementos como As e Hg. O capítulo 3 deste estudo demonstrou exatamente que uma grande carga de elementos traço não essenciais encontrados nos juvenis são oriundos de suas mães, por meio de descarga materna. A captura de *Mustelus higmani* é bastante frequente na fauna acompanhante de diversas pescarias, por ser uma espécie abundande na costa. A utilização de fêmeas grávidas foi essencial para compreender que o Se pode ter um papel protetor contra a toxicidade do Hg durante os estágios iniciais do desenvolvimento de *M. higmani*. Bem como que a dinâmica do suprimento de nutrientes do embrião durante o período gestacional, sendo este um reflexo direto da dieta e habitats frequentados pela mãe.

Concluímos também que o fígado acumula mais oligoelementos não essenciais que o músculo. Apesar de ser um resultado esperado, é deveras preocupante; uma vez que os consumidores de cação no município de Belém possuem preferência pela presença do fígado no ato da compra. Alegando que o fígado é utilizado na preparação da carne de cação, o consumidor belenense estaria na verdade consumindo o órgão que oferece maior risco de exposição a contaminantes.

Apesar da presença de importantes nomes da ecotoxicologia Brasileira desenvolverem pesquisas consolidadas sobre a contaminação de elementos traço na

Amazônia, elas são restritas aos ecossistemas aquáticos continentais. Os estudos sobre contaminação por elementos traço em peixes marinhos da Amazônia ainda são bastante escassos, principalmente no que diz respeito às espécies tradicionalmente consumidas e abundantemente contaminadas como os tubarões. Além dos riscos de exposição aqui mencionados, a escala e os impactos das remoções destes tubarões juvenis do ecossistema nesta região são desconhecidos. Consequentemente, são necessários mais dados para avaliar a sustentabilidade desta pesca, sazonalidade de captura e também investigar a funcionalidade destas espécies na cadeia trófica. Os resultados desta tese podem ser usados por agências ambientais e de saúde pública para desenvolver a conservação de espécies de tubarões e projetos de segurança alimentar.

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Capítulo 1

Trace elements (As, Hg, Pb and Cd) in Amazon marine fish and their implications to human health

Trace elements (As, Hg, Pb and Cd) in marine Amazonian fish and their implications for human health

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ABSTRACT

The Amazon Coast is one of the world's most productive ecosystems, but there few studies are available on its ecotoxicology and the implications of the consumption of the seafood from this region for human health. To address this knowledge gap, and provide a database of basic parameters for future research, the present study evaluated the concentrations of As, Hg, Pb and Cd in marine fish of ecological and commercial importance collected from the Amazon Coast, investigated the variation among habitats, and estimated the health risk through the hazard quotient, hazard index, and target cancer risk. Trace elements were determined by Induced Coupled Plasma Mass Spectrometry (ICP-MS) with the ^{15}N stable isotope being used to determine the trophic position of the fish species. The observed concentrations of As were higher than the assessment guidelines and legal limits in 63.82% of the species, whereas those of Hg, Pb and Cd were generally very low. Reef-associated fish presented concentrations of Hg more than twice as high as those recorded in demersal fish ($p<0.001$). The As, Hg, and Pb concentrations were found to represent a potential non-carcinogenic health risk from the consumption of some cartilaginous species. The sum of the contamination by these elements was considered to be a potential human health hazard.

Key-Words:

Trace elements; Marine fish; Habitat variation; Consumption risk; Amazon

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1. INTRODUCTION

Tropical coastal regions are among the most productive ecosystems in the world (Sigman & Hain, 2012), and have long tended to accumulate human populations, industries, and farming operations. As a result, coastal regions are under increasing pressure from stressors derived from the discharge of terrestrial freshwater, including sediments, nutrients, and contaminants of anthropogenic origin (Kroon et al., 2016; Nkwoji et al., 2020).

Data on the abundance of different classes of contaminants and the rates of accumulation occurring in different organisms can provide important insights into the possible impacts on human health caused by the consumption of potentially hazardous species (Ahmed et al., 2015; Javed & Usmani, 2016; Liu et al., 2018). The study of the relationship between contaminant levels and ecological aspects (size of capture, diet, habitat) of the species is also useful to establish safe measures for the management and exploitation of resources that are intended for consumption (Asante et al., 2009; Azad et al., 2019; Xia et al., 2019).

The Amazon Coast is one of the most productive ecosystems of the world (Vasconcelos, 2005; Isaac & Ferrari, 2017). This coast supports a fishery industry with an annual revenue of $610 million, which is one of the principal components of the economy of both the region and the country as a whole, and also provides food resources for the local populations (FAO, 2014; Sea Around Us, 2017). The estimative of primary production point for one oligotrophic and stratify habit, which the Amazon and Orinoco plumes are the main source of nutrients (Hu et al., 2004).

However, the Amazon basin is known to be a substantial source, both natural and anthropogenic, of elements such as Hg and As (Bundschuh et al., 2012; Arrifano etl al., 2018; Siqueira et al., 2018; da Silva Júnior et al., 2019), which end up in the sea through the enormous discharge of freshwater and sediments from the basin (Scarpelli, 2005; Isaac & Ferrari, 2017). In addition to these impacts, there has been a recent proposal for the extraction of petroleum at the mouth of the Amazon River. If approved, this would likely have a major impact on the ecosystem, and lead to an increase in the quantity of contaminants in the near future, given that any operation of this type will almost inevitably result in accidents involving petroleum spills, which tend to occur frequently during these operations (NAP, 2003), and have highly deleterious effects (Rodrigues et al., 2010; D'Costa et al., 2017).

Despite the considerable importance of the Amazon Coast for the commercial fisheries of South America, few data are available on the marine ecotoxicology of the region or the potential implications of any contamination for the health of the people that consume the seafood produced in this region. To address this knowledge gap and provide a database of local conditions for future research, the present study evaluated the concentrations of As, Hg, Pb and Cd in the marine fish of the Amazon Coast that are either ecologically and/or commercially important. The variation in the accumulation of trace elements among habitats was also investigated, and the health risks for human consumers were estimated through the hazard quotient, hazard index, and target cancer risk.

97 **MATERIAL AND METHODS**

98 2.1. STUDY AREA

99

100 Samples were collected on the Amazon Coast of Brazil (Figure 1). This region is
101 formed by the discharge of the Amazonian rivers to the north of Marajó Island and the
102 Tocantins River to the south of this island (Sioli, 1968), which results in the mixing of
103 approximately 6,300 km^3/year of freshwater and 9.3 x 10^8 t/year of sediments with the
104 coastal waters of the western Atlantic Ocean (Meade et al., 1995). This process, together
105 with the substantial sedimentary deposition caused by local erosion, and the development
106 of islands and floodplains, favors the maintenance of estuarine and mangrove ecosystems.
107 The region's climate is warm and humid equatorial with mean air temperatures of around
108 26°C (Fisch et al., 1998). Mean annual precipitation is approximately 2,300 mm, although
109 it may reach 3,500 mm in some years (Fisch et al., 1998). The region presents a well-
110 defined dry season with mean monthly precipitation of less than 50 mm, which lasts from
111 July to December (Fisch et al., 1998).

112

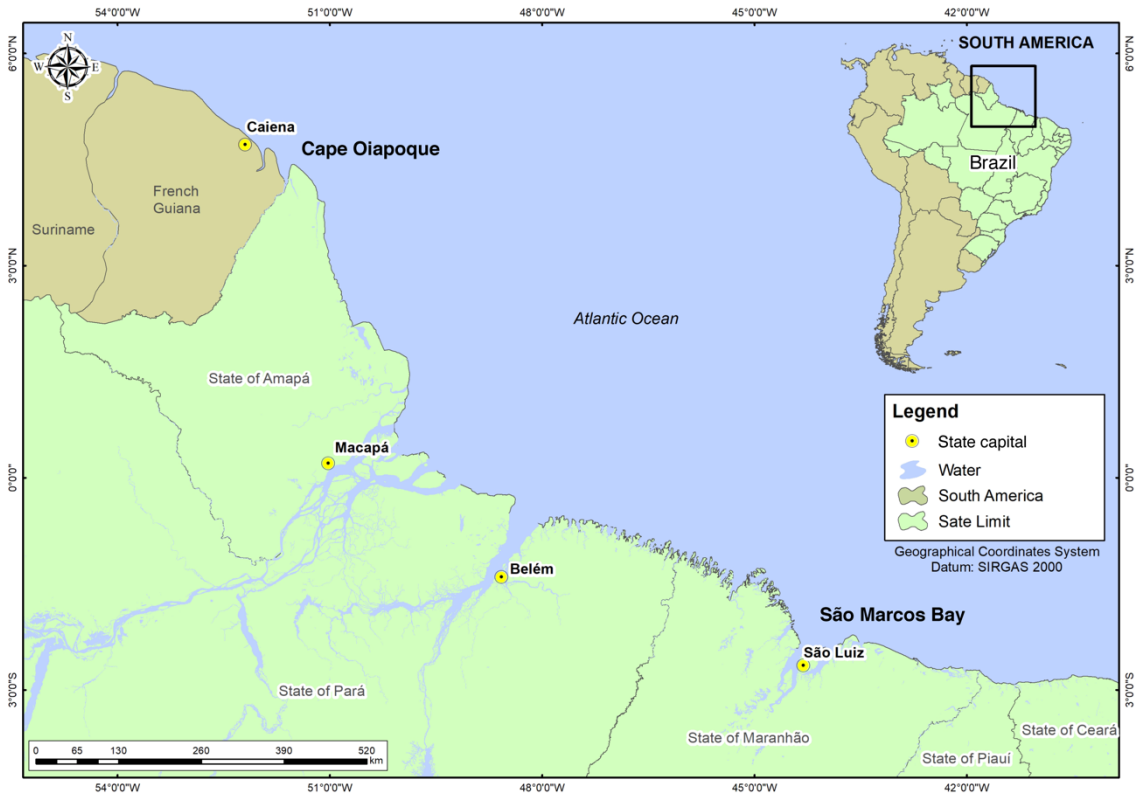


Figure 1: Map of the study area in northern Brazil.

2.2. FISH SAMPLING

Fish were obtained from the bycatch of commercial bottom-trawl shrimp fisheries every two months between 2015 and 2017. Two subsamples of fish were taken from each trawl and kept on ice until landing at a fishing port.

In the laboratory, the specimens were identified to the species level, and their L_T and mass were conferred. Samples of white muscle tissue (10 g) were taken from under the anterior end of the dorsal fin of each individual and stored in polyethylene bags, which were kept frozen until analysis.

2.3. ANALYSIS OF TRACE ELEMENTS

Trace element concentrations were determined by Induced Coupled Plasma Mass Spectrometry (ICP-MS). Each muscle sample was first homogenized. After homogenize, 0.1g were weight in PTFE bottles, 1.5 ml of HNO_3 and after 30 minutes 0.5 ml of H_2O_2 were added. Samples were then heated in a microwave oven (MarsXpress, CEM Corporation) over a temperature ramp and then cooled down for 20 minutes in a cold bath. The digested solutions were transferred to polyethylene bottles, completed to 15 ml with HNO_3 (1%) and stored at 4ºC until the ICP-MS analysis. For quality control, 12 samples of DORM-3 Certified Reference Material (National Research Council of Canada) were analyzed, and the percentage recovery ranged from 76.80% to 88.03% for all the different elements. In addition, 12 blanks were analyzed, with mean recovery ranging from 10.28% to 19.11% of the limit of detection (LD) for all elements. In addition to the five automatic replicates that the equipment makes when reading each sample, 12 triplicates were run, with a difference of less then 10% being found in each element. Inorganic As (iAs, 10% of total As) was estimated because USEPA (2000) suggests using the uptake of inorganic As, rather than total exposure to As, for the assessment of human health risks (USFDA, 1993).

2.4. STABLE ISOTOPES ANALYSIS

The muscle samples were dried at 60°C for 24 hours, and then macerated and homogenized to a fine powder using a porcelain mortar and pestle. Lipids were extracted by shaking this powder into cryovials, in which they were mixed for one minute in 1.9 ml of chloroform-methanol solution (1:2). The cryovials were then left to sit for at least

24 hours in a water bath at 30˚C. After 24h, the cryovials were centrifuged for 4–6 minutes and the solvent was filtered off. New chloroform-methanol solution was added, and the samples were then shaken for one minute and centrifuged once again for 4–6 minutes. The resulting filtrate was left under the fumehood for 24–48 h to evaporate the remaining solvent (Hussey et al., 2012). The urea of the cartilaginous fish samples was extracted after lipid extraction by shaking the powdered sample in a cryovial containing 1.9 ml of de-ionized water for one minute. The criovials were then left to sit for at least 24 hours in a water bath at 30˚C. After 24 h, the samples were centrifuged for 4–6 minutes and the water was removed using a medical syringe. This whole process was repeated three times, and the samples were then dried again.

An aliquot of 400–600 µg of the resulting powder was weighed and compressed into 5 mm x 3.5 mm tin capsules, which had been weighed previously. The ™^{15}N isotope signatures of the samples were determined by burning the samples, one by one, in a Continuous Flow Isotope Ratio Mass Spectrometer (IR-MS, Finnigan MAT Deltaplus, Thermo Finnigan, San Jose, CA, USA) equipped with an elemental analyzer (Costech, Valenica, CA, USA). The isotopic signatures are expressed in delta notation (™) and defined as parts per thousand (‰) in relation to a standard sample, as follows:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R_{Sample} and $R_{Standard}$ are the ratios of stable isotopes ($^{15}N/^{14}N$) in the experimental and standard samples, respectively. The precision of this procedure was assessed by the standard deviation of the replicate analyses of the four standards, i.e., NIST1577c, internal lab standard (tilapia muscle), USGS 40, and Urea (n=68 in all cases), with $\delta^{15}N$

≤0.18‰ in all cases. The accuracy, based on the certified values of USGS 40 (n=68 for ™^{15}N) analyzed throughout runs and not used to normalize samples showed a difference of -0.05‰ for δ^{15}N from the certified value. Instrumentation accuracy was checked throughout the study period, based on NIST standards 8573, 8547, and 8574 for δ^{15}N (n=20 in all cases). The mean differences from the certified values were -0.17, -0.10 and -0.14‰.

2.5. HEALTH RISK ASSUMPTION

The assessment of the human health risk from the trace elements found in the fish meat was estimated using the following equations:

2.5.1. Estimated Daily Intake (EDI)

$$EDI = (te \times DC)/BW$$

where EDI (μg kg^{-1}_{bw} day^{-1}) is the estimated daily intake; *te* is the mean trace element concentration (μg g^{-1}ww); DC is the daily consumption of fish by the Brazilian Amazon population (g day^{-1}), as reported by Mangas et al (2016); BW is the mean human body weight (70 kg for an adult). The per capita daily aquatic products consumption value in Brazil was 39.72g in 2011 (IBGE, 2011), but in Brazilian Amazon population is estimated 73.86g (Mangas et al., 2016), higher than the world average of 54.19g per day in 2014 (FAO, 2016).

2.5.2. Hazard Quotient (HQ)

$$HQ = EDI/RfD$$

where HQ is the hazard quotient, that is, the ratio of the potential exposure to a substance to the level at which no adverse effects are expected; EDI is the estimated daily intake (see above), and RfD is the oral reference dose ($\mu g\ kg^{-1}_{bw}\ day^{-1}$) for the trace elements (Table 2), which is an estimate of a daily dose of contaminants that is likely to have no appreciable risk of deleterious effects for human health (IRIS, 2019; USEPA, 2013). If the value of HQ is <1.0, adverse effects are unlikely, and hazard can thus be considered to be negligible. On the other hand, if >1.0, there is a statistical probability of harm, given that exposure exceeds the reference concentration (RfD), and the consumption of fishery produce may thus constitute a health hazard for the consumer, especially in the case of susceptible individuals, such as pregnant women (Karaminasab et al., 2015).

2.5.3. Hazard Index (HI)

$$HI = HQ\ (iAs) + HQ\ (Hg) + HQ\ (Pb) + HQ\ (Cd)$$

where HI is the hazard index of the HQs, expressed as the sum of the hazard quotients (USEPA, 2011), HQ (iAs) is the hazard quotient for the intake of inorganic As, HQ (Hg) is the quotient for Hg, and so on.

2.5.4. Target Cancer Risk (TR)

$$TR = EDI \times CPSo / 1000$$

where the target cancer risk (TR) represents the carcinogenic risk; EDI is the estimated daily intake (see above), and CPSo is the carcinogenic oral potency slope, oral (mg kg^{bw-} day^{-1}). As there are no CPSo values established for Hg, Cd and Se, the TR was calculated only for the intake of iAs and Pb to estimate the carcinogenic risk. The oral factor slope (CPSo) of iAs is 1.5 mg kg^{bw-} day^{-1} and that of Pb is 0.0085 mg kg^{bw-} day^{-1} (IRIS, 2019).

2.5. STATISTICS

The trophic position (TP) of the fish species was calculated using the stable ^{15}N isotope signature following Jackson et al (2013):

$$Nt = [(\delta^{15}N_{sample} - \delta^{15}N_{c1})/ 2.54] + 2$$

where $\delta^{15}N_{sample}$ is the mean isotope signature of each individual, $\delta^{15}N_{c1}$ is the mean isotope signature of the primary consumer (*Rhinoptera bonasus*), 2.54 is the mean trophic fractionation of the ecosystem and 2 is the trophic position of *Rhinoptera bonasus* (Jackson et al., 2013).

To examine differences in the concentrations of trace elements among species and habitats, a univariate PERMANOVA was run on Euclidean distances matrices with 9999 permutations, including the Monte Carlo correction for small sample sizes (Anderson, 2001). All the analyses were conducted in PERMANOVA+ in the PRIMER-E software (Anderson et al., 2008) and the results were plotted in Rstudio (Version 1.2.5019).

253 ## 3. RESULTS AND DISCUSSION

254 ### 3.1. Concentrations of trace elements

255

256 A total of 314 fish from 47 species were analyzed here (**Table 1**). This is the first
257 study of the concentrations of trace elements in marine teleost fish from the Amazon
258 Coast. The mean concentrations of As, iAs, Hg, Cd, and Pb recorded in each Amazon
259 Coast fish species were compared with the assessment guidelines for assessing fishery
260 products and the legal limits for human consumption (see **Table 2**). The doses of arsenic
261 were higher than both the assessment guidelines and the legal limit in 63.82% of the
262 species. The mean As for the total sample was 5.22 μg/g, and the species with the highest
263 concentrations was *Rhinoptera bonasus* (83.44 ± 4.62 μg/g). The lowest mean
264 concentration was recorded in *Trichiurus lepturus* (0.18 ± 0.04 μg/g).

265

266 In general, marine fishes have 1–10 μg/g more As than freshwater species
267 (Amlund & Berntssen, 2004; Schaeffer *et al*., 2006; Ciardullo *et al*., 2010), although 50–
268 100% of this may be present in the non-toxic arseniobetaine (AsB) form (Amlund et al.,
269 2006; Maher *et al.,* 2011; Zhang et al., 2012). In fact, a number of studies have found that
270 marine fish which absorb iAs may biotransform this element preferentially into AsB,
271 resulting in a high level of bioaccumulation of total As (Francesconi *et* al., 1994;
272 Caumette *et* al., 2012; Zhang et al., 2016). On the other hand, the coastal region close to
273 the mouth of the Amazon River receives an annual input of approximately 5 tons of As
274 in the sediments discharged by the river (Scarpelli, 2005), which may account for the
275 relatively higher level of total As found in the species analyzed in the present study.

276

As the vast majority of the fish species included in the present study are predators, it did not include any systematic analysis of the relationship between trace element concentrations and trophic position. It is nevertheless important to note that higher concentrations of arsenic were found in species in the lower tropic positions (Table 1), which indicates biodilution trough the trophic chain. The biodilution of arsenic in coastal systems has been recorded in the past few years in a number of regions around the world (Meador et al., 2004; Vizzini et al., 2013; Huang, 2016), where predators typically have lower concentrations than primary and secondary consumers. Souza-Araujo *et al* (*in prep*) have also found a clear negative relationship between As and ^{15}N in sharks from the present study area on the Amazon coast.

The Hg concentrations recorded in the present study were generally much lower than either the assessment guidelines or the legal limits. The overall mean concentration was 0.09 μg/g, and *Genyatremus luteus*, *Stellifer microps*, *Pellona harroweri* and *Notarius grandicassis* presented the lowest means, of 0.01 μg/g. Only *Conodon nobilis* presented a mean mercury concentration above the safety limit (0.68 ± 0.10 μg/g). As high Hg concentrations are expected in predator species, these findings reflect the fact that almost 80% of the fish sampled in the present study were juvenile or sub-adult predators. An increase in Hg concentrations is expected as exposure time, that is, the age of the fish, increases (Jinadasa et al., 2013; Sackett et al., 2013).

As an example of this, Costa et al (2009) and Barbosa et al (2011) found a significant positive correlation between total length and Hg in *Trichiurus lepturus*, which also had a higher Hg concentration than the *T. lepturus* specimens analyzed in the present study. A similar pattern was also observed in *Micropogonias funieri* by Carneiro et al

(2013) and Corrales et al (2016), which was also analyzed in the present study. So, the low concentrations of Hg found in most species of our study maybe due the small length of specimens catch.

None of the species analyzed had a mean Pb concentration above the guidelines or legal limits, and only *Mustelus higmani* (0.05 ± 0.01 μg/g) and *Pseudobatos horkelii* (0.07 μg/g) had Cd concentrations above the recommended levels. Of all the trace elements analyzed here, Cd is typically toxic even at relatively low concentrations and can cause adverse effects, such as genotoxicity, in fish (Pavlaki et al., 2016) due to its marked tendency for bioaccumulation (Chandurvelan et al., 2012; 2013). Species at lower trophic levels, such as *M. higmani*, are thought to be more sensitive to the genotoxic effects of trace elements (Pavlaki et al., 2016), so like As, Cd may suffer biodilution through the trophic chain (Espejo et al., 2018). Given this, even though Cd contamination was recorded in a few bottom-dwelling species on the Amazon Coast, it may impact local populations, triggering bottom-up cascade effects in coastal trophic webs.

Only the concentrations of Hg varied significantly among habitats (Pseudo-F = 6.051 P = 0.015). Although demersal species were most abundant group in the fauna sampled, reef-associated fish presented Hg concentrations more than double (p = 0.001) those recorded in the demersal species (**Figure 2**).

The newly-discovered Great Amazon Reef System (GARS) is composed of typical mesophotic reefs at depths of 70–220 m (Francini-Filho et al. 2018). Within the GARS, areas under the direct influence of the Amazon plume have significant amounts of anaerobic organisms, with dissolved oxygen levels of as little as ≤ 3.5 ml liter^{-1} near

the bottom at some points (Moura et al., 2016). Since the species associated with this reef system appear to occupy similar trophic positions to those from other habitats, the presence of higher doses of Hg in reef-associated fish indicates that the GARS is more susceptible to the methylation of inorganic Hg than other habitats, in particular in areas of deeper water (Blum et al., 2013), where accumulation rates will be even higher (Chouvelon et al., 2012; Kiszka et al., 2015).

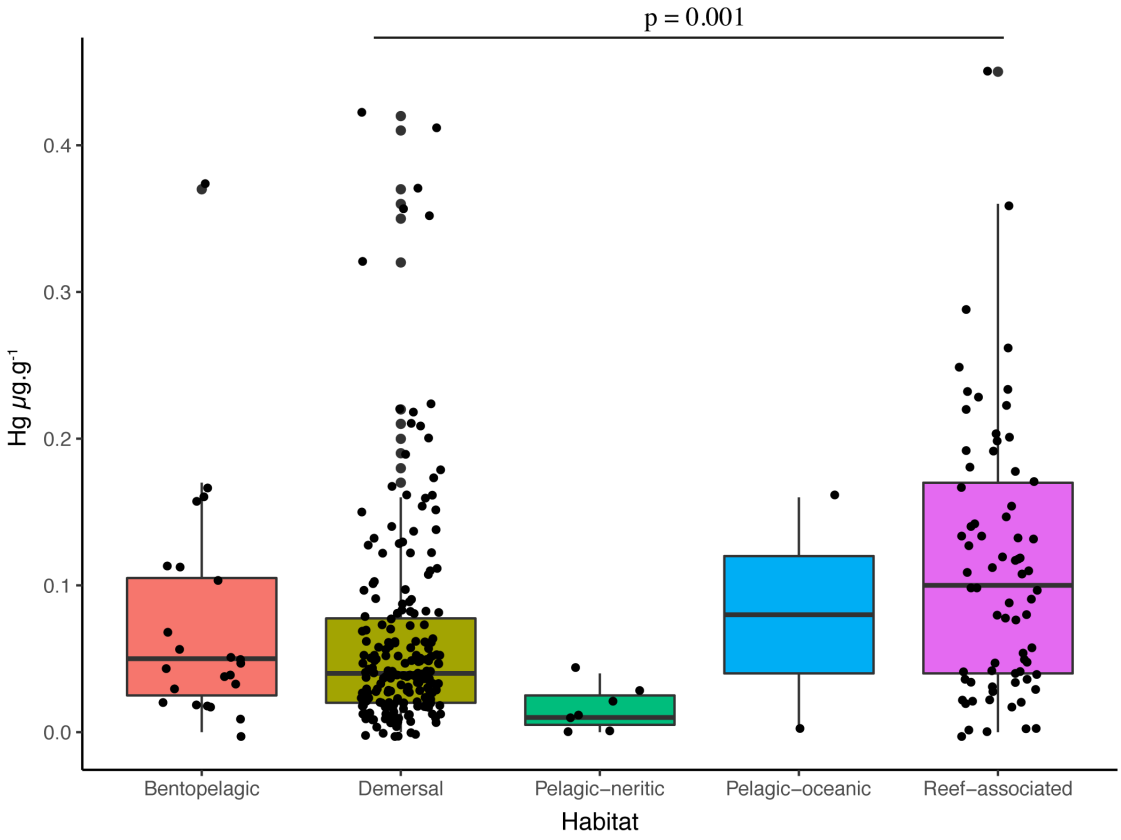


Figure 2: Variation in the Hg concentrations found in marine fish from different habitats off the Amazon Coast.

3.2. Assessment of risk exposure

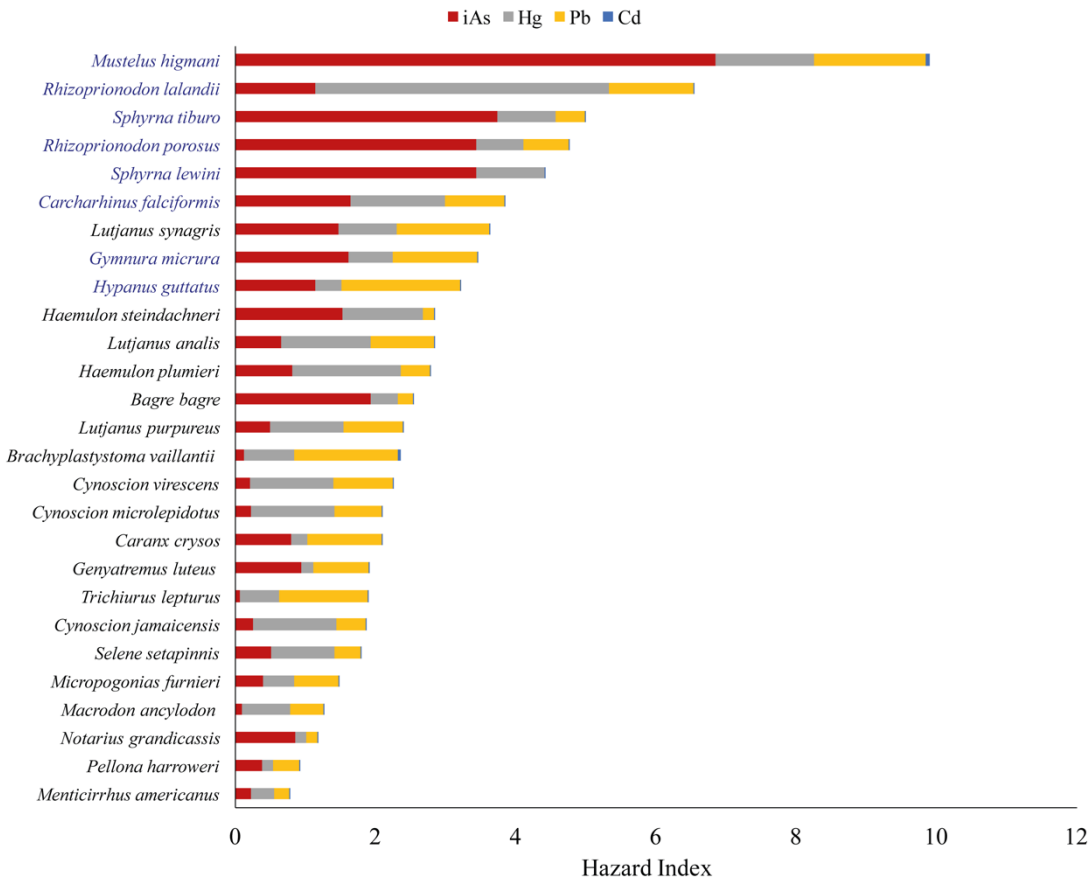
A total of 27 commercially-important fish species were selected here for the assessment of health risk. The estimated daily intake (EDI), hazard quotient (HQ), and target cancer risk (TR) estimated for the different trace elements resulted from the consumption of these fish species are presented in **Table 3**. Overall, the HQ of total As was above 1 in 25 of these 27 commercial species, while the HQ of the iAs remained over 1 in 11 species. The highest HQ values for both iAS (6.84) and Pb (1.58) were recorded in *M. higmani*. The HQ for Pb in *Hypanus guttatus*, *Gymnura micrura*, *Rhizoprionodon lalandii*, *Trichiurus lepturus* and *Lutjanus synagris* were also more than 1, while 10 species were that returned HQ values of over 1 (*Rhizoprionodon lalandii* had the highest 4.18) for Hg. All these species can be considered inappropriate for human consumption, given that their HQ values indicate a potential non-carcinogenic health risk from the ingestion of iAs, Hg or Pb.

The HQ values for Cd were less than 1 in all 27 commercial fish species, indicating the absence of any non-carcinogenic health risk from the ingestion of this trace element through the human consumption of these fish species. Although the individual HQ values for the ingestion of a single trace element were within the acceptable limit (HQ = 1) in most cases, the combined HQ value recorded for the four elements analyzed in the present study was above the acceptable limit in most species (Figure 3).

The iAs and Hg concentrations contributed most to the HI in all the 27 commercial fish species (Figure 3). Once again, *M. higmani* presented the highest health risk in the 27 species. However, all the other cartilaginous fish were also among the species with the

highest HI values. This indicates that the continuous consumption of large amounts of this type of fish, in particular sharks, may have chronic non-carcinogenic effects.

The TR values were estimated only for iAs and Pb, which have known carcinogenic effects. The TR values for iAs ranged from $2.87x10^{-5}$ to $3.08x10^{-3}$, while those for Pb varied from $2.69x10^{-8}$ to $2.86x10^{-7}$ (**Table 3**). As the recorded TR values were higher than the guideline value of 10^{-6} (USEPA 2011), the consumption of large amounts of these fish over a long period of time may have carcinogenic effects. However, while the cartilaginous fish species analyzed in the present study were considered to be unsafe for human consumption, an increased probability of developing cancer would depend on the continuous consumption of these species for at least 76.3 years.



379 **Figure 3:** Relative contribution iAs, Hg, Pb, and Cd to the hazard index (HI) for the
380 consumption of 27 commercial fish species collected on the Amazon Coast. In blue are
381 the cartilaginous and in black the bone fish.

382

383

384 **Table 1:** Descriptions of the fish species from the Brazilian Amazon Coast analyzed in the present study.

Scientific name	Common name	N	TL ± SD (cm)	TP (Mean ± SE)	Feeding habit	Habitat	IUCN
Anisotremus surinamensis	Black margate	7	24.5 ± 1.3	3.21 ± 0.16	Hunting macrofauna (predator)	Reef-associated	DD
Bagre bagre	Coco sea catfish	7	34.2 ± 5.2	3.26 ± 0.10	Hunting macrofauna (predator)	Demersal	LC
Bairdiella ronchus	Ground croaker	6	30.6 ± 3.3	3.20 ± 0.09	Variable	Demersal	LC
Brachyplatystoma vaillantii	Laulao catfish	7	25.9 ± 3.1	2.62 ± 0.12	Mainly animals	Demersal	-
Caranx crysos	Blue runner	7	16.4 ± 8.2	2.85 ± 0.60	Hunting macrofauna (predator)	Reef-associated	LC
Carcharhinus falciformis	Silky shark	1	49	3.63	Hunting macrofauna (predator)	Reef-associated	VU
Chaetodipterus faber	Atlantic spadefish	7	16 ± 3.2	3.16 ± 0.28	Hunting macrofauna (predator)	Reef-associated	LC
Conodon nobilis	Barred grunt	7	26.5 ± 6	3.25 ± 0.05	Hunting macrofauna (predator)	Reef-associated	LC
Ctenosciaena gracilicirrhus	Barbel drum	7	14.7 ± 1.8	3.15 ± 0.10	Hunting macrofauna (predator)	Demersal	LC
Cynoscion jamaicensis	Jamaica weakfish	7	22.7 ± 1.8	3.66 ± 0.09	Hunting macrofauna (predator)	Demersal	LC
Cynoscion microlepidotus	Smallscale weakfish	7	33.5 ± 7.2	3.18 ± 0.20	-	Demersal	LC
Cynoscion virescens	Green weakfish	9	32.3 ± 11	3.33 ± 0.38	Hunting macrofauna (predator)	Demersal	LC

Dactylopterus volitans	Flying gurnard	6	10.6 ± 2.8	2.51 ± 0.10	Hunting macrofauna (predator)	Reef-associated	LC
Decapterus tabl	Roughear scad	7	19.9 ± 2.9	2.73 ± 0.19	Selective plankton feeding	Demersal	LC
Genyatremus luteus	Torroto grunt	7	19.6 ± 3.3	2.74 ± 0.03	Hunting macrofauna (predator)	Demersal	-
Gymnachirus nudus	Naked sole	7	15.5 ± 0.8	3.63 ± 0.48	-	Demersal	LC
Gymnura micrura	Smooth butterfly ray	9	21.7 ± 7.2	3.37 ± 0.11	Hunting macrofauna (predator)	Demersal	DD
Haemulon plumieri	White grunt	7	20.3 ± 1.1	3.33 ± 0.15	Hunting macrofauna (predator)	Reef-associated	LC
Haemulon steindachneri	Chere-chere grunt	6	20.7 ± 1.4	2.66 ± 0.33	Hunting macrofauna (predator)	Reef-associated	LC
Hypanus guttatus	Longnose stingray	5	46.3 ± 34	2.91 ± 0.33	Variable	Demersal	DD
Lutjanus analis	Mutton snapper	8	32.2 ± 5.2	3.28 ± 0.16	Hunting macrofauna (predator)	Reef-associated	NT
Lutjanus purpureus	Southern red snapper	3	41 ± 1.4	3.25 ± 0.14	Hunting macrofauna (predator)	Demersal	-
Lutjanus synagris	Lane snapper	2	28.9 ± 6.7	3.77 ± 0.22	Hunting macrofauna (predator)	Reef-associated	NT
Macrodon ancylodon	King weakfish	7	28 ± 4.3	3.03 ± 0.16	Hunting macrofauna (predator)	Demersal	LC
Menticirrhus americanus	Southern kingcroaker	6	15.1 ± 1.5	3.33 ± 0.08	Hunting macrofauna (predator)	Demersal	LC
Micropogonias furnieri	Whitemouth croaker	7	21.3 ± 0.9	3.03 ± 0.17	Hunting macrofauna (predator)	Demersal	LC

Mustelus higmani	Smalleye smooth-hound	40	41.9 ± 10	2.82 ± 0.16	Hunting macrofauna (predator)	Demersal	LC
Narcine brasiliensis	Brazilian eletric ray	8	40.3 ± 24	2.87 ± 0.15	Hunting macrofauna (predator)	Reef-associated	DD
Notarius grandicassis	Thomas sea catfish	7	18.6 ± 2.5	3.06 ± 0.10	Hunting macrofauna (predator)	Demersal	LC
Paralonchurus brasiliensis	Banded croaker	9	21.6 ± 2.1	2.74 ± 0.25	Hunting macrofauna (predator)	Demersal	LC
Pellona harroweri	American coastal pellona	7	14.4 ± 1.1	3.23 ± 0.14	Hunting macrofauna (predator)	Pelagic-neritic	LC
Peprilus paru	American harvestfish	7	19.7 ± 4	3.30 ± 0.19	Hunting macrofauna (predator)	Bentopelagic	LC
Polydactylus virginicus	Barbu	7	21.1 ± 3.1	3.14 ± 0.23	Hunting macrofauna (predator)	Demersal	LC
Prionotus punctatus	Bluewing searobin	4	23.2 ± 3.2	2.79 ± 0.21	Hunting macrofauna (predator)	Demersal	LC
Pseudobatos horkelii	Brazilian guitarfish	1	23	2.64	Hunting macrofauna (predator)	Demersal	CR
Pseudobatos percellens	Chola guitarfish	3	57.2 ± 6.9	2.90 ± 0.14	Hunting macrofauna (predator)	Demersal	NT
Rhinoptera bonasus	Cownose ray	2	90.5 ± 13	1.99 ± 0.02	Hunting macrofauna (predator)	Bentopelagic	NT
Rhizoprionodon lalandii	Brazilian sharpnose shark	2	74.1 ± 4.4	2.99 ± 0.02	Hunting macrofauna (predator)	Demersal	DD
Rhizoprionodon porosus	Caribbean sharpnose shark	2	38 ± 2.8	3.62 ± 0.07	Hunting macrofauna (predator)	Reef-associated	LC
Selene setapinnis	Atlantic moonfish	7	29.6 ± 5.4	3.27 ± 0.26	Hunting macrofauna (predator)	Bentopelagic	LC

Sphyrna tiburo	Scalloped hammerhead	10	45.5 ± 36	3.25 ± 0.75	Hunting macrofauna (predator)	Reef-associated	LC
Sphyrna lewini	Bonnethead	2	44.1 ± 4.9	3.33 ± 0.15	Hunting macrofauna (predator)	Pelagic-Oceanic	VU
Stellifer micros	Smalleye stardrum	7	11.3 ± 1.7	2.95 ± 0.33	Hunting macrofauna (predator)	Demersal	LC
Stellifer naso	Cabeçudo preto	7	9.66 ± 0.7	2.68 ± 0.08	Hunting macrofauna (predator)	Demersal	LC
Trichiurus lepturus	Largehead hairtail	7	69.5 ± 9.5	3.46 ± 0.21	Hunting macrofauna (predator)	Bentopelagic	LC
Umbrina coroides	Sand drum	7	20.5 ± 1.6	2.96 ± 0.18	Hunting macrofauna (predator)	Demersal	LC
Upeneus parvus	Dwarf goatfish	7	15.3 ± 1	3.27 ± 0.16	Hunting macrofauna (predator)	Demersal	LC

[a] Froese & Pauly (2020)
[b] IUCN (2020)

389 **Table 2:** Concentrations (μg g^{-1}) of the trace elements recorded in 47 fish species from the Amazon Coast, the assessment guidelines set by
390 international institutions, and the legal limits for human consumption established by different countries or economic blocs.

Specie	As		iAs		Hg		Pb		Cd	
	Mean ± SE	Min - Max	Mean ± SE	Min - Max	Mean ± SE	Min - Max	Mean ± SE	Min - Max	Mean ± SE	Min - Max
Anisotremus surinamensis	0.38 ± 0.05	0.2 - 0.52	0.03 ± 0.01	0.02 - 0.05	0.22 ± 0.04	0.11 - 0.45	0 ± 0	0 - 0	0 ± 0	0 - 0
Bagre bagre	5.46 ± 0.17	3.08 - 7.55	0.54 ± 0.15	0.30 - 0.75	0.03 ± 0.02	0 - 0.08	0 ± 0	0 - 0.01	0 ± 0	0 - 0
Bairdiella ronchus	0.98 ± 0.09	0.55 - 1.6	0.09 ± 0.03	0.05 - 0.16	0.02 ± 0.01	0 - 0.03	0 ± 0	0 - 0.01	0 ± 0	0 - 0
Brachyplatystoma vaillantii	0.32 ± 0.04	0.14 - 0.46	0.03 ± 0.01	0.01 - 0.04	0.06 ± 0.02	0.03 - 0.13	0.02 ± 0.01	0.01 - 0.04	0.03 ± 0.01	0.01 - 0.06
Caranx crysos	2.27 ± 0.16	1.03 - 4.18	0.22 ± 0.12	0.10 - 0.41	0.02 ± 0.02	0 - 0.05	0.02 ± 0	0.01 – 0.04	0 ± 0.01	0 - 0.01
Carcharhinus falciformis	4.66	4.66	0.46	0.46	0.12	0.12	0.01	0.01	0	0
Chaetodipterus faber	3.91 ± 0.18	1.95 - 6.21	0.39 ± 0.16	0.19 - 0.62	0.04 ± 0.01	0.02 - 0.09	0.02 ± 0.02	0.01 - 0.06	0 ± 0	0 - 0.01
Conodon nobilis	5.28 ± 0.28	0.77 - 11.9	0.52 ± 0.40	0.07 - 1.19	0.68 ± 0.1	0.1 - 1.34	0.01 ± 0.01	0 - 0.03	0.01 ± 0.01	0 - 0.03
Ctenosciaena gracilicirrhus	0.75 ± 0.08	0.4 - 1.35	0.07 ± 0.03	0.04 - 0.13	0.02 ± 0.01	0.01 - 0.04	0.01 ± 0.01	0 - 0.02	0 ± 0	0 - 0
Cynoscion jamaicensis	0.72 ± 0.12	0.18 - 2.37	0.07 ± 0.07	0.01 - 0.23	0.11 ± 0.03	0.05 - 0.19	0 ± 0.01	0 - 0.01	0 ± 0	0 - 0
Cynoscion microlepidotus	0.61 ± 0.07	0.22 - 0.94	0.06 ± 0.02	0.02 - 0.09	0.11 ± 0.06	0.02 - 0.6	0.01 ± 0	0.01 - 0.01	0 ± 1.92	0 - 0
Cynoscion virescens	0.60 ± 0.07	0.19 - 1.71	0.06 ± 0.04	0.01 - 0.17	0.11 ± 0.02	0.05 - 0.2	0.01 ± 0.01	0 - 0.05	0 ± 0	0 - 0.02
Dactylopterus volitans	2.14 ± 0.18	1.75 - 3.4	0.25 ± 0.06	0.17 - 0.34	0.02 ± 0.02	0.02 - 0.04	0.02 ± 0.01	0.02 - 0.04	0.01 ± 0.02	0 - 0.04

Decapterus tabl	0.94 ± 0.10	0.58 - 2.01	0.09 ± 0.04	0.05 - 0.20	0.02 ± 0.01	0 - 0.05	0 ± 0	0 - 0	0.01 ± 0	0 - 0.01
Genyatremus luteus	2.65 ± 0.21	0.64 - 7.8	0.26 ± 0.23	0.06 - 0.78	0.01 ± 0.01	0 - 0.04	0.01 ± 0	0.01 - 0.01	0 ± 1.92	0 - 0
Gymnachirus nudus	1.29 ± 0.08	0.78 - 1.8	0.12 ± 0.03	0.07 - 0.18	0.02 ± 0	0.01 - 0.03	0.02 ± 0	0.01 - 0.02	0 ± 1.92	0 - 0
Gymnura micrura	4.57 ± 0.21	1.21 - 12.7	0.45 ± 0.37	0.12 - 1.27	0.06 ± 0.02	0.01 - 0.22	0.02 ± 0.01	0.01 - 0.05	0 ± 0	0 - 0
Haemulon plumieri	2.28 ± 0.14	0.88 - 4.14	0.22 ± 0.10	0.08 - 0.41	0.14 ± 0.02	0.11 - 0.18	0 ± 0.01	0 - 0.04	0 ± 0	0 - 0.01
Haemulon steindachneri	4.34 ± 0.20	1.81 - 6.14	0.43 ± 0.15	0.18 - 0.61	0.1 ± 0.02	0.07 - 0.15	0 ± 0	0 - 0	0 ± 0	0 - 0.01
Hypanus guttatus	3.23 ± 0.34	0.7 - 7.37	0.32 ± 0.29	0.07 - 0.73	0.03 ± 0.03	0.01 - 0.07	0.03 ± 0.02	0.01 - 0.04	0 ± 0	0 - 0
Lutjanus analis	1.83 ± 0.17	0.21 - 5.7	0.18 ± 0.20	0.02 - 0.57	0.12 ± 0.04	0 - 0.29	0.01 ± 0.01	0 - 0.02	0 ± 0	0 - 0
Lutjanus purpureus	0.93 ± 0.32	0.93 - 1.88	0.14 ± 0.06	0.09 - 0.18	0.09 ± 0.09	0.13 - 0.16	0.01 ± 0.04	0.02 - 0.02	0 ± 0.01	0 - 0
Lutjanus synagris	4.16 ± 0.64	2.98 - 5.34	0.41 ± 0.16	0.29 - 0.53	0.07 ± 0.14	0.01 - 0.14	0.02 ± 0.01	0.02 - 0.02	0 ± 0.01	0 - 0
Macrodon ancylodon	0.25 ± 0.03	0.19 - 0.31	0.02 ± 0.00	0.01 - 0.03	0.06 ± 0.01	0.04 - 0.08	0 ± 0.01	0 - 0.03	0 ± 0	0 - 0.01
Menticirrhus americanus	0.63 ± 0.08	0.31 - 1.01	0.06 ± 0.02	0.03 - 0.10	0.03 ± 0.01	0.01 - 0.05	0 ± 0	0 - 0	0 ± 0	0 - 0
Micropogonias furnieri	1.13 ± 0.09	0.55 - 1.59	0.11 ± 0.04	0.05 - 0.15	0.04 ± 0.01	0.03 - 0.05	0.01 ± 0.01	0 - 0.04	0 ± 0	0 - 0.01
Mustelus higmani	19.46 ± 0.07	6.19 - 42.9	1.94 ± 0.87	0.61 - 4.29	0.13 ± 0	0 - 0.42	0.03 ± 0	0 - 0.13	0.05 ± 0.01	0 - 1.59
Narcine brasiliensis	8.14 ± 0.33	2.58 - 24.2	0.81 ± 0.72	0.25 - 2.42	0.17 ± 0.04	0.01 - 0.36	0.03 ± 0.01	0.01 - 0.04	0.04 ± 0.02	0 - 0.11
Notarius grandicassis	2.43 ± 0.06	2.14 - 2.7	0.24 ± 0.01	0.21 - 0.27	0.01 ± 0	0.01 - 0.01	0 ± 0	0 - 0	0 ± 0	0 - 0.01

Paralonchurus brasiliensis	0.82 ± 0.09	0.7 - 1.57	0.12 ± 0.03	0.07 - 0.15	0.03 ± 0.01	0.04 - 0.06	0 ± 0.01	0 - 0.02	0 ± 0	0 - 0
Pellona harroweri	1.07 ± 0.08	0.77 - 1.8	0.10 ± 0.03	0.07 - 0.18	0.01 ± 0.01	0 - 0.03	0 ± 0	0 - 0.01	0 ± 0	0 - 0
Peprilus paru	1.54 ± 0.15	0.69 - 3.89	0.15 ± 0.12	0.06 - 0.38	0.02 ± 0.01	0.01 - 0.05	0 ± 0	0 - 0	0 ± 0	0 - 0.01
Polydactylus virginicus	0.47 ± 0.03	0.4 - 0.57	0.04 ± 0.00	0.04 - 0.05	0.03 ± 0.02	0.01 - 0.08	0 ± 0	0 - 0	0 ± 0	0 - 0
Prionotus punctatus	0.69 ± 0.13	0.44 - 1.1	0.06 ± 0.02	0.04 - 0.11	0.02 ± 0.03	0 - 0.03	0.01 ± 0.01	0.01 - 0.02	0 ± 0	0 - 0
Pseudobatos horkelii	2.19	2.19 - 2.19	0.21	0.21 - 0.21	0.03 ±	0.03 - 0.03	0.04 ±	0.04 - 0.04	0.07 ±	0.07 - 0.07
Pseudobatos percellens	4.63 ± 0.38	3.19 - 5.83	0.46 ± 0.13	0.31 - 0.58	0.28 ± 0.15	0.11 - 0.53	0.01 ± 0.02	0.01 - 0.02	0.01 ± 0.03	0 - 0.02
Rhinoptera bonasus	83.44 ± 4.62	23 - 143	8.34 ± 8.53	2.30 - 14.3	0.26 ± 0.19	0.15 - 0.37	0.01 ± 0	0.01 - 0.01	0 ± 0.02	0 - 0
Rhizoprionodon lalandii	3.23 ± 0.77	1.51 - 4.94	0.32 ± 0.24	0.15 - 0.49	0.39 ± 0.32	0.09 - 0.7	0.02 ± 0.05	0.01 - 0.03	0.01 ± 0.06	0 - 0.02
Rhizoprionodon porosus	9.78 ± 1.07	6.5 - 13	0.97 ± 0.46	0.65 - 1.30	0.06 ± 0.13	0.01 - 0.11	0.01 ± 0.05	0 - 0.02	0 ± 0	0 - 0
Selene setapinnis	1.46 ± 0.09	1.01 - 2.33	0.14 ± 0.04	0.10 - 0.23	0.08 ± 0.03	0 - 0.17	0 ± 0	0 - 0.01	0 ± 0	0 - 0
Sphyrna lewini	10.61 ± 0.13	7.01 - 12.9	0.97 ± 1.15	0.16 - 1.79	0.07 ± 0.02	0.02 - 0.22	0 ± 0	0 - 0	0 ± 0	0 - 0.01
Sphyrna tiburo	9.77 ± 1.69	1.63 - 17.9	1.06 ± 0.19	0.70 - 1.29	0.09 ± 0.16	0 - 0.15	0 ± 0.05	0 - 0.01	0 ± 0	0 - 0
Stellifer microps	0.66 ± 0.04	0.54 - 0.85	0.06 ± 0.01	0.05 - 0.08	0.01 ± 0.01	0 - 0.02	0.01 ± 0	0.01 - 0.01	0 ± 0	0 - 0
Stellifer naso	0.70 ± 0.08	0.14 - 1.11	0.07 ± 0.03	0.01 - 0.11	0.04 ± 0	0.03 - 0.04	0.02 ± 0.01	0 - 0.05	0 ± 0	0 - 0.01
Trichiurus lepturus	0.18 ± 0.04	0.11 - 0.41	0.01 ± 0.01	0.01 - 0.04	0.05 ± 0.02	0.03 - 0.1	0.02 ± 0.02	0 - 0.06	0 ± 0	0 - 0

Umbrina coroides	2.27 ± 0.11	0.86 - 2.8	0.22 ± 0.06	0.08 - 0.28	0.04 ± 0.01	0.03 - 0.05	0.01 ± 0.01	0.01 - 0.02	0 ± 0	0 - 0
Upeneus parvus	5.02 ± 0.12	3.76 - 6.1	0.50 ± 0.07	0.37 - 0.61	0.06 ± 0.03	0.01 - 0.13	0.02 ± 0	0.02 - 0.03	0.01 ± 0.01	0 - 0.02
International Limits										
Brazil	1.0				0.5 – 1.0					
European Union	6.0				0.5 – 1.0					
Ireland					0.5 – 1.0		0.3		0.05	
China			0.1		0.5 – 1.0					
United States					0.3					
Russian Federation					0.2					
WHO					0.5					
CODEX STAN 193 - 1995					0.5 – 1.0		0.3			

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392 **Table 3:** The Estimated Daily Intake, EDI (μg kg^{-1}_{bw} day^{-1}), and Hazard Quotient (HQ) of 27 commercially-important fish species from
393 the Amazon Coast and the oral reference dose (RfD) of each trace element. The EDI values above the RfD and the HQ values of over 1 are
394 highlighted in red.

Specie	As		iAs			Hg		Pb			Cd	
	EDI	HQ	EDI	HQ	TR	EDI	HQ	EDI	HQ	TR	EDI	HQ
Bagre bagre	5.76	19.23	0.57	1.92	$8.65x10^{-4}$	0.04	0.39	0	0.21	$3.58x10^{-8}$	0	0
Brachyplastystoma vaillantii	0.34	1.14	0.03	0.11	$5.16x10^{-5}$	0.07	0.72	0.02	1.47	$2.52x10^{-7}$	0.04	0.04
Caranx crysos	2.39	7.98	0.23	0.79	$3.59x10^{-4}$	0.02	0.23	0.02	1.05	$1.79x10^{-7}$	0	0
Carcharhinus falciformis	4.91	16.39	0.49	1.63	$7.37x10^{-4}$	0.14	1.35	0.01	0.84	$1.43x10^{-7}$	0	0
Cynoscion jamaicensis	0.76	2.53	0.07	0.25	$1.14x10^{-4}$	0.12	1.18	0	0.42	$7.17x10^{-8}$	0	0
Cynoscion microlepidotus	0.64	2.14	0.06	0.21	$9.66x10^{-5}$	0.12	1.19	0.01	0.68	$1.16x10^{-7}$	0	0
Cynoscion virescens	0.63	2.11	0.06	0.21	$9.50x10^{-5}$	0.12	1.18	0.01	0.84	$1.43x10^{-7}$	0	0
Genyatremus luteus	3.41	11.37	0.34	1.13	$5.11x10^{-4}$	0.04	0.37	0.03	1.68	$2.86x10^{-7}$	0	0
Gymnura micrura	2.79	9.32	0.27	0.93	$4.19x10^{-4}$	0.02	0.17	0.01	0.79	$1.34x10^{-7}$	0	0
Haemulon plumieri	4.82	16.08	0.48	1.6	$7.23x10^{-4}$	0.06	0.63	0.02	1.21	$2.06x10^{-7}$	0	0
Haemulon steindachneri	2.41	8.03	0.24	0.8	$3.61x10^{-4}$	0.16	1.55	0	0.42	$7.17x10^{-8}$	0	0
Hypanus guttatus	4.58	15.27	0.45	1.52	$6.87x10^{-4}$	0.12	1.15	0	0.15	$2.69x10^{-8}$	0	0
Lutjanus analis	1.93	6.43	0.19	0.64	$2.89x10^{-4}$	0.13	1.28	0.01	0.89	$1.52x10^{-7}$	0	0
Lutjanus purpureus	0.98	3.29	0.14	0.49	$2.22x10^{-4}$	0.10	1.04	0.01	0.84	$1.43x10^{-7}$	0	0
Lutjanus synagris	4.39	14.64	0.43	1.46	$6.58x10^{-4}$	0.08	0.83	0.02	1.31	$2.24x10^{-7}$	0	0
Macrodon ancylodon	0.26	0.88	0.02	0.08	$3.99x10^{-5}$	0.07	0.68	0	0.47	$8.07x10^{-8}$	0	0
Menticirrhus americanus	0.67	2.24	0.06	0.22	$1.01x10^{-4}$	0.03	0.32	0	0.21	$3.58x10^{-8}$	0	0
Micropogonias furnieri	1.19	3.99	0.12	0.4	$1.80x10^{-4}$	0.04	0.43	0.01	0.63	$1.07x10^{-7}$	0	0
Mustelus higmani	20.5	68.44	2.05	6.84	$3.08x10^{-3}$	0.14	1.41	0.03	1.58	$2.69x10^{-7}$	0.06	0.06
Notarius grandicassis	2.56	8.55	0.25	0.85	$3.84x10^{-4}$	0.01	0.14	0	0.15	$2.69x10^{-8}$	0	0
Pellona harroweri	1.13	3.77	0.11	0.37	$1.70x10^{-4}$	0.02	0.15	0	0.36	$6.27x10^{-8}$	0	0
Rhizoprionodon lalandii	3.4	11.36	0.34	1.13	$5.11x10^{-4}$	0.42	4.18	0.02	1.21	2.06x10-7	0.01	0.01
Rhizoprionodon porosus	10.31	34.39	1.03	3.43	$1.54x10^{-3}$	0.07	0.67	0.01	0.63	$1.07x10^{-7}$	0	0

Selene sepapinnis	1.54	5.14	0.15	0.51	$2.31x10^{-4}$	0.09	0.89	0	0.36	$6.27x10^{-8}$	0	0
Sphyrna tiburo	10.31	34.36	1.03	3.43	$1.54x10^{-3}$	0.08	0.83	0	0.42	$7.17x10^{-8}$	0	0
Sphyrna lewini	11.19	37.31	1.11	3.73	$1.67x10^{-3}$	0.10	0.97				0	0
Trichiurus lepturus	0.19	0.63	0.01	0.06	$2.87x10^{-5}$	0.06	0.55	0.02	1.26	$2.15x10^{-7}$	0	0
Rfd ($\mu g\ kg^{-1}_{bw}\ day^{-1}$)	0.3		0.3			0.1		0.02			1	

4. CONCLUSIONS

The findings of the present study indicate that the fish species that occur in the waters of the Amazon Coast accumulate As, Hg, Pb, and Cd. Arsenic was the most abundant trace element in all the fish, in particular in the species in low trophic positions, and in some cases, it was recorded at concentrations higher than the recommended levels. The other three elements were recorded at low concentrations. Reef-associated fish are more susceptible to the accumulation of Hg. The concentrations of iAs, Hg and Pb were each found to pose a potential non-carcinogenic health risk through the consumption of some cartilaginous species. Collectively, these elements reached levels that can be considered to be a potential human health hazard. Given this, individuals who continuously consume cartilaginous fish contaminated with the toxic elements recorded here will likely be under target cancer risk over the long term.

5. Declaration of Competing Interest

The authors declare having no conflicts of interest.

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Capítulo 2:

The consumption of shark meat in the Amazon region and its implications for human health and the marine ecosystem

The consumption of shark meat in the Amazon region and its implications for human health and the marine ecosystem

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Abstract

In certain global regions, the consumption of shark meat is increasing. Sharks, however, as top/mesopredators are highly susceptible to the uptake and biomagnification of trace elements, that can be detrimental to human health. Here, we evaluated the levels of As, Hg, Pb, and Cd in shark meat sold along the Amazon Coast of Brazil and used nitrogen stable isotope values to determine trophic position (TP) and to assess element biomagnification. From shark meat sold in markets, a total of 13 species were identified via molecular analysis, including those listed as endangered and vulnerable by IUCN Red List. Arsenic was present in significantly higher concentrations than all other elements, followed by Hg, with the highest mean concentrations recorded in *M. higmani* (As: 19.46 ± 8.79 µg/g) and *C. acronotus* (Hg: 1.12 ± 0.68 µg/g). Pb and Cd were recorded at much lower levels in all species. The estimated daily intake (EDI) of individual elements were above *Provisional Tolerable Daily Intake* (PTDI) for all species when considering Hg, seven species for iAs, and one species for Pb. The daily consumption of five of 13 species should be reduced to less than 10g, indicating these species should not be eaten. The mean (± SD) $\delta^{15}N$ values of species ranged from 10.7 ± 0.51‰ in *Mustelus higmani* to 14.2 ± 0.59‰ in *Carcharhinus porosus*, indicating feeding over >1 trophic level. Arsenic was negatively correlated with $\delta^{15}N$ values, while Hg was positively correlated indicating biodilution and biomagnification, respectively. Our results indicate that the sale and consumption of shark meat will expose consumers to potentially harmful levels of iAs and Hg, as well as contributing to the population decline of species including those that are currently categorized as threatened.

Keywords: Trace elements; Biomagnification; Biodilution; Elasmobranchi; Amazon

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1. Introduction

Coupled with declines of shark populations associated with the fin trade (Fowler & Séret, 2010; Heithaus et al., 2010), the increasing use of shark meat as a food source is further impacting stocks worldwide (Borrel et al., 2011; Taylor et al., 2014; Ong & Gan, 2016; McKinney *et al*., 2016). Brazil (Barreto et al., 2017; Bornatowiski et al., 2017), where no specific licenses are required to catch sharks, is now among the top elasmobranch fishing nations, and could be considered to be the world's leading importer of shark meat (Dulvy et al., 2014; Barreto et al., 2015; Dent & Clarke, 2015). Since 2012, it is estimated that 8000 boats interacted in Brazilian fisheries targeting pelagic sharks, but this value is likely underestimated, since the number of illegal fishing vessels is unknown (Barreto et al. 2017).

Approximately one third of elasmobranch species targeted by Brazilian commercial offshore fisheries are listed under a threat category assigned by the IUCN; 19 species are listed as Vulnerable (VU), eight as Endangered (EN), and 28 as Critically Endangered (CR) (ICMBio, 2016). A similar proportion (36%) of species are Data Deficient (DD). Globally, these species represent a quarter of the world's threatened sharks (Dulvy et al., 2014). Palmeira et al (2013) for example, reported specimens of *Pristis perotteti*, a critically endangered sawfish, being sold in fish markets on the northern coast of Brazil, while Feitosa et al (2018) used DNA sequences to identify that nine of 17 species obtained from local fisheries were listed under a extinction threat category. In addition to directed shark fisheries, many species are also impacted as bycatch, but this impact is largely unknown.

Aside from the ecological implications of shark fishing, removal and associated population declines, the consumption of shark meat can also expose humans to potential contamination by organohalogenated compounds and trace elements, which are known to

bioaccumulate to high (and potentially harmful) concentrations through the process of biomagnification (Pethybridge et al., 2010; Barrera-García et al., 2012; Lopez et al., 2013; Rumbold et al., 2014; Weijs et al., 2015). While trace metals occur naturally in the environment, and may be introduced into marine ecosystems through a number of natural biogeochemical processes, the recent, ongoing increase in contamination levels is primarily attributed to urban and industrial effluents (Authman et al., 2015; Bosch et al., 2015). A range of anthropogenic pressures, including deforestation, fires, and hydroelectric dams impact the Amazon basin and have raised concerns with regard to the release of metals into the region's rivers (Scarpelli, 2005; Lacerda & Malm, 2008; Sampaio da Silva et al., 2009; Patry et al., 2013; Kasper et al., 2014). This concern is based on the fact that the Amazon River discharges large volumes of water and sediments into the coastal region (Isaac & Ferrari, 2017), in which contaminants may become concentrated and then made available to high order consumers in the marine environment. To date, however, no studies have focused specifically on the presence and concentrations of trace elements in shark species sampled from the Amazon Coast. The concentrations of trace elements, such as Hg (Souza-Araujo et al., 2016b), and even microplastics (Pegado et al., 2018), however, have been recorded in other marine species from this region.

To address this knowledge gap, the present study aimed to (i) evaluate the levels of key trace elements; As, Hg, Pb, and Cd in shark meat sold at the principal fish markets of the Amazon Coast, northern Brazil relative to international standards for human consumption and (ii) examine the relative trophic position of each species using nitrogen stable isotope ($\delta^{15}N$) data and determine the degree of biomagnification of trace elements using combined element concentrations and $\delta^{15}N$ values. Sharks are considered to play a significant role in structuring food webs (Heithaus et al., 2008; Ferreti et al., 2010),

although fishing and the shark fin trade have impacted the conservation status of global populations (Dent & Clark, 2015). In northern Brazil, the number of studies on elasmobranchs, in particular those focused on sharks, is limited, with most research restricted to the more developed regions in the south of the country (Barreto et al., 2015; Bornatowski et al., 2013, 2015 and 2017). Along the northern coast of Brazil, an extremely productive region influenced by the Amazon estuary, these data provide the first measures of element concentrations and δ^{15}N-Hg and δ^{15}N-As dynamics for 13 species of shark, including data on a large number of juveniles.

2. Material and Methods

2.1.Study area

A total of 91 sharks were sampled at fish markets at five ports located along the Amazon Coast. This region is part of the Amazon Continental Shelf, which is known to be one of the world's most productive ecosystems, but is subject to overfishing, pollution, and rising ocean temperatures (Isaac & Ferrari, 2017). The region encompasses the largest continuous tracts of mangrove forest in the world, which cover an area of 8900 km^2 (Kjerfve & Lacerda, 1993). The Amazon rainforest biome covers more than 4.2x10^6 km^2 (Bernardes et al., 2012), and is located within the drainage basins of the Amazon, Orinoco, and other smaller rivers (Figure 1).

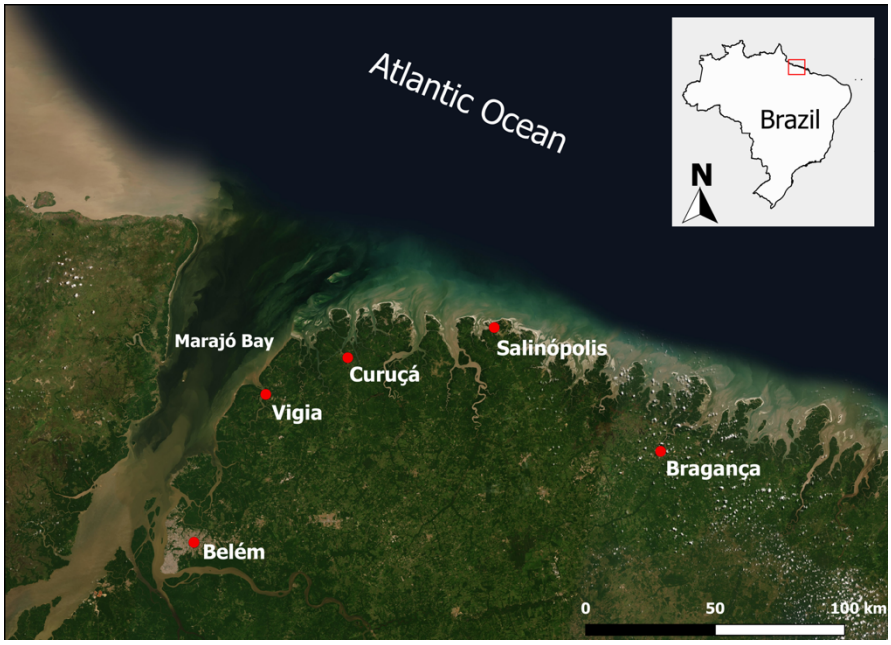


Figure 1: The Pará coast, in Amazon Coastal region, North Atlantic Ocean, and the five most representative landing points for shark meat market (red circles).

2.2.Sampling

Muscle tissue samples taken from the dorsal surface of sharks, known locally as "cação", were obtained from individuals on display for sale in local markets. Samples (n = 91; mass = ~20g) were placed in individual polyethylene bags on ice, transported back to the laboratory and kept frozen (-20º C) until elemental/isotope analysis. A tissue subsample was also reserved for species identification through molecular analysis. Total body length was not recorded because sharks were without head and fins, but most body trunks were less than 100 cm.

2.3. Species identification

To first identify species, total genomic DNA was extracted from muscle tissue using a Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI – USA) following the manufacturer's protocol. A fragment of the Cytochrome C Oxidase I

gene (COI), standardized as DNA Barcoding, were amplified using the primers: COI 5′TCAACCAACCACAAAGACATTGGCAC3′ and COI 5′ TAGACTTCTGGGTGGCCAAAGAATCA 3′ (Ward et al. 2005). The samples were amplified in a final volume of 25 µL, containing 4 µl of DNTP (1.25 mM), 2.5 µl of 10X buffer solution, 1 µl of MgCl2 (25 Mm), 0.25 µl of each primer (200 ng/µl), 1–1.5 µl of genomic DNA (100 ng/µl), 1 U of Taq DNA polymerase (5 U/µl), and purified water to complete the final reaction volume. The Polymerase Chain Reactions (PCRs) were run in a thermocycler (Applied Biosystems) under the following thermal protocol: initial denaturation at 93° C for 3 min; 35 cycles of denaturation at 94° C for 30 s, annealing (at temperatures of 50–60 °C, depending on the species) for 45 s, and extension at 72° C for 45 s, with a final extension of 5 minutes at 72° C. All positive reactions were sequenced in an ABI 3500 automatic sequencer (Applied Biosystems). Following DNA sequencing barcoding, each sample was identified to species level by cross referencing with those held in the following public databases: GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and BoldSystems V4 (http: // www.boldsystems.org).

2.4.Trace elements analysis

Concentrations of the trace elements As, Hg, Pb, and Cd in shark muscle tissue samples were determined by Induced Plasma Coupled Mass Spectrometry (ICP-MS). Muscle tissue samples were first homogenized with surgical scissors or a PTFE stick, and an aliquot of 0.1g (wet weigh) of tissue was placed in a PTFE bottle with 1.5 ml of HNO_3 (65% PA). After 30 minutes, 0.5 ml of H_2O_2 was added and samples were heated in a microwave oven (MarsXpress, CEM Corporation) along a temperature ramp (1st step: 800W, 180º C, 10 minutes; 2nd step: 1200W, 200º C, 5 minutes; 3rd step: 1000W, 100º C,

10 minutes) and then cooled for 20 minutes in a cold bath. The digested solutions were then transferred to polyethylene bottles, which were topped up to 15 ml with HNO_3 (1%), and stored at 4 ºC until analysis by ICP-MS. A quality control sample, DORM-3 (0.05g dw) Certified Reference Material (National Research Council, Canada) was analyzed simultaneously with the study samples with percentage recovery ranging from 76.7 to 88%. Based on EPA methods, percentage recovery must range between 75 and 125% to pass quality control; all elements were between this interval.

2.5. *Health Risk Assumption*

An assessment of the human health risk posed from trace element concentrations recorded in shark meat was estimated using the following equations:

2.5.1. Estimated Daily Intake (EDI)

$$\text{EDI} = \frac{te \times \text{daily consumption of fish}}{\text{body weight}}$$

Where EDI ($\mu g/kg_{bw}$/day) is the estimated daily intake; *te* is the mean trace element concentration recorded per species (µg/g ww); the daily consumption of fish (g/day) is the average consumed, here a value of 416.39g was used according to Isaac et al (2015) and the mean human body weight; a value of 70 kg was used for an average adult. For As, USEPA (2000) suggests using the uptake of inorganic As (iAs) rather than total exposure to As for assessment of human health risks. For As, it was estimated that 10% of total As was iAs (UFSDA, 1993). The obtained EDI values were compared with the *Provisional Tolerable Daily Intake* (PTDI) values determined by the Joint Food and Agriculture Organization Expert Committee of Food Additives (Table 2) (JECFA, 2019).

There are no recommended PTDI values for Pb, however the European Food Safety Authority (EFSA) states a value of 25 μg/kg_{bw} (3.57 μg/kg_{bw} per day) as a regulatory PTWI guideline for the dietary intake of Pb.

2.5.2. Maximum Amount of Shark (MAS)

$$\text{MAS} = \frac{\text{PTDI} \times \text{body weight}}{te}$$

Where MAS is the Maximum Amount of Shark (g) that should be consumed per day to remain within the limits of the *Provisional Tolerable Daily Intake* (PTDI).

2.6. *Stable isotopes analysis*

To determine nitrogen stable isotope values ($\delta^{15}N$), samples were dried at 60°C for 24 hours, macerated and homogenized to a fine powder using a porcelain mortar and pestle. Lipids were extracted by vortexing the homogenized powder in a cryovial with 1.9 ml of chloroform-methanol solution (1:2) for one minute. Cryovials were then placed in a water bath at 30˚C for at least 24 hours, after which, they were centrifuged for 4–6 minutes and solvent was filtered. New chloroform-methanol solution was then added, and the samples were shaken for one minute and centrifuged once again for 4–6 minutes. The resulting filtrate was left under a fume hood for 24–48h to evaporate the remaining solvent (Hussey et al., 2012). Following lipid extraction, urea was removed by shaking the resultant powdered tissue in a cryovial with 1.9 ml of de-ionized water for one minute. Vials were then placed in a water bath at 30˚C for 24 hours, after which, they were centrifuged for 4–6 minutes and water was extracted using a medical syringe. This process was repeated three times, and the samples once again dried. Approximately 710–

890 μg of lipid and urea extracted muscle tissue for each sample was weighed and compressed into 5 mm x 3.5 mm tin capsules. Nitrogen stable isotope values were then determined by combustion in a Continuous Flow Isotope Ratio Mass Spectrometer (IR-MS, Finnigan MAT Deltaplus, Thermo Finnigan, San Jose, CA, USA) equipped with an elemental analyzer (Costech, Valenica, CA, USA). The isotopic signatures are expressed in delta notation (δ) and defined as parts per thousand (‰) in relation to a standard sample, as follows:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R_{Sample} and $R_{Standad}$ correspond to the stable isotope values ($^{15}N/^{14}N$) in the test and standard samples, respectively. The precision of this procedure was assessed by the standard deviation of the replicate analyses of four standards; NIST1577c, internal lab standard (tilapia muscle), USGS 40, and Urea (n=68 in all cases), with $\delta^{15}N \leq 0.18‰$. The accuracy, based on the certified values of USGS 40 (n=68) analyzed throughout runs and not used to normalize samples showed a difference of -0.05‰ for $\delta^{15}N$ from the certified value. Instrumentation accuracy was checked throughout the study period, based on NIST standards 8573, 8547, and 8574 (n=20 for each). The mean differences from the certified values were -0.17, -0.10 and -0.14‰, respectively.

2.7. *Statistical analyses*

To examine differences in the concentrations of trace elements among species, a univariate PERMANOVA based on Euclidean distances matrices with 9999 permutations and including the Monte Carlo correction for small sample size was conducted (Anderson, 2001). Only species with $n \geq 3$ individuals were used in the PERMANOVA. To assess biomagnification profiles of each trace element, the relationship between log transformed element concentrations (As, Hg, Pb and Cd) and $\delta^{15}N$ values was evaluated

using Pearson's correlation coefficients. All analyses were conducted in Rstudio (Version 1.1.383) and PERMANOVA+ in the PRIMER-E software (Anderson et al., 2008).

3. Results

Of the 91 shark muscle samples collected from fish markets along the Amazon coast, DNA barcoding identified 13 species belonging to three families (Carcharinidae, Sphyrnidae and Triakidae). Of these, two species are listed as endangered (EN), three are near threatened (NT), two are vulnerable (VU), four are least concern (LC), and two are data deficient, DD according to the IUCN RedList (Table 1).

The concentrations of trace elements in muscle tissue samples were highly variable across species (Pseudo-F = 128.9 $p < 0.001$; (Table 1)), with significantly higher overall levels of As when compared with the other three elements (**Hg**: $t = 11.1$ $p < 0.001$; **Pb**: $t = 11.4$ $p < 0.001$; **Cd**: $t = 11.4$ $p < 0.001$). The highest concentration of As (42 μg/g) was recorded in a sample of an individual *Mustelus higmani* (Figure 2A). The highest mean (± SD) As concentration (19.46 ± 8.79 μg/g) was also recorded for this species, followed by *Carcharhinus leucas* (15.26 ± 15.80 μg/g), *Sphyrna tiburo* (10.61 ± 1.934 μg/g), *Sphyrna lewini* (9.77 ± 11.50 μg/g), and *Rhizoprionodon porosus* (9.588 ± 5.278 μg/g) (Figure 2A).

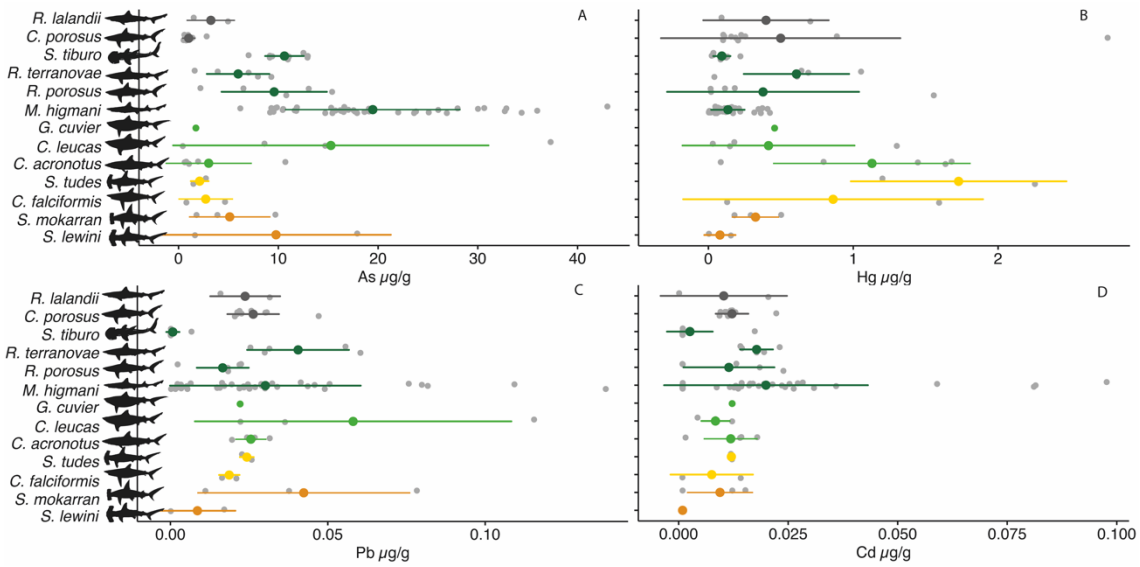


Figure 2: Trace element concentrations recorded in 91 samples of shark meat obtained from fish markets along the Brazilian Amazon Coast in 2017: (A) Arsenic [As]; (B) mercury [Hg], (C) lead [Pb] and; (D) cadmium [Cd]. The grey circles represent element concentrations in individual samples, the central circles are the mean for each species, and the horizontal lines represent the Standard Deviation.

Hg concentrations were significantly higher than those recorded for Pb ($t = 5.7$ $p < 0.001$) and Cd ($t = 6.0$ $p < 0.001$) across all species (Table 1). Mean Hg concentrations ranged from 0.07 ± 0.10 µg/g in *Sphyrna lewini* to 1.72 ± 0.74 µg/g in *Sphyrna tudes* with the highest Hg value (2.75 µg/g) recorded for *Carcharhinus porosus* (Figure 2B). Pb and Cd were recorded at much lower concentrations in all species. Mean Pb concentrations ranged from 0.0007 ± 0.002 in *Sphyrna tiburo* to 0.64 ± 1.37 µg/g in *Carcharhinus acronotus*; the maximum value recorded of 3.10 µg/g was for an individual of the latter species (Figure 2C). *Sphyrna tiburo* had the lowest mean Cd value (0.002 ± 0.005) while *Mustelus higmani* had the highest mean value (0.05 ± 0.25 µg/g), with the maximum concentration recorded (1.59 µg/g) (Figure 2D).

According to EDI (Table 2), the intake of iAs would exceed the PTDI (2.14 μg/kg_{bw}/day) for seven of the species examined, with values ranging from 0.61 to 11.58 μg/kg_{bw}/day. For Hg, EDI exceed the PTDI (0.23 μg/kg_{bw}/day) for all species; values ranged between 0.47 to 10.28 μg/kg_{bw}/day. Only one species, *Carcharhinus acronotus,* exceeded the EFSA guideline for Pb (3.57 μg/kg_{bw}/day), with an EDI of 3.82 μg/kg_{bw}/day, withd the EDI of the remaining 12 species ranging between 0.05 – 1.93 μg/kg_{bw}/day. No species exceed the Cd PTDI (0.83 μg/kg_{bw}/day). According to the estimated MAS values for the ingestion of a single trace element, the consumption of at least seven species should be reduced to stay within the limits of the respective PTDI, with the exception of Cd (Table 2). However, based on the joint analysis of the ingestion of combinations of the four elements in each species – general MAS (the lowest MAS value in each specie), the daily consumption of five species should be reduced to less than 10g (Figure 3).

Table 1: Species identified in the present study, IUCN category (EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient), number of samples (N), mean and standard deviation (SD) of $\delta^{15}N$ values, and the trace element concentrations recorded in 91 samples of shark meat obtained from markets along the Brazilian Amazon Coast in 2017. Concentrations above the limit recommended by the WHO for human consumption are identified in bold.

Species	IUCN	N	$\delta^{15}N$		As		Hg		Pb		Cd	
			Mean ± SD	Min - Max	Mean ± SD	Min - Max	Mean ± SD	Min - Max	Mean ± SD	Min - Max	Mean ± SD	Min - Max
Sphyrna lewini	**EN**	2	-	10.4 - 13.7	-	**1.63 - 17.9**	-	<0.01 - 0.1	-	<0.001 - 0.017	-	<0.001
Sphyrna mokarran	**EN**	3	13.1 ± 0.5	12.5 - 13.5	**5.13 ± 4.09**	**1.81 - 9.70**	0.32 ± 0.16	0.18 - 0.50	0.04 ± 0.03	0.011 - 0.078	0.01 ± 0.007	<0.001 - 0.01
Carcharhinus falciformis	**VU**	2	-	11.7 - 13.2	-	**0.78 - 4.66**	-	**0.12 - 1.59**	-	0.01 - 0.02	-	<0.001 - 0.01
Sphyrna tudes	**VU**	2	-	12.7 - 13.9	-	**1.49 - 2.74**	-	**1.20 - 2.25**	-	0.022 - 0.025	-	0.012

Carcharhinus acronotus	**NT**	5	13.4 ± 2.0	9.8 - 14.7	**3.01 ± 4.32**	**0.60 - 10.70**	**1.12 ± 0.68**	**0.08 - 1.67**	**0.64 ± 1.37**	**0.01 - 3.10**	0.011 ± 0.006	<0.001 - 0.01
Carcharhinus leucas	**NT**	4	12.1 ± 2.0	10.3 - 14.0	**15.26 ± 15.8**	**0.43 - 37.29**	0.41 ± 0.59	**0.02 - 1.30**	**0.32 ± 0.53**	**0.02 - 1.12**	0.008 ± 0.005	<0.001 - 0.01
Galeocerdo cuvier	**NT**	1	12.9		**1.74**		0.45		0.02		0.012	
Mustelus higmani	**LC**	40	10.7 ± 0.5	9.8 - 11.4	**19.46 ± 8.79**	**6.19 - 42.98**	0.13 ± 0.11	<0.01 - 0.42	0.03 ± 0.03	<0.001 - 0.1	**0.05 ± 0.2**	**<0.001 - 1.59**
Rhizoprionodon porosus	**LC**	5	12.3 ± 1.3	10.0 - 13.4	**9.58 ± 5.27**	**2.19 - 15.39**	0.37 ± 0.66	**0.01- 1.55**	0.01 ± 0.008	0.002 - 0.022	0.011 ± 0.010	<0.001 - 0.02
Rhizoprionodon terraenovae	**LC**	5	12.1 ± 0.3	11.8 - 12.6	**5.96 ± 3.13**	**1.61 - 9.30**	**0.60 ± 0.36**	**0.04 - 1.05**	0.04 ± 0.01	0.025 - 0.060	0.017 ± 0.01	0.01 - 0.02
Sphyrna tiburo	**LC**	10	12.3 ± 0.4	11.8 - 13.5	**10.61 ± 1.93**	**7.01 - 12.95**	0.09 ± 0.05	0.02 - 0.22	<0.01 ± 0.002	<0.001 - 0.006	0.002 ± 0.005	<0.001 - 0.01
Carcharhinus porosus	**DD**	10	14.2 ± 0.5	13.4 - 14.9	**1.02 ± 0.67**	**0.50 - 2.81**	0.49 ± 0.82	**0.10 - 2.75**	0.02 ± 0.00	0.02 - 0.04	0.012 ± 0.003	<0.001 - 0.02

Rhizoprionodon lalandii	**DD**	2	-	11.2 - 11.4	-	**1.51 - 4.94**	-	**0.09 - 0.70**	-	0.01 - 0.031	-	<0.001 - 0.02

292

293

294 **Table 2:** Estimated daily intake (EDI) of trace elements in 13 shark species obtained from markets along the Amazon Coastal region in 2017 and
295 the maximum amount of shark (MAS) that can be consumed per species to remain within the limits of the Provisional Tolerable Daily Intake
296 (PTDI).

Specie	**iAs**		**Hg**		**Pb**		**Cd**	
	EDI (μg/kg$_{bw}$/day)	**MAS (g)**	**EDI (μg/kg$_{bw}$/day)**	**MAS (g)**	**EDI (μg/kg$_{bw}$/day)**	**MAS (g)**	**EDI (μg/kg$_{bw}$/day)**	**MAS (g)**
Carcharhinus acronotus	1.79	487.36	6.71[a]	14.26[b]	3.82[a]	389.52[b]	0.07	4890.57
Carcharhinus falciformis	1.62	539.35	5.12[a]	18.70[b]	0.11[a]	13363.64	0.04	7695.36
Carcharhinus leucas	9.08[a]	96.32[b]	2.47[a]	38.81[b]	1.93[a]	771.77	0.05	6896.14
Carcharhinus porosus	1.68	522.00	2.97[a]	32.29[b]	0.16[a]	9509.13	0.07	4770.11
Galeocerdo cuvier	1.04	841.25	2.71[a]	35.28[b]	0.13[a]	11307.69	0.07	4762.30
Mustelus higmani	11.58[a]	75.51[b]	0.80[a]	119.69[b]	0.18[a]	8286.50	0.35	979.23
Rhizoprionodon lalandii	1.92	455.07	2.36[a]	40.52[b]	0.14[a]	10522.11	0.06	5640.78
Rhizoprionodon porosus	5.70[a]	153.30[b]	2.24[a]	42.67[b]	0.10[a]	15036.10	0.07	5060.98
Rhizoprionodon terraenovae	3.55[a]	246.34[b]	3.62[a]	26.49[b]	0.24[a]	6164.28	0.11	3267.72
Sphyrna lewini	5.81[a]	150.42[b]	0.47[a]	203.03[b]	0.05[a]	29058.14	0.01	64555.56
Sphyrna mokarran	3.05[a]	286.37[b]	1.93[a]	49.56[b]	0.25[a]	5898.51	0.06	6159.01

Sphyrna tiburo	6.31[a]	138.49[b]	0.55[a]	173.49[b]	0.00	324545.45	0.02	22784.31
Sphyrna tudes	1.26	693.79	10.28[a]	9.32[b]	0.14[a]	10305.15	0.07	4821.58
PTDI	2.14[c]		0.23[c]		3.57[d]		0.83[c]	

[a] Higher than PTDI
[b] Lower than the average daily consumption rate (416.39g)
[c] JECFA, 2019
[d] EFSA, 2010

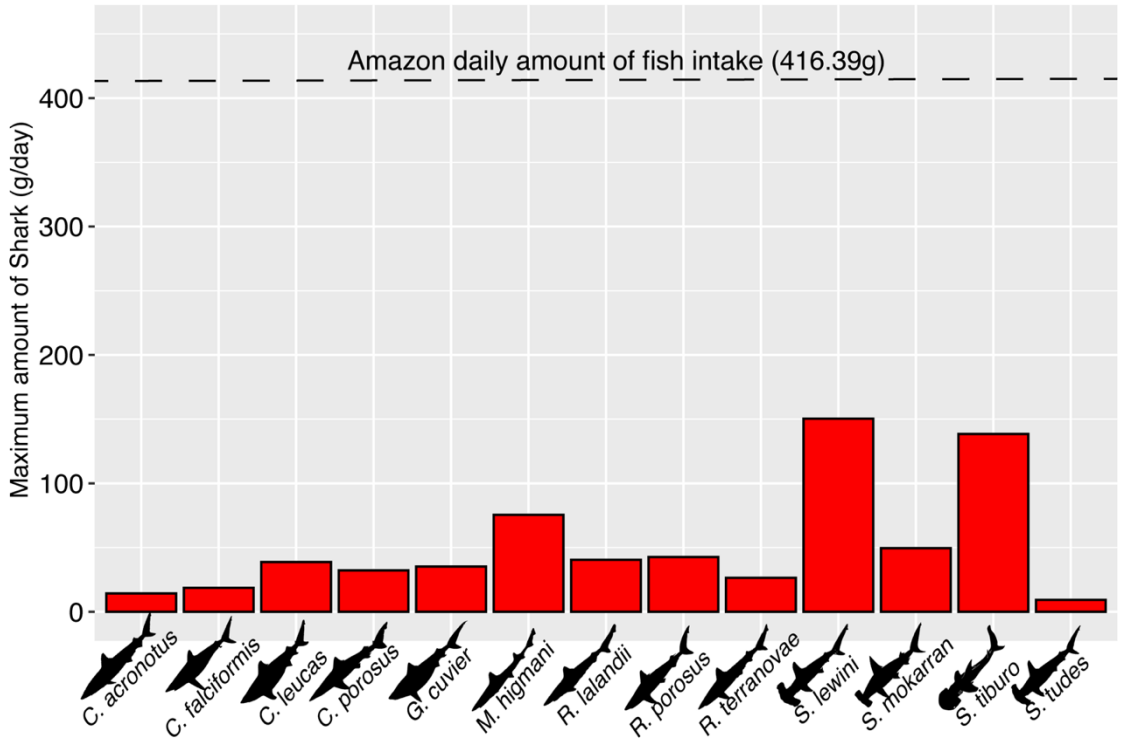
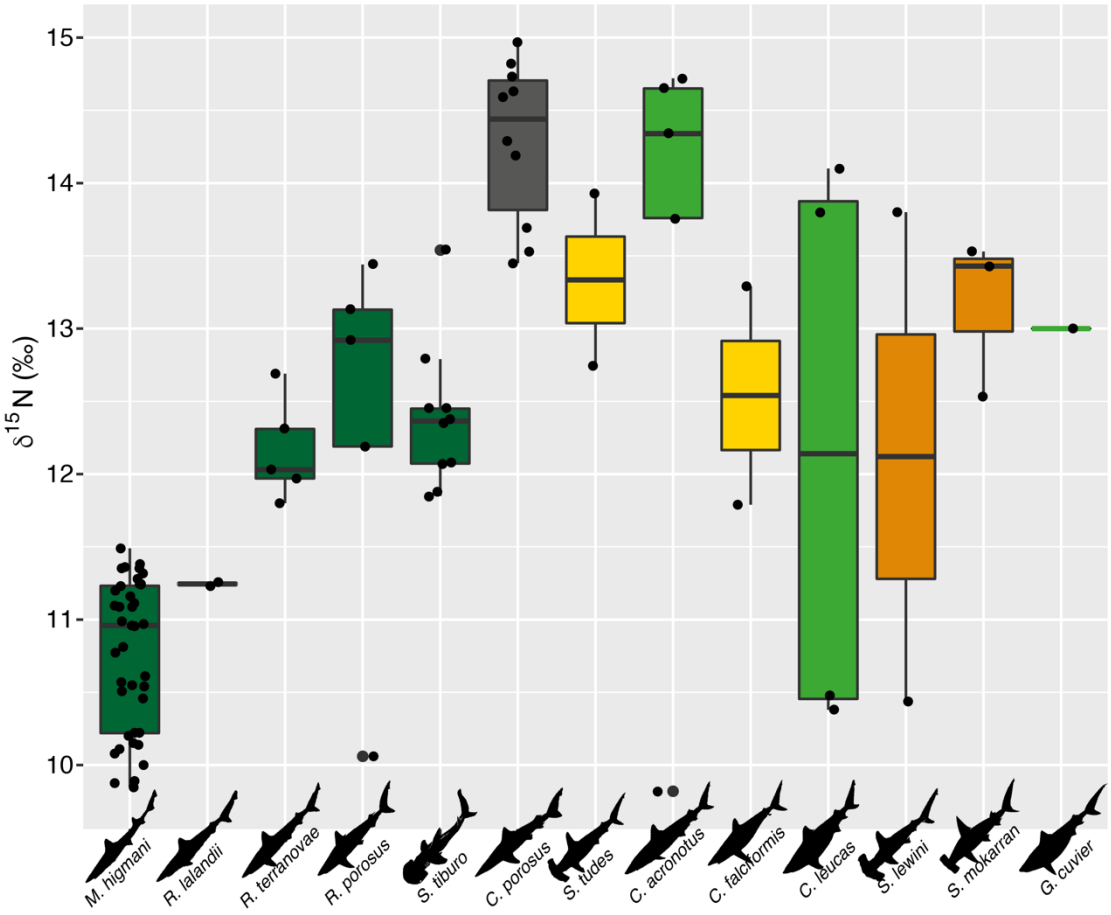


Figure 3: Estimated general maximum amount of shark (MAS) that can be consumed per species without exceeding the lowest Provisional Tolerable Daily Intake (PTDI) among the elements As, Hg, Pb and Cd. The dotted line represents the daily amount of fish typically consumed in the Amazon coastal region in 2017.

Across species, mean (± SD) $\delta^{15}N$ values ranged from 10.7 ± 0.51‰ in *Mustelus higmani* to 14.2 ± 0.59‰ in *Carcharhinus porosus* (Table 1; Figure 4) with significant differences observed among the eight species analyzed (Pseudo-F = 25.65; $p < 0.001$; see Supplementary Material). Pairwise tests revealed that while *Carcharhinus acronotus* had $\delta^{15}N$ values that were only significantly different to *Mustelus higmani; M. higmani* $\delta^{15}N$ values were significantly different from all seven species tested (see Supplementary Material). When considering $\delta^{15}N$ as an absolute measure of trophic position, large variation was observed among the smaller bodied shark complex, while *C. leucas* exhibited the largest intra-species variation (Fig. 4). Arsenic was negatively correlated (r

316 = -0.79; $p < 0.001$) with $\delta^{15}N$ (Table 3) suggesting a biodilution process, whereas Hg was
317 positively correlated ($r = 0.48$; $p < 0.001$) indicating biomagnification. On average, a 1‰
318 enrichment of $\delta^{15}N$ was associated with an As reduction of approximately 4.71 µg/g ($y =$
319 $-4.7126x + 68.412$) (Figure 5A), and a 0.15 µg/g increase in Hg ($y = 0.1503x - 1.4542$)
320 (Figure 5B). No systematic relationships were identified between Pb and Cd
321 concentrations and nitrogen stable isotope values.
322



323
324 **Figure 4:** : Box plots of the $\delta^{15}N$ values recorded in 91 samples of shark meat
325 comprising 13 individual species obtained from fish markets along the Amazon Coastal
326 region in 2017. The central horizontal line is the mean $\delta^{15}N$ value for each shark species,
327 while the boxes contain 50% of the data and the vertical lines correspond to the 95%
328 confidence intervals. Colors represent conservation status of the specie and species are
329 ordered by body size.

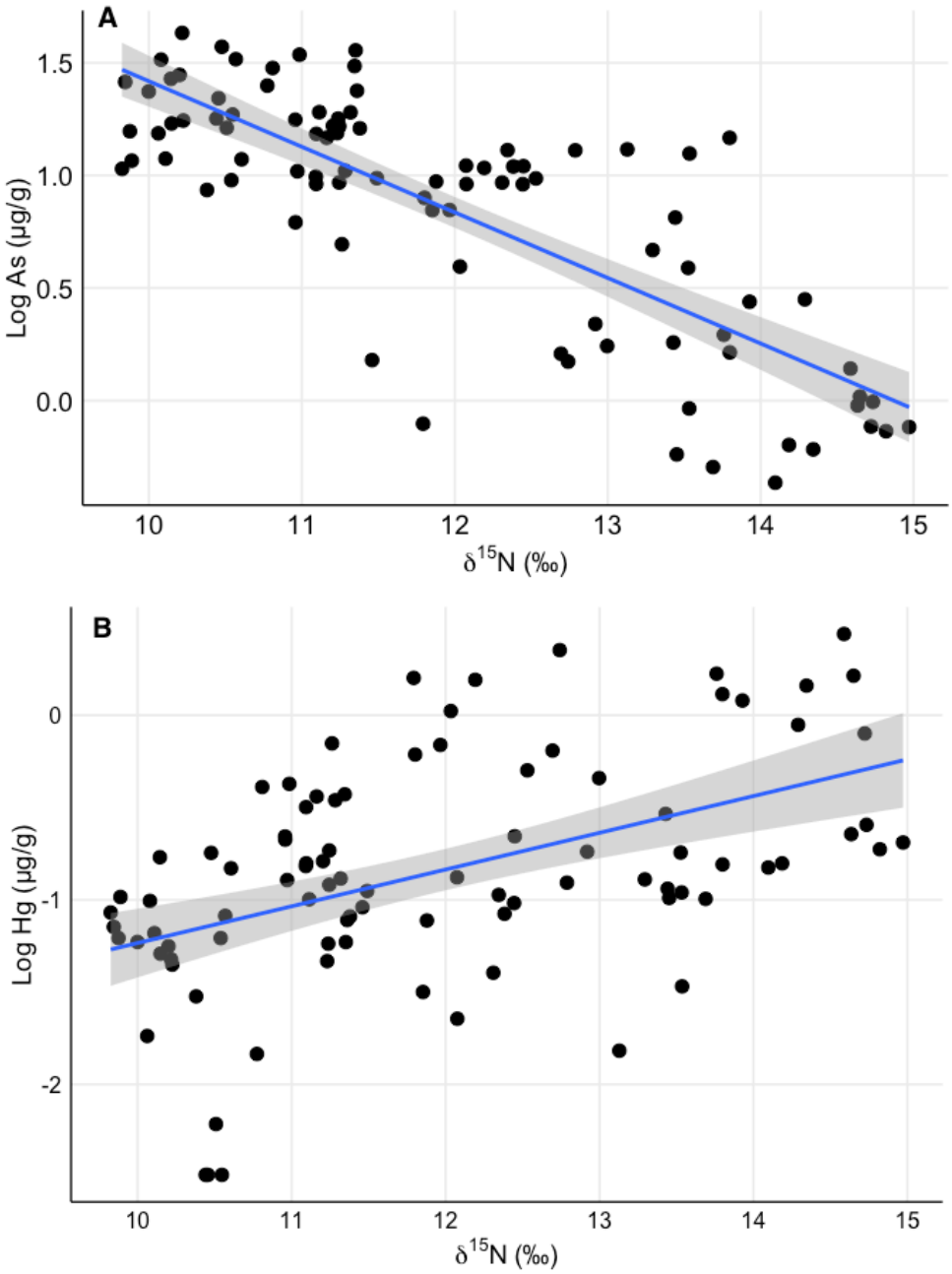


Figure 5: Relationship between log transformed As (**A**) and Hg (**B**) concentrations [µg/g (wet weight)] and $\delta^{15}N$ values (‰) recorded in 91 samples of shark meat obtained from fish markets along the Amazon Coastal region in 2017. Statistically significant correlations are presented (for the r and p values, see Table 3).

Table 3: Pearson correlation coefficients for the relationship between trace element concentrations and the $\delta^{15}N$ values for the 91 samples of shark meat obtained from fish markets along the Brazilian Amazon Coast in 2017.

Element	$\delta^{15}N$	
	r	p
As	-0.79	<0.001
Hg	0.48	<0.001
Pb	-0.17	0.09
Cd	-0.09	0.37

4. Discussion

4.1. Trace element concentrations in shark muscle tissue

The trace element As was recorded at the highest concentrations in all shark species caught along the Amazon coast, with values reported here similar to or higher than those reported for species from other global regions (Southeastern Australia: 60.29 - 115.59 μg/g – Gilbert et al., 2015; Celtic sea: 2.6 - 12.0 μg/g – Nicolaus et al., 2016; South Africa: 28.31 ± 18.79 μg/g – Bosch et al., 2016; Trinidad and Tobago: 0.13 - 6 .15 μg/g – Mohammed & Mohammed, 2017). Despite analyzing total As, our results identify that the discharge of arsenic via sediments transported from the Andes through the Amazon basin interferes with the accumulation of As in marine species that occur on the Amazon coast. Annually, the coastal region at the mouth of the Amazon River receives approximately 5 tons of As via sediments discharged by the river (Scarpelli, 2005), due to the geological features present, but also seasonal effects and certain anthropogenic activities in the Andes region (Bundschuh et al., 2012; Mukherjee et al., 2019; Tapia et

al., 2019). Marine organisms inhabiting the Amazon coast may act as important ecological filters of As sources, metabolizing and mobilizing the element within the coastal food web (Huang, 2016).

M. higmani and *C. leucas* had the highest recorded concentrations of As, but appeared to feed at the lowest trophic level of all examined species, based on their $\delta^{15}N$ values. Unlike contaminants such as MeHg, which is known to biomagnify through the food web, the trophodynamics of As is poorly understood. In a review of published data, Huang (2016) concluded that As tends to be biodiluted in coastal systems, whereby, predators typically have lower concentrations than primary and secondary consumers (Meador et al., 2004; Vizzini et al., 2013). Factors such as food habit or dietary preference may have influenced the accumulation of As in *M. higmani*; this species feeds primarily on lower trophic level crustaceans (Tagliafico et al., 2015). For *C. leucas*, the high As concentrations may relate to proximity to the source given this species commonly occurs close to the Amazon river mouth (Thorson, 1972; Werder & Alhanati, 1981), and parturition and residency of young occurs in estuaries and rivers (Montoya &Thorson, 1982; Compagno et al., 2005; Pillans et al., 2006). As is largely found in the Amazon river (Scarpelli, 2005) supporting this point.

While Hg was the second most abundant element recorded across all species, the concentrations reported here are lower than those found in Southeastern Australia (6.71 - 9.71 μg/g; Gilbert et al., 2015), Kuwait (4.37 ± 3.31μg/g; Moore et al., 2015), Ishigaki Island, Japan (1.32 ng/g; Endo et al., 2015), Korea (0.1 – 7 μg/g; Kim et al., 2016) and in the North-eastern Atlantic (0.12 – 2.57 μg/g; Biton-Porsmoguer et al., 2018). Since most sharks caught along the Amazon Coast are smaller in body size (i.e. smaller bodied species or juveniles of larger species), Hg concentrations may reflect the life-stage examined and present in the region. Smaller individuals are gape limited and

consequently feed on lower trophic level secondary consumers and small tertiary consumers (Lucifora et al., 2009; Grubbs, 2010; Dicken et al., 2017). In contrast to As, there was a positive relationship between Hg and $\delta^{15}N$ values across species. This relationship identifies biomagnification whereby species feeding at a higher trophic level had higher levels of Hg (Bisi et al., 2012; Matulik et al., 2017; Rumbold et al., 2018). Similarly to our results, biomagnification of Hg has also been reported at the species level for *Carcharhinus leucas* and *Carcharhinus acronotus* from Florida Bay (Matulik et al., 2017), and *Carcharhinus leucas* and *Sphyrna lewini* from the southwestern Indian Ocean (Le Bourg et al., 2019).

The levels of Pb and Cd recorded in the present study are below those of concern, but fewer data are available on Cd and Pb concentrations in shark muscle tissue for comparison (Mohamed & Mohamed, 2017). Pb concentrations in the majority of species analyzed were lower than those reported for sharks sampled in Malaysia (0.11 ± 0.02 – 0.43 ± 0.32; Ong & Gan, 2016), the Western coast of Baja California Sur (median: 0.16; Veléz-Alavez et al., 2013) and the Persian Gulf (0.10 ± 0.03 – 0.13 ± 0.04; Adel et al., 2016). Only *C. acronotus* and *C. leucas* had Pb concentrations that were higher than those reported for these locations. Similarly, Cd concentrations were lower than those reported in Southeastern Australia (0.04 – 0.37 μg/g; Gilbert et al., 2015), Ishigaki Island, Japan (0.03 – 7.59 ng/g; Endo et al., 2015) and South Africa (0.04 ± 0.02 μg/g; Bosh et al., 2016).

4.2. Risk Assessment of shark meat consumption

While most shark meat available for sale along the Amazon coast is derived from small bodied species, or juveniles (of larger species), the estimated EDIs of iAs, Hg and Pb were up to 10 times higher than the *Provisional Tolerable Daily Intake* (PTDI). This

408 suggests regular consumption of shark meat poses a risk to human health (IRIS, 2019).
409 Excluding occupational exposure, the primary route of trace element contamination in
410 humans is through the consumption of drinking water and food, in particular fish and
411 shellfish (ATSDR, 1999; 2007; Clarkson *et al*., 2007).

412 In the marine environment the major forms of As in seafood, namely
413 arsenobetaine and arsenosugars, are considered nontoxic (Francesconi, 2010), with only
414 1 - 5% consisting of the iAs form, which is highly carcinogenic (Mandal and Suzuki,
415 2002; Peshut et al., 2007; Gao et al., 2018; Juncos et al., 2019). We emphasize, however,
416 that our EDI estimate is based on expected iAs (10% of the total As), following the
417 recommendations of the USEPA(2000) to use the uptake of inorganic As rather than total
418 As for the assessment of human health risk and that the proportion of iAs can be as much
419 as 9.5% for sharks (USEPA,1997). While consumption of all species would lead to high
420 As exposure, the iAs value was higher than the PTDI when considering seven species,
421 with the estimated iAs intake per 416.39 g serving ranging between 3.05 (*S. mokarran*)
422 and 11.58 μg/kg (*M. higmani*) of body weight. Following the recommendations of the
423 World Health Organization (WHO; 2011a) that the PTDI for As of 2.1 μg/kg bw is no
424 longer a relevant cut off for measuring health risk and that the intake of iAs reported here
425 is higher than this value in more than half of the species analyzed, we suggest that there
426 is an imminent risk of exposure to iAs from the consumption of shark meat along the
427 Amazon coast.

428 Of the four elements analyzed, estimated EDI values for Hg were higher than
429 PTDI for all species. Unlike total As, total Hg (THg) concentrations in fish muscle can
430 be used to assess the risk of human exposure to MeHg, since most THg in fish is MeHg
431 (WHO, 1990; 2008; Zillioux, 2015; Arantes et al., 2016; Souza-Araujo et al., 2016a;
432 Watanabe et al., 2017), including sharks, where more than 95% of THg in muscle tissue

433 is MeHg (USEPA, 1997). The estimated Hg intake per 416.39g serving of shark meat
434 derived from the Amazon coast would range between 0.47 (*S. lewini*) and 10.28 μg/kg (*S.*
435 *tudes*) of body weight. According to WHO, the intake of MeHg up to 0.45 μg/kg bw/per
436 day may not represent a risk for developing neurotoxicity in healthy adults (WHO 2008).
437 However, the intake of MeHg above the PTDI (0.23 μg/kg bw) by women of childbearing
438 age, pregnant females, young children and people with zinc, selenium, glutathione and
439 antioxidant nutritional deficiencies may present a risk and measures of intervention and
440 risk management must be considered (WHO, 2008; Ha et al., 2017; Fuentes-Gandara et
441 al., 2018). As a result, none of the species analyzed could be considered suitable for
442 consumption by healthy adults or the identified risk groups, since the lowest EDI was
443 above the maximum PTDI of no deleterious effect.

444 There are no formal recommended PTDI values for any metal that causes cancer
445 by a mutagenic route; consequently it cannot be assumed that there is any threshold level
446 below which they can safely be consumed (WHO, 2011b; Bat, 2017). As a result, our
447 estimated EDI for Pb was compared with the regulatory PTDI guideline for the dietary
448 intake of Pb (3.57 μg/kg bw; EFSA, 2010). Accordingly, our results showed that the daily
449 intake of Pb was lower than the PTDI for 12 of the 13 species analyzed, and it is
450 approximately less than 16% of the regulatory guideline value. When compared to other
451 European regulations, our values are still lower than the guideline intake of 0.57 μg/kg
452 bw (Bat, 2017). Although consumption of *M. higmani* flesh might lead to some exposure
453 to Cd, the intake per 416.39g serving across all shark species was far less than the PTDI.

454 Given the MAS for each species is calculated for each individual trace element,
455 the daily consumption of meat from most shark species (7 out of 13) would have to be
456 drastically reduced for people to stay within the safe limit of iAs intake, and for all species
457 when considering the safe limit of Hg. However, assessing risk exposure relative to

458 species-specific consumption is problematic for human consumers in the region as an
459 individual often buys shark meat with no knowledge of the species, i.e. if it is a high or
460 low risk. Shark meat available is either from unidentified species or consists of mixed
461 species catches (Rodrigues-Filho et al., 2009; Bornatowski et al., 2013; 2015; Feitosa et
462 al., 2018; Bernardo et al., 2020). Determining species specific health risks of examined
463 elements was only possible here through molecular analysis to identify species. These
464 latter points highlight that if the sale of shark meat is to continue, then regulations need
465 to be established that vendors must label/identify the species for sale.

466 A second important point aside from species specific elements concentration
467 profiles in sharks and associated risks identified here is the amount of fish consumed by
468 the regional population. In the Amazon coastal area, fish consumption rates are above
469 average and are formed of a diverse diet from crustaceans and teleost fish (reef and
470 pelagic) to small bodied and juveniles of large sharks. Consequently, the risk assessment
471 presented here based only on the concentrations of trace elements in sharks could
472 underestimate the true or absolute quantity of elements ingested through overall diet and
473 the health risks caused by chronic exposure.

474 Given this enhanced risk, it is recommend that people, especially pregnant
475 women, breastfeeding mothers, young children, and those who regularly consume large
476 amounts of fish avoid eating fish named “cação”. Additionally, the general public in
477 Brazil should be made aware of the reported element levels in marine resources and
478 provided with recommendations on the risks and benefits of fish consumption relative to
479 established risk guidelines. In the US, for example, the Food and Drug Administration
480 (USFDA, 2020) advises the general public over the risk of contaminant toxicity through
481 classifying fish as "best choice, good choice, or choice to avoid". Moreover, a National
482 Listing of Fish Advisories by state assists people to check how often it is safely eat fish

483 species (EPA, 2020). The Fisheries and Agriculture Organization (FAO/WHO, 2011) and
484 European Food Safety Authority (EFSA, 2014) also recommend that consumers choose
485 fish and seafood with known low pollutant levels, such as salmon, shrimp, cod, and
486 sardines, and to avoid, for example, swordfish, dogfish, marlin, shark, and rays.
487
488 4.3. Relative trophic ecology of sharks along the Amazon Coast
489 Overall, observed variation in $\delta^{15}N$ values among sampled sharks reflected their
490 varying food habits and associated relative trophic position (Cortés, 1999). Among the
491 eight species analyzed, *C. porosus*, *C. acronotus* and *S. mokarran* had the highest $\delta^{15}N$
492 values. Although *S. mokarran* is considered the largest species in the Sphyrnidae family,
493 and an apex predator primarily consuming other sharks and rays (Cliff, 1995; Raoult et
494 al., 2019), its $\delta^{15}N$ values were significantly lower than *C. porosus*, a species that preys
495 on small fish, crustaceans and cephalopods (Lessa & Almeida, 1977). The fact that the
496 sharks sampled in this study were <100 cm TL indicates that *S. mokarran* were all
497 juveniles. These data identify the diet of juveniles is different to adults and support an
498 ontogenetic diet shift reported by Raoult et al. (2019).
499 Among small bodied coastal shark species, there were also marked differences in
500 $\delta^{15}N$ values and hence relative trophic position. *M. higmani* (common length: 55 cm) had
501 low $\delta^{15}N$ values (mean: 10.7‰) when compared to *R. terranovae* (common length: 70
502 cm; mean $\delta^{15}N$ = 12.1‰) and *R. porosus* (common length; mean $\delta^{15}N$ = 12.3‰). These
503 differences can be largely attributed to prey preference and habitat. *M. higmani* occur
504 primarily in muddy, sandy and limestone environments feeding on decapod crustaceans
505 and, occasionally, small fish, stomatopods and cephalopods (Tagliafico et al., 2015). In
506 contrast, *Rhizoprionodon* spp. inhabit bays and estuaries and is classified as an
507 opportunistic predator feeding on small bony fish, but also marine snails, squid and

shrimp (Silva & Almeida, 2001; Drymond et al., 2011; Harrington et al., 2016; Plumlee & Wells, 2016). These combined data indicate these species likely have distinct ecological roles in the Amazonian marine ecosystem, but further work is required to derive $\delta^{15}N$ values for the broader food web.

Of all species examined, *C. leucas* had the most variable $\delta^{15}N$ values, ranging from 10.3 to 14‰. Analysis of stomach contents indicates that juveniles of this species are tertiary consumers, occupying a high trophic position in coastal, estuarine and riverine food webs (Estupiñán-Montaño et al., 2017). In an analysis of 81 juvenile sharks (70–162 cm in total length) in the Shark River estuary in the Everglades National Park, Florida, USA, Matich et al. (2010) reported a similar range of $\delta^{15}N$ values (11.0 to 13.2‰). This variation is likely a result of variation in prey types consumed related to distinct isotopic baselines between riverine, estuarine and marine ecosystems (Fry, 2006; Newton, 2010; Hussey et al., 2012; Olin). These data may therefore suggest that the Amazon Coast plays an important ecological role as a nursery area for this species in a region that is highly exploited by fisheries.

Diversity in trophic roles is important for food web structure and functioning (Hussey et al. 2015; Ferreria et al., 2017). In an analysis of the structure of the trophic web in southern Brazil, Bornatowski et al. (2014) found that species such as *G. cuvier* and *S. lewini* (included in the present study) have important ecological functions, and a major influence on lower trophic levels. Given the productivity of the Amazon Continental Shelf along the northern coast of Brazil and its importance for supporting regional fisheries, further research is necessary to evaluate the trophic role of the local elasmobranch assemblage and their influence on the trophic structure of the local marine-estuarine ecosystems (Myers et al., 2007; Kiszka et al., 2014). Systematic monitoring of biological parameters such as sex ratios and body size, and the seasonality of the catches

of commercially exploited shark species is also necessary for the long-term management of stocks and to provide further context for these nitrogen stable isotope data.

5. Conclusions

The results of the present study indicate that the sale and consumption of shark meat can expose consumers inhabiting the Amazon coastal region and consuming meat on a regular basis to potentially harmful ingestion levels of iAs and Hg that are above those of recommended guidelines. Moreover, consumption of shark meat which promotes fishers to catch individuals to meet market demand is leading to the catch and sale of threatened species including those that are endangered (e.g. *S. lewini*, *S. mokarran*, *S. tudes*, *C. falciformis*, *C. acronotus*, *C. leucas* and *G. cuvier*). Certain species, such as the highly endangered *Sphyrna tudes*, are highly habitat specialized and consequently their affinity to coastal regions will result in high catch rates that will lead to localized population declines and potential extirpation. Moreover, high variability in $\delta^{15}N$ values among the sampled sharks, including multiple smaller bodied species and juveniles of larger species, suggests diverse ecological roles in the coastal environment. In turn, the scale and impacts of shark removals in this region are unknown, consequently more data is required to assess if fisheries are even sustainable. To our knowledge, these are the first data on trace element concentrations and risk assessments for the consumption of shark meat sold along the entire Brazilian North Coast. These combined results can be used by environmental and public health agencies to develop food safety guidelines, to build public awareness and to promote the conservation of threatened shark species in the region.

Declaration of Competing Interest

The authors declare no conflict of interest.

Author contributions

J.S.A. and T.G. conceived of the presented idea. J.S.A. and O.G.S.J. performed the samplings. A.G.C. performed the molecular analysis. M.O.L and N.E.H verified the analytical methods. T.G. supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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Supplementary material

The consumption of shark meat in the Amazon region and its implications for human health and the marine ecosystem

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Supplementary Material

Supplementary material 1 - Results of the PERMANOVA pair-wise test of the $\delta^{15}N$ values among shark species collected from markets in northern Brazil, 2017.

Supplementary Material 1

Results of the PERMANOVA pair-wise test of the $\delta^{15}N$ values among shark species collected from markets in northern Brazil, 2017.

	C. acronotus	*C. leucas*	*C. porosus*	*M. higmani*	*R. porosus*	*R. terraenovae*	*S. mokarran*	*S. tiburo*
C. acronotus								
C. leucas	0.91986							
C. porosus	1.2259	**3.1487***						
M. higmani	**7.1136****	**3.6936****	**19.12****					
R. porosus	1.0027	0.13993	**4.0033****	**5.2066****				
R. terraenovae	1.3835	3.29E-02	**7.7364****	**5.8822****	0.29956			
S. mokarran	0.23476	0.78892	**3.0747****	**7.7854****	0.967	**3.2194***		
S. tiburo	1.6068	0.29646	**8.0525****	**8.972****	7.64E-02	0.89289	**2.3267***	

* $p < 0.05$

** $p < 0.01$

Capítulo 3

Maternal and embryonic trace element concentrations and stable isotope fractionation in the smalleye smooth-hound (*Mustelus higmani*)

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Maternal and embryonic trace element concentrations and stable isotope fractionation in the smalleye smooth-hound (*Mustelus higmani*)

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ABSTRACT

Here, we evaluate maternal offloading of 16 trace elements (Essential: Co, Cr, Cu, Fe, Mn, Ni, Se and Zn; Nonessential: Al, As, Ba, Cd, Hg, Pb, Tl and U) and determine mother-offspring isotopic fractionation of $\delta^{13}C$ and $\delta^{15}N$ in muscle and liver tissue of four pregnant *Mustelus higmani* and 18 associated embryos sampled from the Amazon Coast of Brazil. Embryo muscle tissue had significantly higher concentrations of most trace elements when compared to mothers, with the exception of Hg. Embryo liver accumulated more nonessential elements than muscle (n = 7 *vs*. 0, respectively), while the Se:Hg molar ratio was >1 in liver and muscle of both mothers and embryos. Livers of embryos were moderately enriched in $\delta^{13}C$ and $\delta^{15}N$ when compared to that of their mother. Negative correlations were observed between embryo body length and $\delta^{13}C$ and trace elements concentrations. We conclude that mothers offload a large portion of all essential elements as Al, As and Pb to their young and that the isotopic fractionation of embryos reflects maternal diet and habitat occupied, with $\delta^{13}C$ diluted with embryonic growth. We also show that muscle and liver accumulate trace elements at different rates relative to the body length of embryos. The Se:Hg molar ratio suggests that Se could play a protective role against Hg toxicity during early stages of *M. higmani* embryonic development.

KEY-WORDS: Amazon, Elasmobranch, Maternal offloading, Trace elements, Se:Hg molar ratio; Stable isotopes

1. **INTRODUCTION**

Maternal offloading is the process whereby pregnant females transfer a portion of their body burden of contaminants to their offspring during gestation (Addison and Brodie, 1987; Anas and Wilson, 1970). While maternal offloading of essential trace elements such as Cu, Fe, Se and Zn are critical for embryonic growth and development, the transfer of nonessential trace elements (Hg, As, Cd and Pb) has no known biological function and are considered to have deleterious health and developmental effects even at low concentrations (Bosch et al., 2016). During reproduction of vertebrates, large amounts of lipids are required by females for the formation of eggs or lactation. In elasmobranchs, these lipids are derived from the lipid-dense liver of the mother (Davidson and Cliff, 2010; Hussey et al., 2010; Pethybridge et al., 2011; 2014; Rossouw, 1987), that stores energy for egg production and acts as a detoxification organ through accumulating trace elements at high concentrations (Ardeshir et al*.,* 2017). As a result, trace elements, particularly nonessential trace elements, can be transferred to developing eggs and therefore embryos (Ardeshir et al*.,* 2017; Hall et al., 2001).

Compared to marine organisms such as turtles (Guirlet et al., 2008; 2010; Páez-Osuna et al., 2011) and whales (Borrell et al., 1995; Desforges et al., 2012), little is known with regard to the dynamics of maternal offloading of trace elements in sharks, with most research to date focused on a few toxic elements such as Hg (Frías-Espericueta et al., 2015; Hauser-Davis et al., 2020; Lyons and Lowe, 2013; van Hees and Ebert, 2017) and persistent organic pollutants (POPs) including PCBs, DDTs and pesticides (Lyons et al., 2013; Lyons and Adams, 2015). For example, quantifying the extent of maternal transfer of Hg and POPs has been examined for eleven shark species including the common thresher shark (*Alopias vulpinus*; Lyons and Lowe, 2013; Lyons et al., 2013), white shark (*Carcharodon carcharias*; Lyons et al., 2013; Mull et al., 2013) and bull shark (*Carcharhinus leucas*; Rumbold et al., 2014; Weijs et al.,

2015), while the maternal offloading of a suite of trace elements has only been investigated in the Pacific sharpnose (Cd, Cu, Pb, and Zn; Frías-Espericueta et al., 2014), common thresher shark (essential [Co, Cr, Cu, Fe, Mn, Ni, Se, Zn] and nonessential [Ag, As, Cd, Hg, Pb]; Dutton and Venuti 2019) and in the maternal plasma and uterine fluids of the ragged-tooth sharks (*Carcharhinus Taurus*; Al, As, Cd, Pb and Se; Naidoo et al., 2017)

The application of carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$, respectively) to investigate the diet and movement of sharks is a well-established technique (Estrada et al., 2003, 2006; Fisk et al., 2002; Kerr et al., 2006), which has proven useful for studying nutrient transfer between mothers and young (Le Bourg et al*.,* 2014; McMeans et al*.,* 2009; Vaudo et al., 2010). Given neonatal sharks use energy provided through maternal investment for weeks to months while they develop foraging skills (Hussey et al., 2010), understanding stable isotope fractionation between mother-embryo tissues is fundamental to provide insight into the dynamics of maternal provisioning (de Sousa Rangel et al., 2019 and 2020; McMeans et al*.,* 2009; Olin et al., 2018; Vaudo et al., 2010;).

The smalleye smooth-hound, *Mustelus higmani* Springer and Lowe, 1963, is a small (70 cm max length) placentotrophic shark, whereby the mother nourishes individual embryos (range: 20 to 29 cm total length: L_T) via a vascular placenta-like structure (Tagliafico et al*.,* 2015). The gestation period of *M. higmani* is 10 months producing on average 3-4 offspring per litter (range: 1 to 7) (Heemstra, 1997; Tagliafico et al., 2015). It is an endemic species to South America, occurring in coastal waters ranging from the Gulf of Venezuela, via Curaçao and Trinidad, to southeastern Brazil (Froese and Pauly, 2020; Piorski et al., 2010). The species forages on benthic species in neritic waters, feeding primarily on lobsters and crabs (Cortés, 1999; Heemstra, 1997; Tagliafico et al., 2015). *Mustelus higmani* is often caught as bycatch, with 40% of the species catch a result of drift and bottom gillnet artisanal fisheries in Venezuela

(Tavares et al. 2010), while in Brazilian waters the species is caught in bottom trawl shrimp fisheries (Feitosa et al., 2018).

The current study aimed to examine maternal and embryonic trace element concentrations (essential: Co, Cr, Cu, Fe, Mn, Ni, Se and Zn; nonessential: Al, As, Ba, Cd, Hg, Pb, Tl and U) and determine fractionation of carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in muscle and liver tissue of *M. higmani* obtained from fisheries bycatch off the Brazilian coast. Specifically, we compared (i) the occurrence and concentrations of trace elements and trends in stable isotope values in mothers relative to their embryos and (ii) variation in trace element concentrations and stable isotope values between two tissue types with different turnover rates (fast *vs*. slow: liver *vs*. muscle) and with increasing embryo length.

2. MATERIAL AND METHODS

2.1. STUDY AREA

Specimens of *Mustelus higmani* used in this study were captured off the Amazon Coast of Brazil, which extends over 1,059 km from the Cape of Oiapoque to São Marcos Bay (**Figure 1**). The region is influenced by run-off from the Amazon River to the north of Marajó Island, and that of the Tocantins River to the south, that mixes approximately 6,300 km^3/year of continental waters and 9.3 x 10^8 t/year of sediments with ocean waters (Meade et al., 1979). Together with the high sediment deposition caused by the action of erosion, the development of islands and flooded plains, contributes to the maintenance of estuarine and mangrove ecosystems.

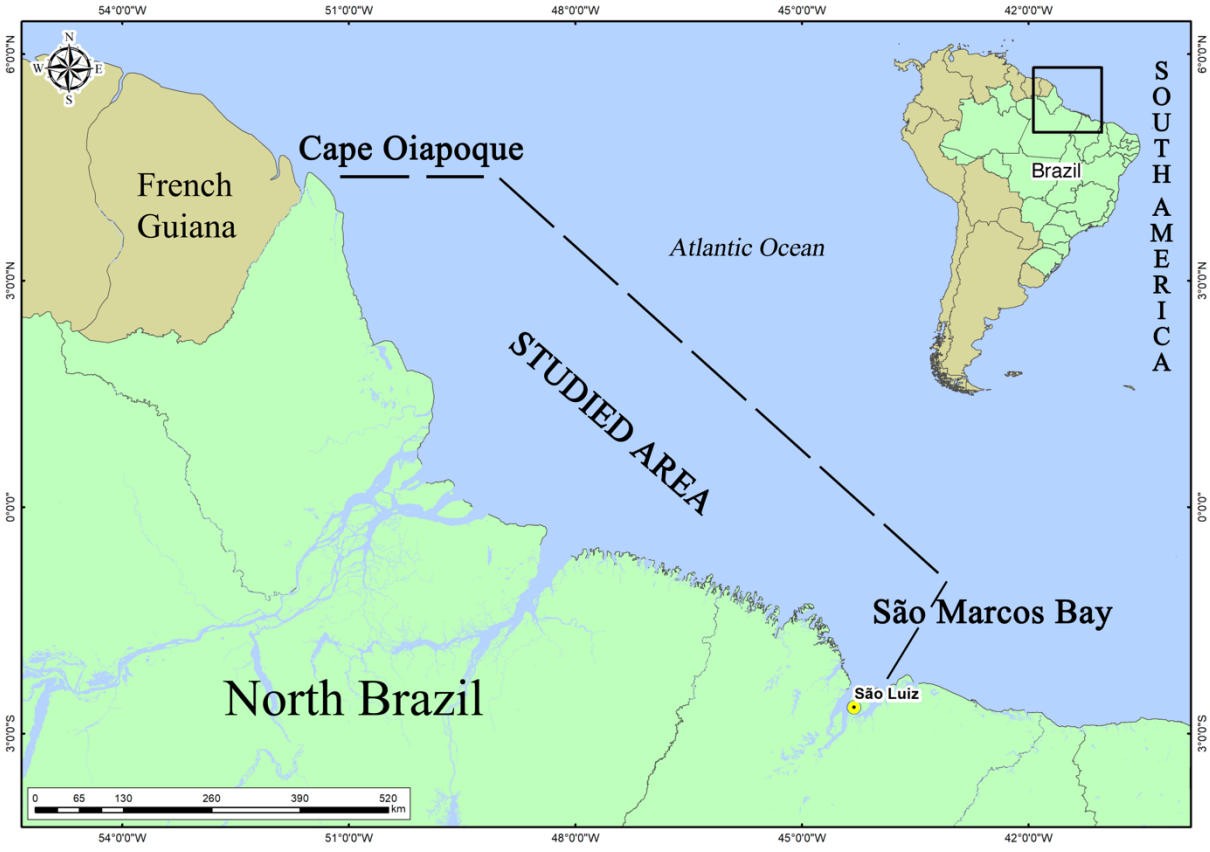

Figure 1: Map of the study area located on the North Coast of Brazil.

2.2. SAMPLING

Samples of four *M. higmani*, assessed to be mid and late-stage gestation, were obtained from bycatch mortalities captured in shrimp trawl fisheries in 2016 (Table 1). Pregnant females and all associated embryos were first measured (L_T) and weighed. Muscle (from the base of the first dorsal fin) and liver samples were taken for trace element and stable isotope (SIA; $\delta^{15}N$ and $\delta^{13}C$) analyses. All tissue samples were kept frozen at -20ºC in polyethylene bags until analysis.

2.3. TRACE ELEMENTS ANALYSIS

The concentrations of 16 trace elements (Essential: Co, Cr, Cu, Fe, Mn, Ni, Se and Zn; Nonessential: Al, As, Ba, Cd, Hg, Pb, Tl and U) were determined using Induced Plasma Coupled Mass Spectrometry (ICP-MS). For the analysis, wet samples were first homogenized and a 0.1 g aliquot of each tissue was transferred to a PTFE bottle with 1.5 ml of HNO_3. Following a 30 minutes period, 0.5 ml of H_2O_2 was added. In the case of one litter with two small embryos, liver samples were pooled to obtain sufficient mass (minimum of 0.05 g) for analysis. Samples were then heated in a microwave oven (MarsXpress, CEM Corporation) over stages of temperature (1º stage: 800W, 180º C, 10 minutes; 2º stage: 1200W, 200º C, 5 minutes; 3º stage: 1000W, 100º C, 10 minutes), and then cooled for 20 minutes in a cold bath. The digested solutions were transferred to polyethylene bottles, topped up to 15 ml with 1% HNO_3, and stored at 4ºC until analysis by ICP-MS. For quality control, certified reference materials [DORM-3 fish protein (n = 3) and DOLT- 4 dogfish liver (n = 2); National Research Council Canada] were used, with the percentage recovery of all elements ranging from 75.7% to 109.9% for DORM-3 and from 75.3 to 90.5% for DOLT-4 (**Table 1**). In addition to the analysis of five automatic replicates of each sample, all samples were weighed, digested and analysed in duplicate. Six blanks were also analysed simultaneously with mother/embryo samples and all were below the detection limit of the respective elements.

Table 1: Analytical recovery of the certified reference material (DORM-3 and DOLT-4) for the quality control of the muscle and liver tissue samples.

Element	DORM-3		DOLT-4	
	Recovery %	Mean ± SD	Recovery %	Mean
Al	93.6	1409.02 ± 704.62	-	-
Cr	92.1	1.72 ± 0.18	-	-
Mn	86.3	2.73 ± 0.38	-	-
Fe	91.7	314 ± 43.5	77.6	1422.81
Co	76.3	0.19 ± 0.01	-	-

Ni	76.2	1.02 ± 0.16	79.2	0.76
Cu	84	13.1 ± 1.16	90.5	28.24
Zn	75.7	39 ± 1.84	80.7	93.66
As	76.2	5.23 ± 0.71	75.3	7.27
Se	87.7	3.02 ± 0.48	78.2	6.49
Cd	76.79	0.22 ± 0.07	84.2	20.47
Ba		4.29 ± 0.34	-	-
Hg	109.9	0.45 ± 0.14	76.3	1.97
Tl		0.005 ± 0.003	-	-
Pb	86	0.34 ± 0.08	76.8	0.12
U	87.3	0.04 ± 0.004	-	-

2.4. STABLE ISOTOPE ($\delta^{15}N$ and $\delta^{13}C$) ANALYSIS

Muscle tissue samples (~1 g) were dried in a standard laboratory oven at 60˚C for 24 hours, and then homogenized to a fine powder using a porcelain mortar and pestle. Lipids were extracted by the addition of 1.9 ml of chloroform-methanol solution (1:2) to powdered muscle tissue into cryovials, and vortexed for one minute. Cryovials were then placed in a water bath at 30˚C for 24 hours, after which, they were centrifuged for 4–6 minutes and the solvent filtered. This process was repeated once. The resulting residue was dried under a fume hood for 24–48h to evaporate off the remaining solvent (Hussey et al., 2012a). For liver tissue, lipid extraction was repeated twice given the known high levels of lipid in this tissue (Hussey et al., 2012a). Following lipid extraction, urea was extracted in both tissues by the addition of 1.9 ml of deionized water and vortexed for one minute. Cryovials were then placed in a water bath at 30˚C for 24 hours, after which, they were centrifuged for 4–6 minutes and the water removed using a medical syringe. The water washing process was repeated three times and the samples dried (Li et al., 2016). Approximately 710–890 µg of muscle tissue for each sample was weighed into 5 mm x 3.5 mm tin capsules of known mass and $\delta^{15}N$ and $\delta^{13}C$ values determined

using a Continuous Flow Isotope Ratio Mass Spectrometer (IR-MS, Finnigan MAT Deltaplus, Thermo Finnigan, San Jose, CA, USA) equipped with an elemental analyzer (Costech, Valencia, CA, USA). Stable isotope values are expressed in delta (δ) notation and are defined as parts per thousand (‰) in relation to a known standard, as follows:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000,$$

Where R_{Sample} and $R_{Standard}$ correspond to $^{13}C/^{12}C$ and $^{15}N/^{14}N$ values in the experimental and standard (control), respectively. Precision, assessed by the standard deviation of replicate analyses of four standards (NIST1577c, internal lab standard - tilapia muscle), USGS 40 and Urea; n=68 for all), measured ≤0.18‰ for $\delta^{15}N$ and ≤0.14‰ for $\delta^{13}C$. Accuracy, based on the certified values of USGS 40 (n=68 for $\delta^{15}N$ and $\delta^{13}C$) analysed throughout runs and not used to normalize samples showed a mean difference of -0.05‰ for $\delta^{15}N$ and -0.07‰ for $\delta^{13}C$ from the certified value. Instrumentation accuracy checked throughout sample runs was based on NIST standards 8573, 8547 and 8574 for $\delta^{15}N$ and 8542, 8573 and 8574 for $\delta^{13}C$ (n=20 for all). The mean difference from the certified values were -0.17, -0.10, -0.14‰ for $\delta^{15}N$ and -0.10, -0.06 and 0.14‰ for $\delta^{13}C$.

2.5. DATA ANALYSIS

The difference between mother trace element concentrations and their respective litters were determined for each tissue type (muscle and liver). Data were log transformed to meet assumptions of normality. One-sample t-tests were used to examine differences in trace element concentrations between each litter and their respective mother; individual mother provided the theoretical values.

The Se:Hg molar ratio was calculated by dividing the concentration in ppm ($\mu g.g^{-1}$) by the molecular weight. For each individual, we divided the Se concentration ($\mu g.g^{-1}$) by 78.96

192 and the Hg concentration ($\mu g.g^{-1}$) by 200.59, and then calculated the Se:Hg ratio separately for
193 mothers and embryos and for both muscle and liver. Pearson's tests were performed to
194 determine correlations between Hg concentrations and Se:Hg molar ratios.

195 Differences between mother and embryo $\delta^{13}C$ and $\delta^{15}N$ values for each tissue ($\Delta\delta^{13}C$
196 and $\Delta\delta^{15}N$, respectively), were calculated for litter–mother pairs (as a method of standardizing
197 litters to facilitate among litter comparisons):

198 $$\Delta\delta^{13}C = \delta^{13}C_{embryo} - \delta^{13}C_{mother}$$

199 $$\Delta\delta^{15}N = \delta^{15}N_{embryo} - \delta^{15}N_{mother}$$

200 The $\delta^{13}C$ and $\delta^{15}N$ values of each litter were also compared to that of their respective
201 mother using one-sample t-tests; individual mother provided the theoretical value. Differences
202 in combined trace elements concentrations and $\delta^{13}C$ and $\delta^{15}N$ values between tissues were then
203 tested using a univariate PERMANOVA run on Euclidean distances matrices with 9999
204 permutations (Anderson, 2001). Analysis was performed in the PRIMER-E software 6.0
205 (Anderson et al. 2008). Finally, we examined Pearson correlation values between total length
206 and $\delta^{13}C$ and $\delta^{15}N$ values and trace element concentrations of embryos to examine the potential
207 effects of maternal shifts in diet and the accumulation of isotopes/elements over gestation. The
208 level of significance for all analysis was designated at $p < 0.05$.
209

210 **3. RESULTS**

211 The mean total length (± standard error; SE) of pregnant *M. higmani* and their respective
212 embryos was 50.6 cm ± 0.39 (range: 48 – 54 cm) and 16.9 cm ± 0.08 (range: 12.5 – 19 cm),
213 respectively (**Table 2)**. Embryos had higher concentrations of most trace elements in muscle
214 tissue when compared to mothers (essential n = 8 [100%]; nonessential n = 4; [66.6%]; **Table**
215 **3**), with the exception of Hg. The mean Hg (± standard error) value in mothers (0.15 ± 0.09

μg.g^{-1}) was almost four times higher than that recorded in the embryos. When considering the percent difference in muscle tissue trace element concentrations, embryos had 822% more Cu, 799% more Cd, and 782% more Pb, but 88% less Hg than their mothers. In contrast, liver concentrations of Al, As, Se, Hg and Cd were significantly higher in mothers compared to embryos (**Table 3**). Embryos had 35% less Al than mothers, 23% less As, 38% less Se, 89% less Hg, and 94% less Cd. Ba, Tl and U, were not detected in muscle, but were present at low doses in liver tissue, and at lower doses in embryos when compared to mothers. Embryos had 5% less Ba than mothers, 15% less Tl, and 31% less U. Overall, embryos concentration of trace elements (essential: Co, Fe and Se; nonessential: Al, As, Ba, Cd, Pb, Tl and U) were higher in liver than muscle (**Table 4**).

For all tissues, the Se:Hg ratio was >1, and embryo Se:Hg molar ratios were higher than their mothers. The mean mother muscle Se:Hg value was 8.99 ± 0.59 (ranging from 4.13 to 16.77), while the mean embryo muscle value was 706.17 ± 1.11 (ranging from 57.91 to 3397.5). In contrast, the mean mother Se:Hg ratio in liver was 50.9 ± 1.08 (range: 29.67 to 75.57) and 559.66 ± 1.08 in embryos (range: 214.10 to 1137.98). The coefficients of the Se:Hg molar ratio in muscle and liver were negatively correlated with Hg concentrations, indicated by the high Pearson's correlation coefficients (range of r^2: 0.72 to 0.98) (**Figure 2**).

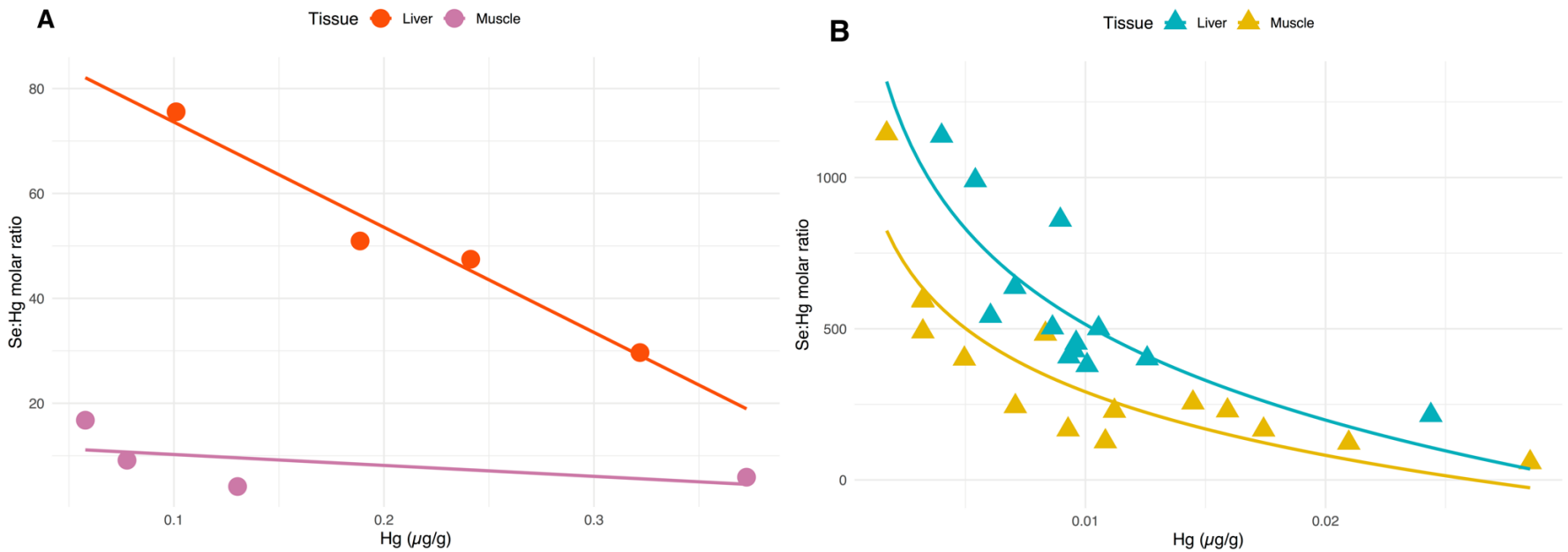

Figure 2: Relationship between Se:Hg molar ratio and mercury concentration in muscle and liver of mothes and embryos of *Mustelus higmani* from the North Coast of Brazil.

240 **Table 2:** Total length (L_T) of the four *M. higmani* mothers and their embryos (n = number of embryos in each litter). For embryos, the $\delta^{13}C$ and $\delta^{15}N$
241 values for muscle and liver tissue are presented as the mean ± SE calculated for each litter, and levels of significance after one-sample t-tests are shown
242 by stars (*: $p < 0.05$, **: $p < 0.01$)

Sample	N	L_T (cm)	Muscle			Liver		
			$\delta^{13}C$ ‰	$\delta^{15}N$ ‰	C:N	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰	C:N
Mother A		50	-15.15	11.31	3.11	-15.72	10.52	3.75
Litter A	2	16	-14.62 ± 0.04	11.26 ± 0.08	3.14 ± 0.08	-15.59*	11.07**	3.47
Mother B		48	-15.42	11.36	3.16	-16.91	10.32	3.88
Litter B	3	13.1 ± 0.33	-14.29	10.66	3.10	-16.28 ± 0.14*	10.68 ± 0.19	3.73 ± 0.04
Mother C		50.5	-14.83	11.23	3.08	-16.24	10.66	3.52
Litter C	5	18.5 ± 0.31	-14.81 ± 0.05	11.18 ± 0.06	3.14 ± 0.02	-15.72 ± 0.11*	11.22 ± 0.05**	3.60 ± 0.07
Mother D		54	-14.72	11.34	3.12	-16.75	10.81	3.62
Litter D	8	17.5 ± 0.32	-14.70 ± 0.03	11.35 ± 0.05	3.13 ± 0.02	-15.59 ± 0.12**	11.59 ± 0.07**	3. 45 ± 0.03

243

The fractionation of carbon between mother and embryos ($\Delta\delta^{13}C$) in muscle tissue ranged from -0.14–1.13‰, and was not significantly different. In liver, carbon fractionation values ranged from 0.12‰ to 1.45‰, with all litters significantly enriched in ^{13}C compared to mothers (**Table 2**). When considering $\delta^{15}N$ in muscle tissue, values were similar between mothers and embryos, with an observed fractionation value ($\Delta\delta^{15}N$) of -0.70–0.23‰. In contrast, 3 of 4 litter's livers were significantly enriched in ^{15}N relative to mothers (**Table 2**), with fractionation values ranging from 0.12–1.06‰. While the $\delta^{13}C$ values of embryo's muscle tissue were significantly enriched in ^{13}C (1.04‰; $t = 10.18$ $p < 0.001$) compared to liver (**Figure 3**), there was a moderate, but significant ($t = 3.42$ $p = 0.002$) enrichment of ^{15}N (*ca.* 0.09‰) in embryo livers compared to muscle (**Figure 3**).

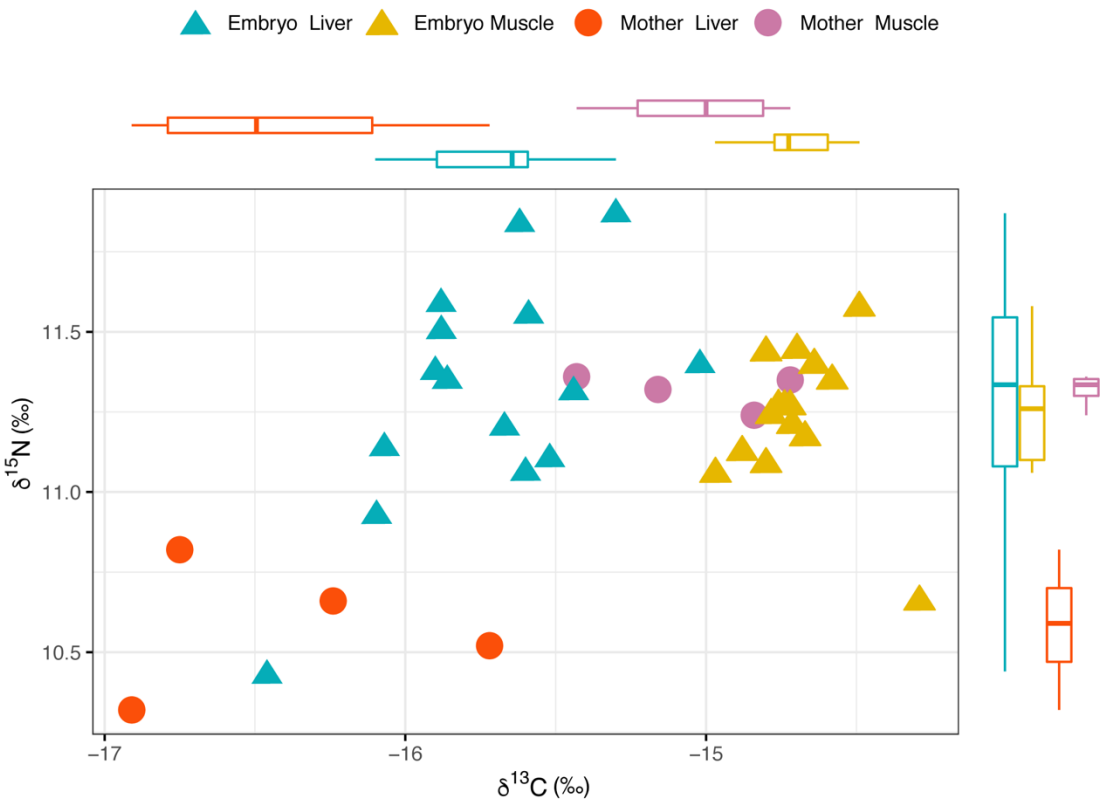

Figure 3: Muscle and liver $\delta^{13}C$ and $\delta^{15}N$ values for four pregnant female *Mustelus higmani* and associated embryos (n = 18) sampled from the Amazon Coast of Brazil in 2016.

259 **Table 3:** Means ± SE (µg.g^{-1}) concentrations of 16 trace elements in muscle (A) and liver (B) tissue of four *Mustelus higmani* mothers caught off the
260 Amazon Coast of Brazil in 2016 in comparison with their respective litters. With the exception of Cd, Ba, Tl and U (which are presented in ng.g^{-1} due to
261 the low concentrations recorded), all values are in µg.g^{-1}. The values presented for Cd, Ba, Tl, and U in muscle tissue are below the detection limit of the
262 equipment. Significance of of the one-way *t* test results between mother and litters are indicated by * except for Mother and Litter A where only two
263 embryos were present.
264
265 A – Muscle

ELEMENT	Mother A	Litter A (N= 2)	One sample T-test	Mother B	Litter B (N= 3)	One sample T-test	Mother C	Litter C (N= 5)	One sample T-test	Mother D	Litter D (N= 8)	One sample T-test
Essential												
Co	1.67	11.25	2.32	3.10	22.57 ± 9.67*	3.50	1.73	4.91 ± 2.20**	3.97	3.17	7.74 ± 3.17**	4.22
Cr	0.29	0.88 *	9.18	0.33	1.023 ± 0.43	2.73	0.26	0.54 ± 0.51	1.18	0.37	1.19 ± 0.57*	4.07
Cu	0.22	3.05	2.19	0.24	6.85 ± 1.79*	6.37	0.23	0.99 ± 0.14**	11.34	0.39	1.58 ± 0.60**	5.54
Fe	5.02	42.06	1.96	6.92	14.22 ± 24.49*	3.63	4.77	19.65 ± 15.16*	2.19	11.06	24.26 ± 5.63**	6.62
Mn	0.13	0.53	2.69	0.11	1.89 ± 1.04*	2.94	0.11	0.22 ± 0.12	1.93	0.22	0.26 ± 0.09	1.06
Ni	0.04	0.19	1.42	0.04	0.29 ± 0.10*	4.09	0.04	0.07 ± 0.01**	3.93	0.05	0.12 ± 0.08*	2.20
Se	0.21	1.05	2.08	0.27	2.60 ± 0.89*	4.48	0.38	0.80 ± 0.17**	5.24	0.86	0.89 ± 0.30	0.30
Zn	4.30	19.48	2.17	4.90	36.63 ± 15.58*	3.52	4.25	8.84 ± 1.13**	9.08	4.52	13.27 ± 3.81**	6.49
Nonessential												

Al	5.34	14.40	1.62	4.32	19.67 ± 8.02*	3.31	4.76	5.83 ± 0.96*	2.48	5.13	7.91 ± 1.58**	4.96
As	19.07	17.09	-0.29	23.78	37.40 ± 7.63*	3.09	17.88	16.90 ± 1.33	-1.63	30.62	22.45 ± 6.71*	-3.43
Hg	0.13	0.02	-15.45	0.07**	0.00 ± 0.002	-30.22	0.05**	0.00 ± 0.001	-167.93	0.37**	0.01 ± 0.005	-187.2
Pb	0.00	0.02	1.67	0.00	0.045 ± 0.02	0.59	0.00	0.01 ± 0.009*	2.392	0.00	0.01 ±0.004**	8.29
Cd	<0.93	<0.93	-	< 0.93	40.12 ± 38.91	1.75	< 0.93	<0.93	-	< 0.93	50.13 ± 83.36	1.13
Ba	<0.44	29.05	1.08	<0.44	33.08 ± 56.55	0.99	<0.44	9.97 ± 21.31	0.99	<0.44	10.1 ± 25.59	1.06
Tl	<0.003	<0.003	-	<0.003	<0.003	-	<0.003	<0.003	-	<0.003	<0.003	-
U	<0.004	<0.004	-	<0.004	<0.004	-	<0.004	<0.004	-	<0.004	<0.004	-

266

267

268 B – Liver

ELEMENT	Mother A	Litter A (N= 2 – pool)	Mother B	Litter B (N= 3)	One sample T-test	Mother C	Litter C (N= 5)	One sample T-test	Mother D	Litter D (N= 8)	One sample T-test
Essential											
Co	61.70	38.01	38.63	20.93 ± 6.41	-3.90	57.71**	13.27 ± 3.69	-26.90	43.32**	14.84 ± 2.46	-32.72
Cr	1.40	1.32	0.76	0.83 ± 0.22	0.45	0.78**	0.65 ± 0.04	-6.99	0.74	0.69 ± 0.14	-1.04
Cu	1.91	14.08	1.08	4.59 ± 0.57	8.64	3.41	6.18 ± 5.23	1.18	2.62**	1.26 ± 0.38	-10.00
Fe	123.88	282.89	102.36	93.17 ± 17.25	-0.75	85.02	60.94 ± 27.54	-1.95	63.06	68.37 ± 24.16	0.62
Mn	0.74	4.71	0.51	0.36 ± 0.14	-1.54	0.9**	0.43 ± 0.21	-5.38	0.66**	0.21 ± 0.03	-39.82
Ni	0.20	0.24	0.10	0.14 ± 0.05	1.15	0.10	0.10 ± 0.02	0.24	0.12**	0.10 ± 0.01	-3.25
Se	3.78	2.05	3.00*	1.35 ± 0.19	-11.78	4.50	3.08 ± 1.90	-1.66	**3.76****	1.92 ± 0.48	-10.71

Zn	6.68	62.05	4.71	8.76 ± 1.05	5.43	9.07	15.89 ± 11.20	1.36	**10.36****	7.53 ± 1.72	-4.64
Nonessential											
Al	35.00	29.87	25.06	17.94 ± 4.75	-2.11	**30.04****	13.16 ± 0.56	-66.66	**26.02****	14.59 ± 3.72	-8.66
As	26.53	16.59	**19.01****	16.33 ± 0.02	-167.60	21.90	18.02 ± 11.46	-0.75	**25.97****	15.82 ± 1.14	-25.12
Hg	0.18	0.02	**0.10****	0.00 ± 0.00	-110.40	**0.24****	0.00 ± 0.00	-71.41	**0.32****	0.00 ± 0.00	-554.53
Pb	0.19	0.21	0.09	0.12 ± 0.03	0.83	**0.09****	0.08 ± 0.00	-5.82	0.09	0.09± 0.02	-0.24
Cd	1039.02	<0.12	597.03	<0.12	-	1391.17	<0.12 ± 0	-	**3187.92****	18.08 ± 49.14	-182.42
Ba	761.53	843.26	411.39	442.71 ± 136.96	0.32	**419.25****	314.97 ± 14.92	-15.62	**425.72***	348.29 ± 83.29	-2.629
Tl	0.41	0.31	0.44	0.35 ± 0.02	-5.34	0.28	0.42 ± 0.35	0.93	**0.67****	0.23 ± 0.03	-32.03
U	1.50	1.74	1.09	0.73 ± 0.26	-1.93	**1.07****	0.55 ± 0.09	-11.83	**1.10****	0.58 ± 0.10	-14.35

269 * $p < 0.05$

270 **$p < 0.01$

Table 4: Mean ± SE values of 16 trace elements in muscle and liver tissue of *M. higamni* embryos from Amazon Coast of Brazil in 2016. Statistical significance, and Pseudo-F values for comparisons between all muscle and liver samples of individuals sampled from the Northern Coast of Brazil are shown; * $p < 0.05$; ** $p < 0.01$

Element	Muscle Mean ± SE	Liver Mean ± SE	Pseudo-F
Essential			
Co	0.00 ± 0.00	0.02 ± 0.00**	15.70
Cr	0.83 ± 0.11	0.81 ± 0.05	0.21
Cu	2.06 ± 0.47	4.16 ± 0.94	25.92
Fe	20.31 ± 3.28	94.70 ± 14.08**	29.45
Mn	0.48 ± 0.14	0.79 ± 0.27	0.26
Ni	0.12 ± 0.02	0.13 ± 0.01	<0.001
Se	1.03 ± 0.16	2.46 ± 0.26**	21.68
Zn	14.42 ± 2.45	14.62 ± 3.52	0.24
Nonessential			
Al	9.08 ± 1.26	18.89 ± 1.58**	49.66
As	22.81 ± 1.80	17.83 ± 1.26*	43.97
Ba	13.17 ± 6.00	0.43 ± 0.03**	136.05
Cd	0.02 ± 0.01	0.28 ± 0.15*	31.06
Hg	0.03 ± 0.01	0.04 ± 0.01	0.29
Pb	0.01 ± 0.00	0.11 ± 0.00**	96.93
Tl	0.00	0.34 ± 0.04**	62.61
U	0.00	0.82 ± 0.08**	6.60

For embryos, Al, Cu, Zn, As, Mn, Ni, Se, Pb and Co concentrations were negatively correlated with total length for muscle tissue, while Ni, Pb and Co concentrations showed a significant positive relationship in liver. One element, Cr, had a negative correlation with body size in liver tissue (**Table 5**). A strong negative correlation was observed between total length

and muscle $\Delta\delta^{13}C$ values of embryos ($r = -0.90$, $p < 0.001$; **Table 5**). There was no observed relationship between $\Delta\delta^{13}C$ values of liver and body size and $\Delta\delta^{15}N$ values for both tissues.

Table 5: Pearson correlations between embryo total length and differences between mother and embryo $\delta^{13}C$ and $\delta^{15}N$ values ($\Delta\delta^{15}N$ and $\Delta\delta^{13}C$) and trace elements in muscle and liver tissue of *Mustelus higmani* sampled from the Amazon Coast of Brazil in 2016. Ba, Tl and U were excluded from this analysis due to values lower than the LD. * = $p < 0.05$

Variable	Muscle		Liver	
	r	p	r	p
$\Delta\delta^{13}C$	-0.90	<0.001*	-0.014	0.96
$\Delta\delta^{15}N$	0.30	0.25	0.24	0.36
Essential				
Co	-0.73	<0.001*	-0.57	0.008*
Cr	-0.091	0.73	-0.55	0.011*
Cu	-0.87	<0.001*	-0.14	0.55
Fe	0.055	0.83	-0.37	0.1
Mn	-0.81	<0.001*	-0.2	0.39
Ni	-0.66	0.004*	-0.56	0.01*
Se	-0.75	<0.001*	0.34	0.14
Zn	-0.75	<0.001*	-0.12	0.61
Nonessential				

Al	-0.74	<0.001*	-0.24	0.3
As	-0.58	0.014*	-0.22	0.35
Cd	-0.031	0.91	0.049	0.84
Hg	-0.11	0.67	-0.17	0.47
Pb	-0.64	0.005*	-0.51	0.02*

290
291
292 **4. DISCUSSION**

293 **4.1. Comparison of maternal and embryo trace element concentrations**

294 Since there is no direct contact between embryos and the external environment during
295 gestation, all trace elements present in embryonic tissues can be considered to be derived
296 through maternal offloading (Lyons and Lowe, 2013). Both essential (Cu, Fe, Se and Zn) and
297 nonessential (Al, As and Pb) elements were transferred from *M. higmani* mothers to embryos,
298 including known hazardous elements, such as Pb and Cd, that have no known biological
299 function. As would be expected, Fe, Zn, Co and Cu, elements that are critical for successful
300 embryonic growth and development (FAO, 1987; Wood et al., 2012), were offloaded at the
301 highest concentrations. Key constituents of metabolic enzymes (e.g., Cu, Co, Fe, Mn, Se, Zn),
302 assisting oxygen transport (e.g. Fe), providing protection against free radical damage (e.g., Se,
303 Zn), and aiding metabolism of carbohydrates (e.g., Cr) are physiological processes that are
304 dependent on concentrations of these trace elements (Wood et al., 2012).

305 In contrast, four trace elements offloaded to *M. higmani* embryos such as Cd, Hg, and
306 Pb are among the most toxic to organisms (ASTDR, 2017). While As concentrations in marine
307 fish are higher (1−10 $\mu g.g^{-1}$) than those in freshwater fish (<1 $\mu g.g^{-1}$) (Ciardullo et al., 2010;
308 Schaeffer et al., 2006), the high doses recorded in *M. higmani* are likely related to the

309 characteristics of the study area; large amounts of total As is transported from the Andes to the
310 ocean via sediment and dissolved in water discharged from the Amazon river basin (Scarpelli,
311 2005). High concentrations of As (up to 100 $\mu g.g^{-1}$) have been recorded in some edible marine
312 species in other locations, but in most cases, the values are total As concentrations, rather than
313 that of the toxic inorganic form, Arsenite (ATSDR, 2007). Typically up to 95% of As in fish
314 muscle is present in the non-toxic arsenobetane form (Zhang et al., 2016).

315 Unlike most elements analysed, Hg was found in higher concentrations in muscle and
316 liver tissue of *M. higmani* mothers compared to embryo tissues. Hg muscle concentrations in
317 pregnant female *M. higmani* were on average ~17 times higher than embryos, while liver
318 concentration were ~19 times higher. These results are in agreement with those reported for the
319 common thresher and leopard shark (Dutton and Venuti, 2019; Lyons and Lowe, 2013; van
320 Hees and Ebert, 2017). While most contaminants preferentially accumulate in liver tissue, Hg,
321 in particular methylmercury (which is the principal form recorded in most fish species with
322 more than 98% recovery of total mercury; Souza-Araujo, et al., 2016; Watanabe et al., 2017;
323 WHO, 1990; 2008), tends to associate with proteins (Mason et al., 1995), and accumulates in
324 different tissue types (Lyons and Lowe, 2013; Mull et al., 2012). Given the liver is the primary
325 energy storage organ from which females draw resources to nourish offspring, and most Hg is
326 held in muscle, only a small proportion of the mother's Hg burden are offloaded to litters.

327 Despite the occurrence of low concentrations of Cd and Pb in pregnant female *M.*
328 *higmani* muscle tissue (the Cd doses were below the LD), they were present in respective
329 embryo tissues. Previous studies investigating maternal offloading of Cd and Pb in the Pacific
330 sharpnose and common thresher shark also found that Cd and Pb accumulated in embryo muscle
331 and liver tissue. For both species and including *M. higmani*, the doses of both elements were

higher in liver compared to muscle tissue (Dutton and Venuti, 2019; Frías-Espericueta et al., 2014).

With the exception of Hg, liver tissue of embryos accumulated higher concentrations of all nonessential elements relative to muscle. This is to be expected given liver tissue is more metabolically active than muscle (Ardeshir et al., 2017). Hussey et al. (2010) reported that neonatal dusky sharks (*Carcharhinus obscurus*) had high hepatic lipid levels, inferred by high hepatosomatic index (HSI) immediately following birth and a decline in HSI values with increasing body size. This indicates maternal allocation of lipid reserves to developing offspring during gestation that facilitates the transfer of non-essential elements. Moreover, during the early phase of *M. higmani* gestation, when maternal allocation of lipid reserves is higher, a negative correlation between trace element concentrations and increasing embryo body size was found. This indicates that trace elements are diluted with growth, and are more concentrated in younger (mid gestation) than near term individuals (late gestation). This trend is identical to that observed for aplacental sharks (Le Bourg, 2014)

There are a growing number of studies examining Se:Hg molar ratios as a measure of toxicity, with the inclusion of this parameter in health risk assessments for the consumption of fish muscle (white) tissue. This is based on the fact that Se is known to neutralize the toxicity of Hg^{2+} (Parizek and Ostradalova, 1967), through high affinity binding to Hg that produces inert mercuric selenide (HgSe) compounds in the bloodstream (Burk et al., 1974). Se, if present at high relative concentrations (Se:Hg > 1), may consequently have a potential protective effect on reducing methylmercury toxicity for consumption of fish muscle, including shark meat (Burger and Gochfeld, 2012; Dutton and Venuti, 2019; Kaneko and Ralston, 2007). In contrast, acute toxicity of methymercury can occur when the Se:Hg molar ratio is < 1 (Peterson et al., 2009; Ralston and Raymond, 2010; Yang et al., 2010). For *M. higmani*, the Se:Hg molar ratio

was much higher than 1 in all tissues of mothers and embryos, suggesting that Se may play a protective role against Hg toxicity during embryonic developmental stages similar to that reported for the common thresher shark (Dutton and Venuti, 2019).

4.2. Maternal-offspring fractionation of stable isotopes

Our results indicate that the mean $\delta^{13}C$ and $\delta^{15}N$ levels (-15.04 ± 0.14 and 11.33 ± 0.05, respectively) for pregnant female *M. higmani* sampled off Northern Brazil are quite variable when compared to other species of the genus *Mustelus* previously studied ($\delta^{13}C$: -15.8 ± 1 and $\delta^{15}N$: 14.5 ± 1.2; Domi et al., 2005; $\delta^{13}C$: -14.1± 0.5 and $\delta^{15}N$: 15.68 ± 0.4; Borrell et al., 2011; $\delta^{13}C$: -16.3 ± 0.4 and $\delta^{15}N$: 12.5 ± 0.8; Endo et al., 2013). While baseline stable isotope values were not sampled in our study area, isotope values across studies suggest *M. higmani* is a secondary consumer (Cortés, 1999; Tagliafico et al., 2015). Neonatal elasmobranchs would be expected to have higher $\delta^{15}N$ and $\delta^{13}C$ values than mothers, as a result of isotopic discrimination of maternal resources throughout development (Pilgrim, 2007; Post et al., 2007). Our relatively small $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values, however, indicates minimal fractionation occurs between *M. higmani* mothers and embryos.

Embryos were more enriched in ^{13}C relative to mothers, but the $\Delta\delta^{13}C$ values was below the upper the limit of the 0 – 2‰ change expected to denote a difference in trophic level (Caut et al., 2008; McMeans et al., 2009). This suggests that the isotopic composition of *M. higmani* embryos (both $\delta^{15}N$ and $\delta^{13}C$) predominantly reflects maternal tissue composition and consequently their foraging patterns/habitat use (Pilgrim, 2007). Similar, but slightly higher $\Delta\delta^{13}C$ values have been reported between mother and embryos for other placentotrophic species including the Atlantic sharpnose ($\delta^{13}C$: 1.2 in both tissues and $\delta^{15}N$: 1.4 and 1.7 ‰ in muscle

and liver, respectively; McMeans et al., 2009), scalloped hammerhead ($\delta^{13}C$: 1.01‰ and $\delta^{15}N$: 0.82‰ in muscle; Vaudo et al., 2010) and blacktip shark ($\delta^{13}C$: -0.26‰ and $\delta^{15}N$: 0.88‰ in muscle; Vaudo et al., 2010). In agreement with Olin et al. (2018), our results show that the degree of fractionation between embryos and mothers of placentatrophic sharks is variable, but typically positive fractionation occurs. Intraspecific variation among mothers could be attributed to physiological differences, if gestation occurs in the same environment and individuals consume the same resources (Barnes et al., 2008) or a result of variable resource use patterns.

As a more metabolically active organ, $\delta^{15}N$ and $\delta^{13}C$ values in liver are known to turn over faster compared to muscle tissue (Kim et al., 2012; MacNeil et al., 2006), allowing examination of recent shifts in diet/habitat use (Hussey et al., 2012b). Observed differences in the stable isotope values of both pregnant female and *M. higmani* embryo muscle and liver tissue may reflect shifts in diet/habitat use of mothers during the gestation period. Alternatively, this may be a result of physiological process driving variation among tissues or varying amino acid composition between tissue types (Lorrain et al., 2012; Pinnegar and Polunin, 1999).

The observed negative relationship between embryo L_T and $\Delta\delta^{13}C$ values could be due to initial yolk phase use as a nutritional source in the early stages of *M. higmani* gestation. This result and the non-significant relationship between $\Delta\delta^{15}N$ values and embryo L_T in muscle and liver, however contrasts with previous findings (McMeans et al., 2009). Alternatively, our results are similar to Le Bourg et al. (2014), whereby $\delta^{13}C$ values in muscle tissue of *Squalus megalops* embryos (an aplacental species) were negatively correlated with L_T. The authors suggested this could be due to higher incorporation rates of heavy isotopes in tissues such as cartilage or kidney in growing embryos. Further comparisons of $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values in

403 embryo-mother pairs in multiple tissues of *M. higmani* as well as other shark species across a
404 range of reproductive strategies is required to fully characterize these isotope dynamics.
405
406 **5. CONCLUSIONS**
407 We conclude that pregnant female *M. higmani* offload both essential and nonessential
408 trace elements to their embryos during gestation and that isotopic fractionation between mother
409 and embryo is minimal, indicating embryo tissues primarily reflect those of the mother's diet
410 and habitat occupied during gestation. It is also evident that the liver accumulates more
411 nonessential trace elements than muscle and that trace elements concentrations in embryos are
412 diluted with growth. Finally, the Se:Hg molar ratio of *M. higmani* tissues suggest that Se may
413 play a protective role against Hg toxicity during the early stages of embryonic development.
414
415 **6. ACKNOWLEDGMENTS**
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422
423 **7. CONTRIBUTIONS**
424 J.S.A and T.G. conceived of the presented idea. R.A. performed the statistics. M.O.L and N.E.H
425 verified the analytical methods. T.G. supervised the findings of this work. All authors discussed
426 the results and contributed to the final manuscript.

8.DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

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Apêndice

CATÁLOGO DE PEIXES DA COSTA NORTE DO BRASIL, 2020

CARCHARHINIFORMES
CARCHARHINIDAE

Carcharhinus acronotus
Fonte: DnREC

Carcharhinus falciformes
Fonte: fishIDER

Carcharhinus leucas
Fonte: fishIDER.org

Carcharhinus porosus
Fonte: biogeodb.stri.si.edu

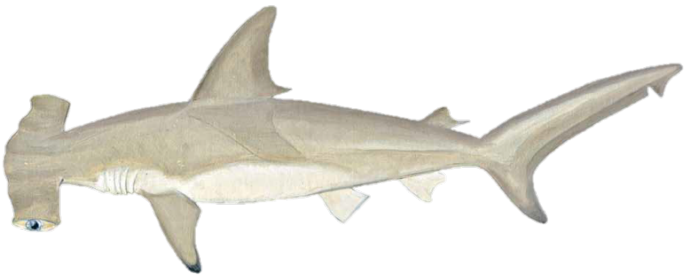
Galeocerdo cuvier
Fonte: fishIDER

Rhizoprionodon lalandii
Fonte: Peixes do Maranhão

Rhizoprionodon porosus
Fonte: Fish of the world wiki

Rhizoprionodon terranovae
Fonte: Wikimedia commons

SPHYRNIDAE



Sphyrna lewini
Fonte: Clima Pesca

Sphyrna mokarran
Fonte: Fishes from Australia

Sphyrna tiburo
Fonte: Fish identification

Sphyrna tudes
Fonte:FAO

TRIAKIDAE

Mustelus higmani
Fonte: biogeodb.stri.si.edu

CLUPEIFORMES
PRISTIGASTERIDAE

Pellona harroweri
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MYLIOBATIFORMES

GYMNURIDAE

Gymnura micrura

Fonte: Florida Museum of Natural History

DASYATIDAE

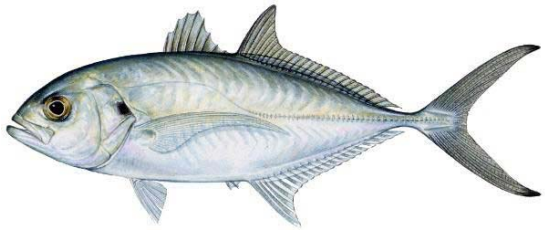
Hypanus guttatus
Fonte: Wikimedia

MYLIOBATIDAE

Rhinoptera bonasus
Fonte: Shark-references

PECIFORMES

CARANGIDAE



Caranx crysos
Fonte: Mexico Saltwater Game Fish Identification

Decapterus tabl
Fonte: Fishes of Australia

Selene setapinnis
Fonte: PET pesca

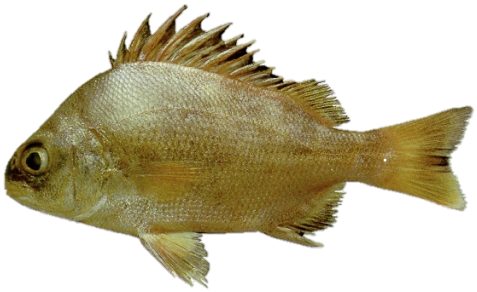
EPHIPPIDAE

Chaetodipterus faber
Fonte: Klima naturali

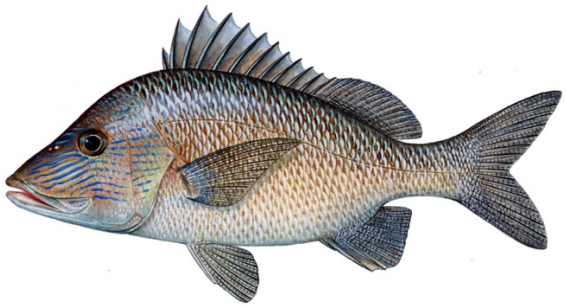
HAEMULIDAE

Anisotremus surinamensis
Fonte: Klima naturali

Conodon nobilis
Fonte: Klima naturali



Genyatremus luteus
Fonte: Fishwisepro.com



Haemulon plumieri
Fonte: Fish Index

Haemulon steindachneri
Fonte: Mexican-fish.com

LUTJANIDAE

Lutjanus analis
Fonte: Wikimedia commons

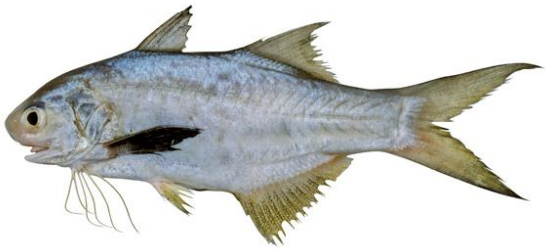
Lutjanus purpureus
Fonte: Wikipédia

Lutjanus synagris
Fonte: Mexican-fish.com

MULLIDAE

Upeneus parvus
Fonte: Wikimedia

POLYNEMIDAE

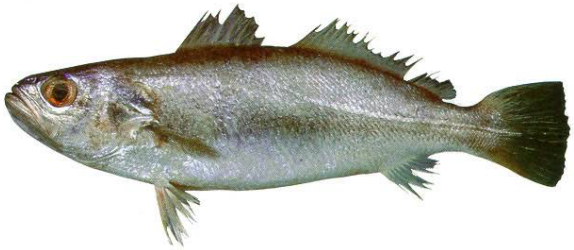


Polydactylus virginicus
Fonte: Alchetron

SCIANIDAE

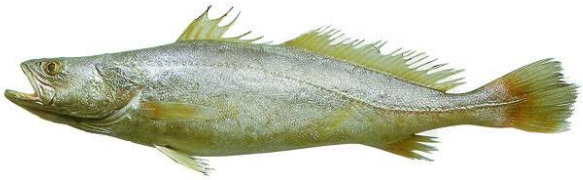
Bairdiella ronchus
Fonte: biogeodb.stri.si.edu

Ctenosciaena gracilicirrhus
Fonte: iucnredlist



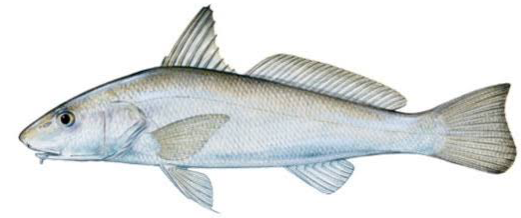
Cynoscion jamaicensis
Fonte: Fishbase

Cynoscion microlepidotus
Fonte: Wikipédia



Cynoscion virescens
Fonte: Fishbase

Macrodon ancylodon
Fonte: teas-tarts-tings



Menticirrhus americanus
Fonte: peixesdesportivosdomundo

Micropogonias furnieri
Fonte: Fao

Paralonchurus brasiliensis
Fonte: usp.br

Stellifer microps
Fonte: flickr.com

Stellifer naso
Fonte: fishwisepro

Umbrina coroides
Fonte: Fishbase

STROMATEIDAE

Peprilus paru
Fonte: alibaba.com

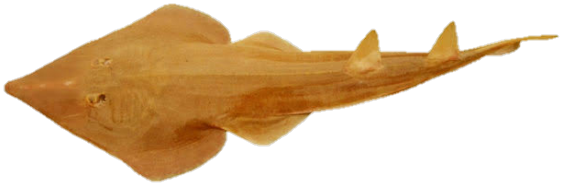
TRICHIURIDAE

Trichiurus lepturus
Fonte: wikimedia.org

PLEURONECTIFORMES
ACHIRIDAE

Gymnachirus nudus
Fonte: fishwisepro.com

RHINOPRISTIFORMES
RHINOBATIDAE

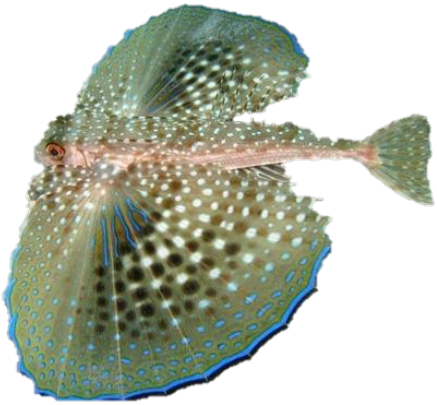


Pseudobatos horkelii
Fonte: Fishbase

Pseudobatos percellens
Fonte: Wikipedia

SCORPAENIFORMES

DACTYLOPTERIDAE



Dactylopterus volitans
Fonte: wikimedia

TRIGLIDAE

Prionotus punctatus
Fonte: Fishbase

SILURIFORMES
ARIIDAE

Bagre bagre
Fonte: guiaboya

Notarius grandicassis
Fonte: ishbiosystem

Brachyplastystoma vaillantii
Fonte: fishbase

TORPEDINIFORMES
NARCINIDAE

Narcine brasiliensis
Fonte: wikipedia

ANEXOS

ANEXO 1

Journal of FISH BIOLOGY

Journal of Fish Biology (2016)
doi:10.1111/jfb.13027, available online at wileyonlinelibrary.com

Mercury and methyl mercury in fishes from Bacajá River (Brazilian Amazon): evidence for bioaccumulation and biomagnification

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This study assessed total mercury (THg) and methyl mercury (MeHg) concentrations, bioaccumulation and biomagnification of THg through the food web in fishes consumed by indigenous communities of Bacajá River, the largest tributary of the right bank of Xingu River. In total, 496 fish (22 species) were sampled. Nine species had THg concentrations above the limit recommended by the World Health Organisation (0·5 μg g^{-1} wet mass), and one exceeded the recommended level for Hg in predatory fishes by Brazilian law (1·0 μg g^{-1}). The average concentration of THg increased significantly with trophic guild (herbivorous to piscivorous) and trophic level, with higher accumulation in fishes with greater total length. Ninety-six per cent of all mercury was methylated. These results suggest that feeding habits determine THg concentrations in fishes and that Hg elimination rate is slow during growth, which allows greater accumulation. These findings show that fishes in the Bacajá River contain high concentrations of THg and MeHg.

Key words: Hg contamination; Hg transfer; indigenous lands; Xingu basin.

INTRODUCTION

Mercury is a major environmental pollutant of current concern. Found in elemental, ionic and methylated forms, it is a highly toxic metal. Overexposure to mercury, especially methyl mercury, can cause health damage (Chan, 2011; Debes *et al.*, 2015). Many cases of mercury contamination have occurred worldwide in areas with historical mining activity, areas exposed to the direct influence of petrochemical and coal combustion plants, and areas with metal smelting industries (Zhang & Wong, 2007; Mieiro *et al.*, 2011; Nevado *et al.*, 2012).

It was thought that mercury contamination of aquatic ecosystems in the Amazon was a result of gold mining; however, it was demonstrated that 90% of the mercury found in soil is of natural origin and associated with iron (Veiga *et al.*, 1994; Barbosa *et al.*, 1998; Maurice-Bourgoin *et al.*, 2000). Mercury present in particulate matter is

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ANEXO 2

International Journal of Ecology and Environmental Sciences 42 (4): 251-255, 2016
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Short Communication

Mercury Levels in Fish Marketed in the Metropolitan Region of Belém, Pará, Brazil

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ABSTRACT

Mercury is an environmental contaminant found in in aquatic ecosystems, derived from both manmade and natural sources. Studies of mercury contamination in fish have focused on areas with a known history of contamination, such as large rivers and their tributaries. As few data are available on the contamination of fish by heavy metals, the major urban centers in the Amazon basin have been surveyed, and in the present study, the mercury levels in the principal fish species marketed in some of the largest retailers in the city of Belém were evaluated. Samples were collected in March 2013 from the city's principal supermarkets and street markets, either in the form of whole fish or processed portions. A sample of 10-20 g of muscle tissue was taken from each specimen for preparation and analysis in a Cold Vapor Atomic Absorption Spectrometer. Only four of the 28 species analyzed presented mercury concentrations higher than those permitted by the World Health Organization (0.5 $\mu g.g^{-1}$). While contamination may be partly related to the feeding habits of the species, environmental variables are the principal determinants of contamination. Given this, there is a clear need for the monitoring of mercury contamination levels in fish supplies, and the careful evaluation of the supply chain, in order to minimize any major future risks to public health.

Key Words: Urban Center, Amazon, Contaminated Fish, Trace Element, Fairs and Supermarkets, Trophic Levels.

ANEXO 3

Biota Neotropica 19(4): e20190792, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Short Communication

Morphological abnormality in a Longnose Stingray *Hypanus guttatus* (Bloch & Schneider, 1801) (Myliobatiformes: Dasyatidae)

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SCHMID, K., ANDRADE. M., MACHADO, F., ARAUJO, J. CORRÊA, E., GIARRIZZO, T. **Morphological abnormality in a Longnose Stingray *Hypanus guttatus* (Bloch & Schneider, 1801) (Myliobatiformes: Dasyatidae)**. Biota Neotropica. 19(4): e20190792. http://dx.doi.org/10.1590/1676-0611-BN-2019-0792

Abstract: A Longnose stingray *Hypanus guttatus* (Bloch & Schneider, 1801) embryo with a major asymmetrical morphological abnormality to its pectoral fin was obtained from commercial shrimp fisher's bycatch, off the coast of the Amazon River Mouth in northern Brazil. The specimen and the deformity, which would presumably have impeded its long-term survival, are described and documented in detail. We herein provide the first report of an abnormal individual of this species for the Brazilian coast.
Keywords: *Fish, Elasmobranchs, Atlantic, Teratology, Deformity.*

Anormalidade morfológica em uma Arraia-bicuda *Hypanus guttatus* (Bloch & Schneider, 1801) (Myliobatiformes: Dasyatidae)

Resumo: Um embrião de Arraia-bicuda *Hypanus guttatus* (Bloch & Schneider, 1801) com uma anomalia morfológica assimétrica grave na nadadeira peitoral foi obtido como captura-acidental de um barco de pesca de camarão da costa da foz do rio Amazonas, no norte do Brasil. O indivíduo e sua deformação, que provavelmente teria impedido sua sobrevivência ao longo prazo, são descritos e documentados em detalhe. Apresentamos com esse estudo o primeiro registro de um indivíduo anormal dessa espécie para a costa do Brasil.
Palavras-chave: *Peixes, Elasmobrânquios, Atlântico, Teratologia, Deformação.*

ANEXO 4

XIV Congresso Brasileiro de Ecotoxicologia
07 - 10. Setembro. Curitiba - PR

Biodisponibilidade, bioacumulação e biomagnificação

Oral

142 - CONCENTRAÇÃO DE MERCÚRIO TOTAL E METILMERCÚRIO EM MÚSCULO DE *Podocnemis unifilis* (TROSCHEL DE 1848) (PODOCNEMIDIDAE: TESTUDINES) DE UMA ÁREA INDÍGENA NO RIO BACAJÁ - AMAZÔNIA, BRASIL

PENICHE, D. M., GIARRIZZO, T., LIMA, M. O., SOUZA, M. B. G., ARAÚJO, J. S.

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Palavras-chave: mercúrio; metilmercúrio; Podocnemis unifilis; Bacajá

INTRODUÇÃO

O mercúrio é um contaminante presente nos ecossistemas aquáticos por motivos antrópicos e naturais. As formas orgânicas têm grande toxicidade aos organismos como quelônios, podendo gerar riscos ao consumo destes. Grande parte das pesquisas sobre contaminação por mercúrio utilizam peixes, sendo a utilização de tartarugas ainda escassos. Estudos com *Podocnemis unifilis* (tracajá) do rio Bacajá são de suma importância pois seu consumo é apreciado pela população indígena, e o rio em questão possui alguns garimpos clandestinos. Sendo assim, o objetivo deste estudo foi verificar as concentrações de Hgt e MeHg em tracajás capturados e consumidos na TI Trincheira do Bacajá.

METODOLOGIA

As amostras de tecido muscular dos quelônios foram coletadas em dezembro de 2011, durante uma expedição científica na Trincheira do Bacajá, terra indígena localizada entre as coordenadas 5° 5 '38,9

RESULTADOS E DISCUSSÃO

Foram capturados e analisados oito indivíduos *Podocnemis unifilis* dos quais possuíam CCL médio de 31,58 ± 5,05 cm, em um intervalo entre 24,5 cm e 39,4 cm. A média de HgT no músculo das tracajás foi de 0,070 ± 0,116 µg.g-1 em um intervalo entre 0,004 µg.g-1 (CCL=26,8cm) a 0,282 µg.g-1 (CCL=33cm). As concentrações são esperadas para essa espécie, tendo em vista que alimentação é predominantemente a base de plantas, insetos, crustáceos e moluscos.

Das oito amostras de mercúrio total analisadas, três foram destinadas para análise de metilmercúrio, representando 98% do mercúrio total detectado (R^2 = 0,987; MeHg = – 0,0014 + 1,093*HgT). Fato este que corresponde à proporcionalidade de 1:1 esperado para a região amazônica. As características físico-químicas do rio Bacajá como: pH= 6,77; condutividade = 55,38 µS/cm; alcalinidade = 23,21 mg-CaCO3/L; N total dissolvido = 0,68 mg/L; P total dissolvido = 62,36 µg/L; sólidos totais dissolvidos = 0,03 g/L; C inorgânico dissolvido = 12,94 mg/L; C orgânico dissolvido = 3,97 mg/L; Na = 3,27 mg - Na/L; K = 1,60 mg - K/L; Mg = 0,85 mg - Mg/L; Ca = 1,67 mg - Ca/L podem estar favorecendo à biodisponibilidade do mercúrio em seu processo de metilação. Além disso, a presença de focos de desmatamento e atividade ilegal de extração de ouro recorrente nas cabeceiras do rio podem ocasionar maior liberação de mercúrio no leito do rio Bacajá. A concentração média de metilmercúrio foi de 0,005 ± 0,002 µg.g-1.

HgT em água foi menor que 0,001 µg.g-1, ou seja, inferior ao limite estabelecido para a resolução CONAMA 357/2005 para a classe 1 de água, que é de 0,2 mg/L. O fator de bioacumulação – BAF – foi igual a 7,77. Isto indica que a maior parte de todo o mercúrio é proveniente da alimentação.

ANEXO 5

XIV Congresso Brasileiro de Ecotoxicologia
07 - 10. Setembro. Curitiba - PR

Integração de ecossistemas e saúde humana

Painel

507 - CONCENTRAÇÃO DE MERCÚRIO NOS *Caiman crocodilus* E *Melanosuchus niger* E SEUS POSSÍVEIS IMPACTOS NEGATIVOS NA DINÂMICA DO MERCÚRIO NOS SISTEMAS AQUÁTICOS DO RIO XINGU. - AMAZONIA , BRASIL

TOURINHO, I. G. R., GIARRIZZO, T., LIMA, M. O., ARAÚJO, J. S.

bellygatti@gmail.com, tgiarrizzo@gmail.com, marcelolima@iec.pa.gov.br, j.araujo.bio@gmail.com

Palavras-chave: Mercúrio; Impactos; Rio Xingu.

INTRODUÇÃO

O mercúrio, além de tóxico é bioacumulável, capaz de acumular-se nos tecidos dos organismos dos níveis mais altos das cadeias alimentares. As espécies pesquisadas, por serem carnívoros, tem elevado potencial de bioacumulação de mercúrio sendo o nível crítico (500 nanogramas por grama - peso úmido), estabelecido para consumo humano pela Organização Mundial de Saúde e pela Agência de Proteção Ambiental do Estados Unidos. Desta forma, o trabalho tem o proposito de mostrar seus possíveis impactos negativos para as comunidades próximas ao Rio Xingu, sendo de suma importância pesquisar para o conhecimento da concentração de mercúrio em jacarés e seus ovos.

METODOLOGIA

Foram coletadas amostras de *Caiman crocodilus* e *Melanosuchus niger*, junto com a coleta de ovos, no Xingu em outubro de 2011. Espécimes foram capturados manualmente, próximo ao município de Senador José Porfírio na região do baixo Xingu, com o auxílio de um pulsar, os animais foram sacrificados por meio do corte da espinal medula. Os animais foram necropsiados e os tecidos foram recolhidos para diversas análises. Recolhemos uma amostra composta de várias peças de músculo do cubo de cada lado da cauda. Um bisturi descartável fresco e luvas foram utilizadas entre amostras para evitar a contaminação, e cada amostra de compósito foi colocada em um saco fechado, marcado, mantido em gelo e congelados para análise posterior. As amostras de tecido de fígado, músculo, gordura e couro da cauda foram excisados a partir de cada animal, buscou-se selecionar crocodilos com uma variedade de tamanhos e incluindo machos e fêmeas. Sete amostras eras de *Caiman crocodilus* (machos) e dez eram de *Melanosuchus niger* (seis machos e quatro fêmeas).

O teste t de Student foi utilizado no laboratório analítico para testar as concentrações e diferenças entre os níveis de mercúrio em macho e fêmeas, e os níveis encontrados nos ovos e cascas.

RESULTADOS E DISCUSSÃO

Ao analisar as amostras, os níveis de mercúrio no músculo dos jacarés variou de 0,010 ppm para 0,066 ppm , todos abaixo do nível de ação da OMS para o consumo humano . Todas as amostras foram combinadas (n = 17) em média 0,015 ppm de mercúrio. Amostras de ambos os sexos de *Caiman crocodilus* foram mais elevadas (0,079 ± 0,066 ppm , n = 7) do que aqueles de *Melanosuchus niger* (0,038 ± 0,003 ppm; n = 10). Nos C. crocodilos os níveis de mercúrio no couro foram de 0,919 ± 0,006 ppm, menor que nos *M. niger* que foram 3,173 ± 0,013 ppm. Nas analises de mercúrio no fígado teve em média 0,042 ppm e de gordura 0,014 ppm. Os níveis de mercúrio para todos os machos foram 0,079 ± 0,003 ppm (n = 13) e para todas as fêmeas 0,038± 0,010 ppm (N = 4). Nas analises dos ovos (n=4) encontrados, os níveis de mercúrio foram de 0,070 ± 0,0005 ppm e nas cascas foram de 0,012 ± 0,0005 ppm. Os resultados até agora sugerem que a carne de jacaré e o ovo próximo ao município de Senador José Porfírio na região do baixo

ANEXO 6

Painel

Biodisponibilidade, bioacumulação e biomagnificação

27 - VARIAÇÃO SAZONAL DE MERCÚRIO EM *Hemiodus unimaculatus* (CHARACIFORMES: HEMIODONTIDAE)(BLOCH, 1794) NO RIO XINGU, AMAZÔNIA, BRASIL

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Palavras-chave: biodisponibilidade; metais pesados; onívora

INTRODUÇÃO

O Rio Xingu tem aproximadamente 1450 km de extensão, nasce no estado do Mato Grosso e desagua pela margem direita do Rio Amazonas. A variação máxima da vazão se dá entre os meses de Outubro (seca) - 2.000 m³/s e Abril (cheia) - 8.000 a 10.000 m³/s. A inundação sazonal da floresta adjacente e concomitantemente as alterações nas condições abióticas podem influenciar na biodisponibilidade de elementos como o mercúrio (Hg). Tendo em vista isso, o nosso objetivo foi verificar a variação sazonal na concentração de Hg em *Hemiodus unimaculatus*, uma espécie onívora do rio Xingu.

METODOLOGIA

A área de estudo está localizada em um trecho do rio Xingu que vai desde a confluência com o rio Iriri até confluência com o rio Bacajá. Foram coletados 15 indivíduos da espécie *Hemiodus unimaculatus* em cada período de seca e cheia respectivamente de 2013 e 2014. Após feita a identificação e biometria, de cada indivíduo foi retirado de 10-20 g de tecido muscular livre de espinhas e escamas, armazenados em gelo e posteriormente congelados até o momento de análise para mensuração do Hg. Para determinação de Hg, as amostras foram homogeneizadas e foi utilizada Espectrometria de Absorção Atômica com Vapor, com controle de qualidade a partir do uso do material de referência certificada DOLT-3. Também foram mensurados parâmetros físico-quimicos como: pH, condutividade, alcalinidade, Oxigênio dissolvido e temperatura, com auxílio de Sonda Multiparamétrica modelo YSI 6600. A variação sazonal na concentração de THg entre os períodos de seca e cheia, foi testada com PERMANOVA univariada em matrizes de distâncias Euclidianas com base em 9999 permutações (ANDERSON, 2001). Os cálculos e testes foram realizados usando o PERMANOVA+ para o software PRIMER-E (ANDERSON et al., 2008).

RESULTADOS E DISCUSSÃO

Os parâmetros físico-químicos mostraram que em ambos os períodos apresentam bons níveis de oxigenação, as concentrações médias de oxigênio dissolvido variaram de 5,14mg/L na cheia a 7,29 mg/L na seca. No período cheio a temperatura da água variou entre 27,2 °C a 27,7 °C, já na seca, devido ao menor volume de água, a temperatura variou de 29,8 °C a 31,1 °C. O pH da água estava na faixa de acidez (abaixo de 7,0) em todas as leituras, e os valores de alcalinidade

ANEXO 7

Painel

Biodisponibilidade, bioacumulação e biomagnificação

29 - NÍVEIS DE MERCURIO EM ESPÉCIES CARNÍVORAS DE IMPORTÂNCIA ALIMENTAR NA BACIA DO RIO XINGU-PA, BRASIL

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Palavras-chave: metais pesados; biomagnificação; bioacumulação

INTRODUÇÃO

A poluição por mercúrio (Hg) pode ocasionar sérios danos à saúde humana, animal e ao meio ambiente, com grande prejuízo a biota aquática devido sua elevada toxidade, particularmente na forma metilmercúrio (MeHg). Rejeitos provenientes da mineração, depositados no ambiente oriundos de garimpos; implantações de barragens e desmatamento ocasionam a acentuação desse contaminante. Além disso, a capacidade de Bioacumulação e Biomagnificação permite que o mercúrio atinja elevadas concentrações em organismos topo de cadeia. Com isso, o objetivo deste trabalho visa mensurar as concentrações de Hg em peixes carnívoros da Bacia do Xingu.

METODOLOGIA

A bacia hidrográfica do Rio Xingu abrange uma área de 509.000 km², possui aguas claras e é pobre em nutrientes e matéria orgânica, sua produtividade depende de fontes externas. O Rio Xingu é caracterizado por existir em seu leito trechos onde ocorre a presença de garimpos clandestinos, barragens e a UHE de Belo Monte. Para análise de Hg foram selecionadas as espécies-chave *Boulengerella cuvieri*, *Phractocephalus hemioliopterus*, *Potamotrygon leopoldi*, *Serrasalmus manueli*, *Serrasalmus rhombeus* e *Tocantinsia piresi*, sendo coletados de 14 a 29 indivíduos de cada espécie totalizando 119 indivíduos entre os anos de 2013-2014. Foram retirados de 10-20 g de tecido muscular livre de espinhas e escamas, armazenados em gelo e posteriormente congelados até o momento de análise para mensuração do Hg. Para determinação de Hg foi utilizada Espectrometria de Absorção Atômica com Vapor. Também foram mensurados parâmetros físico-quimicos como: pH, condutividade, Oxigênio dissolvido e temperatura, com auxílio de Sonda Multiparamétrica modelo YSI 6600. Os resultados obtidos nessa coleta foram comparados a dados coletados em outras localidades também da Bacia Amazônica.

RESULTADOS E DISCUSSÃO

Os resultados apresentados das análises dos parâmetros físico-químico revelaram que trecho estudado apresenta bons níveis de oxigenação, indicando um bom equilíbrio entre a produção fotossintética e o consumo produzido pelos processos metabólicos. A temperatura variou de 27,24 a 31,39 ºC, pH 6,48 a 7,39, Condutividade 0,01 a 0,03 mS/cm, Oxigênio dissolvido 5,14 a 7,29 mg/l, alcalinidade 7,27 a 10,52 mg-CaCO3/l, não sofrendo, portanto, grandes variações entre os períodos de cheia e seca.

ANEXO 8

Painel

Biodisponibilidade, bioacumulação e biomagnificação

46 - MERCÚRIO E ARSÊNIO EM TUBARÕES MARTELO DA COSTA NORTE DO BRASIL

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Palavras-chave: Elasmobrânquios; Elementos traço; Poluição marinha

INTRODUÇÃO

O tubarão martelo habita em regiões tropicais e temperadas, tendo preferência por clima subtropical. Ocorre perto da costa e da plataforma continental. Devido ao processo de biomagnificação, espécies como tubarão martelo tendem a acumular maiores concentrações de mercúrio em seus tecidos, podendo ocasionar danos à saúde reprodutiva, cognitiva dos animais e até mesmo morte. Na costa norte do brasil, efluentes industrias e domésticos contribuem diretamente para o aumento da contaminação do ambiente. O objetivo desse trabalho é apresentar informações sobre contaminação por elementos traço em indivíduos juvenis e neonatos do tubarão martelo.

METODOLOGIA

A área de estudo corresponde à área de atuação da pesca artesanal e industrial na plataforma da costa Norte do Brasil, em um trecho que compreende os estados do Amapá, Pará e parte do Maranhão, desde o cabo do Oiapoque – AP até a baía de São Marcos – MA (04°S; 50°W a 01°S; 44°W). A região em questão é formada pela descarga dos rios Amazonas ao norte e rio Tocantins ao sul da Ilha de Marajó e mistura de aproximadamente 6.300 km3/ano de águas continentais e 9,3 x 108 t/ano de sedimentos com águas oceânicas (MEADE et al., 1979).

Os indivíduos capturados foram identificados e medidos o comprimento total (CT). De cada indivíduo foi coletado uma amostra de aproximadamente 10 g de músculo. A determinação de elementos traço nas amostras foi realizada por Espectrometria de Massas Acoplada com Plasma Induzido (ICP-MS). Todas as análises foram realizadas em duplicata e para o controle de qualidade analítica foram utilizadas amostras de referência certificadas de (CRM) DOLT-3.

RESULTADOS E DISCUSSÃO

Os resultados da análise de 12 indivíduos do Gênero *Sphyrna sp*. Apresentou media para o hg de 0,09± 0,06 mg/kg com min-máx. 0; 0,2 mg/kg. Para o nível de as 10,4 ± 3,89 mg/kg, min-máx. com (1,63 e 17,9 mg/kg). Os resultados encontrados para o nível de hg indicaram baixas concentrações nas espécies da costa Norte do Brasil em relação as demais regiões coletadas. Na Baja Califórnia Sur, México (ESCOBAR-SANCHEZ et al., 2010) a min-máx. Da concentração foi de 0,005 a 1,93, Califórnia,(BERGÉS-TIZNADO et al.,2015), (0,12 a 1,17), norte do Peru, (GONZALEZ PESTANA et al., 2017)(0,13 e 0,86), costa leste da África do Sul (MCKINNE, et al.,2016), (0,1 mg/kg-1), Trinidad e Tobago (MOHAMMED &

ANEXO 9

Painel

Efeito de contaminantes inorgânicos

5 - ASSESSING TOXIC ELEMENTS IN AN ABNORMAL SPECIMEN OF *Hypanus guttatus* (MYLIOBATIFORMES: DASYATIDAE) FROM THE NORTH COAST OF BRAZIL

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Keywords: Trace elements; ICP-MS, Elasmobranchii; Marine toxicology; Amazonian Coast

INTRODUCION

In the last decades, studies have shown that the presence of elements like cadmium and zinc in the aquatic environments can lead to effects on the developing embryo of fishes, due to the permeability of these elements through the chorion of fish eggs, altering egg incubation time and causing eye, jaw, and spinal deformities (ZEITOUN and MEHANA, 2014). The present study shows trace elements concentrations found in muscle tissue of an embryo of the Longnose stingray, *H. guttatus* with morphological abnormality captured off the Amazon coast of northern Brazil.

METHODS

The *H. guttatus* specimen was caught approximately 200 km off the mouth of the Amazon River, in northern Brazil (1°23'54.4" N 48°07'37.2" W) in February 2017. The specimen was immediately stored on ice and later deposited in a frozen condition. For analysis, the embryo was defrosted and weighed, and a sample of the muscle tissue was obtained for the analysis of its metal content. The concentrations of metals in the tissue of the specimen were determined by Induced Plasma Coupled Mass Spectrometry. 0.05 g aliquots were transferred to PTFE bottles with 1 ml of HNO_3 concentrated and 0,5 ml of H_2O_2. The samples were then heated in a microwave oven. The digested solution was transferred to polyethylene bottles, completed to 15 ml with HNO_3 (1%) and stored at 4°C until the ICP-MS analysis. A quality control sample, the DORM-3 (Dogfish Muscle) Certified Reference Material (National Research Council of Canada), was analyzed simultaneously with the the experimental samples and the percentage recovery rate was determined for data validation. The recovery of the trace elements was in average more than 90%.

RESULTS AND DISCUSSION

The ray caught was an embryo and had abnormalities at the head and pectoral fin, which was incompletely fused with the head, beyond a tail long coiled. A total of 16 elements were analyzed: Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Cd, a, Hg, Tl, P, U. Most of these elements, including toxic, non-essential metals such as Hg (0.03 mg kg^{-1}) and As (0.35 mg kg^{-1}), were present at low concentrations when compared to results found in similar species. However, zinc was found at an extremely high concentration, of 37.49 mg kg^{-1}.