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ARISTÓTELES PHILIPPE NUNES QUEIROZ

ECOLOGIA TRÓFICA DE DASIATÍDEOS (MYLIOBATOIDEI: DASYATIDAE) NA COSTA DE PERNAMBUCO, BRASIL

Recife
2023

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Tese apresentada ao Programa de Pós-Graduação em Biologia Animal da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de doutor em Biologia Animal. Área de concentração: Biologia Animal.

Orientadora: Rosângela Paula Teixeira Lessa

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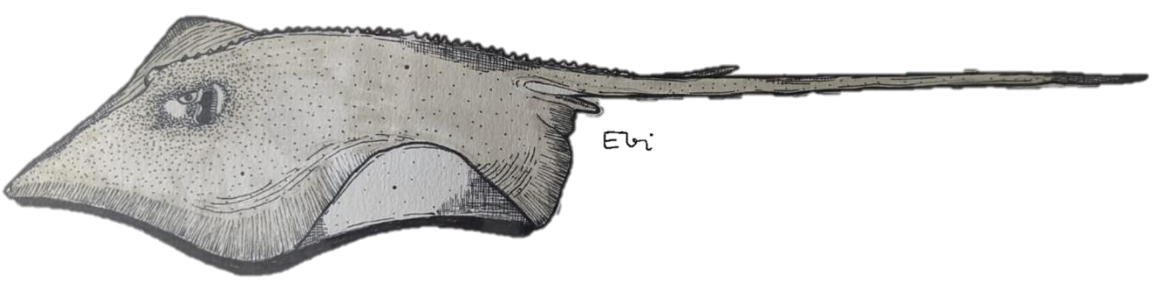
Dedico este trabalho aos meus pais

e à minha orientadora.



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Ao grupo "🍻 🍻 🍻cervejinhes🍻 🍻 🍻", meus amigos do DIMAR e a equipe do @elasmos_br. Vocês mantiveram minha sanidade em níveis aceitáveis, vocês são incríveis.

Ao meu namorado – em breve marido, eu espero – que há 10 anos tem sido meu porto seguro. "And when I felt like I was an old cardigan. Under someone's bed. You put me on and said I was your favorite" (SWIFT, 2022).

“Os lugares mais sombrios do Inferno são reservados
àqueles que se mantiveram neutros em tempos de crise moral”
(BROWN, 2013 apud ALIGHIERI, 1304-1321).

“Se você quer fazer do mundo um lugar melhor,
dê uma olhada em si mesmo e, em seguida, faça uma mudança”
(JACKSON, 1995).

RESUMO

Compreender a dinâmica trófica e espacial de elasmobrânquios comercialmente importantes é fundamental para a gestão pesqueira. Por isso, este trabalho objetivou descrever a ecologia alimentar e uso de hábitat de três espécies de raias demersais simpátricas e testar a importância topológica de uma delas em uma rede trófica estuarina. Três abordagens foram utilizadas neste estudo: I) Através de análises combinadas de conteúdo estomacal e isótopos estáveis ($\delta^{13}C$ e $\delta^{15}N$). Observou-se que *Hypanus guttatus* é um mesopredador com uma dieta diversa de organismos bentônicos e epibentônicos marinhos e estuarinos. *Hypanus berthalutzae* é um predador marinho generalista e ocupa nível trófico mais alto. Em contraste, *Hypanus marianae* é um mesopredador marinho especializado e com baixa amplitude de nicho. Embora ocorra sobreposição de nicho, as três raias utilizam os as presas em proporções diferentes e ocuparam nichos tróficos distintos, potencialmente limitando a competição por recursos e promovendo a coexistência; II) Utilizamos dados de isótopos estáveis de tecido muscular ($\delta^{13}C$ e $\delta^{15}N$) e dados de microquímica de vértebras (^{24}Mg, ^{43}Ca, ^{55}Mn, ^{86}Sr e ^{138}Ba) para analisar o uso do habitat pelas três espécies de raias em diferentes estágios de vida. Nossa abordagem revelou movimentos de entrada e saída em áreas estuarinas por *H. guttatus*, forte especificidade para habitats de recifes costeiros para *H. marianae*, e uso de águas mais profundas por adultos de *H. berthalutzae*. Também revelamos diferenças significativas entre os sexos no uso do habitat para *H. berthalutzae*, especialmente para elementos associados a zonas hipóxicas (^{55}Mn) e variações de salinidade (^{86}Sr e ^{138}Ba); III) Com abordagens topológicas, foi avaliada importância da raia *H. guttatus* em um ambiente estuarino. A raia não foi classificada como espécie-chave e seu potencial de dispersão de efeitos indiretos se limita aos efeitos “top-down”, atuando como um predador de topo da fauna bentônica. Detritos se destacaram como um dos principais nós nas análises topológicas e de espécies-chave, reflexo da importância do suprimento estuarino de matéria orgânica na manutenção de redes alimentares aquáticas costeiras. A pesca atua como um predador de topo, sendo um dos principais nós capazes de afetar a rede de forma rápida e com ampla disseminação e de gerar maior fragmentação. Em uma visão geral, as três raias desempenham papéis ecológicos diferentes nos ecossistemas que ocupam, limitando a redundância funcional. Os ambientes estuarinos e recifes de coral são as áreas mais importantes para *H. guttatus* e *H. marianae*, respectivamente, enquanto *H. berthalutzae* parece utilizar toda a plataforma continental ao longo de seu ciclo de vida.

Palavras-chave: Arraia; cação; nível trófico; ecologia alimentar.

ABSTRACT

Understanding the trophic and spatial dynamics of commercially important elasmobranchs is fundamental for fisheries management. Therefore, this work aimed to describe the feeding ecology and habitat use of three sympatric species of demersal stingrays and test the topological importance of one of them in a estuarine trophic network. Three approaches were used in this study: I) Through combined analyzes of stomach contents and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$). It was observed that *Hypanus guttatus* is a mesopredator with a diverse diet of marine and estuarine benthic and epibenthic organisms. *Hypanus berthalutzae* is a generalist marine predator but occupies higher trophic levels. In contrast, *Hypanus marianae* is a specialized marine mesopredator with a low niche breadth. Although there is niche overlap, the three rays use preys in different proportions and occupied different trophic niches, potentially limiting competition for resources and promoting coexistence; II) We used stable isotope data from muscle tissue ($\delta^{13}C$ and $\delta^{15}N$) and microchemical data from vertebrae (^{24}Mg, ^{43}Ca, ^{55}Mn, ^{86}Sr and ^{138}Ba) to analyze habitat use by three stingray species at different life stages. Our approach revealed movements in and out of estuarine areas by *H. guttatus*, strong specificity for coastal reef habitats for *H. marianae*, and deeper water use by *H. berthalutzae* adults. We also revealed significant sex differences in habitat use for *H. berthalutzae*, especially for elements associated with hypoxic zones (^{55}Mn) and salinity variations (^{86}Sr and 138Ba); III) With topological approaches, the importance of the stingray *H. guttatus* in an estuarine environment was evaluated. The stingray was not classified as a key species and its potential for dispersion of indirect effects is limited to “top-down” effects, acting as a top predator of the benthic fauna. Debris stood out as one of the main nodes in the topological and key species analyses, reflecting the importance of the estuarine supply of organic matter in maintaining coastal aquatic food webs. Fishing acts as a top predator, being one of the main nodes able to affect the network quickly and with wide dissemination and to generate greater fragmentation. In an overview, the three stingrays play different ecological roles in the ecosystems they occupy, limiting functional redundancy. Estuarine environments and coral reefs are the most important areas for *H. guttatus* and *H. marianae,* respectively, while *H. berthalutzae* seems to use the entire continental shelf throughout its life cycle.

Keywords: Stingray; shark; trophic level; food ecology.

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1 INTRODUÇÃO GERAL

Os elasmobrânquios (Subclasse Elasmobranchii), grupo de peixes cartilaginosos que compreende os tubarões e os batóides, desempenham importantes e variáveis papéis ecológicos nos ecossistemas que habitam (BIZZARRO et al., 2007; NAVIA et al., 2010). São, desde predadores de topo que controlam e regulam as populações em níveis tróficos médio-inferiores (EBERT; BIZZARRO, 2007; HEITHAUS et al., 2008; NAVIA et al., 2012; NAVIA; CORTÉS; MEJÍA-FALLA, 2010; PACE et al., 1999; SHERMAN et al., 2020) até mesopredadores, que transferem energia da produção primária bentônica para níveis tróficos superiores (MYERS *et al.*, 2007; SÁNCHEZ; OLASO, 2004; SÁNCHEZ; RODRÍGUEZ-CABELLO; OLASO, 2005; VAUDO; HEITHAUS, 2011). A diversidade de espécies de elasmobrânquios, que abrangem tubarões e batóides de diferentes tamanhos corporais e que ocupam habitats que variam de águas costeiras rasas a oceano aberto em latitudes polares a tropicais, levanta questões sobre a partição de nicho ou o grau de redundância funcional em conjuntos de espécies coocorrentes. Estudos que examinam a ecologia alimentar de várias espécies geralmente identificam um grau de sobreposição alimentar, mas também destacam variáveis preferências de habitat e presas, sugerindo redundância funcional limitada e alta complexidade trófica (HUSSEY *et al.*, 2015).

Dentre as espécies de elasmobrânquios encontradas no Brasil, as que habitam áreas litorâneas estão mais expostas à pesca intensa. No entanto, informações básicas sobre a biologia e uso do habitat ainda são incipientes para muitas dessas espécies (LESSA, R. *et al.*, 1999; MARTINS, A. P. B. *et al.*, 2018). Algumas das informações mais importantes para o gerenciamento eficaz da pesca incluem características reprodutivas, como fecundidade, período de gestação e idade de maturidade, bem como estimativas de idade e parâmetros de crescimento e captura (CAILLIET, 2015). A falta desses dados dificulta avaliações sobre o estado de conservação das espécies e a implementação de medidas de manejo para as pescarias que ocorrem ao longo da área de distribuição das espécies. Outra informação valiosa que pode auxiliar no manejo é a dinâmica espacial dos organismos, principalmente de espécies exploradas comercialmente (FRASER *et al.*, 2018; LEA, 2017).

Ainda assim, em um panorama geral, há uma significativa falta de informações sobre a subclasse Elasmobranchii no mundo, cenário ainda mais crítico para os batóides quando comparados aos tubarões (SHIFFMAN *et al.*, 2020). No Brasil, os dados são escassos para muitas espécies de batóides de importância comercial, como as raias *Hypanus guttatus* (BLOCH; SCHNEIDER, J. G., 1801), *Hypanus marianae* (GOMES; ROSA; GADIG, 2000) e *Hypanus berthalutzae* (PETEAN; NAYLOR; LIMA, 2020). Essas espécies de *Hypanus* são desembarcadas por frotas de pesca artesanal comercial que operam na costa brasileira, onde são capturadas como espécies-alvo primárias ou secundárias (LESSA et al., 1999; LESSA; NÓBREGA; SANTANA, 2009; YOKOTA; LESSA, 2007).

A raia-bicuda, *H. guttatus* é uma espécie demersal que pode atingir até 200 cm de largura do disco (DW) e ocorre desde o centro-oeste até o sudoeste do oceano Atlântico, bem como nas ilhas do Caribe até 70 m de profundidade (CARLSON et al., 2020). Em diversas pescarias ao longo da costa brasileira, *H. guttatus* é a espécie de batóide mais frequentemente capturada (BASÍLIO; FARIA; FURTADO-NETO, 2008; CARMONA et al., 2008; GIANETI et al., 2019; LESSA et al., 2008; MARION, 2015; MELO, 2016; MENESES; SANTOS; PEREIRA, 2005; MENNI; LESSA, 1998; YOKOTA; LESSA, 2007) sendo uma fonte substancial do crescente comércio de carne de elasmobrânquios no país, o que fez com que uma espécie de elasmobrânquio antes descartada, se tornasse uma das mais exploradas (FEITOSA *et al.*, 2021; GEMAQUE *et al.*, 2017).

Endêmica do Brasil, *H. marianae* ocupa ambientes de recifes de corais (BENDER et al., 2013; GOMES; ROSA; GADIG, 2000; YOKOTA; LESSA, 2007) e apresenta características como endemismo, corpo pequeno e alimentação especializada que podem levar a espécie a um alto risco de extinção (BENDER et al., 2013), além do que seus habitats preferenciais são altamente sensíveis às mudanças climáticas (COSTA; PENNINO; MENDES, Liana F., 2017). A espécie apresenta segregação ontogenética onde os indivíduos mais jovens são encontrados próximo à zona de arrebentação os quis migram para regiões de maior profundidade ao longo do crescimento (COSTA; THAYER; MENDES, 2015).

A raia-prego, *H. berthalutzae* é encontrada em águas costeiras tropicais e subtropicais do Atlântico Sudoeste, da foz do Rio Amazonas ao Estado de São Paulo, incluindo as ilhas oceânicas do Nordeste (PETEAN; NAYLOR; LIMA, 2020). Ocorre em ambientes de fundo arenoso ou cascalho e de recifes de coral e algas calcárias, onde, em Fernando de Noronha, jovens são comuns em praias rasas enquanto indivíduos maiores ocorrem desde as praias até águas mais profundas (AGUIAR; VALENTIN; ROSA, 2009). É uma raia generalista oportunista (AGUIAR, 2010; QUEIROZ et al., 2022), havendo registro de predação sobre indivíduos jovens de *H. marianae* (BRANCO-NUNES *et al.*, 2016). Nas avaliações brasileiras, *H. guttatus* é classificado como Pouco Preocupante (LC) e *H. marianae* e *H. berthalutzae* são classificados como Dados Insuficientes (DD) (ICMBIO/MMA, 2016), enquanto seus status globais na IUCN são Quase Ameaçados (NT) (CARLSON et al., 2020), Em Perigo (EN) (POLLOM *et al.*, 2020) e Vulnerável (VU) (CHARVET *et al.*, 2020), respectivamente.

Ao longo das últimas décadas, o aumento da demanda por carne de "cação" (nomenclatura utilizada comercialmente para se referir a tubarões e raias) levou ao desenvolvimento de novas técnicas de pesca que melhoraram a eficiência na captura de raias, especialmente *H. guttatus* (GIANETI *et al.*, 2019; MELO, 2016). Além da pressão da pesca, suas características biológicas, como crescimento lento, maturidade tardia e baixa taxa reprodutiva (GIANETI et al., 2019; NUNES et al., 2019; SILVA et al., 2018), juntamente com a crescente destruição do habitat, conferem a essas espécies uma baixa resiliência contra os impactos humanos. Nesse cenário, uma compreensão ampla e integrada sobre seus hábitos alimentares, movimentação e uso de habitat e funções ecológicas desempenhadas no ecossistema podem orientar medidas de manejo para estas espécies visadas pela pesca.

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2 REFERENCIAL TEÓRICO

No Brasil, a conservação dos elasmobrânquios marinhos ainda é inadequada principalmente por não haver monitoramento da pesca que impacta esses organismos os quais, muitas vezes, apresentam maturação sexual tardia e baixa fecundidade (LESSA; QUIJANO; SANTANA, 2002). Além do mais, entre os elasmobrânquios que ocorrem no Brasil, as espécies que habitam áreas costeiras estão expostas a intensa atividade pesqueira, e informações básicos sobre sua biologia e uso de hábitat ainda são incipientes para muitas delas (LESSA et al., 1999; MARTINS et al., 2018).

Dentre as informações mais importantes para uma gestão pesqueira eficiente estão as características reprodutivas, como fecundidade, período de gestação e idade de maturidade, bem como as estimativas de idade e parâmetros de crescimento e captura (CAILLIET, 2015). A falta destes dados dificulta a avaliação do estado de conservação das espécies e a implementação de medidas de manejo da pesca que atua ao longo de sua área de distribuição. Outras informações valiosas que podem auxiliar tais medidas de manejo são acerca da dinâmica espacial dos organismos, uso de hábitat e dos recursos naturais disponíveis, especialmente de espécies comercializadas (FRASER et al., 2018; LEA, 2017). Ainda assim, ainda há uma carência significativa desse tipo informação dentro da subclasse Elasmobranchii no mundo, mas esse cenário é ainda mais crítico para os batóides se comparados aos tubarões (SHIFFMAN et al., 2020). No Brasil, várias espécies de batóides de importância comercial seguem com poucos dados.

2.1 ESPÉCIES ESTUDADAS

Os batóides são um grupo de elasmobrânquios com alta variedade de formas corporais, com mais espécies válidas do que qualquer outro grupo dentro dos Chondrichthyes (COMPAGNO, 1990; EBERT; COMPAGNO, 2007). As raias ocorrem em todos os ecossistemas aquáticos, ocupando habitats bentônicos a epipelágicos (COMPAGNO, 1990) e as espécies marinhas são encontradas desde áreas litorâneas até profundidades superiores a 3.000 m. Em regiões tropicais o principal grupo da superordem Batoidea são os Myliobatoidea (Superfamília) (ASCHLIMAN; CLEASON; McEACHRAN, 2012; EBERT; COMPAGNO, 2007).

As raias da ordem Myliobatiformes apresentam grande diversidade morfológica e uma alta riqueza de espécies, com 10 famílias, 25 gêneros e 210 espécies (WEIGMANN, 2016). No geral, o número total de espécies de Miliobatiformes representa mais de 22% de todas as espécies conhecidas de Chondrichthyes e cerca de 43% de todos os batóides (EBERT; COMPAGNO, 2007). Segundo Rosa & Gadig (2014), são conhecidas seis famílias, 10 gêneros e 26 espécies de Myliobatiformes em águas marinhas brasileiras, dentre elas as da família Dasyatidae, representada por espécies de médio e grande porte. As espécies desta família ocorrem em áreas de ambientes costeiros e estuarinos (BIGELOW; SCHROEDER, 1953; McEACHRAN; CARVALHO, 2002).

A família Dasyatidae possui quatro subfamílias: Hypolophinae Stromer, 1910; Neotrygoninae Gray, 1851; Urogymninae Gray, 1851; e Dasyatinae Jordan e Gilbert, 1879, esta última com oito gêneros: *Bathytoshia*; *Dasyatis*; *Hemitrygon*; *Hypanus*; *Megatrygon*; *Pteroplatytrygon*; *Taeniurops* e *Telatrygon* (LAST; NAYLOR; SERET, 2016), com 42 espécies (WEIGMANN, 2016). Estando *Hypanus guttatus* (BLOCH; SCHNEIDER, 1801), *Hypanus marianae* (GOMES; ROSA; GADIG, 2000) e *Hypanus berthalutzae* PETEAN; NAYLOR; LIMA, (2020) entre as espécies de raia mais abundantes da costa pernambucana (LESSA; NÓBREGA; SANTANA, 2009).

2.1.1 *Hypanus guttatus*

A raia-bicuda, *H. guttatus* (Fig. 1), possui forma corporal discoide devido às nadadeiras peitorais expandidas e unidas ao corpo, pode atingir até 200 cm de largura de disco (LD), não apresenta nadadeira dorsal e possui cauda longa e fina (CARLSON et al., 2020; CARVALHO NETA; ALMEIDA, 2002). Possui focinho pronunciado, olhos sem pálpebras e os dentes distribuem-se formando placas (CARVALHO NETA; ALMEIDA, 2002). A espécie exibe dimorfismo sexual dentário, onde machos juvenis possuem dentes molariformes e adultos apresentam dentes pontiagudos caniniformes enquanto as fêmeas apenas dentes molariformes ao longo de todo a vida (GIANETI, 2011).

Os indivíduos jovens da espécie apresentam tubérculos em formação na região mediana central dorsal. Já os adultos apresentam também tubérculos secundários paralelos à linha mediana, não evidentes nos jovens. As linhas medianas dos animais adultos apresentam continuidade com formação de tubérculos maiores na região da base da cauda (BIGELOW;

SCHROEDER, 1953; FIGUEREDO; MENEZES, 1977; SILVA; VIANA; FURTADO-NETO, 2001). A região ventral dos indivíduos apresenta-se lisa em todos os estágios de desenvolvimento, onde é observada a presença de lóbulos franjados na porção terminal da membrana nasal e de papilas orais características da espécie, presentes tanto nos indivíduos jovens como nos indivíduos adultos (BIGELOW & SCHROEDER, 1953; FIGUEREDO & MENEZES, 1977; SILVA et al., 2001).

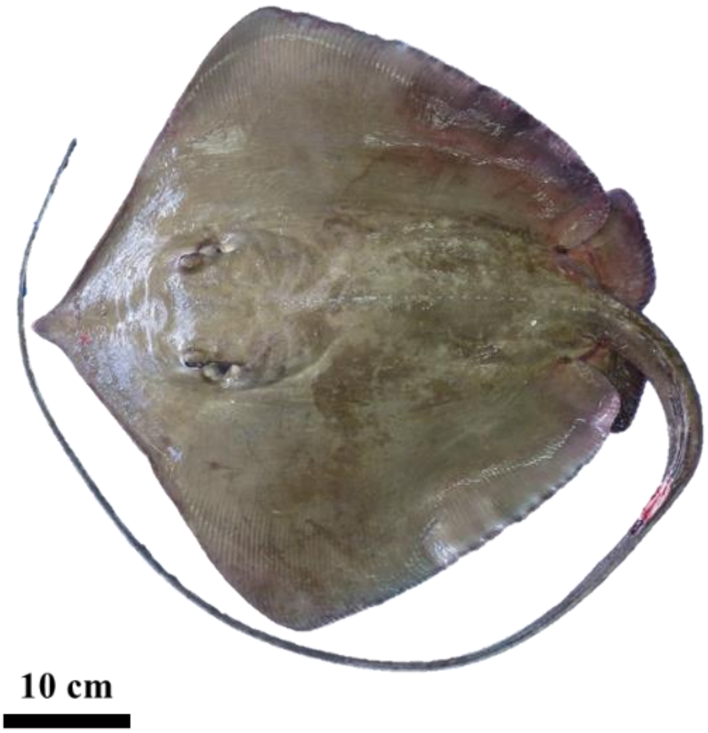

Figura 1 - Vista dorsal de fêmea adulta de *Hypanus guttatus* capturada em Itapissuma, Pernambuco, Brasil no período de julho/2013 a novembro/2016. Foto: QUEIROZ, A.P.N.

Hypanus guttatus ocorre em águas costeiras tropicais do Oceano Atlântico, desde o Golfo do México até a região sul do Brasil, em profundidades de até 70 metros (BIGELOW; SCHROEDER, 1953; CARLSON et al., 2020). A espécie habita áreas costeiras e estuarinas com preferência por fundos de areia e lama sendo capaz de tolerar alta amplitude de salinidade (THORSON, 1983). Os jovens da espécie ocorrem em águas costeiras rasas enquanto adultos são capazes de explorar também áreas estuarinas (FEITOSA et al., 2021; GIANETI, 2011; MELO, 2016; QUEIROZ et al., 2022; SANTOS et al., 2016; YOKOTA; LESSA, 2007).

As fêmeas de *H. guttatus* atingem a maturidade em idades mais avançadas que os machos, 7 e 5 anos, respectivamente (PD médio = 51,3 cm para fêmeas e 43,5 cm para machos) (GIANETI et al., 2019a). O tamanho médio ao nascimento é de 16 cm DW para ambos os sexos e a espécie apresenta crescimento relativamente rápido, mas os machos crescem mais rápido (k = 0,219) do que as fêmeas (k = 0,112) (GIANETI et al., 2019a). Além disso, a gestação por histotrofia matrotrófica dura cerca de 5 meses (YOKOTA; LESSA, 2007) e a fecundidade é baixa (um a cinco embriões por ninhada) (DA SILVA et al., 2018; GIANETI et al., 2019a; MELO, 2016). A raia-bicuda é um predador de hábito alimentar noturno, generalista oportunista, predando essencialmente organismos bentônicos, como: moluscos bivalves, sipunculas, crustáceos decápodes, poliquetas e peixes teleósteos (CARVALHO NETA; ALMEIDA, 2002; GIANETI et al., 2019b; QUEIROZ et al., 2022; SILVA; VIANA; FURTADO-NETO, 2001).

2.1.2 *Hypanus marianae*

Endêmica do nordeste do Brasil, a raia-do-olhão, *H. marianae* (Fig. 2), é uma raia de médio porte que pode chegar a 40 cm de LD. Possui disco romboide, com focinho pouco pronunciado e comprimento do disco aproximadamente igual à largura. Possui olhos grandes e cauda fina e relativamente curta com crista dorsal e dobra ventral terminando aproximadamente na mesma linha vertical (GOMES; ROSA; GADIG, 2000). Possui boca relativamente pequena e heterodontia sexual ontogenética, onde dentes de machos subadultos e adultos apresentam coroas pontiagudas, enquanto dentes de fêmeas e juvenis possuem coroas arredondadas (GOMES; ROSA; GADIG, 2000; ROMAN, 2019).

A raia-do-olhão se distribuição ao longo da costa nordeste do Brasil, do estado do Maranhão ao sul da Bahia, ocorrendo em ambientes de recifes de corais, praias arenosas e bancos de fanerógamas em áreas marinhas costeiras rasas (BENDER et al., 2013; COSTA; PENNINO; MENDES, 2017; COSTA; THAYER; MENDES, 2015; GOMES; ROSA; GADIG, 2000; YOKOTA; LESSA, 2007). A espécie apresenta segregação ontogenética onde indivíduos mais jovens são encontrados próximos a zonas de arrebentação os quis migram para maiores profundidade ao longo do crescimento (COSTA; THAYER; MENDES, 2015).

Figura 2 - Vista dorsal de macho adulto de *Hypanus marianae* capturada em Ponta de Pedras, Pernambuco, Brasil no período de julho/2013 a novembro/2016. Foto: QUEIROZ, A.P.N.

É um predador especialista que se alimenta principalmente de pequenos camarões e poliquetas, sendo sua pequena abertura oral um dos principais responsáveis por limitar sua diversidade alimentar (COSTA; THAYER; MENDES, 2015; QUEIROZ et al., 2022; QUEIROZ; ARAÚJO; LESSA, 2019). Apesar de possuir um período gestacional relativamente curto para uma espécie de elasmobrânquio (quatro a seis meses), a espécie apresente uma baixa fecundidade, geralmente com um embrião por ciclo gestacional (NUNES et al., 2019). Sua biologia reprodutiva combinada a características como endemismo, corpo pequeno e alimentação especializada podem levar a espécie a um alto risco de extinção (BENDER et al., 2013), além do mais, seus habitats preferenciais são altamente sensíveis às mudanças climáticas.

2.1.3 *Hypanus berthalutzae*

A raia-prego, *H. berthalutzae* (Fig. 3), possui focinho angular, nadadeiras peitorais com margens anteriores retas, laterais moderadamente angulares e pontas traseiras livres angulares. Disco mais largo que comprido e aberturas branquiais ventrais sinuosas. Presença de crista caudal dorsal e dobra caudal iniciando abaixo da origem do espinho e terminando aproximadamente na mesma linha vertical. Possui dimorfismo sexual dentário com machos adultos apresentando dentes com coroa cuspidada, enquanto nas fêmeas a coroa se apresenta arredondada e não-cuspidada (PETEAN; NAYLOR; LIMA, 2020).

Os olhos são salientes acima da cabeça e o dorso possui espinhos interorbitais e interespiraculares rostrais e estelares; um espinho escapular e duas fileiras escapulares (de 0 a 14 espinhos de cada lado); disco dorsal coberto por espinhos esparsos próximos à linha média, e uma fileira do meio da escápula ao ferrão caudal (de 0 a 24 espinhos); nadadeira caudal com pequenos espinhos esparsos (PETEAN; NAYLOR; LIMA, 2020).

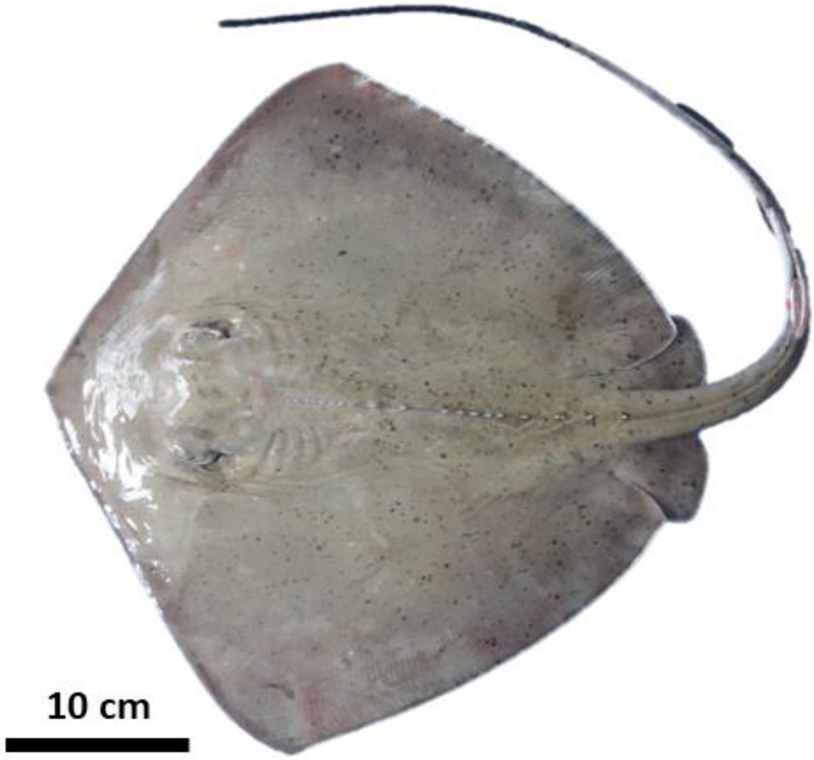

Figura 3 - Vista dorsal de fêmea adulta de *Hypanus berthalutzae* capturada em Itamaracá, Pernambuco, Brasil no período de julho/2013 a novembro/2018. Foto: QUEIROZ, A.P.N.

A espécie é encontrada em águas costeiras tropicais e subtropicais do Atlântico Sudoeste, da foz do Rio Amazonas ao Estado de São Paulo, incluindo as ilhas oceânicas do Nordeste (PETEAN; NAYLOR; LIMA, 2020). Ocorre em ambientes de fundo arenoso ou cascalho e de recifes de coral e algas calcárias, onde, em Fernando de Noronha, jovens são comuns em praias rasas enquanto indivíduos maiores ocorrem desde as praias até águas mais profundas (AGUIAR; VALENTIN; ROSA, 2009). A maior largura do disco registrada para a espécie foi de 680 mm em um macho maduro, contudo, existem relatos de espécimes maiores observados vivos no Arquipélago de Fernando de Noronha, mas não medidos, sugerindo que esta espécie pode atingir tamanhos ainda maiores (PETEAN; NAYLOR; LIMA, 2020). É uma raia generalista oportunista (AGUIAR, 2010; QUEIROZ et al., 2022), havendo registro de predação sobre indivíduos jovens de *H. marianae* (BRANCO-NUNES et al., 2016).

2.2 ECOLOGIA TRÓFICA

Os elasmobrânquios desempenham importantes papeis nos ecossistemas que ocupam e a alta diversidade de morfotipos existente sugere uma ampla variedade de hábitos alimentares (AGUIAR; VALENTIN, 2010; EBERT; BIZZARRO, 2007). Como predadores, os elasmobrânquios estão distribuídos entre níveis tróficos elevados e intermediários, que geralmente estão associados a habitats bentônicos, demersais e pelágicos (NAVIA; CORTÉS; MEJÍA-FALLA, 2010). A importância trófica como predadores de topo faz com que este grupo, atue no controle e regulação das populações em níveis tróficos inferiores, como é o caso da maioria os tubarões (BIZZARRO et al., 2007; HEITHAUS et al., 2008; NAVIA et al., 2012; NAVIA; CORTÉS; MEJÍA-FALLA, 2010; PACE et al., 1999).

Contudo, elasmobrânquios de níveis tróficos intermediários como as raias também exercem importante papel no ecossistema, atuando na transferência de energia da produção primária bentônica para níveis tróficos superiores (MYERS et al., 2007; SÁNCHEZ; OLASO, 2004; SÁNCHEZ; RODRÍGUEZ-CABELLO; OLASO, 2005; VAUDO; HEITHAUS, 2011) ou ainda modificando consideravelmente o habitat, reestruturando comunidades de invertebrados e de algas e promovendo ressuspensão de nutrientes no ecossistema bentônico devido ao seu comportamento alimentar (CARTAMIL et al., 2003; FLOWERS; HEITHAUS; PAPASTAMATIOU, 2020; KARL; OBREBSKI, 1976; ORTH, 1975; TAKEUCHI; TAMAKI, 2014), sendo apontadas como um dos organismos mais potentes em promover a ressuspensão de sedimento no ambiente marinho (TAKEUCHI & TAMACHI, 2014). Entende-se, portanto, que o equilíbrio e a saúde dos ecossistemas podem depender diretamente da integridade das populações locais de elasmobrânquios (AGUIAR; VALENTIN, 2010; BIZZARRO et al. 2007; NAVIA et al, 2012).

Compreender as interações que ocorrem entre as espécies nas redes alimentares não é uma tarefa fácil, contudo estudos que visam estudar as relações tróficas desses predadores são cruciais para o entendimento não só de sua ecologia, mas do ecossistema como um todo sendo uma ótima ferramenta para conservação. Apesar disso, no que concerne à ecologia alimentar, os elasmobrânquios são mais comumente conhecidos como grandes predadores marinhos, negligenciando a existência e a importância das espécies de níveis tróficos inferiores (AGUIAR; VALENTIN, 2010; BORNATOWSKI; ROBERT; COSTA, 2010; HUSSEY et al., 2014).

2.2.1 Conteúdo estomacal

Observando a notável necessidade de promover consistência, facilitar comparações e verificar a aplicação adequada de abordagens metodológicas em estudos de alimentação, Cortés (1997) realizou uma revisão crítica dos métodos e abordagens estatísticas utilizadas em estudos de alimentação de peixes e fez recomendações sobre a aplicação de tal metodologia para os elasmobrânquios que são utilizadas até hoje. Partindo desse princípio, (JACOBSEN; BENNETT, 2013) estimaram o nível trófico (TL) para 67 espécies de raias da subordem Myliobatoidei. Segundo os autores, os crustáceos decápodes e teleósteos foram os itens que mais contribuíram para a dieta padronizada das Myliobatoidei. As estimativas do nível trófico para essas raias variaram de 3,10 para *Potamotrygon falkneri* a 4,24 para *Gymnura australis*. A maioria das espécies examinadas no estudo foram classificadas como consumidores secundários (TL < 4,00) o que era esperado já que, de um modo geral, as raias possuem nível trófico inferior aos tubarões (CORTÉS, 1999).

Segundo Jacobsen & Bennett (2012), as diferentes estratégias de alimentação são o principal fator que influencia as estimativas de TL dos Myliobatoidei. Onde, diferente do que é reportado para Rajoidei (EBERT; BIZZARRO, 2007), não há uma correlação significativa entre o tamanho máximo atingido pelas espécies e seus respectivos níveis tróficos. Entretanto, é comumente reportado que diferentes classes de tamanho dentro de uma espécie podem ser consideradas como espécies funcionalmente diferentes em termos de dinâmica trófica (EBERT; BIZZARRO, 2007; GARRISON, 2000; ROSS, 1986). Desse modo, calcular e comparar a composição da dieta para diferentes classes de tamanho ou fases do ciclo de vida torna-se mais apropriado, resultando em estimativas mais precisas de TL e determinações mais exatas dos papéis tróficos dentro das redes alimentares (EBERT; BIZZARRO, 2007; ELLIS, 2003).

Assim, diversos estudos descreveram mudanças ontogenéticas na dieta de raias (BRICKLE et al., 2003; JACOBSEN; BENNETT, 2011; KYNE; COURTNEY; BENNETT, 2008; LÓPEZ-GARCÍA et al., 2012; MARSHALL; KYNE; BENNETT, 2008; RUOCCO; LUCIFORA, 2017; SISNEROS; TRICAS, 2002; VAUDO; HEITHAUS, 2011; YICK; TRACEY; WHITE, 2011) e associaram essa mudança na alimentação a diversos fatores, tais como mudança de habitat, aumento no tamanho das presas com o aumento no tamanho do corpo do predador, migração e aprimoramento na capacidade de capturar as presas (AGUIAR; VALENTIN, 2010; GRUBBS, 2010; WETHERBEE; CORTÉS, 2004).

Desse modo, analisar a dieta de raias ganhou um aspecto muito mais amplo do que apenas verificar a frequência, a quantidade e o volume de determinados táxons presentes na dieta do predador. Ainda assim, em um panorama geral, os estudos existentes sobre a biologia alimentar de elasmobrânquios são limitados (CORTÉS, 1999; WETHERBEE; CORTÉS, 2004) e mais frequentes para tubarão (AGUIAR; VALENTIN, 2010). As razões para a falta destes estudos são várias e vão desde a falta de recursos para estudar espécies não-alvo da pesca ou de pouco valor econômico ao hábitat de difícil acesso que muitas espécies habitam (EBERT; BIZZARRO, 2007). Por isso, a existência de uma grande lacuna no conhecimento sobre biologia e ecologia alimentar das espécies de elasmobrânquios na costa brasileira é evidente, muitas das quais já se encontram em fortes declínios populacionais (AGUIAR; VALENTIN, 2010).

2.2.2 Isótopos estáveis

A investigação do conteúdo estomacal é uma excelente ferramenta capaz de gerar informações acerca de recursos alimentares utilizados pelas espécies no ambiente, podendo ser usadas para entender desde a história natural até seu papel no ecossistema (WETHERBEE; CORTÉS 2004). Entretanto, embora esta metodologia forneça um alto nível de resolução taxonômica, o conteúdo estomacal representa a alimentação mais recente ingerida pelo consumidor.

A análise de isótopos estáveis ($\delta^{13}C$ e $\delta^{15}N$), embora proporcionando menor resolução taxonômica, é capaz de explicar aspectos que são limitantes para as análises de conteúdos estomacais, como refletir o material assimilado dentro da dieta em oposição ao material ingerido e representar a alimentação a longo prazo de um indivíduo (HUSSEY et al., 2012; PETERSON; FRY, 1987; VAUDO; HEITHAUS, 2011). É um método importante para examinar a dieta, a posição trófica e o movimento/migração de animais aquáticos e terrestres (HUSSEY; MACNEIL; FISK, 2010).

Além disso, o conteúdo estomacal pode gerar estimativas sub-ótimas do nível trófico, pois os itens alimentares são organizados em grupos funcionais muito amplos que não refletem o verdadeiro nível trófico dos organismos utilizados como presas (HUSSEY et al., 2011). Para resolver esses problemas, os isótopos estáveis de nitrogênio ($\delta^{15}N$) tornaram-se um método rotineiro para estimar o nível trófico com base na premissa de que eles integram a assinatura da dieta espacial e temporal de um organismo (ZANDEN; RASMUSSEN, 1999). Partindo desse princípio, a análise de isótopos estáveis vem sendo utilizada como ferramenta para verificar o nível trófico de elasmobrânquios marinhos, oferecendo uma análise da assimilação da matéria ingerida pelo predador e, desse modo, gerando um resultado de longo prazo da dieta do indivíduo (HUSSEY et al., 2012).

Há uma vasta literatura sobre métodos de análise de isótopos estáveis em diferentes taxas. Contudo, por apresentarem características fisiológicas únicas em relação a outros vertebrados como a ausência de tecido adiposo e a retenção de resíduos metabólicos para osmorregulação, os elasmobrânquios necessitam de uma metodologia específica de coleta e preparo do material biológico (KIM; KOCH, 2012). Por exemplo, sabendo que os elasmobrânquios armazenam lipídios no fígado para regular sua flutuabilidade (BALLANTYNE, 1997) e que as variações na sua concentração no músculo podem afetar os valores de $\delta^{13}C$ (PINNEGAR & POLUNIN, 1999; POST et al., 2007), Kim & Koch (2012) sugerem a utilização de éter de petróleo como solvente no processo de extração de lipídeos do tecido muscular, uma substância apolar que, além de minimizar a perda de aminoácidos, extrai de forma eficiente lipídios do tecido (DOBUSH; ANKNEY; KREMENTZ, 1985; NEWSOME; CLEMENTZ; KOCH, 2010). Kim & Koch (2012) verificaram ainda que a concentração de ureia nos tecidos de elasmobrânquios pode influenciar os valores $\delta^{13}C$ e $\delta^{15}N$, comprometendo potencialmente suas interpretações ecológicas. Isso porque ao contrário dos peixes ósseos, os elasmobrânquios mantêm o equilíbrio osmótico por retenção de ureia, $(NH_2)_2CO$. A utilização de água deionizada para a remoção de ureia no tecido se mostrou eficiente segundo os autores.

Apesar desta ferramenta ter se tornado mais acessível na última década, alguns conceitos clássicos e importantes precisam ser levados em consideração para uma boa aplicação do método. Valores de $\delta^{15}N$ e $\delta^{13}C$ de um organismo isolado fornecem poucas informações úteis sobre sua ecologia ou sobre a sua posição trófica e é essencial que essas razões sejam comparadas com outros organismos e fontes de matéria orgânica que compõem o ambiente estudado. Além disso, compreender o fracionamento trófico do isótopo em questão é fundamental para interpretação de dados de isótopos estáveis.

Sabe-se que o fracionamento trófico do $\delta^{13}C$ é 0,39‰ (POST, 2002), geralmente acordado como 0‰ de fracionamento por nível trófico (PETERSON; FRY, 1987; POST, 2002; ROUNICK; WINTERBOURN, 1986; VANDER ZANDEN et al., 1999). Já o fracionamento do $\delta^{15}N$ é de 3,4‰ por nível trófico (POST, 2002), cabendo ainda discussões sobre diferentes valores de enriquecimento em diferentes espécies de elasmobrânquios (CAUT et al., 2013; GALVÁN; JAÑEZ; IRIGOYEN, 2016; HUSSEY; MACNEIL; FISK, 2010; KIM; KOCH, 2012; LOGAN; LUTCAVAGE, 2010; MALPICA-CRUZ et al., 2012). Desse modo, o valor do $\delta^{15}N$ de cada animal torna-se uma estimativa da posição trófica em relação ao valor do $\delta^{15}N$ e da posição trófica conhecidos de um consumidor primário ou *baseline* (BORRELL et al., 2011; CABANA; RASMUSSEN, 1996; POST, 2002). Com essa abordagem, a assinatura isotópica do ^{15}N fornece uma visão geral e integrada do nível trófico no qual a espécie estudada se alimenta, muito embora não forneça informação da dieta específica (BORRELL et al., 2011).

Compreender o fracionamento do $\delta^{13}C$ ao longo da teia trófica também proporciona um importante subsídio para interpretar as assinaturas isotópicas dos organismos a ponto de fornecer informações, por exemplo, acerca da fonte de energia da espécie estudada (BORRELL et al., 2011; BOUTTON, 1991), já que os valores de $\delta^{13}C$ são conservados ao longo da cadeia alimentar. Essa aplicação também se provou útil para identificar onde os organismos em particulares se alimentam, sabendo, por exemplo, que os valores de $\delta^{13}C$ são tipicamente mais negativos (empobrecidos em ^{13}C) em teias tróficas costeiras ou bentônicas do que em teias mais oceânicas. Sendo, dessa forma, mais enriquecidos nos ecossistemas fluviais e estuarinos quando comparadas aos ecossistemas marinhos (FRY, 2006).

Outro conceito importante é o de relógio isotópico. Comumente, diferentes tecidos do mesmo organismo apresentam taxas de troca celular distintas, conhecido como taxas de *turnover*. Se a dieta de um elasmobrânquio muda ao longo do tempo, seus tecidos irão refletir a composição isotópica da nova dieta em tempos diferentes. Por exemplo, o plasma sanguíneo terá uma resposta mais rápida, enquanto o músculo terá uma resposta mais lenta e o fígado seria um meio termo. Além do mais, essas taxas de *turnover* estão intimamente associadas ao crescimento/metabolismo dos organismos, podendo variar entre espécies ou até mesmo sazonalmente e ontogeneticamente em uma mesma espécie (FRY, 2006; POST, 2002; VANDER ZANDEN et al., 2015).

De modo geral, a detecção e a medição da dinâmica da rede alimentar são altamente complexas, tendo em conta ambientes heterogêneos, múltiplos grupos funcionais e interações que ocorrem entre espécies de vários níveis (HUSSEY et al., 2014). Mesmo utilizando análises de isótopos estáveis, se a dieta de um indivíduo muda ao longo do tempo, a assinatura isotópica do tecido irá integrar diferentes períodos de alimentação a depender da taxa de *turnover* do tecido específico (PINNEGAR; POLUNIN, 1999). É possível que a movimentação de predadores entre redes alimentares isotopicamente distintas, mudanças ontogenéticas na dieta e variabilidade temporal da presa e do *baseline* influenciem os resultados de análises de isótopos estáveis (OLIN et al., 2013). Por isso, o máximo de informações pré-existentes sobre as espécies devem ser levadas em consideração ao se interpretar dados de isótopos estáveis.

A análise do conteúdo do estômago continua a ser uma das medidas mais importantes da ecologia alimentar de um animal e, quando associadas à análise de isótopos estáveis, resultam em *insights* ecológicos significativos (OLIN et al., 2013). Por isso, o uso apropriado de isótopos estáveis para complementar os dados de conteúdo estomacal pode revelar interações tróficas que de outra forma seriam indetectáveis. A análise das interações tróficas é um importante passo inicial na formulação de políticas de manejo da pesca e dos ecossistemas (RICHERT; GALVÁN-MAGAÑA; KLIMLEY, 2015).

2.3 USO DE HÁBITAT

Descrever a dinâmica espacial de espécies de elasmobrânquios tem sido um componente importante para entender, de forma mais ampla e complexa, a dinâmica populacional de diversas espécies. Nos últimos anos, a aplicação da telemetria remota, usando transmissores acústicos e conectados por satélite, vem fornecendo informações relevantes sobre o comportamento espacial de espécies de elasmobrânquios, principalmente espécies de tubarões (LEA, 2017; SIMS, 2010). Este tipo de informação é de grande valor para a adoção e gerenciamento de medidas de manejo eficientes para os ecossistemas, uma vez que são capazes de descrever a dinâmica de uso do hábitat e dos recursos naturais disponíveis, bem como revelar a extensão da sobreposição com as atividades humanas focadas na área, por exemplo, pesca (FRASER et al., 2018; LEA, 2017).

2.3.1 Microquímica de estruturas rígidas

Uma metodologia que vem sendo aplicada no estudo do uso do hábitat por espécies de peixes é a análise da composição elementar de estruturas biomineralizadas. Essas análises microquímicas em estruturas calcificadas, como vértebras, podem fornecer informações sobre as condições ambientais – físicas e químicas – nas quais os elementos foram depositados (CAMPANA; GAGNE; MCLAREN, 1995; ELSDON; GILLANDERS, 2003; SMITH; MILLER; HEPPELL, 2013). Desse modo, a composição elementar pode fornecer registros cronológicos permanentes das condições ambientais experimentadas ao longo da vida do indivíduo. Tais como origens natais, pontos críticos biológicos, padrões de movimento, uso de habitat e estrutura populacional, gerando informações importantes para medidas de conservação e manejo (FEITOSA et al., 2021; FEITOSA; DRESSLER; LESSA, 2020; LEWIS et al., 2016; MOHAN et al., 2018; MULLIGAN et al., 1983; SMITH; MILLER; HEPPELL, 2013; TILLETT et al., 2011).

Por exemplo: sabe-se que o estrôncio (Sr) é provavelmente um valioso marcador elementar em elasmobrânquios, particularmente entre as espécies eurialinas. Estudos conduzidos em espécies de peixes que usam tanto o ambiente marinho quanto estuarino têm mostrado grandes oscilações em Sr que refletem migrações entre gradientes de salinidade (RADTKE et al., 1996; SMITH; MILLER; HEPPELL, 2013); já o magnésio (Mg) pode representar um *proxy* para a temperatura ambiente sob salinidade constante, com concentrações mais elevadas em vértebras de elasmobrânquios associadas ao uso de águas mais frias (SMITH; MILLER; HEPPELL, 2013); concentrações mais elevadas de bário (Ba) são conhecidas por indicarem a proximidade a áreas de influência de água doce (McMILLAN et al., 2017); enquanto altas concentrações de manganês (Mn) em estruturas rígidas de peixes têm sido associadas a zonas hipóxicas, como alguns hábitats estuarinos e de águas profundas (LIMBURG et al., 2015; LIMBURG; CASINI, 2018).

É importante mencionar que existem algumas ressalvas com relação à utilização de certos elementos como descritores ambientais. A alimentação e ativação de hormônios sexuais, por exemplo, são citados como fatores que podem influenciar concentrações de Mn em estruturas rígidas de peixes (ELSDON; GILLANDERS, 2006; SMITH; MILLER; HEPPELL, 2013). Também não há um consenso se a temperatura é o principal fator responsável pelas variações nas concentrações de Mg em elasmobrânquios (PISTEVOS et al., 2019). Por isso, a utilização de dados de microquímica de estruturas rígidas aliado a dados de captura, movimentação, alimentação ou reprodução das espécies podem fornecer conclusões mais robustas acerca do uso do hábitat pelas espécies.

2.3.2 Isótopos estáveis

A utilização de isótopos estáveis para detectar migração ou movimentação de organismos marinhos é uma técnica bastante aplicada: Phillips et al. (2009), utilizando análises de isótopos estáveis de $\delta^{13}C$ e $\delta^{15}N$, revelaram diferentes estratégias de migração e preferências de habitat em 15 espécies de aves marinhas no Atlântico Sul; Mackenzie et al. (2012) utilizaram abordagem similar para investigar e descrever a dieta marinha, localização e migração em níveis específicos de estoque e recrutamento do salmão *Salmo salar* (Linnaeus, 1758) no Atlântico Norte, indicando diferentes áreas de alimentação marinha usadas pela espécie; Dierking et al. (2012) utilizaram análises de $\delta^{13}C$ e $\delta^{15}N$ associadas a análise microelementar em otólitos para descrever a movimentação do linguado *Solea solea* (Linnaeus, 1758) entre o ambiente marinho e um lago costeiro no Nordeste do Mar Mediterrâneo; e Trueman, Mackenzie & Palmer (2012), através de uma ampla revisão bibliográfica, sintetizaram a base teórica que sustenta o uso de isótopos estáveis de carbono, nitrogênio e oxigênio em otólitos, escamas e tecidos musculares de peixes marinhos como ferramentas de geolocalização.

As análises de isótopos estáveis de ^{13}C e ^{15}N têm sido amplamente utilizadas neste sentido (DIERKING et al., 2012; MACKENZIE et al., 2012; PHILLIPS et al., 2009; TRUEMAN; MACKENZIE; PALMER, 2012), visto que isótopos funcionam como biomarcadores naturais, capazes de indicar fontes alimentares utilizadas pelos consumidores. Em espécies de metabolismo e crescimento lento, como os elasmobrânquios, estes isótopos representam a alimentação a longo prazo dos indivíduos, refletindo o material ingerido e assimilado ao longo de vários meses (GALVÁN; JAÑEZ; IRIGOYEN, 2016; HUSSEY et al., 2014).

Esse método tem se provado útil para identificar onde espécies de elasmobrânquios em particular se alimentaram no período de até um ano, sabendo, por exemplo, que os valores de $\delta^{13}C$ são tipicamente mais negativos em fontes de alimentos estuarinas do que em fontes mais oceânicas. Sendo, dessa forma, mais empobrecidos em ^{13}C nos ecossistemas fluviais e estuarinos quando comparadas aos ecossistemas marinhos (FRY, 2006). O que torna esta ferramenta comprovadamente útil para diferenciar fontes de alimentação marinhas de fontes estuarinas (ETHERIDGE et al., 2008; MORINVILLE; RASMUSSEN, 2006; SWANSON; KIDD, 2009).

2.4 AMEAÇAS

Presentes no imaginário popular, *H. guttatus* e *H. berthalutzae* possuem importância sócio-econômica e sócio-cultural em Pernambuco, sendo ingrediente principal de pratos típicos como a moqueca de arraia e a caldeirada que, além de serem apreciados localmente, são atrativos turísticos, compondo assim um dos pratos mais tradicionais da culinária local (PEREIRA, 2012; SILVA, 2020). Essa valorização da carne da arraia para o consumo e comercialização levou a pesca na região a desenvolver petrechos específicos para a captura dessas Myliobatiformes, como a “raieira” e o espinhel de fundo (MELO, 2016). Além do mais, sua área de ocorrência as torna facilmente expostas às diversas artes de pesca que atuam na região, sendo capturadas também como fauna acompanhante. Este fato é preocupante sob o ponto de vista conservacionista já que a escassez de manejo pesqueiro ou monitoramento da pesca pode levar essas espécies a declínios populacionais silenciosos (SIMPFENDORFER et al., 2011).

A raia *H. guttatus* é citada como sendo a espécie de batóide mais capturada pela pesca artesanal no Pará, Maranhão, Ceará, Rio Grande do Norte, Sergipe, Bahia e Pernambuco (BASÍLIO; FARIA; FURTADO-NETO, 2008; CARMONA et al., 2008; GIANETI, 2011; LESSA et al., 2008; MARION, 2015; MELO, 2016; MENESES; SANTOS; PEREIRA, 2005; MENNI; LESSA, 1998). Sendo em Pernambuco, espécie alvo de algumas artes de pesca, principalmente no estuário, seu hábitat preferencial onde são capturados principalmente indivíduos adultos da espécie, já os indivíduos jovens podem ser encontrados em ambientes de maior salinidade, como as praias (ETEPE, 1995; MELO, 2016; QUEIROZ et al., 2022). *Hypanus berthalutzae* também é exposta a consideráveis pressões pesqueiras não manejadas ao longo de sua distribuição, tanto por pescarias artesanais quanto industriais, que a conduziram a uma redução populacional estimada em 30-49% nas últimas três gerações (CHARVET et al., 2020).

Apesar do baixo valor comercial, *H. marianae* também é comumente encontrada nos desembarques de diversas pescarias em Pernambuco, principalmente como fauna acompanhante. Além do hábito alimentar especializado, características críticas como endemismo, corpo pequeno e baixa fecundidade podem torná-la mais vulnerável à extinção (BENDER et al., 2013; NUNES et al., 2019). Além disso, os recifes de coral, seu habitat preferencial, estão ameaçados pelo aquecimento global e impactos humanos diretos (LEÃO et al., 2010, 2016; MAGRIS; GRECH; PRESSEY, 2018). A exploração pesqueira sem manejo dessas espécies ao longo do seus ciclos de vida, associada à suas características biológicas como baixa fecundidade, crescimento lento e maturação tardia (CAMHI, 1998; DA SILVA et al., 2018; GIANETI et al., 2019a; MELO, 2016; NUNES et al., 2019), além da degradação ambiental podem, a longo prazo, levar as três *Hypanus* a declínios populacionais, como já ocorre com diversas espécies de elasmobrânquios no mundo (SIMPFENDORFER et al., 2011).

Além do mais, a falta de dados sobre essas espécies dificulta a elaboração de medidas de manejo. Em um panorama mais amplo, ainda há uma carência significativa de informações dentro da subclasse Elasmobranchii no mundo, mas esse cenário é ainda mais crítico para os batóides se comparados aos tubarões (SHIFFMAN et al., 2020). No Brasil, várias espécies de batóides de importância comercial seguem com poucos dados, incluindo *H. guttatus*, *H. marianae* e *H. berthalutzae*, que seguem sendo desembarcadas por frotas da pesca artesanal que atuam ao longo da costa brasileira, onde são capturados como espécies-alvo ou acessórias (LESSA et al., 1999; LESSA; NÓBREGA; SANTANA, 2009; YOKOTA; LESSA, 2007). Enquanto no Brasil *H. guttatus* é classificado como Menos Preocupante (LC), e *H. marianae* e *H. berthalutzae* como Dados Deficientes (DD) (ICMBIO/MMA, 2016), seus status globais são Quase Ameaçado (NT) (CARLSON et al., 2020), Em Perigo (EN) (POLLOM et al., 2020) e Vulnerável (VU) (CHARVET et al., 2020) de acordo com a IUCN, respectivamente.

Em 2019, meses após o término das amostragens do presente estudo, milhares de toneladas de resíduos de petróleo bruto contaminaram aproximadamente 9.000km² de ecossistemas costeiros do Nordeste brasileiro, sudoeste do Atlântico, tornando-se o maior desastre ambiental da história do país (ARAÚJO et al., 2021; BRUM; CAMPOS-SILVA; OLIVEIRA, 2020; ESCOBAR, 2019; IBAMA, 2020; MAGRIS; GIARRIZZO, 2020; SISSINI et al., 2020; SOARES et al., 2020a, 2020b). Manchas de óleo foram encontradas em vários habitats ocupados por *H. guttatus* e *H. berthalutzae*, e praticamente toda a área de ocorrência de *H. marianae*. Seus impactos potenciais para a fauna local ainda não são bem compreendidos.

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3 ARTIGO 1 – TROPHIC ECOLOGY OF THREE STINGRAYS (MYLIOBATOIDEI: DASYATIDAE) OFF THE BRAZILIAN NORTH-EASTERN COAST: HABITAT USE AND RESOURCE PARTITIONING

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Trophic ecology of three stingrays (Myliobatoidei: Dasyatidae) off the Brazilian Northeastern coast: habitat use and resource partitioning

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ABSTRACT

Understanding the ecological role of species with overlapping distributions is central to inform ecosystem management. Here we describe the diet, trophic level and habitat use of three sympatric stingrays, *Hypanus guttatus*, *H. marianae* and *H. berthalutzae*, through combined stomach content and stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analyses. Our integrated approach revealed that *H. guttatus* is a mesopredator that feeds on a diverse diet of benthic and epibenthic marine and estuarine organisms, principally bivalve molluscs, Alpheus shrimp and teleost fishes. Isotopic data supported movement of this species between marine and estuarine environments. *H. berthalutzae* is also a marine generalist feeder, but feeds primarily on teleost fishes and cephalopods, and consequently occupies a higher trophic level. In contrast, *H. marianae* is a mesopredator specialized on shrimps and polychaetas occurring only in the marine environment and occupying a low niche breadth. While niche overlap occurred, the three stingrays utilized the same prey resources at different rates and occupied distinct trophic niches, potentially limiting competition for resources and promoting coexistence. These combined data demonstrate that these three mesopredators perform different ecological roles in the ecosystems they occupy, limiting functional redundancy.

Keywords: elasmobranchs, feeding ecology, stable isotopes, stingray stomach contents.

1 INTRODUCTION

Elasmobranchs are considered to play important, but variable, eco- logical roles in the ecosystems they inhabit (Bizzarro *et al*., 2007; Navia *et al*., 2010). These range from apex predators that control and regulate populations at mid-lower trophic levels (Bizzarro *et al*., 2007; Heithaus *et al*., 2008; Navia *et al*., 2010, 2012; Pace *et al*., 1999; Sherman *et al*., 2020) to mesopredators, which transfer energy from benthic primary production to higher trophic levels (Myers *et al*., 2007; Sánchez *et al*., 2005; Sánchez & Olaso, 2004; Vaudo & Heithaus, 2011). The diversity of elasmobranch species, which encompass sharks and batoids of different body sizes that occupy habitats ranging from shallow coastal waters to the open ocean and polar to tropical latitudes, raises questions over niche partitioning or the degree of functional redundancy in co-occurring species assemblages. Studies examining the feeding ecology of mul- tiple species often identify a degree of dietary overlap, but also highlight variable habitat and prey preferences, suggesting limited functional redundancy and expanded trophic complexity (Hussey *et al*. 2015).

When considering the ecological role of batoids, species have been shown to alter habitat, restructure invertebrate and algae communities, and promote resuspension of nutrients in the benthic environment through their feeding mode (Aguiar, 2010; Flowers *et al*., 2020; Gonzalez-Pestana *et al*., 2021; Sasko *et al*., 2006). The balance and health of ecosystems is therefore partially dependent on the integrity of local elasmobranch populations (Aguiar & Valentin, 2010), with batoids acknowledged as possessing key functional roles, but for which limited comparative data exist.

In the coastal zones of north-eastern Brazil, stingrays from the Dasyatidae family comprise a significant portion of the marine biota and occupy diverse habits such as sandy or pebble beaches, seagrass beds, coral reefs, mud banks and estuaries (Costa *et al*., 2015; Lessa *et al*., 1999, 2008, 2009; Queiroz *et al*., 2019). *Hypanus guttatus* (Bloch & Schneider, 1801), *Hypanus marianae* (Gomes *et al*., 2000) and *Hypanus berthalutzae* Petean *et al*. (2020) are among the most com- mon batoid species of the region and are known to use the same habitats during different stages of their life cycles (Lessa *et al*., 2009). These three *Hypanus* species are landed by artisanal fisheries fleets along the north-eastern Brazilian coast, where they are caught as both target species and bycatch (Lessa *et al*., 1999, 2009; Yokota & Lessa, 2007). While *H. guttatus* is classified as Least Concern (LC) in Brazil (ICMBio/MMA, 2016), and *H. marianae* and *H. berthalutzae* as Vulnerable (VU) (ICMBio/MMA, 2022), they are globally assessed as Near Threatened (NT) (Carlson *et al*., 2020), Endangered (EN) (Pollom *et al*., 2020) and Vulnerable (VU) (Charvet *et al*., 2020), respectively, according to the IUCN. Consequently, improved understanding of the trophic and spatial ecology of stingrays captured by regional fisheries is required to assist fisheries management (Lea, 2017; Sommerville *et al*., 2011).

The combined application of traditional stomach content analysis (SCA) and stable isotope analysis (SIA; $\delta^{13}C$ and $\delta^{15}N$) is a proven approach to elucidate elasmobranch habitat-trophic ecology (Abrantes & Barnett, 2011; Dale *et al*., 2011; Hussey *et al*., 2012) and to examine multispecies ecological roles. Stomach content analysis can directly identify the prey types consumed and the proportion of prey types eaten, but is limited by the temporal scope of sampling, typically revealing only diet recently consumed (Polunin & Pinnegar, 2002; Vaudo & Heithaus, 2011). Complementary to this approach, bulk stable isotopes in muscle tissue can provide information about the material assimilated into the diet over temporal scales as opposed to the material physically ingested, representing an individual's long-term diet (Peterson & Fry, 1987). The combination of these two methodologies allows the taxonomic identification of prey- to-prey assimilation by predators, resulting in a more complete view of energy flow in food webs.

In the current study, we aimed to describe the trophic ecology and habitat use of *H. guttatus*, *H. marianae* and *H. berthalutzae* in the context of determining if they exhibit unique ecological roles or con- fer functional redundancy. Our specific objectives were to use a combined SCA and SIA approach (i) to describe the feeding habits of the three species, (ii) to test the occurrence of dietary overlap and (iii) to understand variation in habitat use (marine *vs*. estuarine).

Based on the premise that co-occurring mesopredators commonly exhibit food partitioning to limit competition and that, within the context of the three study species, only *H. guttatus* is captured by fisheries in estuarine environments, we hypothesized that (i) despite coexisting in coastal habitats, low dietary overlap will occur among the three *Hypanus* species identifying unique trophic roles and (ii) *H. guttatus* is the only one of the three species that migrates between marine and estuarine areas, connecting both environments.

2 MATERIALS AND METHODS

2.1 Focal species

The longnose stingray *H. guttatus* is found from the southern Gulf of Mexico to southern Brazil (Bigelow & Schroeder, 1953), inhabiting estuaries to coastal marine areas up to 70 m deep across its range (Feitosa *et al*., 2021; Melo *et al*., 2021; Weigmann, 2016). It is a generalist opportunistic predator exhibiting slow growth (female k = 0.112 year^{-1}, males k = 0.219 year^{-1}), late maturity (7 years for females and 5 years for males) and low fecundity (average one to two embryos per hatch) (Carvalho Neta & Almeida, 2002; Gianeti, Santana, *et al*., 2019; Gianeti, Yokota, *et al*., 2019; Silva *et al*., 2001).

Endemic to the south-western Atlantic Ocean, the large-eye stingray, *H. marianae*, exhibits a short gestation period (4–6 months) and low fecundity, with usually one embryo per cycle (Nunes *et al*., 2019). The species exhibits sexual and ontogenetic segregation, occupying coral and coralline algae reefs, seagrass beds and sandy beaches (Costa *et al*., 2015; Gomes *et al*., 2000; Queiroz *et al*., 2019; Yokota & Lessa, 2007).

The recently described *H. berthalutzae* inhabits tropical and sub- tropical waters of the south-western Atlantic in shallow areas along the Brazilian coast, from the mouth of the Amazon River to the state of São Paulo, including the oceanic islands of north-east Brazil

(Petean *et al*., 2020). In island regions, it is found in areas with sandy or gravel substrates, as well as coral and coralline algal reefs, with juveniles more common in shallow beaches and larger individuals thought to inhabit deeper waters (Aguiar *et al*., 2009).

2.2 Study area and sampling

The current study was carried out in the coastal region of north-east Brazil, south-west Atlantic Ocean (Figure 1), an area that experiences an overall semiarid continental climate. Few rivers with high fresh- water outflow into the sea occur in the region, most of which have minimal influence on the coastal zone, although seasonality can drive variation (Ekau & Knoppers, 1999); monthly precipitation during the wet season (March to August) may reach up to 400 mm, falling to as little as 100 mm in the dry season (September to February) (Silva, 2004).

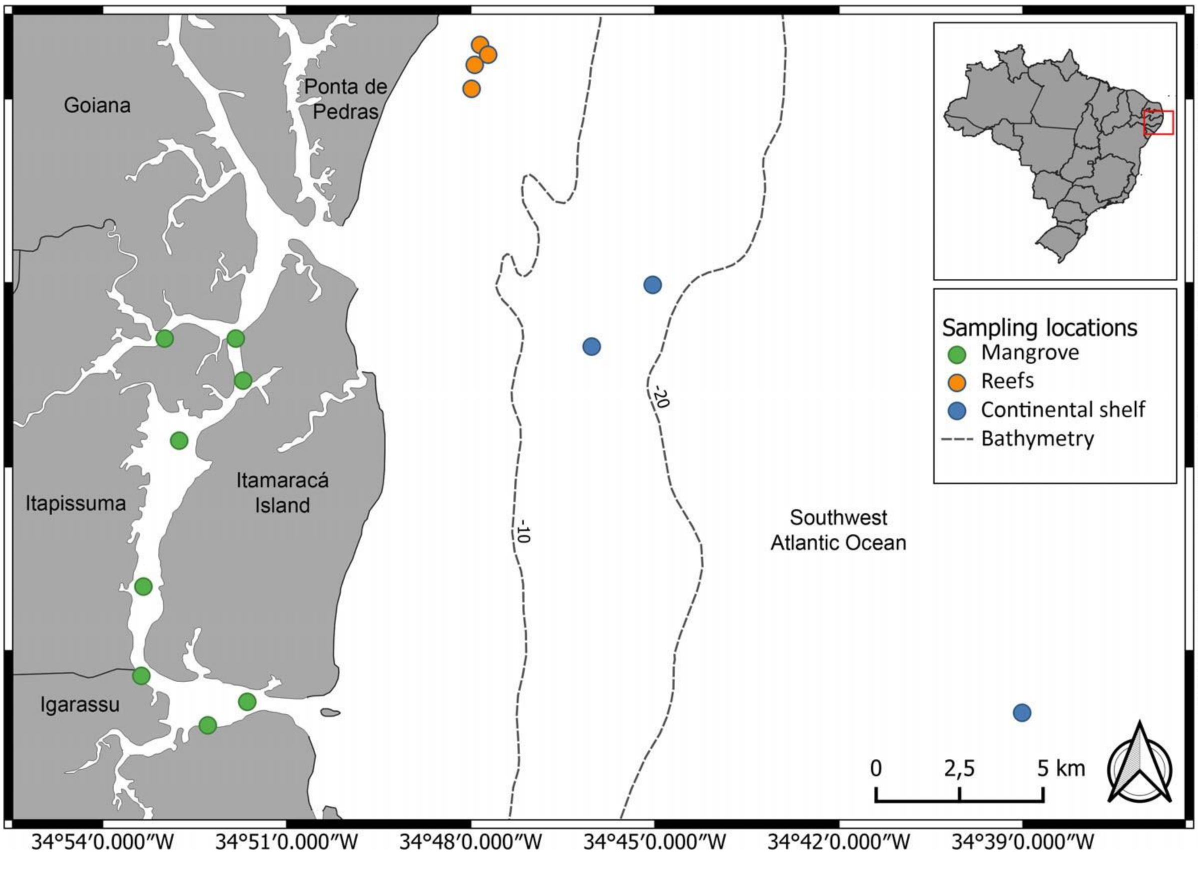

Figure 1 Sampling sites for *Hypanus guttatus*, *H. marianae* and *H. berthalutzae* captured by artisanal fishing in Pernambuco, Brazil: mangrove; coral reef; sea.

The study area comprises a well-defined environmental gradient of habitats, with distinct substrate, depth and salinity characteristics. Specimens of *H. guttatus*, *H. marianae*, *H. berthalutzae* and associated organisms, including known prey, were obtained at artisanal fisheries landings and sampling trips off Pernambuco state, Brazil, between 2016 and 2019, in the following habitats (Figure 1):

I. Mangroves: Comprised mainly of muddy bottoms and the mangrove tree species Rhizophora mangle (Linnaeus, 1753). The Santa Cruz channel (Figure 1) is one of the most important and productive coastal systems in the region (Carneiro et al., 2008; Medeiros & Kjerfve, 1993; Paranaguá et al., 2010), extending across more than 22 km with a variable width of 0.6 to 1.5 km, circumventing Itamaracá Island (Paiva et al., 2017). Depth varies between 10 and 17 m and the complex is connected to the sea at its northern and southern edges (Eskinazi-Leça et al., 1980) with a freshwater outflow of 58m3/s in the wet season and only 0.2 m3/s during the dry season (Medeiros & Kjerfve, 1993). Samples from *H. guttatus* were obtained in this environment, where it is caught as a target species by the 'raieira' fleet using bottom gillnets manufactured using multifilament and a 16 cm mesh between side knots. The 'raieira' has a low selectivity for stingrays, since individuals are caught by the caudal stinger in the multifilament nets, but this leads to the capture of all size classes, with the exception of neonate or young-of-the-year (YOY). Fishing occurs at depths between 5 and 18 m and lasts ~9 h, beginning at sunset and ending at sunrise (Melo, 2016).

II. Reefs: Ponta de Pedras beach, Goiana municipality, Pernambuco state (Figure 1) is a region with little to no estuarine influence, with a predominantly sandy and gravel bottom, several shallow coral and coralline algal reefs, as well as seagrass beds. 'Currais', fixed fishing traps made with wood rods anchored to the ground, are commonly used in the area. These traps are built mainly over sandy bottoms in areas with high tidal influence at depths between 1 and 6 m (Dias, 2019; Lucena *et al*., 2013). The 'currais' fishery has low selectivity, thus catching a wide variety of taxa and size spectra, including species of little commercial interest, such as *H. marianae*. Furthermore, the 'currais' permanently attract and retain fish in their chambers, which are later caught using bottom trawl nets or handheld harpoons. Fish are collected once a day at low tide. Specimens of all life cycles of *H. guttatus* and *H. marianae* were caught in this environment. For *H. berthalutzae* only immature juveniles were caught, including neonates and YOY individuals.

III. Continental shelf: The coastal zone of Itamaracá Island is characterized by sandy bottom beaches, the presence of coral reefs and encrusting coralline algae, as well as seagrass beds and marine macroalgae (Cocentino *et al*., 2004; Magalhães *et al*., 1997; Viana, 2005). The region is under little to no estua- rine influence, even during the wet season, and the continental shelf does not extend beyond 33 km (Viana, 2005). In this environment, specimens of all three species were captured. The fishery takes place at 5.4–18.1 km from the coast at depths of up to 50 m and is directed towards *H. berthalutzae*. Specimens of *H. guttatus* and *H. berthalutzae* were captured through bottom longlines, manufactured using nylon wires (2.5 mm thick) and metal J hooks baited with teleost fish, usually *Pseudupeneus maculatus* (Bloch 1793). Fishing occurs during the night and lasts for ~12 h, with deployment at sunset and retrieval at sunrise. The size of the J hook (4 cm) and the size of the bait (4–8 cm) used are selective and target adult individuals only. Individuals of *H. marianae* were caught using handheld harpoons during the day through dives and active searching by fishers.

In the laboratory, sampled stingrays were identified, sexed and classified according to their life stage: neonates and YOY juveniles, immature or mature (ICES, 2013; Melo, 2016). Stomachs were then excised from each individual and a white muscle tissue (~5 g) excised from the interior portion of the stingrays' pectoral fins.

To provide ecosystem baseline stable isotope data, samples from organisms that inhabit the three distinct environments were sampled: mangrove vegetation (*R. mangle*); and the continental shelf algae (*Padina gymnospora* (Sonder, 1871)) and periphyton; bivalve molluscs removed from the stomachs of *H. guttatus* caught in the mangrove environment; polychaetes in the stomachs of *H. marianae* caught on reefs; and *Octopus vulgaris* (Cuvier, 1797) in the stomachs of *H. berthalutzae* caught on the continental shelf. All samples obtained from stomach contents of stingrays were collected prior to fixation of whole-stomach contents in formalin solution for identification (see below). All biological material obtained was stored in a deep freezer at -80°C. Sampling was carried out in both the dry and wet seasons, with all organisms represented across both seasons.

2.3 Ethics statement

Samples were collected according to Brazilian environmental legislation under licence (Licença SISBIO, 49663–1) and without the need for approval by the ethics committee, since all samples were collected from animals that were already dead, landed or being sold at local markets.

2.4 Stomach content analysis

Extracted stomachs were labelled and fixed in a 4% formaline solution for 24 h, opened and washed in running water. All material retained in a 0.1 cm mesh following washing was preserved in 70% alcohol and analysed using a stereomicroscope. Diet items were identified to the lowest taxonomic level possible. Following identification, prey items were separated, counted and weighed individually (wet weight) using a precision scale (Cortés, 1997). For *H. marianae*, diet data published by Queiroz *et al*. (2019) were also included given these data were obtained at the same sampling locations and over the same period as the present study.

For each of the three stingrays, we first constructed a prey species accumulation curve (Cortés, 1997) through the EstimateS soft- ware version 7.5.1 to identify whether the number of analysed stomachs was sufficient to represent the species' diet, based on the reduction in variation of standard deviations. We then calculated the Relative Importance Index (RII) (Pinkas et al., 1971) for the diet, described by the equation RII = (%N + %W) × %O, where %N is the numeric frequency of a specific prey in relation to the total number of prey, %W is the weight frequency of a specific prey in relation to the total weight of all prey and %O is the frequency of occurrence of a specific prey in all analysed stomachs. The RII was converted to percentage values (RII%) for standardized comparisons (Cortés, 1997): %RII=(RII/∑RII)*100.

The trophic level of each species was calculated based on the stomach content data (Cortés, 1999): $TL_k = 1 + (\sum_{j=1}^{n} Pj * TL_J)$, where: TL_k is the trophic level of species “k”, Pj is the proportion of prey category “j” in the diet of predator “k”, n is the total number of prey categories, and TLj is the trophic level of prey category “j”. We used the estimated trophic level values of prey categories suggested by Ebert & Bizzarro (2007) for these calculations.

The diet niche breadth for each stingray species was then estimated using the Levin Bi index (Krebs, 1999): $Bi = 1/\sum P_j^2$. Where P_j is the fraction of the RII of each item in diet ‘j’. Values were standardized as suggested by (Krebs, 1999): $B_A = (Bi - 1)/(N - 1)$, in which N is the number of classes and B_A varies between 0 and 1. Low B_A values indicate diets composed mainly of a few food items (i.e. specialist feeding behavior), while high values indicate generalist diets.

To test for differences among the diets of the three stingrays, we used the weight of food items (%W), grouped into eight functional prey categories (Ebert & Bizzarro, 2007): AMPH (amphipods), DECA (decapods), OCRUS (other crustaceans), POLY (polychaetes), INVERT (other invertebrates), FISH (teleost fishes), CEPH (cephalopods) and MOLL (bivalves and gastropods). Standardized %W values for prey categories were used to build a similarity matrix using the Bray-Curtis similarity coefficient.

A PERMANOVA was then used, in two steps: first, all data were pooled to assess differences in diet among species (*H. guttatus*, *H. marianae* and *H. berthalutzae*) and habitats (mangrove, reef and continental shelf); second, for each species, differences were tested between life stages (YOY, immature and mature), by season (wet sea- son and dry season), by sex (male and female) and among habitats. Pair-wise PERMANOVA was used to evaluate the degree of significant dietary differences among species, and a SIMPER analysis to estimate the contribution of each prey category to each species diets. Analyses were carried out in the PRIMER v.6 software (Clarke & Gorley, 2005). Nonmetric multidimensional scaling (nMDS) was per- formed to observe possible groupings or separation in diet among stingrays using the vegan package (Oksanen et al., 2019) in R v.4.0.3 (R Core Team, 2020).

2.5 Stable isotope analysis

Muscle tissue samples excised from stingrays were cut into 0.5–1 cm^2 cubes, transferred to test-tubes and lipid extracted by agitating samples in 10 ml of petroleum ether in an ultrasonic cleaner (Ultracleaner 700, Unique, Indaiatuba, Sa˜o Paulo, Brazil) for 15 min following the methods of Kim and Koch (2012). The petroleum ether solution was discarded following agitation and the procedure repeated. Following removal of lipids from samples, 10 ml of deionized water was added to each sample to extract urea; the test-tube was agitated for 15 min, after which the deionized water was discarded. This process of water washing for urea removal from stingray muscle tissue was repeated twice. For prey items, only lipid extraction was undertaken following the above steps.

Following the above processing, all stingray and prey samples were lyophilized, macerated, and analyzed for stable isotopes using a Delta V Isotope Ratio Mass Spectrometer, Advantage, and the ISODAT software. Values (δ) were calculated from carbon and nitrogen reference standards (Pee Dee Belemnite and air, respectively), according to the equation (Peterson & Fry, 1987): $\delta_{sample} = \left(\left(\frac{R_{sample}}{R_{standard}}\right) - 1 * 1000\right)$, where R represents the ratio between the heavier and the lighter isotopes (^{13}C:^{12}C; ^{15}N:^{14}N).

The $\delta^{13}C$ and $\delta^{15}N$ values for the muscle tissue of the three stingray species were used to build a similarity matrix using Euclidian distances. PERMANOVA was then used to test for differences in the isotopic composition among the three species, by season and environment.

Individual PERMANOVAs were then conducted to examine each species separately, including sex, life stage, season and sampling location as factors. Analyses were performed using PRIMER v.6 soft- ware (Clarke & Gorley, 2005).

Considering that an organism's isotopic values reflect both diet and assimilation of baseline carbon, we used the $\delta^{13}C$ and $\delta^{15}N$ values (average, S.D.) of *H. guttatus*, *H. marianae* and *H. berthalutzae* to estimate the proportional contribution of marine and estuarine sources to diet using the Bayesian stable isotopic mixing model SIMMR in R (Stable Isotope Mixing Models in R with simmr, Parnell & Inger, 2021; R Core Team, 2020). Average values of $\delta^{13}C$ and $\delta^{15}N$ (± SD) for producer (*R. mangle*, *P. gymnospora* and Periphyton) and primary (Bivalve) and secondary (Polychaeta, *O. vulgaris*, *H. guttatus*, *H. marianae* and *H. berthalutzae*) consumer species were included to represent marine and estuarine sources (see Table 7). We adjusted source isotope values to account for fractionation; 1.5‰ for $\delta^{15}N$ and 1.3‰ for $\delta^{13}C$ (Post, 2002; Galván *et al.*, 2016).

To visualize the isotopic niche breadth and possible niche overlap among the three stingrays species (*H. guttatus*, *H. marianae* and *H. berthalutzae*) across environments, isotopic ellipses were calculated for each species sampled in each habitat in bi-dimensional space (Jackson *et al.*, 2011) using the SIBER package (Jackson *et al.*, 2011) in R (R Core Team, 2014).

The trophic level of each stingray species (*H. guttatus*, *H. marianae* and *H. berthalutzae*) was calculated through the equation proposed by Post (2002): $TL_{\delta^{15}N} = TL_{prey} + \frac{(\delta^{15}N_{predator} - \delta^{15}N_{prey})}{1.5‰}$, in which $TL_{\delta^{15}N}$ is the predator's trophic position, TL_{prey} is the baseline or prey's trophic position, $\delta^{15}N_{predator}$ is the consumer's nitrogen value, $\delta^{15}N_{prey}$ is the prey's nitrogen value, and 1.5‰ is the expected $\delta^{15}N$ fractionation constant between trophic levels. The $\delta^{15}N$ fractionation constant used was based on Galván *et al.* (2016), who estimated trophic discrimination factors (TDF) and turnover rates in the blood and muscle of the batoid *Sympterygia bonapartii* (Müller & Henle, 1841) through controlled feeding over a 1-year period. Considering that fractionation can vary according to the species studied and diet consumed (Hussey *et al.,* 2014; Post, 2002), and since *S. bonapartii* is a demersal batoid similar to the *Hypanus* genus (Hozbor & Ana, 2015; Jañez *et al.*, 2018), we considered it the most appropriate TDF. Prey used as the baseline ($\delta^{15}N_{prey}$) to estimate the trophic level of *H. guttatus*, *H. marianae* and *H. berthalutzae* were bivalves (TL_{prey} = 2), polychaetes (TL_{prey} = 2.5) and *O. vulgaris* (TL_{prey} = 2.7), respectively, obtained from stomach content analysis.

3 RESULTS

3.1 Quantitative diet analysis

From a total of 330 stomachs (*H. guttatus*, *n* = 163; *H. marianae*, *n* = 105; *H. berthalutzae*, *n* = 62), 230 contained prey: 103 *H. guttatus* (mean disk width [DW] = 44.37 ± 9.06 cm), 89 *H. marianae* (mean DW = 26.53± 4.82 cm) and 38 *H. berthalutzae* (mean DW = 79.57 ± 18.66 cm). For *H. guttatus* that contained stomach contents, 45.8% were male (18.9–58.5 cm DW) and 54.2% were female (15.1–75.3 cm DW). For *H. marianae*, 47.7% were male (15.1–30.7 cm DW) and 52.3% were female (15–37.80 cm DW). For *H. berthalutzae* males (43–83 cm DW) represented 35.3% of the sample, while females accounted for 64.7% (40.4–135 cm DW) (Table 1).

Table 1 Total number of samples collected, number of individuals with stomach content data, sex ratio and disk width of *Hypanus guttatus*, *H. marianae* and *H. berthalutzae* collected on the coast of Pernambuco, Brazil, from 2017 to 2019.

	Number of Samples		**Sex (%)**		**Disk Width (cm)**			
	Total	With stomach contents	Females	Male	Min	Max	Mean	±SD
H. guttatus	163	103	54.24	45.76	15.1	75.3	44.71	12.28
H. marianae	105	89	52.27	47.72	15	37.8	26.53	4.82
H. berthalutzae	62	38	64.71	35.29	40.4	135	79.57	18.66

Min, minimum; Max, maximum; ±SD, standard deviation.

Prey accumulation curves reached an asymptote at approximately 90 and 70 stomachs for *H. guttatus* and *H. mariane*, respectively, indicating that the number of analysed stomachs was sufficient to accurately describe the diet of these two species.

Table 2 Frequency of occurrence (%O), numerical frequency (%N), frequency of weight (%W), percent relative importance index (% RII) and order of importance (IO-RII) of the five main food items of *Hypanus guttatus*, *H. marianae* and *H. berthalutzae* collected on the coast of Pernambuco, Brazil, from 2017 to 2019. Complete worksheet in the Supporting Information Table 1.

	Hypanus guttatus					***Hypanus marianae***					***Hypanus berthalutzae***				
ITEMS	**%O**	**%N**	**%W**	**%RII**	**IO**	**%O**	**%N**	**%W**	**%RII**	**IO**	**%O**	**%N**	**%W**	**%RII**	**IO**
Phylum Annelida						57.3	9.12	26.28	39.27	**1**					
Phylum Arthropoda															
Suborder Dendrobranchiata						38.2	13.73	3.78	12.95	**3**					
Family Alpheidae															
Alpheus sp.	22.92	18.97	12.00	19.05	**2**										
Family Pasiphaeidae															
Leptochela serratorbita						33.71	35.64	6.86	27.73	**2**					
Suborder Brachyura	19.79	5.57	8.70	7.59	**4**						13.16	7.41	0.76	3.02	**5**
Callinectes sp.	19.79	4.11	9.67	7.32	**5**										
Order Isopoda						19.1	10.17	0.95	4.11	**5**					
Meiosquilla schmitti						16.85	5.77	7.8	4.42	**4**					
Phylum Mollusca															
Class Bivalvia	34.38	20.82	16.42	34.37	**1**										
Family Octopodidae															
Octopus vulgaris											23.68	16.05	13.77	19.83	**2**
Phylum Sipuncula											13.16	9.88	7.97	6.59	**3**
Phylum Chordata															
Class Actinopterygii															
Teleost n.i.	27.08	4.38	13.45	12.96	**3**						26.32	38.27	42.93	60	**1**
Haemulon aurolineatum											5.26	2.47	20.6	3.41	**4**

An asymptote in the prey accumulation curve for *H. berthalutzae* was not recorded, suggesting that more samples would be required to properly characterize this species diet (Figure S1).

Stomach content data revealed that *H. guttatus* fed on a diverse diet of benthic and epibenthic marine and estuarine prey. A total of 33 prey items distributed across five functional groups were identified: Arthropoda (Amphipoda, Decapoda, Isopoda and Stomatopoda), Mollusca (Bivalvia), Annelida (Polychaeta), Chordata (Teleostei) and Sipuncula. The principle prey items consumed by *H. guttatus* were molluscs of Class Bivalvia (34.37% RII), followed by *Alpheus* sp. (19.05% RII), fishes of infraclass Teleostei (12.96% RII), unidentified crabs of suborder Brachyura (7.59% RII), *Callinectes* sp. (7.32% RII) and *Upogebia* sp. (5.46% RII) (Table 2 and Supporting Information Table S1).

Table 3 Niche breadth and estimated trophic level of *Hypanus guttatus*, *H. marianae* and *H. berthalutzae* collected on the coast of Pernambuco, Brazil, from 2017 to 2019.

	B_A	TL (SCA)	TL (SIA)
H. guttatus	0.81	3.55	3.33 ± 1.09
H. marianae	0.16	3.61	3.19 ± 0.71
H. berthalutzae	0.29	3.81	4.29 ± 0.98

B_A, niche breadth; TL, trophic level; SCA, stomach content analysis; SIA, stable isotope analysis.

For *H. marianae*, a total of 23 prey items within four functional groups were identified: Arthropoda (Amphipoda, Decapoda, Isopoda and Stomatopoda), Annelida (Polychaeta), Chordata (Teleostei) and Sipuncula. The class Polychaeta was the most represented in the diet of *H. marianae* (39.27% RII), followed by *Leptochela serratorbita* (Spence Bate, 1888) (27.73% RII), suborder Dendrobranchiata (12.95% RII), *Meiosquilla schmitti* (de Castro, 1955) (4.42% RII) and order Isopoda (4.11% RII).

For *H. berthalutzae*, a total of 12 prey items were identified within four main functional groups: Arthropoda (Decapoda, Isopoda and Stomatopoda), Mollusca (Cephalopoda), Cordata (Teleostei) and Sipuncula. The principle prey consumed was infraclass Teleostei (60% RII), followed by the cephalopod *Octopus vulgaris* (Cuvier, 1797) (19.83% RII), the phylum Sipuncula (6.59% RII), the tomtate grunt fish *Haemulon aurolineatum* (Cuvier, 1830) (3.41% RII) and the suborder Brachyura (3.02% RII) (Table 2 and Supporting Information Table S1).

The estimated trophic niche breadth of *H. guttatus* was high (B_A = 0.81), indicating a high diversity of food items in its diet, while corresponding values for *H. marianae* and *H. berthalutzae* were low (B_A = 0.16 and 0.29, respectively) reflecting a less diverse diet

(Table 3 and Supporting Information Figure S2). Trophic level estimates based on stomach content data placed all three Mylobatiformes as mesopredators feeding between trophic levels 3.55 and 3.81 (Table 3).

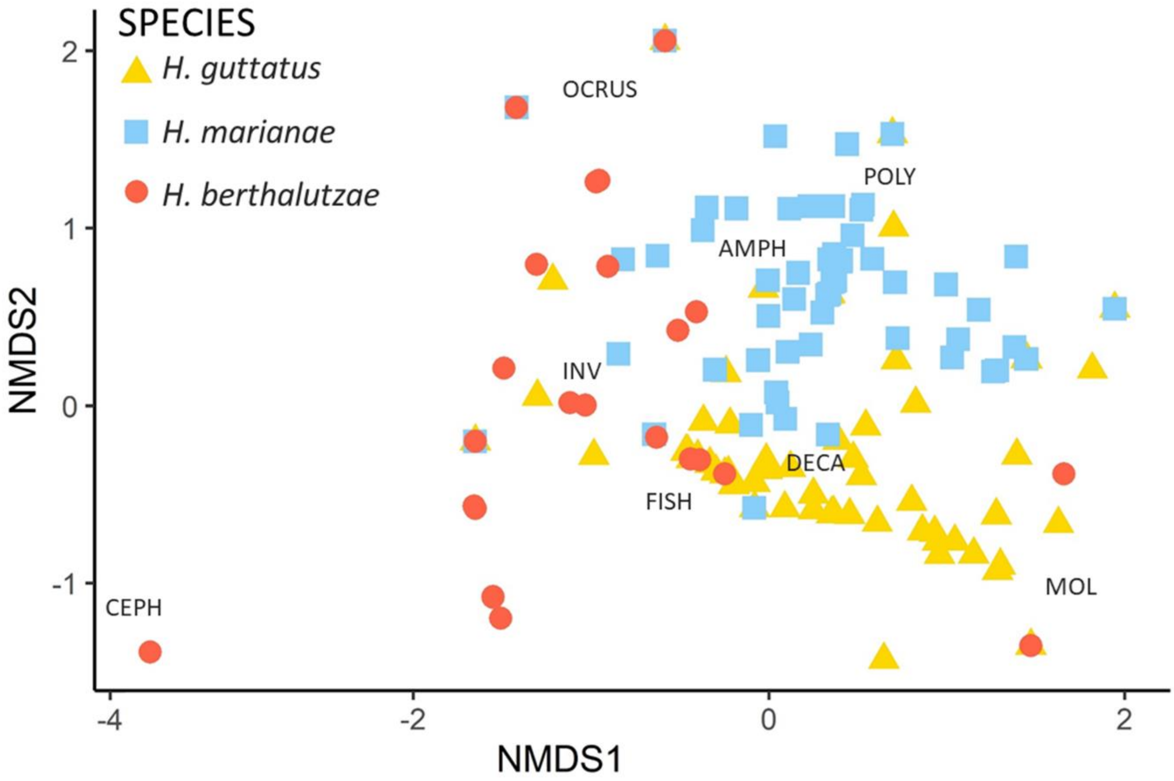

Figure 2 nMDS of the functional prey categories identified in the diet of *Hypanus guttatus*, *H. marianae* and *H. berthalutzae*: FISH, fish; AMPH, amphipod; DECA, decapod crustaceans; MOL, bivalve mollusks; POLY, polychaetes; OCRUS, other crustaceans; CEPH, cephalopods; INV, other invertebrates. Samples collected on the coast of Pernambuco, Brazil, from 2017 to 2019.

The one-way PERMANOVA indicated significant differences among diets of *Hypanus guttatus*, *H. marianae* and *H. berthalutzae* ($P < 0.001$; Table 4). The pair-wise PERMANOVA showed that the mean distances were higher between *H. guttatus* and *H. marianae*, fol- lowed by *H. guttatus* and *H. berthalutzae*, and finally by *H. marianae* and *H. berthalutzae* (Table 5).

Furthermore, the PERMANOVA with all species grouped indicated differences in diet among sampled habitats. Finally, the one- way PERMANOVA conducted for each species separately revealed significant differences in diet by habitat for all three species and among life stages for *H. guttatus* and *H. marianae* (Table 4).

Table 4 Multi-specific PRMANOVA with species and habitat as fixed factors and PERMANOVA of each species with life cycle, season, sex, and habitat as fixed factors. Samples collected on the coast of Pernambuco, Brazil, from 2017 to 2019.

Species	Factor	df	SS	MS	Pseudo-F	*P*	Var	SD
All species	Species	2	1.45	7299	28.35	0.001*	987.97	31.43
	Habitat	2	52272	26136	8.74	0.009*	311.1	17.64
H. guttatus	Life cycle	2	14153	7076.6	2.96	0.019*	164.95	12.84
	Season	1	2676.7	2676.7	1.08	0.33	4.2542	2.07
	Sex	1	2595.2	2595.2	1.05	0.34	2.2896	1.51
	Habitat	2	19294	9647.1	4.13	0.005*	342.92	18.52
H. marianae	Life cycle	2	18816	9407.9	3.73	0.006*	274.84	16.58
	Season	1	10519	10519	4.06	0.06*	180.26	13.43
	Sex	1	4159.4	4159.4	1.56	0.18	33.97	5.83
	Habitat	1	11191	11191	4.33	0.009*	240.44	15.51
H. berthalutzae	Life cycle	2	4812.2	2406.1	0.93	0.43	-16.93	-4.12
	Season	1	3164.9	3164.9	1.23	0.29	32.86	5.73
	Sex	1	3573.9	3573.9	1.39	0.22	56.01	7.48
	Habitat	1	9463.2	9463.2	3.95	0.01*	619.02	24.88

df = degrees of freedom, SS = sums of squares, MS = mean squares, p = statistical significance of hypothesis test, Var = estimated sizes of components of variation, SD = square root.* indicates tests were significant at $p<0.05$.

The nMDS analysis revealed that while there was a degree of diet overlap among the three stingray species, there was evidence for resource partitioning supporting the PERMANOVA results (Figure 2). The Similarity Percentage Analysis (SIMPER) identified that the differences in diet indicated by PERMANOVA were associated with contrasting proportions of the same prey resource used by each stingray species (Table 6).

Table 5 Pairwise PERMANOVA between species. The distance columns show the results of the average distances between sites. Samples collected on the coast of Pernambuco, Brazil, from 2017 to 2019.

Species	t	*p*	Distance
H. guttatus x *H. marianae*	4.3415	0.001	30.268
H. guttatus x *H. berthalutzae*	5.8057	0.001	14.749
H. marianae x *H. berthalutzae*	6.0535	0.001	8.2535

3.2 Stable isotope analysis

A total of 119 samples for the three stingray species and baseline and prey samples including vertebrates (teleost fishes), invertebrates (polychaetes, molluscs and crustaceans), plant material (mangrove leaves and marine macroalgae) and periphyton were analysed. Baseline/prey organisms associated with each discrete sampling habitat had distinct isotopic values, as would be expected. Estuarine samples were depleted in ^{13}C (-17.89 ± 3.01) when compared to reefs (-14.63 ± 1.4) and the continental shelf (-14.29 ± 1.25). In contrast, $\delta^{15}N$ values were higher in organisms sampled from the estuary (11.29 ± 2.32) when compared to reefs (10.95 ± 1.84) and the continental shelf (9.02 ± 1.88) (Table 7).

Table 6 Percentage of prey categories that contributed most to diet similarity within species. Specimens collected on the coast of Pernambuco, Brazil, from 2017 to 2019.

	SPECIES		
	H. guttatus (%)	*H. marianae* (%)	*H. berthalutzae* (%)
N	103	89	38
DECA	84.63	63.16	1.7
MOL	9.96	-	0.33
FISH	4.84	0.59	92.45
POLY	0.24	32.06	-
AMPH	0.17	1.21	-
INV	0.16	0.56	1.84
OCRUS	-	2.42	0.88
CEPH	-	-	2.81

N, number; FISH, fish; AMPH, amphipod; DECA, decapod crustaceans; MOL, mollusks; POLY, polychaetes; OCRUS, other crustaceans; INV, other invertebrates; CEPH, cephalopods.

PERMANOVA showed significant differences among species, sea- son and habitat for both $\delta^{15}N$ and $\delta^{13}C$ (Table 8). When PERMA- NOVA was performed on each species separately, significant differences in $\delta^{15}N$ and $\delta^{13}C$ were found between seasons for *H. guttatus* and *H. marianae*, respectively. Life-stage $\delta^{15}N$ values were significantly different in *H. berthalutzae* (Table 8).

Of the 36 *H. guttatus* captured in the estuary, 11 (mostly mature females) had $\delta^{13}C$ values ranging between -15.69 and -13.55. These values were within the isotopic range of *H. marianae* and *H. berthalutzae* captured in reef and continental shelf environments. In contrast, of seven *H. guttatus* captured in reef habitat, values for one female and two immature males ranged between -19.06 and -17.46, outside the observed range for *H. marianae* and *H. berthalutzae* captured in the same environment, and similar to estuarine fauna (Figure 3).

SIMMR dietary mixing models found that *H. guttatus* captured in the estuary had a large contribution from bivalves, while individuals caught in reef habitat had higher contributions of estuarine (bivalves), reef (polychaete) and continental shelf (*P. gymnospora*) sources. Furthermore, the only *H. guttatus* captured on the continental shelf had contributions from that sampling environment (*P. gymnospora* and periphyton) and from reef habitat (polychaete) (Figure 4 and Supporting Information Table S2).

Table 7 Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) for *Hypanus guttatus*, *H. marianae*, *H. berthalutzae* and other baseline/prey organisms collected in mangrove, reef, and continental shelf. habitat. Specimens collected on the coast of Pernambuco, Brazil, from 2017 to 2019.

	N	$\delta^{13}C$ max	$\delta^{13}C$ min	$\delta^{13}C$ mean	$\delta^{13}C$ ±SD	$\delta^{15}N$ max	$\delta^{15}N$ min	$\delta^{15}N$ mean	$\delta^{15}N$ ±SD
Mangrove									
Rhizophora mangle	3	-26.86	-28.01	-27.31	0.61	6.40	4.51	5.59	0.97
Bivalve	3	-17.27	-20.19	-18.49	1.52	10.45	9.51	10.03	0.48
Alpheus estuariensis	3	-17.81	-19.21	-18.60	0.53	7.97	8.28	8.14	0.11
Hypanus guttatus	36	-13.55	-21.8	-16.99	1.65	14.85	8.38	12.13	1.48
Reefs									
Cassis tuberosa	2	-14.45	-14.47	-14.46	0.01	10.39	10.34	10.36	0.03
Polychaeta	2	-13.50	-14.53	-14.02	0.73	10.59	10.33	10.46	0.18
Penaeus subtilis	2	-14.16	-14.18	-14.17	0.01	10.79	9.86	10.33	0.46
Hypanus guttatus	7	-13.93	-19.06	-16.13	2.03	14.79	8.27	11.66	2.47
Hypanus marianae	8	-13.12	-14.72	-13.81	0.65	12.52	9.73	11.68	1.27
Hypanus berthalutzae	8	-13.41	-15.26	-14.43	0.73	12.77	7.68	10.04	2.06
Continental shelf									
Plocamium brasiliense	2	-14.97	-15.62	-15.29	0.46	5.92	5.79	5.85	0.09
Padina gymnospora	2	-15.29	-15.02	-15.15	0.20	9.15	8.76	8.95	0.27
Dictyota crenulata	2	-13.16	-13.63	-13.40	0.33	7.95	7.89	7.92	0.04
Periphyton	2	-9.56	-9.81	-9.68	0.17	4.13	4.09	4.11	0.03
Octopus vulgaris	3	-15.02	-17.27	-16.22	1.14	7.10	7.01	7.04	0.05
Hypanus guttatus	1	-	-	-14.49	-	-	-	10.73	-
Hypanus marianae	6	-13.77	-14.37	-14.07	0.73	12.11	10.14	11.26	0.73
Hypanus berthalutzae	27	-13.57	-15.79	-14.38	0.59	12.41	7.41	9.27	1.22

N, number; Min, minimum; Max, maximum; ±SD, standard deviation.

For *H. marianae* sampled in reef and continental shelf habitat, reef prey/baseline sources were most important. In contrast, continental shelf prey/baseline sources (*P. gymnospora*, *O. vulgaris* and periphyton) were the most important for *H. berthalutzae* from both environments (Table 2).

Similar to the niche breadth analysis based on stomach content data, *H. guttatus* had a markedly wider isotopic niche when compared to the other two species (Figure 5). Estimates of trophic level for the three *Hypanus* spp. based on $\delta^{15}N$ values revealed trophic level estimates similar to that calculated using stomach content data, assigning stingray species as intermediate consumers (TL range from 3.33 to 4.29; Table 3).

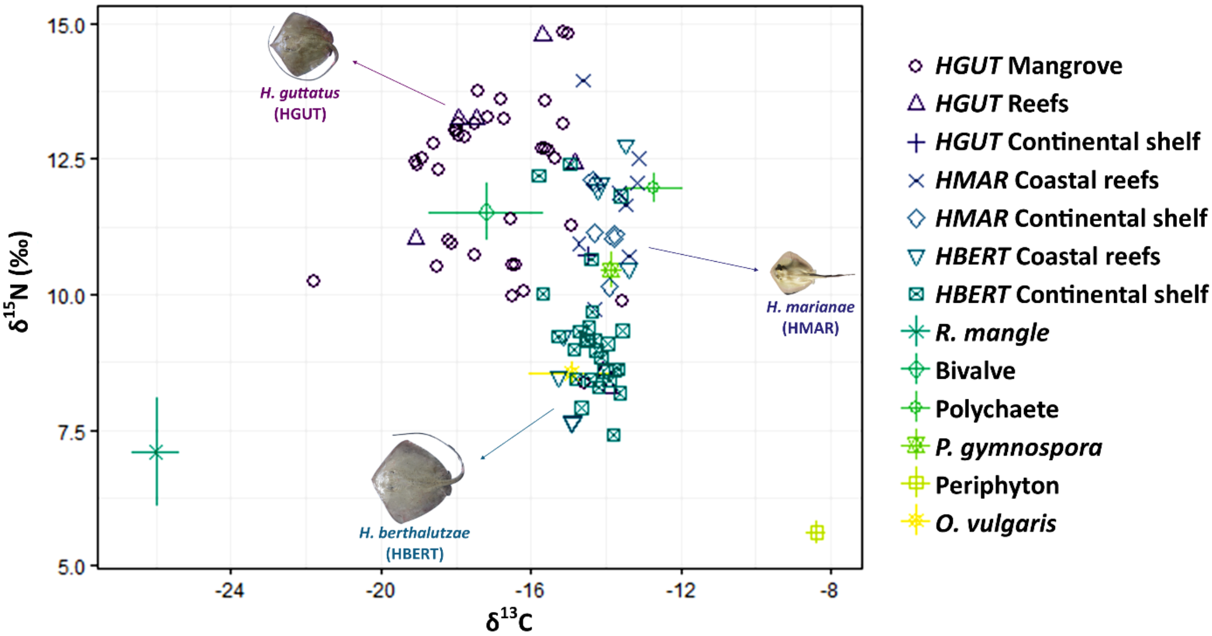

Figure 3 Biplot of $\delta^{13}C$ and $\delta^{15}N$ baseline/prey values applied in the Bayesian isotopic mixture model (SIMMR) including individual stable isotope values for the three stingrays sampled in distinct habitats: HGUT = *Hypanus guttatus;* HMAR = *H. marianae*; HBERT = H. *berthalutzae*. Specimens collected on the coast of Pernambuco, Brazil from 2017 to 2019.

4 DISCUSSION

While the three *Hypanus* species inhabit coastal environments with variation observed in habitat occupied by life stage, diet data revealed they consume a few similar prey items. Despite this, the diets of the three species were statistically different, with each consuming prey resources at different rates. As a result, our combined SCA and SIA approach suggests that only *H. guttatus* uses estuarine areas and even when the three *Hypanus* species co-occur in the marine environment each one exploits available food resources differently, allowing coexistence, reducing competition and ultimately limiting functional redundancy. These results corroborate the two proposed hypotheses.

4.1 Diet and feeding behaviour: SCA

The longnose-stingray *H. guttatus* exhibited a diverse diet, with the highest number of food items among the three studied species (33 items), as well as a high niche breadth value (B_A = 0.81), identifying it as a generalist predator. This generalist feeding strategy is in agre-

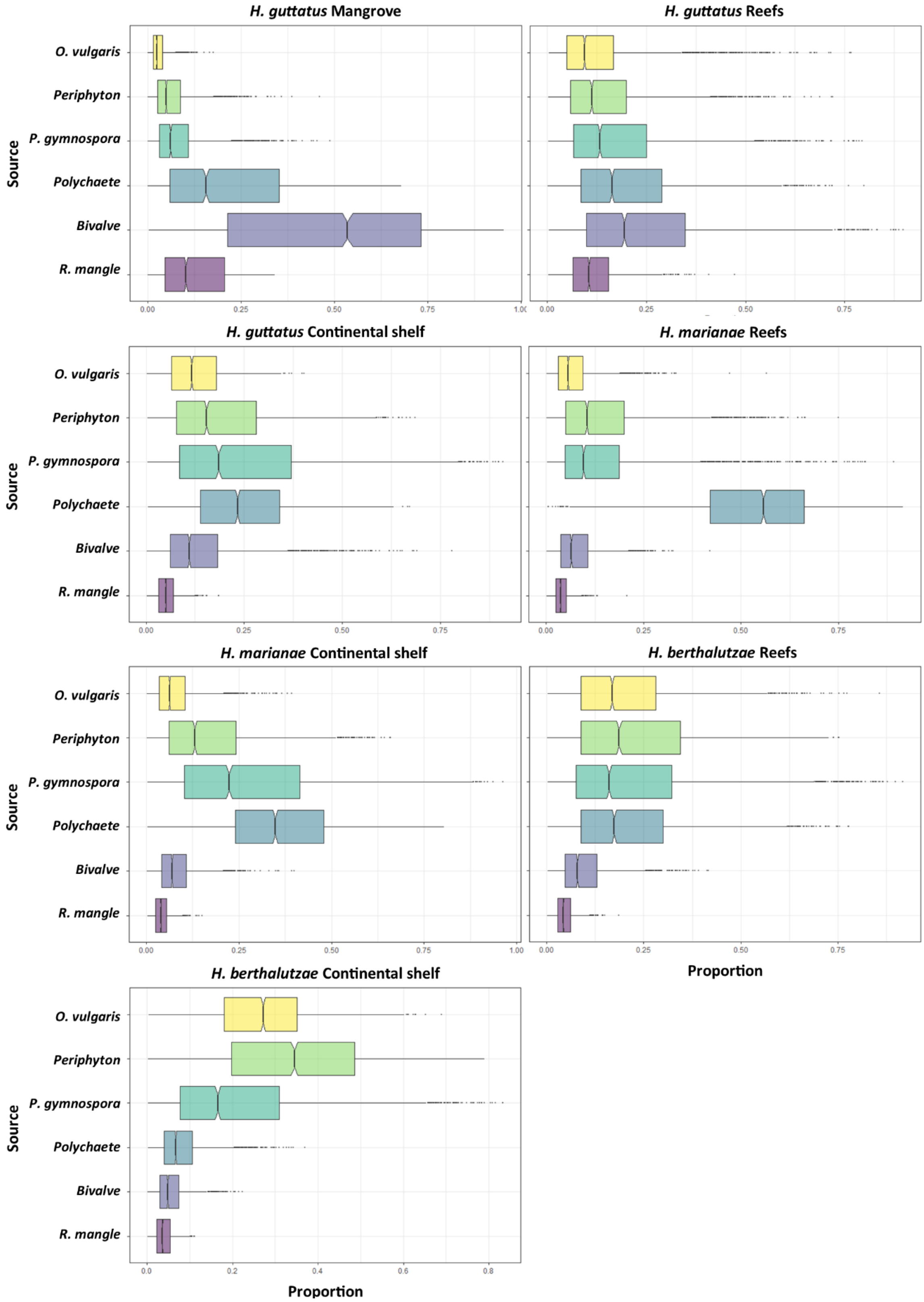

Figure 4 Contribution of different sources to the diet of *Hypanus guttatus*, *H. marianae* and *H. berthalutzae* estimated using Bayesian isotopic mixing model (SIMMR). Results show posterior model estimates (median, interquartile range and max/min values) of source contribution to stingrays' muscle tissue of each specimen captured in each environment. The confidence interval is 97.5%. Specimens collected on the coast of Pernambuco, Brazil, from 2017 to 2019.

-ement with previous studies on *H. guttatus* undertaken at other localities throughout its distribution range along the Brazilian coast (Carvalho Neta & Almeida, 2002; Gianeti, Santana, *et al*., 2019; Silva *et al*., 2001).

Table 8 Multi-specific PERMANOVA with species, seasons, and habitat as fixed factors and PERMANOVA of $\delta^{13}C$ and $\delta^{15}N$ of individual species with sex, life cycle stage, season, and habitat as fixed factors. Specimens collected on the coast of Pernambuco, Brazil, from 2017 to 2019.

Species	Factor	df	SS	MS	Pseudo-F	*p*	Var	SD
All species ($\delta^{13}C$)	Species	2	151.71	75.85	46.9	0.001*	2.61	1.61
	Season	1	17.59	17.59	5.72	0.02*	0.31	0.56
	Habitat	2	139.99	69.99	40.05	0.001*	2.24	1.49
All species ($\delta^{15}N$)	Species	2	134.64	67.32	29.81	0.001*	2.29	1.51
	Season	1	36.18	36.18	10.91	0.002*	0.71	0.84
	Habitat	2	107.33	53.66	20.95	0.001*	1.68	1.29
H. guttatus ($\delta^{13}C$)	Sex	1	8.8	8.8	3.07	0.08	0.27	0.52
	Life cycle	1	2.84	2.84	0.94	0.34	-7.71	-8.78
	Season	1	0.21	0.21	6.79	0.78	-0.14	-0.37
	Habitat	2	9.75	4.87	1.67	0.16	0.29	0.54
H. guttatus ($\delta^{15}N$)	Sex	1	0.32	0.32	0.12	0.74	-0.11	-0.33
	Life cycle	1	1.87	1.87	0.68	0.41	-3.98	-0.19
	Season	1	16.07	16.07	6.72	0.02*	0.66	0.81
	Habitat	2	3.04	1.52	0.55	0.57	-0.19	-0.43
H. marianae ($\delta^{13}C$)	Sex	1	2.27	2.27	7.69	0.77	-3.89	-0.19
	Life cycle	1	0.76	0.76	3.24	0.11	8.15	0.28
	Season	1	1.31	1.31	6.91	0.03*	0.32	0.57
	Habitat	1	0.24	0.24	0.87	0.35	-5.37	-7.33
H. marianae ($\delta^{15}N$)	Sex	1	1.58	1.58	1.46	0.25	7.13	0.27
	Life cycle	1	0.11	0.11	9.02	0.76	-0.17	-0.41
	Season	1	2.06	2.06	1.98	0.19	0.29	0.55
	Habitat	1	0.59	0.59	0.51	0.52	-8.41	-0.29
H. berthalutzae ($\delta^{13}C$)	Sex	1	0.19	0.19	0.51	0.48	-1.11	-0.11
	Life cycle	1	0.26	0.26	0.67	0.43	-7.41	-8.61
	Season	1	0.49	0.49	1.32	1.32	7.23	8.51
	Habitat	1	1.86	1.86	4.77	0.84	-3.01	-0.17
H. berthalutzae ($\delta^{15}N$)	Sex	1	1.51	1.51	6.86	0.98	-0.13	-0.36
	Life cycle	1	11.96	11.96	6.55	0.02*	0.61	0.78
	Season	1	4.42	4.42	2.15	0.15	0.14	0.37
	Habitat	1	3.66	3.66	1.77	0.19	0.13	0.36

df = degrees of freedom, SS = sums of squares, MS = mean squares, p = statistical significance of hypothesis test, Var = estimated sizes of components of variation, SD = square root. * indicates tests were significant at $p<0.05$.

The large-eye stingray *H. marianae*, in contrast, yielded a small niche breadth (B_A = 0.16), indicating a lower diversity of prey items consumed and suggestive of a specialized diet (Queiroz *et al*., 2019). Although 23 prey items were identified, most were rare occurrences, with diet dominated by shrimp and polychaetes (81.63%). The fact that the diet of *H. marianae* reached an asymptote with a lower number of stomachs in the prey accumulation curve, similar to chola guitarfish ray *Pseudobatos percellens* (Walbaum, 1792) sampled in Southern Bahia state (B_A = 0.06) (do Carmo *et al*., 2015), further sup- ports a specialized diet. While *H. berthalutzae* exhibited a low niche breadth (B_A = 0.29), it is possible that the diversity of food resources it consumes is not well represented in our sample, given the accumulation curve did not reach an asymptote.

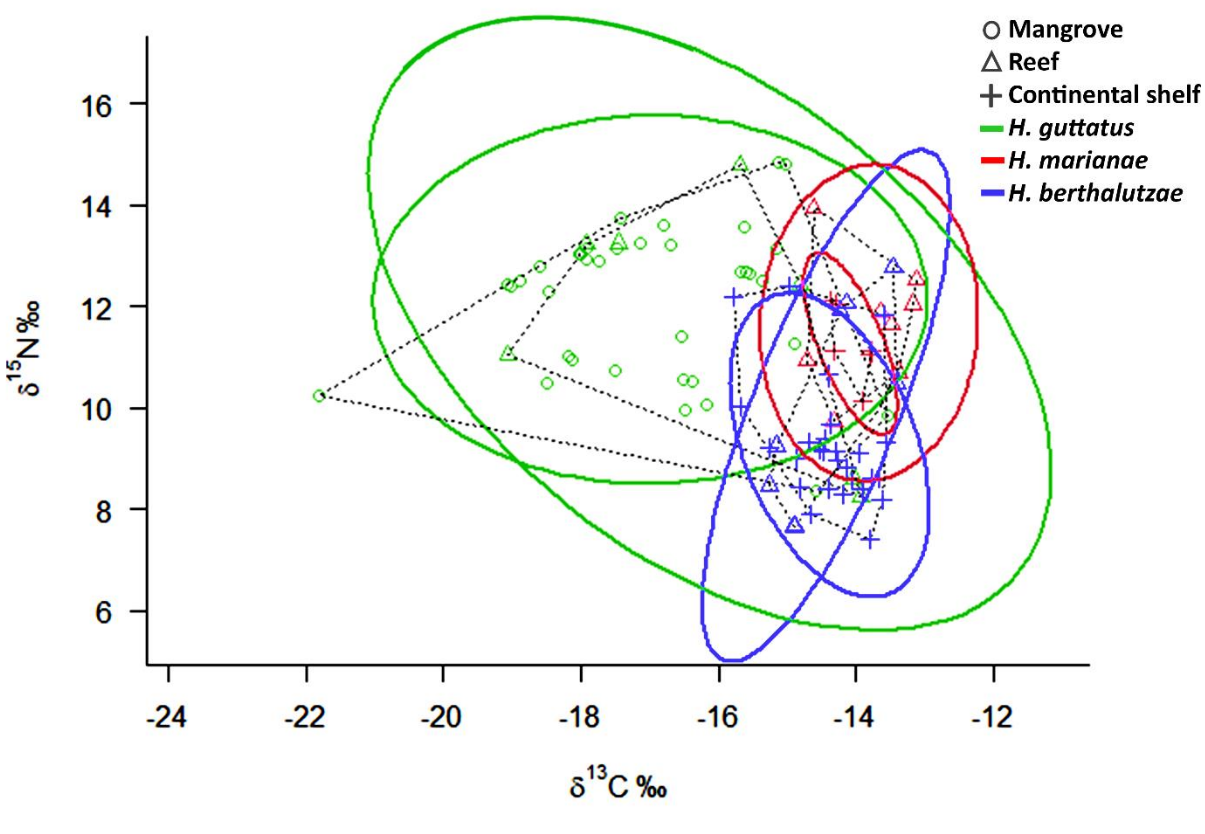

Figure 5 Isotopic niche of *Hypanus berthalutzae*, *H. guttatus* and *H. marianae* in relation to the capture environment. The ellipses represent the isotopic niche of the species in each environment (mangrove, reef, and continental shelf) at the 95% confidence interval. Specimens collected on the coast of Pernambuco, Brazil, from 2017 to 2019.

In mangrove habitat, structures were observed in the estuarine muddy bottom locally known as ‘stingray beds’, marks left by stingrays digging to facilitate foraging. Evidence for this type of foraging behaviour has previously been reported for *H. guttatus*, through using its snout to dig through the sediment when searching for food (Löwemark, 2015; Takeuchi & Tamaki, 2014; Carqueija *et al*., 1995).

The second most important prey item in the diet of *H. guttatus*, Alpheidae shrimps, inhabit marine and estuarine waters of the Brazilian coast and are commonly found in sediment burrows (Atkinson *et al*., 2003; Soledade & Almeida, 2013). Known as snapping shrimp, they produce shockwaves when closing their well-developed cheliped, causing a snap. This characteristic potentially makes them easy prey for *H. guttatus* (Carqueija *et al*., 1995). While olfaction is important during predation in Myliobatiformes (Blonder & Alevizon, 1988), these species use a variety of information related to their environment to navigate and localize prey, including detecting mechanical stimuli, electrical impulses and sounds to locate food (Bedore, 2013; Jordan *et al*., 2013). Furthermore, the symbiotic relationship between gobiid fishes and alpheid shrimps (Karplus, 1979) may explain their simultaneous presence at similar states of digestion in several *H. guttatus* stomachs. In addition to digging through the substrate to locate alpheid shrimp, the stingray promotes a rapid water flux into its mouth when suction feeding (Dean *et al*., 2005). This likely leads to the consumption of gobiid fishes associated with snapping shrimp.

Bivalve molluscs were an abundant prey item in the diet of *H. guttatus*, occurring in nearly half the stomachs analysed, corresponding to their high abundance in the Santa Cruz Canal estuary (Silva, 2004). In environments where this prey species is less abundant (*i.e*., reef habitat and on the continental shelf), it occurred in <10% of stomachs, with the diet of *H. guttatus* more balanced across prey groups. This diet composition could indicate that *H. gutattus* not only feeds on the most abundant resources, but also those that are most easily accessible, acting as a high-level predator in benthic habitats. The adaptability of this species to tolerate variations in salinity tied with its generalist opportunistic feeding behaviour enable it to exploit prey from diverse coastal environments, even overcoming ecological barriers such as the Amazon River mouth.

Both *H. guttatus* and *H. marianae* underwent ontogenetic shifts in diet. This could be a result of the higher predation capacity of more experienced adults (Sisneros & Tricas, 2002), selective predation related to body size or consumption of a broader prey base with increasing body size tied with increasing gape size (Queiroz *et al*., 2019; Tilley *et al*., 2013). While the PERMANOVA did not detect any statistically significant differences in diet between life stages of *H. berthalutzae*, a trend of intensified predation on crustaceans during younger phases and a preference for cephalopods by adults was apparent.

Hypanus marianae neonates and YOYs fed on shrimps, amphipods and isopods, while immature individuals aged >1 years exhibited an increased consumption of shrimps and polychaetes, with the occurrence of crabs, stomatopods and other invertebrates in smaller numbers. While adults continued to consume shrimps and polychaetes, the occurrence of stomatopods increased, and it was the only life stage in which teleost fish were predated, especially by pregnant females. Costa *et al*. (2015) suggested the consumption of lobsters by adult females was tied to their higher energetic value during pregnancy. In the current study we suggest increased gape size and strength of *H. marianae* leads to increased consumption of teleost fishes and stomatopods (Queiroz *et al*., 2019; Tilley *et al*., 2013).

In contrast to *H. berthalutzae* feeding predominantly on higher trophic level organisms such as teleost fishes and cephalopods in both benthic and epibenthic environments, Aguiar (2010) describes the species as a generalist opportunist at the Fernando de Noronha Archipelago. In the later study, this species primarily consumed polychaetes and crustaceans. This suggests trophic plasticity in *H. berthalutzae*. Alternatively, the discrepancy in results could be a consequence of methodological bias, since Aguiar (2010) caught specimens of *H. berthalutzae* during the day, while in our study captures occurred during the night. We note, however, that the importance of cephalo- pods to the diet of *H. berthalutzae* may be underestimated in stomach contents. This prey item was represented by small tissue portions associated with the beak or only by the presence of beaks. Therefore, although it is the second most important item in the species' diet, underestimation of mass consumed may have masked its importance as a prey group.

Although the use of shallow waters (up to 1 m) by adult individuals of *H. berthalutzae* is widely reported in island regions (Aguiar *et al*., 2009; Branco-Nunes, Veras, *et al*., 2016), there were no records of adult *H. berthalutzae* in coastal captures up to 6 m deep in our study area (Figure 1, Reefs). This could suggest ontogenetic habitat segregation in this species in the south-west Atlantic. Such size-based habitat segregation may have influenced the differences observed in the diet, since the different composition of available prey in each environment is commonly reflected in generalists' diets.

4.2 Trophic level: SIA and SCA

The estimated trophic levels for *H. guttatus* (SIA = 3.33 ± 1.09, SCA = 3.55; Table 3) and *H. marianae* (SIA = 3.19 ± 0.71, SCA = 3.61; Table 3) characterize these species as mesopredators, within the expected range (3.16–4.08) for Dasyatidae stingrays (Jacobsen & Bennett, 2012; Tilley *et al*., 2013). Similar results have been reported for other Myliobatiformes such as *Urotrygon rogersi* (Jordan & Starks, 1895) in Colombia (3.5; Navia *et al*., 2016), *Urotrygon microphthalmum* Delsman 1941 in Pernambuco (3.5; Santander- Neto *et al*., 2021), *Hypanus americanus* (Hildebrand & Schroeder, 1928) in the Caribbean (3.52; Tilley *et al*., 2013), *Neotrygon kuhlii* (Müller & Henle, 1841), *Neotrygon annotata* (Last, 1987) and *Neotrygon picta* Last & White 2008 in Australia (3.58, 3.57 and 3.55, respectively; Jacobsen & Bennett, 2012). This is driven primarily by their consumption of benthic and epibenthic macroinvertebrates such as crustaceans, molluscs and polychaetes, but including some teleost fishes.

Although trophic level estimate based on stomach contents found *H. berthalutzae* fed at TL < 4, the trophic level estimated based on stable isotope analysis exceeded the expected value for the Dasyatidae family (Jacobsen & Bennett, 2012; Tilley *et al*., 2013). The reported predation of *H. berthalutzae* on *H. marianae* (Branco-Nunes, Albuquerque, *et al*., 2016) was not observed in this study, but may have been reflected in its isotopic composition, elevating its trophic level to that observed for benthic feeding sharks, such as *Sphyrna zygaena* Linnaeus, 1758, *and Rhizoprionodon lalandii* (Müller & Henle, 1839) (Bornatowski *et al*., 2014). This discrepancy highlights the importance of using a combined stable isotope and stomach content approach to estimate trophic position.

Trophic level estimates indicate that all three species, although demersal Myliobatiformes, act at different levels of the trophic net based on variable prey consumption rates, rendering each important in structuring the food webs they inhabit. Although the ecological role of mesopredators is evident through linking benthic primary producers and higher trophic levels (Karl & Obrebski, 1976; Myers *et al*., 2007; Orth, 1975; Sánchez *et al*., 2005; Sánchez & Olaso, 2004), *H. guttatus*, *H. marianae* and *H. berthalutzae* act as a key linkage between infaunal and higher trophic level species.

4.3 Habitat use and diet: SIA

The isotopic composition of muscle tissue of *H. guttatus* specimens caught in the mangroves was the most negative, as would be expected if they were resident in that system (Fry, 2006). Likewise, *H. guttatus* caught in reef habitat had a more negative isotopic composition than expected for foraging in that environment. The apparent contributions of marine sources to individuals caught in the estuary (polychaetes), and estuarine contributions to reef individuals (bivalve molluscs) likely reflect movements undertaken by *H. guttatus* between both environments. For elasmobranchs, controlled experiments have shown that it can take several months for a new diet to be reflected in consumer muscle tissue isotope values (Caut *et al*., 2013; Galván *et al*., 2016; Hussey *et al*., 2010; Kim *et al*., 2012; Logan & Lutcavage, 2010; Malpica-Cruz *et al*., 2012). Thorson (1983) suggested that female *H. guttatus* give birth in brackish waters (2.8– 16 ppt) in the Caribbean, with neonates rapidly moving to higher salinities (16–32 ppt), given their suggested intolerance of low salinities during the first months of life. In our study area, *H. guttatus* have been previously shown to exhibit ontogenetic segregation between marine and estuarine environments (Melo *et al*., 2021; Santos *et al*., 2016), but parturition take place in the marine environment (Melo, 2016). Data on trace elements concentrations (Ba:Ca, Mg:Ca, Mn:Ca and Sr:Ca) in vertebrae also suggest the use of higher-salinity areas during the first months of life (Feitosa *et al*., 2021).

In this study, captures of *H. berthalutzae* occurred in coastal areas near beaches (juveniles) and near the continental shelf break (adults), indicating ontogenetic segregation between these life stages. However, $\delta^{13}C$ values were similar for both size classes, and *H. berthalutzae* sampled in coastal and continental shelf environments had higher isotopic contributions from sources located on the continental shelf. Furthermore, a few young *H. berthalutzae* exhibited similar $\delta^{15}N$ values to adults, despite stomach contents indicating feeding on prey at a different trophic level. This could be a result of maternal isotopic enrichment whereby embryos and neonates exhibit enriched $\delta^{13}C$ and $\delta^{15}N$ isotopic values relative to the mother (Garzón-Peña *et al*., 2020; Rangel *et al*., 2020). In addition, Myliobatiformes are born with an internal vitelum and liver reserves, which aids nutrition during this early life stage but is likely representative of the mother's isotopic signature. In both cases, it would take several months for muscle tis- sue to reflect the isotopic composition of their own diet (when reaching tissue-diet balance) depending on the organism's metabolism and growth. Neonates or YOYs were not included in the analysis, but some individuals were just over a year old. Given the length of time to use the internal vitelum following birth and its influence on isotopic values is unknown (*e.g*., see Niella *et al*. 2021), it is not possible to state if muscle tissue in these young animals directly reflects their diet. Comparing these results to $\delta^{13}C$ and $\delta^{15}N$ values in plasma and liver would allow a better understanding of maternal influence on juvenile carbon sources.

Hypanus marianae caught in reef habitat and on the continental shelf had isotopic compositions with the highest contributions from reef sources (Polychaete). This suggests a degree of connectivity of this species between these two environments. Despite this, the isotopic niche of *H. marianae* was the smallest among the three species. This species is an endemic stingray that is considered to have a relatively limited distribution, food and habitat specificity (Costa *et al*., 2015, 2017; Queiroz *et al*., 2019). The isotopic niche breadth and overlap of *H. guttatus* by life stages reflect its generalist feeding habit and the different environments it occupies. Given individuals of different size classes were analysed, representing different life stages, the overlap of predicted estuarine and marine niches is influenced by movements of individuals between both environments. In contrast, *H. marianae* and *H. berthalutzae* exhibited considerably lower isotopic niche breadths, interspecific and between-environment overlap.

While isotopic niche analysis indicated a degree of niche overlap between the three *Hypanus* species, SCA indicated differential exploitation rates of prey types, trophic position estimates were variable among species and a previous predation record between *H. marianae* and *H. berthalutzae* (Branco-Nunes, Albuquerque, *et al*., 2016) demonstrate these three demersal meesopredators play different functional roles in the ecosystems they inhabit.

5 CONCLUSIONS

Stingrays *H. guttatus*, *H. marianae* and *H. berthalutzae* occur in the same environments (although different life stages occupy different components of the total environment) and share common food resources. Despite this overlap in diet and habitat, functional redundancy is unlikely, since each species showed preferential prey choice and fed at variable trophic levels. Our data indicate connectivity between estuarine and marine environments mediated by *H. guttatus* throughout its life cycle, while *H. marianae* and *H. berthalutzae* had more similar isotopic niches even though SCA identified distinct feeding habits. The habitats occupied and the predator–prey relationships for these two species are factors that make these them more similar when compared to *H. guttatus*. The three *Hypanus* species studied in this work play important ecological roles as predators in the benthic ecosystem and through transferring energy up the food chain as prey to higher predators act to stabilize the ecosystem they inhabit and promote energy flow.

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SUPPORTING INFORMATION

Table 1 Frequency of occurrence (%O), numerical frequency (%N), frequency of weight (%W), percentage relative importance index (%IIR) and order of importance (OI-IRI) of the food items of *Hypanus guttatus*, *H. marianae* and *H. berthalutzae* collected on the coast of Pernambuco, Brazil.

	Hypanus guttatus						*Hypanus marianae*						*Hypanus berthalutzae*					
Itens	**%O**	**%N**	**%W**	**IRI**	**%IRI**	**OI - IRI**	**%O**	**%N**	**%W**	**IRI**	**%IRI**	**OI - IRI**	**%O**	**%N**	**%W**	**IRI**	**%IRI**	**OI - IRI**
Filo Annelida																		
Class Polychaeta																		
Polychaeta n.i.	10.42	1.99	1.11	32.29	0.87	**9**	57.3	9.12	26.28	2028.69	39.27	**1**						
Filo Arthropoda																		
Class Crustacea																		
Crustacea n.i.	2.08	0.27	0.00	0.55	0.01	**23**												
Order Decapoda																		
Decapoda n.i.	12.50	4.51	0.10	57.66	1.55	**8**												
Suborder Pleocyemata																		
Family Palinuridae																		
Palinuridae							2.25	0.21	0.05	0.58	0.01	**19**						
Family Scillaridae																		
Scyllarides sp.							1.12	0.31	0.23	0.61	0.01	**19**						
Suborder Dendrobranchiata																		
Dendrobranchiata n.i.	3.13	2.39	0.12	7.85	0.21	**17**	38.2	13.73	3.78	669.07	12.95	**3**						
Family Penaeidae																		
Penaeidae n.i.	5.21	2.39	0.39	14.45	0.39	**14**												
Rimapenaeus constrictus	2.08	0.66	0.11	1.62	0.04	**22**	7.84	2.41	3.17	43.92	0.85	**10**						
Penaeus schmitti	1.04	0.27	0.02	0.29	0.01	**24**												
Family Solenoceridae																		
Pleoticus muelleri	3.13	0.80	0.84	5.12	0.14	**19**	3.37	0.42	1.51	6.5	0.13	**15**						
Family Sicyoniidae																		
Sicyonia sp.													5.26	3.7	0.04	19.72	0.55	**9**
Sicyonia typica	2.08	0.27	0.08	0.73	0.02	**23**												

Sicyonia parri							2.25	0.42	0.11	1.18	0.02	**18**						
Family Ogyridae																		
Ogyrides sp.	1.04	1.59	0.02	1.67	0.04	**21**												
Family Alpheidae																		
Alpheus sp.	22.92	18.97	12.00	709.72	19.05	**2**												
Alpheus chacei	3.13	1.59	1.27	8.95	0.24	**16**												
Alpheus estuariensis	2.08	0.80	0.57	2.85	0.08	**20**												
Family Pasiphaeidae																		
Leptochela serratorbita	2.08	0.93	0.01	1.95	0.05	**21**	33.71	35.64	6.86	1432.72	27.73	**2**						
Family Sergestoidea																		
Sergestoidea							3.37	9.96	0.46	35.11	0.68	**13**						
Acetes americanus							1.12	0.1	0.01	0.13	<0.01	**20**						
Suborder Brachyura																		
Brachyura n.i.	19.79	5.57	8.70	282.52	7.59	**4**	13.48	1.78	6.19	107.47	2.08	**7**	13.16	7.41	0.76	107.43	3.02	**5**
Family Portunidae																		
Portunidae n.i.	7.29	1.06	1.47	18.46	0.50	**13**							5.26	4.94	4.48	49.55	1.39	**8**
Callinectes sp.	19.79	4.11	9.67	272.82	7.32	**5**	16.85	2.2	8.05	172.78	3.34	**6**						
Callinectes exasperatus							2.25	0.52	2.52	6.84	0.13	**15**						
Microphrys bicornutus							1.12	0.1	1.25	1.52	0.03	**17**						
Charybdis hellerii	4.17	1.72	4.48	25.87	0.69	**12**												
Family Calapidae																		
Calapídeo n.i.	1.04	0.13	0.08	0.22	0.01	**24**							2.63	1.23	1.6	6.03	0.17	**12**
Hepatus sp.																		
Family Pinnotheridae																		
Pinnotheridae n.i.	1.04	0.13	0.06	0.20	0.01	**24**												
Family Xanthidae																		
Acantholobulus caribbaeus	3.13	0.53	0.06	1.83	0.05	**21**												
Family Panopeidae																		
Panopeus sp.	2.08	0.40	0.26	1.37	0.04	**22**												
Family Epialtidae																		

Epialtus sp.	1.04	0.13	0.01	0.15	<0.01	**25**												
Family Upogebiidae																		
Upogebia sp.	17.71	7.16	4.33	203.43	5.46	**6**	7.87	0.84	0.85	13.3	0.26	**14**						
Upogebia omissa	6.25	7.96	17.37	158.32	4.25	**7**												
Family Axianassidae																		
Axianassa australis	1.04	0.13	0.36	0.51	0.01	**24**												
Order Isopoda																		
Isopoda n.i.	9.38	2.79	0.11	27.13	0.73	**11**	19.1	10.17	0.95	212.38	4.11	**5**						
Odem Amphipoda																		
Amphipoda n.i.	4.17	2.79	0.06	11.85	0.32	**15**	3.37	1.05	0.09	3.82	0.07	**16**						
Class Cephalocarida																		
Cephalocarida							1.12	0.1	0.01	0.13	<0.01	**20**						
Order Stomatopoda																		
Stomatopoda n.i.							12.36	2.52	2.08	56.85	1.1	**9**	7.89	6.17	1.25	58.63	1.65	**7**
Family Squillidae																		
Squillidae n.i.	2.08	0.27	0.10	0.77	0.02	**23**							7.89	4.94	6.87	93.23	2.62	**6**
Meiosquilla schmitti							16.85	5.77	7.8	228.57	4.42	**4**						
Filo Mollusca																		
Class Bivalvia																		
Bivalvia n.i.	34.38	20.82	16.42	1280.29	34.37	**1**							5.26	2.47	0.13	13.7	0.38	**11**
Class Cephalopoda																		
Order Octopoda																		
Family Octopodidae																		
Octopus vulgaris													23.68	16.05	13.77	706.2	19.83	**2**
Filo Sipuncula																		
Sipuncula n.i.	7.29	1.99	1.79	27.58	0.74	**10**	8.99	1.36	3.22	41.21	0.8	**11**	13.16	9.88	7.97	234.77	6.59	**3**
Filo Chordata																		
Class Actinopterygii																		
Teleósteo n.i.	27.08	4.38	13.45	482.69	12.96	**3**	7.87	0.73	7.20	62.43	1.21	**8**	26.32	38.27	42.93	2136.88	60	**1**
Family Gobiidae																		

Gobiidae n.i.	1.04	0.53	4.57	5.31	0.14	**18**												
Family Holocentridae																		
Holocentrus adscensionis							2.25	0.52	17.32	40.1	0.78	**12**						
Family Haemulidae													5.26	2.47	0.15	13.78	0.39	**10**
Haemulon aurolineatum													5.26	2.47	20.6	121.39	3.41	**4**
TOTAL				3724.61						5165.91						5056.338		

Table 2 Percentage contribution of associated fauna with each studied environment to the isotopic composition of *Hypanus guttatus*, *H. marianae* and *H berthalutzae* in the 97.5% confidence interval. Specimens collected on the coast of Pernambuco, Brazil, from 2017 to 2019.

	Hypanus guttatus			***Hypanus marianae***		***Hypanus berthalutzae***	
	Mangrove	**Reefs**	**Continental shelf**	**Reefs**	**Continental shelf**	**Reefs**	**Continental shelf**
Mangrove							
Rhizophora mangle	28.7%	26%	11.1%	8%	8.4%	10%	8.7%
Bivalve	89.1%	67.3%	42.1%	20.3%	20.5%	24.7%	13.5%
Reefs							
Polychaete	56.4%	53.7%	51.7%	80.1%	67.5%	57.1%	21.2%
Continental shelf							
Padina gymnospora	26.6%	54.2%	73.1%	52.6%	76.2%	69.9%	63.8%
Periphyton	19%	44.3%	51.9%	46.5%	48.1%	60.3%	70.2%
Octopus vulgaris	8.4%	42.6%	28.7%	20.6%	20.5%	52.6%	51%

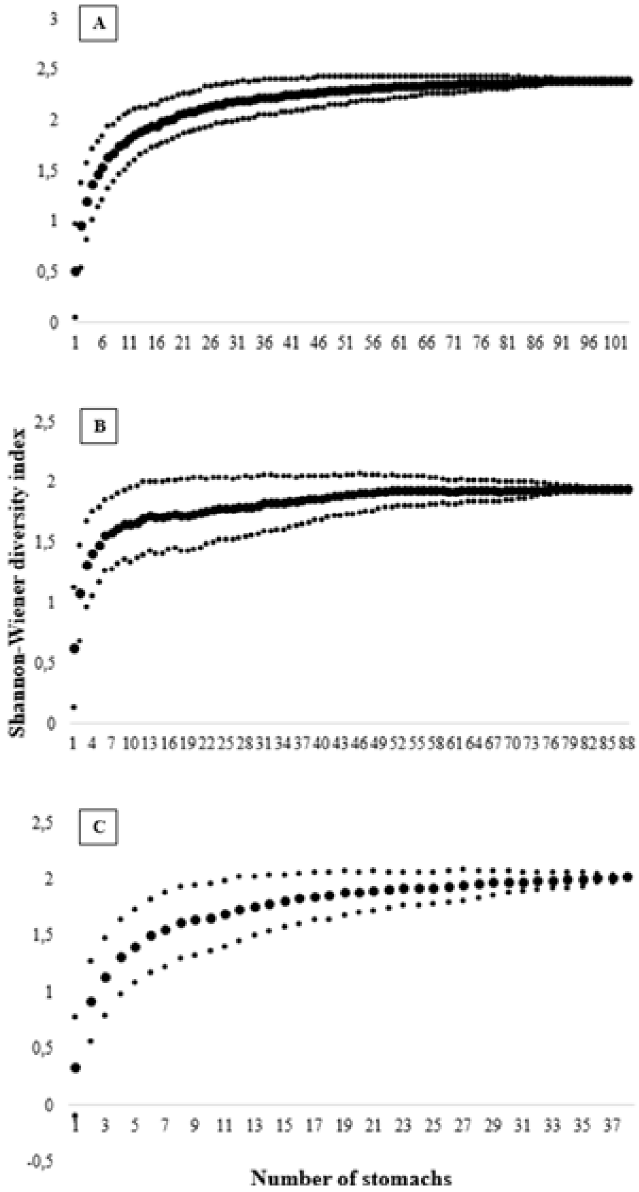

Figure 1 Accumulated mean and standard deviation of the Shannon-Wiener diversity index of food items found in the stomachs of Hypanus guttatus (A), H. marianae (B) and H. berthalutzae (C) collected on the coast of Pernambuco, Brasil.

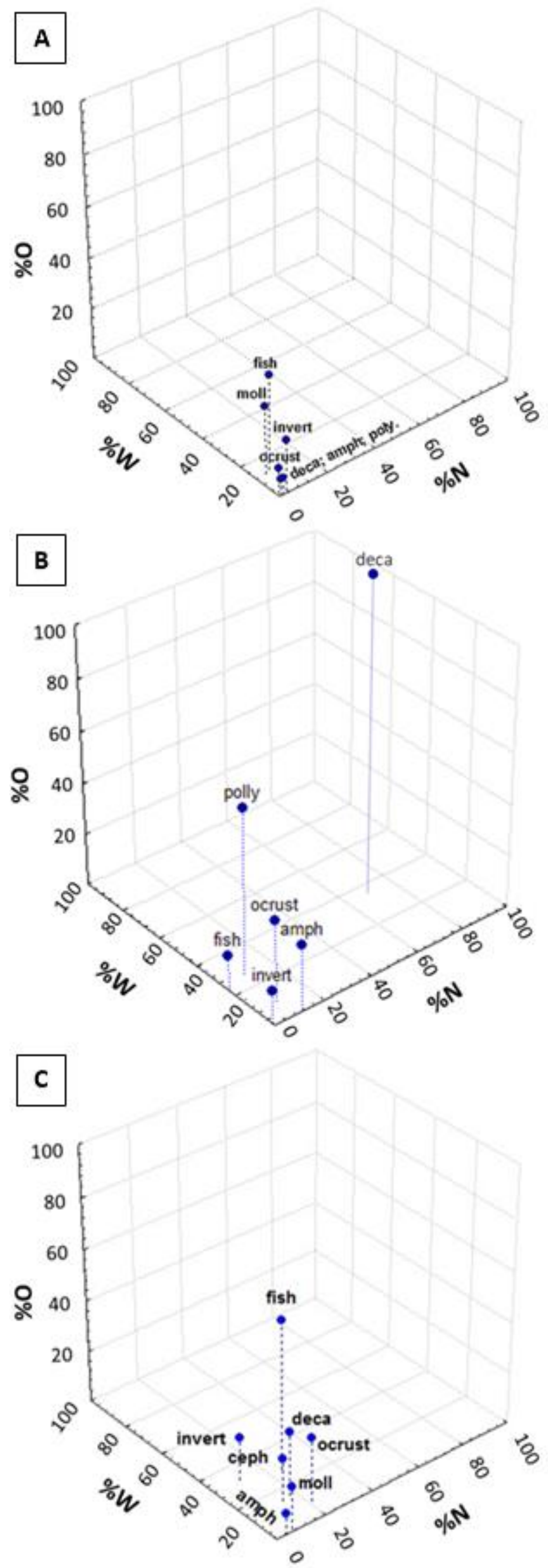

Figure 1 (a) *Hypanus guttatus*, (b) *H. marianae* and (c) *H. berthalutzae* three-dimensional graphical representation of the relationship between the frequency of occurrence (O%), numerical percentage (N%) and percentage by weight (W%) of the prey categories present in the diet: fish, fish; amph, amphipod; deca, decapod crustaceans; mol, mollusks; poly, polychaetes; ocrus, other crustaceans; inv, other invertebrates; ceph, cephalopods.

4 ARTIGO 2 – COMBINING STABLE ISOTOPES AND VERTEBRAE MICROCHEMISTRY DEMONSTRATES CRUCIAL HABITATS FOR THREE HEAVILY FISHED COASTAL STINGRAYS IN THE WESTERN ATLANTIC OCEAN

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Combining stable isotopes and vertebrae microchemistry demonstrates crucial habitats for three heavily fished coastal stingrays in the Western Atlantic Ocean

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ABSTRACT

Demand for ray consumption has been increasing considerably in Brazil, especially in the Northeast region. Species previously caught as bycatch are now targeted by fisheries but the available information on these species remains scarce. In the present study, we provide the first application of stable isotopes from muscle tissue ($\delta^{13}C$ e $\delta^{15}N$) and vertebrae microchemistry (^{24}Mg, ^{43}Ca, ^{55}Mn, ^{86}Sr, ^{138}Ba) data to analyze the habitat use of elasmobranchs. We employ these techniques on three sympatric demersal ray species across different life stages. Our approach revealed entry and exit movements in estuarine areas by *Hypanus guttatus*, a strong specificity for coastal reef habitats for *Hypanus marianae*, and the use of deeper waters by adults of *Hypanus berthalutzae*. We also revealed significant between-sex differences in habitat use for *H. berthalutzae*, especially for elements associated with hypoxic zones (^{55}Mn) and salinity variations (^{86}Sr and ^{138}Ba). The stable isotope data associated with the microchemistry data in elasmobranchs were considered good descriptors for the various environments these species are found in. Our results suggest that mangroves and coral reefs are the most important areas for *H. guttatus* and *H. marianae*, respectively, while *H. berthalutzae* seems to use the entire continental shelf throughout its life cycle.

Key words: elasmobranchs, tropical stingrays; spatial dynamics; habitat use.

INTRODUCTION

Among elasmobranch species found in Brazil, those inhabiting coastal areas are more exposed to intense fishing. Nevertheless, basic information on the biology and habitat use are still incipient for many of those species (Lessa et al., 1999; Martins et al., 2018). Some of the most important information for effective fisheries management include reproductive features such as fecundity, gestation period and age at maturity, as well as age estimates and growth and catch parameters (Cailliet, 2015). Lack of such data hampers assessments on species conservation status and the implementation of management measures for fisheries occurring along species' distribution range. Another valuable information which may aid in management is the spatial dynamics of organisms, especially of commercially exploited species (Fraser et al., 2018; Lea, 2017).

Overall, there is still a significant lack of information regarding the Elasmobranchii subclass (sharks and rays) in the world, a scenario which is even more critical for batoids when compared to sharks (Shiffman et al., 2020). In Brazil, data are scarce for many batoid species of commercial importance, such as the stingrays *Hypanus guttatus* (Bloch and Schneider, 1801), *Hypanus marianae* (Gomes et al., 2000), and *Hypanus berthalutzae* (Petean et al., 2020). These *Hypanus* species are landed by commercial artisanal fishing fleets operating off the Brazilian coast, where they are caught as either primary or secondary target species (Lessa et al., 2009, 1999; Yokota and Lessa, 2007). While *H. guttatus* is classified as Least Concern (LC) and *H. marianae* and *H. berthalutzae* are classified as Data Deficient (DD) in Brazil (ICMBio/MMA, 2016), their IUCN global statuses are Near Threatened (NT) (Carlson, J. et al., 2020), Endangered (EN) (Pollom et al., 2020), and Vulnerable (VU) (Charvet et al., 2020), respectively.

Hypanus guttatus is the most caught stingray in the artisanal and semi-industrial fishing fleets across over 2,000 km along the Northern and Northeastern Brazilian coasts (Basílio et al., 2008; Carmona et al., 2008; Gianeti, 2011, 2019; Lessa et al., 2008; Marion, 2015; Melo, 2016; Meneses et al., 2005; Menni and Lessa, 1998; ETEPE, 1995). On the other hand, *H. berthalutzae* and *H. marianae* and endemic species (Petean et al 2020, Gomes et al 200?) and are caught mainly as bycatch by the artisanal fishing fleet throughout their entire distribution ranges (Aragão, 2018; Barbosa, 2016; Branco-Nunes, 2015; Costa and Chaves, 2006; Costa et al., 2015; Lessa et al., 2008; Silva et al., 2007), with *H. berthalutzae* being a target species for the artisanal fisheries operating off Pernambuco state.

Furthermore, the increased demand for ray meat led to the development of new fishing techniques that improved the efficiency of catching stingrays, especially *H. guttatus* (Gianeti et al., 2019; Melo, 2016). In addition to fishing pressure, their biological features, such as slow growth, late maturity, and low reproductive rate (da Silva et al., 2018; Gianeti et al., 2019; Nunes et al., 2019), together with the increasing habitat destruction, render these species a low resilience against human impacts. In this scenario, data on movement and habitat use may guide management measures for species targeted by fisheries.

Analyses of ^{13}C e ^{15}N stable isotopes have been widely used for this purpose (Dierking et al., 2012; Mackenzie et al., 2012; Phillips et al., 2009; Trueman et al., 2012), since isotopes are natural biomarkers, capable of indicating dietary sources used by consumers. In species with slow metabolism and growth such as elasmobranchs, these isotopes represent an individual's long term dietary habits, reflecting the ingested and assimilated content over the course of several months (Galván et al., 2016; Hussey et al., 2014).

Another tool that provides important information on habitat use is the analysis of trace elements in biomineralized structures. This technique is based on the premise that some hard structures reflect the physico-chemical properties of the environment in which the fish lived for a certain period (Campana et al., 1995; Elsdon and Gillanders, 2003; Smith et al., 2013). Thus, the trace-element composition of these structures may represent a permanent chronological record of the environmental conditions present throughout an individual's life cycle (Mulligan et al., 1983; Santana et al., 2018). This technique has been increasingly used in habitat use studies for elasmobranch species (Feitosa et al., 2021, 2020; Lewis et al., 2016; Mohan et al., 2018; Tillett et al., 2011).

Here we provide the first application of both stable isotope and vertebrae microchemistry in elasmobranchs to obtain a more holistic and robust understanding of their habitat use patterns. Trace elements from vertebrae and ^{13}C and ^{15}N stable isotopes from muscle tissue was used to explore habitat use patterns in *H. guttatus*, *H. marianae* and *H. berthalutzae* across life cycle stages and between sexes to understand how these species use various habitats along a marine-estuarine environmental gradient. Our hypotheses are, I) Although co-occurring, each ray species uses the environment differently across their ontogenetic development; II) *H. guttatus* is the only among the three ray species studied capable of exploring estuarine habitats; III) *H. marianae* exhibits the highest habitat specificity; IV) *H. berthalutzae* is the species that explores deeper and farther areas from the coast among the three species studied. We further discuss the implication of our results to each species' geographical distribution range and their potential exposure to fishing pressure.

MATERIAL AND METHODS

Sampling area

The study was carried out in the coastal zone of Northeastern Brazil, southwest Atlantic Ocean, in an area comprising a well-defined environmental gradient with distinct substrates, depth and salinity features (Figure 1). The area exhibits a semi-arid continental climate and few rivers with low freshwater runoff and, thus exerting little influence on the coastal zone (Ekau and Knoppers, 1999). However, their influence increases seasonally with the rainy season (March to August), which may have up to 400 mm of precipitation and dropping to 10mm in the dry season (September to February) (Silva, 2004).

Sample collection

Hypanus guttatus, *H. marianae* and *H. berthalutzae* specimens were obtained at artisanal fisheries landing sites and during sampling trips along the coast of Pernambuco state, Brazil, from 2016 to 2019, in the following locations: Ponta de Pedras, a coastal region with little to no estuarine influence, where *H. guttatus*, *H. marianae* and *H. berthalutzae* specimens were obtained from fishing weirs at depths between 1 and 6 meters; Itapissuma, an estuarine region at Canal de Santa Cruz, where *H. guttatus* samples were caught by the "*raieira*" fisheries at depths between 5 and 18 meters; Itamaracá island, a region with little estuarine influence, in which *H. guttatus*, *H. marianae* and *H. berthalutzae* specimens were caught through bottom longlines operating between 5 and 18. km off the coast at depths of up to 50 m, and São José da Coroa Grande, a region with little to no estuarine influence, where *H. berthalutzae* specimens were caught by longline fisheries at depths of approximately 100 meters (Figure 1).

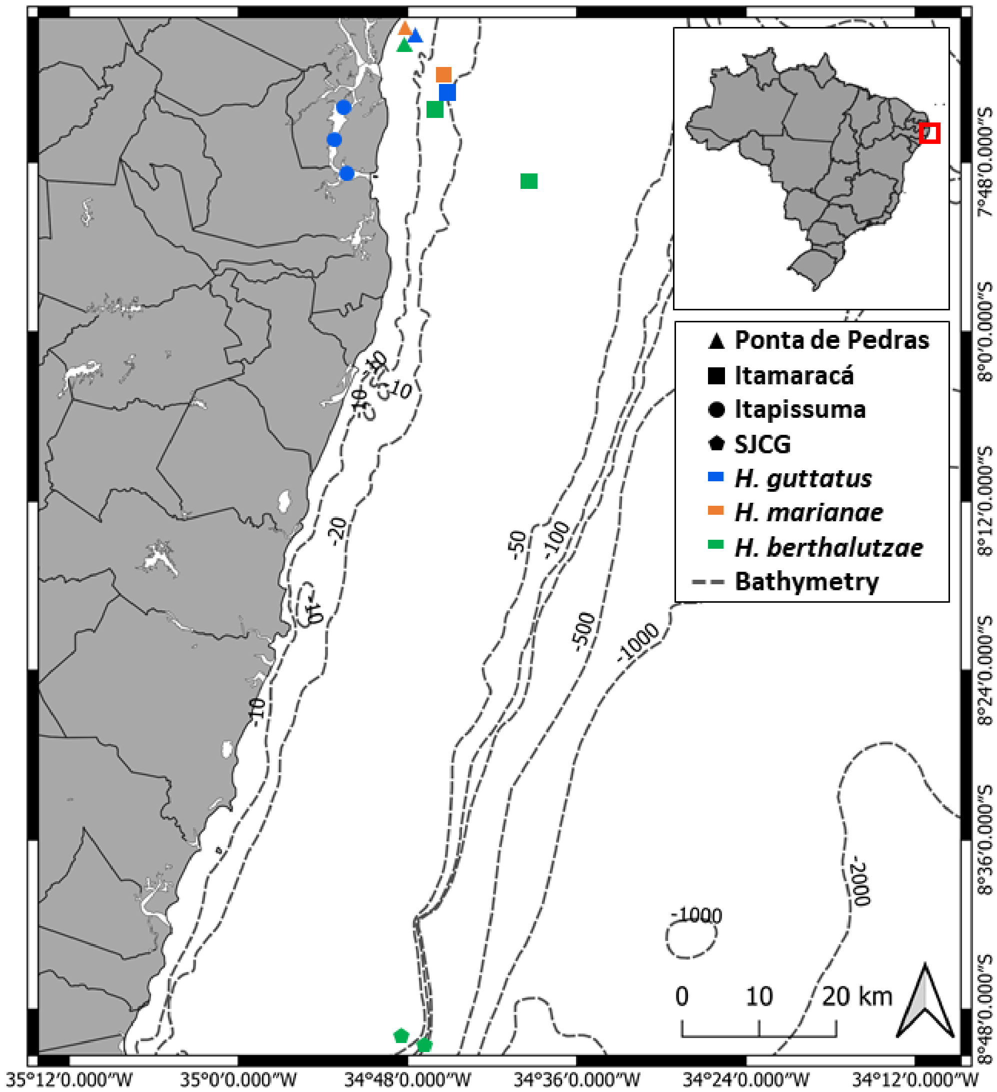

Figure 1. Sampling sites of *Hypanus guttatus*, *H. marianae* and *H. berthalutzae* caught by artisanal fishing in Pernambuco, Brazil. SJCG = São José da Coroa Grande. Symbols represent the sampling points (triangle - Ponta de Pedras; square - Itamaracá; circle - Itapissuma and pentagram - SJCG) and colors represent the species caught (blue - *H. guttatus*; orange - *H. marianae* and green - *H. berthalutzae*).

Samples of muscle and vertebrae of the three studied were obtained opportunistically at artisanal fisheries landing sites: Ponta de Pedras (3 *H. guttatus*; 5 *H. marianae* and 5 *H. berthalutzae*); Itamaracá (1 *H. guttatus*; 6 *H. marianae* and 5 *H. berthalutzae*); Itapissuma (10 *H. guttatus*) and São José da Coroa Grande (2 *H. berthalutzae*) (Table 1). All stingrays were identified at the species level and had disc width (DW, cm), maturity (identified from ICES, 2013 and Melo, 2016) and sex recorded. To provide baselines for analysis of stable isotope data, we obtained samples of bivalve mollusks removed from the stomachs of *H. guttatus* caught in the mangrove environment, polychaetes from the stomachs of *H. marianae* caught in reefs, and *Octopus vulgaris* (Cuvier, 1797) from the stomachs of *H. berthalutzae* caught in the continental shelf.

Since all samples were collected from dead specimens landed and commercialized in local markets, no ethics committee approval was needed. Nevertheless, sampling was carried out in accordance with Brazilian environmental laws under license nº 49663-1 (SISBIO).

Muscle tissue processing

From the 37 specimens collected, 3 samples of young of the year (YOY) *H. marianae* were excluded from isotopic analysis due to uncertainties associated with maternal contribution. (Garzón-Peña et al., 2020; Rangel et al., 2020). White muscle tissue samples weighing 5 g were obtained from each ray and cut into 0.5 to 1 cm² cubes in the laboratory.

These were then transferred to test tubes and underwent lipid extraction by agitating samples in 10 ml petroleum in an ultrasonic cleanser (Ultracleaner 700 - Unique) for 15 minutes following the methods described by Kim and Koch (2012). The petroleum ether solution was discarded following agitation, and the procedure repeated another time. Following the lipid removal from samples, 10 ml of deionized water were added to each sample to extract urea; the test tube was agitated for 15 minutes, after which the deionized water was discarded. This process of water washing for urea removal of ray muscle tissue was repeated twice. For prey items, only lipid extraction was performed following the above steps.

Once samples were clean of lipids and urea, both ray and prey samples were lyophilized, macerated, and analyzed for stable isotopes using a Delta V Isotope Ratio Mass Spectrometer, Advantage, and the ISODAT software. Values (δ) were calculated from carbon and nitrogen reference standards (Pee Dee Belemnite and air, respectively), according to the equation:

$\delta_{sample} = \left(\left(\frac{R_{sample}}{R_{standard}}\right) - 1 * 1000\right)$ (Peterson and Fry, 1987), where R represents the ratio between the heavier and the lighter isotopes of each element (^{13}C:^{12}C; ^{15}N:^{14}N).

Vertebrae processing

Post-cephalic vertebrae blocks were obtained from the posterior synarchual and fixated in a 4% formaldehyde solution for 24 hours, followed by 70% ethanol for another 24 hours. Despite this chemical treatment, there is no evidence that it affects the concentrations of the trace elements used in this study except for Mg, which tends to decrease slightly after formaldehyde treatment (Mohan et al., 2017). Nevertheless, we consider this potential effect negligible since we employed the same treatment to all samples.

We took two vertebrae from each block, removed their connective tissue, and air-dried them for 48 hours. For further processing, we embedded the vertebrae in polyester resin and air-dried for 48 hours at room temperature. Then, the vertebrae were transversely sectioned using an Isomet™ (Buehler) low speed diamond saw. Once sectioned, the distance of each growth band to the vertebra focus was measured to obtain the exact position of element concentrations determined by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). After that, the profile of each analyzed element was established by year of life. We considered pairs of translucent and opaque to be formed yearly for all species (Gianeti et al., 2019).

However, because specimens were of different ages and information on habitat use by life stage is more significant than on an annual scale, we analyzed data by life stages divided according to the age and data on growth or size at maturity available for each species. For *H. guttatus*, we used age and growth data (Gianeti et al., 2019), considering the neonatal portion of vertebrae to be from the birthmark until the end of the first year of life, followed by the juvenile phase between the beginning of the second year of life and the fourth pair of rings for males, and the sixth pair of rings for females. Readings in adults began in the fifth year until the end of vertebrae for males, and from the seventh ring until the end of vertebrae for females.

Since there are no age and growth studies for *H. berthalutzae* nor *H. marianae*, data on size at first maturity for both species (Nunes et al., 2019; Silva et al., 2007) were compared to vertebrae readings of individuals in the same size classes and used to estimate age at maturity. As such, for *H. marianae*, we considered that the neonatal portion of vertebrae went from the birthmark up until the first year of life, followed by the juvenile stage during the second year of life, and the adult stage from the third year until the end of vertebrae for both males and females.

As for *H. berthalutzae*, the neonatal stage in vertebrae went from the birthmark until the end of the first year of life, followed by the juvenile stage between the beginning of the second year until the fourth pair of rings for males and the sixth pair of rings for females. Readings in adults began in the fifth year until the end of vertebrae for males and from the seventh ring until the end of vertebrae for females. It is important to highlight that these are probable maturity data used due to the lack of age and growth data for *H. marianae* and *H. berthalutzae* and should not be used as growth parameters.

Prior to the LA-ICP-MS analyses, vertebrae sections were polished using a silicon carbide sandpaper (nº 8000), washed in ultrasound with 30% hydrogen peroxide for 10 minutes, rinsed and washed in ultrasound again with ultrapure water for 40min (Milli-Q, Millipore). Clean samples were dried at room temperature inside sterile plastic vials for 48 hours and stored individually in hermetic plastic bags until laser ablation. Laser ablation was performed in transects along the corpus calcareum of each vertebra.

We used an Nd:YAG laser ablation system with a 213 nm switched Q-pulse with a quadruple frequency (LSX-266 Teledyne CETAC Technologies, EUA) coupled to a inductively coupled plasma mass spectrometer ELAN DRC II (PerkinElmer Sciex, EUA) (LA-ICP-MS). The laser was operated according to the settings used by (Feitosa et al., 2020), with a 20 Hz pulse frequency, a scanning rate of 30 µm s-1, a 50 µm point diameter, and an energy output of 0.2 to 0.3 mJ per pulse. The ablated material was conducted by a Teflon-lined tube to the ICP-MS using Argon as a carrier gas (1.20 L min-1). The plasma was operated with power ratings of 1,300 W, with an external and intermediate gas brightness of 15.0 and 1.1 L min-1, respectively. The measurement of each element was performed using calcium as an internal element following the pattern element:Ca. The certified reference material NIST 612 was used to obtain signal intensity values (counts per second - CPS) for each element. Limit of Detection (LOD) values were calculated according to (Longerich et al., 1996). All analytical measures were performed at the Atomic Spectrometry Laboratory (Universidade Federal de Santa Maria - UFSM, Brazil) and at the Research Institute for the Development of Marine Biodiversity, Exploitation and Conservation (MARBEC/IRD - Université de Montpellier, France).

Data analyses

Stable isotope data

Considering that an organism's isotopic values reflect both food source and assimilation and can be used as a biological marker, we used the $\delta^{13}C$ and $\delta^{15}N$ values (mean and standard deviation) of the three batoid species to estimate the proportional dietary contribution of marine and estuarine sources using the Bayesian stable isotopic mixing model SIMMR in R (Stable Isotope Mixing Models in R with simmr, Parnell & Inger, 2021; R CoreTeam, 2020). Average mean values of $\delta^{13}C$ and $\delta^{15}N$ (±SD) for prey species (Bivalve, Polychaeta, and *O. vulgaris*) were included to represent marine and estuarine sources. We adjusted source isotope values to account for fractionation of 1.5‰ to $\delta^{15}N$ and 1.3‰ to $\delta^{13}C$ values (Galván et al., 2016; Post, 2002).

Vertebrae microchemistry data

Samples were analyzed in a multi-element LA-ICP-MS for ^{24}Mg, ^{43}Ca, ^{55}Mn, ^{86}Sr, ^{138}Ba. Given that Ca is the baseline element in elasmobranch vertebrae, we calculated element:Ca ratios for each element in counts per second (CPS), and later transformed them to concentrations (ppm) as per Longerich et al., (1996). We assumed a 35% Ca concentration to convert element readings from CPS to ppm, which were then scaled by a square root transformation. In subsequent analyses, we only used elements which remained above the limit of detection (LOD) and that have been validated as proxies for environmental properties: such as Sr and Ba for salinity, Mg and Mn for temperature variability or hypoxic zones (Limburg et al., 2015; Limburg and Casini, 2018; McMillan et al., 2017; Smith et al., 2013).

Since our data did not follow normal distribution for any element:Ca ratio, we calculated median values per sample for each life stage for subsequent statistical analyses. With the median values we performed single and multi-element permutational ANOVAs (PERMANOVA) based on a similarity matrix calculated using Euclidean distances and 1000 permutations to investigate differences between life stages, sexes, and species (fixed factors). In addition, we also performed the single and multi-element PERMANOVAs within each species to test differences in habitat use between life stages and sexes. Finally, we performed a non-metric multidimensional scale (NMDS) analysis with Euclidean distances to visualize potential clusters. All PERMANOVAs were performed using the PRIMER6 software, while exploratory data analysis and graphs, as well as the NMDS analysis using the vegan package (Oksanen et al., 2019) were done in the R software version 4.0.3 (R Core Team, 2020).

RESULTS

We sampled a total of 37 specimens, of which 14 were *H. guttatus* (DW varied from 42.1 to 53.5 cm for males, and from 32.6 to 86.2 cm for females), 11 *H. marianae* (DW varied from 15.1 to 27.8 cm for males and 15.9 to 30.2 cm for females), and 12 *H. berthalutzae* (DW varied from 43 to 88 cm for males and 35.4 to 135 cm for females) (Table 1).

Table 1. Total number of samples collected, number of individuals with stomach content data, sex ratio and disk width of *Hypanus guttatus*, *H. marianae* and *H. berthalutzae*.

Hypanus guttatus	**N**	**Sex**		**Disk Width (cm)**			
		♀	**♂**	**min**	**max**	**mean**	**±SD**
Ponta de Pedras	3	2	1	42.1	45	44	1.32
Itamaracá	1	1	-	-	-	75.3	-
Itapissuma	10	6	4	23.6	86.2	51.9	15.03
SJCG	-	-	-	-	-	-	-
Total	14	9	5	32.6	86.2	51.86	14.64
Hypanus marianae	**N**	**Sex (%)**		**Disk Width (cm)**			
		♀	**♂**	**min**	**max**	**mean**	**±SD**
Ponta de Pedras	5	4	1	23.2	30.2	26.9	2.3
Itamaracá	6	3	3	15.1	29.5	21.48	5.96
Itapissuma	-	-	-	-	-	-	-
SJCG	-	-	-	-	-	-	-
Total	11	7	4	15.1	30.2	23.95	5.39
Hypanus berthalutzae	**N**	**Sex (%)**		**Disk Width (cm)**			
		♀	**♂**	**min**	**max**	**mean**	**±SD**
Ponta de Pedras	5	2	3	35.4	53.5	43.78	5.75
Itamaracá	5	2	3	79	135	99	20.95
Itapissuma	-	-	-	-	-	-	-
SJCG	2	2	-	83.9	86	84.95	1.05
Total	12	6	6	35.4	135	73.65	29.28

Stable isotopes

The 34 muscle tissue samples from rays and prey presented varying isotopic compositions between sampled environments. Overall, samples collected in the estuarine environment exhibited more negative $\delta^{13}C$ values when compared to samples from reefs and the continental shelf. As for $\delta^{15}N$ values, these were higher among samples collected in the estuary and reefs when compared to samples collected in the continental shelf (Table 2).

A mature male and an immature female of *H. guttatus* caught in coastal reefs presented similar carbon and nitrogen values to those of the estuarine fauna ($\delta^{13}C$ = -17.92; $\delta^{15}N$ = 13.23

and δ^{13}C = -15.69; δ^{15}N = 14.79, respectively). Similarly, a mature and an immature male of *H. guttatus* caught in the estuary exhibited carbon and nitrogen values within the range observed for the reef fauna (δ^{13}C = -14.59; δ^{15}N = 8.38 and δ^{13}C = -15.02; δ^{15}N = 14.82, respectively; Figure 2). Additionally, figure 2 clearly shows the importance of mangrove food sources to *H. guttatus* stable isotope signature, while the coastal reefs and continental shelf habitats have a more intertwined representation for *H. berthalutzae* and *H. marianae*.

Table 2. Isotopic composition of δ^{13}C (‰) and δ^{15}N (‰) for *Hypanus guttatus*, *H. marianae*, *H. berthalutzae* and organisms collected in the mangrove, reef, and continental shelf habitats.

	N	δ^{13}C				δ^{15}N			
		max	**min**	**mean**	**±SD**	**max**	**min**	**mean**	**±SD**
Mangrove									
Bivalve	3	-17.27	-20.19	-18.49	1.52	10.45	9.51	10.03	0.48
Hypanus guttatus	10	-14.59	-18.46	-16.55	1.22	14.82	8.38	12.01	1.82
Reefs									
Polychaeta	2	-13.50	-14.53	-14.02	0.73	10.59	10.33	10.46	0.18
Hypanus guttatus	3	-14.82	-17.97	-16.14	1.31	14.79	12.42	13.48	0.98
Hypanus marianae	5	-13.39	-14.72	-14.11	0.56	12.52	9.73	11.65	0.93
Hypanus berthalutzae	5	-13.41	-15.16	-14.08	0.64	12.77	9.25	11.3	1.26
Continental shelf									
Octopus vulgaris	3	-15.02	-17.27	-16.22	1.14	7.10	7.01	7.04	0.05
Hypanus guttatus	1	-	-	-14.49	-	-	-	10.73	-
Hypanus marianae	3	-13.76	-14.29	-13.99	0.22	12.04	10.14	11.1	0.77
Hypanus berthalutzae	7	-13.6	-15.68	-14.57	0.69	12.41	8.57	9.98	1.42

N, number; Min, minimum; Max, maximum; ±SD, standard deviation.

A mature male and an immature female of *H. guttatus* caught in coastal reefs presented similar carbon and nitrogen values to those of the estuarine fauna (δ^{13}C = -17.92; δ^{15}N = 13.23
and δ^{13}C = -15.69; δ^{15}N = 14.79, respectively). Similarly, a mature and an immature male of *H. guttatus* caught in the estuary exhibited carbon and nitrogen values within the range observed for the reef fauna (δ^{13}C = -14.59; δ^{15}N = 8.38 and δ^{13}C = -15.02; δ^{15}N = 14.82, respectively; Figure 2). Additionally, figure 2 clearly shows the importance of mangrove food sources to *H. guttatus* stable isotope signature, while the coastal reefs and continental shelf habitats have a more intertwined representation for *H. berthalutzae* and *H. marianae*.

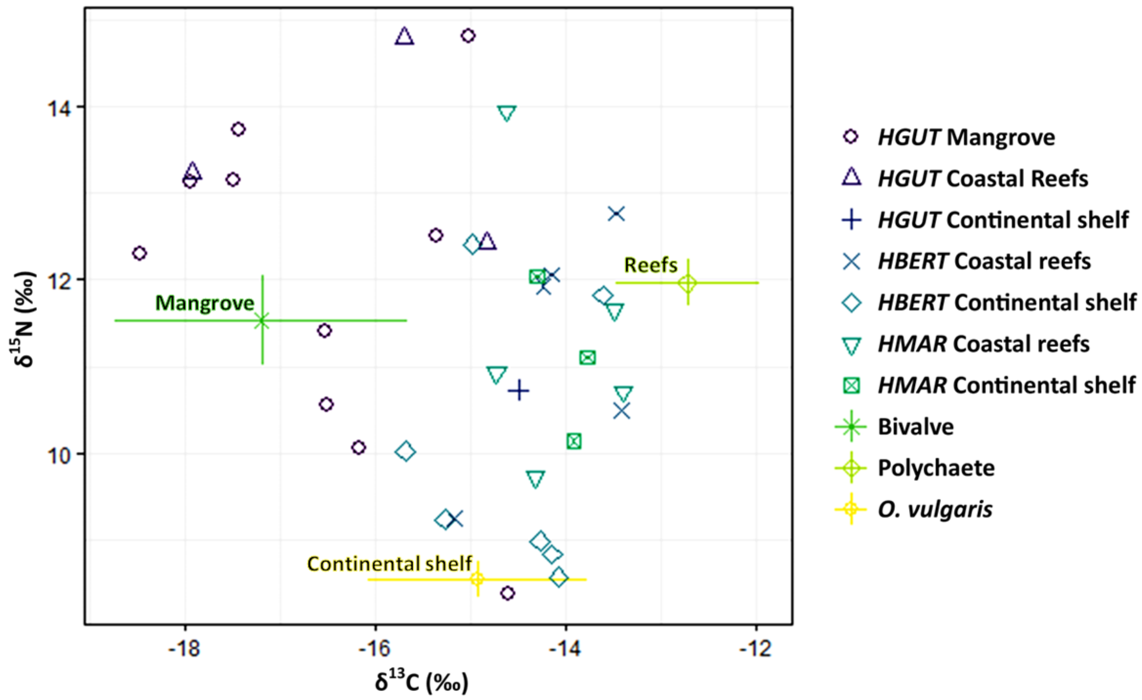

Figure 2. Biplot of $\delta^{13}C$ and $\delta^{15}N$ prey values applied in the Bayesian isotopic mixture model (SIMM) including individual stable isotope values for the three stingrays sampled in distinct habitats: HGUT = *Hypanus guttatus*; HMAR = *H. marianae*; HBERT = *H. berthalutzae*.

This is further shown in table 3, with bivalve mollusks representing the highest percentages of isotopic contributionfor *H. guttatus* sampled in the estuary, reefs, and the continental shelf. As for the analyzed *H. marianae*, polychaetes had the highest percentages of isotopic contributions for individuals collected in reefs and the continental shelf. Finally, *H. berthalutzae* individuals sampled in reefs had polychaetes as their most important prey items, whereas the highest percentages of isotopic contributions were represented by *O. vulgaris* in individuals from the continental shelf (Table 3).

Table 3. Percentage contribution of associated fauna with each studied environment to the isotopic composition of *Hypanus guttatus*, *H. marianae* and *H berthalutzae* in the 97.5% confidence interval.

	Hypanus guttatus			***Hypanus marianae***		***Hypanus berthalutzae***	
	Mangrove	**Reefs**	**Shelf**	**Reefs**	**Shelf**	**Reefs**	**Shelf**
Mangrove							
Bivalve	91.7%	87.6%	67.4%	42.6%	58.4%	43.5%	40.1%
Reefs							
Polychaete	32.4%	77.3%	63.5%	78.4%	79.2%	78.4%	57.5%
Continental shelf							
Octopus vulgaris	32.5%	72.1%	42.3%	57.3%	59.7%	56.2%	78.8%

Vertebrae microchemistry

All elements exhibited concentrations above the LOD for all samples. After calculating element:Ca ratios, Mg:Ca was the most abundant ratio followed by Sr, Mn, and Ba, respectively (Figure 3). Element:Ca ratios varied according to species, life stage and sex, with Mg:Ca exhibiting the widest range (Supporting information).

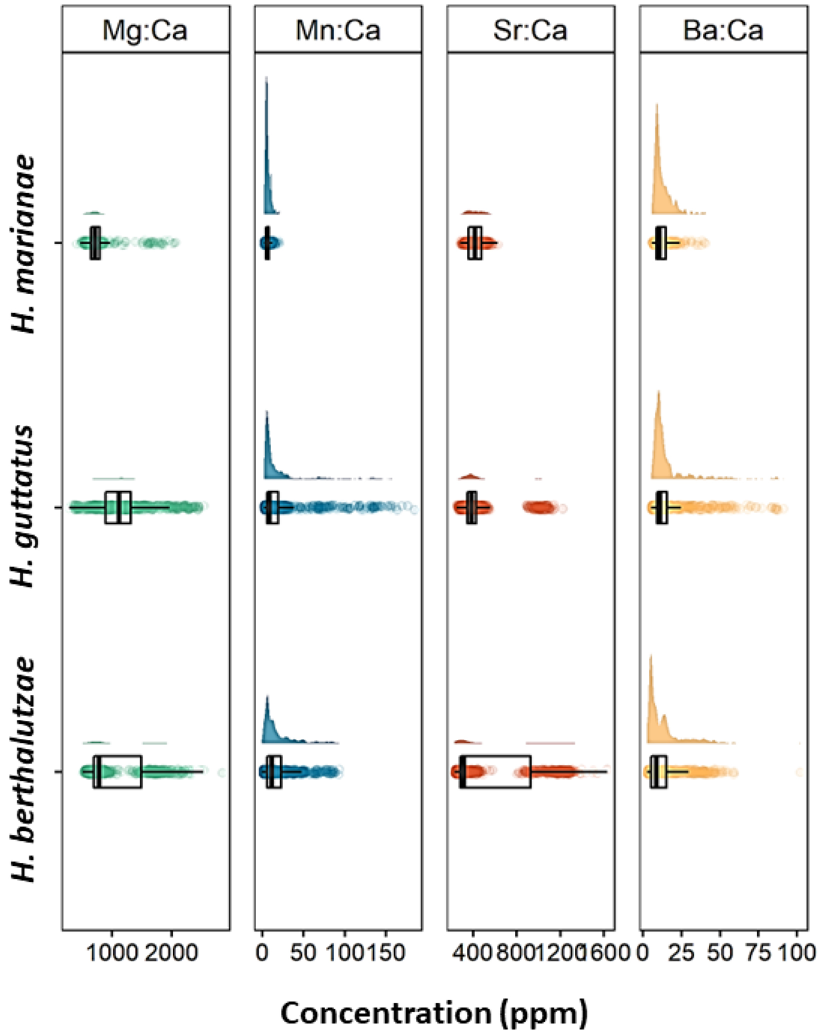

Figure 3. Range and distribution of element:Ca ratios for Mg, Mn, Sr, and Ba for *Hypanus guttatus*, *H. marianae* and *H. berthalutzae*.

Our data suggest that larger females in each location presented habitat use overlap in specific life stages (Figure 4). For example, the Mn:Ca pattern was somewhate similar for all species in the neonate phase. Given that *H. marianae* individuals are considerably smaller than the other two species studied, we have less data points for this species. Nevertheless, it is clear that the largest females inhabited similar areas with low concentrations of Mn, which suggest areas with little presence of mangroves. Further in life, *H. guttatus* shows a strong signal of moving into mangrove habitats, while *H. berthalutzae* stabilizes in a transition habitat, consistent with the continental shelf. This pattern is reinforced by the Sr.Ca and Ba:Ca reads. Sr:Ca exhibited overlaps between the initial stages of *H. guttatus* and *H. marianae*, with a subsequent increase in concentrations for *H. marianae* and a decrease for *H. guttatus*. *H. berthalutzae* exhibited initially low concentrations (around 100 ppm) followed by an increase and an overlap with H. guttatus between 1-2.5 mm, with a subsequent decrease (Figure 5). Ba:Ca initial concentrations were slightly lower, reaching the concentrations of *H. guttatus* and *H. marianae* between 1-2 mm of the vertebra radius, dropping again after that (Figure 5). Regarding Mg:Ca, the three species overlapped during the initial stages, with a constant increase in concentrations for *H. guttatus* starting at 1 mm of the vertebra radius.

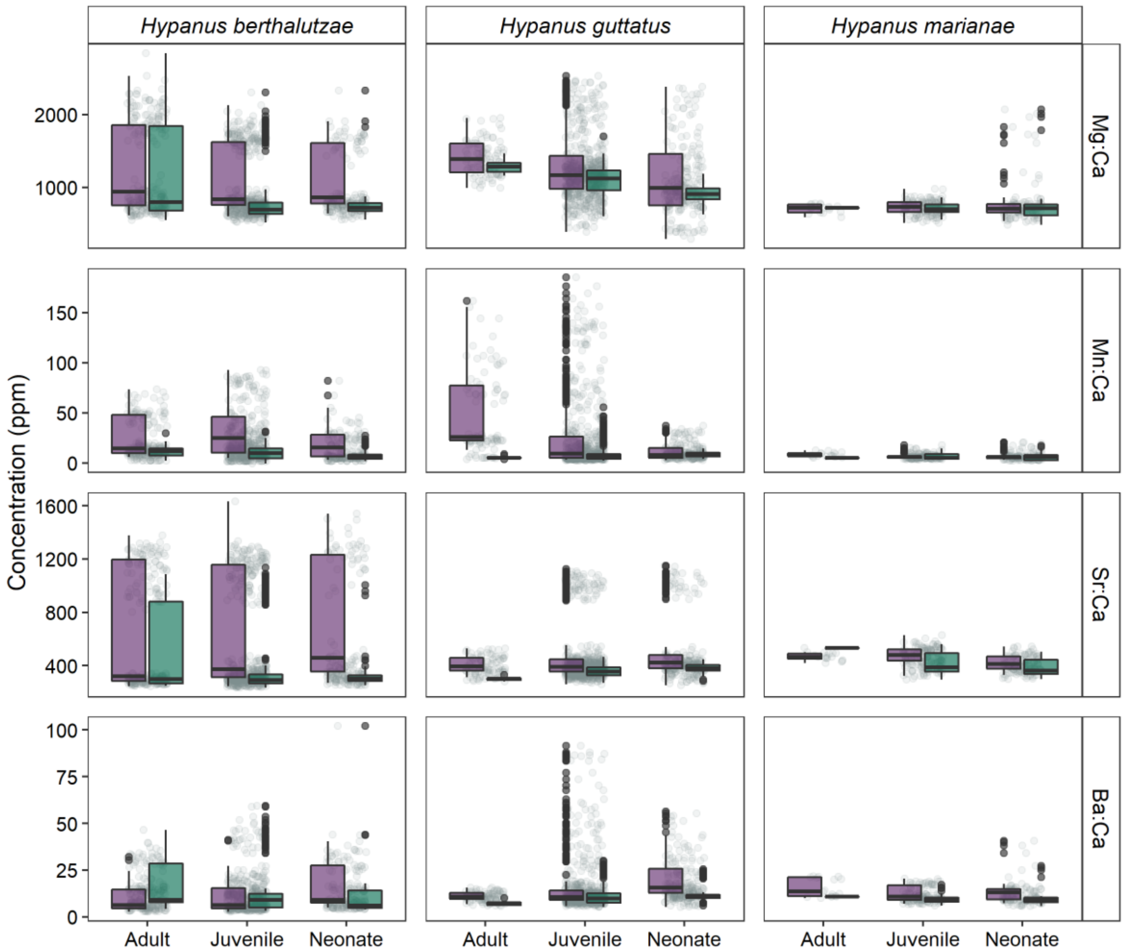

Figure 4. Range and distribution of element:Ca ratios for Mg, Mn, Sr, and Ba per life stage for each species and sex. Males are shown in purple and females in green, while jittered points represent the ratios from transect points for all samples.

When evaluated together, multi-element PERMANOVAs yielded statistically significant results for between sexes and species. As for single-element PERMANOVAs, results were significant only for species as a fixed factor for Mg:Ca and Mn:Ca concentrations, and between sexes as a fixed factor for Mn:Ca and Sr:Ca (Table 4).

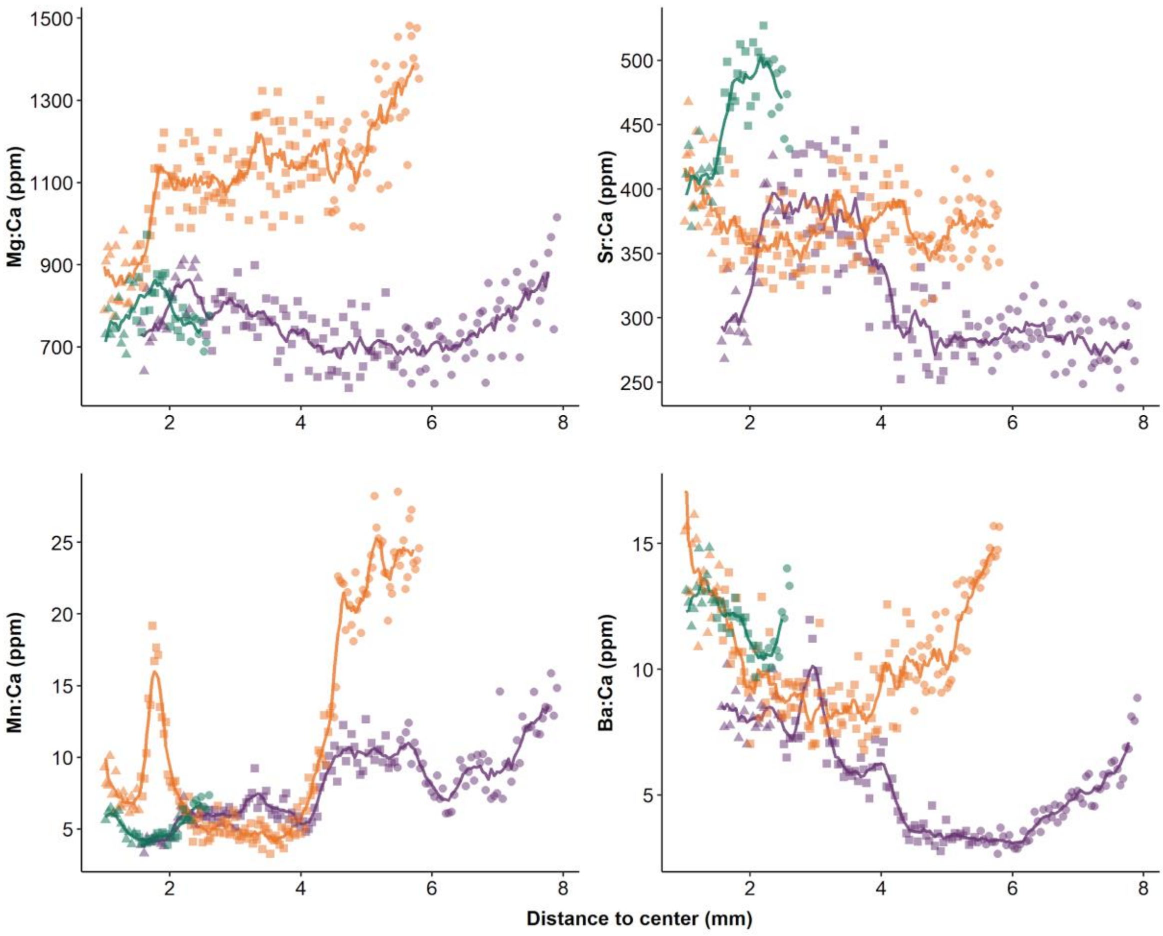

Figure 5. Lifetime Mg:Ca, Sr:Ca, Mn:Ca and Ba:Ca concentrations in ppm for the oldest female from each species with seven point rolling average (solid lines). Purple = *H. berthalutzae*; orange = *H. guttatus*, and green = *H. marianae*. Life stages are shown in point shapes, with triangles representing the neonate phase, squares representing the juvenile phase, and circles representing the adult phase of each individual.

When PERMANOVAs were carried out for each species separately, significant results were obtained only between sexes for Sr:Ca in *H. guttatus* and for Mg:Ca, Mn:Ca, Sr:Ca, and multi-elements for *H. berthalutzae* (Table 5). We did not find any significant differences for H. marianae. These data are also in accordance with the line graphs plotted for all samples by element:Ca (Supporting information). These graphs reveal little variation for all elements between species. Once we obtained significant values for the multi-element PERMANOVAs using species as a fixed factor, we decided to carry out a pairwise PERMANOVA to investigate which species differed from each other and at what level. Only *H. guttatus* vs. *H. berthalutzae* did not yield significant results, while *H. guttatus* vs. *H. marianae* and *H. berthalutzae* vs. *H. marianae* produced statistically significant results, with average distances of 9.2 and 10.38, respectively (Table 6).

Table 4. Multi and single-element PERMANOVA with all species gathered and sexes, life stages and species as fixed factors. df = degrees of freedom, SS = sums of squares, MS = mean squares, p = statistical significance of hypothesis tests, Var = estimated sizes of components of variation, which are analogous to ANOVA effect sizes, SD = square root of these values in Euclidian units.

Element	Factor	df	SS	MS	Pseudo-F	*p*	Var	SD
Multi	Sex	1	471.32	471.32	6.07	**0.006**	9.41	3.06
	Life stage	2	35.65	17.82	0.21	0.91	-2.43	-1.56
	Species	2	492.41	246.21	3.14	**0.03**	5.94	2.43
Mg:Ca	Sex	1	128.4	128.4	2.96	0.08	2.03	1.45
	Life stage	2	19.11	9.55	0.21	0.8	-1.31	-1.14
	Species	2	364.8	182.4	4.45	**0.01**	5.01	2.23
Mn:Ca	Sex	1	25.32	25.32	9.15	**0.003**	0.53	0.73
	Life stage	2	12.77	6.38	2.16	0.11	0.12	0.35
	Species	2	22.04	11.02	3.88	**0.02**	0.28	0.53
Sr:Ca	Sex	1	311.97	311.97	10.37	**0.005**	6.73	2.59
	Life stage	2	1.53	0.76	2.25	0.98	-1.23	-1.11
	Species	2	104.23	52.11	1.58	0.21	0.67	0.82
Ba:Ca	Sex	1	5.61	5.61	3.89	0.06	9.97	0.31
	Life stage	2	2.22	1.11	0.73	0.48	-1.44	-0.12
	Species	2	1.34	0.67	0.44	0.61	-2.97	-0.17

We also performed an NMDS analysis to evaluate how samples of the three species formed clusters (Figure 6). Our NMDS analysis yielded a stress of 0.03, which is considered a reliable ordination (Clarke, 1993). Figure 5 shows the similarities in multi-elements ratios between *H. guttatus* and *H. berthalutzae* when compared with *H. marianae*. Since the PERMANOVAs for each element showed significant differences between species for Mg:Ca and Mn:Ca, and non-significant differences for Sr:Ca and Ba:Ca, the NDMS provides a clear picture of this partial overlap in PERMANOVA results between species.

Table 5. Multi and single-element PERMANOVAs per species with sex and life stages as fixed factors. df = degrees of freedom, SS = sums of squares, MS = mean squares, p = statistical significance of hypothesis tests, Var = estimated sizes of components of variation, which are analogous to ANOVA effect sizes, SD = square root of these values in Euclidean units.

Element	Factor	df	SS	MS	Pseudo-F	*p*	Var	SD
				Hypanus guttatus				
Multi	Sex	1	115.53	115.53	1.81	0.15	3.65	1.91
	Life stage	2	76.58	38.29	0.56	0.6	-3.21	-1.79
Mg:Ca	Sex	1	39.22	39.22	0.93	0.31	-0.18	-0.42
	Life stage	2	50.35	25.17	0.58	0.56	-1.96	-1.41
Mn:Ca	Sex	1	5.06	5.06	1.58	0.25	0.13	0.36
	Life stage	2	12.45	6.22	2.04	0.14	0.35	0.59
Sr:Ca	Sex	1	67.17	67.17	3.88	**0.04**	3.51	1.87
	Life stage	2	12.03	6.01	0.3	0.81	-1.53	-1.23
Ba:Ca	Sex	1	4.06	4.06	2.94	0.09	0.18	0.43
	Life stage	2	1.74	0.87	0.57	0.56	-7.12	-0.26
				Hypanus marianae				
Multi	Sex	1	5.01	5.01	0.15	0.79	-2.61	-1.61
	Life stage	2	22.53	11.26	0.34	0.74	-3.14	-1.77
Mg:Ca	Sex	1	1.01	1.01	3.61	0.84	-2.66	-1.63
	Life stage	2	10.56	5.28	0.18	0.86	-3.56	-1.88
Mn:Ca	Sex	1	0.11	0.11	0.43	0.52	-1.41	-0.11
	Life stage	2	0.28	0.14	0.56	0.55	-1.66	-0.12
Sr:Ca	Sex	1	2.71	2.71	1.11	0.29	2.53	0.15
	Life stage	2	11.28	5.64	2.64	0.08	0.52	0.72
Ba:Ca	Sex	1	1.17	1.17	1.64	0.23	4.53	0.21
	Life stage	2	0.39	0.19	0.24	0.8	-8.89	-0.29
				Hypanus berthalutzae				
Multi	Sex	1	700.66	700.66	6.79	**0.01**	36.24	6.02
	Life stage	2	30.76	15.38	0.11	0.92	-10.4	-3.22
Mg:Ca	Sex	1	185.13	185.13	4.09	**0.04**	8.49	2.91
	Life stage	2	15.89	7.94	0.15	0.87	-4.06	-2.01
Mn:Ca	Sex	1	41.56	41.56	14.11	**0.002**	2.34	1.53
	Life stage	2	5.13	2.56	0.6	0.56	-0.15	-0.39
Sr:Ca	Sex	1	473.22	473.22	8.94	**0.009**	25.49	5.04
	Life stage	2	7.97	3.98	5.67	0.93	-6.06	-2.46
Ba:Ca	Sex	1	0.74	0.74	0.35	0.55	-8.25	-0.28
	Life stage	2	1.77	0.88	0.41	0.66	-0.11	-0.33

DISCUSSION

Based on our stable isotopes and vertebrae microchemistry analyses, the three *Hypanus* species exhibited different movement patterns across environments. Overall, we identified *H. guttatus* as the only species capable of moving between marine and estuarine areas, whereas *H.*

marianae was restricted to reef areas, and *H. berthalutzae* shows a pattern of moving further to deeper waters as it grows (Aguiar et al 2009). Therefore, *H. marianae* seems to have the most specialized habitat use pattern between these three species (Tables 2 and 5). Nevertheless, all species show some overlap in habitat use during their ontogenetic development as shown by the NMDS analysis (Figure 6). However, we observed a wider variance in element:Ca concentrations between *H. marianae* and the other two species, which further indicates the use of less heterogeneous habitats and is also corroborated by our stable isotope analysis results. These are further reinforced by the largest female lifetime patterns shown in figure 5. Together, these evidences suggest that these species likely share common areas consisting of shallow coastal waters and their niche likely widens during their growth.

Table 6. Pairwise PERMANOVA between locations. df = degrees of freedom, SS = sums of squares, p = statistical significance of hypothesis tests. The distance column shows the results of the average distances between species.

Species pairs	df	SS	F	R^2	*p*	*p*-adjusted
H. berthalutzae* vs *H. guttatus	1	5.0335	1.0498	0.0166	0.3396	0.3396
H. berthalutzae* vs *H. marianae	1	13.946	3.6159	0.0639	0.0369	0.0739
H. guttatus* vs *H. marianae	1	10.815	3.9496	0.0719	0.0129	0.0389

On a species-specific basis, we also show that *H. berthalutzae* is the only one that clearly displays sex seggregation. This pattern has also been demonstrated for the same region, with females being much more abundant on coastal areas than males (Branco-Nunes et al 2021). Furthermore, the lack of differences between life stages suggest that this sexual segregation behavior occurs throughout ontogeny and that sexes may co-occur solely during the mating season. Branco-Nunes et al (2021) found a 16:1 sex ratio towards females in a shallow coastal area off Pernambuco state, which thus suggests that males are likely to occupy deeper and farther areas from the coast. This is a well known pattern of ontogenetic habitat use for coastal elasmobranchs, but seems to be more common for sharks than batoids (Knip et al 2010).

Similarly, *H. guttatus* also seems to use a wide variety of habitats. In fact, it is probably the most generalistic of the *Hypanus* species studied, given that it is the only euryhaline one and was found in all three habitats we analyzed (Grant et al 2021; Queiroz et al., 2022). Nevertheless, it still seems to demonstrate a preference for coastal areas with some degree of riverine influence. Feitosa et al (2021) showed that *H. guttatus* also seems to display a high degree of plasticity along its distribution range, being the dominant stingray in both estuarine and marine areas of North and Northeast Brazil. Our study reinforces the strong role that *H. guttatus* plays on the energy flow between estuarine and marine areas in the Southwestern Atlantic Ocean, which corroborates the findings of Queiroz et al (2022).

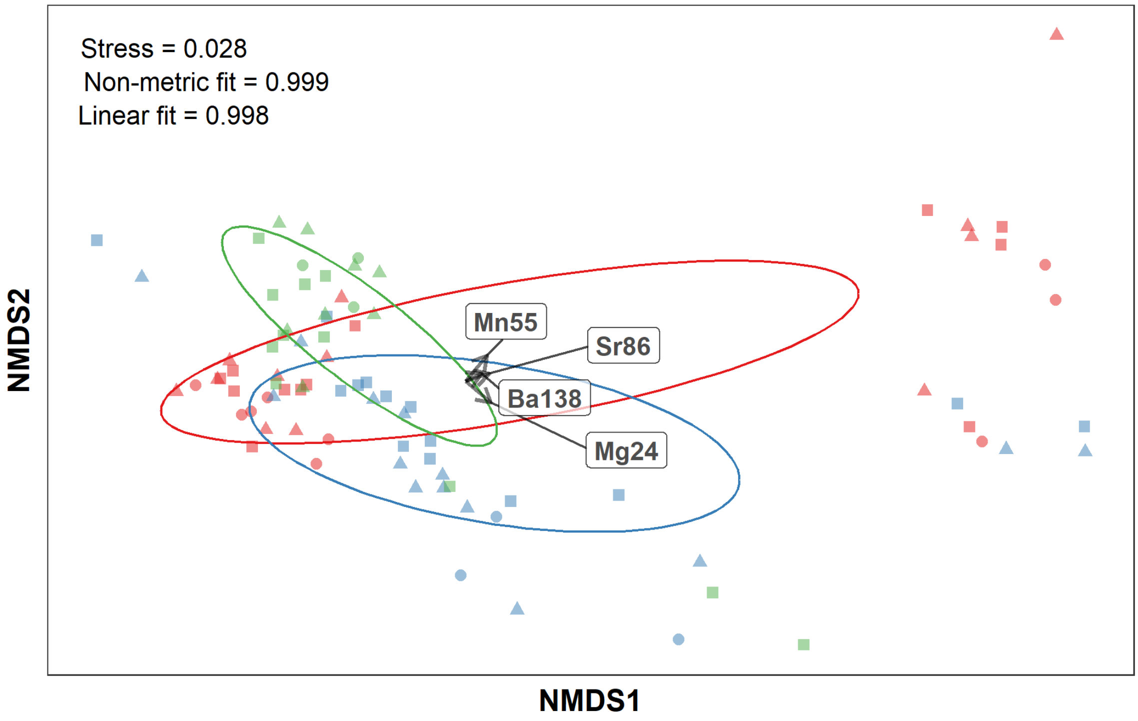

Figure 6. Multi-element non-metric multidimensional scaling (NMDS) plot with species and life stages as factors. Blue points represent *H. guttatus*, green points represent *H. marianae*, and red points represent *H. berthalutzae*. The adult stage is depicted by circles, while the juvenile stage is depicted by triangles, and the neonate stage is depicted by squares.

In contrast, *H. marianae* seems to have the most restricted habitat use pattern among the studied species. It is the only species that has a strong difference between the *Hypanus* studied here and the smallest variance for all elements (Table 5; Figure 3). Other studies also point to this conclusion using different sorts of data, including genetic haplotypes (Costa et al 2022), distribution modeling (Costa et al 2017), rectal gland morphology (Melo et al 2021), diet and isotopic niche (Queiroz et al 2019; 2022). The lack of differences between sexes also corroborate the lack of sexual segregation found for northern areas of its distribution (Costa et al 2015). Therefore, we argue that *H. marianae* has the most specialized habitat use of these three stingray species.

Finally, by interpreting the data we present in this study on a broader level, we argue that these habitats use patterns we describe also help to explain their geographical distribution ranges. *H. guttatus* is the only non-endemic species, while both *H. marianae* and *H. berthalutzae* have large estuarine areas as geographic barriers to their distribution (São Francisco and Parnaíba rivers, and Amazon and La Plata rivers, respectively) (Petean et al 2020, Costa et al 2017). This suggests that *H. guttatus* capacity to explore estuarine habitats and to feed on a diverse range of prey items enabled it to occupy a much larger area than any other coastal stingray in the Southwestern Atlantic Ocean.

While this potentially means that *H. guttatus* is less likely to become an endangered species than *H. marianae* and *H. berthalutzae,* its distribution also overlaps with areas of intense coastal fishing and where demand for its meat is increasing (Rodrigues-Filho et al 2020). In addition, by using both estuarine and marine habitats, it is also more exposed to a wide array of coastal fisheries, encompassing both small to industrial scales (Feitosa et al 2021). Similarly, *H. berthalutzae* may be significantly impacted by these coastal fisheries, with documented consumption in both Northern and Northeastern Brazil (Martins et al 2021, Queiroz et al. 2022). Furthermore, fisheries operating in Brazilian coral reefs are likely to pose a major threat to *H. marianae* and bycaught specimens should be released alive and unharmed as much as possible. Therefore, we highlight estuarine and shallow coastal areas as key habitat for *H. guttatus*, while deeper coastal areas (> 50 m) of Brazil as key for *H. berthalutzae* and the Brazilian coral reefs as essential habitat for *H. marianae*.

Stable isotopes

The analyzed prey (bivalve mollusks, polychaetes and *O. vulgaris*) provided a good representation of the isotopic signature of the sampled areas. We expected more negative $\delta^{13}C$ values in estuarine samples compared with reef environments and the continental shelf, since trophic networks in estuarine environments may be based on terrestrial carbon sources, which tend to be more negative than marine carbon sources (Fry, 2006). Nitrogen also provided a good representation of coastal influence in the environmental gradient sampled, with higher $\delta^{15}N$ values being found among estuarine samples, and lower values in continental shelf samples.

Bivalve mollusks showed the highest percentages of isotopic contribution for *H. guttatus*, even for specimens caught outside the estuary. This finding may indicate *H. guttatus* movements between the estuary and the sea, since individuals that recently came from the estuary could carry the estuarine isotopic signature for months in their muscle tissue due to the low turnover rates (Galván et al., 2016). The two *H. guttatus* males caught in the coastal reefs, whose carbon and nitrogen values fell within the range observed for the estuarine fauna provide evidence that this movement also occurs in the opposite direction.

The scenario that the organic matter carried out to sea by the estuary influenced the isotopic compositions of the two *H. guttatus* caught at sea is unlikely for two reasons. First, river runoff is low even in the rainy season (Medeiros and Kjerfve, 1993). Second, the estuarine isotopic signal was not detected in any other consumer analyzed outside of the estuary, not even in the *H. marianae* and *H. berthalutzae* caught in the same environment and time. Furthermore, the hypothesis of movement between marine and estuarine environments by *H. guttatus* was raised in other studies, generally associated with the species reproductive cycle (Queiroz et al., 2022; Feitosa et al., 2021; Melo et al., 2021; Santos et al., 2016; Thorson, 1983).

Among the three ray species studied, *H. marianae* showed the shortest ranges of $\delta^{13}C$ e $\delta^{15}N$, which reflect a limited dietary niche (Queiroz et al., 2022). For individuals caught in coastal reefs and in the continental shelf, reef polychaetes represented the highest percentages of isotopic contribution. This suggests a potential connectivity between these two environments or the movement of *H. marianae* between them. Indeed, there are records that the species can move between different coastal marine habitats throughout its life cycle, presenting sexual and ontogenetic segregation in a relatively restricted area (Costa et al., 2015).

The *H. berthalutzae* sample caught in coastal reefs comprised young individuals, exhibited reef sources with higher percentages of isotopic contribution, while individuals caught in deeper areas, all adults, had *O. vulgaris* as the highest percentage contribution. These data suggest a behavior of ontogenetic segregation for the species, which is probably born in shallow waters, where it stays in the initial stages, later migrating towards deeper waters along its development. This pattern of ontogenetic movement is widely reported for coastal cartilaginous fish species (Speed et al., 2010).

Vertebrae microchemistry

Overall, our microchemistry results suggest that the three *Hypanus* species analyzed here display significantly different habitat use patterns, with *H. guttatus* and *H. marianae* showing the greatest differences (Table 6). The three *Hypanus* seem to share the same birth area, since element concentrations right after birth were very similar in most samples (Figures 4 and 5). Similarly, *H. berthalutzae* samples caught in São José da Coroa Grande presented similar concentrations between each other, albeit different from samples collected further north

Pernambuco state (Figure 1). Furthermore, the differences in Mn:Ca and Sr:Ca for sexes with all species combined suggest that habitat use differences are likely related to sex seggregation behavior. This has been shown to occur for *H. guttatus* (Thorson, 1983), *H. marianae* (Costa et al., 2015) and *H. berthalutzae* (Branco-Nunes et al., 2021).

Overall, Mg:Ca ratios were the highest, followed by Sr:Ca (Figures 4 and 5). Similar results were obtained by Feitosa et al. (2021) for *H. guttatus*, Feitosa et al. (2020) for *Carcharhinus porosus*, and McMillan et al. (2017) for various elasmobranch species. In addition, Mg:Ca exhibited an increasing or stable trend as individuals got older, and statistically significant differences between the three species, but not for any other fixed factor, within and between species (Tables 4 and 5).

Mg could represent a proxy for environmental temperature, with higher concentrations in vertebrae of elasmobranchs being associated with the occupation of colder waters (Smith et al., 2013). In this study, Mg:Ca concentrations in vertebrae are lower closer to birth for the three *Hypanus* (Figure 4), remaining stable and with little variation in *H. marianae* vertebrae (Figure 5), which could represent the use of shallower and warmer waters by these species throughout their life cycles. Contrarily, *H. berthalutzae* vertebrae showed an increase in Mg:Ca concentrations between the end of the immature stage and the beginning of maturity (Figure 5), which may represent a period of movement into deeper and colder waters. Vertebrae of *H. guttatus* showed a constant increase in Mg:Ca concentrations up until the first year of life (Figure 5). It is important to remember that Mg may represent changes in environmental temperature under constant salinity (Smith et al., 2013), and that this species likely uses estuarine areas starting at age one. As such, these variations should not be associated with temperature changes in an euryhaline stingray.

Similarly, Sr:Ca concentrations in most vertebrae for the three species exhibited similar values right after birth, which then remained constant with little oscillations in vertebrae of *H. guttatus* (Figure 5). The PERMANOVA detected significant differences in Sr:Ca concentrations between male and female (Table 5). *H. guttatus* is known to move between marine and estuarine environments in this region (Feitosa et al., 2021; Melo, 2016; Queiroz et al., 2022) and our stable isotopes analyses detected this behavior in at least four of the specimens caught. However, oscillations in Sr:Ca concentrations were very subtle in the analyzed vertebrae, hampering the identification of periods in which the species used the estuary.

With an opposite pattern, *H. marianae* vertebrae showed an increase in Sr:Ca concentrations after birth (Supporting information Figure 3c-d), which could indicate the use of high salinity areas after birth. This pattern is further reinforced by stable isotope analyses (Table 2). Contrary to what was described for the species by Gomes et al. (2000), there are no records of occurrence for *H. marianae* in estuaries in the region, and our data support this information.

Most of the analyzed *H. berthalutzae* samples showed a constant or decreasing pattern in Sr concentrations across the vertebra (Supporting information Figure 3e-f), although the largest female showed a decrease in Sr concentrations during the adult stage (Figure 5). Despite it being a marine ray species, some individuals may use areas under the influence of the Canal de Santa Cruz estuary (Figure 1), although this was not the pattern seen in the other specimens used in this study. Furthermore, stable isotope analyses suggest marine dietary sources are the most important ones for the species, with a low likelihood of estuarine contribution (Table 3).

Higher concentrations of Ba:Ca ratios indicate a proximity to areas with freshwater influence (McMillan et al., 2017). The PERMANOVA analysis did not show any significant differences between species regarding Ba:Ca ratios. However, higher concentrations were recorded in *H. guttatus* vertebrae, likely due to the use of estuaries in part of its life.

Higher Mn concentrations in fish hard structures have been associated with hypoxic zones, as is the case of some estuarine and deep-water habitats (Limburg et al., 2015; Limburg and Casini, 2018). Significant differences in Mn:Ca concentrations were detected by the PERMANOVAs between species (Table 4), with concentrations being much higher in *H. guttatus* and *H. berthalutzae* samples compared with *H. marianae* (Supporting information Figure 2a-f). However, while in *H. marianae* vertebrae these concentrations remained stable with little variation, in the vertebrae of *H. guttatus* and *H. berthalutzae* there was an increase pattern during ontogenetic development (Supporting information Figure 2a-b, e-f). This could indicate the use of estuarine habitats by *H. guttatus* and of deeper waters by *H. berthalutzae*.

It is important to note that there are caveats to using Mn as a proxy for hypoxia. Feeding and the activation of sexual hormones, for example, are factors that can influence Mn concentrations in fish hard structures (Elsdon and Gillanders, 2006; Smith et al., 2013). Yet, Mn grows substantially in *H. guttatus* vertebrae as soon as the first year of life (Figure 5), much earlier than the age at first maturity, coinciding with the period in which the species likely starts moving into the estuarine environment (Feitosa et al., 2021; Melo, 2016; Thorson, 1983;

Queiroz et al., unpublished data). As for *H. berthalutzae* vertebrae, Mn concentrations increase in the years before the age at maturity (Figure 5). This leads us to believe that, in these vertebrae, changes in Mn concentrations may be more strongly associated with environmental changes than with feeding or maturity.

CONCLUSIONS

Overall, the combination of stable isotope and microchemistry data represented a good descriptor of the different habitats species were found in., We reinforce that the application of both techniques together enables a better expanding the understanding of on habitat use patterns and the establishment of more robust conclusions. The information presented here supports the hypothesis of multiple movements between estuarine and marine environments for *H. guttatus* throughout its life cycle (Feitosa et al., 2021; Melo, 2016; Thorson, 1983; Queiroz et al., unpublished data).

We also reinforce the habitat specificity of *H. marianae*, which may not explore deeper or estuarine areas (Costa et al., 2017), being limited to shallow and constant coastal zones. Contrarily, *H. berthalutzae* showed the potential to explore deeper waters, especially in its adult stage. Among the three species studied here, *H. berthalutzae* is the only one found in island regions such as the Fernando de Noronha archipelago and Atol das Rocas (Aguiar et al., 2009; Branco-Nunes et al., 2016).

Although we suggest limited longitudinal movements for the three species, considering the affinity of *H. berthalutazae* for deeper waters, some rate of connectivity between individuals of insular regions and those of the Brazilian coast likely exists. Therefore, we highlight the need to manage each species separately and that management actions for *H. guttatus* must focus on estuarine and shallow coastal habitats, while those for *H. marianae* should focus on reef fisheries, and for *H. berthalutzae* should focus on a wider area of the continental platform spanning to deeper water (> 100 m).

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SUPPORTING INFORMATION

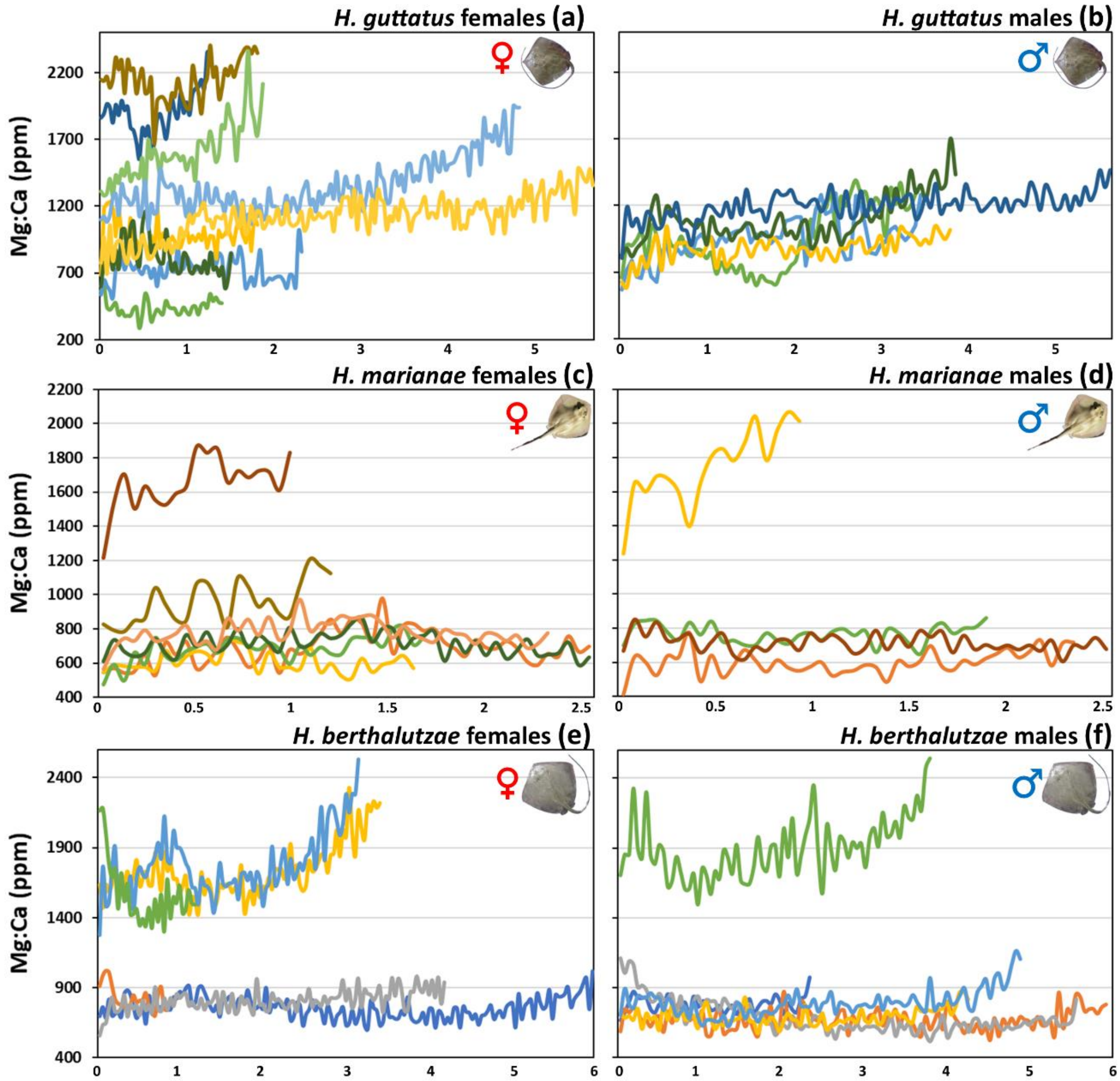

Figure 1 - Lifetime Mg:Ca concentrations in ppm for *H. guttatus* females (a), *H. guttatus* males (b), *H. marianae* females (c), *H. marianae* males (d), *H. berthalutzae* females and *H. berthalutzae* males. Individuals collected on the coast of Pernambuco, Brazil, from 2016 to 2019.

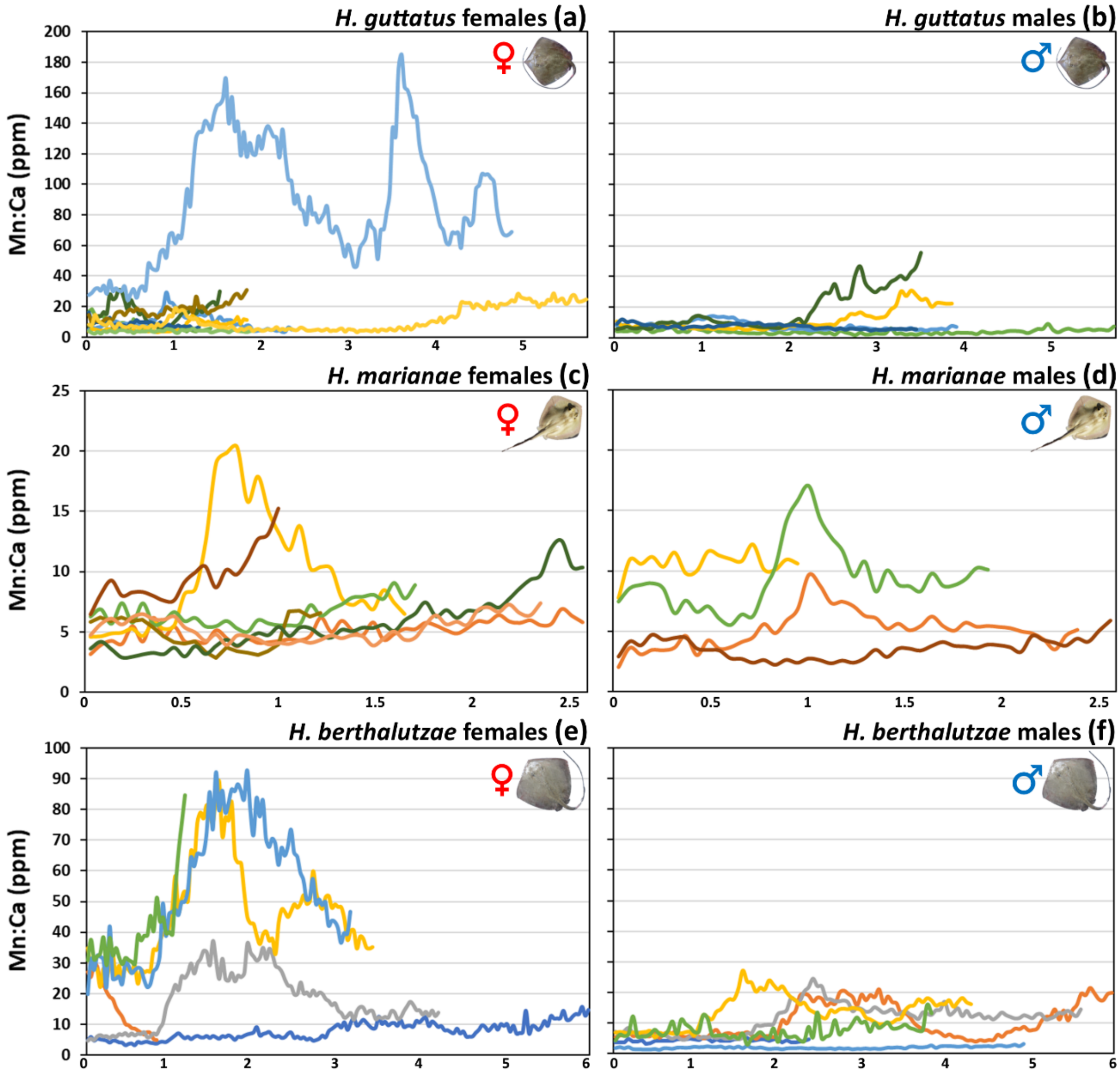

Figure 2 - Lifetime Mn:Ca concentrations in ppm for *H. guttatus* females (a), *H. guttatus* males (b), *H. marianae* females (c), *H. marianae* males (d), *H. berthalutzae* females and *H. berthalutzae* males. Individuals collected on the coast of Pernambuco, Brazil, from 2016 to 2019.

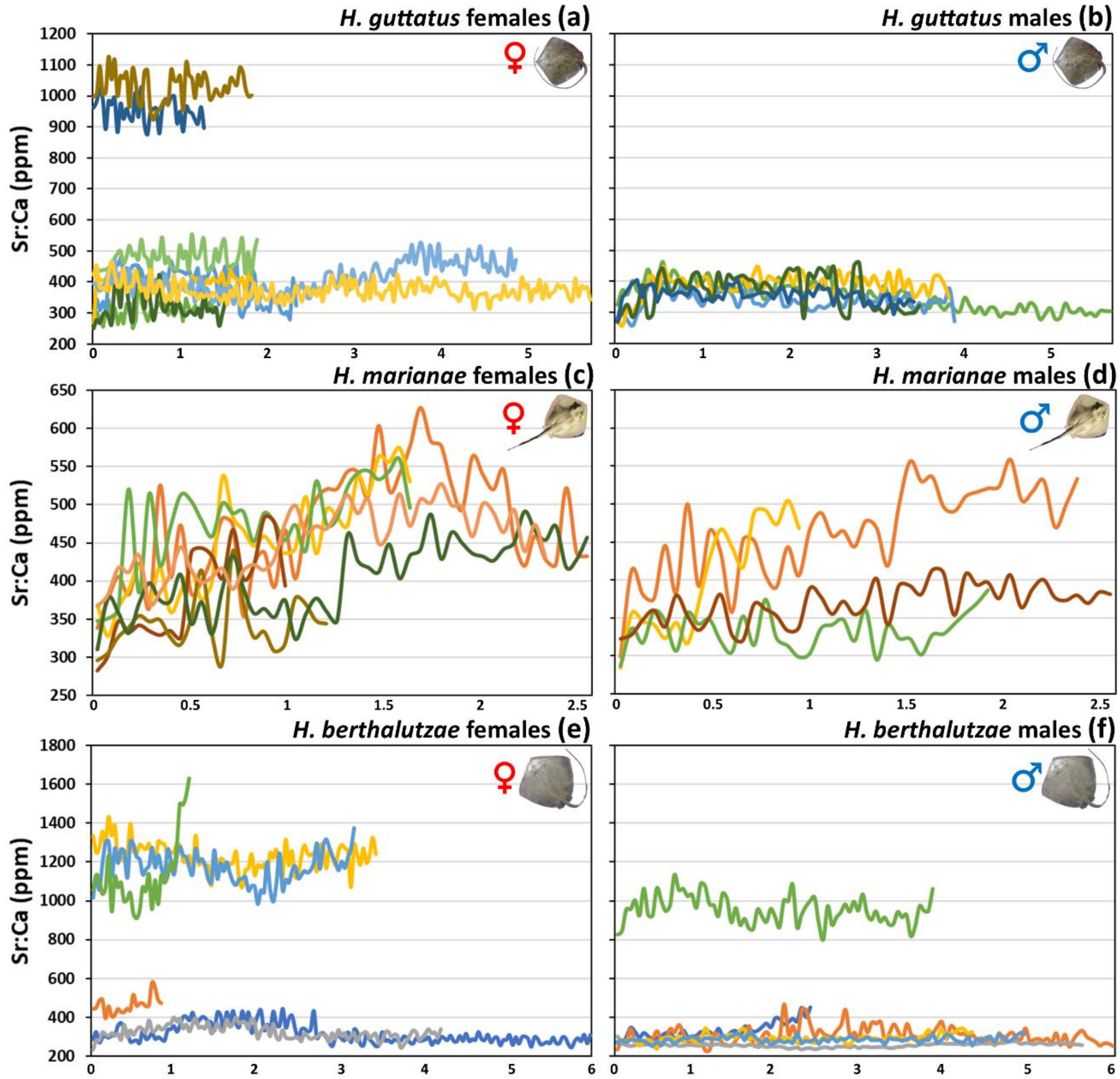

Figure 3 - Lifetime Sr:Ca concentrations in ppm for *H. guttatus* females (a), *H. guttatus* males (b), *H. marianae* females (c), *H. marianae* males (d), *H. berthalutzae* females, and *H. berthalutzae* males. Individuals collected on the coast of Pernambuco, Brazil, from 2016 to 2019.

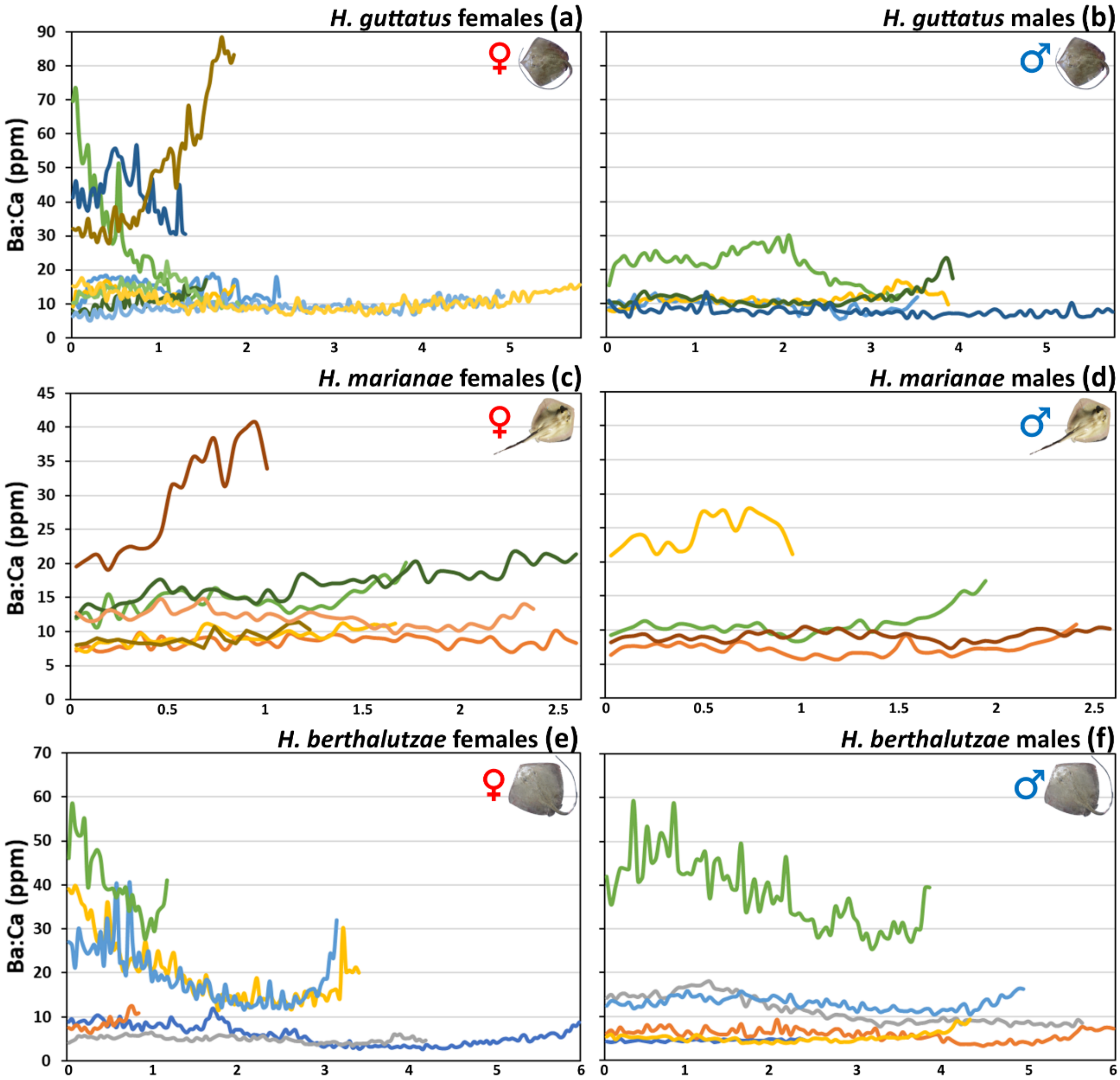

Figure 4 - Lifetime Ba:Ca concentrations in ppm for *H. guttatus* females (a), *H. guttatus* males (b), *H. marianae* females (c), *H. marianae* males (d), *H. berthalutzae* females and *H. berthalutzae* males. Individuals collected on the coast of Pernambuco, Brazil, from 2016 to 2019.

5 ARTIGO 3 – IMPORTÂNCIA TOPOLÓGICA DA RAIA *Hypanus guttatus* (MYLIOBATOIDEI: DASYATIDAE) EM UMA REDE TRÓFICA ESTUARINA TROPICAL: ELASMOBRÂNQUIOS SÃO SEMPRE ESPÉCIES-CHAVE?

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Importância topológica da raia *Hypanus guttatus* (Myliobatoidei: Dasyatidae) em uma rede trófica estuarina tropical: elasmobrânquios são sempre espécies-chave?

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RESUMO

Estudos de redes tróficas têm demonstrado a importância das populações de elasmobrânquios na transmissão de efeitos *"bottom-up"* e *"top-down"* em teias alimentares aquáticas. Neste sentido, o presente estudo avaliou a importância topológica da raia *Hypanus guttatus* em uma rede trófica tropical do estuário do Canal de Santa Cruz, Brasil. Apesar de ser a única espécie de elasmobrânquio no sistema, *H. guttatus* não foi classificada como espécie-chave e seu potencial de dispersão de efeitos indiretos se limita aos efeitos "top-down", atuando como um predador de topo da fauna bentônica. O peixe *Eucinostomus argenteus*, detritos, pesca e material vegetal estão entre os nós-chaves do sistema capazes de gerar maior fragmentação na rede (KPP1). Enquanto no cenário de expansão (KPP2), os nós Actinopterygii e Crustacea são capazes de aumentar as distâncias médias do maior número de pares, enquanto Detritos e Fitoplâncton são responsáveis, principalmente, por desconectar completamente outros nós da rede. Detritos se destaca como um dos principais nós nas análises topológicas e de espécies-chave, reflexo da importância do suprimento estuarino de matéria orgânica na manutenção de redes alimentares aquáticas. A pesca atua como predador de topo na rede trófica analisada, sendo um dos principais nós capazes de afetar a rede de forma rápida e com ampla disseminação e de gerar maior fragmentação. Um maior conhecimento da atividade pesqueira da região é altamente recomendado, tendo em vista a influência que a pesca exerce sobre a estabilidade da teia trófica do ambiente.

Palavras-chave: Teia alimentar; Arraia; Manguezal; Pesca.

INTRODUÇÃO

A importância de tubarões como grandes predadores de cadeia, atuando no controle e regulação das populações em níveis tróficos inferiores é bem documentada na literatura (Bizzarro et al., 2007; Heithaus et al., 2008; Navia et al., 2012, 2010; Pace et al., 1999). Contudo, elasmobrânquios de níveis tróficos intermediários como as raias também exercem importante papel no ecossistema, sendo citadas como grupos chave por transferirem a energia da produção primária bentônica para níveis tróficos superiores (Myers et al., 2007; Sánchez et al., 2005; Sánchez and Olaso, 2004; Vaudo and Heithaus, 2011) ou ainda modificando consideravelmente o habitat, reestruturando comunidades de invertebrados e de algas e promovendo ressuspensão de nutrientes no ecossistema bentônico (Orth, 1975; Takeuchi and Tamaki, 2014).

Estudos de redes tróficas têm revelado fortes efeitos de cascatas tróficas (*top down*) influenciados pelo declínio de predadores de topo, demonstrando a importância das populações de mesopredadores na transmissão desses efeitos para baixo da rede alimentar (Ajemian, 2011; Vaudo and Heithaus, 2011). Outros trabalhos que avaliam relações predador-presa descrevem como a perda de espécies têm provocado extinções secundárias em teias alimentares marinhas (Dunne et al., 2004; Eklöf and Ebenman, 2006) e influenciado mecanismos de controle ecossistêmico (*bottom up*) (Navia et al., 2010). Assim, embora a compreensão da dinâmica de redes tróficas seja complexa, é essencial para compreender como os efeitos do controle *top down* e *bottom up* se de dispersam ao longo das teias e influenciam os ecossistemas aquáticos.

Entende-se, portanto, que o equilíbrio e a saúde dos ecossistemas podem depender diretamente da integridade das populações locais de elasmobrânquios (Aguiar and Valentin, 2010). Tendo em vista os desequilíbrios nos ecossistemas que a redução na abundância desses organismos pode causar (Bizzarro et al., 2007; Navia et al., 2012), é de grande importância a geração de dados acerca da dinâmica trófica dessas espécies, especialmente daquelas que sofrem intensa pressão pesqueira (Bornatowski et al., 2014; Navia et al., 2016; Worm et al., 2013).

Entre os elasmobrânquios que ocorrem no Brasil, as espécies que habitam ambientes costeiros são as que mais são afetadas pela pesca e, uma grande parte dessas espécies carecem de informações biológicas básicas (Lessa et al., 1999; Martins et al., 2018). Essa falta de dados tem dificultado avaliações do seu estado de conservação, como é o caso da raia *Hypanus guttatus*, que é a espécie de batóide mais pescada no país (Basílio et al., 2008; Carmona et al., 2008; Gianeti et al., 2019a; Lessa et al., 1999, 2008; Marion, 2015; Martins et al., 2018; Melo, 2016; Meneses et al., 2005; Menni and Lessa, 1998; Rodrigues Filho et al., 2020).

No Nordeste do Brasil, *H. guttatus* é capturada por diversas artes de pesca que atuam nos ambientes marinho e estuarino, tanto como fauna acompanhante quanto como espécie alvo. Levando em consideração suas características biológicas, a pesca se torna uma ameaça ainda maior. As fêmeas de *H. guttatus* crescem mais devagar e amadurecem aos sete anos, enquanto os machos amadurecem aos cinco, denotando sua maturidade sexual tardia. Além disso, a espécie tem crescimento lento (fêmeas $W\infty = 98{,}61$ cm, $k = 0{,}112$ ano $^{-1}$; machos $W\infty = 60{,}22$ cm, $k = 0{,}219$ ano $^{-1}$) e baixa taxa de fecundidade (média de 1-2 embriões por gestação) (da Silva et al., 2018; Gianeti et al., 2019a). Tais fatores, juntamente com a degradação do habitat podem conferir à espécie baixa resiliência a impactos antrópicos.

Hypanus guttatus é considerada um predador generalista usando uma grande diversidade de espécies bentônicas como itens alimentares, como moluscos, sipunculas, crustáceos, poliquetas e peixes (Carvalho Neta and Almeida, 2002; Gianeti et al., 2019b; Queiroz et al., 2022; Silva et al., 2001). Esse comportamento oportunista confere à espécie um potencial adaptativo para diferentes ambientes, alimentando-se dos itens mais abundantes em cada ecossistema onde ocorre (Knipp et al., 2010). Contudo, expõe a espécie a diferentes artes de pesca que atuam ao longo de sua ocorrência, podendo levá-la a fortes declínios populacionais, similar aos que já ocorrem com diversos elasmobrânquios no mundo (Simpfendorfer et al., 2011).

Este trabalho objetivou avaliar a importância ecológica de *H. guttatus* em uma rede estuarina através de análises topológicas estruturais e testar se ela atua como espécie-chave topológica dispersando os efeitos diretos e indiretos ao longo da teia em que está inserida. Nossos objetivos específicos foram construir uma rede topológica do ambiente estuarino do Canal de Santa Cruz onde *H. guttatus* ocorre para, a partir dela, (i) quantificar a importância relativa de *H. guttatus* no sistema estuarino analisado utilizando os índices: Grau do nó (D), Índice de intermediação (BC) e Índice de proximidade (CC); e (ii) verificar se *H. guttatus* se posiciona entre as três espécies mais importantes para a manutenção da rede (espécie-chave) através do Key Player Problem (KPP).

Baseado na premissa de que elasmobrânquios tendem a atuar como espécies-chave nos ambientes que ocorrem e, sendo *H. guttatus* a única espécie de elasmobrânquio que passa boa parte de seu ciclo de vida no estuário do Canal de Santa Cruz, levantamos as hipóteses de que: I) *H. guttatus* possui grande importância topológica posicional na rede trófica analisada, sendo capaz de propagar efeitos indiretos ao longo da rede e; II) *H. guttatus* se classifica como uma das espécies-chave do sistema, desempenhando papel decisivo na manutenção do equilíbrio e da integridade da rede trófica a qual está inserida.

MATERIAL E MÉTODOS

Área de estudo

Este estudo foi realizado na região costeira do nordeste do Brasil, sudoeste do Oceano Atlântico (Figure 1). De modo geral, a costa Nordeste apresenta poucos rios com grande vazão de água doce, capazes de influenciar drasticamente o ambiente marinho costeiro. Isso acontece tanto pelo clima continental semiárido quanto pela plataforma continental rasa. Portanto, a maioria dos rios exercem pouca influência sobre a região costeira a depender da sazonalidade

(Ekau and Knoppers, 1999). A precipitação mensal na região durante a estação chuvosa (março a agosto) pode chegar a 400 mm^3, enquanto cai para até 100 mm^3 na estação seca (setembro a fevereiro) (Silva, 2004). O Nordeste também é composto por importantes e diversificados manguezais, embora sejam escassos e representem apenas cerca de 2% da cobertura total de manguezais do Brasil (Diniz et al., 2019).

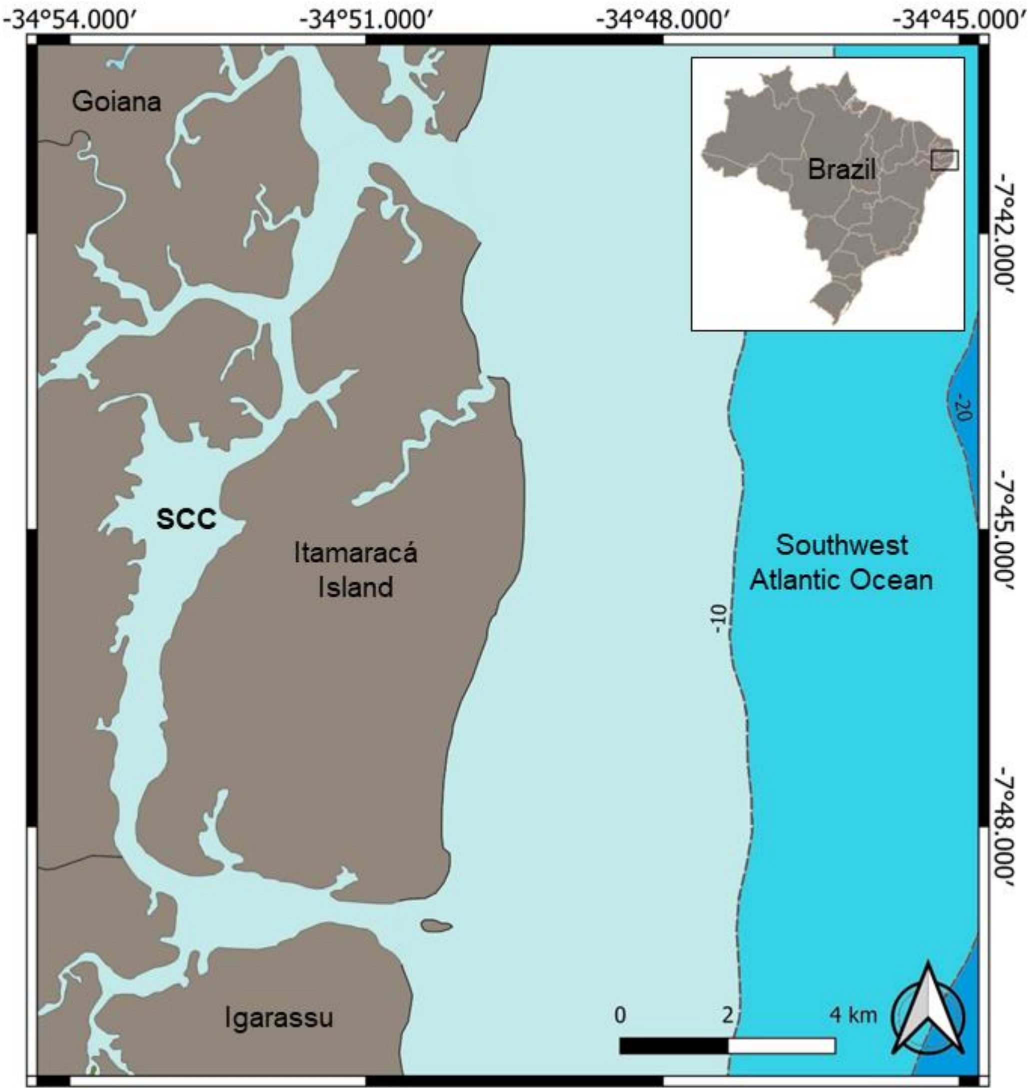

Figure 1. Canal de Santa Cruz (SCC), localizado geograficamente nas porções Oeste, Norte e Sul no entorno da Ilha de Itamaracá, Pernambuco, Brasil.

A área de estudo compreende um ambiente estuarino localizado em torno da Ilha de Itamaracá, Pernambuco, conhecido como Canal de Santa Cruz (SCC). Composto predominantemente por fundo lamoso e vegetação de mangue, o Canal de Santa Cruz (Figure

1) é um dos sistemas costeiros mais importantes e produtivos da região (Carneiro et al., 2008; Medeiros and Kjerfve, 1993; Paranaguá et al., 2010), o qual se estendendo por mais de 22 km com largura de 0,6 a 1,5 km contornando a Ilha de Itamaracá (Paiva et al., 2017). A profundidade varia entre 10 e 17 m e o complexo se conecta ao mar em suas bordas norte e sul (Eskinazi-Leça et al., 1980) com um escoamento total de água doce de 58 m³/s na estação chuvosa e apenas 0,2 m³/s durante a estação seca (Medeiros and Kjerfve, 1993).

Construção da rede

Para construção do banco de dados necessário às análises topográficas da rede trófica, foi realizada uma ampla revisão bibliográfica (incluindo artigos publicados em periódicos, anais de eventos, Teses e Dissertações) sobre hábitos alimentares e registros de interações tróficas de espécies que ocorrem no estuário do Canal de Santa Cruz. A utilização de informações obtidas na literatura é comum para este tipo de análise e tem se mostrado bastante útil (Bornatowski et al., 2014; Navia et al., 2012, 2010). A coleta de dados se deu em 3 fases: 1) Foram obtidos dados de interação trófica e nível trófico a partir de estudos realizados ao longo do Canal de Santa Cruz; 2) Quando uma espécie de consumidor foi inserida no banco de dados como presa, mas suas interações tróficas estavam ausentes (devida a falta de dados sobre sua dieta na área de estudo), foram utilizados dados de interação trófica da espécie em estuários próximos ao Canal de Santa Cruz, contudo, esses dados foram limitados às categorias taxonômicas de Classe, Ordem e Família; 3) Espécies sem dados de nível trófico disponíveis tiveram seu nível trófico estimado a partir da fórmula de (Cortés, 1999) modificada: $TL = 1 + \frac{\sum TLp}{\mathrm{N}}$, onde TL é o nível trófico do consumidor, TLp é o nível trófico da presa e N é o número de itens alimentares.

Com os dados obtidos através da revisão bibliográfica e através da análise de conteúdos estomacais *de H. guttatus* coletadas na área de estudo foi gerada uma matriz binária onde os predadores foram organizados nas colunas e as presas nas linhas (espécie x espécie). A matriz foi baseada na presença ou ausência de interações tróficas sendo, na célula onde havia interação predador-presa foi registrado “1” e onde não havia interação trófica foi registrado “0”. Esta matriz é baseada somente na presença ou ausência de interações entre as espécies e, portanto, serve unicamente para analisar características estruturais da teia (Márquez-Velásquez, 2017).

Com base na matriz binária, foi gerada uma matriz quadrada. Essa segunda matriz incluiu todas as presas e predadores nos títulos das linhas bem como nas colunas, desse modo, todas as interações foram duplicadas, sendo registradas no sentido predador-presa e no sentido oposto presa-predador. Com essa matriz foi possível medir as forças de interação entre as espécies e sua função topológica na rede.

Para a visualização gráfica da teia trófica, a matriz quadrada foi importada para o programa Pajek v. 5.16 (de Nooy et al., 2018). O grau do nó (D) foi utilizado como vetor para ser representado através do tamanho do nó e o nível trófico (TL) foi utilizado como partição, sendo representado pelas cores (verde ≤ 1; azul = 2 a 2,9; laranja = 3 a 3,9; vermelho ≥ 4) e organização dos nós na teia.

Características da rede

Para descrever as propriedades da rede trófica criada, o primeiro índice calculado foi o número de interações médias por espécie ou Densidade (D), que consiste na fração entre o número de interações (L) e o número de nós da rede (S). Em seguida foi calculado o índice de Conectância Direta (C), este índice representa a fração entre todas as possíveis interações da rede e é calculado como L/S^2, onde L é número de interações e S o número de nós da rede (Jordán et al., 2006).

Análise topológica da rede

Para avaliar a participação de cada espécie ou grupo taxonômico (nó), foram utilizados índices que refletem sua importância topológica posicional na rede trófica, em outras palavras, sua capacidade de propagar efeitos indiretos (extinções locais ou grande flutuações na abundância de uma espécie) ao longo da rede analisada. Esses índices apresentam uma variedade de quantificações da importância posicional dos nós na teia, desde nós muito simples, dependentes apenas de características muito locais ao nó focal, até nós que interagem de forma mais ampla e incluem informações sobre recursos da teia em escalas topológicas maiores (Jordán et al., 2006).

Tal abordagem foi utilizada por Bornatowski et al. (2014) para avaliar a importância ecológica de tubarões e raias em uma teia alimentar no sul do Brasil, e tem sido aplicada em diversos ambientes marinhos costeiros (Jordán et al., 2006; Márquez-Velásquez, 2017; Navia et al., 2010). Dentre os índices descritos por (Jordán et al., 2006), utilizamos:

Grau do nó (D)

É o índice mais local e menos informativo sobre a topologia da rede, reflete o número de outros nós conectados diretamente ao nó *i*. Em uma teia alimentar, o grau de um nó *i* (D_i) é a soma de suas presas (in-degree, *Din*, *i*) e seu predadores (out-degree, *Dout*, *i*) e foi calculado usando o software NetDraw (Borgatti, 2021):

$$Di = D_{in,i} + D_{out,i}$$

Índices de centralidade

Betweenness centrality (BC): É uma medida de importância posicional e quantifica a frequência com que um nó *i* está no caminho mais curto entre cada par de nós da rede (*j* e *k*). Este índice é calculado usando o programa UCINET IV (Borgatti et al., 1996), sua forma padronizada para um nó *i* (*BCi*) é:

$$BC_i = \frac{2 \times \sum_{j<k} g_{jk(i)}/g_{jk}}{(\mathrm{N}-1)(\mathrm{N}-2)}$$

onde $i \neq j$ e *k*. g_{jk} é o número de passos igualmente mais curtos entre os nós *j* e *k*, e $g_{jk}(i)$ é o número desses passos mais curtos nos quais o nó *i* é incidente (é claro, g_{jk} pode ser igual a 1). O denominador é duas vezes o número de passos de nós sem o nó *i*. Este índice mede o quão central um nó é no sentido de ser incidente a muitos caminhos mais curtos na rede. Se *BCi* é grande para o grupo trófico *i*, significa que a exclusão desse grupo afetará a rede de forma rápida e com ampla disseminação.

Closeness centrality (CC): É uma medida que quantifica quão curtos são os caminhos mínimos de um determinado nó para todos os outros (Wasserman and Faust, 1994) e foi calculada usando UCINET IV (Borgatti et al., 1996). O índice padronizado para um nó i (*CCi*) é:

$$CC_i = \frac{\mathrm{N}-1}{\sum_{j-1}^{N} d_{ij}}$$

onde $i \neq j$ e d_{ij} é o comprimento do caminho mais curto entre os nós *i* e *j* na rede. Esse índice mede o quão perto um nó está dos outros. Quanto maior *CCi* é para o grupo trófico, maior a capacidade desse grupo afetar diretamente a maioria dos outros grupos, caso seja excluído.

Key Player Problem (KPP)

Esta abordagem é usada para determinar a importância de diferentes combinações de espécies em manter a integridade de uma rede. Segundo Borgatti (2003), existem duas razões básicas para tentar encontrar um conjunto ideal de nós-chave: 1) Identificar nós que, uma vez excluídos, causariam uma paralização na rede; 2) Identificar nós bem conectados que provavelmente possuem uma grande quantidade de informações e que, por causa de suas conexões, estão em posição de influenciar outros. Estes dois tipos de nós são os que merecem atenção ao se procurar por espécies-chave em uma rede trófica.

Neste trabalho, a abordagem foi aplicada para avaliar se *H. guttatus* pertence aos grupos de elementos chave do ecossistema estudado. Para isso, foram utilizados dois cenários, o primeira de fragmentação (KPP1) onde, basicamente, são contados o número de componentes separados na rede após excluir os nós-chave e a proporção de todos os nós contidos em cada componente. A soma dos quadrados dessas proporções dá uma medida da extensão em que os nós estão agrupados em apenas alguns componentes, e um menos essa soma dá o grau de fragmentação, onde um valor próximo a 1 é bom (muitos pequenos grupos) e um valor próximo a 0 é ruim (a maioria dos nós continuam conectados). O segundo cenário, de distanciamento (KPP2), objetiva identificar os nós-chave que, ao serem excluídos, aumentam as distâncias médias entre os pares de nós da teia. Frequentemente, a exclusão de um nó não apenas aumentará a distância entre alguns pares de nós, como também os desconectará completamente, isto também é levado em consideração nesta análise. Todas as análises de espécies-chave foram realizadas com o software Key Player 1.1 (Borgatti, 2003).

RESULTADOS

Banco de dados

Foram utilizados 131 trabalhos com informações sobre dieta ou interações tróficas de espécies que habitam o estuário do Canal de Santa Cruz (Apêndice 1). Dos quais foram obtidas informações de 385 nós (incluindo algas, plantas, invertebrados e vertebrados), sendo 200 espécies, 185 grupos taxonômicos (Classe, Ordem, Família e Gênero) (Tabela 1).

Tabela 1 - Espécies com informações de dieta obtidas na literatura e utilizada para a construção da rede trófica do estuário do Canal de Santa Cruz, Itamaracá-PE e seu respectivo número do nó. *Hypanus guttatus* (177) está destacada em negrito.

Nó	Componente trófico	Nó	Componente trófico	Nó	Componente trófico
1	*Acantholobulus bermudensis*	130	Echinodermata	259	*Pandion haliaetus carolinensis*
2	*Acartia lilljeborgi*	131	Echinoidea	260	*Panopeus lacustris*
3	*Achirus lineatus*	132	*Edotea sublittoralis*	261	*Panopeus occidentalis*
4	*Acteocina candei*	133	*Encope emarginata*	262	*Panopeus* sp.
5	Actinopterygii (teleostei)	134	Engraulidae	263	Pantopoda
6	*Actitis macularius*	135	*Epialtus* sp.	264	*Panulirus argus*
7	*Aegathoa linguifrons*	136	*Erichthonius hunteri*	265	*Paracalanus* sp.

8 *Agytria leucogaster bahiae*
9 *Alpheopsis* sp.
10 *Alpheus chacei*
11 *Alpheus estuariensis*
12 *Alpheus intrinsecus*
13 *Alpheus nuttingi*
14 *Alpheus* sp.
15 Amphibia
16 *Amphiglena* sp.
17 Amphinomidae
18 Amphipoda
19 Ampithoidae
20 *Anas b. bahamensis*
21 *Ancistrosyllis* sp.
22 *Anomalocardia brasiliana*
23 Anomura
24 Anthozoa
25 Anthuridea
26 *Antinoe* sp.
27 Arachnida
28 *Aratus pisonii*
29 *Arcopsis adamsi*
30 *Arenaria interpres marinella*
31 *Aricidea* sp.
32 *Arius parkeri*
33 *Arius proops*
34 *Armases* sp.
35 Ascidiacea
36 *Assiminea succinea*
37 *Astropecten articulatus*
38 *Astropecten marginatus*
39 *Astropecten* sp.
40 *Atherinella brasiliensis*
41 Aves
42 *Axianassa australis*
43 Balanomorpha
44 *Bathygobius soporator*
45 Bivalvia
46 *Boccardia* sp.
47 Brachyura
48 *Branchiostoma platae*
49 Bryozoa
50 Calanoida
51 *Calanopia americana*
52 *Calappa ocellata*
137 *Eucinostomus argenteus*
138 *Eucinostomus gula*
139 *Eugerres brasilianus*
140 *Eulalia* sp.
141 Eunicidae
142 *Eusyllis* sp.
143 *Euterpina acutifrons*
144 *Exogone* sp.
145 Fabricinae
146 *Falco peregrinus*
147 *Falco sparverius cearae*
148 *Farranula gracilis*
149 Annelida
150 Fitoplancton
151 Flabellifera
152 Foraminíferos
153 *Fregata magnificens*
154 *Frikea lewisiana*
155 Gammaridae
156 Gastropoda
157 *Globigerina* sp.
158 Glyceridae
159 *Glycinde* sp.
160 Gobiidae
161 *Gobionellus oceanicus*
162 *Gobionellus stomatus*
163 Goniadidae
164 Gymnolaemata
165 Halacaridae
166 *Haploscoloplos* sp.
167 Harpacticoida
168 Hemiptera
169 *Hemiramphus brasiliensis*
170 *Hepatus* sp.
171 *Heteromastus* sp.
172 *Hexapanopeus caribbaeus*
173 Hippocampus
174 Hirudinea
175 *Hyale* sp.
176 Hydrozoa
177 ***Hypanus guttatus***
178 *Hyporhamphus unifasciatus*
179 Insecta
180 *Iphigenia brasiliana*
181 *Isolda* sp.
266 *Paradoneis* sp.
267 Paraonidae
268 *Parvocalanus crassirostris*
269 Pattelinidae
270 Penaeidae
271 *Penaeus brasiliensis*
272 *Penaeus schmitti*
273 *Penaeus subtilis*
274 *Periclimenes longicaudatus*
275 *Petrolisthes armatus*
276 *Petrolisthes* sp.
277 Phoronida
278 *Phyllodoce* sp.
279 Phyllodocidae
280 Pilargidae
281 Pinnotheridae
282 *Pitangus sulphuratus maximiliani*
283 Pitar fulminatus
284 Platyhelminthes
285 *Pleoticus muelleri*
286 *Pluvialis squatarola*
287 *Poecilia vivipara*
288 *Poecilochaetus* sp.
289 Polychaeta
290 *Polycladida* sp.
291 Polynoidae
292 Porifera
293 Portunidae
294 *Potamilla* sp.
295 *Prionospio* sp.
296 *Processa* sp.
297 *Progne chalybea*
298 *Protodorvillea* sp.
299 Protozoa
300 *Pseudosphaeroma mourei*
301 *Pugilina morio*
302 Pycnogonida
303 Quinqueloculina
304 Radiolários
305 Reptilia
306 *Rimapenaeus constrictus*
307 *Robertsonia mourei*
308 *Salmoneus ortmanni*
309 Scaphopoda
310 *Scoloplos* sp.

53 Calappidae
54 Calidris alba
55 *Calidris canutus rufa*
56 *Calidris fuscicollis*
57 *Calidris pusilla*
58 *Callinectes danae*
59 *Callinectes exasperatus*
60 *Callinectes larvatus*
61 *Callinectes ornatus*
62 *Callinectes sapidus*
63 *Callinectes* sp.
64 *Callithrix jacchus*
65 *Capitella* sp.
66 *Capitellides* sp.
67 Capitomastus
68 Caprellidae
69 *Caracara plancus*
70 *Caranx latus*
71 Caridea
72 *Caryocorbula swiftiana*
73 *Cassidinea lunifrons*
74 *Cathartes aura ruficollis*
75 *Catoptrophorus s. semipalmatus*
76 *Centropomus parallelus*
77 *Centropomus undecimalis*
78 Ceratopogonidae
79 Ceriantharia
80 *Chaetodipterus faber*
81 *Charadrius collaris*
82 *Charadrius semipalmatus*
83 *Charadrius w. wilsonius*
84 *Charybdis hellerii*
85 *Chione cancellata*
86 *Chirona amaryllis*
87 *Chloroscombrus chrysurus*
88 Cicadidae
89 *Cirolana* sp.
90 Cirripedia
91 *Citharichthys spilopterus*
92 *Cletocamptus deitersi*
93 *Clibanarius sclopetarius*
94 *Clibanarius vittatus*
95 Clupeidae
96 Cnidaria

182 Isopoda
183 *Langerhansia* sp.
184 *Latreutes parvulus*
185 *Leander paulensis*
186 *Leiocapitellides* sp.
187 *Leitoscoloplos* sp.
188 *Leptochela serratorbita*
189 *Leptochelia dubia*
190 Leptocheliidae
191 *Leucothoe* sp.
192 *Lile piquitinga*
193 *Limnodromus g. griseus*
194 *Litopenaeus vannamei*
195 *Littoraria angulifera*
196 *Longipedia* sp.
197 *Lucifer faxoni*
198 *Lucifer* sp.
199 *Lucina* sp.
200 *Luidia clathrata*
201 *Luidia senegalensis*
202 *Lumbrineris* sp.
203 *Lurocalis semitorquatus*
204 *Lycengraulis grossidens*
205 *Macoma* sp.
206 *Macrosetella gracilis*
207 *Magelona* sp.
208 Mammalia
209 *Marphysa* sp.
210 *Mediomastus* sp.
211 *Megalops atlanticus*
212 *Melita sexiesperforata*
213 *Menippe nodifrons*
214 *Mesochra* sp.
215 *Metis holothuriae*
216 *Micromaldane* sp.
217 *Microphrys antillensis*
218 *Microspio* sp.
219 *Minyocerus angustus*
220 Mollusca
221 *Molossus* sp.
222 Monoplacophora
223 *Mugil brasiliensis*
224 *Mugil curema*
225 *Mugil liza*

311 *Sicyonia dorsalis*
312 *Sicyonia laevigata*
313 *Sicyonia parri*
314 *Sicyonia typica*
315 *Sigambra* sp.
316 Sipuncula
317 *Sphaerosyllis* sp.
318 *Sphoeroides testudineus*
319 *Spio* sp.
320 Squillidae
321 *Stercorarius pomarinus*
322 *Sterna h. hirundo*
323 *Sterna superciliaris*
324 *Sternaspis* sp.
325 Stomatopoda
326 *Streblospio benedicti*
327 Syllidae
328 *Symphurus plagusia*
329 *Syngnatus* sp.
330 *Synodus foetens*
331 Tabanidae
332 *Tachycineta Albiventer*
333 Tagelus sp.
334 Tanaidacea
335 *Tangara cayana*
336 *Tellina* sp.
337 Terebellidae
338 *Thalasseus sandvicensis acuflavidus*
339 *Thalasseus sandvicensis eurygnathus*
340 Thalassinidea
341 *Tharyx* sp.
342 Thoracica
343 *Tigriopus* sp.
344 *Transenella stimpsoni*
345 *Trichiurus lepturus*
346 *Trichodina* sp.
347 *Tricollia affinis*
348 *Tringa flavipes*
349 Trochominidae
350 Turbellaria
351 *Turbonilla* sp.
352 *Ucides cordatus*
353 *Upogebia omissa*
354 *Upogebia* sp.

97	*Coereba flaveola chloropyga*	226	*Mugil* sp.	355	*Vanellus chilensis lampronotus*
98	*Columba livia*	227	*Mugil trichodon*	356	Material Vegetal
99	*Conodon nobilis*	228	*Myiozetetes similis*	357	*Vitrinella filifera*
100	Copepoda	229	Mysidacea	358	Xanthidae
101	*Corbula caribbaea*	230	*Mytella charruana*	359	*Zoaps ostreus*
102	*Corbula cubaniana*	231	*Mytella guyanensis*	360	Zooplâncton
103	*Corbula lyon*	232	*Nassarius vibex*	361	Fishery
104	Corophiidae	233	Nematoda	362	*Pomadasys corvinaeformis*
105	*Crassostrea rhizophorae*	234	*Nemertea* sp.	363	*Lutjanus jocu*
106	*Crotophaga ani*	235	*Neomediomastus* sp.	364	*Cetengraulis edentulus*
107	Crustacea	236	Nereidae	365	*Anchoa filifera*
108	*Ctenodrillus serratus*	237	*Neritina virginea*	366	*Amphichthys cryptocentrus*
109	*Ctenogobius smaragdus*	238	*Ninoe* sp.	367	*Anchovia* sp.
110	*Ctenoplana* sp.	239	*Notolopas brasiliensis*	368	*Anchoa januaria*
111	Cumacea	240	*Nudibranchia* sp.	369	*Cynoscion* sp.
112	*Cyclinella tenuis*	241	*Numenius phaeopus hudsonicus*	370	*Epinephelus itajara*
113	Cyclopoida	242	*Odostomia laevigata*	371	*Caranx hippos*
114	*Cyclostremiscus carababoensis*	243	*Ogyrides alphaerostris*	372	*Callianassa* sp.
115	*Cylichna* sp.	244	*Ogyrides* sp.	373	*Cloridopsis dubia*
116	*Cymothoa excisa*	245	*Oithona hebes*	374	*Strongylura marina*
117	*Dactyloscopus tridigitatus*	246	*Oithona osvaldocruzi*	375	*Caranx crysos*
118	Dendrobranchiata	247	*Oithona* sp.	376	*Chloroscombrus chrysurus*
119	*Dentalium* sp.	248	Oligochaeta	377	*Oligoplites saurus*
120	Detritos	249	*Oligoplites palometa*	378	*Harengula clupeola*
121	*Diapterus auratus*	250	*Oncaea* spp.	379	*Anchovia clupeoides*
122	*Diapterus olisthostomus*	251	*Ophiothrix* sp.	380	*Anchoa tricolor*
123	*Diapterus rhombeus*	252	Ophiuroidea	381	*Cetengraulis edentulus*
124	*Diopatra* sp.	253	*Opisthonema oglinum*	382	*Bairdiella ronchus*
125	*Diplodonta* sp.	254	Orbiniidae	383	*Scomberomorus brasiliensis*
126	*Diploneis bombus*	255	Decapoda	384	*Anchoa spinifer*
127	*Dissodactylus crinitichelis*	256	Ostracoda	385	*Mytilus falcata*
128	*Dorvillea* sp.	257	*Paguristes triangulopsis*		
129	*Echinaster echinophorus*	258	*Pagurus criniticornis*		

Características da rede

A rede trófica da área estudada foi formada por 480 nós, que inclui predadores e presas, com um total de 2.519 interações (Figura 2). O número de interações médias por espécie ou Densidade (D) foi de 5,25 e o valor de Conectância Direta (C) foi de 0,01. Com relação ao grau do nó (D), Detritos foi o nó que obteve valor mais elevado (nó: 120; D: 151), seguido por Crustacea (nó: 107; D = 100), Fitoplâncton (nó: 150; D = 98), Material vegetal (nó: 356; D = 95) e Pesca (nó: 361; D = 64). *Hypanus guttatus* aparece entre os 20 componentes tróficos com valor de grau do nó mais elevados (nó: 177; D = 37), sendo a décima segunda entre os nós (Tabela 2).

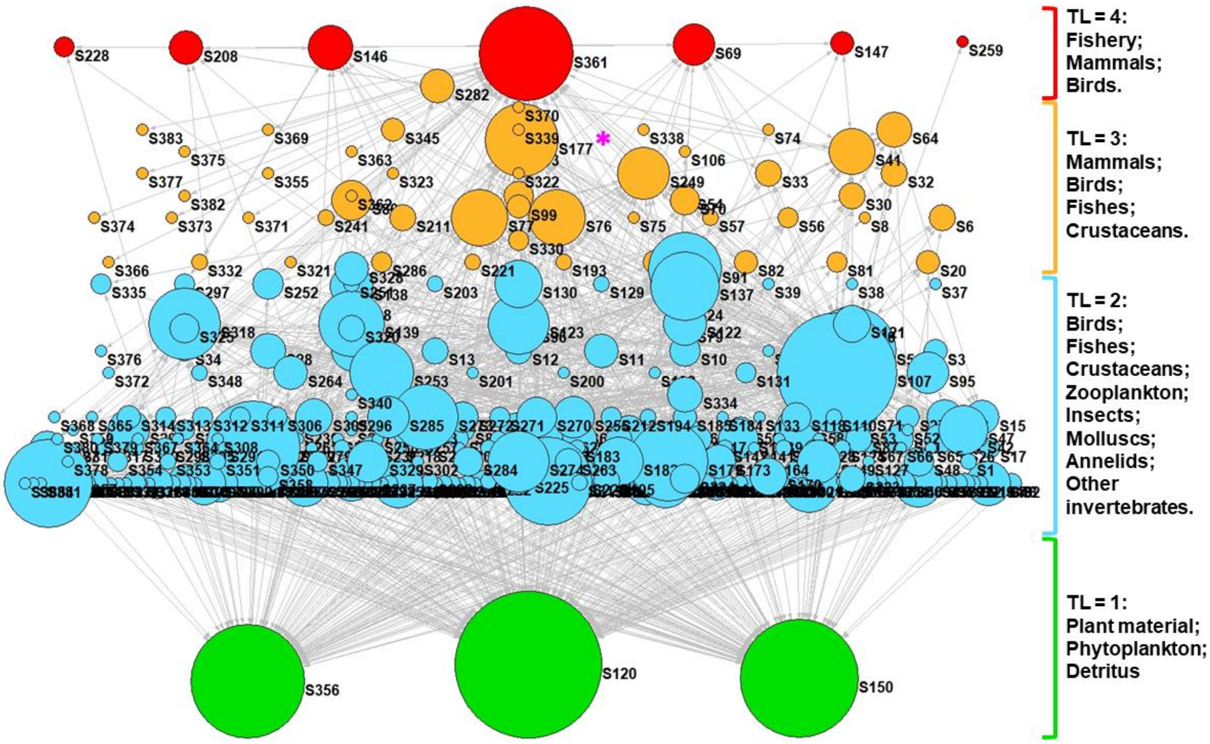

Figure 2. Rede topológica do estuário do Canal de Santa Cruz, Pernambuco, Brazil. O tamanho do nó é proporcional ao Grau do Nó (D). A cor do nó indica o nível trófico (verde ≤ 1; azul = 2 a 2,9; laranja = de 3 a 3,9; vermelho ≥ 4). O nó S177 (*Hypanus guttatus*) está destacado com um asterisco rosa (*).

Índices de centralidade

Sobre o índice de intermediação ou *"Betweenness centrality"* (BC), Detritos também obteve o maior valor (nó: 120; BC = 27.502.137), seguido por Crustácea (nó: 107; BC = 11.493.251), Fitoplâncton (nó: 150; D = 10.334.181), Pesca (nó: 361; D = 10.213.311) e Material vegetal (nó: 356; D = 8.132.210). Neste índice *H. guttatus* ocupa a posição 14 (nó: 177; BC = 1.964.127) (Tabela 2).

Tabela 2. Grau do nó (D), índice de intermediação (BC) e índice de proximidade (CC) dos 20 primeiros componentes da rede trófica do estuário do Canal de Santa Cruz, Pernambuco, Brasil. *Hypanus guttatus* está destacada em negrito.

Posição	Nó	Componente trófico	Grau do nó (D)	Nó	Componente trófico	Índice de intermediação (BC)	Nó	Componente trófico	Índice de proximidade (CC)
1	120	Detritos	151	120	Detritos	27,502,137	200	*Luidia clathrata*	1,364,000
2	107	Crustacea	100	107	Crustacea	11,493,251	201	*Luidia senegalensis*	1,364,000
3	150	Fitoplancton	98	150	Fitoplancton	10,334,181	164	Gymnolaemata	1,350,000
4	356	Material vegetal	95	361	Fishery	10,213,311	351	*Turbonilla* sp.	1,341,000
5	361	Fishery	64	356	Material vegetal	8,132,210	154	*Frikea lewisiana*	1,340,000
6	289	Polychaeta	63	5	Actinopterygii (teleostei)	5,430,973	185	*Leander paulensis*	1,340,000
7	5	Actinopterygii (teleostei)	55	137	*Eucinostomus argenteus*	4,445,053	126	*Diploneis bombus*	1,332,000
8	360	Zooplâncton	55	179	Insecta	4,159,631	217	*Microphrys antillensis*	1,321,000
9	220	Mollusca	54	289	Polychaeta	4,100,251	244	*Ogyrides* sp.	1,309,000
10	179	Insecta	44	220	Mollusca	3,831,177	260	*Panopeus lacustris*	1,309,000
11	91	*Citharichthys spilopterus*	38	91	*Citharichthys spilopterus*	3,345,058	71	Caridea	1,301,000
12	177	***Hypanus guttatus***	37	273	*Penaeus subtilis*	2,258,881	192	*Lile piquitinga*	1,287,000
13	318	*Sphoeroides testudineus*	36	318	*Sphoeroides testudineus*	2,176,468	287	*Poecilia vivipara*	1,267,000
14	137	*Eucinostomus argenteus*	33	177	***Hypanus guttatus***	1,964,127	136	*Erichthonius hunteri*	1,254,000
15	139	*Eugerres brasilianus*	30	139	*Eugerres brasilianus*	1,884,971	184	*Latreutes parvulus*	1,254,000
16	273	*Penaeus subtilis*	30	253	*Opisthonema oglinum*	1,877,911	243	*Ogyrides alphaerostris*	1,254,000
17	253	*Opisthonema oglinum*	29	360	Zooplâncton	1,659,359	296	*Processa* sp.	1,254,000
18	123	*Diapterus rhombeus*	26	274	*Periclimenes longicaudatus*	1,428,877	308	*Salmoneus ortmanni*	1,254,000
19	274	*Periclimenes longicaudatus*	26	58	*Callinectes danae*	1,131,534	301	*Pugilina morio*	1,244,000
20	100	Copepoda	24	76	*Centropomus parallelus*	1,063,972	302	Pycnogonida	1,241,000

Em relação ao índice de proximidade ou Closeness centrality (CC), *H. guttatus* ocupou a posição 322 (nó: 177; CC = 926), sendo as primeiras posições ocupadas por espécies de invertebrados bentônicos, algas e fitoplâncton: *Luidia clathrata* (nó: 200; CC = 1.364.000); *Luidia senegalensis* (nó: 201; CC = 1.364.000); Gymnolaemata (nó: 164; CC = 1.350.000); *Turbonilla* sp. (nó: 351; CC = 1.341.000) e *Frikea lewisiana* (nó: 154; CC = 1.340.000) (Tabela 2).

Key Player Problem (KPP)

Na análise de espécies-chave foram identificadas sete nós com potencial para atuar como espécie chave na teia analisada. Com o critério de fragmentação (KPP1), Detritos, *Eucinostomus argenteus* e Pesca foram os nós responsáveis por gerar o máximo de fragmentação na rede, com um índice de fragmentação de 0.39. Enquanto com o critério de fragmentação ponderada por distância (KPP1), Detritos, Material vegetal e Pesca causaram mais fragmentações, com um índice de não-coesão de 0.76.

Tabela 3. Conjuntos de espécies chave identificadas para a rede trófica do estuário do Canal de Santa Cruz, Pernambuco, Brasil: KPP1 = método de fragmentação; KPP2 = método de distanciamento.

KPP1	**Critério de fragmentação**		
	Componente	**Fragmentação**	**Heterogeneidade**
	Detritos	0.393	0.392
	Eucinostomus argenteus		
	Pesca		
	Critério de fragmentação ponderada por distância		
	Componente	**Índice de não-coesão**	
	Detritos	0.759	
	Material vegetal		
	Pesca		
KPP2	**Vários tamanhos de grupo**		
	Componente	**Nº de espécies atingidas**	**% de espécies atingidas**
	Actinopterygii (teleostei)	374	97.1
	Crustacea	383	99.5
	Fitoplancton	385	100
	Critério de alcance ponderado por distância		
	Componente	**Índice de distância recíproca (%)**	
	Crustacea	84.5	
	Detritos		
	Fitoplancton		

Já no cenário de distanciamento (KPP2), os três nós que atingiram a maioria dos demais nós da rede com o número mínimo de passos foram Actinopterygii (teleostei), Crustacea e Fitoplâncton. No cenário de distanciamento com critério de alcance ponderado por distância (KKP2), Crustacea, Detritos e Fitoplâncton foram os nós que mais atingiram outros nós na teia, com um índice de distância recíproca de 84,5%.

DISCUSSÃO

Análises topológicas de redes tróficas vêm sendo cada vez mais utilizadas para tentar entender de forma detalhada o papel que os elasmobrânquios desempenham na formação da estrutura, dinâmica e estabilidade dos ecossistemas marinhos (Navia et al., 2010). Neste estudo, aplicamos esta abordagem para compreender a importância topológica estrutural de uma raia mesopredadora demersal em uma rede trófica estuarina onde ela é a única espécie de elasmobrânquio de ocorrência constante no ambiente.

Com relação à Densidade (D) e Conectância (C) da teia, os valores encontrados neste trabalho foram similares aos descritos para diversas teias alimentares marinhas e fluviais (Bornatowski et al., 2014; Dunne et al., 2002; Gaichas and Francis, 2008; Márquez Velásquez, 2017; Navia, 2013), sugerindo se tratar de uma teia alimentar de alta complexidade estrutural. A raia *H. guttatus* se posicionou entre as 20 espécies mais importantes com relação aos valores de Grau do nó (D) e índice de Intermediação (BC), mas não em relação ao Índice de proximidade (CC). Estes resultados são reflexo de sua dieta generalista e oportunista (Queiroz et al., 2022) que confere a este predador 37 conexões na rede trófica analisada. Desse modo, a remoção de *H. guttatus* do sistema pode afetar a rede de forma rápida e com ampla disseminação, dispersando os efeitos a outros nós principalmente de forma indireta.

Em um estudo similar realizado em uma área marinha costeira próxima ao da presente pesquisa, raias predadoras de níveis tróficos intermediários e com hábitos alimentares similares a *H. guttatus*, apresentaram grande importância estrutural para a rede trófica analisada, se destacando-se entre os índices de importância topológica e de espécies chave (Bornatowski et al., 2014). Isso não foi observado para *H. guttatus* neste estudo, que não aparece entre os 20 nós mais importantes do índice de proximidade (CC), nem entre as três espécies chave do sistema em nenhum dos cenários analisados. É importante mencionar que, na rede trófica analisada por Bornatowski et al., (2014), diferentes espécies de tubarões atuam como predadores de topo de grande importância estrutural, predando inclusive raias, o que não ocorre na rede analisada neste estudo.

Por outro lado, com um cenário parecido com o da presente pesquisa, (Márquez-Velásquez, 2017) estudou a importância topológica de *Potamotrygon magdalenae* (Duméril, 1865) em uma rede trófica fluvial, onde a espécie não possui predadores naturais e, apesar de *P. magdalenae* apresentar valores altos de Grau do nó (D) e índice de Intermediação (BC), e valor intermediário do Índice de proximidade (CC), não se destacou entre as espécies chave do sistema. Tais resultados são atribuídos à ausência de predadores naturais para a espécie, com *P. magdalenae* promovendo apenas efeitos “top-down”, sem participação nos efeitos “bottom-up”, o que limita sua intervenção na dispersão de efeitos indiretos aos demais componentes de rede (Márquez-Velásquez, 2017).

Nossos resultados também mostram a ausência de predadores naturais para *H. guttatus* no estuário do Canal de Santa Cruz, tendo apenas a pesca atuando como predador da espécie no sistema. Desse modo, apesar de apresentar um número alto de interações na rede trófica, a maioria dos efeitos promovidos pela espécie são do tipo “top-down”, e sua única interação com um predador (pesca) limita a transmissão de efeitos “bottom-up”. Outro ponto é que, apesar de ser uma raia generalista e com alta amplitude de nicho alimentar, sua habilidade de cavar e acessar presas infaunais tornam *H. guttatus* um predador essencialmente bentônico (Carqueija et al., 1995; Löwemark, 2015; Queiroz et al., 2022; Takeuchi and Tamaki, 2014), concentrando e limitando seus efeitos sobre espécies associadas ao substrato. Neste sentido, *H. guttatus* promove um controle nas comunidades bentônicas análogo aos predadores de topo em redes alimentares.

Diversos trabalhos evidenciam a importância de espécies de elasmobrânquios na manutenção e estrutura de redes tróficas, especialmente tubarões predadores de topo. Teoriza-se, inclusive, a possibilidade de ocupação de níveis tróficos mais altos (de predador intermediário a predador de topo) por elasmobrânquios mesopredadores em cenários de desaparecimento dos tubarões predadores de topo (Bornatowski et al., 2014b; Ferretti et al., 2010; Frid et al., 2008; Heithaus et al., 2008; Myers et al., 2007; Ruppert et al., 2013). Contudo, diferente da rede trófica do Canal de Santa Cruz, todos estes trabalhos estudaram ambientes que apresentam múltiplas espécies de elasmobrânquios, distribuídos em diferentes níveis da rede e exercendo diferentes funções ecológicas. Portanto, é improvável que *H. guttatus* seja capaz de atuar como predador de topo ou espécie chave em um cenário de desaparecimento dos predadores de topo da rede trófica do Canal de Santa Cruz. Outro fator que reforça esta hipótese é a ausência de redundância ecológica entre *H. guttatus* e outros mesopredadores da rede analisada (Navia, 2013).

Eucinostomus argenteus (Baird and Girard, 1855) foi a única espécie entre os conjuntos de nós chave do sistema, apresentando um total de 33 interações na rede trófica, sendo 28 delas com presas e outras cinco com predadores. Com uma dieta onívora que inclui peixes, crustáceos, moluscos, poliquetas, nematoides, material vegetal e detritos (Leão, 2016), *E. argenteus* não se destaca entre os índices de grau do nó (D), intermediação (D) ou proximidade (CC), mas faz conexão com a maioria dos nós mais importantes do sistema, o que explica a inclusão da espécie entre os nós capazes de gerar maior fragmentação na rede (KPP1). Detritos, pesca e vegetal também estão entre os nós chave do sistema, uma vez que representam a base e o topo da rede. Já no cenário de expansão (KPP2), os nós Actinopterygii e Crustacea são capazes de aumentar as distâncias médias do maior número de pares de nós da teia. Ambos são nós centrais, de posição trófica intermediária e, dessa forma, são responsáveis por ligar predadores de níveis tróficos superiores à base da rede alimentar. Já os nós Detritos e Fitoplancton são responsáveis, principalmente, por desconectar completamente outros nós da rede.

Ambos os cenários de avaliação de espécies chave contrastam com resultados encontrados por (Navia, 2013) para a região centro sul do Oceano Pacífico colombiano, onde os nós chave do sistema foram representados predominantemente por nós intermediários, incluindo espécies de elasmobrânquios. Neste sistema, a remoção dos predadores de topo fragilizaria a rede, tornando-a mais vulnerável a efeitos antrópicos, por exemplo. Contudo, do ponto de vista estrutural, a eliminação de predadores de topo não produziria efeitos importantes sobre os mesopredadores, uma vez que os papéis dessas espécies são desempenhados por um amplo conjunto de espécies, redundantes entre si (Navia, 2013).

Detritos foi o nó com maiores valores de Grau do nó (D) e índice de Intermediação (BC), além de aparecer em três dos quatro cenários de espécies chave analisados. Se tratando de um ambiente estuarino onde a decomposição de matéria orgânica é uma das principais fontes alimentares para os consumidores primários (Giarrizzo et al., 2011; Pelage et al., 2022), este nó se conecta de forma direta ou indiretamente à maioria dos demais nós da teia. Estes resultados evidenciam a importância destes ambientes transicionais no suprimento de matéria orgânica para diversas teias alimentares aquáticas. Não à toa, estes ambientes são amplamente classificados como áreas de berçário para diversas espécies (Heupel et al., 2019; Swift and Portnoy, 2021; Vasconcelos et al., 2011; Whitfield, 2020).

Apesar de sua importância, os ambientes marinhos costeiros e estuarinos vêm sendo impactados pelas atividades humanas ao longo das décadas, comprometendo sua integridade ecológica a longo prazo. Entre os fatores que mais afetam estes ambientes estão o rápido crescimento populacional humano, a poluição, a perda e alteração do habitat e a pesca. Segundo Kennish (2002), tendências sugerem que até 2025 os estuários serão significativamente afetados pela perda e alteração de habitat associada a uma crescente população humana costeira em muitas regiões do mundo. A qualidade da água nos estuários, particularmente nos sistemas urbanizados, também é frequentemente comprometida pela sobrecarga de nutrientes e matéria orgânica de fontes terrestres, o influxo de patógenos e o acúmulo de contaminantes químicos (Chi et al., 2018; Kennish, 2002). As atividades pesqueiras nestas áreas também têm impactado o ambiente, seja de forma direta, afetando as populações de vertebrados e invertebrados alvos da pesca ou capturados como *bycatch*; ou de forma indireta, quando o declínio de espécies pescadas cria efeitos de cascata que afetam as populações de espécies não pescadas (Defeo and de Alava, 1995; DeMaster et al., 2001; Dulvy et al., 2004; Williams et al., 2008).

Nestes ambientes, as atividades pesqueiras exercem um forte controle sobre populações de espécies capturadas, atuando como um predador de topo supergeneralista, amplificado pela diversidade e intensidade das pescarias locais. Por isso, a inclusão da pesca como um nó em abordagens de análises de teias tróficas possibilita, desde uma visão mais ampla e realista da estrutura da teia, à compreensão do nível de influência e controle que as atividades pesqueiras exercem nas mais diversas teias aquáticas (Ávila-Thieme et al., 2021; Dunne et al., 2016; Glaum et al., 2020; Márquez-Velásquez et al., 2021; Pérez-Matus et al., 2017). No ambiente estuarino de Canal de Santa Cruz, a pesca foi o predador com o maior número de interações (64), se destacando entre os cinco nós mais importantes quanto ao Grau do Nó (D) e Índice de Intermediação (BC), em outras palavras, é um dos principais nós capazes de afetar a rede de forma rápida e com ampla disseminação. Além disso, a pesca também se destaca entre os três nós-chave do sistema capazes de gerar maior fragmentação na rede (KPP1), sendo o único predador de topo da rede analisada classificado como nó-chave. Esta abordagem topológica mostra a importância do manejo pesqueiro na preservação das espécies aquáticas e do ecossistema, baseando-se principalmente no grau de controle e influência que a pesca exerce sobre a estabilidade da teia trófica. Por isso, um manejo eficiente das atividades pesqueiras na região é essencial para garantir o equilíbrio do ecossistema e, consequentemente, a manutenção da segurança alimentar e econômica das comunidades que dependem destes recursos.

CONCLUSÕES

A rede trófica do Canal de Santa Cruz é um sistema de alta complexidade estrutural. Nesta rede, a raia *H. guttatus* é capaz de dispersar efeitos indiretos a outros nós de forma rápida e com ampla dispersão. Contudo, apesar de ser um predador generalista e com alta amplitude de nicho alimentar, sua alimentação essencialmente bentônica e ausência de múltiplos predadores naturais limitam seus efeitos sobre espécies associadas ao substrato (top-down). Assim, apesar de ser o único elasmobrânquio na rede analisada, *H. guttatus* não foi classificada como espécie-chave em nenhum dos cenários de fragmentação ou expansão.

O peixe *E. argenteus* foi a única espécie entre os conjuntos de nós chave do sistema, principalmente por fazer conexão com a maioria dos nós mais importantes da rede. Detritos, Pesca e Material vegetal também estão entre os nós chave do sistema capazes de gerar maior fragmentação na rede (KPP1). Já no cenário de expansão (KPP2), os nós Actinopterygii e Crustacea são capazes de aumentar as distâncias médias do maior número de pares, enquanto Detritos e Fitoplancton são responsáveis, principalmente, por desconectar completamente outros nós da rede. Detritos se destacou entre os índices de Grau do nó (D) e índice de Intermediação (BC), além de aparecer em três dos quatro cenários de nós-chave analisados, reforçando a importância do suprimento estuarino de matéria orgânica na manutenção de redes alimentares aquáticas.

A pesca atua como predador de topo na rede trófica do estuário do Canal de Santa Cruz, sendo um dos principais nós capazes de afetar a rede de forma rápida e com ampla disseminação e de gerar maior fragmentação (KPP1). Um maior conhecimento da atividade pesqueira da região é altamente recomendado, tendo em vista a influência que a pesca exerce sobre a estabilidade da teia trófica deste ambiente.

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CONSIDERAÇÕES FINAIS

Aqui, descrevemos a dieta, nível trófico, uso de habitat e importância topológica de raias demersais simpátricas através da combinação de análises de conteúdo estomacal, isótopos estáveis de tecido muscular ($\delta^{13}C$ e $\delta^{15}N$), microquímica de vértebras (^{24}Mg, ^{43}Ca, ^{55}Mn, ^{86}Sr e ^{138}Ba) e modelagem de teia trófica.

Embora ocorram nas mesmas áreas, as arraias *Hypanus guttatus*, *H. marianae* e *H. berthalutzae* exploram os mesmos ambientes e recursos de maneira singular ao longo de seus ciclos de vida. Apesar desta sobreposição na dieta e no habitat, a redundância funcional é improvável, uma vez que cada espécie mostrou hábitos alimentares, níveis tróficos e espacializações distintas. Nossos dados indicam conectividade entre os ambientes estuarino e marinho mediada por *H. guttatus* ao longo de seu ciclo de vida, enquanto *H. marianae* e *H. berthalutzae* apresentaram nichos isotópicos mais semelhantes. Os habitats ocupados e as relações predador-presa para essas duas espécies são fatores que as tornam mais próximas quando comparadas a *H. guttatus*. As três espécies de *Hypanus* estudadas neste trabalho desempenham papéis ecológicos únicos como predadores nos ecossistemas bentônicos.

Embora esta abordagem seja nova em espécies de elasmobrânquios, no geral, a combinação de isótopos estáveis e dados microquímicos representou um bom descritor dos diferentes habitats em que as espécies foram encontradas. Reforçamos que a aplicação de ambas as técnicas em conjunto permite uma melhor compreensão dos padrões de uso do habitat e o estabelecimento de conclusões mais robustas. A informação aqui apresentada suporta a hipótese de múltiplos movimentos entre ambientes estuarino e marinho para *H. guttatus* ao longo do seu ciclo de vida. Reforçamos também a especificidade do habitat de *H. marianae*, que pode não explorar áreas mais profundas ou estuarinas, limitando-se a zonas costeiras e rasas. Ao contrário, *H. berthalutzae* mostrou potencial para explorar águas mais profundas, principalmente na fase adulta.

Dentre as três espécies aqui estudadas, *H. berthalutzae* é a única encontrada em regiões insulares como o arquipélago de Fernando de Noronha e o Atol das Rocas. Embora sugiramos movimentos longitudinais limitados para as três espécies, considerando a afinidade de *H. berthalutazae* por águas mais profundas, é provável que exista alguma taxa de conectividade entre indivíduos de regiões insulares e aqueles da costa brasileira. Portanto, destacamos a necessidade de manejo de cada espécie separadamente e que as ações de manejo para *H. guttatus* devem focar em habitats estuarinos e costeiros rasos, enquanto as de *H. marianae* devem focar na pesca recifal, e para *H. berthalutzae* devem focar em uma área mais ampla da plataforma continental que se estende até águas mais profundas (> 100 m).

A rede trófica do Canal de Santa Cruz é um sistema de alta complexidade estrutural. Nesta rede, a raia *H. guttatus* é capaz de dispersar efeitos indiretos a outros nós de forma rápida e com ampla dispersão. Contudo, apesar de ser um predador generalista e com alta amplitude de nicho alimentar, sua alimentação essencialmente bentônica e ausência de múltiplos predadores naturais limitam seus efeitos sobre espécies associadas ao substrato (*top-down*). Assim, apesar de ser o único elasmobrânquio na rede analisada, *H. guttatus* não foi classificada como espécie-chave em nenhum dos cenários de fragmentação ou expansão.

O peixe *Eucinostomus argenteus* foi a única espécie entre os conjuntos de nós chave do sistema, principalmente por fazer conexão com a maioria dos nós mais importantes da rede. Detritos, Pesca e Material vegetal também estão entre os nós chave do sistema capazes de gerar maior fragmentação na rede (KPP1). Já no cenário de expansão (KPP2), os nós Actinopterygii e Crustacea são capazes de aumentar as distâncias médias do maior número de pares, enquanto Detritos e Fitoplancton são responsáveis, principalmente, por desconectar completamente outros nós da rede. Detritos se destacou entre os índices de Grau do nó (D) e índice de Intermediação (BC), além de aparecer em três dos quatro cenários de nós-chave analisados, reforçando a importância do suprimento estuarino de matéria orgânica na manutenção de redes alimentares aquáticas.

A pesca atua como predador de topo na rede trófica do estuário do Canal de Santa Cruz, sendo um dos principais nós capazes de afetar a rede de forma rápida e com ampla disseminação e de gerar maior fragmentação (KPP1). Um maior conhecimento da atividade pesqueira da região é altamente recomendado, tendo em vista a influência que a pesca exerce sobre a estabilidade da teia trófica deste ambiente.

As raias *H. guttatus*, *H. marianae* e *H. berthaluzae* já eram tradicionalmente capturadas por diferentes artes de pesca que operam ao longo da costa brasileira, mas o surgimento de pescarias direcionadas a Myliobatiformes e o desenvolvimento de novas técnicas de pesca aumentaram ainda mais as capturas dessas arraias, especialmente *H. guttatus*. No entanto, há uma escassez de dados pesqueiros disponíveis para essas espécies no Brasil, principalmente devido à falta de regulamentação ou monitoramento da pesca. O que torna os dados apresentados neste estudo de grande relevância, não só pela escassez de estudos deste tipo para estas espécies como também pela importância deste tipo de informação para auxiliar a implementação de futuras propostas de manejo, principalmente as informações sobre uso do habitat discutido nos manuscritos.

Em 2019, meses após o término das amostragens do presente estudo, milhares de toneladas de resíduos de petróleo bruto contaminaram aproximadamente 9.000km² de ecossistemas costeiros do Nordeste brasileiro, sudoeste do Atlântico, transformando-se no maior desastre ambiental da história do país. Manchas de óleo foram encontradas em vários habitats ocupados por *H. guttatus e H. berthalutzae*, e praticamente toda a área de ocorrência de *H. marianae*. Seus impactos potenciais para a fauna local ainda não são bem compreendidos. Nesse aspecto, nosso trabalho oferece informações prévias ao evento que podem servir de comparação para trabalhos futuros que pretendam avaliar o impacto desse evento na fauna local, incluindo espécies endêmicas.

APÊNDICE A - JFB INSTRUCTIONS FOR AUTHORS

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Contents

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The title page must contain the following information:

3.1.1.1. **Title of the paper**, which should be short, informative and avoid any geographical or regional references, unless they are fundamental to the scientific thrust of the paper. If a species name is used in the title, we require a common name (if available) followed by the full scientific name. Avoid the use of abbreviations unless they include the name of a group that is best known by its acronym (e.g., CONSORT statement). See Wiley's tips for search engine optimization: **https://authorservices.wiley.com/author-resources/Journal-Authors/Prepare/writing-for-seo.html**;
3.1.1.2. **The family (or formal) name** by which each author is known plus the **given or familiar names** and any initials (see Section 6 for criteria on author eligibility);
3.1.1.3. The **address in full of each author's primary affiliation** (research institute, university, city, state/province, country) as a numbered list below the Author list;
3.1.1.4. The **corresponding author's name, full postal address** and **email address**.
3.1.1.5 An author's **current address** can be listed here if different from that at the head

of the page.
3.1.1.6 **Funding Information** is listed here.
3.1.1.7 **Joint first and/or senior authorship** can be indicated by stating in a footnote that 'X and Y should be considered joint first author' or '… made an equal contribution to this work'.

3.1.2 Abstract and Key Words

The **Abstract** must be a concise and accurate summary of the **significant findings** of the paper without any introductory or contextual information. Abstracts should not be structured with headings. Methods can be identified only as part of a result (e.g., Respirometry revealed that exercise increased…; GWAS identified a significant number of SNPs…). A species name in the Abstract appears as in the title, a common name (if available) followed by the full scientific name.

Provide a list of up to 6 descriptive **Key Words** (maximum 100 characters) in alphabetical order. Specific geographical (e.g., Baffin Island, Amazon Basin) or regional references (e.g., south-east Asia) can be included here. Keywords are listed underneath the abstract and separated by commas.

3.1.3 Introduction

The **Introduction** alerts readers to literature relevant to the research discovery so that the originality of the research cannot be easily assigned. Also, the Introduction must state the intent of the research in the form of a research question or hypothesis so that no confusion arises as to what advance in fish biology is being sought. Footnotes to the text are not allowed.

3.1.3.1 **Text citations of references:** we recommend that you use the style "author, date" and multiple references are list in alphabetical order. However Wiley will update the formatting for you into journal style when your manuscript is accepted for publication.

For example: '…as demonstrated by McKenzie (2001) and by McKenzie and Farrell (2010)'; '…as suggested previously in some works (Sloman, 2010), but not others (McKenzie and Farrell, 2010)'; '…consistent with earlier studies (Blaber, 1975, 1988; Lujan, 2011a,b; Prodöhl, 1988)'. Three or more authors are cited with the name of the first author followed by et al. (in italics): e.g., (Sloman et al., 2002) or Sloman et al. (2002). Authors sharing the same surname and year of publication are distinguished by their initials: e.g., (Young, L., 2012; Young, T., 2012).

3.1.4 Materials and Methods

The **Materials and Methods** may contain up to two levels of sub-headings and must provide sufficient detail so that the work can be replicated by others. Established methods can be simply referenced, preferably acknowledging the original work (rather than a recent user of that method), even if minor methodological changes were made (which should be described). Materials and Methods must also include information on how observations were analysed to derive the quantitative results. Statistics should be based on independent biological samples. Technical replicates should be averaged before statistical treatment and not used to calculate deviation parameters. In the case of multiple comparisons (e.g., microarray data), the probability of false positives should be considered in the analysis. Journal style is for citations to tables, figures, and equations to becapitalized and not contracted (e.g., Table 1, Figure 3, Equation 5). Parts of figure should be in lowercase (a), (b), etc., in legend as well as in the figure. For example: Figure 1; Figure 2a; Figure 1a–c; Figures 2a–d and 5.

3.1.5 Results

The **Results** section presents a concise and accurate description of the results of the research. It may contain up to two levels of sub-headings. Figures and Tables, which are numbered consecutively in order of their mention in the text, increase the clarity and conciseness of the result presentation; excessive duplication of material in text, figures and tables is not permitted. All statements concerning quantitative differences between experimental conditions require quantitative data and adequate statistical treatment. The deviation parameter, the number of biological samples and the statistical procedures should be provided for each dataset either in the main text or as part of a Figure or Table.

3.1.6 Discussion

The **Discussion**, which may contain up to two levels of sub-headings, places the results of the study into a broader context so that the significance, quality and novelty of the work can be established with respect to existing literature. The Discussion should directly address the original research question or hypothesis, as stated in the Introduction. Excessive repetition of results is not permitted. The potential for future work or a brief perspective on the findings can be included.

3.1.7 Acknowledgements

Contributions from anyone who does not meet the criteria for authorship should be listed here. Thanks to editors and anonymous reviewers are not appropriate. Authors are responsible for the accuracy of their funder designation. If in doubt, please check the Open Funder Registry for the correct nomenclature: **https://www.crossref.org/services/funder-registry/**

3.1.8 Contributions

The contributions of each author, including ideas, data generation, data analysis, manuscript preparation and funding, must be listed here using their initials only, e.g., V. T. F..

3.1.9 References

All published citations mentioned in the text, tables or figures must be listed in the reference list, which includes all key elements of each reference, including the names of journals in full (not abbreviated). Authors are responsible for checking the accuracy of their references. *JFB* uses APA style referencing and examples of APA style are shown below. However, references may be submitted in any style or format, as long as it is consistent throughout the manuscript. Wiley will update the formatting of references to APA style for you when your manuscript is accepted for publication.

Journal Article:

Flowers, K. I., Henderson, A. C., Lupton, J. L., & Chapman, D. D. (2017). Site affinity of whitespotted eagle rays *Aetobatus narinari* assessed using photographic identification. *Journal of Fish Biology*, **91**, 1337– 1349 Gill, A. B. (2003).

Gill, A. B. (2003). The dynamics of prey choice in fish: The importance for prey size and satiation. *Journal of Fish Biology*, **63**, 105– 116

Online Article Not Yet Published in an Issue:

Mussen, T. D., & Cech Jr, J. J. (2018). Assessing the use of vibrations and strobe lights at fish screens as enhanced deterrents for two estuarine fishes. Advance online publication. https://doi.org/10.1111/jfb.13776

An online article is cited by its Digital Object Identifier (DOI), which remains valid and allows article tracking even after its allocation to an issue. It has no volume, issue or page numbers.

Book:

Halver, J. E., & Hardy, R. W. (2002). *Fish nutrition*. San Diego, CA: Academic Press

Chapter in a Book:

Mench, J. A., & Mason, G. J. (1997). Behaviour. In M. C. Appleby & B. O. Hughes (Eds.), *Animal Welfare* (pp. 127– 142). New York, NY: CAB International

Docotral Thesis: These must have a permanent record of where they are held (e.g., thesis has been lodged at the individual's University or Institution Library as a permanent addition to the collection there), e.g., Al-Badran, A. A. (1987). *Factors influencing river bank stability in the Tigris and Shatt Al- Arab water ways, Iraq* (Doctoral thesis, University of Dundee, UK). Retrieved from **https://ethos.bl.uk/Home.do;jsessionid=DA702A005B56B131D4E776CD6A605544**

Master's Thesis: These must be readily available electronically and the URL provided, e.g., Cox, G. K. (2010). Anoxic survival and cardiovascular responses of the Pacific hagfish, Eptatretus stoutii (Masters thesis). Available from UBC Library **https://open.library.ubc.ca/** .

Electronic References: These include references not subject to peer review and formal publication and can be set out as shown given below. ICES (2016). Report of the Baltic salmon and trout assessment working group (WGBAST). ICES CM 2016/ACOM:09. Available at:

http://ices.dk/sites/pub/Publication%20Reports/Expert%20Group%20Report/acom/2016/WGBAST/wgbast_2016.pdf

Marshall, A., Bennett, M. B., Kodja, G., Hinojosa-Alvarez, S., Galvan-Magana, F., Harding, M., Stevens, G. & Kashiwagi, T. (2011). *Manta birostris*. In *IUCN Red List of Threatened Species* Version 2013.2. Available at http://www.iucnredlist.org/details/198921/0 (last accessed 9 December 2013).

3.1.10 Tables

Tables complement but do not duplicate information contained in the text. Tables should contain **no** vertical lines and are numbered consecutively in order of appearance in the text. The table caption is concise and descriptive, and understandable without reference to the main text. It includes the full scientific name(s) of the species to which the table relates. Statistical measures, such as SD or SE, should be identified in the caption. Dimensions for the units should appear in parentheses in the column headings and not in the legend or body of the table. All abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶, should be used (in that order) and *, **, *** should be reserved for P-values.

3.1.11 Figures

Figures complement information contained in the text, but without unnecessary duplication. Figures that contain data are intended to accurately, clearly and concisely represent the research results, while other figures may better orientate the reader, e.g., maps.

3.1.11.1 **Preparing Figures** Figures are submitted in digital format. Figures are numbered consecutively in order of appearance in the text. A wide variety of formats, sizes and resolutions of high quality figures are accepted for initial peer review. More information is found at: **https://authorservices.wiley.com/asset/photos/electronic_artwork_guidelines.pdf** **Line artwork** (vector graphics) are prepared in black and white with shades of grey, unless colour is essential for clarity. Error bars must be included and the method used to derive them explained in the caption. Line artwork must be saved as Encapsulated PostScript (EPS) file.

Photographs should illustrate something that cannot adequately be displayed in any other manner. Electron and light microscope photographs must embed a magnification as a **scale bar**. Staining techniques should be described in the caption. Photographs must be saved as bitmap files (half-tones or photographic images) as Tagged Image Format (TIFF) file. **Maps and charts** should be contained within a frame and show either a latitude and longitude or a single co-ordinate (N, S, E or W). *JFB* use The Times Concise Atlas of the World. London: Times Books as its standard for geographical names, countries, seas, rivers, etc.

3.1.11.2 **Figure captions**

A Figure caption is a concise and self-contained description of the figure that can be understood without reference to the main text. They begin with a short title for the figure, which **include the full scientific name(s) of the species** to which the illustration relates. Any lines fitted through data points in the figure must be statistically significant and be supported by the mathematical equation and statistical information (P-values and R2 or R values). Keys to the symbols, formulae and regression values can be included in the figure itself or the caption, but not both. The minimum reduction for a figure may be indicated. If material has previously been published, authors must obtain permission from the copyright owner (usually the publisher) to use such material and cite the author in the caption (or text), e.g., ‘Reproduced with permission from Blaber (1975).’. (This requirement also applies to the reproduction of a previously published Table or an extended quotation from material.)

3.1.12 Supporting Information

When appropriate, submissions may include **Supporting Information** specifically files containing videos and animations, and long datasets, tables and figures. Supporting Information contains information that is not essential to the article but is a valuable addition by providing greater depth and background. Supporting Information will be reviewed, will appear without typesetting and be hosted only online. The availability of Supporting Information is indicated in the main text after the Acknowledgements, headed “Supporting Information”. Short captions list the titles of all supporting material. Supporting Information should be supplied as separate files, and not incorporated into the main manuscript text file. Wiley’s FAQs on Supporting Information is found at: **https://authorservices.wiley.com/author-resources/Journal-Authors/Prepare/manuscript-preparation-guidelines.html/supporting-information.html**

3.2 Preparing a Brief Communication

A **Brief Communication** is confined to a **single point or issue of progress** such as an unusual occurrence, an interesting observation, a particularly topical and timely finding or an important technical advance. The point or issue must have relevance beyond the species or locality under consideration. First records should adhere to best practices proposed by Bello et al. (2014. A proposed best practice approach to overcome unverified and unverifiable "first records" in ichthyology. *Cybium* 38, 9-14) and should strive to aggregate and report regional historical records for the same species. *JFB* no longer considers short technical notes describing molecular markers (e.g., microsatellites). A **Brief Communication** is limited in length (**no more than 5 printed pages**; c. 2500 words of text) and normally includes no more than **one (multi-panel) figure and one table**. It follows the same format as Research Articles with respect to the Title, Authors and Affiliations, Abstract, Key Words, Statement of Significance, Acknowledgements and References (see Section 3.1), but the main text is written in freeform **without any headings**. The Abstract is **no more than 90 words.**

3.3 Preparing a Review Article

Prospective authors will submit a synopsis (two pages maximum) of their article to the Editor-in-Chief. The synopsis should outline why the review is topical, its main points and objectives, and how it will stimulate debate and research. When the proposal has been accepted, the authors will submit a manuscript within a mutually agreed upon time and page limit.

3.4 Preparing an Opinion Piece

An **Opinion Piece** presents a brief, personal view on a topical or emerging issue in fish biology that has broad readership appeal. It may be offered to or commissioned by the Editor-in-Chief. The submission includes a Title page, Main Text and References. It contains no Abstract or Key Words but can contain Tables or Figures. It will be peer reviewed.

3.5 Preparing a Comment to the Editor

Comments are no more than c. 750 words of text and deal with single significant finding or point for discussion concerning a recent published paper in *JFB* and needs rapid publication. The submission includes a Title page, Main Text and References (maximum four). It contains no Abstract, Key Words, Tables or Figures. After satisfactory peer review, it will be sent to the original corresponding author for a Reply. The reply will take the same form and will be peer reviewed. Publication will end the debate.

4. ETHICAL CONSIDERATIONS

4.1 Ethical considerations for the use of animals

JFB takes its responsibility towards animal welfare very seriously, whether it concerns fish collection, predator-prey interactions or invasive surgical procedures. At the same time *JFB* recognizes that permitting requirements for animal collections and animal welfare have regional differences and therefore may not be exactly the same as those stipulated in the United Kingdom, which is the home of *JFB*.

Therefore, when a research paper that involves animal experimentation or harm is submitted to *JFB*, authors are accepting and acknowledging that appropriate permits for animal collections and animal welfare issues were sought and approved by the local committee(s) responsible for such permits. If a submission is received from a country where no such permitting is required, then any decision with regards to ethics rests solely with the Editor-in-Chief, who will seek advice from the Editorial Team, referees and other qualified scientists as needed.

Furthermore, as specific evidence of the permitting, a clear ethical statement must be provided in the Materials and Methods under a subheading Ethical Statement for any submissions to the *JFB*. This statement may take a form similar to the following:

The care and use of experimental animals complied with [Insert the local or national body] animal welfare laws, guidelines and policies as approved by [Insert the local or national permitting authority and the permit reference number].

Independent of any such permits, the *JFB* still reserves the right to reject papers on an ethical basis should valid concerns emerge from the contents of the research paper.

Therefore, it is essential that within their ethical statement authors clearly identify any welfare implications arising from their experimental design including steps taken to minimise impact on fish welfare. Studies which may require additional information in the ethical statement include (but are not limited to) those where: fishes were collected as part of faunal surveys; experimental conditions caused severe distress or lasting harm to sentient fishes (e.g. predation studies, toxicity testing, disease trials); surgical procedures were used; sentient un-anaesthetised animals were subjected to chemical agents that induce neuromuscular blockade, such as muscle relaxants. In addition, ethical statements should say whether fishes were killed at the end of the experiment (e.g. for tissue sampling).

Ahead of submission, authors will benefit greatly from reading our Editorials on animal welfare: **http://onlinelibrary.wiley.com/doi/10.1111/j.0022-1112.2006.01035.x/full** (2006) and **http://onlinelibrary.wiley.com/doi/10.1111/j.1095-8649.2010.02900.x/full** (2011).

If the research did not involve animal experimentation or harm, and required no permits then no ethical statement is required.

4.2 Publication Ethics

The Fisheries Society of the British Isles (FSBI) considers that scientists should avoid research threatening the conservation status of any species of fish that is already regarded as threatened according to the IUCN Red List of Threatened Species and the associated current Red List Categories and Criteria (**http://www.iucnredlist.org/technical-documents/categories-and-criteria**) or which is listed as such in a Red Data Book appropriate to the country or geographical area concerned. In accordance with this view, papers based on such research will not be accepted, unless the work had clear conservation objectives.

4.3 Authorship

The list of authors should accurately illustrate who contributed to the work. Any person listed as an author, by definition, will have contributed substantially to the article’s conception and design, or acquisition of data, or analysis and interpretation of data. All listed authors will be contacted by email after a manuscript is submitted to confirm their contribution. Listed authors should meet the following criteria:

- Have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; given final approval of the version to be published and have participated sufficiently in the work to take public responsibility for appropriate portions of the content;
- Been involved in drafting the manuscript or revising it critically for important intellectual content; and
- Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Contributions from anyone who does not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgments section (for example, to recognize contributions from people who provided technical help, collation of data, writing assistance, acquisition of funding, or a department chairperson who provided general support). Prior to submitting the article all authors should agree on the order in which their names will be listed in the manuscript. (https://www.crossref.org/services/funder-registry/).

How individual authors specifically contributed to the work is listed in the **Contributions** statement (see Section 3.8).

5. EDITORIAL POLICIES AND JOURNAL STYLE

The following is general advice on formatting and style. Wiley will update the formatting for you into journal style when your manuscript is accepted for publication. As *JFB* serves an international community of fish biologists, some conventions are required that deviate from the APA style. For a full explanation of style requirements for *JFB*, with examples, please click **here.**

5.1 Abbreviations and acronyms:

All abbreviations and acronyms in the text and in all figure and table captions must be given in the fully expanded form on first mention and abbreviated thereafter, except for the small number of abbreviations and acronyms that are scientifically accepted, e.g., DNA. Useful resources are:

BSI (1967). *Recommendations for Letter Symbols, Signs and Abbreviations:* BS 1991, Part I. London: British Standards Institute.
Baron, D. N. (Ed.) (1977) *Units, Symbols and Abbreviations. A Guide for Biological and Medical Editors and Authors*, 3rd edn. London: The Royal Society of Medicine.

5.2 Units: Physical measurements only use metric units in accordance with the Systeme International d'Unites (SI), e.g., m, mm3, s (h and day are acceptable), g, m s−1, g l−1, mg l−1 (not ppm), J (not calories).
The 24-h clock is used for time of day, e.g., 1435 hours, not 2.35 p.m. Calendar dates use day month year, e.g., 15 June 1998. Salinity has no units; do not use psu, ‰ or similar. Ship's speed is given in km h−1; knots (nautical miles h−1) can follow in parentheses. Latitude and longitude can be given either as degrees minute seconds, or decimal degrees, at a level of precision proportionate to the accuracy of the fix. (0.1 second of latitude is equivalent to 185 m, but this decreases for longitude by the cosine latitude).

5.3 Statistics, equations & mathematical expressions: A useful resource for equations and mathematical expressions:
Journal of Fish Biology **8**2, 1771–1772 DOI: 10.1111/jfb.12146 (2013); A useful resource for reporting statistics: *Journal of Fish Biology* **78**, 697–699 DOI: 10.1111/j.1095-8649.2011.02914.x (2011)

Where decimal values are given, the number of decimal places must reflect the accuracy of the work. Thus, means and error (S.D., S.E., 95% C.L., etc.), should have the same number of decimal places, e.g., 15.1 + 0.2 and not 15.1 + 0.19. In mathematical expressions, italicized single letters are used for dimensions, qualified by subscripts (roman) as required, e.g., mass (not weight) M, wet mass (M_W), length L, fork length L_F (not FL), standard length L_S, index I, gonadosomatic index I_G, hepatosomatic index I_H, etc.

Statistics are presented as follows: name of test, test statistic with associated degrees of freedom (d.f.; *N.B.* an F distribution has two d.f. values) and probability level (P). Although ANOVA and regression are robust, the real P-values are likely to be different from the precise values provided by the statistics program, because of violations of the assumptions. If the manuscript clearly states that data conform fully to all the assumptions of the statistical method used, then precise *P*-values can be cited with three decimal places. Otherwise, *P*-values are normally limited to: > 0.05, 0.05, 0.01 and 0.001. Confidence intervals (95% C.I.) can be provided for parameters estimated by ANOVA and regression analysis. Where numerical resampling (e.g. bootstrapping) is used to assess the statistical significance of a given parameter (e.g. F_{ST}), in addition to resulting confidence intervals, the number of replicates should be also provided (e.g. 1000 bootstrap replicates).

5.3 Species nomenclature, authority and nomenclature: The plural of more than one individual of a single species is 'fish', but it is 'fishes' if there is more than one species. After its first mention, a fish species is **only** referred to by its scientific name. There should then be no further reference to the common name, describing author or date. The genus name can be abbreviated to a single letter (e.g., *C. carpio* and *O. mykiss*), except either at the start of a sentence, or where confusion arises from multiple genera with the same first letter, when either the genus is given in full, or the first three letters of the genus is used to provide a clear distinction.

First use of a fish species name in the Title and Abstract must include common (if available) and scientific name without describing the authority and date of authorship. First mention of a fish species in the main text must include the common name (if available), the binomial scientific name (in italics) **and the describing authority and date of authorship**, e.g., rainbow trout *Oncorhynchus mykiss* (Walbaum 1792), not (Walbaum, 1792). Naming authorities must appear in full except Linnaeus, 1758, e.g., Atlantic salmon *Salmo salar* L. No commas are necessary to separate either the common name from the species, or the authority from the date. **The use or absence of parentheses around the naming authority's name and date is covered by strict scientific rules.** If the current accepted genus and species name is the same as that given by the original naming author, the name appears without parentheses, e.g., *Pleuronectes platessa* L. 1758, but if the current accepted scientific name differs from that given by the original naming author, the original author's name appears within parentheses, e.g., *Platichthys flesus* (L. 1758).

For **correct scientific names and formatting of naming author** please use the following:

Eschmeyer, W. N. (Ed.) *Catalog of Fishes* electronic version (15 November 2013). http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp

For **accepted common names of fishes**:

Wheeler, A. (1992). A list of the common and scientific names of fishes of the British Isles. *Journal of Fish Biology* **41**(Suppl. A), 17–26. doi: 10.1111/j.1095-8649.1992.tb05644.

Wheeler, A. C., Merrett, N. R. & Quigley, D. T. G. (2004). Additional records and notes for Wheeler's (1992) List of the Common and Scientific Names of Fishes of the British Isles. *Journal of Fish Biology* **65**(Suppl. B), 1–40. doi: 10.1111/j.0022-1112.2004.00583.x

Nelson, J. S., Crossman, E. J., Espinosa-Perez, H., Findley, L. T., Gilbert, C. R., Lea, R. N. & Williams, J. D. (2004). Common and scientific names of fishes from the United States, Canada, and Mexico, 6th edn. Special Publication 29. Bethesda, MD: American Fisheries Society.

Froese, R. & Pauly, D. (Eds) (2013). *FishBase*. World Wide Web electronic publication. Available at http://www.fishbase.org/Search.php

FAO (2013). A*SFIS List of Species for Fishery Statistics Purposes*. Rome: Fisheries & Aquaculture Department, FAO. Available at http://www.fao.org/fishery/collection/asfis/en

5.4 Synonyms for a species

Synonyms require the following style: *Eptatretus cirrhatus* (Forster 1801) *Homea banksii* Fleming 1822: 375 (original description; type locality: South Seas; holotype: unknown); *Bdellostoma heptatrema* Muller 1836: 79 (original description; type locality: South seas; holotype: unknown); *Bdellostoma forsteri* Muller 1836: 80 (original description; type locality: Queen Charlotte Sound, New Zealand; holotype: unknown). Conel, 1931: 76 *Bdellostoma forsteri* var. *heptatrema*. Muller, 1838: 174 (new combination); *Bdellostoma cirrhatum*. G¨unther, 1870: 511 (in part). Hutton, 1872: 87 (in part). Putnam, 1874: 160 (in part); Gunther, 1880: 27. (Note that species names that are modifications of an existing binomial, rather than an original description, are separated from the author name by a full stop, *Bdellostoma cirrhatum*. Gunther, 1870: 511 (in part).

[based in part on: Mincarone, M. M. & Fernholm, B. (2010). Review of the Australian hagfishes with description of two new species of *Eptatretus* (Myxinidae), *Journal of Fish Biology* **77**, 779–801. doi: 10.1111/j.1095-8649.2010. 02661.x]

5.5 New species: The International Code of Zoological Nomenclature (Article 8.5, amendment) requires that a work bearing a new taxonomic name, issued and distributed electronically must be registered in the Official Register of Zoological Nomenclature (ZooBank) and contain evidence in the work itself of such registration. Any manuscript dealing with the description of new species, genera, or family submitted to *JFB* must be registered in ZooBank and the name of each new taxonomic name (e.g., new family, genus or species) should be added to ZooBank. Read http:**//zoobank.org/** and associated video tutorials (**http://zoobank.org/VideoGuide**) and the Editorial on this subject in ***JFB*** **90, 1167–1169.**

5.6 Curation of taxonomic specimens

Name-bearing type specimens of taxa that are described in *JFB* as new to science must be deposited in recognized national or international institutions that can meet ICZN (2012) criteria for Recommendations 72F.1-5 into the foreseeable future: ICZN (2012). *The International Code of Zoological Nomenclature*, 4th edn. London: The International Trust for Zoological Nomenclature 1999.

Other specimens used for taxonomic analyses should, wherever possible, be deposited in appropriate scientific collections (e.g., museums and university collections, or private collections when there is good evidence that these are adequately maintained), with identifying catalogue numbers, so that they are accessible to the scientific community for subsequent examination and taxonomic revision **http://iczn.org/iczn/index.jsp**. Distribution of paratype series among more than one recognized national or international institution is at the discretion of the authors, but is encouraged for paratype series whenever the paratype series can be split into two or more representative samples for deposit at different institutions. Institutions and their official abbreviations are listed in Eschmeyer's *Catalog of Fishes Online* : **https://www.calacademy.org/scientists/projects/catalog-of-fishes** and in Poss, S. G. & Collette, B. B. (1995). Second survey of fish collections in the United States and Canada. *Copeia* 1995, 48–70. **http://www.jstor.org/stable/1446799**

5.7 Genetic nomenclature: Authors are responsible for ensuring correct style for naming genes, etc. to avoid delay publication at the final proofreading stage. To differentiate genes, proteins etc., by fish origin, *JFB* uses the zebrafish system: **https://wiki.zfin.org/display/general/ZFIN+Zebrafish+Nomenclature+Guidelines**. On first mention, the name of a gene, etc. should be given in full (roman) with its abbreviated form immediately after in parentheses. Thereafter, an abbreviated format should be used, as shown below.

Full name	**Abbreviated form**
Heat-specific protein-I gene	*hspI* (all lower case italic)
Heat-specific protein-I	Hsp1 (all roman, capital first letter)
Xxx-x microsatellite locus	Gsp-1 (all roman, capital first letter of genus, followed by: two first initials of species name and the clone number; *e.g.* Gmo-1 for *Gadus morhua*)
zzz exon	*zzz-ex1* (all lower case italic followed by *-ex* also italics and a number for the exon number)
aaa intron	*aaa-in1* (*cf.* exon)
Enhanced fluorescent green protein-N3 plasmid	pEGFP-N3 (all roman capitals preceded by roman lowercase p)
Bbb-x primer	*Gsp-1* (all italic, capital first letter of genus, followed by: two first initials of species name and the clone number; *e.g. Gmo-1* for *Gadus morhua*)
Plasmids	All roman
Mammalian heat-specific protein-I gene	*HSPI* (all capital italic)
Mammalian heat-specific protein-I	HSPI (all capital roman)

5.8 Sequence data: Descriptions of novel amino-acid sequences of proteins or novel nucleotide sequences (e.g., primer sequences) are only be accepted if they carry a statement that all the data have been deposited with an appropriate data bank, e.g., the European Molecular Biology Laboratory (EMBL) or GenBank Data Libraries, and the database accession number must be given in the Materials and Methods. Data deposited in genetic data banks should include: specimen catalogue numbers (for specimens preserved in collections); a note identifying sequences that are derived from type specimens; and the collection locality data. For taxonomic papers that refer to sequences derived from specimens preserved in collections, authors should include a Table that clearly links each sequence accession number with the specimen from which it was derived. Sequences from type specimens should be clearly identified by bold text in this table and the significance of the bold text explained as a table footnote. For appropriate nomenclature for genetic sequences of type specimens please see: Chakrabarty, P. (2010). Genetypes: a concept to help integrate molecular phylogenetics and taxonomy. *Zootaxa* **2632**, 67–68. **http://www.mapress.com/zootaxa/2010/f/zt02632p068.pdf**. Sequences from holotypes are identified as hologenetypes, those from topotypes are topogenetypes, and the genetic marker(s) used are incorporated into the nomenclature (e.g., paragenetype ND2). Lengthy nucleotide sequences will only be published in the text if, in the judgement of the Editorial Team, these results are of general interest and importance. Where sequences are already published, reference to the original source will suffice.

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APÊNDICE B - ECSS INSTRUCTIONS FOR AUTHORS

Link: https://www.elsevier.com/journals/estuarine-coastal-and-shelf-science/0272-7714/guide-for-authors

ESTUARINE, COASTAL AND SHELF SCIENCE

In association with the Estuarine Coastal Sciences Association (ECSA)

AUTHOR INFORMATION PACK

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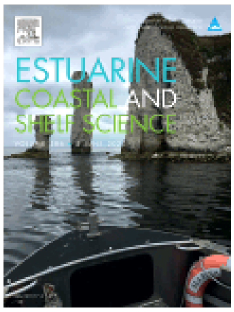

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- Species distribution in relation to varying environments
- Effects of waste disposal
- Groundwater runoff and Chemical processes
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Estuarine, Coastal and Shelf Science is an international multidisciplinary journal devoted to the analysis of saline water phenomena ranging from the outer edge of the continental shelf to the upper limits of the tidal zone. The journal provides a unique forum, unifying the multidisciplinary approaches to the study of the oceanography of estuaries, coastal zones, and continental shelf seas. It features original research papers, review papers and short communications treating such disciplines as zoology, botany, geology, sedimentology, physical oceanography. Data reports of mainly local interest are discouraged. An original research paper should not contain more than 8000 words, and no more than 8 figures and 3 tables. A **research note/short communication** should not contain more than 4,000 words and no more than 3 figures and 1 table. The Journal also welcomes suggestions from leading and internationally renowned scientists for in-depth **Reviews and Invited Feature Articles** on wide-ranging and contemporary topics. These Reviews can be approx. 12,000 words but the suggestions should be discussed with one of the Editors-in-Chief in the first instance.

Research areas include: Numerical modelling of estuarine and coastal marine ecosystems; Species distribution in relation to varying environments; Effects of waste disposal; Groundwater runoff and Chemical processes; Estuarine and fjord circulation patterns; Meteorological and oceanic forcing of semi-enclosed and continental shelf water masses; Sea-surface and sea-bed processes; Estuarine and coastal sedimentary processes and geochemistry; Brackish water and lagoon phenomena; Transitional waters.

Up-front rejections of papers submitted to Estuarine, Coastal and Shelf Science

ECSS handles about 1000 papers per year and over 3000 reviewers are involved in assisting the journal each year.

As editors we follow the declared guidelines for the journal and we also receive advice and comments from the publishers, and members of the editorial board as well as reviewers. The consistent advice that we have received from everyone is that the editors should reject papers which are likely to be rejected at the beginning of the process rather than sending them out for review, knowing what the answer is likely to be. Over 25% of papers are now rejected at the editorial submission phase.

The papers are subject to an initial technical pre-screening process by the publisher. This process checks on submission format and examines matters such as the provision of suitable keywords and legible figures. It also tries to check up on the standard of English, as it is totally inappropriate to expect a reviewer to undertake linguistic revision.

The pre-screening process however makes no judgement on the suitability of the paper for ECSS. This judgement is made by one of the editors who will up-front reject a paper judged unsuitable without going to review. These up-front rejections are due to three principal reasons:

Firstly, we receive several papers each year that have been submitted to the "wrong journal". We have received, for example, papers on inland freshwater lakes or palaeontology, and other topics which are clearly beyond the scope of the journal. As a simple guide, if there is no mention of any previous ECSS paper in the reference list, it strongly suggests that the paper has been submitted to the wrong journal.

Secondly, papers that are "data reports" or "reports of local interest" will be rejected up-front. Papers in this category may describe a particular estuary in great detail, but fail to advance estuarine, coastal and shelf science. The overwhelming feeling when reading such a paper is "so-what!"

Thirdly, other reasons for up-front rejection can be a lack of a valid Discussion which integrates the study with the peer-reviewed literature or else relies on excessive self-citation, or a lack of appropriate statistical analysis, or purely statistical analyses without considering processes.

We at ECSS seek that all papers are based on hypothesis testing and that the hypotheses should be of general and international interest. We are interested in contributions that add to general knowledge, and move the field forward.

By up-front rejection we hope to give the authors a chance to quickly submit to a more appropriate journal. We do accept that we will sometimes make mistakes in this process, but we do this to protect the reviewers by offering them only relevant papers that are potentially publishable in ECSS. Up-front rejected papers will not be reconsidered for publication and we have a similar policy for papers rejected after review.

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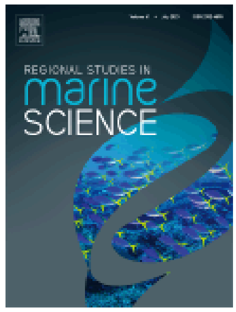

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