UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

Utilização de ferramentas biológicas e geoquímica como indicadoras do status de saúde de dois estuários tropicais no Nordeste do Brasil

Ítala Gabriela Sobral dos Santos

Tese a ser apresentada ao Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura da Universidade Federal Rural de Pernambuco como exigência para a obtenção do título de Doutor.

Prof. Dr^{a}. Flávia Lucena Frédou
Orientadora

Prof. Dr^{a}. Rossineide Martins da Rocha
Co-Orientadora

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1. Prof. Dra. Flávia Lucena-Frédou (Membro Titular e Orientadora);
2. Prof. Dr. William Severi (Membro Interno – Titular);
3. Dra. Latifa Pélage (Membro Interno – Titular);
4. Prof. Dra. Lílian Lund Amado (Membro Externo – Titular);
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Resumo

Os ecossistemas marinhos e estuarinos da costa brasileira formam um mosaico de ambientes interconectados através do fluxo de energia e por espécies que ocupam diferentes habitats ao longo de seu ciclo de vida, sustentando inúmeras atividades econômicas como pesca ou turismo. O Nordeste é uma das regiões costeiras mais densamente povoadas do Brasil, destacando-se pela urbanização e degradação dos ecossistemas, resultado de múltiplos impactos, principalmente devido à poluição doméstica, atividade agro-industrial, e modificação do habitat em torno dos principais centros urbanos. O presente estudo teve como objetivo o uso de diferentes técnicas complementares para diagnosticar a qualidade ambiental de dois estuários tropicais em Pernambuco, Nordeste do Brasil: o estuário da Barra de Sirinhaém (SIR), no litoral sul de Pernambuco e o estuário do Canal de Santa Cruz (ITAP), litoral norte. Como ferramentas para este diagnóstico foram utilizados a integração de ferramentas biológica e geoquímica através da mensuração dos metais nos peixes e no sedimento, o uso de biomarcadores histopatológicos nas brânquias e no fígado, e aplicação do isoscape associado a isótopos $\delta^{13}C$ e $\delta^{15}N$ como biomarcadores de poluição através do matéria orgânica no sedimento (MOS). No Capítulo 1, avaliamos as concentrações de metais traços nos peixes (*Bardiella ronchus*, *Caranx latus*, *Centropomus undecimalis*, *Centropomus parallelus* e *Gobionellus stomatus*) e no sedimento, a partir da matriz abiótica observamos que, em SIR, a contaminação está a nível de alerta apenas para Cu de acordo com o Guia de qualidade de sedimentos do Canadá, e os demais metais (Cd, Cr Hg, Pb e Zn) são classificados como com baixa probabilidade de causar efeitos na biota. Em ITAP, os metais Cd, Cr, Pb e Zn no sedimento estiveram classificados como baixa probabilidade de causar efeitos deletérios na biota. Adicionalmente, ao utilizar vários índices aplicados no sedimento e através da concentração de metais nos peixes, também foram observados baixo risco através da avaliação de risco ecológico. Entretanto, foi observada contaminação por Cu e principalmente o Hg em ITAP, e este impacto persistente do Hg foi refletido com valores acima do permitido para ingestão de pescado nas espécies *Centropomus undecimalis* e *Bardiella ronchus.* No Capítulo 2, com o uso de biomarcadores histopatológicos, permitiu observar que os efeitos desses metais e dos demais agentes contaminantes presentes nos estuários, SIR e ITAP, resultaram em um impacto com grau e severidade de lesões graves nos peixes. No capítulo 3 usamos isótopos estáveis $\delta^{13}C$ e $\delta^{15}N$ com os modelos de mistura e isoscape aplicados em sedimentos e peixes para avaliar os impactos antropogênicos presentes em SIR. Através destas técnicas foi observado que a assinatura isotópica da SOM para $\delta^{13}C$ era mais alta no estuário superior próximo da indústria de cana de açúcar e de outros efluentes domésticos, em comparação com o estuário inferior próximo do mar. O $\delta^{15}N$ do SOM variou espacialmente no estuário sendo mais alta no estuário inferior, devido aos efluentes da indústria canavieira transportados da parte superior do estuário e à descarga de efluentes. Estas ferramentas foram adequadas como forma de biomonitoramento, especialmente o modelo de mistura para avaliar a contribuição de regiões com maior aporte de contaminantes na dieta das espécies *C. undecimalis*, *C. paralleus e Caranx latus*. Baseados nos três capítulos, as espécies supracitadas possuem atributos ideais para serem utilizadas como biomonitoras de contaminação, especialmente as espécies *C. undecimalis e C. paralleus*. Todas as metodologias e análises realizadas convergiram para o mesmo resultado. O ITAP está potencialmente mais contaminado do que o SIR, sendo este último considerado uma região com baixo impacto. A presente tese utilizou técnicas eficientes e facilmente aplicáveis e replicáveis em outras áreas de estudo, com o intuito de fornecer subsídios à gestão ambiental dos estuários e os efeitos da poluição na biota, além da relevância no âmbito dos avanços nas pesquisa na área de recursos pesqueiros e aquicultura.

Palavras-chave: metais traços; isotópos estáveis; histopatologia; fator de risco ecológico; *Centropomus undecimalis.*

Abstract

The marine and estuarine ecosystems of the Brazilian coast form a mosaic of environments interconnected through energy flow and by species that occupy different habitats throughout their life cycle, sustaining numerous economic activities such as fishing or tourism. The Northeast is one of the most densely populated coastal regions of Brazil, standing out for urbanization and degradation of ecosystems, the result of multiple impacts, mainly due to domestic pollution, agro-industrial activity, and habitat modification around major urban centers. The present study aimed to use different complementary techniques to diagnose the environmental quality of two tropical estuaries in Pernambuco, Northeast Brazil: the estuary of Barra de Sirinhaém (SIR), on the southern coast of Pernambuco and the estuary of Canal de Santa Cruz (ITAP), northern coast. As tools for this diagnosis were used the integration of biological and geochemical tools through the measurement of metals in fish and sediment, the use of histopathological biomarkers in gills and liver, and application of isoscape associated with $\delta^{13}C$ and $\delta^{15}N$ isotopes as biomarkers of pollution through organic matter in sediment (SOM). In Chapter 1, we evaluated the concentrations of trace metals in fish (*Bardiella ronchus, Caranx latus, Centropomus undecimalis, Centropomus parallelus* and *Gobionellus stomatus*) and in sediment, from the abiotic matrix we observed that, in SIR, the contamination is at the alert level only for Cu according to the Canadian Sediment Quality Guide, and the other metals (Cd, Cr Hg, Pb and Zn) are classified as with low probability of causing effects in biota. In ITAP, the metals Cd, Cr, Pb and Zn in the sediment were classified as low probability to cause deleterious effects on biota. Additionally, by using various indices applied to the sediment and through metal concentration in fish, low risk was also observed through the ecological risk assessment. However, Cu and especially Hg contamination was observed in ITAP, and this persistent impact of Hg was reflected with values above the allowable for fish ingestion in the species *Centropomus undecimalis* and *Bardiella ronchus*. In Chapter 2, using histopathological biomarkers, it was observed that the effects of these metals and the other contaminants present in the estuaries, SIR and ITAP, resulted in an impact with a degree and severity of severe injury to fish. In chapter 3 we used stable isotope $\delta^{13}C$ and $\delta^{15}N$ with the mixing and isoscape models applied to sediment and fish to assess the anthropogenic impacts present in SIR. Using these techniques, it was observed that the isotopic signature of the SOM for $\delta^{13}C$ was higher in the upper estuary near the sugarcane industry and other domestic effluents, compared to the lower estuary near the sea. The $\delta^{15}N$ of the SOM varied spatially in the estuary being higher in the lower estuary due to the sugarcane industry effluents transported from the upper part of the estuary and the effluent discharge. These tools were suitable as a form of biomonitoring, especially the mixing model to evaluate the contribution of regions with higher inputs of contaminants in the diet of *C. undecimalis, C. paralleus* and *Caranx latus*. Based on the three chapters, the species have ideal attributes to be used as biomonitors of contamination, especially the species *C. undecimalis* and *C. paralleus*. All methodologies and analyses performed converged to the same result. The ITAP is potentially more contaminated than the SIR, the latter being considered a region with low impact. This thesis used efficient techniques that are easily applicable and replicable in other areas of study in order to provide subsidies for the environmental management of estuaries and the effects of pollution on biota, as well as relevance to the advancement of research in the area of fishery resources and aquaculture.

Key words: Trace metals; stable isotopes; histopathology; ecological risk factor; *Centropomus undecimalis*.

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Sumário

INTRODUÇÃO

Os estuários estão entre os ecossistemas que mais recebem aporte de contaminantes oriundos das atividades antropogênicas, que afetam negativamente a saúde dos seres humanos e da biota (Carr et al., 2017; Salas et al., 2017). A contaminação de ecossistemas aquáticos vem aumentando exponencialmente nas últimas décadas, especialmente em estuários (Ramos e Silva et al., 2017;Cabral et al., 2019;), onde a dinâmica das marés favorece o deslocamento de contaminantes como metais pesados, esgotos e efluentes industriais e efluentes (Dang et al., 2015; Wolanski et al., 2019; Perina and Abessa, 2020; Souza et al., 2021).

A poluição estuarina tem relevância global devido à multiplicidade de atividades potencialmente poluidoras presentes em suas margens, como pecuária, portos, agricultura, e o lançamentos de esgoto doméstico e efluentes industriais (Elliott et al., 2019). Dentre eles, as descargas de efluentes domésticos, industriais e agrícolas possuem o maior potencial de contaminação e eutrofização nestes ecossistemas (Nie et al., 2018; Saldarriaga-Hernandez et al., 2020). As indústrias de curtimento de couro, cimento, soda cáustica, mineração, anticorrosivo, fertilizantes e lavanderia têxtil estão entre as que mais ameaçam o desequilíbrio dos ecossistemas com o lançamentos de metais traços (Pacyna et al., 2010; Aslam and Yousafzai, 2017; Becker et al., 2018; Silva et al., 2018; Vieira et al., 2019). Além disto, pesticidas e herbicidas utilizados na agricultura, como o cultivo de cana-de-açúcar, disponibilizam Pesticidas e herbicidas, utilizados no cultivo da cana-de-açúcar, disponibilizam os metais Cd, Cu, Hg e Zn presentes como impurezas nestes produtos para o ambiente, favorecendo o aumento do impacto da atividade nos estuários (Yadav et al., 2010;Chavan and Muley, 2014; Coelho et al., 2018).

Particularmente os metais, quando lançados no ambiente, assumem duas vias: no sedimento e na biota (Ali et al., 2019). O sedimento atua como repositório final e os metais depositados podem ficar indisponíveis ou ser redisponibilizados para coluna de água (Masindi and Muedi, 2018; Yan et al., 2020). O sedimento, além de ser um compartimento ativo devido à conectividade sedimento-água, pode ser considerado um arquivo de ações poluidoras ao longo do tempo (Bakary et al., 2015; Martín-Torre et al., 2017; Rodgers et al., 2019). A acumulação dos poluentes no sedimento ocorre devido à sua alta estabilidade e baixa variabilidade , permitindo o monitoramento da contaminação temporal e espacialmente (Costa et al., 2016). Os metais no sedimento sofrem adsorção, dessorção, redução-oxidação, atividade biológica e processos diagenéticos (Mondal et al., 2018; Ranjan et al., 2018).

Os metais são adsorvidos na fracção fina do sedimento (< 63µm), tornando-se parte do ciclo sedimentar em suspensão por ação da maré, favorecendo o movimento de elementos de um local para outro (Melo et al., 2015). Esta flutuação dos poluentes no sedimento favorece a sua incorporação ao longo da cadeia alimentar, quer diretamente por bioacumulação ou indiretamente dependendo da espécie química biodisponível por biomagnificação, tornando o monitoramento dos sedimentos um alvo importante nos estudos de poluição (Hsu et al., 2016; Ramachandra et al., 2018). Consequentemente, o estudo dos impactos na biota tem relação com a biodisponibilidade dos metais, pois sua fração biodisponível sofre influência direta das variáveis ambientais (Adams et al., 2020). Especialmente nos peixes, estas diferentes espécies químicas dos contaminantes que bioacumulam, o fazem diretamente através do exercício das suas funções vitais, como a respiração e metabolização de nutrientes, sendo acumulados nas brânquias e no fígado, respectivamente, e através da biomagnificação com o MeHg (Filote et al., 2021; Savoca and Pace, 2021). Desta maneira, é fundamental o estudo dos dois compartimentos, biótico e abiótico para o monitoramento ambiental.

Entre os meios para avaliar a contaminação nos sedimentos, o mais utilizado e amplamente difundido na legislação nacional e internacional é a quantificação da concentração de contaminantes como metais, nutrientes e matéria orgânica, avaliando posteriormente a probabilidade destes causarem efeitos deletérios na biota (CCME, 2001; CONAMA, 2012). Entretanto, existem outras metodologias para avaliar a qualidade ambiental dos sedimentos, tais como o fator de contaminação (CF), índice de geo-acumulação (Igeo), multi-fator Nemerow (Pi) e, fator de risco ecológico (ERi), que estão entre os índices mais utilizados para estabelecer a fonte, magnitude da poluição por metais e a avaliação do risco ecológico destes poluentes no ambiente. (Wei and Yang, 2010; Ghrefat et al., 2011; Khuzestani and Souri, 2013). No entanto, no Brasil, estes métodos não são exigidos pela legislação.

Biomonitor tem por definição o organismo (ou uma parte de um organismo) que integram informações acerca das variáveis quantitativas da qualidade do ambiente, também podem evidenciar os efeitos através de respostas especificas, como alterações em características morfológicas, histológicas ou estrutura celular, frente à exposição a um determinado agente tóxico ou elementos (Market et al., 2003).

O uso de biomonitores fornece informações sobre a qualidade do ambiente (Sumudumali e Jayawardana, 2021; Karydis, 2022), uma vez que os organismos sofrem os efeitos acumulativos das variações ambientais, resposta dos efeitos diretos de poluente, e bioacumulam e biomagnificam poluentes (Bonanno and Orlando-Bonaca, 2018; Tashla et al., 2018). Um bom biomonitor tem como características básicas, ocorrência natural no ambiente e, facilidade de identificação e amostragem, , sensitividade ao contaminante estudado e estabilidade na resposta a contaminação (Karydis, 2022).

Os peixes são considerados bons biomonitores para avaliar a qualidade dos ambientes aquáticos (Kroon et al., 2017), por serem um componente transicional importante desde a ciclagem de nutrientes até a transferência de energia ao longo da cadeia trófica. Devido às diferentes formas de acumulação de contaminantes de acordo com o seu nível trófico, o uso de organismos com guildas tróficas distintas é aconselhável como biomonitor de contaminação (Viana et al., 2013). O uso de peixes como biomonitor é vasto na ecotoxicologia, devido à sua elevada sensibilidade às variáveis ambientais e vulnerabilidade aos efeitos deletérios dos poluentes (Zaynab et al., 2022). A principal medida de controle utilizada é a concentração de metais no músculo, como forma de mensurar a probabilidade de efeito na biota e assegurar seu uso para consumo humano (ANVISA, 2013).

Biomarcadores são ferramentas sensíveis para avaliar a contaminação ambiental; uma vez que permitem avaliar os efeitos subletais como resposta dos organismos aos poluentes (Athira and Jaya, 2018). Estas respostas podem ser mensuradas através de órgãos, células, alterações fisiológicas (Van der Oost et al., 2003) e histológicas (Cantanhêde et al., 2014; Borges et al., 2018; Montes et al., 2020), isótopos δ13C e δ15N (Bowen, 2010; Martino et al., 2019) e, enzimas (Lopes, 2018; Simionov et al., 2021).

Os biomarcadores histopatológicos são alvos importantes na investigação dos efeitos da contaminação nos organismos, pela possibilidade de identificar lesões nas células, tecidos e órgãos (Hook et al., 2014; Van der Oost et al., 2003). As brânquias e o fígado são muito utilizados como órgão-alvo como resposta dos efeitos da exposição a metais, devido à alta acumulação de poluentes nestes órgãos (Nyeste et al., 2019). O fígado é o mais utilizado, pois além de ser essencial para o funcionamento fisiológico do animal, possui a capacidade de desintoxicar e excretar substâncias tóxicase pelos efeitos da biotransfromação de poluentes (Borges et al., 2018). As brânquias, pelo seu papel fundamental em vários processos vitais dos peixes, como as trocas gasosas e regulação osmótica, principalmente por ser o primeiro órgão de contato com o ambiente e portanto com os poluentes, também são recomendadas para o biomonitoramento ambiental (Ameur et al., 2015).

O uso do fígado e brânquias para avaliar os efeitos subletais nos peixes tem metodologias robustas e consolidadas (Poleksic and Mitrovic-Tutundzic, 1994; Bernet et al., 1999), aplicadas para ambientes estuarinos e de água doce (Cantanhêde et al., 2016; Saleh e Marie, 2016; Borges et al., 2018; Carvalho et al., 2020; Montes et al., 2020). O tamanho do fígado, pode ser utilizado como ferramenta para monitorar a saúde dos peixes em ambientes aquáticos afetados (Van der Oost et al., 2003), pois seu aumento é uma resposta do organismo à exposição a poluentes químicos, através da atividade metabólica durante o processo de desintoxicação de contaminantes (Schmitt e Dethloff, 2000).

Outra forma de avaliação das possíveis fontes de emissão e o efeito trófico dos contaminantes no ambiente é a análise de isótopos estáveis como biomarcadores da poluição para investigar impactos antropogênicos, tais como o rastreio de fontes de poluição (Pataki et al., 2010; Özdilek et al., 2019). Isótopos de $\delta^{13}C$ e $\delta^{15}N$ são bastante utilizados para caracterizar o nível trófico dos organismos, bem como avaliar fluxos de energia e ciclo de nutrientes em ecossistemas aquáticos, sendo extremamente relevante para o entendimento da rede trófica (Fry, 2006). Estes isótopos também podem ser utilizados para o estudo de contaminação, através da associação de isótopos estáveis de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$) no matéria orgânica no sedimento (SOM), sedimentos e na biota (Sena-Souza et al., 2019; Thibault et al., 2020; Carvalho et al., 2021). A assinatura isotópica de $\delta^{15}N$ é geralmente mais enriquecida em ambientes poluídos que naqueles com baixo impacto ou áreas preservadas, devido ao maior aporte de fontes de nitrogênio (Olsen et al., 2011; Moynihan et al., 2012; Connolly et al., 2013), enquanto os valores de $\delta^{13}C$ indicam a origem da matéria orgânica (de plantas C3 e C4), efluentes com maior aporte orgânico, e o Carbono varia entre os produtores primários(Kopprio et al., 2014; Perkins et al., 2014; Liu et al., 2020). Embora a sazonalidade e as variações ambientais possam interferir na composição da matéria orgânica e no fracionamento isotópico do organismo aquático (Reuss et al., 2013; Kopprio et al., 2014), estas assinaturas podem indicar o deslocamento de poluentes, tais como esgotos, numa escala espacial e sazonal, permitindo a localização da fonte poluidora e e sua entrada na cadeia trófica, através do biomonitoramento nos peixes (Connolly et al., 2013; Souza et al., 2018).

Atualmente, uma das abordagens mais robustas no uso de isótopos $\delta^{13}C$ e $\delta^{15}N$, é a utilização de modelos isoscape (estatísticos ou mecanicistas) (Bowen, 2010) para diferentes fins, tais como migração animal, estudos ecológicos, alterações climáticas e biogeoquímica (Ceriani et al., 2014; Sena-Souza et al., 2019; Winter et al., 2021). A aplicação dos isoscapes na biomonitoramento de ambientes aquáticos facilita a visualização e análise de dados para realizar o monitoramento ambiental de forma mais eficiente (Bowen, 2010; Sena-Souza et al., 2019). O isoscape é mais utilizado no monitoramento da migração de peixes em ambientes costeiros (Radabaugh et al., 2013; Ohshimo et al., 2019; Glew et al., 2021). Em ambientes tropicais, em zonas estuarinas, não há registo de utilização desta abordagem, principalmente devido à falta de dados isotópicos espaciais, bem como à ausência de dados geográficos sobre os ecossistemas.

O Brasil possui um dos mais extensos litorais do mundo e tem a segunda maior cobertura de manguezais do planeta (Primavera, 2019). Devido a seus múltiplos usos e serviços ecossistêmicos, os recursos costeiros e marinhos são fundamentais para o desenvolvimento do País (Cabral et al., 2005; Diniz et al., 2019). Entretanto, a ocupação dessas áreas resulta em inúmeros impactos antrópicos no meio ambiente (Elliott et al., 2019). O Nordeste é uma das regiões costeiras mais densamente povoadas do Brasil, com o Estado de Pernambuco destacando-se como o epicentro dessa concentração, a intensa urbanização e degradação dos ecossistemas costeiros têm resultado em múltiplos impactos, principalmente devido à poluição doméstica, atividade industrial e perda de habitat (Mérigot et al., 2017; Lacerda et al., 2021).

No litoral sul de Pernambuco, o estuário do Rio Sirinhaém (SIR), localizado entre a Área Marinha Protegida de Guadalupe (Lira et al., 2010; Silva et al., 2011;) e a Área de Proteção Ambiental de Sirinhaém, sofre inúmeros impactos derivados da atividade agrícola, principalmente cana de açúcar, cultivo de camarão e pesca (Lira; Fonseca, 1980). Concentrações elevadas de mercúrio no sedimento, superior aos limites estabelecidos pela resolução CONAMA 454/2012, foram observadas nesta região (Lopes, 2018). Adicionalmente, a partir do ano 2000, foi registrado o uso do pesticida fipronil em decorrência da cultura da cana de açúcar na área (Masutti and Mermut, 2007).

No litoral norte, o estuário do Canal de Santa Cruz (ITAP), localizado no município de Itapissuma, é o maior e altamente produtivo estuário do litoral pernambucano (Macêdo et al. 2000). O estuário tem forma de U, com 22 km de comprimento e largura entre 0,6 e 1,5 km, localizado dentro da Área de Preservação Ambiental de Santa Cruz (APA de Santa Cruz). Uma indústria cloro-alcalina foi responsável pelo lançamento de 22 a 35 toneladas de mercúrio inorgânico num importante tributário do complexo estuarino do Canal de Santa Cruz, o rio Botafogo, entre 1963 a 1987 (Meyer et al., 1998; Meyer and Medeiros, 2017). Outras atividades antrópicas também impactam esta área como carcinicultura, pesca artesanal, cultivo de cana-de-açúcar, e diferentes tipos de indústria, tais como química e produtos alimentares e bebidas (CPRH, 2010; Coutinho et al., 2018).

O presente estudo teve como objetivo, através de abordagens integradas, o uso de diferentes técnicas complementares para diagnosticar a qualidade ambiental de dois estuários tropicais em Pernambuco. As técnicas utilizadas, compreendem o uso de biomarcadores histopatológicos nas brânquias e no fígado de peixes, utilização integrada de ferramentas biogeoquímicas e, a aplicação do isoscape associado a isótopos $\delta^{13}C$ e $\delta^{15}N$ no SOM, num estudo de caso em apenas um dos estuários em Sirinhaém.

Para atender o objetivo proposto, a presente tese é composta por três capítulos. O primeiro intitulado “Assessment of trace metals contamination through biogeochemical tools in two estuaries in northeastern, Brazil impacted by multiple pollution sources”, tem como finalidade avaliar a qualidade ambiental de duas zonas estuarinas tropicais, o estuário do Canal de Santa Cruz e o estuário do Rio Sirinhaém, utilizando a integração de ferramentas biológica e geoquímica, através da concentração de metais traços (Cu, Cr, Cd, Hg, Pb e Zn) em sedimentos e biota (*Bardiella ronchus, Caranx latus, Centropomus undecimalis, Centropomus parallelus* e *Gobionellus stomatus*). A utilização integrada destas metodologias fornece informações que permitem compreender as perturbações humanas no ambiente e os seus efeitos sobre a biota, o que pode fornecer subsídios para a gestão da monitoramento ambiental.

O segundo, intitulado “Histopathological biomarkers as indicators of the environmental quality of two estuaries in northeastern Brazil”, tem o objetivo de avaliar a qualidade ambiental nos nos estuários do Canal de Santa Cruz e o estuário do Rio Sirinhaém utilizando biomarcadores histopatológicos no fígado e as brânquias como em peixes (*Bardiella ronchus, Caranx latus, Centropomus undecimalis, Centropomus parallelus* e *Gobionellus stomatus*)de diferentes níveis tróficos. Estes resultados permitiram identificar o grau do dano e a severidade que os impactos antrópicos emambos os estuários resultaram nas espécies estudadas.

O terceiro, intitulado “The use of isoscape maps and stable carbon and nitrogen isotopes as pollution markers in a tropical estuary, northeastern Brazil”, propõe uma abordagem espacial pioneira integrando o uso associado de isótopos estáveis $\delta^{13}C$ e $\delta^{15}N$ e o modelo isoscape aplicados em sedimentos e peixes para avaliar os impactos antropogênicos presentes no estuário tropical do Rio Sirinhaém. As informações obtidas neste estudo permitiram avaliarcomo as fontes de contaminação podem ser diretamente refletidas nas assinaturas isotópicas do sedimento e da biota ao longo do estuário.

A conclusão final propõe integrar os resultados obtidos nos três capítulos da tesecom a finalidade de consolidar o diagnóstico sobre a qualidade ambiental dos dois ecossistemas estuarinos avaliados, associando à análise do uso integrado das metodologias propostas e a perspectiva de serem replicadas em outros estuários ou zonas costeiras tropicais.

CAPÍTULO 1

Assessment of trace metals contamination through biogeochemical tools in two estuaries in northeastern, Brazil impacted by multiple pollution sources

Capítulo 1. Assessment of trace metals contamination through biogeochemical tools in two estuaries in northeastern, Brazil impacted by multiple pollution sources.

Santos, Itala G. S. et al.

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Abstract

The environmental quality of two tropical estuarine areas, Santa Cruz Channel Estuary (ITAP) and Sirinhaém River Estuary (SIR), were assessed using the integration of biological and geochemical tools, through the concentration of trace metals in sediments and biota. The concentration of environmentally available trace metals in the sediments of ITAP (Cd, Cr, Pb and Zn) and SIR (Cd, Cr, Hg, Pb and Zn) were below Threshold Effect Levels (TEL) values, indicating that these metals have a low probability of causing effects on biota. However, Hg showed a high concentrations (>Probable Effect Levels (PEL) values) in ITAP at points located in the Botafogo River. Sediment environmental quality indices indicated contamination by Hg in ITAP, and by Cu for both areas. The ecological risk (Ri) and the Nemerow multi-factor (Pi) indexes showed that SIR had low ecological risk in relation to metal pollution considering the elements evaluated, but suffers the greatest influence of Cu. In ITAP, the opposite was observed, a very high potential ecological risk, with the greatest influence of Hg and Cu. Among the fish species and trace metals analyzed, only those from ITAP (*Bairdiella ronchus* and *Centropomus undecimalis*) were contaminated by Hg, with concentrations above the limit allowed by legislation. The use of multiple indices allowed a holistic view of the state of health of studied estuaries, highlighting the severity with which these areas are subjected to anthropic impacts, especially in ITAP, being necessary the efficient application of public policies of pollution control and food safety.

Keywords: fish; sediment; trace metals; anthropic impacts; Brazilian coast

1.INTRODUCTION

Contamination by trace metals in aquatic ecosystems has become an environmental concern worldwide, especially in estuarine zones (Ramos e Silva et al., 2017).The natural interactions between river and saline waters, influenced by tidal dynamics, exert a decisive role regarding the transport and destination of pollutants, especially trace metals (Liu et al., 2018; Guimarães et al., 2020;. Souza et al., 2021). These metals, when present in the aquatic ecosystems, quickly become part of the food web, aggravating the deleterious effects on the aquatic biota, and hence for the human population that consume these resources (Alyahya et al., 2011) .

Due to the dynamic sediment-water connectivity, the upper layers of the sediment are the first to be affected by changes in the aquatic environment, since they act as a deposit or source of trace metals, as contaminants return to the water column through suspended sediments (Bakary et al., 2015; Martín-Torre et al., 2017). Due to the interaction between the sediment and the benthic organism and the higher uptake of trace metals in the dissolution phase and consequently other organisms such as shrimp and fish (Pandiyan et al., 2021). Sediments can be considered as compartments for the accumulation of chemical species due to their high stability and low variability, allowing for a reliable assessment of temporal and spatial contamination (Costa et al., 2016). These metals present in the sediment undergo adsorption, desorption, reduction-oxidation, biological activity and diagenetic processes (Mondal et al., 2018; Ranjan et al., 2018).

Among the numerous existing methodologies to evaluate sediment contamination, environmental quality indices aim at identifying and assessing the degree of sediment contamination to evaluate possible deleterious effects on biota (Chapman et al., 1999; CONAMA, 2012). Indices for assessing the environmental quality of sediments are used to establish the source, the magnitude of metal pollution and the ecological risk of these pollutants , being an easily applicable approach for environmental monitoring (Wei and Yang, 2010; Ghrefat et al., 2011; Khuzestani and Souri, 2013).

Metals are adsorbed on the fine fraction in of sediments, becoming part of the suspended sediment cycle by tidal action, favoring the movement of elements from one place to another (Melo et al., 2015). This fluctuation of pollutants favors their incorporation along the food chain, either directly by bioaccumulation or indirectly by biomagnification, making sediment an important component for pollution monitoring (Hsu et al., 2016; Ramachandra et al., 2018). Moreover, fish suffer directly from the effects of estuarine pollution, through processes such as bioaccumulation and biomagnification (Yarsan and Yipe, 2013). They are also good for assessing the quality of aquatic environments given that they are (i) susceptible to physicochemical variations of the environment (ii) source of food for the local community and (iii) of economic relevance (Authman, 2015; Kroon et al., 2017; Saleh and Marie, 2016; Tesser et al., 2021).

In this study, we assessed the environmental quality of two tropical estuarine areas, the Santa Cruz Channel Estuary (ITAP) and Sirinhaém River Estuary (SIR), using the integration of biological and geochemical tools, through the concentration of trace metals in sediments and biota, the first study with this approach for both areas. Both areas have high biological and economic relevance, and the integrated use of these methodologies provides information that allows the understanding of human disturbances in the environment and their effects on the biota, which may provide subsidies for environmental monitoring management.

2.MATERIALS AND METHODS

2.1 Study area

The study area includes two estuarine systems along of the coast of Pernambuco state, northeastern Brazil (Fig. 1a). The Santa Cruz Channel Estuary (ITAP) is the largest and most productive estuary in Pernambuco coast (Macêdo et al. 2000) (Fig 1a). It is a 22 km long U-shaped channel with width ranging from 0.6 to 1.5 km, located within the Santa Cruz Environmental Preservation Area (APA Santa Cruz). This area receives different sources of impacts, such as shrimp farming, artisanal fishing, sugarcane cultivation, and different types of industry, such as chemical and food and beverage products (Coutinho et al., 2018; CPRH, 2010).

The Sirinhaém River Estuary (SIR) is a small, shallow, coastal plain estuary, 9.5 km long, 350 m wide increasing up to 800 m at the river´s mouth and with maximum depth ranging from 1.2 to 4.5 m (Silva et al., 2011). Located between the Marine Protected Area of Guadalupe and the Marine Protected Area of Sirinhaém, SIR is characterized by a high mangrove cover (Maia et al., 2006) (Fig. 1b).

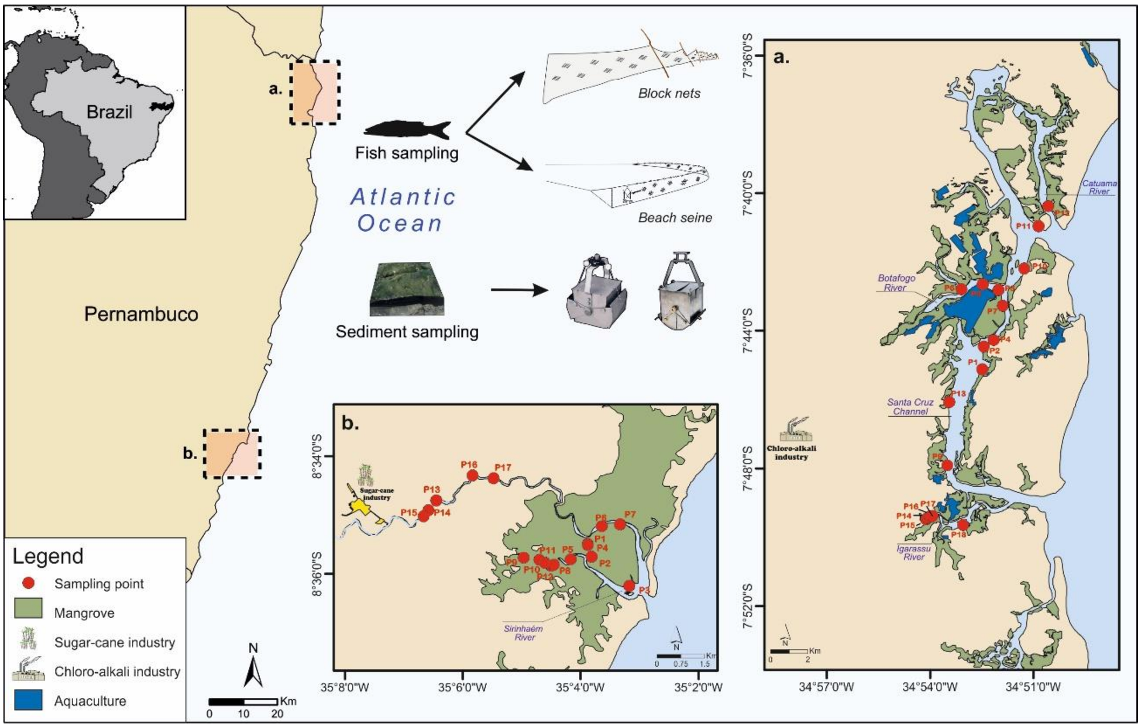

Figure 1. Map of the study areawith the sampling points andthe equipment used during the sampling of sediments and fish, a: Santa Cruz Channel Estuary (ITAP); b: Sirinhaém River Estuary (SIR).

2.2 Collection and preparation of sediment and fish samples

In this study, we evaluated the trace metal contamination by Cd, Cr, Cu, Pb, Zn and Hg. Bottom sediment samples (0-5 cm), collected either manually or using Ekman collector. Punctual samples were obtained in May 2019 (two samplings) and October 2020 (one sampling) for ITAP and November 2018 (one sampling), January to May 2019 (three samplings) for SIR. Samples were stored on ice and transported to the laboratory. Subsequently, they were sieved through 63 µm mesh, and dried in an oven at 60 ºC for 48 hours.

Fish were collected using beach trawl nets (20 × 1.9 m, 20 mm mesh, punctual samples during 2018 (two samplings) and 2019 (six samplings). Fish species were chosen considering their availability in each area, and by the following criteria: (a) importance to the local community as a source of food and income and (b) different trophic guilds. The trophic guild of each species was classified according to Elliott et al. (2007) and Mourão et al. (2014). *Bairdiella ronchus* (n :, Piscivore PV), *Centropomus undecimalis* (n : 5, PV) and *Centropomus parallelus* (n : 5, Piscivore/Zoobenthivore PVZB), *Gobionellus stomatus* (n : 23, Detritivore DV) were sampled in ITAP, and *Caranx latus* (n : 21, PVZB), *Centropomus undecimalis* (n : 24, PV) and *Centropomus parallelus* (n : 22, PVZB) were collected in SIR. All samples were stored on ice and transported to the laboratory. Subsequently, for each individual fish, muscle sample from the dorsal part was removed, washed with distilled water and dried in an oven at 60 ºC for 48 hours.

2.3 Analysis of metal in fish and sediment samples

The analysis of HgT (mg Hg.kg^{-1}, dry weight) were measured using DMA-80 (Milestone, USA) and AMA-254 (Leco, Europe) analyzers for sediment (mg.kg^{-1}) and biological samples, respectively. The concentrations in the biological samples were posteriorly converted to wet weight (wet mg.kg^{-1}) by applying a moisture factor, previously determined for each sample.

To investigate the robustness, accuracy, and precision of measurements, two biological and one sediment certified reference materials (CRM), with varying proportions of HgT levels, were tested: IAEA-436 (tuna tissue), SRM2976 (mussel tissue) and Mess-3 (marine sediment). For each batch of ten samples, three blanks, and three CRMs were analyzed.

For the other metals, the processing was carried out according to the environmentally available levels (T-AD) of Cd, Cr, Cu, Pb and Zn for sediment and fish. Sediment samples were subjected to 3051A acid digestion (U.S.EPA, 2007). A sample of 500 mg of sediment previously dried at 35ºC and pulverized (0.15 mm) was digested with nitric acid (HNO_3) and hydrochloric acid (HCl) at 175 ºC for 4'30” minutes in a closed system. After digestion, the extracts were filtered and diluted to 25 ml with ultrapure water (18.2 mΩ.cm) in certified flasks. The levels of these metals in the extracts were measured by optical emission spectroscopy with dual observation mode (axial and radial) and solid-state detector, with an introduction system via automatic sampler AS 90 plus. The Hg contents in the extracts were determined by an atomic absorption spectrophotometer (AAnalyst 800 Perkin Elmer) coupled to a hydride generator (FIAS 100/Flow Injection System/Perkin Elmer) with electrodeless discharge lamps (EDL), wavelength of 253.7 nm, using 0.2% sodium borohydride as the reducing agent. Tissue samples (0.5 g) were weighed and pre-digested with nitric acid (8 mL) and hydrogen peroxide (2 mL), and then digested in a microwave oven for 10 minutes at 180 °C. The extracts were filtered and diluted in 25 mL flasks with the addition of 5% potassium permanganate and stored in borosilicate flasks until the time of determination.

To assess the potential impact of trace metal levels, concentrations recorded in sediments were first compared to each metal in the guidelines proposed by the “Canadian Sediment Quality Guidelines for the Protection of Aquatic Life” (CCME, 2001). These guidelines were developed to evaluate the degree of contamination and its likely impact on the environment. They consist of Threshold Effect Levels (TEL) and Probable Effect Levels (PEL): below TEL- the minimal effect range within which adverse effects rarely occur; between TEL and PEL - the possible effect range within which adverse effects occasionally occur; above PEL-the probable effect range within which adverse effects frequently occur (Macdonald et al., 1996). Trace metals (Pb, Cd and Hg) levels recorded in biological samples were compared according to the Brazilian Health Regulatory Agency (ANVISA, 2013).

2.4 Assessment of the environmental quality of the sediment

2.4.1. Local background versus guidelines values

To assess the extent of pollution in each area, it is common to use on-site concentrations of heavy metals to calculate the geochemical background. However, the literature is deficient for all metals analyzed for both areas. Thus, the Canadian Sediment Quality Guidelines- SQG (CCME, 2001), threshold effect levels (TEL) were chosen as the standard basis for all metals.

2.4.2. Metal Contamination and Ecological risk assessment in sediment samples

For all metals analyzed (Cd, Cr, Cu, Pb, Zn and Hg), the status of pollution was evaluated through five indexes on sediment samples (Table 1): (a) the contamination factor (CF_i), commonly applied to assess the degree of contamination by each element at every point collected; (b) the Geoaccumulation index (I_{geo}), that quantifies the severity of contamination degree in seven classes (Barbieri, 2016) (Muller, 1969); (c) the Nemerow multi-factor (P_i), an integrated index that takes into account the average of the Contamination Factors of the set of trace metals studied ($CF_{iaverage}$) and the impact that can be caused by the maximum Contamination Factor (CF_{imax}) represented by a single pollutant, among all those studied (Ogunkunle and Fatoba, 2013); (d) the ecological risk assessment carried out using the Ecological Risk Factor (ER_i) and Ecological Risk Index (R_i), proposed by Hakanson(1980), that shows the toxicity for each metal; and (e) the RI that integrates the overall contamination (all metals) and their potential ecotoxicological effect.

Table 1.Metal contamination and ecological risk assessment index applied to the sediments collected in Santa Cruz Channel Estuary (ITAP) and Sirinhaém (SIR).

Index	**Calculation**	**Criteria**
Contamination factor (CF_i) (Hakanson, 1980)	$CFi = \frac{Ci}{Bi}$ C_i: concentration of metal in the sediment B_i: Background value of metal	$CF_i < 1$ low $1 \leq CF_i < 3$ moderate $3 \leq CF_i < 6$ considerable $CF_i \geq 6$ very high contamination factor
Geoaccumulation Index (I_{geo}) (Muller, 1969)	$I_{geo} = \log_2 \frac{C_{metal}}{1.5 \times B_{metal}}$ C_{metal} : concentration of metal in the sediment B_{metal}: Background value of metal 1.5: constant to correct lithogenic effects	$I_{geo} \leq 0$ uncontaminated $0 < I_{geo} \leq 1$ uncontaminated to moderately contaminated $1 < I_{geo} \leq 2$ moderately contaminated $2 < I_{geo} \leq 3$ moderately to highly contaminated

		3 <I_{geo} ≤ 4 highly contaminated 4 <I_{geo} ≤ 5 highly to extremely contaminated I_{geo} > 5 extremely contaminated
Nemerow multi-factor (Pi) (Ogunkunle and Fatoba, 2013)	$Pi = \sqrt[2]{\frac{(CF_{imax}^2 + CF_{iave}^2}{2}}$ CF_{imax}^2: maximum value of the single- factor index CF_{iave}^2: average value of the single- factor index	Pi < 1 unpolluted 1 ≤ P_i < 2.5 low 2.5 ≤ P_i < 7 moderate 7≥ P_i high polluted
Ecological risk factor (ER_i) (Hakanson, 1980)	$ER_i = TR_f \times CF_i$ TR_f: toxic- response factor CF_i: contamination factor	ER_i < 40 low 40 ≤ ER_i <80 moderate 80 ≤ ER_i <160 considerable 160 ≤ ER_i <320 high ER_i ≥ 320 very high potential ecological risk
Ecological risk Index (R_i) (Hakanson, 1980)	$R_i = \sum_{i=1}^{n} ER_i$ $ER_{i:}$ ecological risk fator	R_i < 150 low 150 ≤ R_i <300 moderate 300 ≤ R_i <600 considerable R_i ≥ 600 very high potential ecological risk

[a]TR_f is the metal toxicity factor (Cd = 30, Cu = Pb= 5, Cr =2, Zn = 1 and Hg = 40).

3.RESULTS

3.1. Metal Concentration

A total of 34 sampling points were analyzed, 17 in both The Santa Cruz Channel Estuary (ITAP) and Sirinhaém River Estuary (SIR) (Fig. 1). According to the Sediment Quality Guidelines (SQG) the concentration of trace metals for ITAP (Cd, Cr, Pb and Zn) and SIR (Cd, Cr, Hg, Pb and Zn) were below TEL values. However, in ITAP, for mercury (Hg), 42.10% of analyzed sites, located on the Botafogo River and close to shrimp farm, were above the PEL (mercury concentration >0.7 mg.Kg^{-1}), with the highest values at points P5 and P6 (Fig. 2). The other elements showed concentrations below the TEL level; although differences were observed within the sampled points (Fig.2). Cu level was highest at P9 to P18 near the most urbanized area. Cr was present at all sampled points, with the highest concentration at points P13 and P5. The concentrations of Cd, Pb and Zn were highest near urbanized areas.

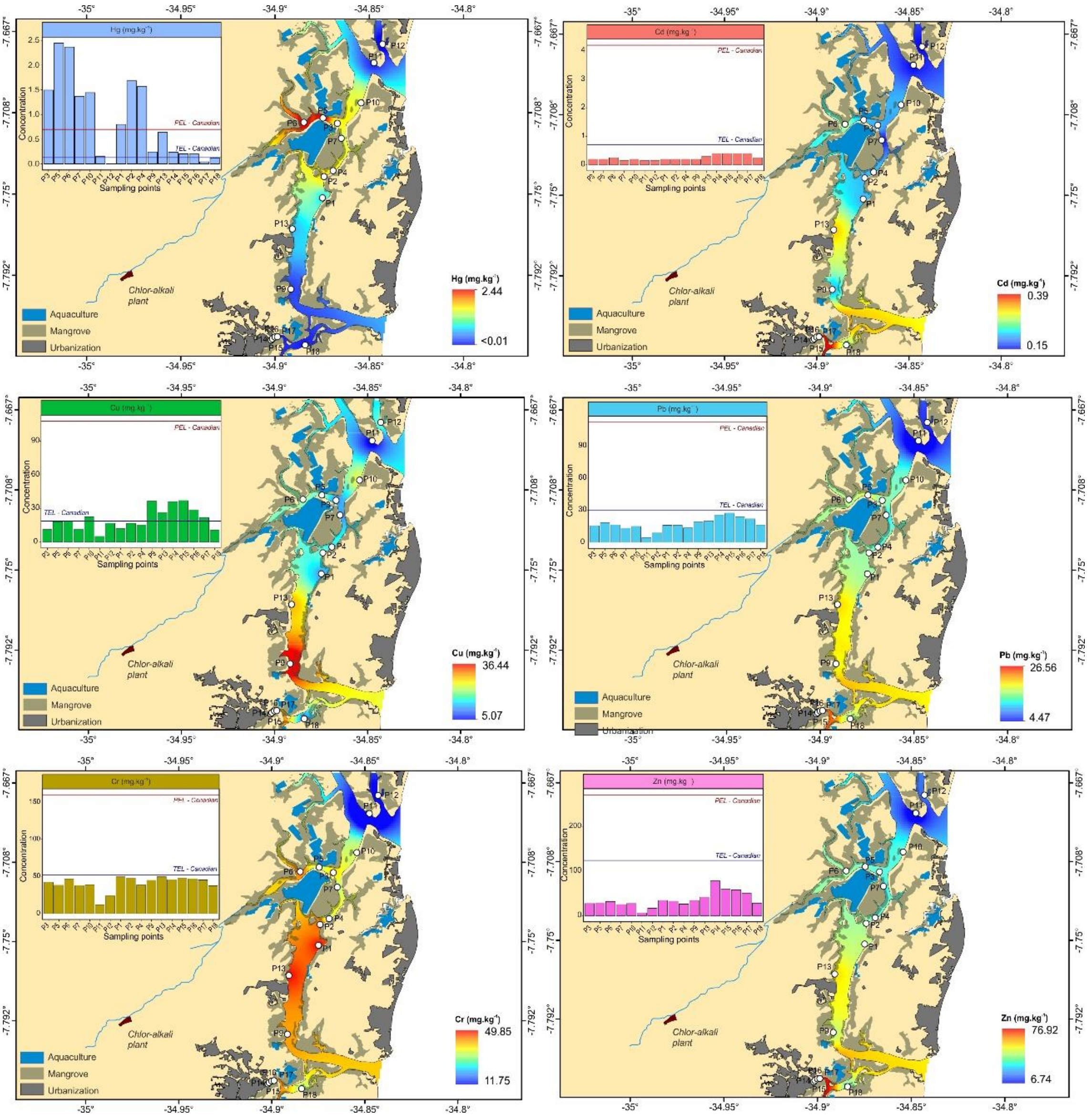

Figure 2. Trace metal concentration (mg.kg-1) distribution map and barplot with the classification criteria representing the Santa Cruz Channel Estuary (ITAP).

In the SIR, the average Cu values were above the TEL values. The highest concentration of Hg (Fig.3), with a few points above the TEL value (0.13), was observed near the sugarcane industry. At the points, with greater maritime and urban influence, Cr and Pb values were higher. However, the points closest to the sugar cane industry showed the highest concentrations o of Hg, Cd and Zn. The element Cu suffers direct anthropic influence from urbanization and the sugarcane industry (Fig. 3).

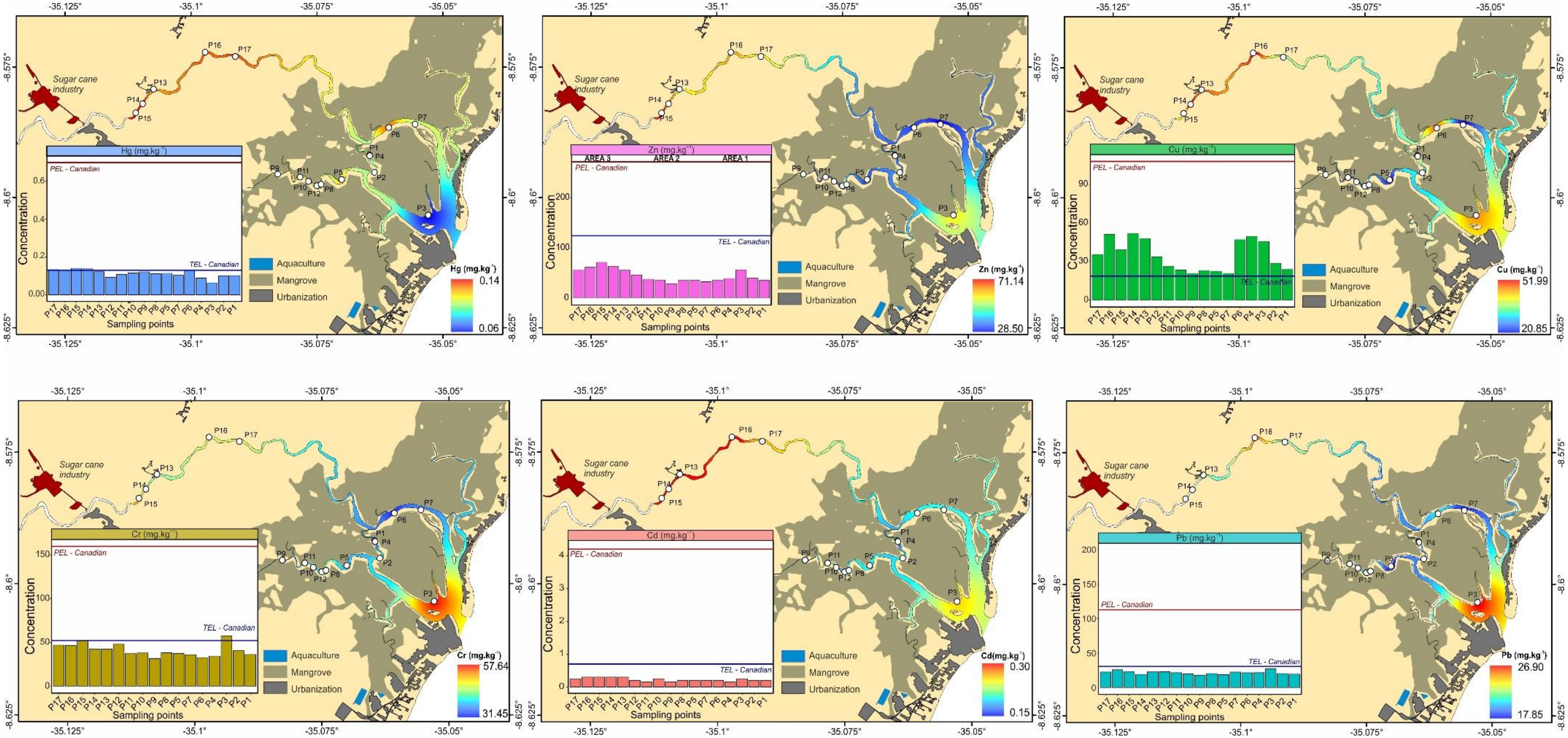

Figure 3. Trace metal concentration distribution map and barplot with the classification criteria representing Sirinhaém River Estuary (SIR).

3.2. Contamination assessment

The trace metal contamination factors in ITAP for Cd, Cr, Pb and Zn were categorized with low contamination (CF< 1) and Cu varied from low to moderate contamination (Fig.4a). Hg fluctuated from moderate to very high factor contamination (CF>6, Fig.4b). This index indicates higher mercury contamination in ITAP than in SIR (Figs. 4a and 5a). In SIR, the average values of Cd, Cr, Zn, Hg and Pb were classified with low contamination (CF< 1) (Fig. 5a). The amounts of Cu was moderate in all points, being the most representative element in SIR (see Fig. 5b).

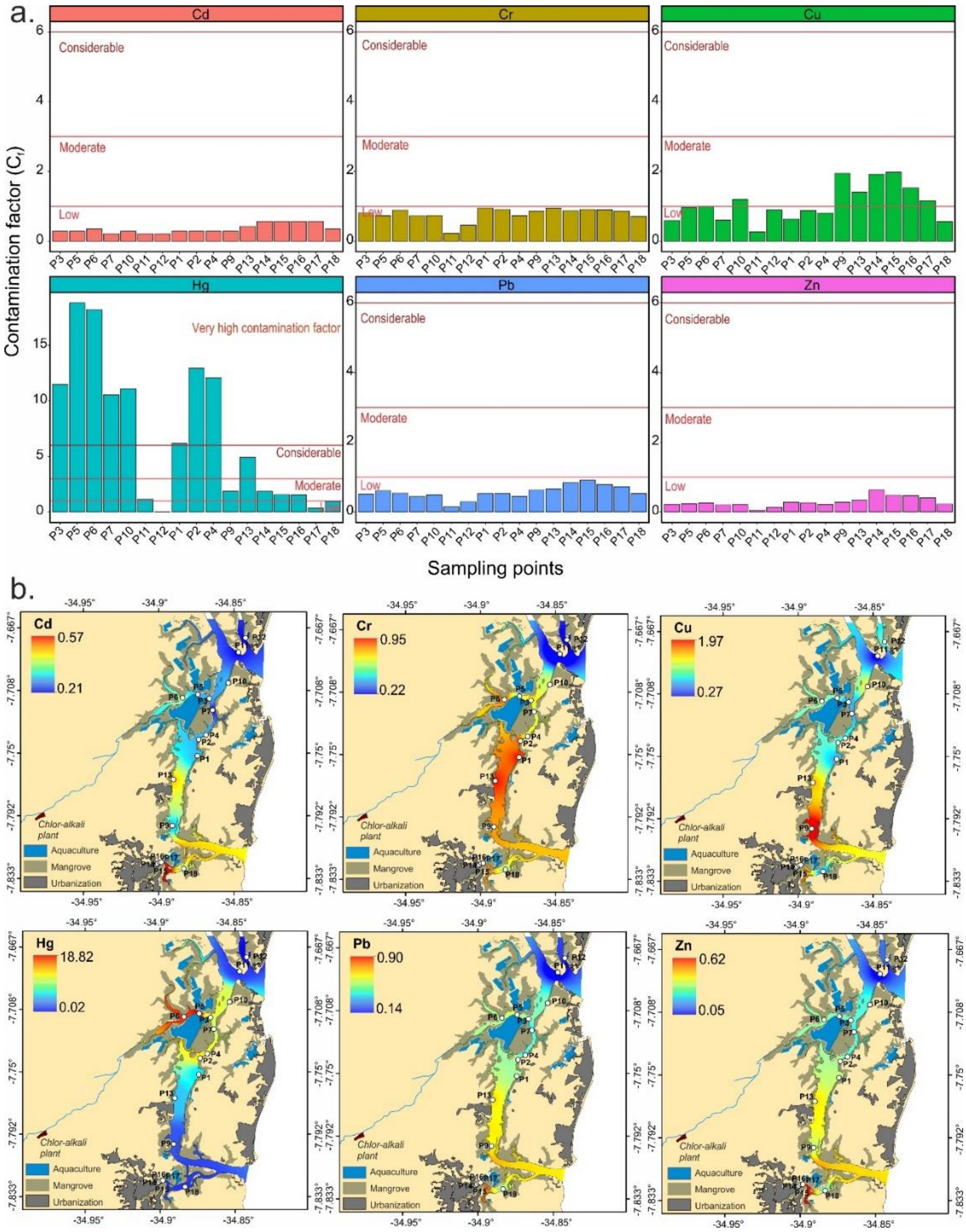

Figure 4. Contamination factor (Cf) for trace metals. a: Cf per sampling point, b: map of distribution Cf in Santa Cruz Channel Estuary (ITAP).

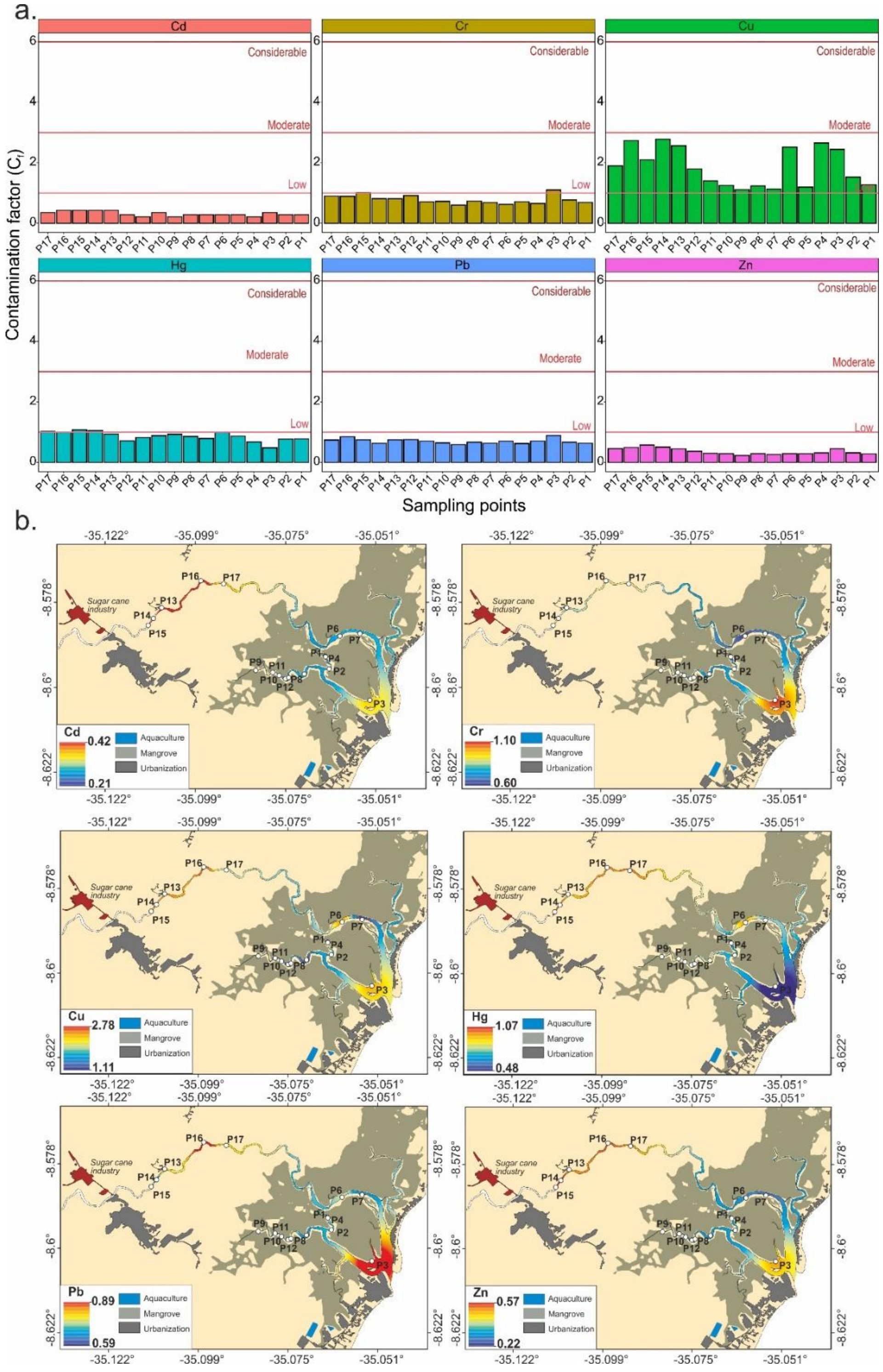

Figure 5. Contamination factor (Cf) for trace metals. a: Cf per sampling point, b: map of distribution of Cf in Sirinhaém River Estuary (SIR).

Considering the geo-accumulation index, ITAP was classified as uncontaminated by Cd, Cr, Pb and Zn, uncontaminated to moderate contaminated by Cu, and uncontaminated to highly contaminated by Hg (Fig.6). SIR was classified as uncontaminated by all metals, except by Cu that moderately contaminate some sites (Fig.6).

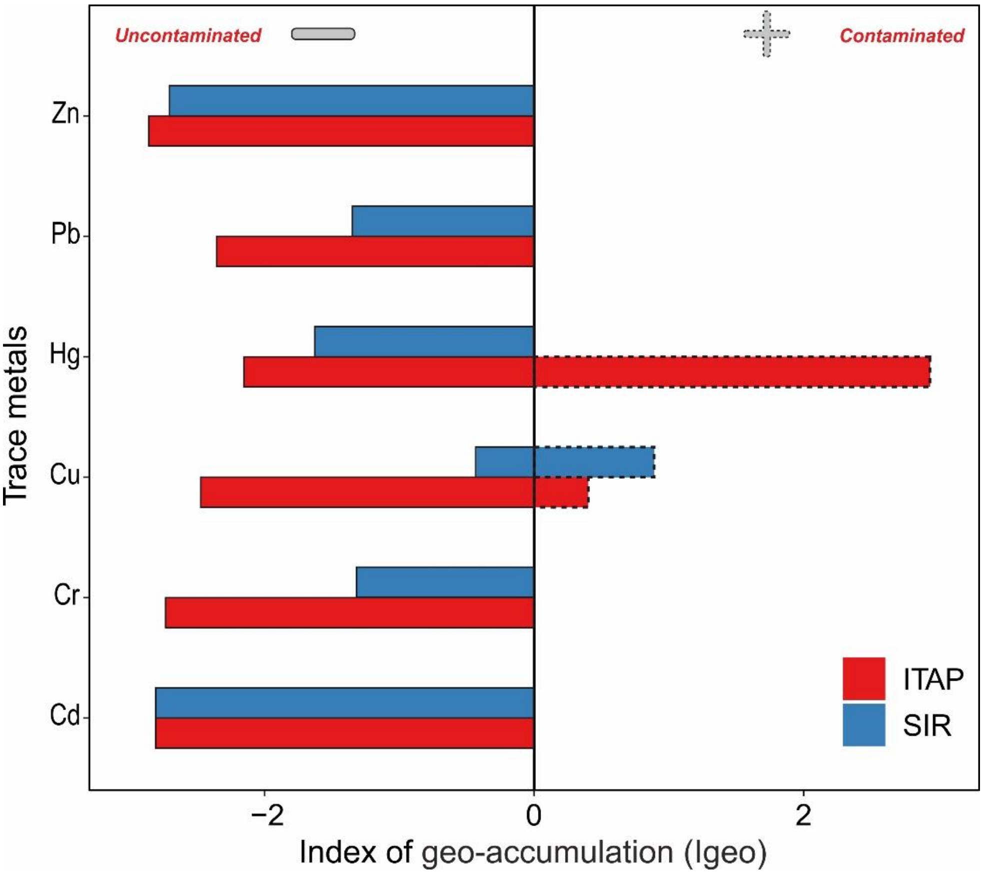

Figure 6. Index of geo-accumulation (Igeo) in the Santa Cruz Channel Estuary (ITAP) and Sirinhaém River Estuary (SIR).

According to the Nemerow multifactorial index, the degree of contamination of all points in ITAP (4.69 ± 3.17) is moderate. However, this method can be applied in another perspective by dividing the points analyzed into three groups, taking into account the main source of contamination. Hence, it is possible to identify the predominant trace metal per estuarine session: the first group (G1: P3, P5, P6, P7, P10, P11, P12), the second group (G2: P1, P2, P4, P9, P13), and third group (G3:P14 to P18). The groups varied from low to highly polluted, in order of severity: G1 (7.36) > G2 (5.52) >>> G3 (1.19), groups 1 and 2 were mainly influenced by mercury and group 3 by Cu. In SIR, the first group (G1: P1, P2, P3, P4, P6, P7), the second group (G2: P5, P8, P9, P10, P11, P12), and third group (G3:P13 to P17). The mean value of the three groups (1.36 ± 0.53) were classified with low contamination and were mostly influenced by Cu.

3.3. Ecological risks assessment

The ecological risk factor (ERi) evaluated the toxicity for each metal, for all sediment samples analyzed. A low ecological risk was found for Cd, Cr, Cu, Pb and Zn (ERi < 40) in both areas (Figs.7 and 8). However, 41.2% of the points in ITAP had a very high potential ecological risk (ERi >320) for Hg. These points were under the greatest influence of the Botafogo River (Figs.1 and 7).

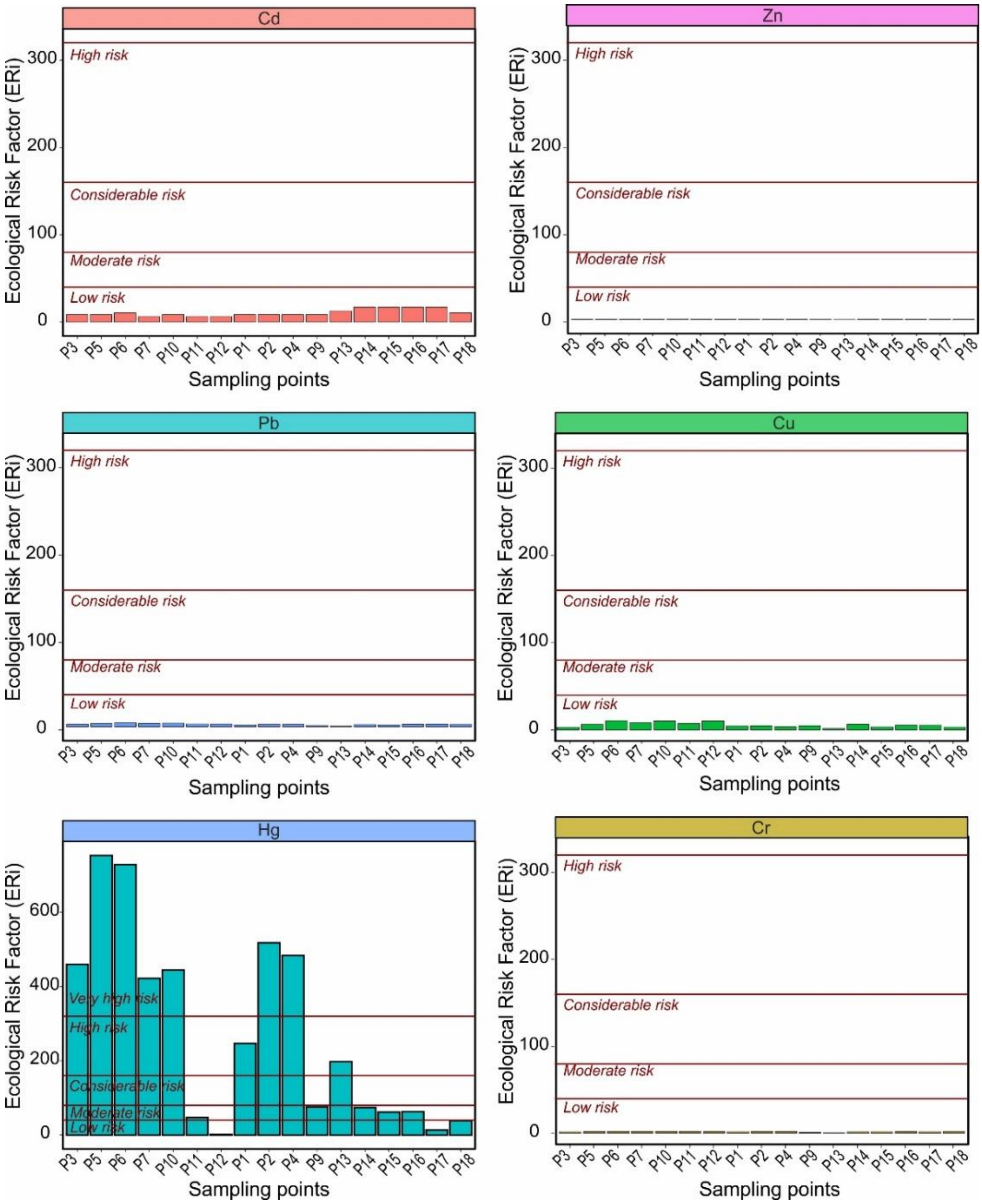

Figure 7. Ecological Risk Factor (Eri) and the risk qualification criteria for Santa Cruz Channel Estuary (ITAP).

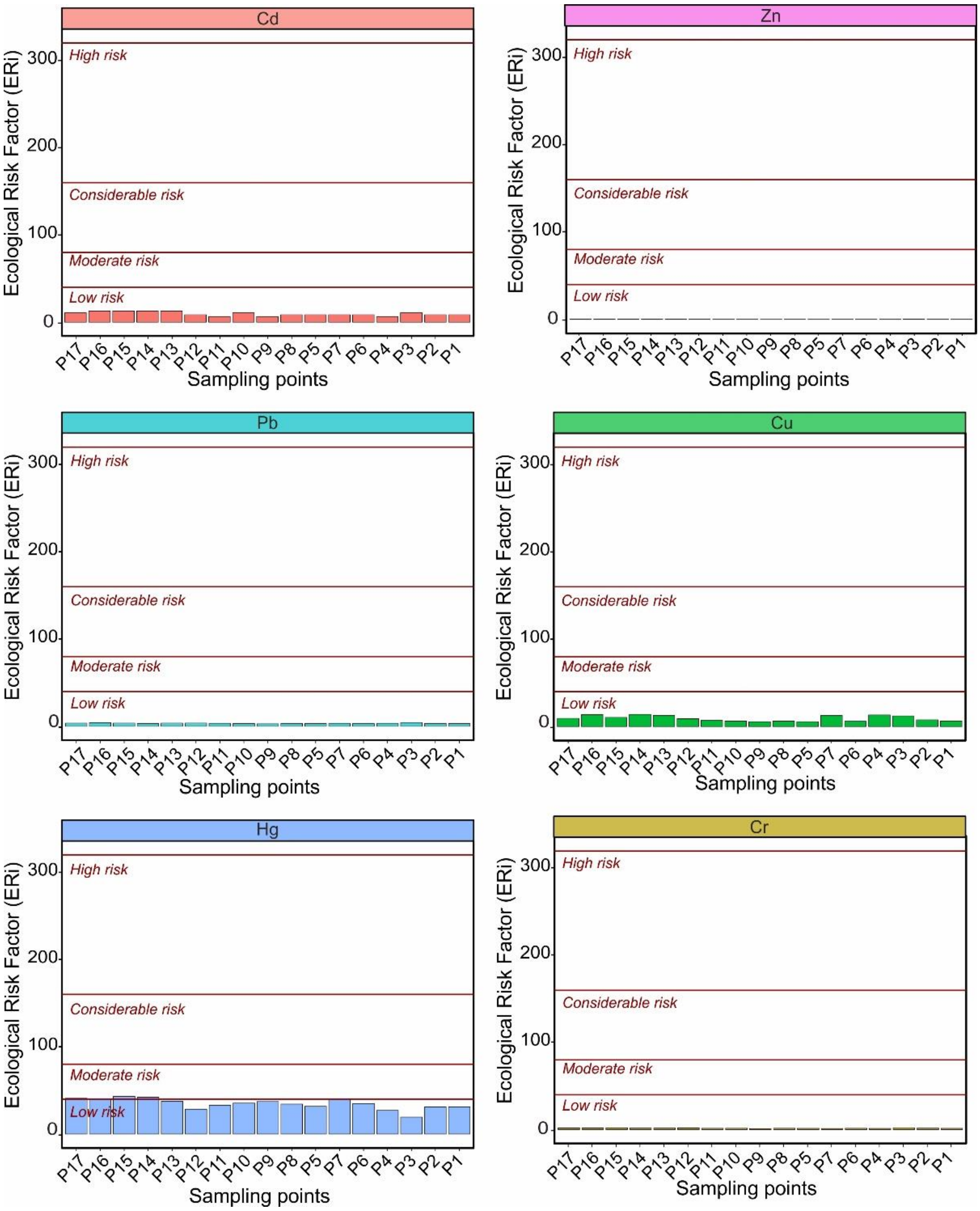

Figure 8. Ecological Risk Factor (Eri) and the risk qualification criteria for Sirinhaém River Estuary (SIR).

Based on the Ecological risk Index (R_i) it was observed that, 44% of the analyzed points in ITAP were of low risk (Ri <150) and the others points had moderate to very high potential ecological risk (Fig. 9b). Meanwhile, all points in SIR had low contamination (Fig. 9a).

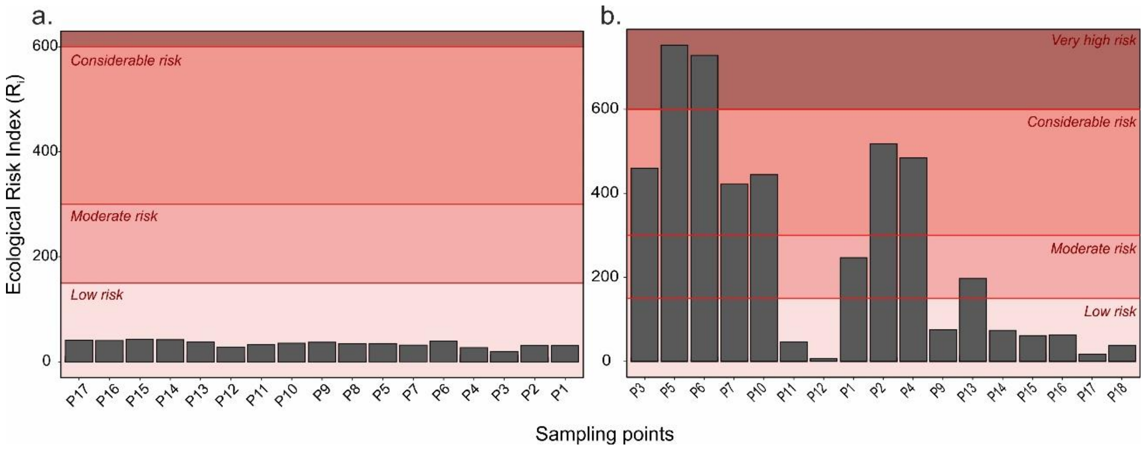

Figure 9. Ecological risk Index (Ri), a: Sirinhaém River Estuary (SIR), b: Santa Cruz Channel Estuary (ITAP).

3.3. Metals in fish

The concentration of Cd, Cr, Cu, Hg, Pb, and Zn in the analyzed fish is shown in Fig.10. For all species (*Bairdiella ronchus, Caranx latus, Centropomus undecimalis, Centropomus parallelus and Gobionellus stomatus), Cd* (Cd< 0.05) and Pb (Pb< 0.3) were below the tolerance limit reported by the Brazilian health regulatory agency (ANVISA, 2013), meaning that their consumption will not lead to risks for human health. Cd was detectable only in *B. ronchus* in ITAP, while the highest concentration of Pb was found in *C. latus* in SIR. Cr, Cu and Zn have no specific regulations. No contamination by Hg was observed in SIR. In ITAP, only *B. ronchus* and *C. undecimalis* had mercury concentration above the allowed limit (Hg >0.5 mg/kg, wet weight) considered by Brazilian health regulatory agency (ANVISA, 2013).

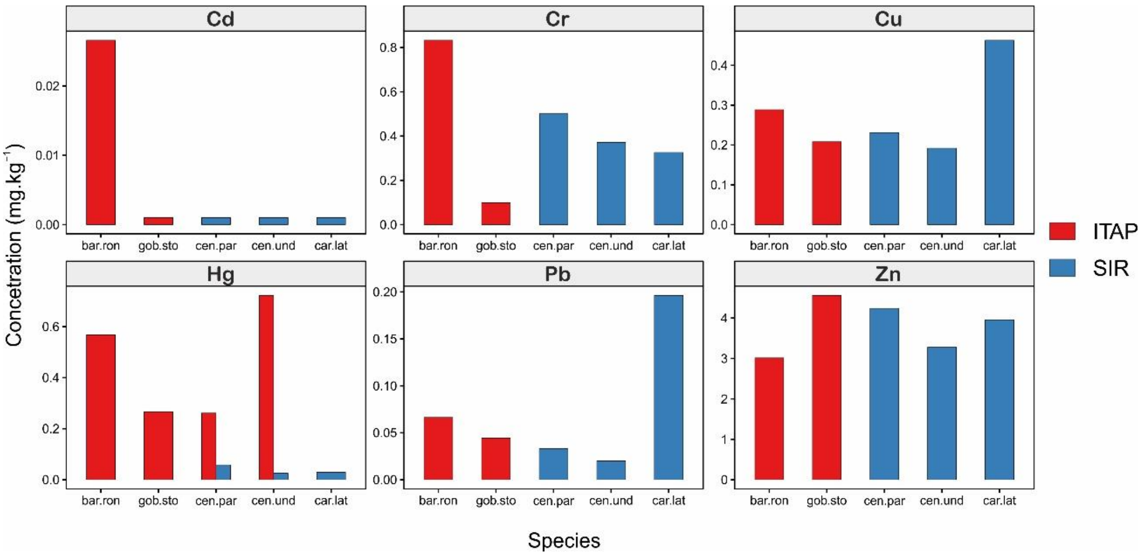

Figure 10. Concentration of trace metals (wet weight mg.Kg^{-1}) in *Bairdiella ronchus, Gobionellus stomatus, Centropomus parallelus, Centropomus undecimalis* and *Caranx latus* from Santa Cruz Channel Estuary and Sirinhaém River Estuary.

4.DISCUSSION

Estuaries are transitional environments of extreme ecological importance due to their high biological productivity, providing support for fish development and refuge for species in sensitive stages of their life cycle (Wolanski et al., 2019). Estuarine pollution has global reach and, agriculture, port, sugarcane industry, sewage and industrial effluents are the main anthropogenic disturbances (Elliott et al., 2019). In Brazil, and particularly in the Northeast region, estuaries are also disturbed by contaminants (CPRH, 2011, 2010), but the control and enforcement measures to ensure the environmental quality of estuarine ecosystems are still ineffective (Barletta et al., 2019). The use of more sensitive tools for environmental monitoring, such as indices and specific techniques like geochronology, enrichment factor, contamination factor or ecological risk assessment are not required by environmental legislations (Albuquerque et al., 2019; Barbieri, 2016; Looi et al., 2019) and are still barely used for control and enforcement measures. In this study, we provide a pioneering study with a comprehensive monitoring of sediment and biota in two tropical estuaries in Brazil, integrating the use of five trace metals and different indexes to evaluate the health status of the environment.

Metal concentration and Index sediment quality in SIR and ITAP

Monitoring estuarine sediment pollution, through the total concentration of trace metals in soil and sediment, allows the identification of the health status of an area and the likely deleterious effects on biota (CONAMA, 2012). It is the metric required by Brazilian and international environmental legislation, given its ease of use and relative efficiency in diagnosing environmental pollution (Vareda et al., 2019) .

The concentration of environmentally available trace metals in the sediment of Santa Cruz Channel Estuary (ITAP) (Cd, Cr, Pb and Zn) and Sirinhaém River Estuary (SIR) (Cd, Cr, Hg, Pb and Zn) were below the TEL values, indicating that these metals have a low probability of causing effects on the biota, suggesting the goog health status of the areas (CCME, 2001). However, Hg had a high concentration in ITAP at points located in Botafogo River. This high Hg concentration in this area is related to the industrial effluents released between 1963 and 1987 by a chlor-alkali plant i into the Botafogo River (Meyer et al., 1998).

The variation in Hg concentrations reported in this study was within the range observed by other authors in the surroundings of the study area (Table 2). Our study showed higher concentrations than those observed by Lopes (2018) and Albuquerque et al. (2019). However, the values of trace metals in this study were lower than those found by other authors in the Santa Cruz Channel and Botafogo River, our study area, because they were closer to the chlor-alkali industry (Araújo et al., 2021, 2019; Meyer et al., 1998; Meyer and Medeiros, 2017). Moreover, shrimp farming in the area adjacent to the Botafogo River may be another contributing source to the increase of mercury in this region. The entry of this metal into the system is related to the impurities present in the food and chemical inputs used in aquaculture. This was already observed and quantified by Lacerda et al. (2011) in Jaguaribe River Estuary, Northeast Brazil. Cu and Zn presented in ITAP may be influenced by urbanization, industrial boom and intensive shrimp farming production around the sampled points (Lacerda et al., 2011; León-Cañedo et al., 2017; Monte et al., 2021).

Table 2. Total mercury concentrations (mg Hg.kg-1, dry weight) in the sediment of this study area and other areas adjacent to chlor-alkali plants.

Location	THg (mg.kg^{-1})	Sampled year	Authors
Study area - Brazil			
Santa Cruz Channel	**0.044-2.44**	**2019-2020**	**This study**
Santa Cruz Channel	0.3 – 20.5	1993–1994	Meyer et al (1998)
Santa Cruz Channel	0.04 - 6.20	1993	Meyer &Medeiros (2017)
Botafogo River	0.13–0.24	2004–2006	Lima et al., (2009)
Botafogo River	0.13-10.44	2017	Araújo et al (2019)
Botafogo River	0.1-14.4	2015	Araújo et al (2021)
Itapessoca Estuarine Complex	0.17-1.29	2017	Albuquerque et al (2019)
Santa Cruz Channel	0.307–1.55	2016	Lopes (2018)

Very little information concerning environmental pollution is available for SIR. The only reference related to the concentration of trace metals in the sediment of this study area (Lopes, 2018), reported Hg and Zn higher than those obtained in this research. However, the elements Cd, Cu, and Pb were far below the values obtained in this study, and this may be related to the different methodologies used and the points sampled.

The high concentration of the metals Cd, Cu, Hg and Zn may be correlated with the use of different types of fertilizers in the sugar cane industry in SIR (Yadav et al., 2010). Cd is an impurity in phosphatic fertilizers (Barzegar et al., 2005; Rayment, 2005), and Hg is present in the form of the organomercurial compound in fungicides (Yadav et al., 2010). Cu and Zn present in SIR may be strongly influenced by agricultural production around the sampled points (Sadeghi et al., 2020). The highest concentrations of Zn and Cu were found near urbanized area, as these metals are common in sewage (Fiori et al., 2020).

The three indices used, CF, I_{geo} and Nemerow (Pi), indicated contamination by the same trace metals, Hg in ITAP and Cu in both areas. The ecological risk index (R_i) showed that SIR presents low ecological risk regarding metal pollution, and very high potential risk for Hg and low risk for others trace metals in ITAP. I_{geo} is the only index that does not depend on the contamination factor (CF), unlike Nemerow and ecological risk assessment, which use CF values as a basic principle. CF is the most often index used to evaluate the individual contribution to the state of soil contamination (Seifi et al., 2019). These indices are complementary for the diagnosis of estuarine sediment contamination, due to their differential approaches for evaluate degree of pollution (Ogunkunle and Fatoba, 2013).

The values of the indices in ITAP were low, when compared to areas with similar or higher impacts. In this study, the I_{geo} values for Hg were lower than the range obtained by Araújo et al. (2021) in the same study area. The Nemerow multi-factor index applied in both areas detected which pollutant sources interfere in the contamination of the sampled points. In ITAP, the first group (G1: P3, P5, P6, P7, P10, P11, P12) may be influenced by the chlor-alkali plant and urbanization; the second (G2: P1, P2, P4, P9, P13) and third group (G3:P14 to P18) by urbanization. In SIR, Cu element outstood, probably given the sugar cane industry and urbanization.

The tools Ecological Risk Factor (Er_i) and Ecological Risk Index (R_i) indicated that SIR is submitted to low ecological risk for all metals. In ITAP, the scenario was similar for all metals except for Hg. Given the high concentration of Hg in the sediment in ITAP, it was observed moderate to very high potential ecological risk for R_i. A similar result was obtained by Araújo et al. (2021) in the Botafogo river, area adjacent to ITAP, since 98% of samples had an Er_i>320 value. The Er_i values in ITAP were lower than that obtained by Relić et al. (2019) when monitoring estuarine pollution directly impacted by a Petrochemical complex in the Republic of Serbia, where Hg, Cu, Pb, Zn, Cd, Se, and Cr were mainly responsible for the increase in the contamination of the area.

Estuarine contamination has beenreported in other estuaries around the world, and it is more alarming in emerging countries (i.e. Brazil, China, India, Ghana) due to increasing economic production models, intensive agriculture, industrial activities, and untreated sewage (Häder et al., 2020). Consequently, the concern of these impacts as the release of trace metals in the ecosystem are still feeble (Briffa et al., 2020). Pobi et al. (2019) assessed estuarine contamination caused by activities in the Durgapur industrial zone (including chlor-alkali industries) in India, with the I_{geo} >7 for Hg, which corresponds to extremely contaminated status. In Changjiang River Estuary, China, 50% of the sampling sites (area with industrial residue) were detected as moderately polluted, using I_{geo} as index (Zhang and Wang, 2021). Truchet et al. (2021) identified sediment pollution by Cu with possible effects on biota from the release of untreated sewage in Patagonian estuarine system, Argentina. Swain et al. (2021) reported Cd contamination in the Mahanadi estuary resulting from wastewater (industrial) and sewage effluents. Akita et al. (2020) observed a high contamination factor for Pb ($3 < CF \leq 6$) reflecting pollution from industrial and domestic effluents and agrochemicals in the sediments of the Densu Estuary, Ghana. In our study, considering all methods used, it is important to highlight the high levels of Hg in ITAP.

Hg is a major problem because it is considered a non-essential toxic element commonly found in aquatic ecosystems, and can remain inert in the environment for an indefinite time (Cardoso et al., 2008; Chen et al., 2008). It can also be found in the environment in the form of methylmercury

(MeHg), a bioaccumulative neurotoxin produced in aquatic ecosystems from divalent inorganic mercury (HgII42) (Mason and Reinfelder, 1995). The methylation process occurs naturally in the environment and is related to its biogeochemical conditions, so the transformation of inorganic mercury through microbial activity can occur into methylmercury, i.e., the organic form of this metal (Condini et al., 2017; Tremain and Schaefer, 2015). This form can be easily accumulated in fish and biomagnified in the trophic chain, resulting in high concentrations, especially in fish of high trophic level, and may contaminate part of the human population through the consumption of these resources (Adams and Paperno, 2012; Lavoie et al., 2013).

There is a general deficiency of biomonitoring of contaminants through biota and especially concerning trace metals in fishes in Brazil. The monitoring of trace metals in fishes in Brazil is still largely on a research scale, and considering the exported products, the resolution N°. 42 of the Brazilian Health Regulatory Agency determines the maximum limits of inorganic contaminants, such as the metals As, Cd, Pb, Sn and Hg (ANVISA, 2013). In the studied areas, there are few reports available using the biota as indicator. In ITAP, Cavalcanti (2003), using the oyster as a biomonitor of contamination due to the high consumption by the local market and riverside population, observed Hg contamination, although below the permitted limit. Araújo et al. (2019), used oysters collected in the Botafogo River to proof the reliability of these animals as biomonitor, showing concentrations up to 7 times higher when compared to the control area.

Others approaches may also contribute to the integrated assessment of the effects of trace metals on fish, likethe use of biomarkers, such as histopathological and enzymatic, characterizing sublethal effects on fish (Shahjahan et al., 2022); and the holistic approach to mercury chemical contamination, such as MeHg neurotoxin, can investigate how this metal behaves in fish and possible effects on humans (Lavoie et al., 2013; Medieu et al., 2022). Another way to assess the deleterious effects of Hg on biota is through specific biomarkers, such as inhibition of AChE activity. Lopes (2018) detected that Hg present in the Santa Cruz Channel, interfered with neural physiological activities in the snook species *C. undecimalis*.

Two main inferences are here reported for the study area.:Firstly, that in general, ITAP is much more contaminated than SIR because of the average of concentration of trace metals for all species; and secondly, fish of the piscivore trophic guild is found to be more contaminated than the other guilds, as reported for *C. undecimalis*, which can be considered a good biomonitor of contamination in the area. In Ciénaga Grande de Santa Marta, Colombia, the highest concentrations of trace metals (Cu, Hg, and Pb) were in carnivorous fish, such as *C. undecimalis*, *Elops smithi* and *Cathorops mapale*, than in non-carnivores species as *Eugerres plumieri* and *Mugil incilis* (Pinzón-Bedoya et al., 2020).

Other studies, also in northeast Brazil, corroborate that *C.undecimalis* is a good biomonitor of trace metal contamination, observing higher concentrations than this study: 1.24±0.16 mg.kg^{-1}

(Sergipe) and 0.70 ± 0.01 $mg.kg^{-1}$ (Bahia) (da Silva et al., 2021; Silva et al., 2019). However, it was not possible to identify a direct and specific source of pollution influencing the contamination of these individuals, since they were purchased from regional fish markets or supermarkets, with no precise source of origin.

The concentration of Cd for all fish from both areas was lower than that obtained by Silva et al. (2019) and higher for Pb when evaluating trace metals in fish market in Northeast Brazil (da Silva et al., 2021). However, in both studies, they are below the limit of the Brazilian legislation. For Cu, Cr and Zn, the presence of these trace metals in fish is justified by their importance in physiological and biochemical functions, but high concentrations of these elements result in deleterious effects for the biota (Ali et al., 2018; Sauliute and Svecevičius, 2015).

Fish contamination by trace metals is reported for other estuaries around the world using different methodologies. Feng et al. (2019), when assessing Hg concentration in catfish *Claria gariepinus* in Sagua La Grande River, Cuba, observed lower values than those obtained in the present study (mean values around 1 mg.kg-1), attributed to the contamination from chlor-alkali plants. Mason et al. (2022) noted high Hg contamination in Cote d'Ivoire (Africa) in small species such as *Chrysichthys maurus, Elops lacerta, Hepsetus sp., Arius africanus* and *Polydactylus quandrifilis,* which were exposed to unsafe levels of MeHg, considering the artisanal and small-scale gold mining, alarming the population that ingests these fish. Pinzón-Bedoya et al. (2020) reported concentrations of trace metals in fish below what is allowed by legislation in Ciénaga Grande de Santa Marta, Colombia and, by using another metric, such as potential risk (HQ) estimation, they found that, over time, Cu and Hg could generate negative effects in the population, especially in groups of women of childbearing age, because they exceeded their related reference doses. Rubalingeswari et al. (2021) monitored the main fish species used as food source for the population in the Adyar estuary, India. The species *Arius parkii* presented high bioaccumulation values for Cr, Pb, and Zn, and *Gerres oyena* for the trace metals Ni, Cu, and Co, highlighting the consequences of fish ingestion for the population. Gabriel et al. (2020) evaluated the impacts on biota two years after the mine tailings disaster in the Doce River estuary, Brazil, and observed that bioaccumulation of trace metals As, Cr, Mn, Hg, and Se was most evident in *Cathorops spixii* and *Genidens genidens*. Keshavarzi et al. (2018) evaluated the contamination of three commercial fish species (*Anodontostoma chacunda*, *Johnius belangerii*, and *Cynoglossurs arel*) in the Persian Gulf, through the target risk (TR) of arsenic, showing that the consumption of these species contaminated by this metal over a long period has carcinogenic possible consequence in humans.

The environmental quality indices of sediments indicated contamination by Hg in ITAP, and by Cu for both areas. The ecological risk index (Ri) and the Nemerow multifactorial (Pi) indicated that SIR had low ecological risk regarding metal pollution by most elements evaluated, but suffers the greatest influence of Cu. In ITAP, the opposite was observed, a very high potential ecological risk for Hg, with the great influence of Hg and Cu. In our study, the use of multiple indexes allowed a holistic view of the state of health of the estuarine environment. The evaluation of the degree of contamination of the sediment is essential to understand the entry of these trace metals into the food chain. In ITAP, Hg contamination was detected in *Bairdiella ronchus* and *Centropomus undecimalis*, with concentrations above the limit allowed by legislation for ingestion of these fish as food. Regarding the severity of contamination in fish, we can state that ITAP is more contaminated than SIR, ensuring that even if the presence of Hg is detected in fish from SIR, they are guaranteed by ANVISA legislation to be consumed. This implies that the trophic guild has direct interference in the bioaccumulation of these metals and that *G. stomatus* is also part of the diet of *C. undecimalis*, corroborating the enrichment of mercury in the species through biomagnification. It is worth mentioning that these species are relevant to the region. *B. ronchus* is used as a food source by the riverine population, and *C. undecimalis* is a highly commercialized species due to its economic value. This study emphasizes the importance of monitoring sediment pollution and the use of biomonitoring tools, due to the possible problems that biomagnification can cause to public health. Fish consumption, even at low concentrations of trace metals, does not prevent from causing significant effects on the population, depending on the amount of fish ingested over long periods of time, given the bioaccumulation and biomagnification processes. Clearly, for both areas and probably for many others estuaries of Northeast Brazil, public policies to control pollutants are deeply encouraged in all areas, in order to guarantee safe food for the population, and avoid deleterious effects on human health, which are still barely known.

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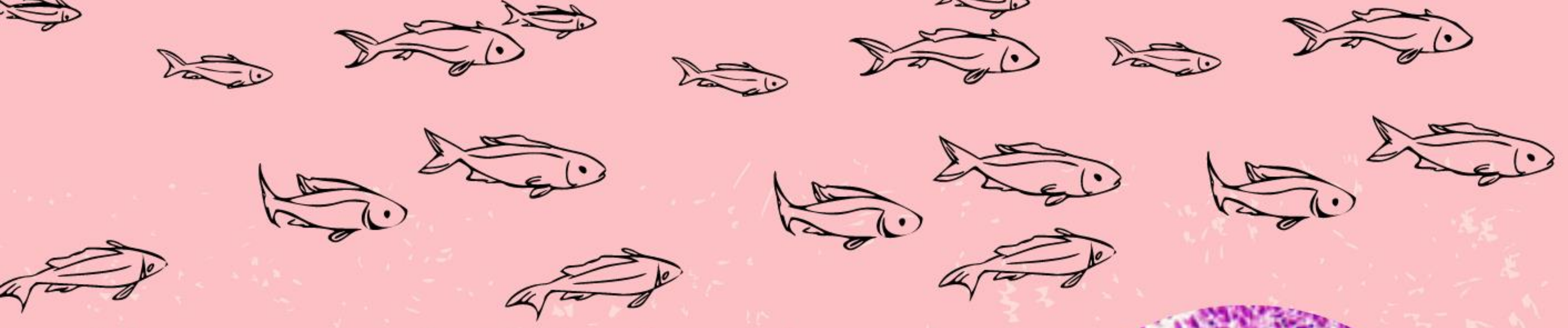
Principais conclusões do capítulo e perspectivas da tese

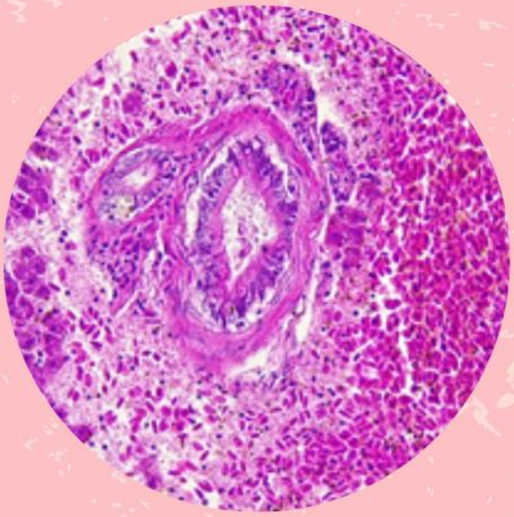
Neste primeiro capítulo, foi realizado um estudo integrado aplicando um conjunto de ferramentas biológicas e geoquímicas para avaliar a qualidade ambiental do estuário do Canal de Santa Cruz em Itapissuma (ITAP) e o estuário do rio Sirinhaém (SIR), litoral norte e sul de Pernambuco, respectivamente. Ambas as áreas estão submetidas a diferentes fontes e níveis de poluição, sendo observado quando : (i) mensuradas as concentrações dos metais traços Cd, Cr, Cu, Pb, Zn e Hg no sedimento na fração fina (63 µm) e no tecido dos peixes em cinco espécies de peixes estuarinos (*Centropomus undecimalis, C. parallelus, Caranx latus, Bairdiella ronchus e Gobionellus stomatus*), e (ii) utilizados índices de qualidade ambiental do estuário (fator de contaminação, índice de geoaculumação e o multifatorial de Nemerow, e avaliação de risco ecológicos dos metais traços).

Os índices de qualidade ambiental do sedimento permitiram avaliar que os pontos amostrados se encontram- em graus de contaminação distintos. Dentre os metais traços analisados os que tem concentrações mais altas são Hg em ITAP e Cu em ambas as áreas. Entretanto, o Hg apresenta um nível preocupante pois, além de estar acima do limite permitido pela legislação, apresenta elevado risco ecológico devido ao aumento da sua toxicidade através da espécie química na forma orgânica, o metilmercúrio, favorecendo a entrada deste contaminante na cadeia trófica resultante dos processos de bioacumulação e biomagnificação.

Dos 100 indivíduos analisados de ambas as áreas, apenas as espécies de ITAP apresentaram concentração do Hg acima do limite permitido pela ANVISA, estando em ordem de severidade de contaminação: *C. undecimalis> B. ronchus> C. parallelus e G. stomatus*, corroborando a importância da guilda trófica no monitoramento ambiental, devido à sua interferência direta na bioacumulação destes metais. Adicionalmente, *G. stomatus* também faz parte da dieta de *C. undecimalis*, confirmando o enriquecimento de mercúrio na espécie através da biomagnificação.

O Capítulo 1 também forneceu uma descrição geral das fontes poluidoras e possíveis origens da presença dos metais traços estudados. Evidenciou que a descarga de Hg pela indústria de soda caústica no rio Botafogo, mesmo após um longo período que cessou esta atividade, ainda apresenta níveis preocupantes no sedimento e nos peixes, com alta probabilidade de causar efeitos deletérios na biota. Em SIR, os metais não estiveram em níveis alarmantes, mas é possível observar a contribuição da indústria da cana de açúcar no aumento das concentrações de Cd, Cu, Hg e Zn. No próximo capítulo (Capítulo 2), utilizamos biomarcadores histopatológicos nas brânquias e fígado para as mesmas cinco espécies avaliadas neste capítulo. O uso destes biomarcadores permitirá a avaliação de uma resposta direta dos efeitos subletais das misturas de fontes poluidoras a que estes animais estão expostos.





CAPÍTULO 2

Histopathological biomarkers as indicators of the environmental quality of two estuaries in northeastern Brazil

Capítulo 2. Histopathological biomarkers as indicators of the environmental quality of two estuaries in northeastern Brazil

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Santos, Itala G. S. et al.

Artigo a ser submetido

Abstract

The present study aimed to assess the environment quality of the Santa Cruz Channel Estuary (ITAP) and Sirinhaém River Estuary (SIR), Northeastern Brazil, using histopathological biomarkers of liver and gills of fishes of different trophic level as indicators. Liver and gills samples were collected from: *Bairdiella ronchus* (n=24) and *Gobionellus stomatus* (n=34) in the ITAP; and *Caranx latus* (n=35), *Centropomus undecimalis* (n=24) and *C. parallelus* (n=29) from SIR. The liver samples had several damages, such as, hepatic steatosis, necrosis, vacuolar degeneration, and infiltration. The highest Histopathological Index of Liver (HIL) was recorded in *C. undecimalis.* Gills exhibited moderate to severe alterations for all species, such as the lifting of epithelial cells, lamellar aneurysm, and rupture of the lamellar epithelium. In SIR *C. undecimalis* and in ITAP *G. stomatus* had the highest number of alterations in liver and gills. Both areas have been historically affected by mercury (Hg) pollution in ITAP, and by the sugarcane industry in SIR and the species used as biomonitors has proven to be severely damaged in both estuaries. The species chosen in this study were considered good biomonitors of pollution and the combination of biomarkers methodologies in two organs, pioneering in Northeastern Brazil, was efficient in diagnosing the health status of the area using fish as biomonitor.

Keywords: gill; liver; biomarkers; histology; anthropic impacts; Brazilian coast

1. INTRODUCTION

Discharges of domestic, industrial and agriculture effluents are among the activities with the greatest potential for contamination in tropical coastal ecosystems (Saldarriaga-Hernandez et al., 2020). The first challenge is to identify chemicals that potentially present a risk to resident fish (Naidu et al., 2016). Industrial activities are responsible for many impacts and the discharge of trace metals, has become of worldwide concern (Ali et al., 2018; Boran and Altinok, 2010). Industries of leather tanning, production of cement, chlor-alkali, mining, anticorrosive agent, and fertilizer production (Aslam and Yousafzai, 2017; Becker et al., 2018; Chellaiah, 2018; Cipurkovic et al., 2014; Pacyna et al., 2010; Vieira et al., 2019) are among the main industrial activities that threaten the balance of ecosystems. Pollutants, such as pesticides and herbicides, used in the cultivation of sugar cane, are also of great concern given their impact on estuaries, consequently on biota (Chavan and Muley, 2014; Coelho et al., 2018).

Estuaries are transitional ecosystems characterized by ecological cycles that receive high inputs of organic and inorganic contaminants, such as pesticides and trace metals (Salas et al., 2017) from multiple coastal anthropogenic activities, which negatively affect human health and biota (Carr et al., 2017; Dellamatrice and Monteiro, 2014). These contaminants are discharged into the water and accumulate mainly in the sediment (Kameli et al., 2017; Willacker et al., 2017).

Fish are considered good monitors for assessing the quality of aquatic environments since they bioconcentrate and bioaccumulate contaminants by direct absorption from water or in some case indirectly by consumption of contaminated preys (Kroon et al., 2017), consequently can reach the upper trophic levels used as food by humans (Wang, 2002). The choice of species to be used as an monitors of contamination is essential (Van der Oost et al. 2003), and those from different trophic guilds and with distinct trophic position are recommended because they use different absorption/accumulation routes, directly related to the type of food consumed (Terra et al., 2008).

Histopathological biomarkers are widely used to investigate the effects of contamination in organisms, due to the possibility of identifying lesions in cells, tissues, and organs (Hook et al. 2014; Van der Oost et al. 2003; Viana et al., 2013). On of the most used means to apply these biomarkers for environmental assessment is through histopathological changes in the tissue, as it is a direct response of sublethal effects, allowing the detection of the effect of pathogens in an efficient and easy-to-apply manner (Bernet et al., 1999; Fonseca et al., 2016). Among the organs used for histopathological studies, the liver is often chosen since it is essential for the physiological functioning of the animal, by detoxifying and excreting toxic substances, and bioaccumulating pollutants (Borges et al., 2018). Gills, the first organ in contact with pollutants, play a key role in gas exchange and osmotic regulation in fish, similarly recommended for environmental biomonitoring (Ameur et al., 2015). This approach is widely used to assess water quality in polluted environments (Dalzochio et al., 2016).

In this study, for the first time in northeastern Brazil, we evaluate the environment quality of the Santa Cruz Channel Estuary (ITAP) and Sirinhaém River Estuary (SIR) using liver and gills as histopathological biomarkers for fishes from different trophic levels. These areas are strongly impacted by intense domestic and industrial effluent discharge, shrimp farming, and artisanal fishing (David and Fontanetti, 2009; Medeiros et al., 2001; Moura and Candeias, 2009).

2. MATERIALS AND METHODS

2.1. Study area

The Santa Cruz Channel Estuary (ITAP) is the largest and most productive estuary in Pernambuco coast (Macêdo et al. 2000) (Fig 1a). It is a 22 km long U-shaped channel with width ranging from 0.6 to 1.5 km, located within the Santa Cruz Environmental Preservation Area (APA Santa Cruz). A chloro-alkali industry was responsible for launching 22 to 35 tons of inorganic mercury in an important tributary of the SCC complex for approximately 24 years of operation (Meyer 1996). These impacts such as shrimp farm, discharge industry and domestic may have contributed for the reduction of up 10% of mangrove coverage in this area (Pelage et al., 2019).

The Sirinhaém River Estuary (SIR) is a small, shallow, coastal plain estuary, 9.5 km long, with maximum depth ranging from 1.2 to 4.5 m (Silva et al., 2011). Located between the Guadalupe Marine Protected Area and the Sirinhaém Marine Protected Area, SIR is characterized by a high density of mangroves, occupying 18.7 km2 (Maia et al., 2006) (Fig. 1b). Spatial land cover is predominantly urbanization, vegetation, mangrove and bare soil (Pelage et al., 2019). Although it is of extreme biological importance, multiple impacts are reported in the SIR estuary such as the sugar cane and domestic and industry effluent (CPRH, 2011).

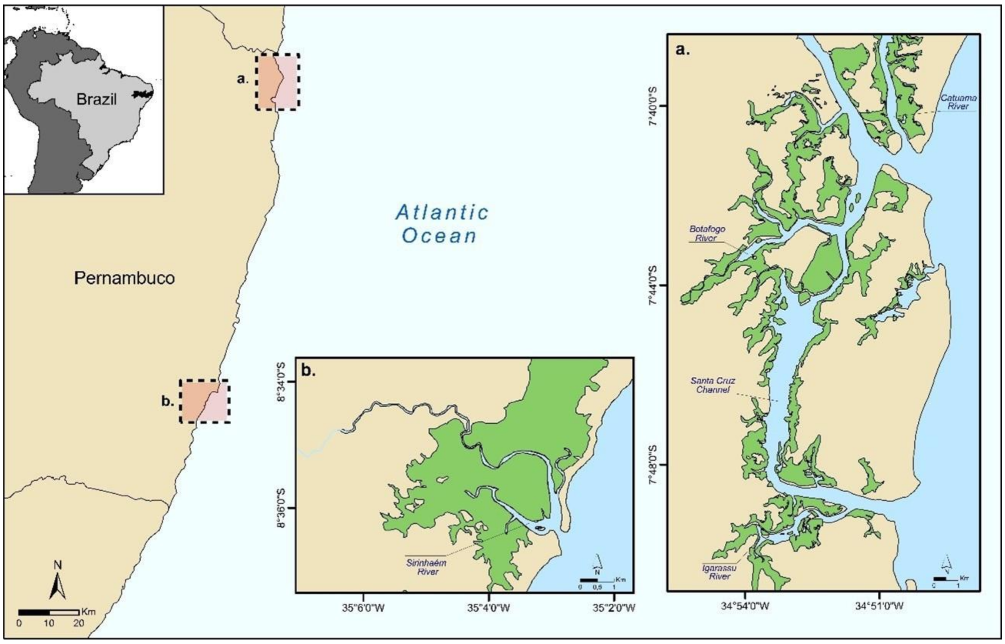

Figure 1- Study area (Pernambuco, northeast Brazil. a: Santa Cruz Channel Estuary (ITAP), b: Sirinhaém River Estuary (SIR).

2.2. Fish samples

Fishes were collected using block nets set close to mangrove creeks (350×2.9 m, 70 mm mesh) and beach seine trawls (20×1.9 m, 20 mm mesh), in 2018 and 2019. The fish species were chosen considering their importance for the local community as a source of food and income and are from the different trophic guilds. The trophic guild of each species was classified according to Elliott et al. (2007) and Mourão et al. (2014) as Detritivore (DV), Piscivore/Zoobenthivore (PVZB) and Piscivore (PV). In ITAP *Bairdiella ronchus* (n=24, PV) and *Gobionellus stomatus* (n=34, DV) were sampled, and *Caranx latus* (n=35, PVZB), *Centropomus undecimalis* (n=24, PV) and *C. parallelus* (n=29, PVZB) were collected in SIR. After collection the specimens were stored on ice and transported to the laboratory. In the laboratory, all fishes were measured (Standard Length – cm) and weighed (g). Posteriorly, an abdominal incision was made to assess the liver, and the second branchial arch from the right side was removed. The liver was weighted, and had its median portion removed.

2.3. Histology

The collected tissues were fixed in 10% formalin for 24 hours, preserved in 70% ethanol for histopathological analysis and subjected to routine histological processing for paraffin embedding (Prophet et al. 1995). Samples were subsequently sectioned into 5-µm-thick sections using a RM2245 microtome (Leica Microsystems, Germany). For both organs 6 sections were analyzed in all species The liver was stained with hematoxylin and eosin (HE), hematoxylin and eosin-phloxine B (HE-P), and Mallory Trichrome. The gills were stained with (HE) and (HE-P). Stained sections were analyzed, and photomicrographs were obtained using an Eclipse Ci-S light microscope (Nikon, Japan) connected to a Nikon DS-Ri1 digital camera.

2.4. Histopathological indicators

2.4.1. Histopathological Index of the liver (HIL)

Liver changes were semi-quantitatively assessed according to the adapted protocol described by Bernet et al. (1999). The Histopathological Index of the Liver (HIL) was calculated for each fish by multiplying the importance factor by the occurrence value of each alteration (see Table 1 for the-List of alterations). According to this index, an occurrence value was attributed to each change by taking into consideration the following tissue injury levels:

(1) minimum – easily reversible change; (2) moderate - reversible if the stressor is neutralized; and (3) severe - often irreversible, thus leading to decreased liver function. It was possible to assign an occurrence value to the changes after they were identified. This value corresponds to the level and extent of the tissue changes, namely: (0) unchanged; (2) occasional; (4) moderate and (6) severe (diffuse lesion).

High HIL values indicate more severe injuries in the affected organs. These indices are indicative of the extent and intensity of histological alterations in tissue morphology expressed into a quantitative value. Because of this, the method recommends using the frequency of the histological alterations, calculated from the occurrence of an alteration divided by the total HIL of the sample multiplied by 100, in all fishes and for each site.

The Hepatosomatic Index (HSI) (Eq.1) (Slooff et al., 1983) and Condition Factor (K)(Eq.2) (Smolders et al., 2002) were also used to assess the status of the fish health, since the variation in the weight of the organ in relation to body weight is a response of the individual when exposed to contaminants (da Mata Pavione et al., 2019). The HSI was calculated as:

$$HSI = 100 \times \frac{\text{liver weight (g)}}{\text{body weight (g)}} \qquad (1)$$

While the condition factor calculated by Eq. 2:

$$K = 100 \times \frac{W}{L^3} \qquad (2)$$

Where W = body weight (g) and L = total length of fish (cm).

2.4.2. Fish health Index (FHI)

The Fish Health Index (FHI) (Zimmerli et al., 2007) was used to classify liver injury severity. This index is divided into five classes, as follows: Class I (<10), normal/healthy tissue structure without impairments or pathological alterations; Class II (11 - 20), normal tissue structure with slight histological alterations; Class III (21-30), moderate modifications of normal tissue and morphology; Class IV (31 - 40), pronounced histological changes (in the liver); and Class V (>40), severe histological alterations of normal tissue and morphology.

2.4.3. Histopathological Index of the gill (HIG)

The gill histopathological alterations were calculated from the adaptation of the Poleksic and Mitrovic-Tutundzic (1994) classification. The type and location of the damaged gill tissue are divided into five groups: *a.* hypertrophy and hyperplasia of gill epithelia and related changes; *b.* changes in the mucous cells; *c.* gill parasites; *d.* blood vessel changes, and *e.* last terminal stages. After that, the method determines the stage of each group of alteration (Table 1). The HIG was calculated as Eq.3:

$$HIG = \sum I + (10 \times \sum II) + (100 \times \Sigma\ III) \quad (3)$$

Where I, II, and III are the number of stages for lesions. An overall score is used to classify the organ, being 0-10: functioning normally, 11-20: slightly to moderately damaged, 21-50: moderately to heavily damaged and > 100 irreparably damaged.

Table 1. Classification of histopathological changes of gill and liver in relation to the type, location and stage of lesions in which they occur. Modified from Poleksić and Mitrovic - Tutundzic (1994) for gills (stage) and Bernet et al. (1999) for liver (importance factor).

GILL/LIVER HISTOPATHOLOGY	STAGE/IMPORTANCE FACTOR
Hypertrophy and hyperplasia of gill epithelium	
Hypertrophy of respiratory epithelium	I
Lifting of epithelial cells	I
Lamellar epithelial hyperplasia	I
Focal hyperplasia of epithelial cells	I
Leukocyte infiltration of gill epithelium	I
Derangement lamelar	I
Incomplete fusion of some lamellae	I
Parasites	I
Complete fusion of all lamellae	II
Rupture of the lamellar epithelium	II
Uncontrolled proliferation of tissue	II
Necrosis	III
Changes in mucous cells	
Hypertrophy and hyperplasia of mucous cells	I
Changes in blood vessels	
Filament blood vessel enlargement	I
Vessel blood congestion	II
Hemorrhages with rupture of epithelium	II
Lamellar telangiectasis	II
Aneurysm Lamellar	II

Changes in hepatocytes	
Cytoplasm alteration	I
Hepatic steatosis	I
Hepatic parenchyma	I
Nuclear alterations	II
Vacuolar degeneration	III
Necrosis	III
Cell hypertrophy	I
Cell hyperplasia	II
Infiltration of leucocytes	II
Parasites	III
Wall proliferation of bile ducts	I
Changes in blood vessels	
Haemorrhage/aneurysm	I

2.5. Statistical analysis

The Kruskal-Wallis non-parametric test, followed by Nemenyi test for multiples comparisons (post-hoc), was performed to compare the HIL and HIG values among fish species, trophic guild and sites. These tests were also used for comparisons between the lesion classes HIL (4 stages) and HIG (3 stages) for each species.

To assess the degree of resemblance in the histopathological alterations among species for liver and gills, multidimensional scaling (MDS) based on a Euclidian distance matrix was applied using the frequency of occurrence of histopathological alterations (%) as input, with the specimens considered as the sampling units. Wisconsin double standardization was applied to improve the gradient detection ability of the dissimilarity indices (Bray et al., 1957). To compare the whole set of histopathological alterations among sites and fish species, a two-way PERMANOVA was applied (Wang et al., 2009). All analyses were performed in the R environment (Core Team R 2020), using vegan (Oksanen et al., 2017) and rrcov packages (Todorov and Filzmoser, 2009). For all analyses, a significance level of 0.05% was considered.

3. RESULTS

3.1. Fish analysis

A total of 146 individuals were analyzed. *Gobionellus stomatus* (3.82) and *Bairdiella ronchus* (1.13) in Santa Cruz Channel Estuary (ITAP) had the highest HSI values (Table 2). In terms of condition factor (K), the highest values were observed in *Caranx latus* (2.68) and *B. ronchus* (1.97), and the lowest in *G. stomatus* (1.01).

Table 2. Morphometric data and gross index. Mean ± standard deviation. n: number of individuals analyzed, HSI: hepatosomatic index, K: condition factor, ITAP: Santa Cruz Channel Estuary, SIR: Sirinhaém River Estuary.

Species	**n**	**Area**	**Standard Length (cm)**	**Body mass (g)**	**HSI**	**K**
Bairdiella ronchus	24	ITAP	14.17±1.38	34.74±11.66	1.13±0.42	1.97±0.15
Gobionellus stomatus	34	ITAP	14.36±3.18	11.96±5.91	3.82±3.31	1.01±0.25
Centropomus undecimalis	24	SIR	24.5 ±3.05	101.9±37.38	0.91±0.28	1.23±0.17
Centropomus parallelus	29	SIR	17.9 ±2.1	55.18±21.85	0.90±0.17	1.85±0.16
Caranx latus	35	SIR	12.49±1.1	27.32±8.8	1.06±0.35	2.68± 0.22

3.2. Histopathology of liver

A total of 13 types of alterations in the liver were observed: circulatory (1), inflammatory (3), progressive (1) and regressive (8), summarized by the Histopathological Index of Liver (HIL - Fig. 2). Given the reaction pattern, these alterations are divided into four categories: (1) circulatory disturbances (pathological condition of blood and tissue flow), including hemorrhage and aneurysm; (2) regressive changes (causes reduction or loss or the organ function) which involve cytoplasm alteration, hepatic steatosis, hepatic parenchyma, necrosis, nuclear alterations, and vacuolar degeneration; (3) progressive changes (increased activity of cells or tissues) represented by hypertrophy and hyperplasia in hepatic parenchyma; and, (4) inflammation (consequence of other reaction patterns), such as infiltration and parasites. The more relevant alterations are regressive changes. The six most important alterations, present in all fishes with different magnitudes were: hemorrhage (97.26%), structural alterations in liver tissue (100%), hepatic steatosis (92.46%), necrosis (72.60%), vacuolar degeneration (95.89%), and infiltration (94.52%).

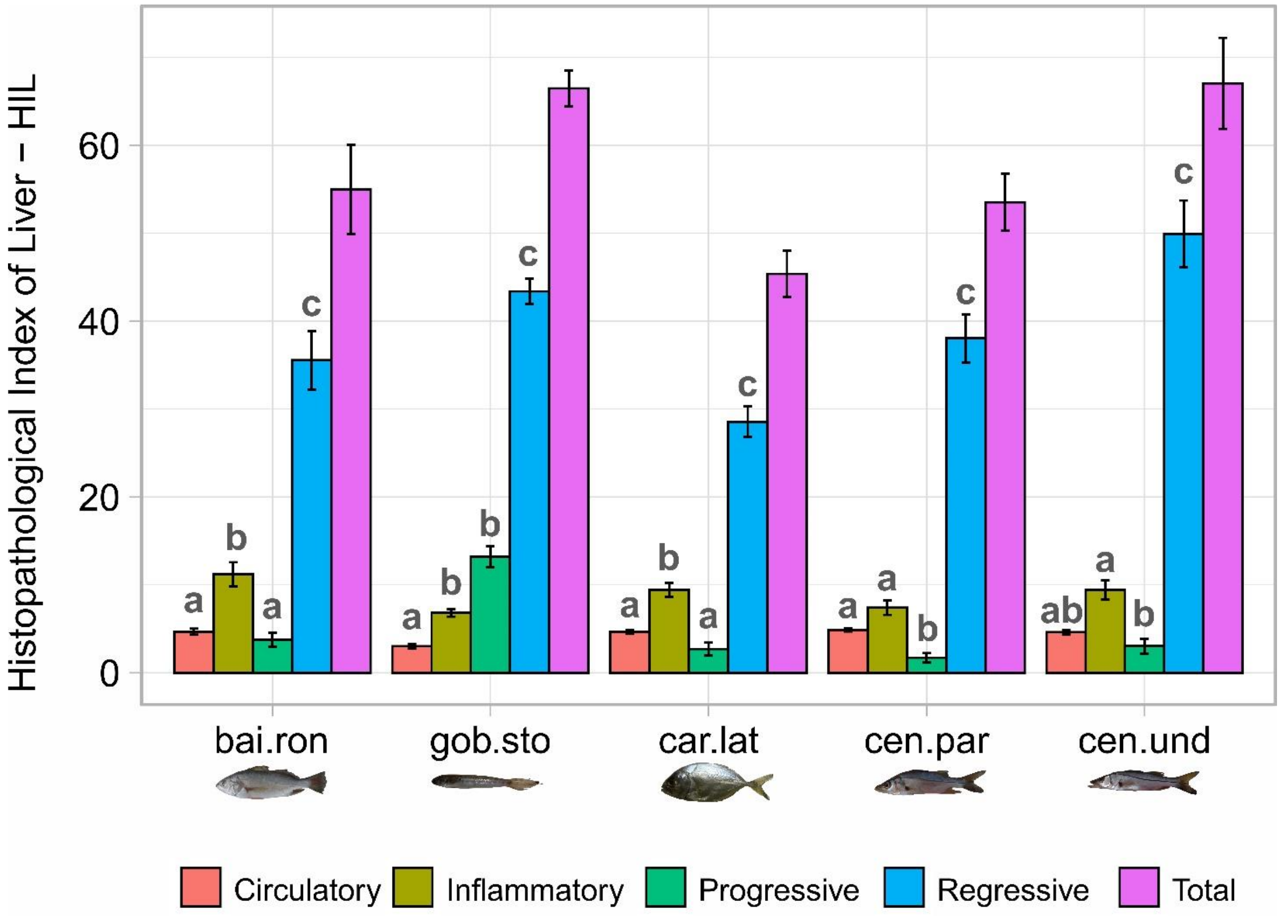

Figure 2 Histopathological index of liver (mean± SD) by studied species. Letters indicate the results of Nemenyi tests among species. bai.ron- *Bairdiella ronchus* and gob.sto- *Gobionellus stomatus* from Santa Cruz Channel Estuary (ITAP), car.lat- *Caranx latus*, cen.par- *Centropomus parallelus* and cen.und- *Centropomus undecimalis* from Sirinhaém River Estuary (SIR).

The alterations were significantly different in ITAP and SIR. *C. undecimalis* in SIR and *G. stomatus* in ITAP had the most severe changes. The HIL index of *G. stomatus* in ITAP was significant higher (66.47 ± 3.32) than those observed in *B. ronchus* (55.21 ± 5.85) (Fig. 2- HIL total). In SIR the HIL values were significantly different between *Centropomus undecimalis* (67.08 ± 5.97), *Caranx latus* (45.4 ± 3.44) and *C. parallelus* (52.10 ± 4.26). Based on Fish

Health Index (FHI), all species were considered with severe alterations given the morphology and hepatic parenchyma.

The rank of the histopathological index of liver (HIL), in severity order, was *C. undecimalis* > *G. stomatus* > *B. ronchus* > *C. parallelus* > *C. latus*. Although *C. undecimalis* and *G. stomatus* were more impacted in absolute values of HIL, *G. stomatus* had more severe histological changes, such as fatty degeneration. The HIL values were significant different between piscivore and piscivore/zoobenthivore. *C. undecimalis* and *C. latus*, and detritivore versus piscivore/zoobenthivore, corresponding to *G. stomatus* and *C. latus.* In general, piscivores (*C. undecimalis* and *B. ronchus*) and detritivores (*G. stomatus*) were more affected by local contamination.

In the MDS plot based on liver alterations, it was possible to observe a clear separation of *G. stomatus* from the other species (Fig. 3a). There were significant alterations in all histopathological alterations between *G. stomatus* and other species (pseudo-F = 9.89; p < 0.001), and there was significant difference between areas (pseudo-F = 21.84; p < 0.001), mainly due to the high occurrence of changes (Fig. 3b), in the vacuolar degeneration (Fig.4g), architectural and structural alteration, hyperplasia, and hepatic steatosis, in comparison with the other species. A high frequency of some alterations by species was observed, such as necrosis in *C. undecimalis* (Fig. 4e), inflammation by leukocyte infiltrate in *C. latus* (Fig. 4d), and parasites in *B. ronchus.*

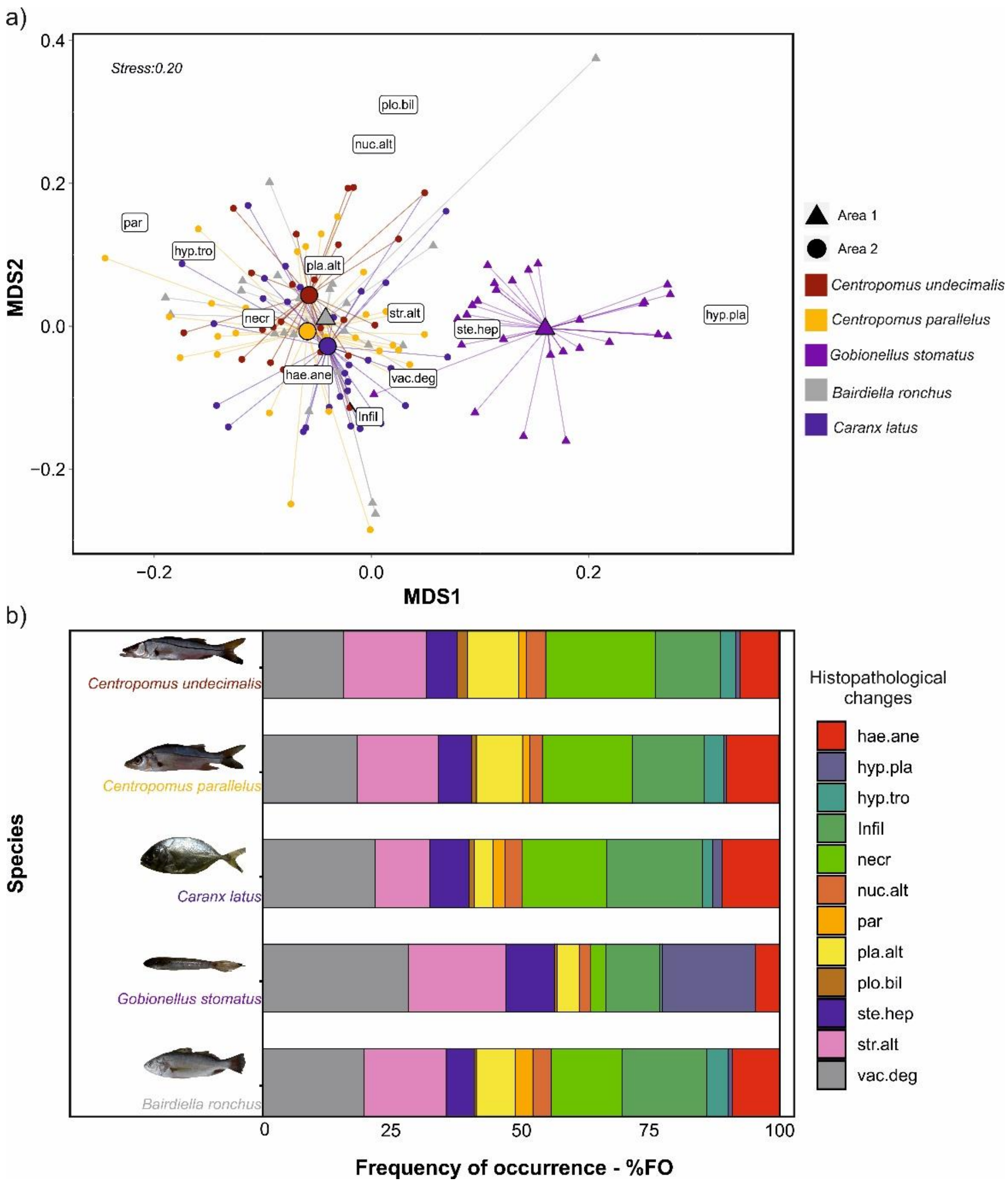

Figure 3. Plot of multidimensional scaling (MDS) with histopathological changes of the liver (a) and the frequency of occurrence of changes (b), hae.ane (haemorrhage/hyperaemia), hyp.tro (hypertrophy), hyp.pla (hyperplasia), Infil (infiltration), necr (necrosis), nuc.alt (nuclear alterations), par (parasite), pla.alt (cytoplasm alterations), ste.hep (hepatic steatosis), plo.bil (wall proliferation of bile ducts), str.alt (Architectural and structural alteration), vac.deg (vacuolar degeneration), area 1 (Santa Cruz Channel Estuary, ITAP), area 2 (Sirinhaém River Estuary, SIR).

Hepatic steatosis (Fig. 4b-c) was noted with a varying degree of intensity, indicating accumulation of lipids. However, particularly to *G. stomatus*, the liver parenchyma completely filled with fat accumulation was observed (Fig. 4g).

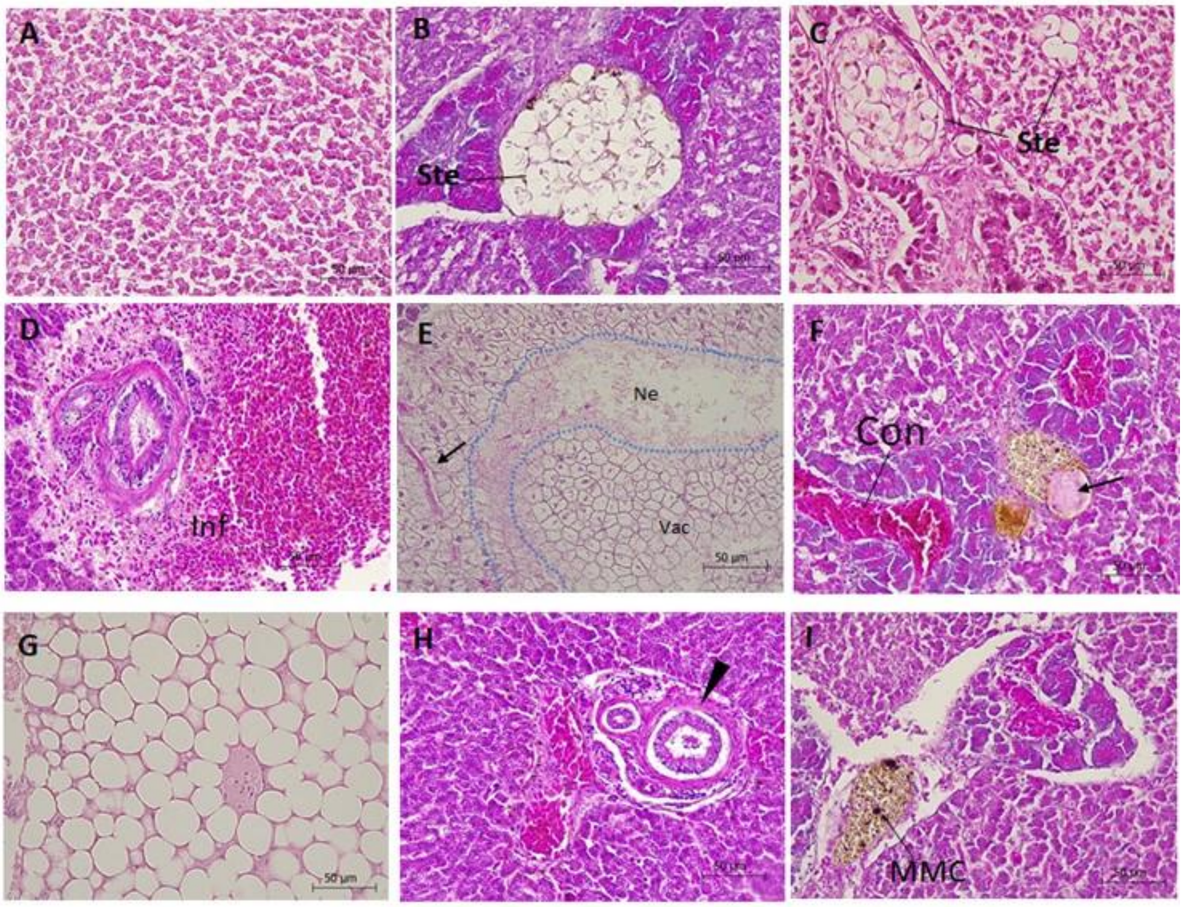

Figure 4. Histopathological changes in the liver of the analyzed species, C. parallelus (a,c), B. ronchus (e), C. undecimalis (b, f, i), C. latus (d, h), G. stomatus (g), Bar= 50µm; H & E-P stain. Photomicrograph hepatic tissue [A] Normal structural architecture ; [B] extensive hepatic steatosis (Ste); [C] hepatic steatosis (Ste) allocated to exocrine tissue and dispersed in the hepatic parenchyma,; [D] Inflammation by leukocyte infiltrate (Inf); [E] leukocyte infiltrate (arrow), vacuolated hepatocytes (Vac) and necrosis (Ne); [F] granuloma (red arrow), blood cell congestion within the exocrine pancreas (Con); [G] severe fatty degeneration; [H] hypertrophy of bile duct,; [I] melano-macrophage centers.

Parasites were present in 12.32% of the individuals of all species, except in *G. stomatus*, that have no parasites. The most affected species were *B. ronchus* and *C. latus*, with 29.16% and 17.14% of livers infected (Fig. 3b). Parasites were identified as metacercariae of digenetic trematodes (Fig. 5b-d) and myxozoan parasite (Fig. 5a). Metacercaries were found within the exocrine pancreas and hepatic parenchyma. Its presence has a clear separation within the blood congestion (Fig. 5b) and presents a central portion of indefinite shape, with a circular tendency in pink and branches in yellow. These pathogens were surrounded by a capsule of connective tissue (Fig. 5c). Myxozoan paratise (Fig. 5a) were observed in the bile ducts of *B. ronchus* that presented granulomas with inflammatory response, the parasite's morphology is mainly composed of collagen fibers, blue in color stained with Mallory trichrome. It occupies the entire bile duct and multiplies within it, increasing the size of this inflammatory capsule.

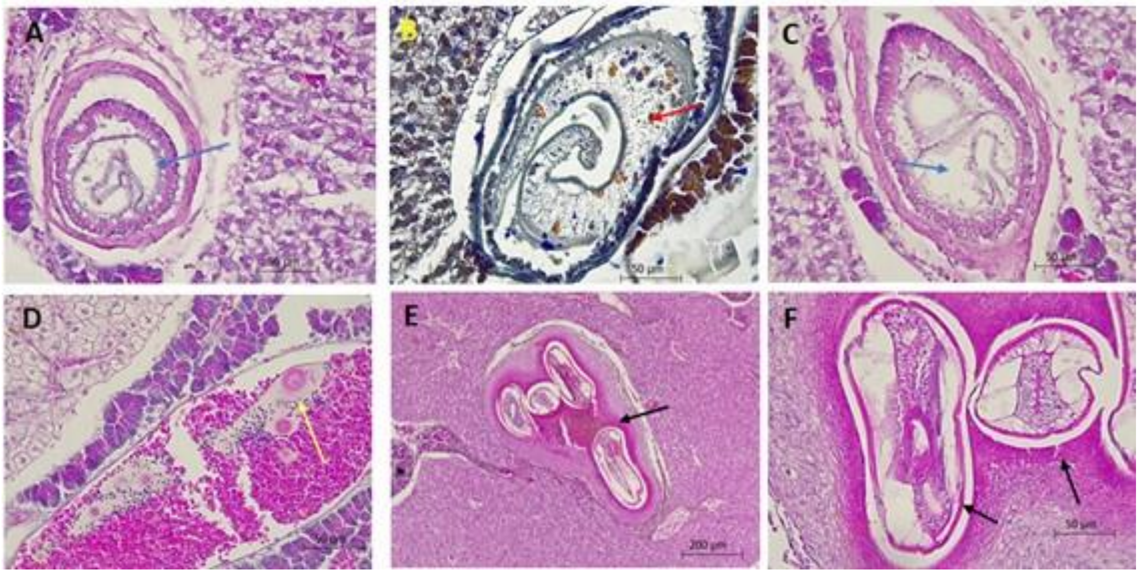

Figure 5. Histopathological changes in the liver of the analyzed species, *C. parallelus* (a,c), *B. ronchus* (e), *C. undecimalis* (b, f, i), *C. latus* (d, h), *G. stomatus* (g), Bar= 50µm; H & E-P stain. Photomicrographs of hepatic tissue [A] Normal structural architecture ; [B] extensive hepatic steatosis (Ste); [C] hepatic steatosis (Ste) allocated to exocrine tissue and dispersed in the hepatic parenchyma,; [d] Inflammation by leukocyte infiltrate (Inf); [E] leukocyte infiltrate (arrow), vacuolated hepatocytes (Vac) and necrosis (Ne); [F] granuloma (red arrow), blood cell congestion within the exocrine pancreas (Con); [G] severe fatty degeneration; [H] hypertrophy of bile duct,; [I] melano-macrophage centers.

3.3. Histopathology of gills

There were 18 types of alterations in the gills, distributed in 3 stages: stage I (10), stage II (7) and stage III (1) (Fig 6). The most abundant changes were: lifting of epithelial cells (65.06%) and fusion of several secondary lamellae (33.56%) in stage I, lamellar aneurysm (63.01%), vessel blood congestion (46.57%) and rupture of the lamellar epithelium (56.16%) in stage II, and necrosis (34.93%) in stage III.

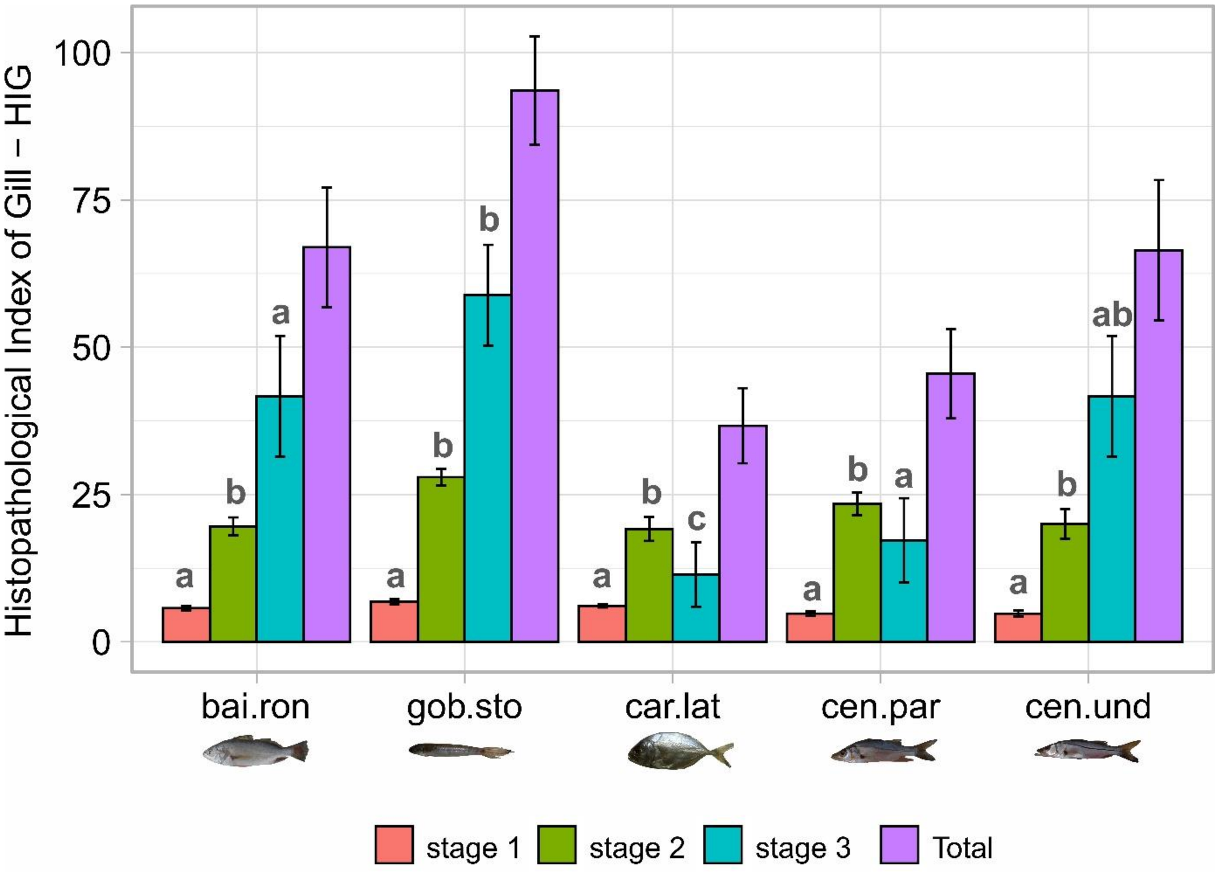

Figure 6. Histopathological index of gills (mean± SD) by studied species. Letters indicate the results of Nemenyi tests among species**.** bai.ron- *Bairdiella ronchus* and gob.sto- *Gobionellus stomatus from* Santa Cruz Channel Estuary (ITAP), car.lat- *Caranx latus*, cen.par- *Centropomus parallelus* and cen.und- *Centropomus undecimalis from* Sirinhaém River Estuary (SIR).

The histopathological index of gill (HIG) (Fig. 6) in ITAP was significant different than SIR. In ITAP the HIG values were significantly higher in *Gobionellus stomatus* (93.55 ± 10.36) than in *Bairdiella ronchus* (66.95 ± 12.21). In SIR the HIG did not significantly vary among species. In terms of severity, the rank of HIG was *G. stomatus* > *B. ronchus* > *Centropomus undecimalis* > *C. parallelus* > *Caranx latus*. Although all species had moderate to heavy damaged gills, *C. undecimalis* in SIR and *G. stomatus* in ITAP had more damages. Moreover, clearly, the species caught in ITAP were more contaminated in relation to the SIR species. Thus, the anthropized environment were more decisive in relation to the severity of the lesions than the species trophic level.

The frequency of changes was generally divided into four classes, 6.16% of fishes had normally functioning gills (HIG: 0-10) and 13.1% had slightly to moderately damaged gills (HIG: 11-20). The third class (HIG: 21-50) was the most abundant (45.89% of fishes), and corresponds to moderately to heavily damaged gills, with more severe changes that can affect normal functioning. In the latter class (33.56% of fishes) (HIG values > 100), there were irreparably damaged gills.

In the MDS plot based on gills damage (Fig. 7a) it was observed spatial differentiation of *G. stomatus* and *B. ronchus* compared to other species (pseudo-F = 2.23; $p < 0.05$), showing significant difference between areas (pseudo-F = 8.06; $p < 0.001$). There was predominance of specific alterations in some species. For example, *ronchus* had mostly necrosis, in *C. parallelus* hemorrhage with rupture of epithelium was most abundant, and complete fusion of all secondary lamellae was most common in *C. undecimalis* (Fig. 8c). In general, changes such as necrosis, lamellar aneurysm, rupture of the lamellar epithelium and hemorrhages with rupture of epithelium were present in all fishes in significant proportions (Fig. 7b and 8).

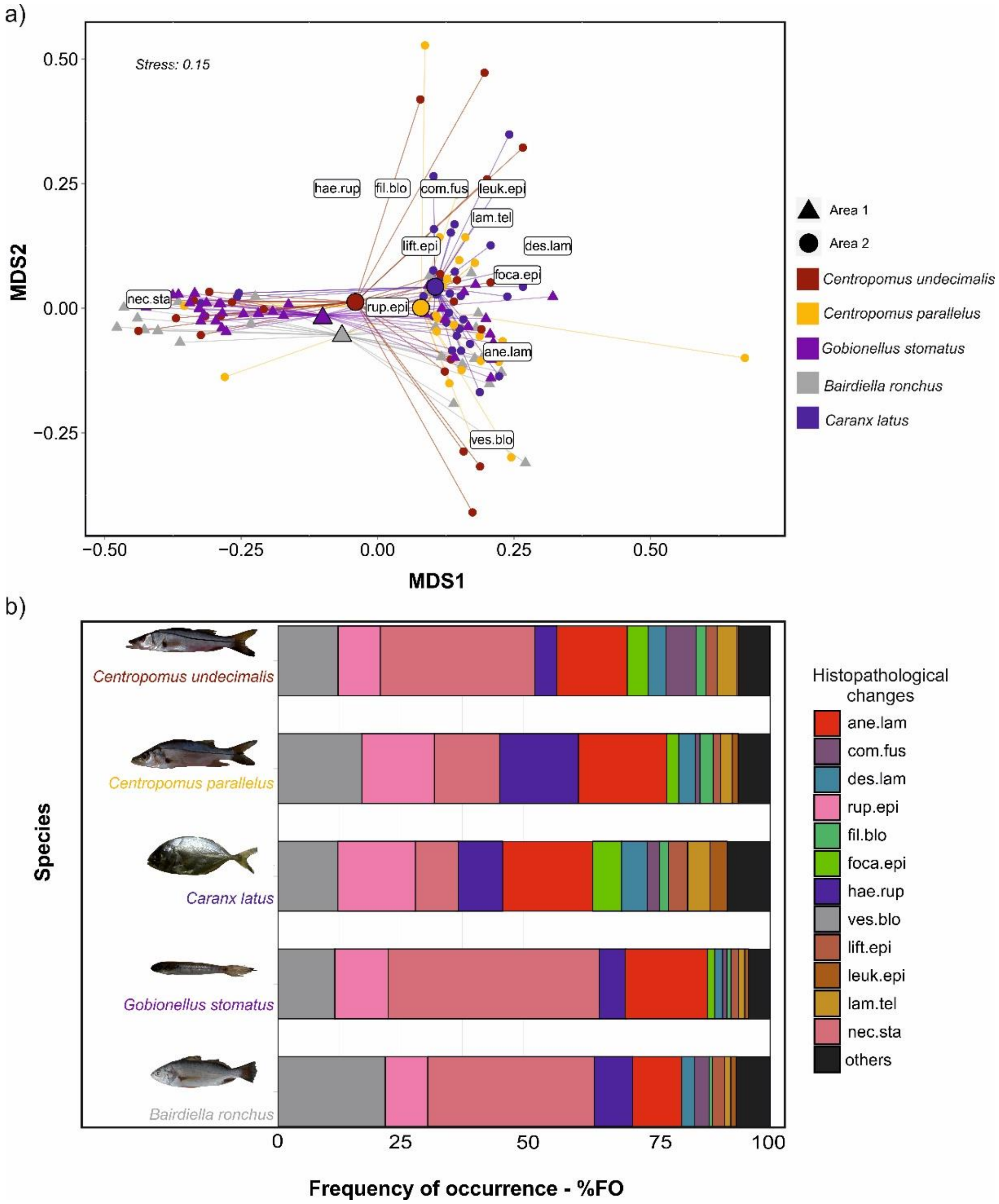

Figure 7. Plot of multidimensional scaling (MDS) with histopathological changes of the gills (a) and the frequency of occurrence of changes (b), ane.lam (aneurysm lamellar), com.fus (complete fusion of all the secondary lamellae), des.lam (derangement lamellar), rup.epi (rupture of the lamellar epithelium), fil.blo (filament blood vessel enlargement), foca.epi (focal hyperplasia of epithelial cells), haem.rup (hemorrhages with rupture of epithelium), ves.blo (vessel blood congestion), lift.epi (Lifting of epithelial cells), leuk.epi (Leukocyte infiltration of gill epithelium), lam.tel (lamellar telangiectasis), nec.sta (necrosis), others (minor changes involving hypertrophy and hyperplasia of gill epithelia), area 1 (Santa Cruz Channel Estuary, ITAP), area 2 (Sirinhaém River Estuary, SIR).

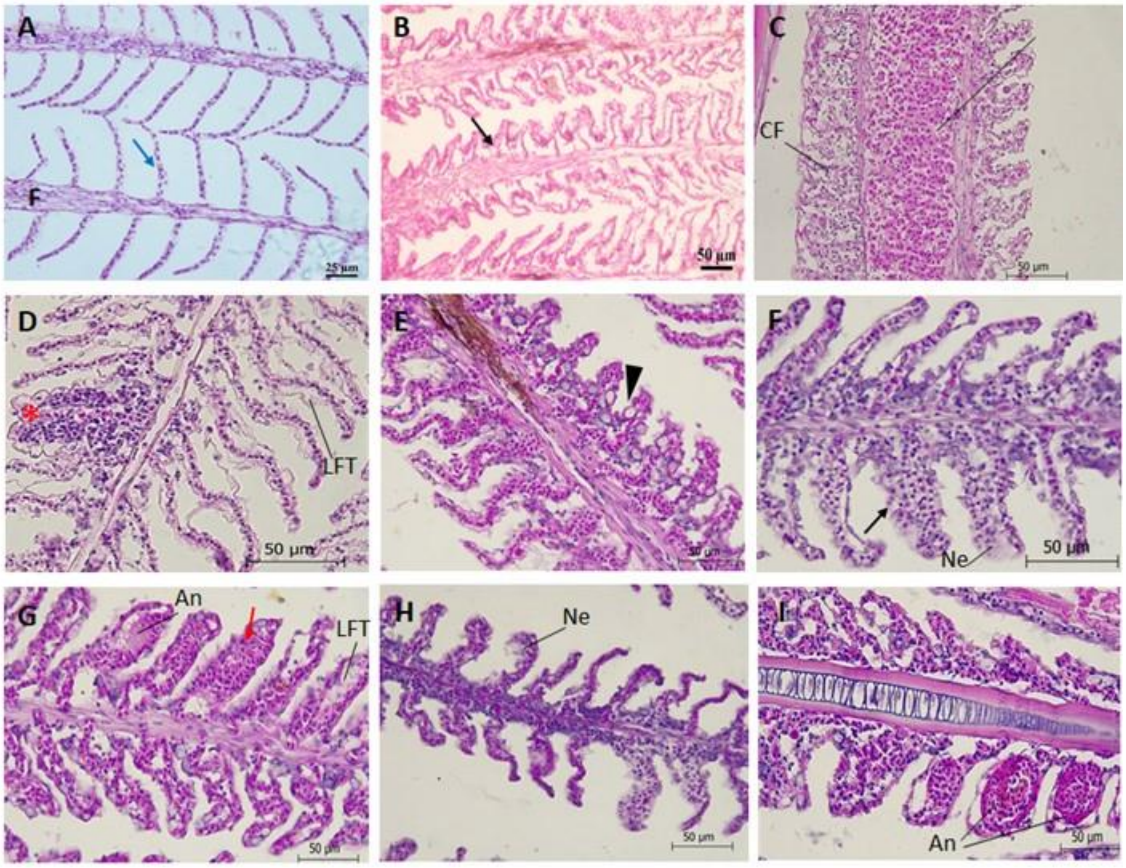

Figure 8. Histopathological gills of analyzed fish, *Bairdiella. ronchus* (a); *Caranx. latus* (d), *Centropomus. undecimalis* (f, h), *Gobionellus stomatus* (e, g, i). [A] normal structure for teleost gills with little lamellar derangement, filament (F) and second lamella (blue arrow) ; [B] Fusion of several secondary lamellae (black arrow); [C] complete fusion of several lamellae (CF), vessel blood congestion (VBC); [D] Lifting of respiratory epithelial cells (LFT), focal hyperplasia of epithelial cells (*), [E] hypertrophy of mucus cell (head arrow) [F] epithelial rupture (arrow), necrosis (Ne), [G] aneurysm (An), hyperplasia of epithelial cells (red arrow), epithelial lifting (LFT) with some intraepithelial edema, [H] necrosis (Ne), [I] aneurysm lamellar (An), H & E-P stain.

4. DISCUSSION

Histopathological biomarkers are widely used to assess the health status of fish in anthropized environments, since they allow the detection of sublethal effects, being a low-cost and easy-to-apply tool. In accordance with our findings obtained with the use of liver and gills and biomonitors, the species evaluated were effective for the diagnosis of environmental impact in the study areas, based on the degree of histological damage in individuals.

High values of hepatosomatic index (HSI) were observed in all species. The liver is an organ commonly used to assess contaminant effects and anthropic impact in fish, mainly through morphometric indices such as HSI and condition factor (K). Several studies have already evaluated the positive correlation between liver enlargement and the exposure to chemical pollutants, which can be a metabolic activity during the process of detoxifying contaminants (Schmitt and Dethloff, 2000; Van der Oost et al. 2003). The size of the liver can be used as a tool to monitor the health of fish in impacted aquatic environments (Van der Oost et al., 2003). Higher HSI values are associated to the presence of toxic compounds and, the increase of liver size is a result of hypertrophy and hyperplasia processes, as well as the increasing ability to metabolize xenobiotics (Araújo et al., 2018; da Mata Pavione et al., 2019). This relationship between a high HSI and accumulation of lipids was also reported by Fåhraeus-Van Ree and Spurrell (2003) for *Limanda ferruginea* from Witless Bay, Newfoundland and Labrador.

As for the HSI, the lowest K value among the analyzed species was observed in *G. stomatus*. K is often associated to water quality and food availability, hence an indicator of fish health (Rossi et al., 2020; Van der Oost et al., 2003). This index may be reduced by the presence of pollutants given the increased metabolic activity and demand of energy for detoxification process, altering the fish feeding rate (Heath, 1995).

All species had high values of histopathological alteration index of the liver (HIL) (HIL=45.44 - 67.8). Several authors have stated the efficiency of using HIL in fishes for environmental monitoring for environmental monitoring (Borges et al., 2018; Kostić et al., 2017; Viana et al., 2013). The HIL values in ITAP and SIR were higher than those recorded in other estuarine and coastal areas submitted to anthropic impacts, for example: *Solea senegalensis* had HIL<10 in contaminated sediments from south of the Gulf of Gabes, Tunisia (Ghribi et al., 2019); *Solea senegalensis* (HIL<12) in sediment from Sado estuary (Costa et al., 2009); and *Solea* spp. (HIL<12) in Bilbao estuary (Bay of Biscay) (Briaudeau et al., 2019). In Brazilian estuaries, our indices were much higher than those reported for *Atherinella* *brasiliensis* (HIL<30) in the Estuarine-Lagoon Complex of Iguape-Cananéia, São Paulo (Salgado et al. 2018) and *Cathorops spixii* (HIL<12) in Paranaguá Bay, Brazil (dos Santos et al., 2014).

Severe histological changes in the tissue (HIL>40) may be categorized as irreparable, like necrosis, that can lead to mortality due to the loss of vital tissue function or increase of susceptibility to disease (Zimmerli et al., 2007). The HLI and the HSI are directly related, and possibly correlated - high HSI values leading to high HIL, and vice-versa. High HSI is commonly associated to sublethal impacts of effluents, such as retention zones for contaminants, exposure time and bioavailability of contaminants (da Mata Pavione et al., 2019).

The liver alterations observed ranged from mild to severe, with loss of tissue function, and liver tissue and bile duct necrosis were the most serious injury (72.60%). Santos et al. (2014) observed necrosis alteration in 100% of liver of *Cathorops spixii* when evaluating the effects of bioaccumulation of butyltins in Paranaguá Bay. Khoshnood (2017) reported that different sources of pollution can result in moderate to severe damage to liver cells. Our findings were similar to those obtained by Oliva et al. (2013) with *Solea senegalensis* in Huelva, Spain; *Cathorops spixii* in Paranaguá Bay (dos Santos et al., 2014), and *Arius thalassinus* in Hodeida, Yemen Republic (Saleh and Marie, 2016). In general, all the studies presented above reported alterations as observed in our study, the liver had an increased vacuolar degeneration, necrotic foci, hemorrhage, leukocyte infiltration, granulomas and parasites when submitted to an anthropified environment. Among these alterations, the granuloma is the most complex due to the origin of its formation.

Granulomas observed for all species in this study were associated with inflammatory processes, which are capsules surrounded by multiple fibrotic layers involving an inflammatory response (Fricke et al., 2012). This alteration may be a response to environmental stress, or a process initiated by parasites (Araújo et al., 2019), as observed in our study. Parasites affect structure of tissue, increase granulomas, and can cause irreversible damage (Melo et al., 2014). These reaction patterns were similarly observed in all fishes of our study, associated with the presence of parasites. Highly anthropized environments directly affect the immunity of fish that inhabit this ecosystem, making them more susceptible to parasite infestations (Byers, 2020; de Melo Souza et al., 2019; Falkenberg et al., 2019).

All fish specimens evaluated in both areas presented around 95.89% vacuolization in the liver. It usually occurs due to the storage of lipids or glycogen as an energy source when found in low proportion or minimums, but it is possible to increase if subjected to some stressors like contaminant exposure and nutritional status of the species (Wolf et al., 2015). Oliva et al.

(2013) found 100% vacuolization in *Solea senegalensis*, from a polluted estuary in Huelva, Spain.

Gobionellus stomatus had a steatosis in higher degree of intensity than other species. The steatosis or fatty degeneration found in all species in this study may be derived from the toxic exposure to pollutants, such as organic compound and heavy metals (Wolf and Wolfe, 2005) . The severity of this pathology is due to the direct impact on liver functions, enabling an increase in the individual's susceptibility to poison by contaminants (Roberts and Rodger, 2012). This pathology in *G. stomatus* may be associated with a specific response of this species to contaminants. Similar results were observed indegenerative processes associated with excessive fat storage in fish from heavily polluted river in *Zoarces viviparus* of the Baltic Sea (Fricke et al., 2012).

In general, the studied fishes had moderately to heavily damaged degree of gill damage. Some individuals had permanent lesions, such as necrosis, resulting in irreparable damage, indicating that these fish are being subjected to long exposure to toxic agents. The presence of these lesions indicates that a complex mixture of contaminants is present in natural environments affecting the fishes and their health condition (da Silva Montes et al., 2020). Mucous and chloride cells are the cells mostly affected by toxic substances, as they act in the defense and transport mechanism of chloride ions, respectively. These stress-inducing factors can induce pathological changes(David and Fontanetti, 2009; Nimet et al., 2020).

In this study, the histopathological index of gills (HIG) ranged from 36.68 to 93.55. Fish as biomonitor for histopathological alterations to assess environmental contamination are being used by several authors. Carvalho et al. (2020) evaluated *Micropogonias furnieri* and *Menticirrhus americanus* (HIG = 100) from the Bay of Sepetiba-Rio de Janeiro, an area known for the disposal of materials and contaminants and recorded higher indexes than those reported in the present study. *Centropomus undecimalis*, with an average HIG of 66.45, is frequently used as a bioindicator of water quality in impacted areas. Several authors in Maranhão (Brazil) (Cantanhêde et al., 2018, 2016, 2014; Santos et al., 2014) found values lower than observed in our study (HIG = 19.11 to 53).

In both estuaries, all fishes had several gill alterations. In general, the histological changes observed in the liver and gills are not of specific origin, being associated with their response when exposed to toxic agents, which can vary from trace metals, and vinasse to pesticides (Chavan and Muley, 2014; Coelho et al., 2018). Even though the studied areas are impacted by different anthropic sources and different severities, such as the launch of 22 to 35 ton of Hg in the ITAP for 24 years and the sugar industry established in SIR since the 19th century (Meyer 1996; Pelage et al. 2019), it is difficult to determine the main source that causes histopathological changes in the liver and gills. Trace metals are responsible for several deleterious effects in fish tissues leading to changes in metabolism, such as hypertrophy (Cr), hyperplasia, lamellar fusion, rupture of epithelium lamellar (Zn), hypertrophy and hyperplasia of primary and secondary lamellae; and liver hepatocytes vacuolations (Cd) (Aslam and Yousafzai, 2017; Labarrère et al., 2012; Naïja et al., 2016). Moreover, the release of vinasse in the estuary can favor the appearance of lesions, such as rupture and hemorrhage in the gills(Correia et al., 2017). The pollution of these areas was studied by Lopes (2018) which evaluated the pollution in sediments and biota, finding Hg values above the level 2 established by Brazilian environmental legislation (CONAMA, 2012), which means a high probability of toxic effect to biota. The presence of this metal resulted in alteration in neurological physiology, measured by inhibition of AChE activity in *C. undecimalis,* used as biomonitors species.

Alterations were more severe in fish from ITAP and in piscivorous and detritivores. *Gobionellus stomatus* had fatty degeneration with a magnitude of severity, totally different from the other species. This species and *C. undecimalis* (or of similar genus) are already assigned as keystone species in these estuaries (Lira et al., 2018), and according to our findings, can also certainly be considered as efficient biomonitors in tropical estuaries. The combination of methodologies pioneering in the Northeast of Brazil, using different species and trophic guilds, was efficient in diagnosing the health status of the area using fish as biomonitor. There is no study of how these potential damages, evident by the histological alterations, can impact human health, mainly by trophic biomagnification, since the studied species are of great importance to the local community, as source of food and income. This study serves as a warning to the population that feed on fish of impacted estuarine areas, such as the present case, and public policies and constant environmental monitoring are needed to investigate the effects of aquatic pollution and consequently, assure food security to the population.

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Principais conclusões do capítulo e perspectivas da tese

No capítulo 2, utilizamos biomarcadores histopatológicos (em brânquias e fígado) como ferramentas complementares para o diagnóstico ambiental do estuário do Canal de Santa Cruz em Itapissuma (ITAP) e o estuário do rio Sirinhaém (SIR), descrevendo os efeitos sub-letais dos contaminantes presentes nestas áreas (Fig.1). Conforme observado no Capítulo 1, a maioria dos metais traços estavam em muitos pontos para ambas as áreas abaixo do TEL. Entretanto, outras fontes de poluição ou a assimilação destes metais podem causar efeitos na biota. Sendo assim, neste Capítulo, investigamos os efeitos de poluentes através das lesões das células de ambos os órgãos.

A contaminação em SIR é predominantemente orgânica devido ao efluentes da indústria da cana de açúcar com a presença de pesticidas e os maiores efeitos na biota estão associados com o período do lançamento desses efluentes no estuário, podendo ser caracterizado como uma fonte pontual com efeito agudo. ITAP, entretanto, está sujeito aos efeitos de um misto de contaminantes, como os advindos das indústrias de diferentes segmentos, efluentes domésticos e a persistência do mercúrio proveniente da indústria de soda caustica, impossibilitando identificar o causador das alterações.

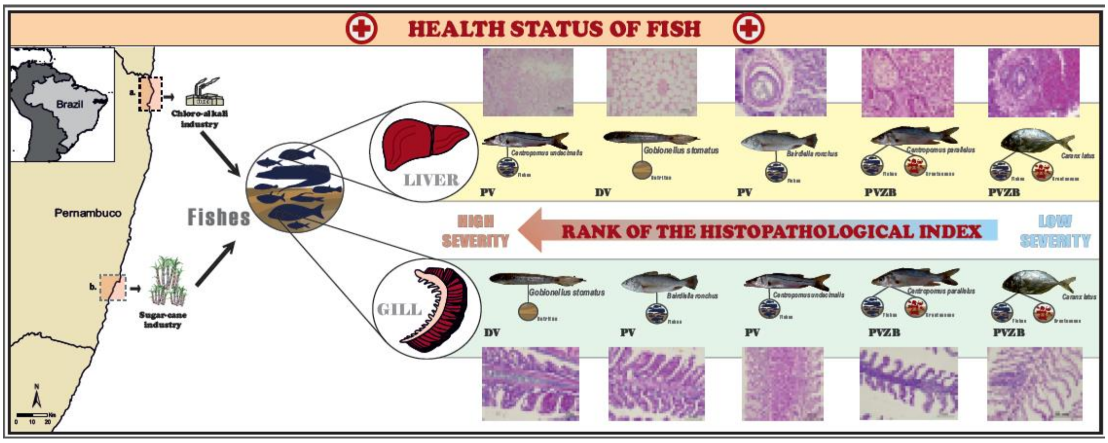

Figura 1. Graphical abstract of health status of fish

Considerando os dois estuários, a classificação do índice histopatológico do fígado (HIL), em ordem de gravidade é,*Centropomus undecimalis*> *Gobionellus stomatus*> *Bairdiella ronchus*> *C. parallelus*> *Caranx latus* e para o índice histopatológico das brânquias (HIG) em ordem de gravidade, é: *G. stomatus*> *B. ronchus*> *C. undecimalis*> *C. parallelus*> *C. latus* (Fig. 1). As alterações mais severas foram observadas para os peixes coletados na área 1 (ITAP), sendo correlacionadas com a guilda trófica, uma vez que piscívoros e detritívoros foram os mais afetados. *Gobionellus stomatus*, considerada a espécie com alterações mais severas, apresentou degeneração gordurosa com magnitude de gravidade diferente das demais espécies, seguida do *C. undecimalis* (ou de gênero similar). As espécies escolhidas neste estudo foram consideradas boas biomonitoras de poluição e a combinação de metodologias de biomarcadores em dois órgãos, pioneira no Nordeste do Brasil, foi eficiente no diagnóstico do estado sanitário da área utilizando peixes como bioindicadores. No próximo capítulo, iremos utilizar outra ferramenta de uso integrado numa abordagem espacial pioneira integrando o uso associado de isótopos estáveis δ13C e δ15N e modelos de mistura e isoscape aplicados em sedimentos e peixes para avaliar os impactos antropogênicos presentes no estuário tropical do rio Sirinhaém, nordeste do Brasil.

CAPÍTULO 3

Using stable isotopes and isoscape to detect pollution in a tropical estuary in Northeastern Brazil

Capítulo 3. Using stable isotopes and isoscape to detect pollution in a tropical estuary in Northeastern Brazil

Santos, Itala G. S. et al.

Artigo a ser submetido a Science of theTotal Environment

Abstract

The present study aimed to evaluate the environmental quality of the Sirinhaém River, Northeast Brazil, using the isoscape associated with the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) as biomarkers of pollution in the Sediment Organic Matter (SOM) and fish (*Centropomus undecimalis*, *Centropomus parallelus* and *Caranx latus*). The isotopic signature of the SOM for $\delta^{13}C$ was higher in the upper estuary near industry and other effluent input, compared to the lower estuary near the sea. The isotopic signature of the SOM for $\delta^{15}N$ varied spatially in the estuary, being highest in the lower estuary, due to the sugarcane industry effluents transported from the upper part of the estuary and the sewage discharge. Overall, the contribution of SOM through multiple mixing model to the diet of the species ranged from 21% (*C. latus*) to 30.7% (*C. parallelus*). Spatially, higher contributions was observed in the upper part of the estuary, mainly for *C. undecimalis* and *C. paralllelus*, while the same species suffered influence from middle to lower estuary. The isoscape showed enriched values for $\delta^{13}C$ and $\delta^{15}N$ in the upper and lower estuary, respectively corroborating mixture models the influence of SOM for fish diet, highlighting that the use of stable isotopes can be used to an efficient environmental control of the estuary and replicated to other regions.

Keywords:fish; biomarkers; anthropic impacts; tropical estuary, , sediment organic matter

1. INTRODUCTION

The contamination of aquatic ecosystems has increased exponentially, especially in transient environments influenced by tidal dynamics as estuarine zones (Ramos e Silva et al., 2017; Cabral et al., 2019), favoring the displacement of pollutants such as trace metals, sewage, industrial discharges, and domestic effluents (Dang et al., 2015; Xu et al., 2015; Elliott et al., 2019; Souza et al., 2021;). The sediment acts as a sink for contaminants and, the natural water, turbulence allows their resuspension into the water column (Masindi and Muedi, 2018). In the biota, especially in fish, different chemical species of the contaminants accumulate directly through their vital functions and indirectly through biomagnification (Filote et al., 2021; Savoca and Pace, 2021).

Contaminant monitoring can be carried out by many approaches, such as by measuring the amount of trace metals in sediments and biota (CCME, 2001; CONAMA, 2012; ANVISA, 2013;), using algae, plants, molluscs and fish populations and communities as biomonitors (Hu et al., 2019; Gutierrez and Agudelo, 2020; Hong et al., 2020; Saadatmand et al., 2022), enzymes (Aljahdali and Alhassan, 2020; Moreira et al., 2020; Oliveira et al., 2020), histopathological (Coelho et al., 2018; Gusso-Choueri et al., 2022; Jesus et al., 2021) hematological and genetic damage (Cardoso et al., 2018; Alimba et al., 2019; Ek-Huchim et al., 2022) as biomarkers.

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios has been widely used to understand the transfer of matter and energy throughout food chains (Funes et al., 2019; Durante et al., 2022). In addition, they are also efficient biomarkers to investigate anthropogenic impacts, such as a tracer of pollution sources in contaminated areas (Pataki et al., 2010; Kopprio et al., 2018; Özdilek et al., 2019;). Seasonality and environmental variations can interfere in the composition of organic matter and isotopic fractionation of the aquatic organism (Reuss et al., 2013; Kopprio et al., 2014). However these signatures can indicate the entrainment of pollutants, such as sewage, on a spatial and seasonal scale, allowing the localization of this input into the trophic chain (Connolly et al., 2013; Souza et al., 2018). The isotopic ratio of $\delta^{15}N$ is generally more enriched in polluted environments than in unpolluted ones (Olsen et al., 2011; Moynihan et al., 2012; Connolly et al., 2013). Meanwhile, enrichment of $\delta^{13}C$ has been reported in fish from impacted areas (Prado, 2020).

Currently, one of the most robust applications of $\delta^{13}C$ and $\delta^{15}N$ isotopes is the use of isoscape (statistical or mechanistic) models (West et al., 2010) for different purposes such as animal migration, ecological studies, climate change and biogeochemistry (Ceriani et al., 2014; Sena-Souza et al., 2019; Winter et al., 2021). The application of isoscape in biomonitoring of aquatic environments provides a spatial overview of isotopic ratios, turning environmental monitoring more efficient (Bowen, 2010; Sena-Souza et al., 2019). Isoscape has been recurrently used for studying monitoring fish migration in coastal environments (Radabaugh et al., 2013; Ohshimo et al., 2019; Glew et al., 2021;). In estuarine areas, there is no record of the usage of this approach.

In Northeasten Brazil, specifically in the southern coast of Pernambuco, the tropical estuary of Barra de Sirinhaém, suffers numerous impacts derived from agricultural activity, mainly sugarcane, domestic sewage, shrimp farming and fishing (Lira;and Fonseca, 1980). High values of mercury concentration in sediments above the limits established by CONAMA resolution 454/2012 (Lopes, 2018), have been observed in this region. In addition, from 2000, the use of the pesticide fipronil was recorded as a result of sugarcane cultivation in the region (Masutti and Mermut, 2007). All these impacts are likely to interfere directly with biota, causing loss of biodiversity and/or reduced fisheries productivity, directly affecting the local economy and the health status of riverside population due to the ingestion of these contaminated organisms (CPRH, 2011; Pelage et al., 2019; Araújo et al., 2021).

This study proposes a pioneering spatial approach integrating the use of stable δ^{13}C and δ^{15}N isotopes from the mixing and isoscape models applied in sediment and fish to assess the anthropogenic impacts in the Sirinhaém River estuary (Northeastern Brazil). It is expected to i) identify the distribution of δ^{13}C and δ^{15}N stable isotope signatures in the sediment along the river-ocean gradient; ii) evaluate the contribution of SOM in fish; and iii) evaluate the pollution in the whole gradient of the Sirinhaém River estuary. The information obtained in this study is efficient for the understanding of the dynamics of estuarine contamination, providing important insights on how the sources of contamination can be directly reflected in the isotopic signatures of the sediment and biota along the estuary. It is also an innovative integrated approach used in tropical estuaries that can be replicated in other similar environments.

2. METHODOLOGY

2.1. Study area

The Sirinhaém River Estuary (SIR) is a small, shallow, coastal plain estuary, 9.5 km long, wide increasing up to 800m at the river´s mouth and with maximum depth ranging from 1.2 to 4.5 m (Silva et al., 2011). Located between the Marine Protected Area of Guadalupe and the Marine Protected Area of Sirinhaém, SIR is characterized by a high density of mangrove, occupying 18 km^2 (Maia et al., 2006) (Fig. 1) and a narrow connection to the ocean. Although it is considered to be of extreme biological importance, multiple impacts are reported in the SIR estuary like sugar cane and domestic and industrial effluent (CPRH, 2011) which provide large inputs of inorganic compounds into the system.

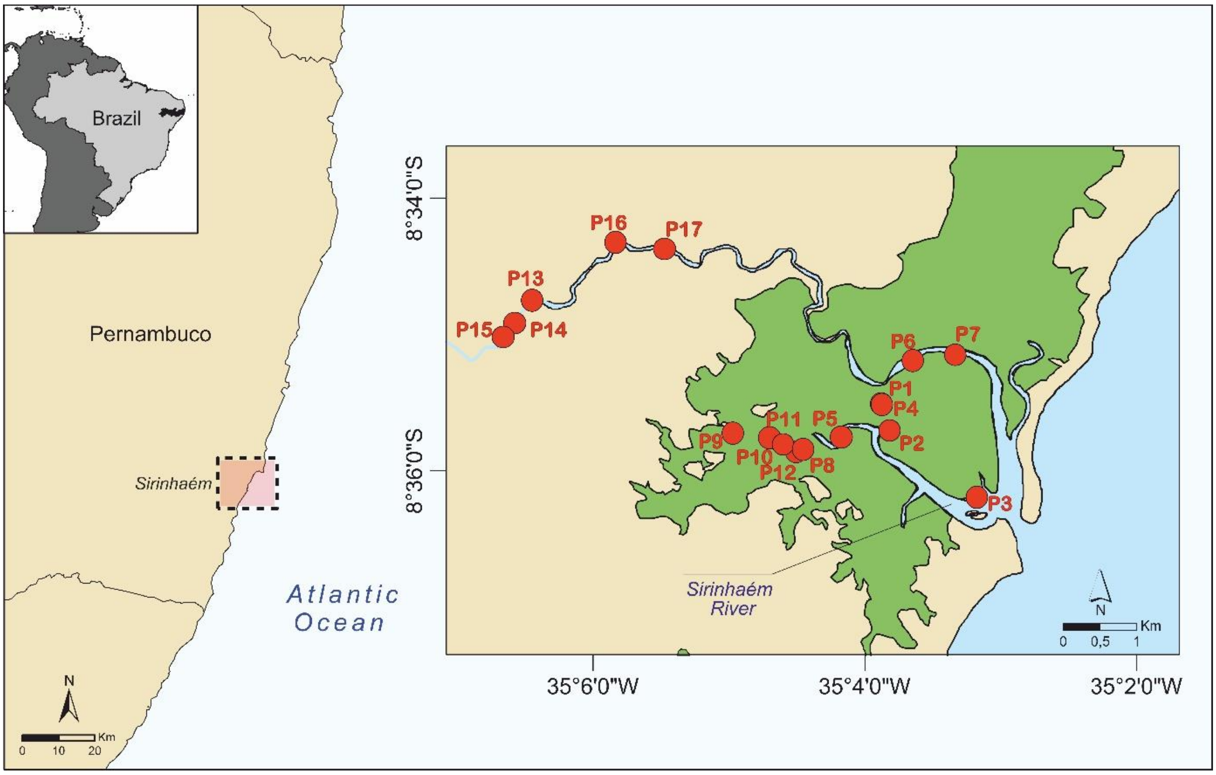

Figure. 1 Sirinhaém River Estuary located in Pernambuco state, northeastern Brazil. Mangrove areas are shown in dark green and sample locations are represented by red dots.

2.2. Sampling procedures

Fishes were collected in 2015, 2018 and 2019 during both the dry (January to March) and the rainy (July to September) seasons (APAC, 2019), so their isotopic ratios should reflect the temporal (inter and intra-annual) variabilities in fish diet and food web composition. Specimens sampled (5 to 10 for each year/season) reflected the common sizes observed for each species in the area: 7 to 12 cm (*Caranx latus,* Piscivore/Zoobenthivore), 15 to 25 cm (*Centropomus undecimalis,* Piscivore) and 12 to 24 cm (*C. parallelus,* Piscivore/Zoobenthivore). These species were chosen considering their different trophic guilds, are keystone species for the trophic structure of the ecosystem (Lira et al., 2018), and are socio-economically important, being widely consumed in the region. Representative estuarine organic matter sources in the study area were gathered according tothe literature (Pelage et al., 2021, Gonzalez et al., 2021), and comprised benthic algae (*Sargassum* spp.and *Lobophora variegata*), mangrove trees (rotten leaves of *Rhizophorae mangle*), and the organic matter present in the surface sediment (SOM) and in the water column (POM). They were all collected in 2015, both during the dry (January to March) and the rainy (July to September) seasons (Pelage et al. 2021). Additional SOM sampling were carried out in 2019 (January to May) along the estuary (from the Upper to the Lower section) (Fig. 1). Mangrove tree leaves and algae were collected manually at low tide. POM was obtained by filtering water (0.5–1 L) on precombusted fiberglass filters (0.75 μm), whereas SOM was sampled from the 2 mm surface layer of the sediment with Ekman collector or manually. All sampled source points were georeferenced.

2.3. Stable isotope analysis

White muscle from each fish specimen were extracted, rinsed with distilled water to remove exogenous materials (e.g., remaining scales or bones), and dried in an oven at 60 °C for 48 h. The whole sample of basal sources was used for the analysis. Dried samples were ground into a fine powder with a mortar and pestle. POM and SOM samples were duplicated. One subsample was acidified to remove inorganic carbon prior to the $\delta^{13}C$ analysis. The other sub-sample was used for the $\delta^{15}N$ analysis (Pinnegar and Polunin, 1999). Each sample weighed between 0.35 and 0.45 mg and was analyzed by continuous flow on a Thermo Scientific Flash EA 2000 elemental analyzer coupled to a Delta V Plus mass spectrometer at the Pôle Spectrométrie Océan (Plouzané, France). Results were expressed in standard δ notation based on international standards (Vienna Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$) following the equation:

$$\delta^{13}\text{C or }\delta^{15}\text{N} = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 10^{3} \text{ (in ‰), where R is } {}^{13}\text{C}/{}^{12}\text{C or } {}^{15}\text{N}/{}^{14}\text{N (eq.1)}$$

Reference materials of known $\delta^{15}N$ and $\delta^{13}C$ were analyzed: USGS61, USGS62 and USGS63. The recommended values of the standards were reproduced within the confidence limits. For every six samples, a home standard (Thermo Acetanilide) of experimental precision (based on the standard deviation of the internal standard replicates) was used, indicating an analytical precision of ± 0.11‰ for $\delta^{13}C$ and ± 0.07‰ for $\delta^{15}N$.

2.4. Data analysis

Isoscape models for $\delta^{13}C$ and $\delta^{15}N$ were performed along the estuary (17 sampling points) in order to identify the spatial distribution of SOM using the R package IsoriX (Courtiol et al. 2019) according to Winter et al. (2021). A geostatistical mean model from linear mixed-effects model (LMM) was used according to the protocol of Winter et al. (2021). Linear river distance to the sea (km from source; DIST) and absolute latitude (latitude point; LAT) were set as fixed effects and sample site as a random effect. Matérn correlation function (Matérn, 1986) was included in the mean model as a random effect to account for spatial autocorrelation. In a further gamma generalized LMM (GLMM; the residual dispersion model), the variance of the residual error in the mean model was assumed to be spatially structured according to the random effects of sample site and a Matérn correlation structure. A detailed account of the model structure is available in Courtiol and Rousset (2017) and Winter et al. (2021). The best-fitting mean models for $\delta^{13}C$ and $\delta^{15}N$ were evaluated by the minimum conditional Akaike's information criterion values (cAIC; Vaida and Blanchard, 2005), provided by the AIC function in package IsoriX (Courtiol et al. 2019). Single isoscapes were generated for SOM using a structural raster of the study system (approximate cell resolution = 0.0004 km^2), containing linear river distance data (distance from source) measured to the sea in km. For each raster cell (r), the predicted mean, prediction and residual variances for $\delta^{13}C$ and $\delta^{15}N$ were derived directly from the mean model outputs. Prediction variances directly quantifies the isotope uncertainty of the predicted isoscape, decreasing in areas that are dense in source data and increasing where data is sparse (Courtiol et al. 2019).

Bayesian stable isotope mixing models, using the MixSiar package (Stock et al., 2018), were built to estimate the relative contribution of the SOM supporting fish species diet. In a first step, one mixing model for each group of species (*C. latus*, *C. undecimalis* and *C. parallelus*) and year of collection (2015, 2018 or 2019) was developed using the representative organic matter sources gathered from the literature and fixing Season as a random effect. This allowed to provide an overall estimate of the relative contribution of SOM and match part of our fish samples to the same year of collection as organic matter sources. Subsequently, multiple mixing models were performed for each group of species and year of collection to evaluate the contribution of SOM from the distinct sampling locations along the estuary. Accordingly, in each of the multiple models, the isotope ratios of SOM obtained from the literature were replaced to those collected in this study since no differences were observed between the years of 2015 and 2019 for $\delta^{13}C$ (Kruskal-Wallis: $\chi2 = 0.4906$, p-value = 0.4837) nor $\delta^{15}N$ values of SOM (Kruskal-Wallis: $\chi2 = 0.4230$, p-value = 0.5154) (Figure S1). Although the isotope ratios of organic matter sources in estuaries are expected to vary according to the environmental dynamics of the system (Bouillon et al., 2008), particularly on a seasonal basis, this approach presume that the variation at the year level is negligible. Moreover, we assumed that the organic matter sources other than SOM were equally available throughout the estuarine system and exhibited similar isotope ratios. These models accounted for Season as a random effect and used the isotope ratios obtained from organic matter sources and fish of each respective season, with exception of SOM samples collected across the estuary that were presumed to have no seasonal differences in $\delta^{13}C$ nor $\delta^{15}N$ (Gonzalez et al., 2019a). All mixing models considered the studied species as secondary consumers (Gonzalez et al., 2021a; Lira et al., 2017) and used a trophic enrichment factor (TEF, mean ± standard deviation of 0.8 ± 1.7‰ and 4.6 ± 2.26‰, for $\delta^{13}C$ and $\delta^{15}N$ respectively), reflecting the TEF between organic matter sources and organisms in aquatic environments (McCutchan et al., 2003; *i.e.* $TEF_{model} = 2 \times TEF_{literature}$).

All statistical analyses were performed with the software R version 4.1.0 (R Core Team, 2021), using the packages vegan (Oksanen et al., 2017), MixSiar (Stock et al., 2018) and IsoriX (Courtiol et al., 2019).-

3. RESULTS

A total of 96 fish specimens (30 *C. undecimalis*, 32 *C. parallelus*, 18 *C. latus*), 90 samples of basal sources (6 samples of *Rhizophorae mangle*, 3 *Sargassum* sp., 2 *Lobophora variegata*, 6 POM and 73 SOM) were collected for stable isotope analyses. Stable isotope ratios ranged across the period analyzed in *C. undecimalis* for δ^{13}C (−27.84 to −20.88‰) and δ^{15}N values (8.77–10.71‰), in *C. parallelus* for δ^{13}C (−25.38 to −22.05‰) and δ^{15}N (8.68–12.54‰), and in *C. latus* for δ^{13}C (−24.36 to −19.88‰) and δ^{15}N (6.23–11.58‰) (Supplementary Table S1). Stable isotope ratios of basal resources ranged from −29.25 to −15.10‰ and from −1.03 to 7.06‰ for δ^{13}C and δ^{15}N, respectively (Supplementary Table S1). δ^{15}N and δ^{13}C values for SOM showed a spatial variability along the latitude and longitude gradient (Fig. 2). Enriched values were observed closer to the sea (-8.60 to -8.59 of latitude and -35.07 to -35.05 of longitude), which suffer from impacts by domestic effluents and shrimp farming, mainly for δ^{15}N (Fig. 2). In contrast, the least enriched values were found farther from the sea and closer to the influence of the river, in the areas closest to near output from sugar cane industry (Fig. 2).

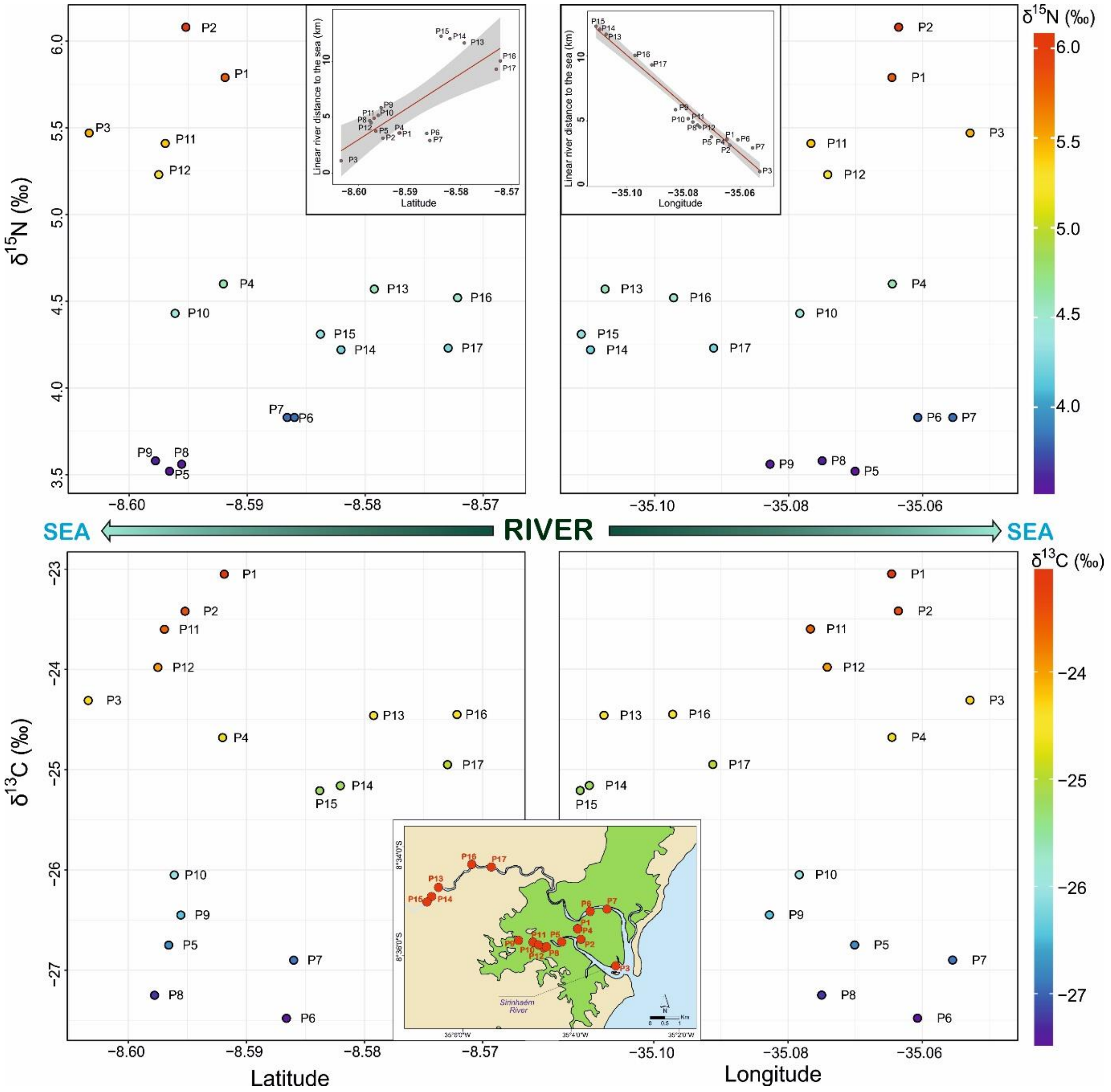

Figure 2. Biplot showing the $\delta^{15}N$ (Top) and $\delta^{13}C$ (Bottom) of organic matter in the sediment (SOM) along the latitude (Left) and longitude (Right) gradient of Sirinhaém River Estuary. The inner scarplot in the upper portion of the figure represents the relationship between the linear distance from the river to the sea with latitude and longitude.

Considering the geostatistical models for SOM isotope values along the Sirinhaém river, the best-fitting for $\delta^{13}C$ mean model (cAIC=34.84) with river distance and absolute latitude as fixed effects had the predicted enrichment for SOM of -0.021‰ per km and latitude fractionation of 19.75‰ (Table 1), whereas for $\delta^{15}N$ mean model (cAIC=35.58), it was reported a predicted enrichment of 0.02‰ and 13.45‰ per km and latitude respectively (Table 1).

Table 1. Linear mixed-effects model coefficient estimates (±standard error) for the geostatistical mean model fixed effects predicting $\delta^{13}C$ (a) and $\delta^{15}N$ (b) from linear river distance to the sea (DIST) and absolute latitude (LAT). ΔcAIC is conditional Akaike Information Criterion.

Model	Intercept	DIST	LAT	cAIC
(a) $\delta^{13}C$	-195.4716±507.66	0.08319±0.1525	19.75842±59.0194	34.84
(b) $\delta^{15}N$	-110.94±282.85	-0.02106±0.0848	13.45603±32.8831	35.58

From both fixed and random effects (Table 2) were performed predictions of $\delta^{13}C$ and $\delta^{15}N$ for SOM resulting in different isoscapes (Fig. 3). A clear variation from river to the sea in nitrogen ($\delta^{15}N$) was observed, with lower values predicted in upper estuary and higher in the lower estuary closer to the sea (Fig. 3). Meanwhile, with a smoother gradient, more enriched $\delta^{13}C$ values were predicted in upper estuary compared to lower zone (Fig. 3). However, a small distinct prediction on $\delta^{13}C$ values was observed between the tributaries of the Sirinhaém River which are further north (-26 to -25.5‰) in comparison to the south (great than -25.5‰). Prediction variance was higher for the estimates of carbon, ranging from 1 to 3.5‰, while nitrogen varied from 0.4 to 1‰. The greatest values were observed in reaches of the river where few samples were taken for both $\delta^{13}C$ and $\delta^{15}N$ isoscapes (Fig. 3).

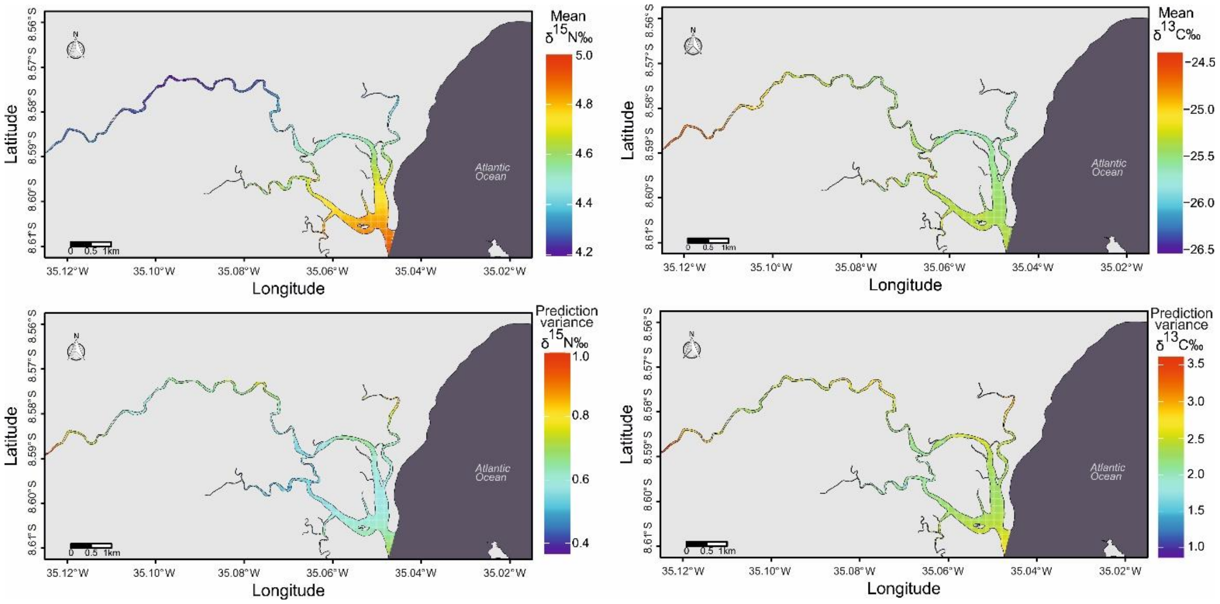

Figure 3. $\delta^{15}N$ (top-left) and $\delta^{13}C$ (top-right) isoscapes mean based on best-fitting linear mixed-effects models for organic matter in the sediment (SOM) in the Sirinhaém River Estuary. Prediction variance (bottom) is also displayed.

In general, basal sources had overlapping isotopic signatures between seasons, except for POM (Figure 4). Algae ratios were close to those of the POM in dry season, meanwhile, in the rainy season, it was similar to SOM in both seasons (Fig. 4). Most of the fish ratios were close to SOM in both seasons and POM in rainy season, but distant from the other sources, mainly mangrove (Fig. 4). Overall, the global relative contributions of the SOM were similar between species (Fig. 4, Supplementary Table 2).

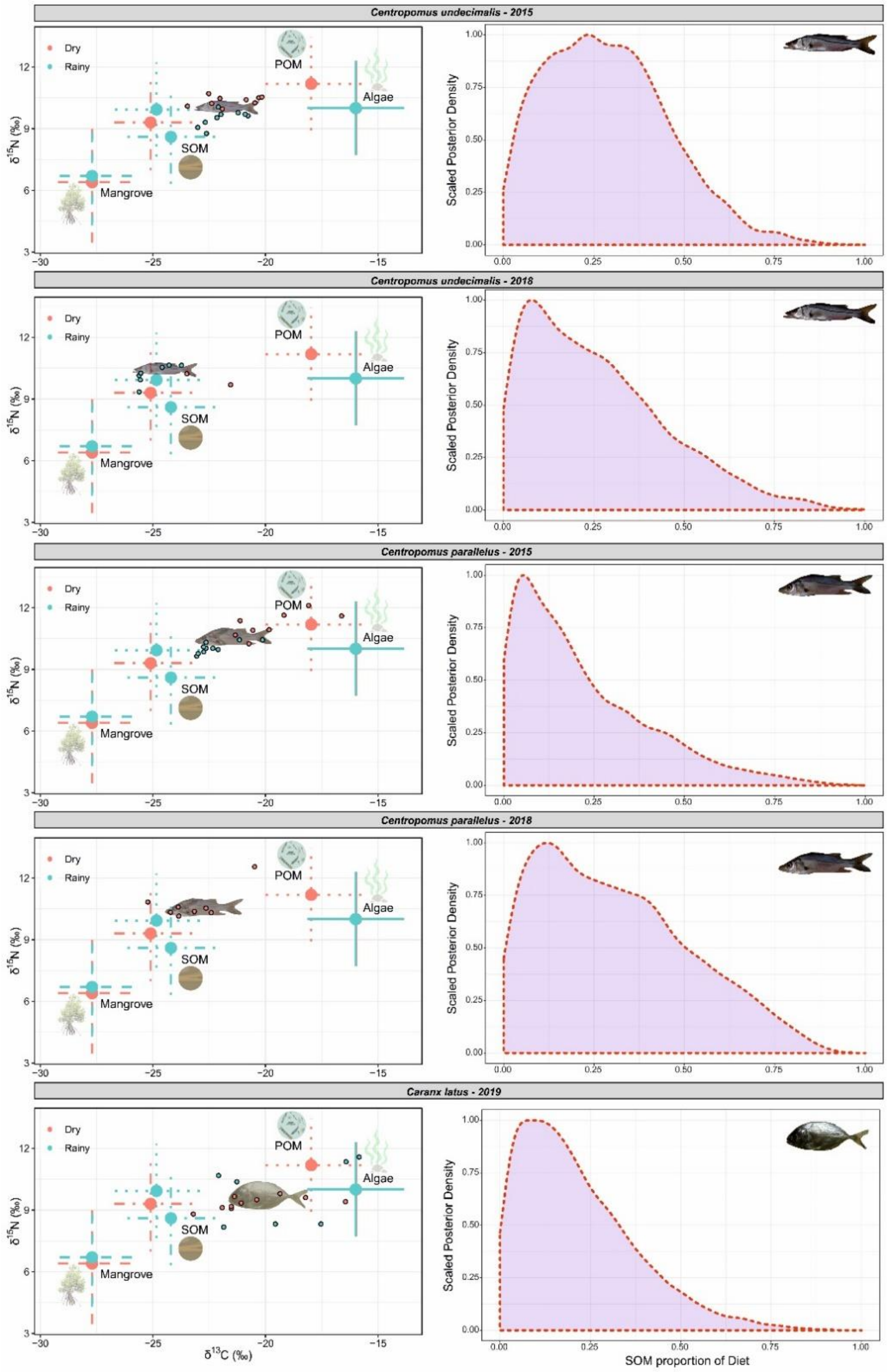

Figure 4. Left frame: carbon (δ13C) and nitrogen (δ15N) isotope ratios of the sources (represented by the circles and bars showing mean values ± SD) and the consumers (represented by the points) by year

sampled (2015, 2018 and 2019) after applying the trophic enrichment factor to the basal sources in the Sirinhaém River Estuary. Right frame: density plots representing global contribution of the SOM in the diet of consumers (*Caranx latus, Centropomus undecimalis* and *Centropomus parallelus*) from mixing model.

The maximum density proportion of SOM contribution to the species diet was below 50% regardless of the year, mainly from 21 to 30% (Fig. 4, Supplementary Table 2). Highest value of SOM contribution was observed in *C. parallelus* – 2018 (30.7%), while the smallest was found for *C. latus* (Fig. 4, Supplementary Table 2). The remaining sources contributed between 12 and 39% (Supplementary Table 2).

From the spatial SOM distribution, multiples mixing model were performed to find stretches of the river with the greatest SOM contribution to the species' diet (see details in section 2.4). Overall, highest proportions of SOM contribution to the diet of the species were observed in the upper stretch of the river (>25%), except for *C. parallelus*-2015 and *C. undecimalis*-2015 whose SOM contributions were higher in the middle and lower stretches (Fig. 5). Although *C. latus* exhibited a similar spatial pattern, with higher values in the upper stretch and lower in the lower stretch of the river, they presented the lowest contributions in overall (Fig. 5).

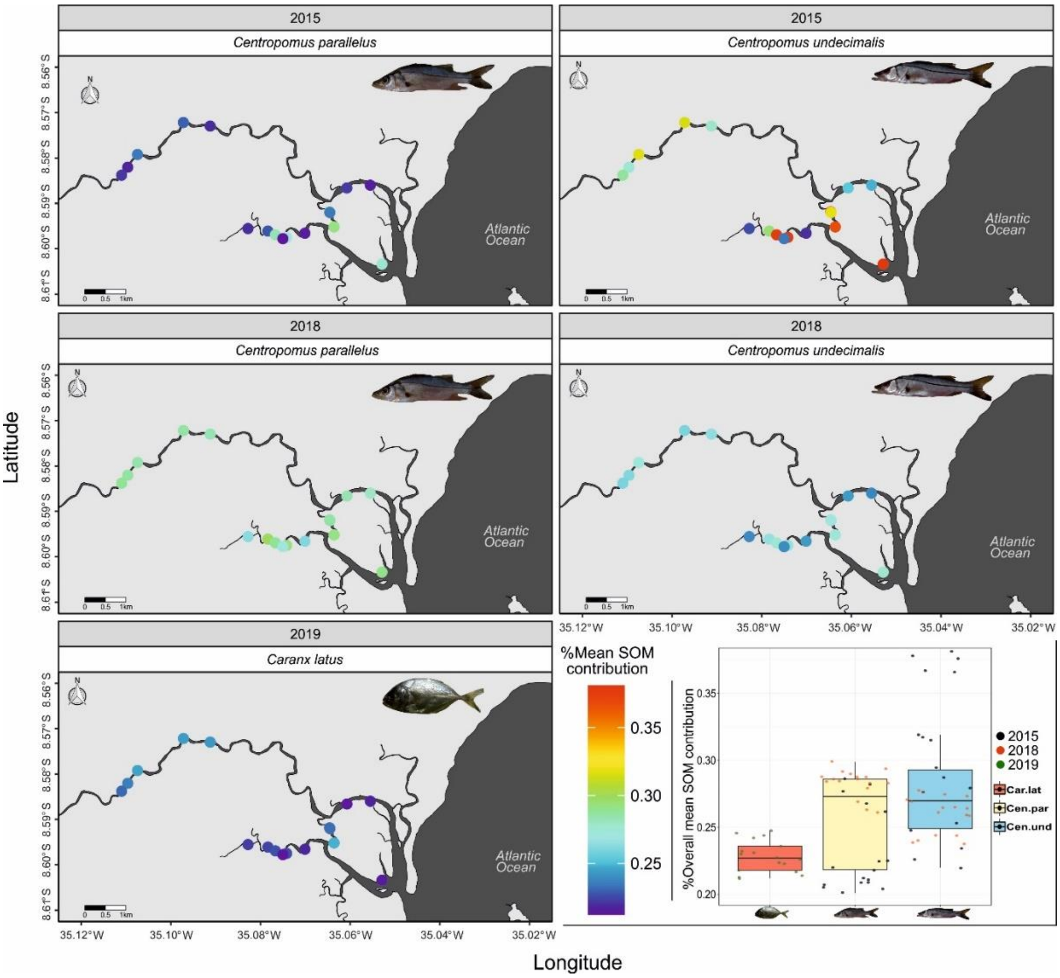

Figure 5. Spatial global contribution of the SOM in the diet of consumers (*Caranx latus, Centropomus undecimalis* and *Centropomus parallelus*) from multiple mixing model along of Sirinhaém River Estuary.

4. DISCUSSION

The use of integrated tools is efficient for assessing the ecosystem health. Stable isotopes analyses for contamination monitoring are normally applied in biomagnification models which involve the increase in mercury concentration and $\delta^{15}N$ isotope ratio (Lavoie et al., 2013). Moreover, the use of isoscape through geospatialization can provide subsidies for biomonitoring and especially in the location of point sources of pollution (Bowen, 2010; Murphy et al., 2022; Vokhshoori and McCarthy, 2014). Both approaches were used in this study. The Sirinhaém Estuary (SIR) is considered a closed lagoon with low salinity and depth, having a reduced interconnectivity with the coast (Pelage et al., 2021). This estuary receives different sources of pollution in the upper part of the estuary, the most significant being sugar cane industry and shrimp farm and sewage (CPRH, 2011).

In terms of the use of carbon and nitrogen isotopes, in our study, enriched values were found for $\delta^{13}C$ and $\delta^{15}N$, respectively in the upper and lower estuary, respectively in Sirinhaém. The two isotopes can be used to distinguish land uses such as industrial, rural, and urban areas (Hong et al., 2019). Enriched values of $\delta^{15}N$ can be observed more often observed in polluted rather than in unpolluted environments, while $\delta^{13}C$ enrichment has been reported in fish from more impacted than pristine areas (Prado et al., 2020). $\delta^{13}C$ enriched values, besides suffering natural environmental and photosynthetic interferences, was efficient to trace the pollutant sources in the industrial complex of Donghae port in Korea (Kim et al., 2021). $\delta^{13}C$ isotope is also a powerful biomarker since it can provide information about the biology, life histories and diet composition (Martino et al., 2019). Wang et al. (2022) used isotopes of $\delta^{13}C$ and $\delta^{15}N$ to identify the sources of organic matter in sediments, to assess the influence of aquaculture cage wastewater in Lake Poyang, China. They observed that the influence of these wastes on sediment organic matter was present at sites 1500 m away from the activity. Gorman et al. (2017) assessed the sensitivity of the alga *Ulva lactuca* and hermit crab *Clibanarius vittatus* to the influence of sewage from Araçá Bay, São Paulo using $\delta^{15}N$ isotopes, and observed that both species were sensitive to this anthropogenic pollution.

Basal source values collected in this study ranged from -29.25 to -15.10‰ for $\delta^{13}C$ and -1.03 to 7.06‰ for $\delta^{15}N$, like those obtained by other studies in Brazilian estuaries subjected to different pollution sources (Table 2). The values obtained in the basal source ($\delta^{15}N$) confirm the anthropogenic influence in the Sirinhaem River estuary, when compared with values of areas with less impact from organic origin, such as domestic effluents and the sugarcane industry (Table 2). As reported by Souza et al. (2021), a value of -2‰ $\delta^{15}N$ in the mangrove plant is a reference for low contaminated sites. Another hypothesis for low nitrogen values is atmospheric nitrogen fixation by mangrove plants, such as -1.5‰ to 3.2‰ in the mangrove plant for $\delta^{15}N$ in Tanzania (Muzuka and Shunula, 2006). In our case, high values can be associated with impacts present in estuaries, such as sewage, vinasse and fertilizers (Constanzo et al., 2001; Prado et al., 2020; Sáez et al., 2020).

Table 2. Values of isotopic carbon and nitrogen ratios for basal sources in different estuaries in Brazil and their respective polluting activities

Location	Source	$\delta^{13}C$	$\delta^{15}N$	Impacts	Authors
Estuaries in northeastern, Brazil	Oysters and basal resource	−29.24 to −14.50‰	−1.03 to 10.11‰	Effluents from port, sewage, mercury, industrial effluents	Gonzalez et al. (2019)
Victoria bay estuary, Brazil	Sediments	-25.7‰ to -25.6 ‰	5.1- 6.8‰	untreated effluents from nearby metropolitan areas	Varzim, 2019
Patos Lagoon, Brazil	Organic sources	-28.7‰ to -10.1‰	1.8-8.2‰	Port, fertilizers, navigation	Claudino (2013) Costa (2021)
Mamanguape River estuary, Brazil	SOM	-1.26‰ to -24.24‰	1.43-4.57‰	No direct impact (Pristine area)	Claudino (2015)
Santos bay, Brazil	Basal food sources	− 28.17‰ to − 9.64 ‰	0.15-1.81‰	Trace metals, POPs	Santos (2020) Neto,2020
Southeastern, Brazil	Sediment	− 29.4‰ to – 26.5‰	2.4-5.8‰	Mercury pollution	Fragoso (2018)
Manguaba lagoon, northeast Brazil	Soil samples;Sugar cane plant; Sugar cane molasses; Vinhasse	-18.1 ± 1.5‰ -11.8‰ -14.9‰ -15.4‰		sugar cane fields	Brockmeyer & Spitzy (2011)
Espirito Santo, Brazil	Base sources (plants)		-1.7 to 9.36‰		Souza et al. (2021)

The isotopic ratio of $\delta^{13}C$ for the three species evaluated (*Centropomus undecimalis, C. parallelus* and *Caranx latus*) ranged from -27.84 ‰ to -19.88 ‰. This variation is mainly due to the multiple sources of carbon present in the estuary of the Sirinhaem River, ontogenetic shifts and resource partitionning between species (Layman, 2007). The $\delta^{15}N$ values for the species analyzed were within the expected range for their corresponding trophic level. They were also within the range found for these species other studies (Gonzalez et al., 2019b, 2021b; Pelage et al., 2022a). *Centropomus parallelus* was considered an adequate biomonitor of anthropogenic contaminants when monitoring bioaccumulation and biomagnification of aggregated trace metals using stable $\delta^{13}C$ and $\delta^{15}N$ isotopes (Souza et al., 2021), highlighting the relevance of this species in studies of contamination. In our study, (See Chapters 1 and 2), *C. paralellus*, as well as *C. undecimalis* and *C. latus*, are also indicated as good biomonitor for monitoring the impacts of the study area.

Our study followed the recommendation of other studies which indicated the use of isoscape approaches as a useful tool for identifying and tracking pollution in estuaries (Kendall et al., 2010; West et al., 2010). The organic matter present in the surface sediment (SOM) was the main parameter of the isoscape. This tool provides subsidies for the use of SOM as a valuable metric in contamination studies because it is the entrance of contaminants into food chains, favoring bioaccumulation and biomagnification processes (Dominik et al., 2014; Fey et al., 2019; Schneider et al., 2015). The variation of SOM with respect to river-sea gradient, its entry into the trophic chain and the association to the proximity of potential pollutant sources was observed. The spatial pattern of the isotopic signature for SOM in SIR, influenced by the sugarcane industry and sewage, was different along the river-sea gradient. The isoscape analysis in this work permitted to observe that isotopic signature of SOM for $\delta^{15}N$ varied spatially in the estuary, being higher in the lower estuary, due to the sugarcane industry effluents transported from the upper part of the estuary. This may be related to the release of vinasse, the main waste product of the sugar-ethanol industry, characterized by unpleasant odors, dark brown color, high concentration of nitrogen, organic matter, fertilizer and pesticide residues (Dellamatrice and Monteiro, 2014; Godoi et al., 2019). Similarly, Carvalho et al. (2015) in Paranaíba River, Brazil and Souza et al. (2018) in Vitória Bay, Brazil,also reported high δ15N values in basal sources and fish from areas located closer to greater anthropogenic influences, such as sugarcane waste and sewage.

The isotopic signature of SOM for $\delta^{13}C$ was higher in the upper estuary near industry and input from other effluents, compared to the lower estuary near the sea. Enriched $\delta^{13}C$ values sewage discharge were also reported by Carvalho et al. (2019). Carbon is a complex natural marker due to the wide availability of organic sources in natural and impacted environments, which directly interfere its isotopic signature, but it is extremely relevant in monitoring punctual source pollutants by inferring about anthropogenic organic matter entering the trophic chain (Varzim et al., 2019). This element is widely used as a tracer of the main organic matter sources that contribute with, such as mangroves, terrestrial plants, sewage, coal, land use and activities with potential pollutant impact (Guo et al., 2010; Liu et al., 2020).

There are few applications of isoscape in environmental biomonitoring. Thibault et al. (2020) used this tool for $\delta^{15}N$ to evaluate the potential risk of eutrophication by sewage considering the residence time of bivalves in an environment exposed to this pollutant in New Caledonia. Murphy et al. (2022) used isoscape to identify the location and potential sources of nutrient input to Ambergris Caye Lagoon, Belize, through the seagrass *Thalassia testudinum,* showing satisfactory results for monitoring biogeochemical and anthropogenic nutrient dynamics patterns. In Brazil, although Troina et al. (2020) have used isoscape associated to $\delta^{13}C$ and $\delta^{15}N$ isotopes in zooplankton to track feeding habits, habitat use and trophic interactions of marine animals in south-southeast Brazil, there are no studies involving combination of isoscape and isotopes, like the approach proposed in this study, for monitoring estuarine contamination.

The isoscape of SOM in SIR contributed to identify the spatial ratios of $\delta^{13}C$ and $\delta^{15}N$ present throughout the estuary to the sea. Bayesian modeling was carried out to evaluate the contribution of basal source to the consumer diet, in terms of SOM. We found SOM contribution greater than 25% in some regions of the estuary, indicating its importance in the composition of what is incorporated in the fish diet. Both tools, allowed us to infer a greater possibility of direct contamination for the species in each region. Bayesian models are considered a good method and mixing models allows the quantification of the relative contribution of sources to a diet, but the weakness of the method lies in the complexity of isotopic overlaps frequently found in estuarine environments (Parnell et al., 2010; Phillips et al., 2014). Pelage et al. (2022) used the same methodology and observed that the contribution of basal sources (SOM + POM) was greater than 40% in the diet of fishes with piscivore, zoobenthivore and zooplanktivore trophic guilds. Carvalho et al. (2020) in Rio das Velhas, Brazil applied this methodology to evaluate the contribution of different basal sources and sewage in Rio das Velhas, Brazil, and observed that the basal source is the main carbon contribution for the species but, in points with higher contamination incidence, the sewage contribution varied from 40% to 70% for omnivorous and dentritivorous fish, respectively.

We conclude that $\delta^{13}C$ and $\delta^{15}N$ isotopes as biomarkers of pollution associated with isoscape maps and SOM contribution, can be used as a biomonitoring tool for estuarine ecosystem health. This was a pioneering approach for the assessment of contamination in Brazil. Anthropogenic impacts present in Sirinhaém showed similar isotopic signatures to areas with similar impacts. With the application of isoscape we observed areas with the highest isotopic signature for $\delta^{13}C$ and $\delta^{15}N$, confirming the influence of the main impacts of in area, namely sugar cane industry and sewage. SOM contributed significantly to biomass incorporation through the diet of the species, considered as a vehicle for contaminant entry into the food chain. Given the integrated approach of this study, *C. undecimalis, C. parallelus* and *C. latus* can be considered bioindicators of pollution in the

Sirinhaem River estuary, as previously observed in others studies. The results here obtained may be associated with the monitoring of pesticides, fertilizers and metals in fish and other organisms in this ecosystem. Moreover, the use of other isotopes such as Hg to identify specific sources of contaminants can be useful to refine the identification of pollutant sources, making the application of measures more reliable. However, based on the results of this study, a regular and periodic monitoring and appropriate regulation of the estuary is recommended, in particular near to those polluting sources where high isotopic signatures have been found.

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ANEXO I – MATERIAL SUPLEMENTAR (CAPÍTULO 3)

Table S1.Number of samples (n) and isotopic means (± S.D.) of carbon ($\delta^{13}C$ ‰) and nitrogen ($\delta^{15}N$ ‰) of basal sources and consumers by the year sampled during and rainy seasons in in the Sirinhaém River Estuary.

Groups/species	n	$\delta^{13}C$ ‰	Min-Max	$\delta^{15}N$ ‰	Min-Max
2015					
Consumer					
Centropomus undecimalis	18	-21.76±0.98	[-23.47 to -20.17]	9.93±0.53	[8.77 to 10.70]
Centropomus parallelus	18	-21.11±1.81	[-23.05 to -16.61]	10.55±0.71	[9.64 to 12.10]
Source					
Particulate Oganic Matter - POM	6	-22.20±1.19	[-26.35 to -17.22]	5.95±0.33	[4.85 to 6.78]
Sedimentary Organic Matter - SOM	12	-25.45±0.85	[-26.84 to -25.18]	4.52±0.21	[3.46 to 4.91]
Algae	6	-16.79±1.30	[-23.05 to -16.61]	5.4±0.40	[-23.05 to -16.61]
Mangrove	6	-28.5±0.30	[-23.05 to -16.61]	1.95±1.35	[-23.05 to -16.61]
2018					
Consumer					
Centropomus undecimalis	18	-24.47±1.29	[-25.63 to -21.56]	10.19±0.43	[9.34 to 10.64]
Centropomus parallelus	9	-23.34±7.49	[-25.20 to -20.47]	10.47±3.44	[-8.68 to 12.54]
2019					
Consumer					
Caranx Latus	17	-19.82±2.30	[-23.20 to -15.85]	9.42±1.10	[7.18 to 11.58]
Source					
Sedimentary Organic Matter - SOM		-25.18±6.08	[-27.48 to -23.05]	4.54±1.32	[3.52 to 6.08]

Table S2. Mean of the relative contributions of the basal sources for each species by year

Centropomus parallelus - 2015

Source	Mean±SD	CI (2.5 - 97.5%)
SOM	0.215±0.184	(0.007 – 0.679)
Mangrove	0.198±0.181	(0.003 – 0.649)
POM	0.341±0.219	(0.022 – 0.792)
Algae	0.246±0.181	(0.013 – 0.682)

Centropomus parallelus – 2018

Source	Mean±SD	CI (2.5 - 97.5%)
SOM	0.307±0.207	(0.016 – 0.753)
Mangrove	0.221±0.179	(0.006 – 0.647)
POM	0.257±0.181	(0.015 – 0.678)
Algae	0.215±0.179	(0.006 – 0.661)

Centropomus undecimalis – 2015

Source	Mean±SD	CI (2.5 - 97.5%)
SOM	0.288±0.169	(0.024 – 0.651)
Mangrove	0.121±0.41	(0.002 – 0.520)
POM	0.360±0.172	(0.054 – 0.707)
Algae	0.231±0.146	(0.023 – 0.598)

Centropomus undecimalis – 2018

Source	Mean±SD	CI (2.5 - 97.5%)
SOM	0.260±0.192	(0.012 – 0.712)
Mangrove	0.209±0.183	(0.005 – 0.666)
POM	0.316±0.209	(0.019 – 0.762)
Algae	0.215±0.181	(0.006 – 0.664)

Caranx latus – 2019

Source	Mean±SD	CI (2.5 - 97.5%)
SOM	0.215±0.160	(0.010 – 0.613)
Mangrove	0.185±0.144	(0.011 – 0.543)
POM	0.208±0.162	(0.010 – 0.611)
Algae	0.393±0.191	(0.045 – 0.751)

Principais conclusões do capítulo e perspectivas da tese

A aplicação de isótopos em estudos de contaminação associada a modelos de isoscape vem se expandindo no mundo. No nosso trabalho, pudemos visualizar que a assinatura isotópica do SOM para $\delta^{15}N$ variou espacialmente no estuário, sendo mais elevada na porção inferior, devido aos efluentes da indústria canavieira transportados desde a parte superior e o maior aporte do lançamento de esgotos nesta área. A assinatura isotópica do SOM para δ13C foi maior no estuário superior próximo da indústria da cana-de-açúcar e a entrada de outros efluentes, em comparação com o estuário inferior próximo do mar. Utilizamos o SOM para avaliar essas contribuições para os peixes e observamos que, dentre as espécies, *Centropomus parallelus*, foi a que apresentou a maior contribuição desta fonte basal.

O uso do isoscape e isótopos no estudo na investigação de contaminação no Brasil ainda é escasso, sendo o presente o primeiro registrado, mas pode ser replicado facilmente em outros ambientes e ecossistemas. A dificuldade de sua aplicação gira em torno da espacialização dos pontos e, consequentemente, da escolha de biomonitores que possam refletir o status de saúde do ambiente.

CONCLUSÃO GERAL

Esta tese utilizou ferramentas diferenciadas com abordagem integrada visando o diagnóstico ambiental dos estuários do Canal de Santa Cruz (ITAP) e do rio Sirinhaém (SIR), localizados no Nordeste do Brasil, indicando, de maneira convergente, que os mesmos estão contaminados em grau e severidade distintos (Fig. 1).

No primeiro capítulo, foi avaliada a qualidade ambiental de duas zonas estuarinas tropicais, o Estuário do Canal de Santa Cruz e o Estuário do Rio Sirinhaém, utilizando a integração de ferramentas biogeoquímicas, através da concentração de metais traços (Cu, Cr, Cd, Hg, Pb e Zn) em sedimentos e nas especies *Bairdiella ronchus*, *Centropomus undecimalis e Centropomus parallellus*, *Caranx latus e Gobionellus stomatus*. ITAP é potencialmente mais contaminada que SIR, sendo esta última considerada como uma região com baixo impacto (Fig. 1). Em ITAP, os metais Cd, Cr, Pb e Zn no sedimento foram classificados como com baixa probabilidade de causar efeitos deletérios na biota e, através de todos os índices aplicados no sedimento e na concentração de metais nos peixes, observou-se baixo risco. Entretanto, observou-se o risco de Cu é moderado e o de Hg elevado. A contaminação por Hg é decorrente do lançamento de 22 a 35 t de Hg no rio Botafogo durante 1963 e 1987, proveniente de uma indústria de soda caústica de 22 a 35 t de Hg no rio Botafogo durante 1963-1987. Este impacto persistente foi refletido nas espécies *C. undecimalis e B. ronchus* com elevadas concentrações de Hg. Em SIR, os metais Cd, Cr, Hg, Pb e Zn foram classificados como com baixa probabilidade de causar efeito deletério e baixo risco ecológico e de contaminação. O Cu esteve com maior probabilidade de causar efeitos na biota e os índices de qualidade ambiental do sedimento indicaram uma contaminação moderada. Nos peixes em SIR, ocorreu a presença de todos os metais analisados, mas a concentração não é preocupante considerando a legislação em vigência.

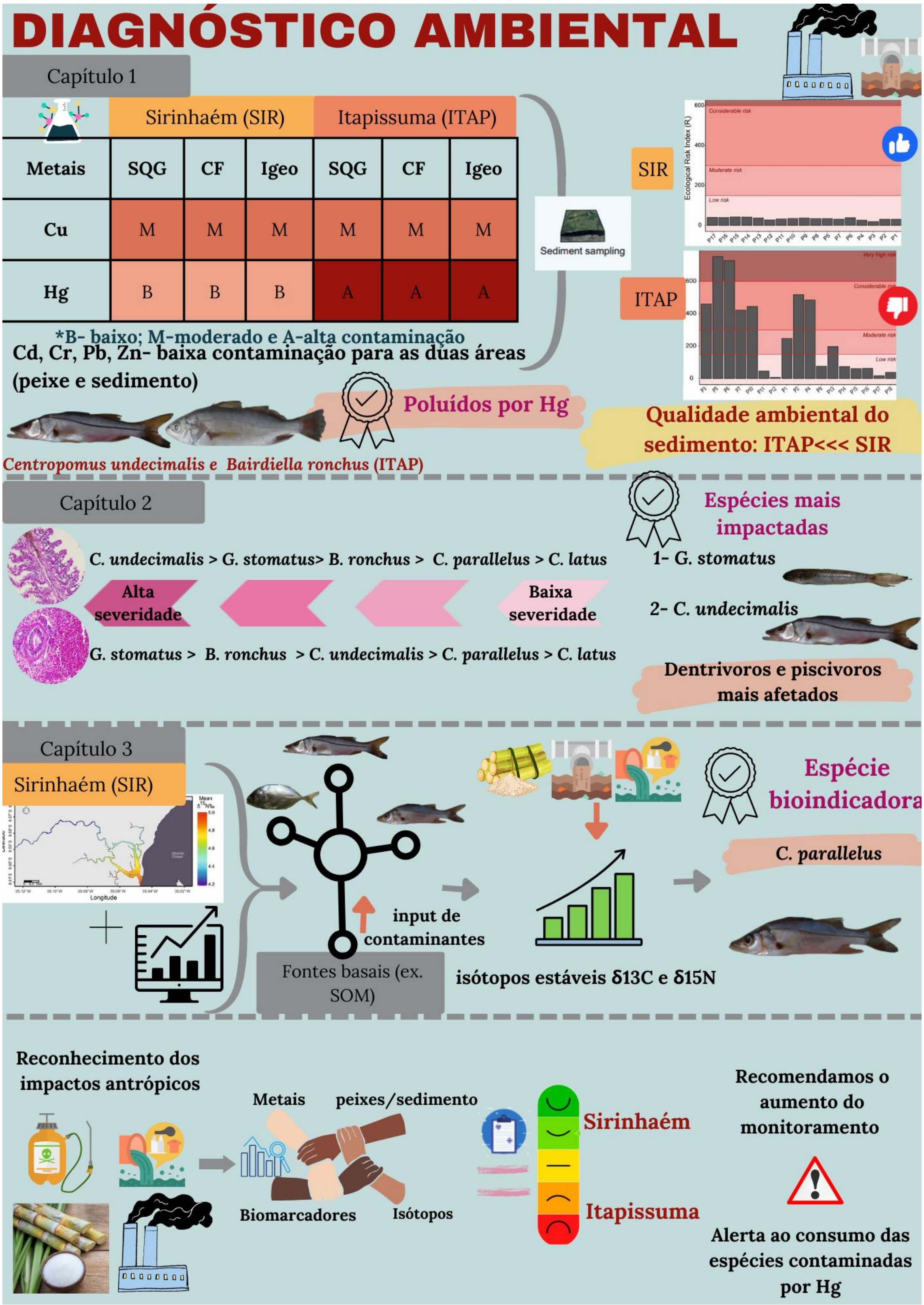
No segundo Capítulo, avaliamos a qualidade ambiental nos mesmos estuários supracitados utilizando o fígado e as brânquias como biomarcadores histopatológicos em peixes de diferentes níveis tróficos (piscívoros, detritívoros e piscívoro/zoobentívoro). Os biomarcadores histopatológicos mostraram como esses poluentes, e eventualmente outros não mensurados nesta tese, estão afetando a saúde dos peixes. Todas espécies estão altamente impactadas, mas a resposta histológica mais pronunciada ocorreu em *G. stomatus,* com degeneração gordurosa de elevada severidade e *C. undecimalis* (ou de gênero similar), com necrose. As espécies em SIR, que se mostraram no Capítulo anterior com baixa concentração de metal conforme a legislação em vigor, são as mesmas que apresentam lesões severas no fígado e nas brânquias, reforçando a necessidade de uma abordagem integrada nos estudos de contaminação.

O Capítulo 3, usamos os isótopos estáveis δ^{13}C e δ^{15}N com modelos de mistura e isoscape aplicados em sedimentos e peixes (*C. undecimalis*, *C. parallelus* e *C. latus*) para avaliar os impactos antropogênicos no estuário do rio Sirinhaém, nordeste do Brasil. Esse conjunto de ferramentas permitiu, observar que a assinatura isotópica da SOM para $\delta^{13}C$ era mais alta no estuário superior, próximo da indústria e de outros efluentes domésticos, em comparação com o estuário inferior próximo do mar. A assinatura isotópica do SOM para $\delta^{15}N$ variou espacialmente no estuário, sendo mais alta no estuário inferior, devido aos efluentes da indústria canavieira transportados da parte superior do estuário e à descarga de efluentes. Apesar de ainda pouco utilizado, o uso de isótopos demonstrou ser uma boa ferramenta de biomonitoramento, e SOM mostrou ser um parâmetro adequado para avaliar a contribuição de regiões com maior aporte de contaminantes na dieta das espécies *C. undecimalis, C. paralleus e C. latus.*

Considerando as abordagens utilizadas, constatamos que as espécies- utilizadas nesta tese (*C. undecimalis, C. parallelus, C. latus, B. ronchus e G. stomatus*) são indicadas para utilização como bioindicadores de contaminação ambiental, principalmente *C. undecimalis*. Estas espécies possuem atributos ideais, uma vez que foram avaliadas por várias métricas, apresentando resultados convergentes, demonstrando sensibilidade às variações ambientais e antropogênicas de ambas as áreas de estudo. Os resultados da presente tese, evidenciaram a persistência do Hg em ITAP, mesmo após 35 anos da interrupção do lançamento direto dos efluentes industriais contaminados no Rio Botafogo, componente desse complexo estuarino. Em SIR, os metais Cd e Hg estavam com maior concentração nos pontos que tem maior proximidade com os efluentes da industria canavieira, indicando essa fonte como contribuidora da poluição na área.

As duas áreas de estudo são de relevância e importância econômica para a produtividade pesqueira do estado, com problemas ambientais identificados e negligenciados pelos órgãos ambientais responsáveis. Apesar de termos observado baixas concentrações da maioria dos metais traços e espécies, quanto ao consumo de peixes em geral, mesmo em baixas concentrações, pode causar efeitos significativos na população, dependendo da quantidade de peixe ingerido (kg) e tempo de ingestão, levando em conta dados os processos de bioacumulação e biomagnificação. Efeitos deletérios de metais na saúde de humanos já foram observados em outras áreas que sofrem os efeitos da contaminação por Hg, como na poulação ribeirinha com inteferência da mineração do ouro no norte do Brazil, demostrando portanto a urgência no controle, monitoramento e mitigação dos efeitos da poluição por metais.

A associação das técnicas aplicadas nesta tese para o monitoramento ambiental se mostrou eficiente em ecossistemas estuarinos, sendo de fácil aplicação, dando subsídios científico e tecnológico para o controle ambiental das áreas de estudo, podendo ser replicado em outros locais do Brasil e do mundo. A preocupação global quanto à contaminação por Hg foi apresentada através da Convenção de Minamata (Decreto Nº 9.470/2018), com o objetivo de proteger a saúde humana e o meio ambiente dos efeitos do Hg. O conjunto de técnicas abordadas nessa tese contribuem para o atendimento de premissas básicas como monitoramento geográfico do Hg no meio ambiente e no compartimento biótico dos peixes, visando propor melhorias no uso das técnicas ambientas para o controle e monitoramento eficiente dos impactos decorrentes da contaminação deste metal. A Convenção, junto ao governo Brasileiro, determinou que, até 2025, seja efetuada a eliminação de Hg na produção de cloro-álcalis para assegurar a qualidade da saúde do meio ambiente. A preocupação com as consequências da poluição vem crescendo e tem uma pauta importante na Agenda 2030 da ONU, com o foco na conservação dos oceanos, através da 'ODS 14: vida debaixo d'água'. Umas das metas desta ODS é a redução da poluição marinha e a segurança alimentar, principalmente da população em geral,e especialmente das pessoas que vivem da pesca de subsistência. O uso de ferramentas adequadas é essencial para colaborar com o país no atendimento deste objetivo pois, para reduzir e assegurar a qualidade ambiental e a segurança alimentar, é necessário o conhecimento minucioso dos impactos antrópicos e seus efeitos na biota. Finalmente, sob o ponto de vista local, recomendamos o aumento do monitoramento de ambas as áreas, principalmente no compartimento biótico e em ITAP, alertando a população quanto ao risco do consumo de espécies contaminadas por Hg. A necessidade de abordagem de educação ambiental junto à população para reduzir a poluição com lixo e plástico e o descarte correto de produtos, também é aqui destacada como essencial.



DIAGNÓSTICO AMBIENTAL

Capítulo 1

	Sirinhaém (SIR)			Itapissuma (ITAP)		
Metais	**SQG**	**CF**	**Igeo**	**SQG**	**CF**	**Igeo**
Cu	M	M	M	M	M	M
Hg	B	B	B	A	A	A

*B- baixo; M-moderado e A-alta contaminação

Cd, Cr, Pb, Zn- baixa contaminação para as duas áreas (peixe e sedimento)

Poluídos por Hg

***Centropomus undecimalis* e *Bairdiella ronchus* (ITAP)**

Sediment sampling

SIR

ITAP

Qualidade ambiental do sedimento: ITAP<<< SIR

Capítulo 2

C. undecimalis* > *G. stomatus*> *B. ronchus* > *C. parallelus* > *C. latus

Alta severidade

Baixa severidade

G. stomatus* > *B. ronchus* > *C. undecimalis* > *C. parallelus* > *C. latus

Espécies mais impactadas

1- *G. stomatus*

2- *C. undecimalis*

Dentrivoros e piscivoros mais afetados

Capítulo 3

Sirinhaém (SIR)

input de contaminantes

Fontes basais (ex. SOM)

isótopos estáveis δ13C e δ15N

Espécie bioindicadora

C. parallelus

Reconhecimento dos impactos antrópicos

Metais

peixes/sedimento

Biomarcadores

Isótopos

Sirinhaém

Itapissuma

Recomendamos o aumento do monitoramento

Alerta ao consumo das espécies contaminadas por Hg

Figura. 1. Diagnóstico ambiental das áreas de estudo, mediante as metodologias utilizadas

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