Universidade Federal do Rio Grande
Instituto de Ciências Biológicas
Pós-graduação em Biologia de
Ambientes Aquáticos Continentais

Estrutura e conectividade trófica das assembleias de peixes em um estuário subtropical e seus sistemas fluvial e marinho adjacentes

Adna Ferreira Silva Garcia

Orientador: João Paes Vieira

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RESUMO

Investigar as mudanças na estrutura trófica e na partição de recursos alimentares entre espécies ao longo dos gradientes ambientais ajuda a revelar a organização das comunidades biológicas e o funcionamento dos ecossistemas. Nesse contexto, o objetivo principal da presente tese foi investigar como as relações tróficas de consumidores aquáticos mudam ao longo de um gradiente rio-estuário-mar e em períodos hidrológicos contrastantes (seco e chuvoso). Mais especificamente, foram investigados (i) mudanças na organização trófica e na importância relativa de fontes autotróficas sustentando assembleias de peixes ao longo de um transecto rio-estuário-mar (Capítulo 1), (ii) a assimilação de material alóctone continental, carreado pelas fortes chuvas associadas ao evento *El Niño* 2015, por consumidores estuarinos (Capítulo 2) e (iii) o grau de sobreposição e amplitude de nicho trófico de espécies congenéricas de tainhas (*Mugil curema* e *M. liza*) entre ambientes estuarinos e marinhos (Capítulo 3). Esses tópicos foram investigados a partir da análise de conteúdos estomacais dos peixes e das razões isotópicas de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$) das fontes alimentares e consumidores no Complexo Estuarino Tramandaí-Armazém (29°S), o rio e a zona de arrebentação marinha adjacentes. Houve uma maior complexidade na estrutura trófica da ictiofauna no estuário, o que poderia ser explicado pelo maior número de componentes (fontes primárias, presas e predadores) associados às cadeias alimentares pelágicas e bentônicas no estuário. A maioria dos consumidores estuarinos apresentou valores médios menores de $\delta^{13}C$ durante o período chuvoso associado ao *El Niño* de 2015, o que parece refletir o aumento na assimilação do material alóctone continental carreado do rio para o interior do estuário nesse período. O tamanho e grau de sobreposição dos nichos isotópicos das tainhas variaram entre os ambientes, com nichos maiores no estuário e maior sobreposição no mar. Estes padrões parecem refletir os níveis contrastantes de disponibilidade de alimentos entre os ambientes; com maior diversidade e distinção na dieta entre as espécies de tainhas no estuário, que abriga uma maior diversidade de alimentos (microalgas). Os resultados obtidos na Tese reforçam a necessidade de considerar (e.g., em planos de mitigação de impactos nos ambientes costeiros) as mudanças nas relações alimentares dos organismos em nível de comunidades e espécies que podem ocorrer ao longo de gradientes ambientais e por influência de fenômenos climáticos globais.

Palavras-chave: isótopos estáveis; conteúdo estomacal; sobreposição de nicho alimentar; redes bipartidas; tainhas; *El Niño*; Complexo Estuarino Tramandaí-Armazém

ABSTRACT

To investigate changes in trophic structure and niche partitioning among species along environmental gradients is helpful to reveal the organization of biological communities and ecosystem functioning. In this context, the main objective of this Thesis was to invetigate how trophic relationships of aquatic consumers change along a river-estuary-sea transect and in contrasting hydrological periods (dry and rainy). More specifically, it was studied (i) changes in trophic organization and in the relative importance of authochthnous sources sustaining fish assemblages along a river-estuary-sea transect (Chapter 1), (ii) the assimilation of continental allochthonous material carried out by excessive rain associated with the 2015 *El Niño* event by estuarine consumers (Chapter 2) and (iii) the breadth and overlap of trophic niches of two congeneric mullet species (*Mugil liza* and *M. curema*) between estuarine and marine habitats (Chapter 3). These topics were investigated based on the analyses of stomach food content of fishes and carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios of food sources and consumers in the Tramandaí-Armazém Estuarine Complex (29°S) and its adjacent river and marine surf-zone. There was a higher complexity of the trophic structure of the ichthyofauna in the estuary, which coud be explained by a higher number of components (primary sources, preys and predators) associated with the pelagic and benthonic food chains in the estuary. The majority of estuarine consumers had average lower $\delta^{13}C$ values during the rainy period associated with the 2015 *El Niño*, which could reflect higher assimilation of continental allochthonous material carried out from the river into the estuary during this period. The breadth and overlap of isotopic niches of mullets changed between habitats, with broader niches in the estuary and higher niches overlap in the sea. These patterns seemed to reflect the contrasting levels of food avalibility between habitats; with higher diversity and dissimilarity in the diet of both mullet species in the estuary, which harbour a greater diversity of food sources (microalgae). The findings obtained in this Thesis highlight the need to take into account (e.g., in management plans to mitigate impacts in coastal systems) the changes in trophic relationships at the community and species level that may occur along environmental gradients and due to climate global phenomena.

Key-words: stable isotopes; stomach content; food niche overlap; bipartite networks; mullets; *El Niño*; Tramandaí-Armazém Estuarine Complex

APRESENTAÇÃO

A presente tese está estruturada da seguinte forma:

(1) Introdução Geral, com citações e referências bibliográficas, abordando o tema central (Ecologia Trófica), a contextualização das Hipóteses e as ferramentas analíticas utilizadas (principalmente Isótopos Estáveis).

(2) Corpo central composto por três Capítulos: o 1º Capítulo (Garcia et al., in press), se intitula "CHANGES IN FOOD WEB STRUCTURE OF FISH ASSEMBLAGES ALONG A RIVER TO OCEAN TRANSECT OF A COASTAL SUBTROPICAL SYSTEM", aceito para publicação e formatado de acordo com as normas da revista **'Marine and Freshwater Research'** (Fator de Impacto 1,757; Qualis B1), o 2º Capítulo intitulado "ASSIMILATION OF ALLOCHTHONOUS MATTER BY ESTUARINE CONSUMERS DURING THE 2015 *EL NIÑO* EVENT" está formatado segundo as normas da revista **'Estuaries and Coasts'** (Fator de Impacto 2,421; Qualis A2), e se encontra na segunda rodada de revisão, e o 3º Capítulo (Garcia et al., in press), se intitula "SPATIAL DIET OVERLAP AND FOOD RESOURCE IN TWO CONGENERIC MULLET SPECIES REVEALED BY STABLE ISOTOPES AND STOMACH CONTENT ANALYSES", aceito para publicação e formatado segundo as normas da revista **'Community Ecology'** (Fator de Impacto 0,782; Qualis B2).

(3) Considerações Finais e Perspectivas.

A formatação do texto (com exceção dos capítulos) esta de acordo com as normas da Associação Brasileira de Normas Técnicas (ABNT).

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INTRODUÇÃO GERAL

Variações na estrutura trófica em ambientes aquáticos continentais e costeiros

Desde o trabalho pioneiro de ELTON (1927), o modo pelos quais as relações alimentares entre os organismos estão estruturadas (e.g. números de níveis tróficos, quantidade e intensidade de elos tróficos) têm sido um importante foco de estudos em ecologia trófica (WINEMILLER; LAYMAN, 2005; MOORE et al., 2017). Isso porque a descrição da estrutura trófica de uma comunidade biológica e, especialmente, o entendimento de como os fatores abióticos e/ou perturbações antrópicas modificam essa organização tem se revelado uma ferramenta importante na compreensão do funcionamento dos ecossistemas e na gestão dos recursos naturais (BEGON; TOWNSEND; HARPER, 2007; LAYMAN et al., 2015; ULLAH et al., 2018). Atualmente, existe grande interesse em entender como as variações na composição e riqueza de espécies influência a estrutura trófica de comunidades em diferentes ecossistemas e seu papel na manutenção da biodiversidade (ESTES et al., 2011; KLARNER; MARAUN; SCHEU, 2013).

Uma teia alimentar é considerada a representação das relações alimentares entre predadores e presas numa comunidade ecológica (COHEN, 1978; PIMM, 1982). Como os recursos alimentares e os predadores estão entre os principais fatores limitantes do crescimento populacional de qualquer espécie, as interações tróficas são componentes essenciais para o entendimento da dinâmica das populações, coexistência e diversidade nos ecossistemas (LEVIN, 1970; HOLT; GROVER; TILMAN, 1994; CHESSON, 2000; GIACOMINI, 2007). A caracterização das teias alimentares é fundamental para avaliar a organização, descrever e quantificar a complexidade dos ecossistemas (MAY, 1972; MONTOYA; PIMM; SOLÉ, 2006; DUNNE, 2009), bem como uma forma de integrar o papel trófico das espécies que compõem as comunidades biológicas (BASCOMPTE, 2009). Usualmente, teias alimentares são construídas com base em dados de dieta através da análise de conteúdo estomacal (GARRISON; LINK 2000). Dentre as principais propriedades das teias alimentares usadas como medidas de sua estruturação e complexidade estão, por exemplo, tamanho da rede alimentar, número de ligações, densidade de elos tróficos e conectância (PIMM, 1982; WINEMILLER; LAYMAN, 2005; THOMPSON et al., 2012). No entanto, a dinâmica dessas propriedades, suas correlações ambientais e sua possível associação com a diversidade e estabilidade da comunidade ainda permanecem pouco compreendidas (PERALTA-MARAVER et al., 2017).

A coexistência de espécies e sua partição alimentar em diferentes habitats vem sendo investigadas desde meados do século XX (PIANKA, 1976; COHEN, 1978), mas ainda permanecem como um tópico de pesquisa importante em ecologia trófica (WINEMILLER et al., 2015; DUPKE et al., 2017; FITZGERALD et al., 2017). Várias teorias são usadas para explicar os principais mecanismos que controlam as forças motrizes dessas interações (PIANKA, 1976; WINEMILLER; LAYMAN, 2005). A teoria de partição de nicho, por exemplo, prevê que a coexistência de espécies competidoras ocorrerá por meio da diferenciação de nicho que reduz a sobreposição entre elas. Segundo essa teoria, espera-se que os consumidores ajustem a alimentação (e.g., apresentando uma dieta mais seletiva) para reduzir a sobreposição de nicho com os competidores em habitats com relativa escassez de alimentos (PIANKA, 1976). Evidências obtidas em estudos de campo parecem corroborar essas predições. Por exemplo, peixes de riachos tropicais podem apresentar nichos tróficos diferentes e mais reduzidos durante períodos de menor disponibilidade de recursos (estação seca), porém apresentam nichos alimentares maiores em períodos mais produtivos (estação chuvosa) (e.g., CORREA; WINEMILLER, 2014).

No entanto, as previsões da teoria do particionamento de nicho trófico são difíceis de serem observadas na natureza, porque a variabilidade dos recursos alimentares no espaço ou no tempo pode influenciar a dieta dos consumidores (GERKING, 1994; BASTOS et al., 2017). Uma espécie pode explorar um número restrito de recursos alimentares (dieta especializada) em um habitat simplesmente porque esses itens são os mais abundantes ou porque não há recursos alternativos disponíveis. Em contraste, esta mesma espécie pode ter um amplo nicho alimentar (dieta generalista) em um habitat que possui uma maior variedade de recursos alimentares disponíveis. Este efeito espacial (ou temporal) da disponibilidade de recursos no comportamente de forrageio do consumidor nem sempre é levado em conta ao se investigar o uso de recursos alimentares pelas espécies (FOX; MORROW, 1981). Nesse contexto, a comparação de dietas e sobreposição alimentar de espécies coexistentes entre habitats com níveis contrastantes de disponibilidade de alimentos (em termos de abundância e diversidade de presas) é útil para entender melhor os mecanismos que controlam a variação da dieta dos consumidores e a estrutura trófica das comunidades biológicas em diferentes ecossistemas (WINEMILLER; LAYMAN, 2005).

Uma das abordagens analíticas que facilitam a comparação da estrutura trófica entre ecossistemas é o uso de guildas funcionais. ROOT (1967) definiu guilda como grupo de espécies que exploram de forma semelhante à mesma classe de recursos ambientais (e.g. guildas de uso de habitats e/ou guildas tróficas). O conceito de guilda trófica foi utilizado pela primeira vez por SYLVA (1975), que definiu agrupamentos com base nas preferências alimentares das espécies. A utilização de guildas tróficas funcionais (GTFs) fornece informações sobre a estrutura e conectividade entre consumidores e seus alimentos com intuito de simplificar ecossistemas complexos (ELLIOTT; DEWAILLY, 1995; GARRISON; LINK, 2000; LOBRY et al., 2003; FRANCO et al., 2006). Vários estudos têm empregado GTFs para descrever e comparar a estrutura trófica de assembléias de peixes dentro e entre ecossistemas (GARRISON; LINK, 2000; ELLIOTT et al., 2007; ALBOUY et al., 2011; BUCHHEISTER; LATOUR, 2015; EGAN et al., 2017). É importante ressaltar que na classificação e agrupamento das GTFs geralmente se utiliza atributos relacionados com o tipo das presas consumidas por esses predadores, os quais são agrupados independentemente de sua identidade taxonômica (ELLIOTT et al., 2007; PESSANHA et al., 2015). A classificação das espécies em guildas funcionais torna mais viável e facilita a comparação do papel funcional de grupos de consumidores e das comunidades entre diferentes ecossistemas em escala global (PURVIS; HECTOR, 2000). Isso porque o uso de guildas tem como foco o papel funcional da espécie (o modo como utiliza o ambiente ou seu tipo de presa) e não sua identidade taxonômica (BUCHHEISTER; LATOUR, 2015). Por isso o uso de GTFs tem se mostrado crescente devido a necessidade de padronização e melhor compreensão do uso dos recursos alimentares pelos consumidores e suas possíveis interações com recursos oriundos de ecossistemas adjacentes (WINEMILLER; LAYMAN, 2005).

Efeitos ambientais e climáticos sobre a estrutura e a conectividade trófica

Dentre os fatores abióticos que podem estruturar as relações tróficas das comunidades biológicas podemos mencionar o pulso hidrológico associado às variações na precipitação sobre as bacias de drenagem costeira, acarretando em variações sazonais na descarga continental de água doce nos estuários (DAY et al., 2012). O elevado aporte de água doce influencia não só a salinidade, mas também a entrada de nutrientes e macrodetritos de origem límnica (e.g., matéria orgânica particulada em suspensão) (LIVINGSTON et al., 1997; BISHOP; MCCLELLAND; DUNTON, 2017). Movimentos de nutrientes, detritos, presas e consumidores entre habitats ocorrem em diversos biomas, podendo influenciar fortemente a dinâmica das populações e comunidades na medida em que podem promover a conectividade trófica entre ecossistemas (POLIS; ANDERSON; HOLT, 1997; MOONEY; MCCLELLAND, 2012; GARCIA et al., 2017a). O aumento súbito da descarga de água doce e de fontes alóctones continentais pode alterar a dinâmica populacional e a estrutura da comunidade nos estuários (GARCIA et al., 2004; ABRANTES et al., 2013; ODEBRECHT et al., 2017), podendo afetar a diversidade e a abundância relativa dos peixes (GARCIA et al., 2003), porém os reflexos sobre a organização trófica deste grupo ainda são pouco conhecidas (LIVINGSTON et al., 1997; ABRANTES; BARNETT; BOUILLON, 2014; POSSAMAI et al., 2018).

Em ecossistemas aquáticos como rios é bem conhecida a importância do período chuvoso no transporte de material alóctone oriundo da paisagem adjacente, e sua subsequente assimilação por consumidores (e.g. LOWE-MCCONNELL, 1987; WINEMILLER, 1990). Por exemplo, estudos realizados em rios amazônicos (e.g., WINEMILLER, 1990) e em planícies de inundação da Bacia do Rio Paraná (LUZ-AGOSTINHO et al., 2008) revelaram que mudanças hidrológicas associadas aos períodos secos e chuvosos afetam a dinânmica e a estruturação trófica das assembleias de peixes. No entanto, os efeitos das alterações hidrológicas na estrutura trófica da biota estuarina têm sido pouco investigadas (LIVINGSTON et al., 1997; ABRANTES et al., 2013), especialmente em latitudes subtropicais (POSSAMAI et al., 2018).

Estuários subtropicais e sua ictiofauna como modelos de investigação em ecologia trófica

As zonas de transição entre ambientes de água doce e marinhos abrigam uma elevada abundância de espécies de peixes (principalmente nas formas juvenis) e são consideradas áreas fundamentais para sobrevivência e manutenção dessas populações, pois funcionam como zonas de berçário, refúgio e alimentação (MCLUSKY; ELLIOTT, 2004; BARLETTA et al., 2010; DAY et al., 2012; BLABER, 2013). A diversidade de peixes nessas zonas de transição (estuários) pode ser elevada (VILAR; JOYEUX, 2018; ARAÚJO et al., 2018), pois além de conter as espécies residentes que completam todo o seu ciclo de vida no estuário (estuarinas residentes), esses ecossistemas podem receber espécies transeuntes estuarino-relacionadas (estuarinas dependentes) que utilizam os estuários como berçários ou zona de reprodução (MCLUSKY; ELLIOTT, 2004). Também ocorrem nos estuários, de modo esporádico e ocasional, espécies de origem marinha ou de água doce dependendo do balanço entre as intrusões de água salgada ou das descargas de água doce dos rios (VIEIRA; CASTELLO, 1997; WOOTTON, 1999).

As mudanças na diversidade e composição das espécies estão associadas principalmente a limitações fisiológicas dos organismos em relação aos efeitos osmóticos da salinidade, sendo bastante pronunciada em assembleias de peixes (HARRISON;

WHITFIELD, 2006; BARLETTA; BLABER, 2007; MOURA; VIEIRA; GARCIA, 2012; WHITFIELD et al., 2012; CAREDDU et al., 2015; CLAUDINO et al., 2015). Por exemplo, MOURA; VIEIRA; GARCIA (2012) demonstraram que tanto a abundância como a riqueza de espécies de peixes muda ao longo do gradiente ambiental englobando ecossistemas de água doce e estuarinos, especialmente devido às marcadas variações em fatores abióticos como salinidade, granulometria do sedimento e transparência da água entre esses ecossistemas. Esses peixes de origens variadas que ocorrem nos estuários exploram os abundantes recursos alimentares com hábitos tróficos bastante diversificados, como herbívoros, fitoplanctívoros, zooplanctívoros, zoobentívoros, piscívoros, detritívoros, entre outros (BUCHHEISTER; LATOUR, 2015).

O padrão de distribuição e dominância dos produtores primários em corpos aquáticos também pode influenciar a estruturação trófica da comunidade de peixes nesses ambientes (PETERSON; HOWARTH; GARRITT, 1985; KANAYA et al., 2007; CLAUDINO et al., 2015). O ambiente estuarino tende a apresentar uma maior diversidade de produtores primários, tais como marismas, mangues, macroalgas, gramíneas, macrófitas e microalgas (DAY et al., 2012), quando comparado, por exemplo, com a região costeira adjacente, que apresenta como principal fonte de produção primária as microalgas (ODEBRECHET et al., 2014), principalmente nas regiões subtropicais como é o caso do sul do Brasil. A elevada produtividade primária e disponibilidade de presas encontrada nos estuários (SEELIGER; ODEBRECHT, 2010; DAY et al., 2012), juntamente com a elevada abundância de peixes residentes ou oriundos dos ambientes adjacentes (BARLETTA; BLABER, 2007; BARLETTA et al., 2010; POTTER et al., 2013), podem influenciar na organização e complexidade das relações tróficas das comunidades biológicas nesses ambientes de transição entre o continente e o mar (GARCIA et al., 2007; CLAUDINO et al., 2015; BERGAMINO; RICHOUX, 2015; MONT"ALVERNE; PEREYRA; GARCIA, 2016). Contudo, características da paisagem atreladas à morfologia dos ecossistemas e dos hábitats (POLIS et al., 1997; TURNER et al., 2001), especialmente em ambientes estuarinos, podem afetar a diversidade de espécies (KANDRATAVICIUS et al., 2015) e a composição e abundância relativa de produtores primários (BOSCUTTI et al., 2015). Estudos realizados na bacia do Rio Tramandaí (área de estudo da presente tese) demonstraram a influência das características da paisagem (e.g., características morfológicas) sobre as assembleias de peixes com efeitos sobre composição de espécies, diversidade e abundância (GUIMARÃES; HARTZ; BECKER, 2014; VASCONCELOS et al., 2015) e estrutura trófica (HOEINGHAUS et al., 2011). O estudo realizado por HOEINGHAUS e colaboradores (2011) revelou que no Complexo Estuarino

Lagunar Tramandaí-Armazém, que é um estuário do tipo estrangulado (*chocked lagoons*, *sensu* KJERFVE, 1986), caracterizado por extensas zonas mixohalinas, elevada razão entre zonas rasas:profundas, baixa declividade e extensas áreas de marisma (*Spartina* spp.) nas suas margens, a ictiofauna parece possuir maior dependência trófica de carbono orgânico oriundo de macrófitas estuarinas (e.g., *Spartina* spp., *Ruppia maritima*). Habitats costeiros como estuários e zonas de arrebetação marinha geralmente apresentam níveis contrastantes de disponibilidade de alimentos, especialmente em termos de diversidade de presas. Na região sul no Brasil os estuários são caracterizados por apresentar alta biomassa de vegetação aquática submersa (principalmente gramíneas marinhas e macroalgas flutuantes), elevada diversidade de invertebrados bentônicos e grandes quantidades de microalgas e detritos (ODEBRECHT et al., 2010a, 2014; HOEINGHAUS et al., 2011). Em contraste, a zona de arrebentação marinha ao longo desta costa arenosa é caracterizada pela alta produção fitoplanctônica *in situ* e floramentos de microalgas, dominada por frequentes e densas acumulações de diatomáceas (ODEBRECHT et al., 2010b, 2014).

Os estuários do sudoeste do Atlântico sob influência do fenômeno *El Niño* são bons modelos para investigar os efeitos das mudanças hidrológicas na organização trófica das comunidades estuarinas (ODEBRECHT et al., 2017; GARCIA et al., 2017a). O *El Niño* é um fenômeno climático global de interação atmosfera-oceano caracterizado pelo aquecimento anômalo das águas superficiais do Pacífico Equatorial Oriental por períodos prolongados, geralmente mais de cinco meses, ocorrendo aparentemente em intervalos irregulares de três a sete anos (PHILANDER, 1990; GLANTZ, 2001). As mudanças oceanográficas associadas com o *El Niño* têm forte correlação com um fenômeno atmosférico conhecido desde 1924, a Oscilação Sul. Este fenômeno é formado pelas oscilações interanuais nas diferenças na pressão atmosférica ao longo da bacia do Oceano Pacífico. A interação oceano-atmosfera entre estes fenômenos é denominada de *El Niño* Oscilação Sul (ENOS) ou, na língua inglesa, como *El Niño Southern Oscilation* (ENSO) (CAVIEDES, 2001). No sul do Brasil, o *El Niño* (a fase „quente‟ do ENOS) está associado com excesso de chuvas, principalmente na primavera do ano em que o fenômeno *El Niño* se inicia, e no outono-inverno do ano seguinte, abrangendo mais intensamente as áreas próximas ao oceano (KOUSKY; ROPELEWSKI, 1989; KANE, 1997; GRIMM; FERRAZ; GOMES, 1998; GRIMM; BARROS; DOYLE, 2000). Por sua vez, esse excesso de chuvas esta associado com o aumento da descarga continental, acarretando alterações nos padrões de salinidade e circulação nos estuários localizados em latitudes subtropicais da América do Sul (ROBERTSON; MECHOSO, 1998; GRIMM; BARROS; DOYLE, 2000; SUN et al., 2015).

Um dos mais fortes eventos de *El Niño* já registrados foi o *El Niño* de 2015, que foi associado a episódios de chuvas excessivas em várias regiões do globo, incluindo a América do Sul (ZHAI et al., 2016; PAEK; YU; QIAN, 2017). A precipitação excessiva sobre a bacia de drenagem de estuários subtropicais na América do Sul tem sido associada a mudanças marcantes na biota estuarina (ODEBRECHT et al., 2017). No estuário da Lagoa dos Patos (32°S), por exemplo, o alto influxo de água doce associada a eventos de *El Niño* de moderados a fortes está associado a mudanças na composição de espécies, padrões de abundância e diversidade (GARCIA; VIEIRA; WINEMILLER, 2001; GARCIA et al., 2004; VIEIRA; GARCIA; GRIMM, 2008), bem como na organização trófica de assembleias de peixes (POSSAMAI et al., 2018). No entanto, os efeitos potenciais das mudanças hidrológicas associadas a eventos do *El Niño* sobre a ocorrência e intensidade das conexões tróficas entre fontes alimentares basais (i.e., produtores primários e outras fontes primárias de matéria organica) e os consumidores estuarinos permanecem em grande parte desconhecidas.

Ferramentas de estudos em ecologia trófica: conteúdo estomacal e isótopos estáveis

A análise de isótopos estáveis (AIE) (PASQUAUD et al., 2008; HANCOCK et al., 2018; MORAES; HENRY-SILVA, 2018) e a análise de conteúdo estomacal (ACE) (BUCHHEISTER; LATOUR, 2015; GARCIA; VENDEL, 2016) são ferramentas comumente utilizadas na investigação dos elos tróficos basais e intermediários que sustentam consumidores em comunidades aquáticas. Mais recentemente, os estudos de caracterização trófica tem combinado o uso das duas técnicas para diferentes grupos taxonômicos, como anfíbios (HUCKEMBECK et al., 2018), peixes (LÓPEZ et al., 2016; MURO-TORRES et al., 2017; WEIDNER et al., 2017; GARCIA et al., 2018), tartarugas (VÉLEZ-RUBIO et al., 2016; PETITET; BUGONI, 2017), aves (POLITO et al., 2011; FARIA et al., 2016) e mamíferos (GOETZ et al., 2017; SECCHI et al., 2017). Os peixes têm sido considerados um modelo adequado para investigar mudanças espaciais no uso de recursos alimentares e a sobreposição da dieta devido à sua diversidade e alta abundância em praticamente todos os sistemas aquáticos (GERKING, 1994). Muitos peixes têm histórias de vida complexas que abrangem o uso de sistemas aquáticos contrastantes com reflexos marcantes na sua ecologia trófica ao longo de seu ciclo de vida (WOOTTON, 1999).

A ACE é uma abordagem tradicionalmente utilizada para descrever a composição da dieta, determinar a amplitude do nicho trófico dos consumidores (NIELSEN et al., 2018) e descrever e quantificar a estrutura trófica de teias alimentares (GIACOMINI; PETRERE JR., 2010). Dentre as métricas mais utilizadas para caracterizar uma teia alimentar estão: tamanho da teia alimentar (S: número de espécies ou consumidores presentes), densidade de elos tróficos (D: razão entre o número total de ligações tróficas e o número de espécies), conectância (C: razão entre número de ligações tróficas realizadas e as ligações possíveis), posição trófica dos consumidores (PT), intensidade dos elos tróficos (I: número de ligações fortes e fracas), número de componentes tróficos de topo (NT), intermediários (NI) e de base (NB) (elementos de topo não possuem predadores, os de base não possuem presas e os intermediários possuem ambos), número de onívoros (NO: que se alimentam em mais de um nível trófico) (PIMM, 1982; WINEMILLER; LAYMAN, 2005; GIACOMINI; PETRERE JR, 2010). Embora a ACE possa alcançar alta resolução taxonômica, este método pode ser tendencioso devido à dificuldade em determinar a origem dos alimentos parcial e/ou totalmente digeridos e a impossibilidade de avaliar a verdadeira assimilação dos nutrientes derivados dos alimentos ingeridos (CONDINI; HOEINGHAUS; GARCIA, 2015).

Nas últimas décadas, a análise dos isótopos estáveis (AIE) tem sido considerada marcadores naturais úteis para reconstruir a dieta dos consumidores e os padrões de assimilação de alimentos (LAYMAN et al., 2012). Isótopos estáveis são átomos do mesmo elemento químico que diferem em número de nêutrons, resultando em átomos mais pesados (e.g., ^{13}C, ^{15}N) e mais leves (e.g., ^{12}C, ^{14}N) que ocorrem naturalmente como razões isotópicas (e.g., $^{13}C/^{12}C$, $^{15}N/^{14}N$) nos materiais biológicos e não biológicos (PETERSON; FRY, 1987). O pressuposto fundamental da AIE é de que os consumidores refletem as razões isotópicas dos alimentos assimilidados e, portanto, podem ser utilizados como marcadores do fluxo de matéria e energia que ocorrem nas relações alimentares (FRY, 2006). As razões isotópicas de alguns elementos como o carbono ($\delta^{13}C$) variam relativamente pouco (entre 0,5 e 1,0 permil) na medida em que o alimento é digerido e assimilado pelo consumidor e, desse modo, são usados principalmente para reconstruir a dieta dos consumidores. Em contrapartida, o nitrogênio aumenta de modo mais significativo (entre 3 e 4 permil) a sua razão isotópica ($\delta^{15}N$) quando o alimento é assimilado pelo consumidor, sendo essas diferenças nas razões isotópicas do $\delta^{13}C$ e $\delta^{15}N$ no consumidor é denominada „fracionamento isotópico". Devido ao aumento mais marcado no valor de $\delta^{15}N$ a cada nível trófico, esse marcador é utilizado para inferir a posição trófica do consumidor (PETERSON; FRY, 1987; BOECKLEN et al., 2011). Os isótopos estáveis de carbono e nitrogênio ($\delta^{13}C$ e $\delta^{15}N$, respectivamente) são os marcadores naturais mais utilizados na AIE para reconstruir a dieta do consumidor e inferir ligações tróficas em redes alimentares (LAYMAN et al., 2012). A AIE permite estimar a contribuição relativa de fontes primárias de alimento para sustentar não apenas os consumidores primários (por exemplo, herbívoros e detritívoros), mas também consumidores posicionados em níveis tróficos superiores (por exemplo, onívoros e carnívoros) (FRY, 2006; CONDINI; HOEINGHAUS; GARCIA, 2015; GARCIA et al., 2017b).

Mais recentemente, métricas de nicho isotópico foram propostas como *proxies* úteis para avaliar a amplitude e a sobreposição de nichos alimentares (BEARHOP et al., 2004; NEWSOME et al., 2007; JACKSON et al., 2011). Assim, a partir da variabilidade e do posicionamento das razões isotópicas dos consumidores no espaço isotópico ($\delta^{13}C$-$\delta^{15}N$) é possível utilizar medidas quantitativas (métricas) que permitam descrever a diversidade trófica de uma comunidade (NEWSOME et al., 2007; LAYMAN et al., 2007). Apesar das suas limitações (HOEINGHAUS; ZEUG, 2008), essas métricas quantitativas vêm sendo usadas de modo crescente para comparar a variação espacial na estrutura trófica de ecossistemas aquáticos (e.g., ABRANTES; BARNETT; BOUILLON, 2014). Dentre as métricas isotópicas mais utilizadas para descrever a diversidade trófica estão à amplitude do carbono ($\delta^{13}C$ *range*, CR) e do nitrogênio ($\delta^{15}N$ *range*, NR), área total (TA) e distância média do centroide (CD). Trabalhos recentes propuseram aprimoramentos (e.g., inclusão de intervalos de credibilidade nas estimativas) e novas métricas mais robustas para descrever o nicho isotópico (e.g., SEA-elipses isotópicas em substituição ao TA) (JACKSON et al., 2011).

Embora a AIE tenha se tornado atualmente um dos principais métodos para investigar relações tróficas, a técnica possui limitações inerentes (LAYMAN et al., 2012). Por exemplo, baixa resolução taxonômica já que as diferenças isotópicas não são espécie-específicas (e.g., entre espécies de produtores primários), mas são mais comumente observadas em grupos de organismos com fisiologias diferentes (e.g., entre produtores com vias fotossintéticas do tipo C_3 e C_4) (FRY, 2006). Por isso o método torna-se mais efetivo quando usado em combinação com abordagens tradicionais como a análise de conteúdo estomacal (ACE), que permite uma descrição mais detalhada (e.g., do ponto de vista taxonômico) das relações alimentares entre macroconsumidores (CONDINI; HOEINGHAUS; GARCIA, 2015). Consequentemente, a combinação de ambas as técnicas (ACE e AIE) fornecem representações mais precisas da dieta dos consumidores e do uso de recursos alimentares (HADWEN; RUSSELL; ARTHINGTON, 2007; LAYMAN et al., 2012; CONDINI; HOEINGHAUS; GARCIA, 2015).

JUSTIFICATIVA E OBJETIVO GERAL

Considerando que a investigação dos fatores que controlam as relações tróficas em ambientes aquáticos costeiros é uma condição primordial para compreender as variações na produção secundária em estuários, especialmente de peixes que tem sido historicamente a base de importantes pescarias (e.g., tainhas), esse estudo tem como objetivo geral investigar como variações ambientais (gradiente salino) e hidrológicas (excesso de chuvas) se relacionam com a estrutura das relações tróficas dos consumidores ao longo de ambientais continentais e marinhos adjacentes.

Objetivos Específicos:

1. Determinar guildas tróficas funcionais (GTF) utilizando como base a composição da dieta dos peixes ao longo de um transecto rio-estuário-mar.

2. Caracterizar a complexidade da estrutura trófica da assembleia de peixes ao longo de um transecto rio-estuário-mar baseado em métricas obtidas com conteúdo estomacal (e.g., Tamanho da rede trófica (S), número de ligações (L), densidade de ligações (D), diversidade Shannon‟s de interações (H‟) e conectância (C)).

3. Comparar a importância relativa e diversidade de fontes autotróficas (e.g., C_3, C_4, POM e SOM) contribuindo como fontes primárias de nutrientes (C, N) para a ictiofauna ao longo de um transecto rio-estuário-mar.

4. Verificar a assimilação de material alóctone continental, carreado pelas fortes chuvas associadas ao evento *El Niño* 2015, por consumidores estuarinos.

5. Comparar o grau de sobreposição e amplitude de nicho trófico de espécies congenéricas de tainhas (*Mugil curema* e *M. liza*) entre o ambiente estuarino e marinho.

HIPÓTESES

1 – Há uma maior diversidade de elos tróficos basais sustentando a assembleia de peixes estuarinos quando comparado aos ambientes de água doce e a zona de arrebentação na região costeira; (Capítulo 1)

2 – Ocorre maior complexidade nas relações tróficas da ictiofauna (e.g., número e densidade de ligações) no estuário do que nas regiões adjacentes; (Capítulo 1)

3 – O aumento do excedente hídrico associado ao *El Niño* de 2015 acarreta um aumento no transporte de material alóctone da região límnica para o estuário, o qual é assimilado pelos macroinvertebrados e peixes; (Capítulo 2)

4 – Espécies congenéricas de tainhas têm menor sobreposição e maior amplitude de nicho trófico em ambientes estuarinos caracterizados pela maior disponibilidade de alimento quando comparado com regiões marinhas menos produtivas (Capítulo 3).

ÁREA DE ESTUDO

A bacia hidrográfica do rio Tramandaí fica na planície costeira do Rio Grande do Sul. A área de drenagem é de 2.697 km^2 com um litoral de 115 km e uma área inundada de 500 km^2. A bacia é constituída por um sistema de lagoas paralelas à costa conectadas por canais. As lagoas estão situados em uma planície costeira de origem holocênica (ca. 5000 BP) e foram formadas como um sistema de barreira lagunar após uma série de regressões e transgressões marinhas (SCHWARZBOLD; SCHÄFER, 1984; TOMAZELLI; DILLENBURG; VILLWOCK, 2000). Os principais rios que entram no sistema são os rios Maquiné e Três Forquilhas. O sistema de canais que conecta as lagoas é uma complexa rede de canais naturais e artificiais (GUIMARÃES; HARTZ; BECKER, 2014). O canal mais importante é o rio Tramandaí, que recebe água de várias lagoas localizadas na parte norte da bacia e deságua no complexo lagunar Tramandaí-Armazém. O complexo lagunar apresenta uma saída única para o Oceano Atlântico (LOITZENBAUER; MENDES, 2012). A área de estudo esta localizada nos limites entre os municípios de Imbé e Tramandaí situado na região norte do estado do Rio Grande do Sul, Brasil (29°58‘‘S; 50°08‘W).

As coletas foram conduzidas ao longo de um gradiente salino representado pelo rio Tramandaí, o Complexo estuarino lagunar Tramandaí-Armazém e a zona de arrebentação da região marinha adjacente (praia de Tramandaí) (vide mapa da área de estudo nos capítulos que compõem a tese). O rio Tramandaí deságua na porção norte da laguna Tramandaí, apresenta fundo lamoso, macrófitas aquáticas flutuantes (e.g., *Eichhornia crassipes*, *Pistia stratiotes*, *Salvinia herzogi*) e emergentes (e.g., *Rhynchospora gigantea*, *Scirpus californicus*, Poacea) ao longo das suas margens (MALABARBA et al., 2013; AFSG, observação pessoal), bem como a presença de serrapilheira sobre o sedimento de fundo (AFSG, observação pessoal).

O complexo estuarino inclui a laguna Tramandaí, a laguna Armazém e o canal permanente (1,5 km de comprimento e 0,3 km de largura) de conexão com o mar, por onde ocorre a entrada de água salgada, pela ação do vento sul e também é favorecida pela influência do regime de micro-marés (LOITZENBAUER; MENDES, 2012). O Estuário é do tipo estrangulado com profundidade variando de 1,5 m nas zonas rasas e 5 m no canal principal, com uma área de 30 km^2 (SCHWARZBOLD; SCHÄFER, 1984; TABAJARA; DILLENBURG, 1997). Os produtores primários mais representativos encontrados ao longo das margens e próximo a desembocadura são as vegetações do tipo marisma (e.g., *Juncus acutus*, *Rhynchospora gigantea*, *Scirpus olneyi*) e gramíneas terrestres, bem como macrófitas aquáticas flutuantes (*Eichhornia crassipes*) e fanerógamas submersas (*Ruppia maritima*) nas suas enseadas rasas (COSTA et al., 2004; HOEINGHAUS et al., 2011).

A zona de arrebentação marinha adjacente tem características de praia do tipo dissipativa sendo exposta diretamente à ação de ondas de energia média a elevada, podendo receber o aporte de águas continentais através dos riachos litorâneos (localmente conhecidos como sangradouros) presentes na linha de costa e do estuário (TOMAZELLI; VILLWOCK, 1991). Esta região marinha é caracterizada pela dominância do fitoplâncton e florações de micoalgas, sendo ausentes outros produtores primários como macrófitas aquáticas, bancos de macroalgas ou fanerógamas marinhas (BROWN; MCLACHLAN, 2010; ODEBRECHT et al., 2010b, 2014).

As atividades antrópicas potencialmente impactantes na área de estudo são crescentes e incluem atividades industriais (especialmente moveleira), silvicultura, orizicultura, pecuária, extração de areia, ocupação humana desordenada e, mais recentemente, implantação de estações eólicas para geração de energia elétrica (MALABARBA et al., 2013). As lagoas costeiras da bacia do rio Tramandaí são de grande importância, pois são uma fonte exclusiva de água doce para a planície costeira do litoral norte do Estado do Rio Grande do Sul. O uso da água é bastante diversificado na região e inclui a irrigação de arroz, atividades relacionadas com a temporada de férias de verão, abastecimento público de água e, particularmente perto de áreas urbanas, a diluição de resíduos domésticos e industriais. As atividades humanas podem ser muito prejudiciais ao alterar adversamente as características hidráulicas do sistema. Nas últimas décadas, tanto a população regional quanto a atividade econômica, principalmente a agricultura irrigada, cresceram consideravelmente, aumentando o consumo de água e levando a efeitos danosos sobre os mananciais naturais (LOITZENBAUER; MENDES, 2012).

Diante do exposto, a área de estudo constitui um modelo propício para avaliar as hipóteses e objetivos propostos, pois apresenta características ambientais, morfológicas e hidrológicas (conexão rio, estuário e mar) ideais para testar essas hipóteses, e ainda possui padrões contrastantes de distribuição, abundância e riqueza de produtores primários e consumidores que utilizam este ambiente.

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CAPÍTULO 1

Manuscrito aceito para publicação na revista *Marine and Freshwater Research.*

CHANGES IN FOOD WEB STRUCTURE OF FISH ASSEMBLAGES ALONG A RIVER TO OCEAN TRANSECT OF A COASTAL SUBTROPICAL SYSTEM

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Abstract

There is an urgent need to understand how food web structure changes along environmental gradients. Here, we investigated changes in trophic organization and the relative importance of autotrophic sources sustaining fish assemblages along a transect from river to ocean. In order to address these topics, we analyzed fish stomach contents and isotopic composition of consumers and food sources. Our findings revealed a greater number of autotrophic sources sustaining fish assemblages in the continental systems (especially in the river) than in the adjacent marine system. Bipartite networks depicting trophic relationships between fish and prey also changed along the transect, showing comparatively higher complexity in the estuary. These findings could be explained by the greater number of food web components (autotrophic sources, fish trophic guilds and preys) associated with pelagic and benthic food chains within the estuary in comparison with the adjacent systems studied. Our findings highlight the need to take into account river to ocean changes in food web structure of fish assemblages in management plans to mitigate man-made impacts in coastal systems.

Additional Keywords: trophic guilds; bipartite networks; stable isotopes; stomach content; trophic topological metrics; estuary

Introduction

The study of trophic relationships and food web structure in biological communities goes back to the pioneer work of Elton (1927) showing that animals are trophically linked to each other and ultimately dependent on primary producers. Since then, the way biological communities are structured (e.g., number and intensity of feeding links, trophic levels) across spatiotemporal scales has been one of the main themes in ecology (Winemiller and Layman 2005). Knowledge of food web structure and how abiotic factors may affect its organization are crucial, both for an understanding of ecosystem functioning and our ability to manage the exploration of natural resources properly (Layman *et al*. 2015; Pérez-Matus *et al*. 2017). There is increasing need to understand how food web structure changes along environmental gradients, especially in the face of environmental change associated with human activities and climate change (Ruiter *et al*. 2005).

Salinity variations in coastal systems are an important factor structuring trophic relationships in biological communities (e.g., Careddu *et al*. 2015; Claudino *et al*. 2015). This is especially apparent for fish assemblages because interspecific differences in physiological tolerance of salinity among these organisms result in marked changes in diversity and species composition along the riverine-estuarine-marine continuum (Harrison and Whitfield 2006; Moura *et al*. 2012; Whitfield *et al*. 2012). For instance, Moura and collaborators (2012) have shown that both species richness and fish abundance change along a freshwater to estuarine transect, mainly due to variations in abiotic factors such as salinity, sediment granulometry and water transparency.

The transitional zone between freshwater and marine systems (i.e. estuaries) supports an abundance of fish species and has been considered a crucial area for survival and growth of fish populations (McLusky and Elliott 2004; Barletta *et al*. 2010; Blaber 2013). The diversity of fish in estuaries can be substantial because they contain both resident species that complete their life cycles within the estuary and marine and freshwater species using them as nursery or spawning grounds (Elliott *et al*. 2007). Moreover, marine and freshwater fish vagrants sporadically enter estuaries, dependent on the net balance between salinity intrusion and freshwater discharge (Wootton 1990; Vieira *et al*. 1997). This diverse fish fauna exploits the abundant food resources available in estuaries via diverse feeding modes (Elliott *et al*. 2007; Buchheister and Latour 2015).

Patterns of dominance and distribution of primary producers in aquatic systems may also influence the trophic structure of fish assemblages (Kanaya *et al*. 2007; Claudino *et al*.

2015). Along the coastal sand plains of the southwestern Atlantic, for instance, estuaries may harbor a higher diversity of primary producers (e.g. salt marshes, mangroves, macroalgae, seagrass, phytoplankton, microphytobenthos) than adjacent marine surf-zones, where phytoplankton usually is the dominant autotrophic source (Seeliger *et al*. 1997; Odebrecht *et al*. 2014; Olds *et al*. 2017). Hence, high primary productivity and prey availability found in estuaries (Day *et al*. 2012; Odebrecht *et al*. 2017), associated with a rich fish fauna comprised of residents and other species (Barletta and Blaber 2007; Potter *et al*. 2013), may influence the food web structure of estuarine fish assemblages (e.g., Claudino *et al*. 2015; Mont‟Alverne *et al*. 2016).

Food webs are representations of trophic relationships between predators and prey (Cohen 1978; Pimm 1982) and it is crucial to unravel these links to better understand population dynamics, species coexistence and ecosystem diversity (Levin 1970; Holt *et al*. 1994; Chesson 2000). Descriptions of trophic networks are a key step towards understanding the organization and complexity of ecosystems (May 1972; Montoya *et al*. 2006; Dunne 2009) and the trophic role of species in a biological community (Bascompte 2009). Among the most common topological metrics used to determine structure and complexity of trophic networks are network size, number of links, link density and connectance (Pimm 1982; Winemiller and Layman 2005; Thompson *et al*. 2012). However, the dynamics of these properties, their correlation with environmental factors, and the diversity and stability of food webs are still a matter of debate (Winemiller and Layman 2005; Peralta-Maraver *et al*. 2017).

Several studies have described trophic relationships in fish assemblages using trophic guilds (Garrison and Link 2000; Elliott *et al*. 2007; Albouy *et al*. 2011; Buchheister and Latour 2015; Egan *et al*. 2017; Zhang *et al*. 2018), which can be defined as a group of organisms using the same type of resources in a similar way (Root 1967). The use of trophic guilds summarizes trophic relationships between consumers and their food sources, allowing simplification of highly complex ecosystems (Elliott and Dewailly 1995; Garrison and Link 2000; Lobry *et al*. 2003; Franco *et al*. 2006). The use of trophic guilds also facilitates between-ecosystem comparisons of the structure and dynamics of communities at a regional and global scale, which are more difficult or unfeasible at species level (Purvis and Hector 2000; Buchheister and Latour 2015). Hence, trophic guilds have been increasingly employed to understand the use of food resources by consumers and trophic connectivity between adjacent systems (Winemiller and Layman 2005).

Stable isotope analysis has been considered an effective tool to investigate indirect and direct food linkages sustaining fish assemblages (e.g., Bănaru and Harmelin-Vivien 2009;

López *et al*. 2016; Garcia *et al*. 2017). The fundamental assumption of the technique is that consumers reflect the stable isotope composition of assimilated food sources. Stable isotopes have been largely employed as natural markers to trace the flow of matter in food webs (Fry 2006; Boecklen *et al*. 2011; Layman *et al*. 2012). Although stable isotopes have become a standard method to infer trophic relationships, this method has some limitations (Boecklen *et al*. 2011; Layman *et al*. 2012). For instance, differences in isotopic composition are usually found among groups of primary producers with distinct photosynthetic physiological pathways (e.g., C_3 vs. C_4 plants), rather than at the species level (Layman *et al*. 2012). Traditional methods, such as stomach content analysis, also have drawbacks. For instance, it is difficult to distinguish among prey in an advanced stage of digestion and to estimate assimilation of ingested food (Jepsen and Winemiller 2002). The combination of stable isotope and stomach content analyses can provide a more effective approach to investigate trophic links in fish assemblages (Hadwen *et al*. 2007; Winemiller *et al*. 2011; Condini *et al*. 2015).

In this context, our hypotheses are: (i) There are a greater number of autotrophic sources sustaining fish assemblages in the estuary compared with adjacent river and marine surf-zone systems; (ii) The complexity of trophic networks depicting food relationships between fish and their prey is higher in the estuary than in the adjacent systems.

Materials and methods

Study área

Sampling was carried out along a salinity gradient formed by the Tramandaí River, the Tramandaí-Armazém estuarine complex and its adjacent marine surf-zone (hereafter referred as river, estuary and surf-zone, respectively) located in southern Brazil (29°58′S; 50°08′W) (Fig. 1). Regarding the adjacent marine area, we sampled only the beach surf-zone because it constitutes the dominant marine habitat occurring adjacent to estuaries along this coastal plain (Tomazelli and Villwock 1991); other marine habitats (e.g. seagrass meadows, mangroves, rocky bottoms, coral reefs) do not occur along this extensive (~600 Km long) coastal plain (Asmus *et al*. 1997).

The river had a muddy bottom and its vegetation was mainly floating (e.g., *Eichhornia crassipes*, *Pistia stratiotes*, *Salvinia herzogii*) and emergent macrophytes (e.g., *Rhynchospora* *gigantea*, *Scirpus californicus*, Poacea) along the margins (Malabarba *et al*. 2013). Coarse macrodetritus was commonly found over the bottom sediment.

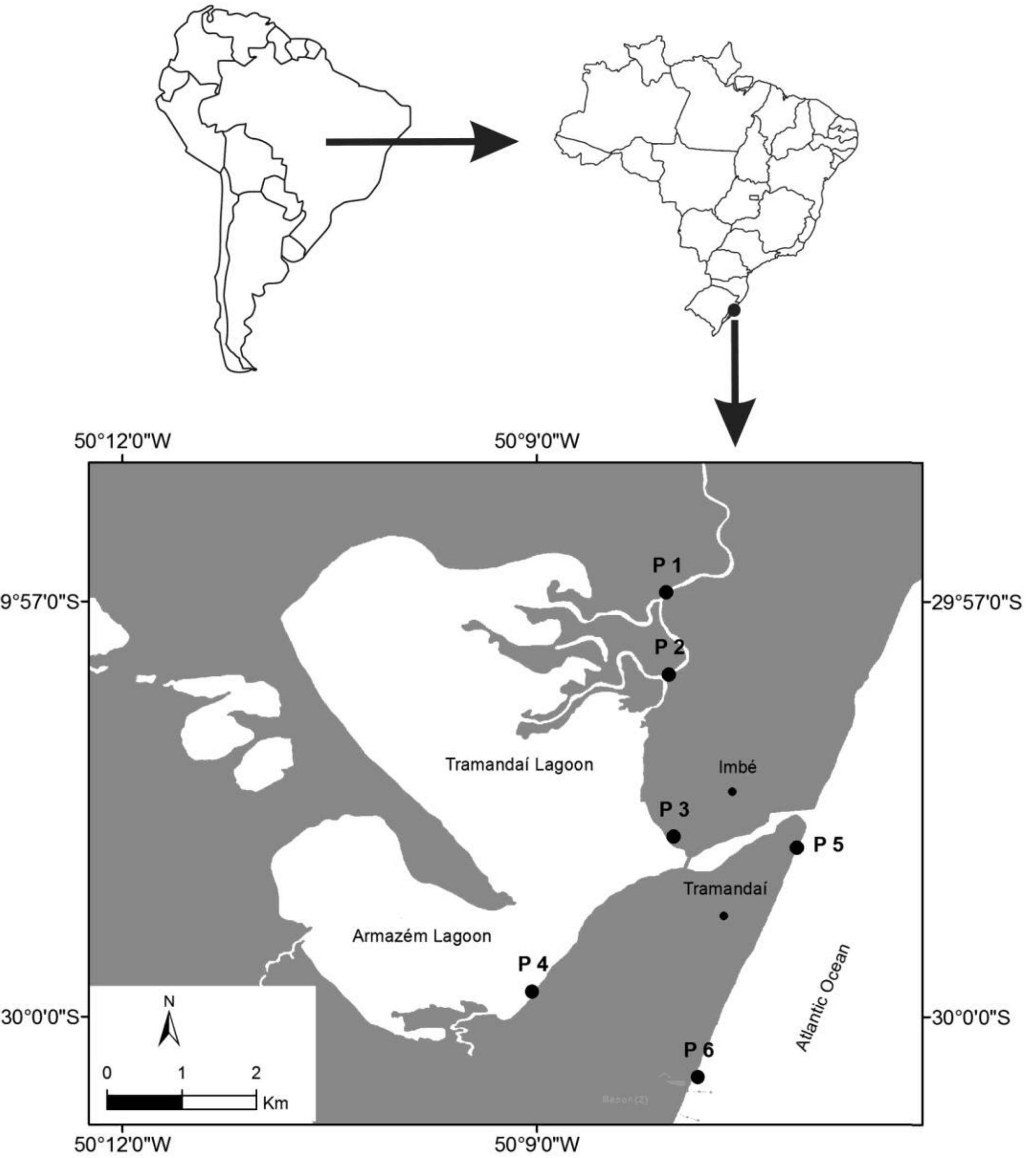


Fig. 1. Map showing the study area (Tramandaí-Armazém estuarine complex) and the locations of the sampling stations at the river (P1, P2), estuary (P3, P4) and the adjacent marine surf-zone (P5, P6).

The estuary had an area of approximately 30 km^2 and comprised two embayments and a main channel, from which high salinity water entered, especially with the predominance of southerly winds. The estuary was of a choked type (*sensu* Kjerfve 1986) with depths ranging from 1 to 5m, and it was under influence of a micro-tidal regime (Loitzenbauer and Mendes 2012). The vegetation comprised marsh plants (e.g., *Juncus acutus*, *Rhynchospora gigantea*, *Scirpus olneyi*), widgeon grass (*Ruppia maritima*), floating macrophytes (e.g., *Eichhornia crassipes*) and terrestrial grasses along the estuarine margins (Hoeinghaus *et al*. 2011).

The marine surf-zone had characteristics of a dissipative beach, being directly exposed to waves with medium to high energy, and received freshwater discharge from coastal streams draining freshwater wetlands (Tomazelli and Villwock 1991). In contrast with the river and the estuary, the surf-zone was characterized by the dominance of phytoplankton; other primary producers, such as floating aquatic macrophytes, seagrasses and macroalgae beds, were absent (Odebrecht *et al*. 2010).

Regarding anthropogenic impacts, the study area faces increasing environmental pressures from the furniture industry, pine afforestation, rice irrigation, cattle farming, sand extraction and unplanned human occupation of the coastal zone (Malabarba *et al*. 2013).

Field collections

We carried out field sampling on March 2015 at two stations in the river (P1, P2), estuary (P3, P4) and surf-zone (P5, P6) (Fig. 1). Fish were caught using various sampling gear, such as beach seine, bottom trawl, cast net and gillnets (see Garcia *et al*. 2006 for dimensions and operation procedures). Besides fish fauna, we collected autotrophic sources (marsh plants, aquatic macrophytes, seagrasses, terrestrial grasses along the margins) and particulate organic matter in suspension (POM) and in the sediment (SOM) at each sampling station. Marsh plants, macrophytes, seagrasses and terrestrial grasses were collected by hand. We obtained POM by filtering approximately 1.5 liters of water onto a pre-combusted (450°C for 4 h) Whatman glass-fiber filter (0.75 μm), and SOM by removing the top 2 cm from the surface of the sediment using a plastic core (10 cm diameter). Whenever possible, we sampled each autotrophic and organic matter source in triplicate at each sampling station. Fish and autotrophic sources were kept frozen until processed in the laboratory, where each food web component was identified to the lowest taxonomic level possible. Concomitant with biological sampling, we measured the following abiotic parameters at each sampling station: salinity, water temperature and transparency using an optical refractometer, mercury thermometer and *Secchi* disk, respectively.

Fish's stomach contents

We measured the total length (TL, mm) and weight (g) of each individual fish, removed its stomach by dissection, which was fixed with formaldehyde 10% and later stored in 70% alcohol. The contents of each stomach were identified to the lowest taxonomic level possible using a microscopic stereoscopic at x40 (Ruppert and Barnes 1994; Brusca and Brusca 2007). We pooled items found by food categories, which were quantified using frequency of occurrence (%F) and area (%A) occupied by the item (mm^2) using graph paper. In order to calculate the area, each item was spread evenly over a Petri dish with no empty space between them and with a standard height of 1 mm. In case of an item with height greater than 1 mm, the item was broken down in to smaller pieces in order to maintain the standard height of 1 mm (Hellawell and Abel 1971). We used the parameters %F and %A to calculate the Alimentary Index (IAi), according to the formula: $IAi = \%F * \%A/\Sigma(\%F * \%A)$ (Kawakami and Vazzoler 1980). We used this index to determine the most representative food sources in the fish diets and in the determination of trophic guilds. We did not carry out stomach content analysis of mullet species (*Mugil liza* and *M. curema*), because a specific protocol regarding stomach content manipulations and observation at a higher resolution (e.g., at x100) would be necessary (Cardona 2016). Therefore, we used diet information from the literature to classify these species into trophic guilds (see references on Table 1 and Table supplementary 1).

The food categories were (i) Decapoda (DECAPO): Penaeidae, Decapoda Brachyura; (ii) Insecta (INSECT): Adults of Coleoptera, Diptera, Ephemeroptera, Hymenoptera, Odonata, insect remains; (iii) Teleostei (TELEOS): Atherinidae, Carangidae, Clupeidae, Engraulidae, Mugilidae, fish"s fragments like scales, eyes, eggs and others not identified); (iv) Zoobenthos (ZOOBEN): Nematoda, Oligochaeta, Polychaeta, Bivalvia, Gastropoda, Harpacticoida, Ostracoda, Amphipoda, *Emerita* sp. Isopoda, Hydracarina, Chironomidae larvae and other insect larvae; (v) Zooplankton (ZOOPLA): Copepoda, Cladocera, Larvae and Zoea of Decapoda; (vi) Microalgae (MICROA): Diatoms; (vii) Macroalgae (MACROA): filamentous algae; (viii) Vegetation (VEGETA): leaves, stalks, seeds and identified fragments; (ix) Organic detritus: remains and organic matter of unidentified origin, which was not included in the data analysis to determine the diet and trophic guilds.

Stable isotope analysis

Samples of approximately 5 g of tissue were dissected from each primary producer and consumer comprising leaves of widgeon seagrass, marsh plants, macrophytes, and terrestrial grasses, and anterodorsal muscular tissues from fish. We rinsed each tissue with distilled water, placed in a sterile Petri dish, and dried in an oven at 60°C to a constant weight (minimum of 48 h). Dried samples were ground to a fine powder with a mortar and pestle and stored. Subsamples were weighed (~1 mg for animal tissues, 25 to 30 mg for SOM, and approximately 3 mg for other autotrophic sources), pressed into ultra-pure tin capsules (Costech Analytical Technologies), and analyzed for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios. The carbon standard was Pee Dee Belemnite limestone, and the nitrogen standard was atmospheric nitrogen. We expressed results in delta notation (parts per thousand deviation from a standard material): $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard})-1]*1000$, where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Standard deviations for $\delta^{13}C$ and $\delta^{15}N$ replicate analyses of internal standards were 0.11‰ and 0.10‰, respectively.

Samples with higher lipid contents can have lower $\delta^{13}C$ (DeNiro and Epstein 1977). In order to control for this effect we applied mathematical normalization using C:N and %C as a proxy for lipid content in animal and plant samples, respectively. Following Post and collaborators (2007), we corrected fish samples with C:N higher than 3.5 using the equation $\Delta\delta^{13}C = -3.32 + 0.99 * C{:}N$ and vegetation samples with %C higher than 40% using the equation $\Delta\delta^{13}C = -5.83 + 0.14 * \%C$ (Post *et al.* 2007).

Data analyses

Classification of trophic guilds based on stomach contents

We classified the different fish species into trophic guilds according to the similarity of their diets based on cluster analysis using Bray-Curtis distances, which we determined using stomach content analysis of all sampled individuals (Elliott *et al.* 2007; Buchheister and Latour 2015; Zhang *et al.* 2018). Before the analysis, we transformed the data using Hellinger procedure, which gives a more accurate, unbiased estimate of variance partition (Rao 1995; Peres-Neto *et al.* 2006). We carried out a correlation between the original distance matrix and the cophenetic matrix to evaluate how well the dendrogram preserved the pairwise distances between the original unmodeled data points (Rohlf and Fisher 1968). We ran these analyses using the Vegan package (Oksanen *et al.* 2017) in R (R Core Team 2017).

Stomach content analysis was based on fish sampled on one occasion. In order to minimize potential inaccuracies in the determination of the trophic guilds due to this lack of temporal (e.g., seasonal) replication, we compared our dietary estimations with reported diets of our analyzed fish (see references on Table 1). In a few cases, where all sampled individuals of a species had empty stomachs we used diet information reported in the literature. The classification of trophic guilds was based on Elliott *et al*. (2007). We also included insectivore (IN) to denote the consumption of post-metamorphic stages of winged insects presumably through feeding at the water surface (Ou and Winemiller 2016; Possamai et al. 2018).

Bipartite networks based on fish stomach contents

We derived bipartite predator-prey networks based on fish‟ stomach contents to compare the trophic structure of fish assemblages along the salinity gradient (Dormann et al. 2008). A bipartite network was derived for each region (river, estuary, surf-zone) using average values of the Alimentary Index (IAi) as a quantitative proxy for the strength of each trophic link. We used the following topological metrics to compare the networks (Pimm 1982; Dunne 2009): total size of the network (i.e. number of nodes; S), number of trophic links among consumers and resources (L), link density (D=L/S), Shannon‟s diversity of interactions (H‟) and connectance (i.e. realized proportion of possible links; C=2L/S(S-1)). This last metric provides an estimative of how interconnected the consumers are in the network and, together with S and H‟, are indicative of communities‟ complexity (Pimm 1982).

Spatial patterns in isotopic composition and correlations with fish body sizes

We conducted a correlation between total length (TL, mm), and δ^{13}C and δ^{15}N of each individual in order to reveal relationships between fish body sizes and their isotopic composition. When we found statistically significant correlation (α = 5%), we classified the species in small and large size groups based on the assumption that size groups with distinct isotopic composition would have distinct diets. We used two criteria to distinguish between both size groups: a) frequency distribution of body sizes, based on our measurements of individual TL, and sexual maturity, based on visual inspections of development stages of their gonads and complemented by reproductive information on FishBase (Froese and Pauly 2018).

We used permutational multivariate analysis of variance (PERMANOVA) to evaluate possible differences in average δ^{13}C and δ^{15}N of fish and autotrophic sources among regions (river, estuary, surf-zone). We transformed the data using square root and used a Bray-Curtis similarity matrix (Anderson *et al*. 2008). We employed a *post-hoc* par-wise test to determine differences among means after considering Bonferroni corrections. We used the betadisper test (n=9,999 permutations) to evaluate the assumption of homogeneity dispersion among groups (Anderson 2006). We carried out these analyses using the Vegan package (Oksanen *et al*. 2017) in R (R Core Team 2017).

Isotope mixing models to estimate autotrophic contributions to fish guilds

We used $\delta^{13}C$-$\delta^{15}N$ biplots to visualize spatial trends in isotopic composition of trophic guilds and autotrophic sources (Fry 2006) and Bayesian mixing models to estimate contributions of autotrophic sources to consumers (Parnell *et al*. 2013). We considered as end-members in the mixing models representative autotrophic sources in each region: C_3 and C_4 plants, POM and SOM in the river and estuary, and POM and SOM in the surf-zone. We ran the isotope Bayesian mixing models with the SIMMR (*Stable Isotope Mixing Models in* R) package (Parnell 2016) in R (R Core Team 2017), which is an updated version of the SIAR (*Stable Isotope Analysis in* R) package that employs Gaussian likelihood and fits the model to the data via Markov chain Monte Carlo (MCMC). One of the benefits of this model is that it takes into account uncertainty and variation in consumers, sources, and trophic enrichment factors (TEF) (Parnell *et al*. 2010). We used average (±standard deviation) TEF values of 2.54 ± 0.11 for $\delta^{15}N$ (Vanderklift and Ponsard 2003) and 0.47 ± 1.23 for $\delta^{13}C$ (Vander Zanden and Rasmussen 2001). TEF values were multiplied by the average trophic position of each species in order to take isotopic fractionation along the food chain into account (Feng *et al*. 2014; Raoult et al. 2018). Trophic positions (TP) were calculated according the formula $TP = \lambda + (\delta^{15}N_{Consumer} - \delta^{15}N_{baseline})/F$, where λ is the baseline trophic level which is set at 1 for primary producers; $\delta^{15}N_{Consumer}$ is the $\delta^{15}N$ value of each specimen; $\delta^{15}N_{baseline}$ is the average $\delta^{15}N$ value from primary producers at the corresponding site; and F is the trophic discrimination factor for $\delta^{15}N$, which was 2.54 (Vanderklift and Ponsard 2003). For each mixing model, we ran 500,000 iterations, followed by a burn-in and thinning of 50,000 and 5,000, respectively (Parnell 2016). We evaluated the assumption of model convergence using the diagnostic tests of Gelman-Rubin and Geweke (Parnell 2016).

A fundamental assumption of isotope mixing models is that the isotopic variability of consumers, after accounting for fractionation, are contained within the variability of the assimilated food sources (Phillips *et al*. 2014). We tested this assumption using isotope mixing polygon simulations that quantitatively determine boundaries of possible source values in the $\delta^{13}C$-$\delta^{15}N$ biplot space that can explain the isotope mixture (Smith *et al*. 2013).

We ran this analyses using the packages *sp* (Pebesma and Bivand 2005; Bivand *et al.* 2013) and *splancs* (Rowlingson and Diggle 2017) in R (R Core Team 2017). Individual consumers located outside the 95% mixing polygon region were excluded from the analysis (see Fig. Supplementary 1) (Smith *et al.* 2013; Phillips *et al.* 2014).

Results

Trophic guilds and bipartite networks based on stomach content

There was a wide range in salinity (PSU) (0 to 38) along the studied gradient, with lower average values in the river (0.5 ± 0.5), intermediate in the estuary (8.5 ± 6.0) and higher in the surf-zone (37.5 ± 0.5). A total of 25 fish species was sampled, with a higher number of species in the estuary (19), followed by the surf-zone (11) and the river (4). Size structure of fish assemblages changed across regions. Average TL (mm) ranged from 27.1 to 385.0 mm in the surf-zone, from 29.3 to 230.0 mm in the estuary, and from 53.0 to 251.0 mm in the river (Table 1).

Table 1. Species composition of each fish trophic guild, number of individuals (N), average (±SD) values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios and total length (TL - mm)of each fish species sampled in the river, estuary and surf-zone.

Codes for trophic guilds are: piscivore (PV), zoobenthivores of epifauna-infauna (ZB-EI), zoobenthivores of hyperbenthos (ZB-H), zooplanktivore (ZP), omnivore (OV), herbivore of phytoplankton (HV-P); herbivore of macroalgae/macrophytes (HV-M), detritivore (DV) and insectivore (IN). The number in superscript denotes the literature reference used to corroborate the trophic guild classification. The letters „S" and „L" after some species names stands for small and large individuals (see M&M for more details). The numbers in superscript refers to the literature consulted to obtain complementary diet information to corroborate our classification of fish guilds based on stomach content analysis (see Table Supplementary 1 for these references). *denote individuals that had their $\delta^{13}C$ values corrected to control for higher lipid contents (see M&M).

GUILDS	Species	N	$\delta^{13}C$	$\delta^{15}N$	TL (mm)
			RIVER		
IN	*Astyanax jacuhiensis* [1]	4	-24.50 ± 3.39	7.56 ± 0.30	53.00 ± 3.74
ZB-H	*Lycengraulis grossidens* L [2]	2	-19.06 ± 1.00	12.38 ± 0.29	91.50 ± 7.78
ZP	*Lycengraulis grossidens* S [2]	8	-22.22 ± 1.50	8.51 ± 0.48	57.50 ± 16.01
OV	*Geophagus brasiliensis* [3]	14	-22.24 ± 2.63	9.12 ± 1.08	120.36 ± 39.92
DV	*Mugil liza* [4. 5]	2	-17.38 ± 2.42	8.93 ± 0.62	251.00 ± 8.49
			ESTUARY		
IN	*Atherinella brasiliensis* [6]	10	-19.18 ± 0.93	11.52 ± 0.53	80.00 ± 30.23
PV	*Oligosarcus jenynsii* [7]	3	-18.04 ± 0.19	11.83 ± 0.28	152.67 ± 12.34
ZB-EI	*Citharichthys spilopterus* [8]	13	-19.06 ± 1.02	9.62 ± 0.54	63.62 ± 22.85
	Diapterus rhomeus [9] *	11	-18.98 ± 0.86	11.23 ± 2.04	104.00 ± 59.36
	Eucinostomus argenteus [11. 12]	16	-17.80 ± 0.47	10.58 ± 0.45	81.00 ± 19.30
	Symphurus tesselatus [8]	2	-19.56 ± 0.28	9.71 ± 0.18	85.00 ± 14.14

	Trachinotus carolinus [10] *	4	-18.02 ± 1.10	11.62 ± 1.24	178.50 ± 10.21
ZB-H	*Lycengraulis grossidens* L [2]	15	-18.27 ± 1.77	13.53 ± 1.67	134.53 ± 23.02
ZP	*Brevoortia pectinata* L [14] *	5	-16.42 ± 0.91	11.99 ± 0.90	230.00 ± 8.15
	Eucinostomus melanopterus [13]	15	-17.25 ± 2.13	11.44 ± 0.47	65.87 ± 13.18
OV	*Genidens barbus* [14. 16] *	19	-17.22 ± 1.74	15.40 ± 2.27	124.11 ± 80.63
	Genidens genidens [15] *	25	-18.18 ± 1.18	12.95 ± 0.71	159.60 ± 88.70
	Geophagus brasiliensis [3]	14	-17.41 ± 2.16	9.30 ± 0.97	92.14 ± 57.55
	Micropogonia furnieri [14. 17] *	17	-18.46 ± 1.08	11.41 ± 1.58	119.12 ± 65.80
HV-P	*Anchoa marinii* [14]	7	-19.23 ± 0.89	10.87 ± 0.66	56.86 ± 6.59
	Jenynsia multidentata [18]	3	-13.97 ± 2.49	7.22 ± 2.64	29.33 ± 4.04
HV-M	*Astyanax fasciatus* [1]	5	-20.48 ± 0.98	11.30 ± 0.40	148.80 ± 5.54
DV	*Mugil curema* [4. 5] *	9	-18.94 ± 0.41	8.04 ± 1.32	197.00 ± 117.77
	Mugil liza [4. 5] *	9	-15.28 ± 2.52	7.00 ± 2.10	32.44 ± 1.59
			SURF-ZONE		
PV	*Macrodon atricauda* [14] *	3	-16.54 ± 0.18	15.44 ± 0.43	385.00 ± 38.00
ZB-EI	*Menticirrhus littoralis* [19]	10	-14.96 ± 0.68	15.21 ± 0.62	139.50 ± 134.73
	Trachinotus carolinus [10]	12	-17.04 ± 0.80	12.80 ± 1.00	79.10 ± 79.01
ZB-H	*Genidens barbus* [20]	2	-15.91 ± 0.71	14.63 ± 0.81	310.00 ± 9.90
	Trachinotus marginatus L [21]	15	-16.05 ± 0.72	14.57 ± 0.49	73.53 ± 16.47
ZP	*Harengula clupeola* [23]	3	-17.20 ± 0.55	12.99 ± 0.12	69.33 ± 4.04
	Micropogonia furnieri [22]	3	-16.54 ± 0.86	13.62 ± 0.28	56.67 ± 17.24
	Trachinotus marginatus S [21] *	10	-17.54 ± 1.06	12.58 ± 0.82	27.07 ± 9.40
HV-P	*Brevoortia pectinata* S [14] *	6	-14.56 ± 0.25	13.88 ± 0.30	78.67 ± 9.83
	Sardinella brasiliensis [24] *	5	-17.30 ± 0.44	12.84 ± 0.37	72.60 ± 10.88
DV	*Mugil curema* [4. 5] *	10	-18.60 ± 0.78	10.29 ± 1.54	32.15 ± 2.01
	Mugil liza [4. 5] *	13	-17.52 ± 3.13	9.97 ± 1.78	112.08 ± 161.13

The cluster analysis (cophenetic correlation = 0.80) revealed 10 trophic guilds at a 41% dissimilarity level (Fig. 2). After considering diet information of each species reported in the literature (Table Supplementary 1), we reclassified *Citharichthys spilopterus* caught in the estuary and *Geophagus brasiliensis* in the river into different trophic guilds. Moreover, all specimens of *Lycengraulis grossidens* caught in the river and individuals of *Brevoortia pectinata* L, *Symphurus tesselatus* and *Trachinotus carolinus* sampled in the estuary had their empty stomachs and they were classified into trophic guilds based on the literature (Table Supplementary 1).

Nine trophic guilds were identified (Fig. 2 and Table supplementary 1): (i) insectivore (IN): dominance of adult winged insects (94.8%), (ii) piscivore (PV): teleosteans (100%), (iii) zoobenthivores of epifauna-infauna (ZB-EI): dominance of zoobenthos (91.6%), (iv) zoobenthivores of hyperbenthos (ZB-H): dominance of decapods (69.8%), but including a substantial amount of teleosteans (22.6%), (v) zooplanktivore (ZP): dominance of zooplankton (95.4%), (vi) omnivore (OV): similar amounts of vegetation (38.4%) and zoobenthos (37.1%), and teleosteans (19.9%), (vii) herbivore of phytoplankton (HV-P): dominance of microalgae (98.5%), (viii) herbivore of macroalgae/macrophytes (HV-M):

dominance of macroalgae (86.1%) and (ix) detritivore (DV): dominance of detritus and microalgae (inferred from literature).

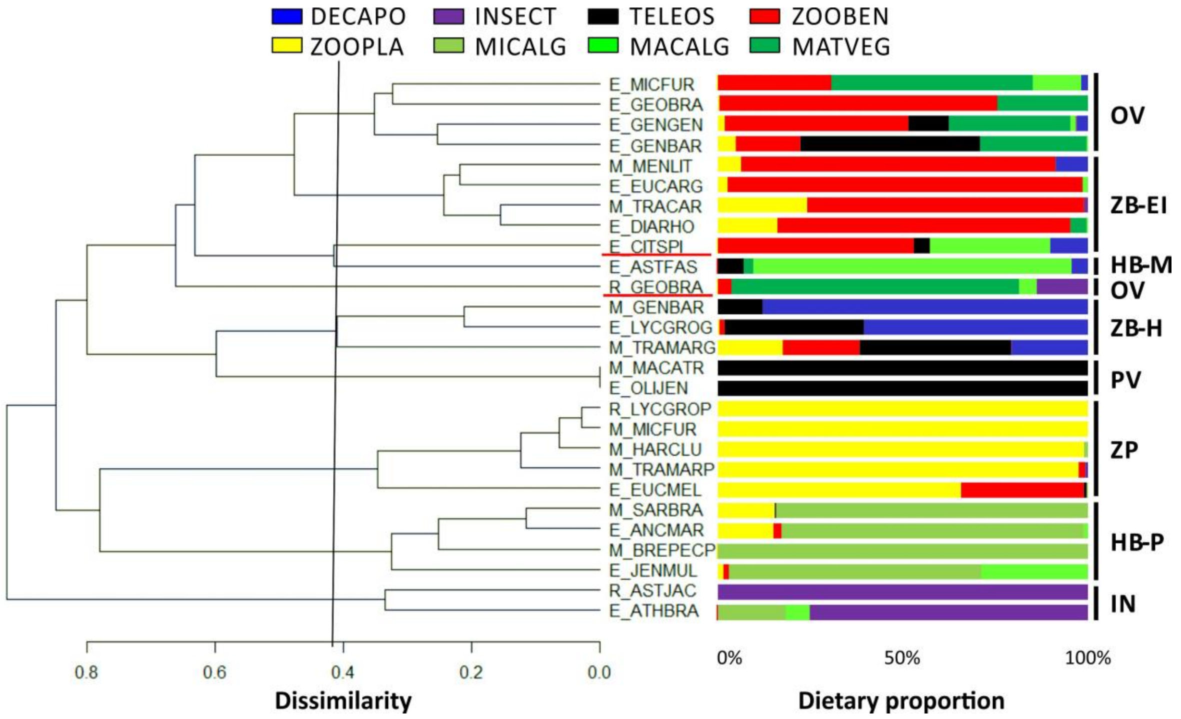
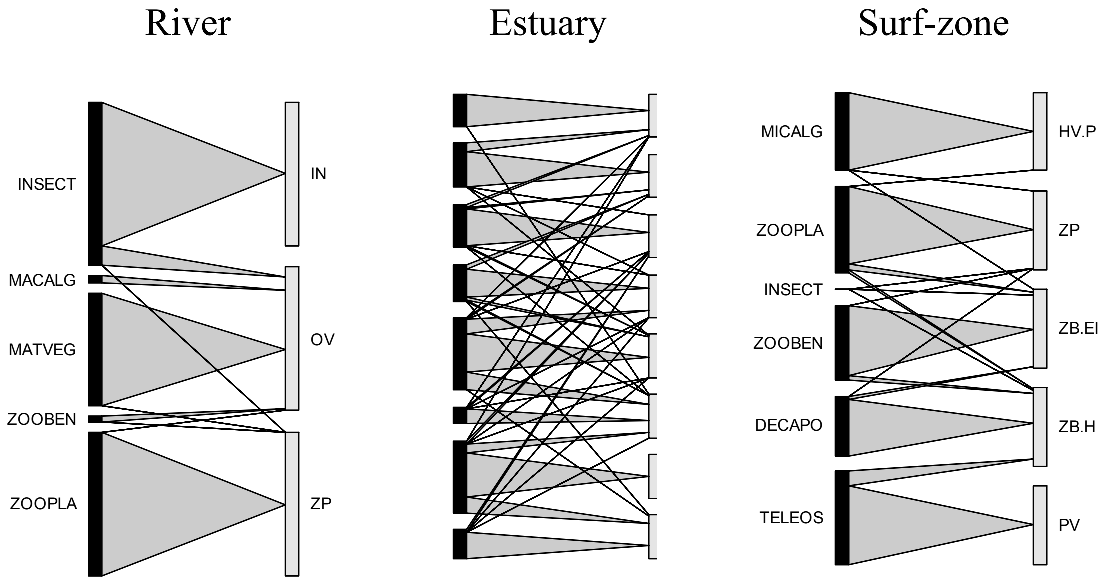


Fig. 2. Results of the cluster analysis showing the classification of the trophic guilds based on dissimilarity in fish diet. Dietary proportions were based on the relative importance (Alimentary Index, IAi) of food items found in stomach contents. The vertical dark line denotes the level of dissimilarity considered to classify the trophic guilds. The horizontal red lines denote two species (CITSPI, GEOBRA) that were reclassified into other guilds (ZB-EI, OV) after considering evidences on the literature. The letters M, E and R before each species name denote the regions marine, estuarine and riverine regions, respectively. See legend of Table 1 for codes of trophic guilds. Species codes are ANCMAR (*Anchoa marinii*), ASTFAS (*Astyanax fasciatus*), ASTJAC (*A. jacuhiensis*), ATHBRA (*Atherinella brasiliensis*), BREPEC (*Brevoortia pectinata*), CITSPI (*Citharichthys spilopterus*), DIARHO (*Diapterus rhomeus*), EUCARG (*Eucinostomus argenteus*), EUCMEL (*E. melanopterus*), GENBAR (*Genidens barbus*), GENGEN (*G. genidens*), GEOBRA (*Geophagus brasiliensis*), HARCLU (*Harengula clupeola*), JENMUL (*Jenynsia multidentata*), LYCGRO (*Lycengraulis grossidens*), MACATR (*Macrodon atricauda*), MENLIT (*Menticirrhus littoralis*), MICFUR (*Micropogonia furnieri*), OLIJEN (*Oligosarcus jenynsii*), SARBRA (*Sardinella brasiliensis*), SYMTES (*Symphurus tesselatus*), TRACAR (*Trachinotus carolinus*), TRAMAR (*T. marginatus*).

The topological metrics of bipartite trophic networks differed along the river-estuary-surf-zone transect suggesting changes in the trophic structure of fish assemblages (Fig. 3). The total size of the network (S = 16), total number of trophic links between consumers and resources (L = 42), link density (D = 2.6), Shannon‟s diversity of interactions (H‟ = 2.7) and connectance (C = 0.35) were higher values in the estuary compared with the river (S = 8, L = 10, D = 1.2, H‟ = 1.3, C = 0.36) and surf-zone (S = 11, L = 18, D = 1.6, H‟ = 1.9, C = 0.33). These findings suggest higher complexity in the trophic bipartite network of the estuarine fish assemblage compared with the other regions.



S=8, L=10, D=1.2, H‟=1.3, C=0.36   S=16, L=42, D=2.6, H‟=2.7, C=0.35   S=11, L=18, D=1.6, H‟=1.9, C=0.33

Fig. 3. Bipartite network representations of trophic relationships between fish assemblages and their preys based on stomach content analysis at each region. The width of each link in the bipartite graphs reflects relative interaction strength estimated by the Alimentary Index (IAi). See Table 1 for legend of trophic guild codes. Prey category codes: Decapoda (DECAPO), Insecta (INSECT), Teleostei (TELEOS), Zoobenthos (ZOOBEN), Zooplankton (ZOOPLA), Microalgae (MICALG), Macroalgae (MACALG), Vegetation (MATVEG). Topological network metrics are denote as S (total size of the network), L (total number of trophic links among consumers and resources), D (link density), H‟ (Shannon‟s diversity of interactions) and C (connectance, i.e. realized proportion of possible links).

Spatial patterns in isotopic composition and correlations with fish body sizes

A few fish species had a statistically significant positive correlation between body size and isotopic composition, both for $\delta^{13}C$ and $\delta^{15}N$, and were classified in two group sizes (small, S and large, L): *Brevoortia pectinata* ($\delta^{13}C$: $r^2 = 0.73$, $p < 0.001$; $\delta^{15}N$: r^2 = 0>74, $p < 0.001$), *Lycengraulis grossidens* ($\delta^{13}C$: $r^2 = 0.73$, $p < 0.000$; $\delta^{15}N$: $r^2 = 0.81$, $p < 0.000$) and *Trachinotus marginatus* ($\delta^{13}C$: $r^2 = 0.62$, $p < 0.000$; $\delta^{15}N$: $r^2 = 0.63$, $p < 0.000$).

PERMANOVA test revealed statistically significant differences in isotopic composition among regions both for fish (δ^{13}C: Pseudo-$F_{2.111}$ = 56.0; $p < 0.000$; δ^{15}N: Pseudo-$F_{2.111}$ = 55.3; $p < 0.000$) and autotrophic sources (δ^{13}C: Pseudo-$F_{2.50}$ = 43.2; $p < 0.000$; δ^{15}N: Pseudo-$F_{2.50}$ = 28.2; $p < 0.000$). For fish, both average δ^{13}C and δ^{15}N increased along the salinity gradient: -22.0 and 9.0 in the river, -18.0 and 11.4 in the estuary, and -16.4 and 12.9 in the surf-zone (posteriori test, α=0.05). We observed an overall similar increasing trend in δ^{13}C and δ^{15}N average values along the river-estuary-surf-zone transect, but the differences between river and estuary were not statistically significant (Fig. 4).

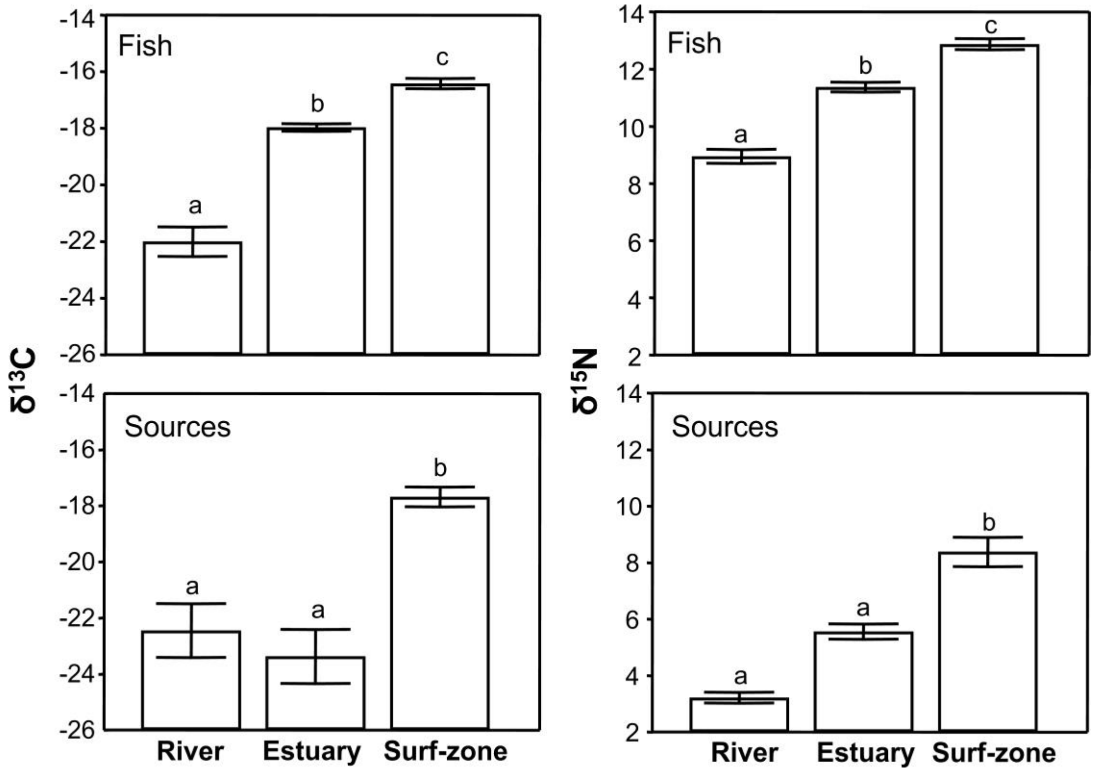


Fig. 4. Average (±SD) values of carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope ratios of fish and autotrophic sources sampled in the river, estuary and surf-zone. Distinct letters denote statistically significant differences ($p < 0.05$) among average values.

Ranges in average δ^{13}C values of trophic guilds changed across regions, with higher values in the river (from -24.5±3.4 to -17.4±2.4) compared with the estuary (from -20.5±1.0 to -17.0±1.9) and surf-zone (from -17.9±2.4 to -15.8±1.5). In contrast, we observed the widest range for δ^{15}N in the estuary (from 7.5±1.8 to 13.5±1.7), followed by the surf-zone (from 10.1±1.7 to 15.4±0.4) and river (from 7.6±0.3 to 12.4±0.3). It is worth noting that some autotrophic sources had the highest δ^{15}N values at surf-zone (POM: 7.0±1.3 and SOM: 10.5±0.4), which was also observed in this region for fish guilds (Fig. 5; Tables 1 and 2).

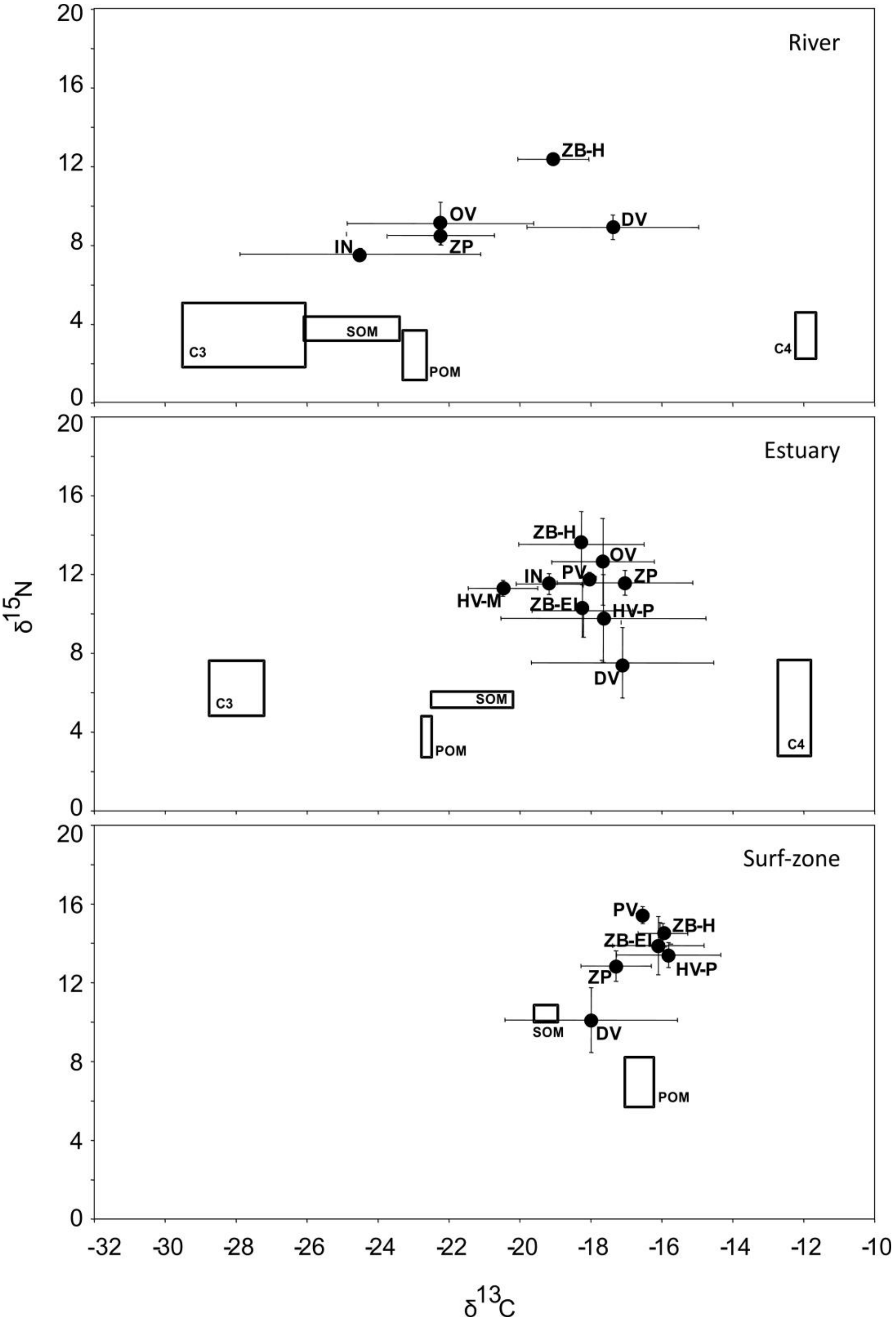


Fig. 5. Average (±SD) values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of trophic guilds (closed circles) and autotrophic sources (open boxes) sampled in the river, estuary and surf-zone. See Tables 1 and 2 for legends of trophic guilds and autotrophic sources codes.

Table 2. Number of samples (N), average (±SD) values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of autotrophic sources sampled in the river, estuary and surf-zone. Primary producers with distinct photosynthetic physiological pathways are denotes C_3 and C_4. POM = particulate organic matter in suspension, SOM = particulate organic matter in the sediment. *denote individuals that had their $\delta^{13}C$ values corrected to control for higher lipid contents (see M&M).

SPECIES	SOURCES	GROUP	N	$\delta^{13}C$	$\delta^{15}N$
			RIVER		
Eichornia crassipes	macrophyte	C_3	6	-27.95 ± 0.66	4.21 ± 0.37
Pistia stratiotes	macrophyte	C_3	3	-30.79 ± 0.46	3.59 ± 0.35
Salvinia herzogii *	macrophyte	C_3	6	-28.43 ± 1.29	1.10 ± 0.83
Rhynchospora gigantea *	marsh plant	C_3	3	-25.88 ± 0.32	5.77 ± 0.63
Scirpus californicus *	marsh plant	C_3	6	-26.35 ± 0.86	3.88 ± 1.10
Poacea (morphotype 1) *	terrestrial grass	C_4	6	-11.96 ± 0.32	3.54 ± 1.56
Poacea (morphotype 2)	terrestrial grass	C_4	3	-11.76 ± 0.21	3.84 ± 0.28
Poacea (morphotype 3)	terrestrial grass	C_4	3	-12.22 ± 0.28	2.37 ± 0.49
Poacea (morphotype 4) *	terrestrial grass	C_4	2	-11.81 ± 0.09	3.77 ± 0.48
Organic matter in suspension	POM		6	-22.97 ± 0.32	2.42 ± 1.27
Organic matter in sediment	SOM		7	-24.77 ± 1.33	3.81 ± 0.62
			ESTUARY		
Eichornia crassipes *	macrophyte	C_3	6	-28.26 ± 0.31	5.83 ± 0.47
Juncus acutus *	marsh plant	C_3	3	-26.55 ± 0.36	5.61 ± 1.10
Rhynchospora gigantean *	marsh plant	C_3	3	-27.93 ± 0.20	8.19 ± 0.31
Scirpus olneyi *	marsh plant	C_3	6	-28.45 ± 0.65	5.97 ± 1.57
Ruppia maritima	seagrass	C_4	3	-12.09 ± 0.54	3.07 ± 0.47
Poacea (morphotype 5) *	terrestrial grass	C_4	3	-12.45 ± 0.03	7.35 ± 0.30
Organic matter in suspension	POM		6	-22.64 ± 0.14	3.81 ± 1.05
Organic matter in sediment	SOM		6	-21.36 ± 1.14	5.68 ± 0.39
			SURF-ZONE		
Organic matter in suspension	POM		6	-16.63 ± 0.43	6.85 ± 1.53
Organic matter in sediment	SOM		6	-19.26 ± 0.29	10.45 ± 0.36

Autotrophic contributions to fish trophic guilds estimated by mixing models

Overall, the relative contribution of autotrophs to trophic guilds changed along the transect from river-estuary-surf-zone (Table 3). POM was the autotrophic source with highest contribution at the estuary (with mean values ranging from 30% for PV to 70% for OV), followed by C_4 plants (9% for HV-M and 44% for DV). The autotrophic sources with the lowest contributions were SOM (ranging from 2% for OV to 18% for PV) and C_3 plants (ranging from 1% for OV to 13% PV). In contrast, all autotrophic sources (C_3, C_4, POM, SOM) contributed in similar proportions in the river to the guild IN (27%, 10%, 29%, 21%, respectively) and ZB-H (18%, 24%, 26%, 19%), and higher contribution of POM to the guilds OV (75%) and ZP (42%). At the surf-zone, there was a predominance of a contribution of POM to all fish guilds (PV = 59%, ZB-EI = 87%, ZB-H = 90%, ZP = 81% and HV-P = 85%), with exception of DV (48% for POM and 52 for SOM) (Table 3).

Table 3. Results of the Bayesian isotopic mixing models (SIMMR) showing the median (50%) and 95% credibility interval (within parentheses) contributions of autotrophic sources (C_3, C_4, POM, SOM) to trophic guilds at the river, estuary and surf-zone.

Codes for trophic guilds are: piscivore (PV), zoobenthivores of epifauna-infauna (ZB-EI), zoobenthivores of hyperbenthos (ZB-H), zooplanktivore (ZP), omnivore (OV), herbivore of phytoplankton (HV-P); herbivore of macroalgae/macrophytes (HV-M), detritivore (DV) and insectivore (IN).

GUILDS	C3		C4		POM		SOM	
				RIVER				
IN	0.27	(0.03 - 0.67)	0.10	(0.02 - 0.32)	0.29	(0.03 - 0.81)	0.21	(0.02 - 0.74)
ZB-H	0.18	(0.02 - 0.60)	0.24	(0.03 - 0.58)	0.26	(0.03 - 0.76)	0.19	(0.02 - 0.63)
ZP	0.24	(0.03 - 0.61)	0.08	(0.01 - 0.21)	0.42	(0.05 - 0.86)	0.16	(0.02 - 0.68)
OV	0.08	(0.01 - 0.42)	0.08	(0.01 - 0.24)	0.75	(0.15 - 0.93)	0.07	(0.01 - 0.42)
				ESTUARY				
IN	0.05	(0.01 - 0.22)	0.19	(0.08 - 0.30)	0.68	(0.27 - 0.84)	0.07	(0.01 - 0.39)
PV	0.13	(0.02 - 0.37)	0.32	(0.07 - 0.51)	0.30	(0.04 - 0.69)	0.18	(0.02 - 0.63)
ZB-EI	0.02	(0.01 - 0.07)	0.28	(0.25 - 0.32)	0.66	(0.55 - 0.72)	0.04	(0.01 - 0.12)
ZB-H	0.06	(0.01 - 0.22)	0.32	(0.21 - 0.42)	0.50	(0.18 - 0.67)	0.10	(0.02 - 0.36)
ZP	0.03	(0.01 - 0.12)	0.34	(0.27 - 0.40)	0.57	(0.35 - 0.67)	0.05	(0.01 - 0.21)
OV	0.01	(0.00 - 0.03)	0.27	(0.24 - 0.31)	0.70	(0.64 - 0.74)	0.02	(0.00 - 0.05)
HV-P	0.07	(0.01 - 0.30)	0.23	(0.07 - 0.39)	0.56	(0.09 - 0.81)	0.11	(0.01 - 0.56)
HV-M	0.09	(0.01 - 0.41)	0.09	(0.02 - 0.28)	0.66	(0.10 - 0.91)	0.12	(0.01 - 0.56)
DV	0.06	(0.01 - 0.21)	0.44	(0.27 - 0.57)	0.41	(0.11 - 0.62)	0.08	(0.01 - 0.34)
				SURF-ZONE				
PV	-		-		0.59	(0.09 - 0.95)	0.41	(0.05 - 0.91)
ZB-EI	-		-		0.87	(0.73 - 0.97)	0.13	(0.03 - 0.27)
ZB-H	-		-		0.90	(0.74 - 0.98)	0.10	(0.02 - 0.26)
ZP	-		-		0.81	(0.50 - 0.96)	0.19	(0.04 - 0.50)
HV-P	-		-		0.85	(0.53 - 0.98)	0.15	(0.03 - 0.47)
DV	-		-		0.48	(0.18 - 0.82)	0.52	(0.18 - 0.82)

Discussion

Relative contributions of autotrophic sources to fish guilds along the salinity gradient

Our findings did not corroborate our first hypothesis of a greater number of autotrophic sources sustaining fish assemblages in the estuary compared with the river and marine surf-zone. In contrast with our prediction, a higher number of autotrophic sources contributed substantially for fish guilds at the river (POM, C_3 plants, SOM and, at lesser extent, C_4 plants) compared with the estuary (predominance of POM followed by C_4 plants). A great diversity of autochthonous and allochthonous food sources sustain fish assemblages from headwater to downstream reaches of rivers (Vannote *et al.* 1980; Junk *et al.* 1989). Stable isotope evidence suggests that the trophic links sustaining consumers in rivers may derived from the consumption of microalgae and detritus in the sediment via microbial loop (Hamilton *et al.* 1992; Thorp and Delong 2002) or C_3 plants and suspended particulate organic matter (Hoeinghaus *et al*. 2007). A great variety of autotrophic sources (mainly authochthonous) are also available in estuaries, such as saltmarshes, mangroves, seagrasses meadows and associated epiphyte algae, drifting macroalgae, planktonic and benthic microalgae (Day *et al*. 2012; Copertino *et al*. 2016). However, stable isotope studies have shown that some autotrophic sources highly abundant in some estuaries may not assimilated by aquatic consumers. For example, Claudino and collaborators (2013) have shown that, despite their high biomass, C_3 marsh plants (e.g. alkali bulrush *Scirpus maritimus*) had contributed little to fish and macroinvertebrates at Patos Lagoon estuary. Others authors have also found similar low contribution of C_3 plants to consumers in estuaries of the southwestern Atlantic (Rodríguez-Graña et al. 2008) and Australia (Raoult *et al*. 2018). It is possible that a similar pattern occurred in our studied estuary where C_3 marsh plants (*Juncus acutus*, *Rhynchospora gigantean*, *Scirpus olneyi*), despite being abundant along the margins of the estuary, also had contributed little to fish guilds.

In contrast with the greater number of autotrophic sources sustaining fish assemblages in the river and, at lesser extent, in the estuary, our results showed that fish guilds in the marine surf-zone were trophically dependent on POM. This could be explained by the physiographic characteristics of the studied coastline that apparently did not favor the occurrence of other primary producers. This coastline is characterized by wide sandy beaches with a gentle slope, moderate to high wave energy and an absence of seagrass meadows, drifting macroalgae and coralline algae (Calliari 1997; Garcia and Gianuca 1997). Moreover, this marine surf-zone is characterized by high *in situ* phytoplankton production and microalgae blooms, dominated by frequent and dense accumulations of diatoms (Odebrecht *et al*. 2010; 2014). A greater contribution of POM to fish assemblages in marine surf-zones has been reported in other studies conducted in subtropical latitudes of the southwest Atlantic coast (Bergamino *et al*. 2011; Oliveira *et al*. 2014). Prior works had also shown a contribution from POM to invertebrate consumers in surf-zones (Heymans and McLachlan 1996; McLachlan and Brown 2006; Allan *et al*. 2010). These findings suggest that the pelagic food chain may be the prevalent trophic pathway linking basal resources with consumers in subtropical marine surf-zones.

It is worth noting that we used POM as a proxy for particulate organic matter in suspension and phytoplankton. This approach is common in studies using stable isotopes as natural markers to infer trophic links (Deegan and Garritt 1997; Sato *et al*. 2006) due to the technical and logistic difficulties of obtaining the isotopic composition of „puræ‟ samples of phytoplankton (Fry 2006). We believe our POM data values are good predictors of the isotopic composition of phytoplankton in our study site: average isotopic ratios for POM (δ^{13}C: -16.63 and δ^{15}N: 6.85) matched closely the isotopic composition of a few samples of „pure" phytoplankton (mostly the diatom *Coscinodiscus* sp.) that we obtained in the marine surf-zone (δ^{13}C: -16.64 and δ^{15}N: 7.33).

Trophic structure changes along the river-estuary-surf-zone transect

The comparison of the structure of bipartite networks depicting trophic relationships between fish guilds and their preys corroborated our second hypothesis of higher food web complexity in the estuary than in the adjacent systems. This finding was directly influenced by a comparatively greater number of food web components (fish guilds and preys) and trophic links in the estuary. Despite the marked variability in abiotic conditions limiting the number of species that complete their life cycles within estuaries (McLusky and Elliott 2004; Whitfield *et al*. 2012), this transitional system holds a diverse fish fauna and prey resources (Whitfield 1990; Elliott *et al*. 2007; Barletta *et al*. 2010; Moura *et al*. 2012).

Marine surf-zones may function as nursery areas for some fish species, which juveniles reside temporarily before their recruitment into estuaries for growth or towards offshore marine areas for reproduction (Clark 1997; Layman 2000; Monteiro-Neto *et al*. 2003; Pessanha and Araújo 2003). This could explain why the bipartite trophic network of the marine surf-zone showed intermediate complexity in comparison with the estuary and the river. Finally, the more simplified bipartite trophic network found in the river could be related with comparatively low number of food web components and simple physical conditions (Careddu *et al*. 2015).

It is important to highlight that coastal systems are highly variable (Valiela 1995; Day *et al*. 2012) and, therefore, generalizations based on a single river to ocean transect are limited. For instance, differences in hydrogeomorphologic features influence the occurrence and distribution of primary producers and consumers both within estuaries and their adjacent marine surf-zones (Hoeinghaus *et al*. 2011; Petry *et al*. 2016; Araújo *et al*. 2018). Moreover, in contrast with our studied coastal plain dominated by dissipative sandy beaches (Tomazelli and Villwock 1991; Odebrecht *et al*. 2010), other marine areas adjacent to estuaries may harbor a greater variety of primary producers (e.g. seagrass and macroalgae beds) and habitats with higher heterogeneity (e.g. coral reefs, rocky bottoms) (Valiela 1995; Copertino *et al*. 2016). Hence, overall predictions of food web changes along river to ocean transects would need further studies in different latitudes including factors as hydrogeomorphology, climate, tidal amplitude, functional composition and distribution of the consumers and their movement patterns across freshwater, estuarine, and marine environments.

Conclusions

Our findings revealed that the relative importance of autotrophic sources sustaining fish assemblages changes markedly along a transect from river to ocean in a coastal system of the southwestern Atlantic. A greater number of primary food sources, integrating pelagic (POM) and benthic (C_3 plants, SOM and, at lesser extent, C_4 plants) trophic pathways, sustained the riverine fish assemblage, when compared with the estuary (mainly POM, followed by C_4 plants) and the marine surf-zone (POM). In contrast, higher richness of fish guilds and preys resulted in a more complex food web structure in the estuary, than in the adjacent systems. The riverine fish assemblage showed a more simplified food web structure with a lower number of guilds and trophic links. The vulnerability of biological communities to natural and man-made impacts are closely related with their trophic organization, with several works suggesting that more simplified food webs are more vulnerable to perturbations than complex ones (Pimm 1982; Winemiller and Layman 2005; Peralta-Maraver *et al*. 2017). Therefore, management and conservation actions aiming to mitigate increasing anthropogenic impacts in coastal systems (Smith 2003; Lotze *et al*. 2006) should take into account changes in food web structure of fish assemblages occurring along the river-estuarine-marine continuum. However, the great variability in coastal systems demand additional studies to allow predictions on food web changes along river to ocean transects.

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SUPPLEMENTARY MATERIAL

Table Supplementary 1. References cited in Table 1 and used to obtain complementary diet information to corroborate the classification of fish guilds.

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Table Supplementary 2. Number of stomach content analyzed (N) and relative contributions of prey categories (Alimentary Index, IAi) for each trophic guild in the study area.
Codes for trophic guilds are piscivore (PV), zoobenthivores of epifauna-infauna (ZB-EI), zoobenthivores of hyperbenthos (ZB-H), zooplanktivore (ZP), omnivore (OV), herbivore of phytoplankton (HV-P); herbivore of macroalgae/macrophytes (HV-M), detritivore (DV) and insectivore (IN). Codes for preys categories are Decapoda (DECAPO), Insecta (INSECT), Teleostei (TELEOS), Zoobenthos (ZOOBEN), Zooplankton (ZOOPLA), Microalgae (MICALG), Macroalgae (MACALG), Vegetation (MATVEG). ** The alimentary index (IAi) was not computed to the guild detritivore (DV) because we did not carry out stomach content analysis of mullet species. This guild was classified according to the literature (see Table 1 and Table Supplementary 1).

GUILD	N	DECAPO	INSECT	TELEOS	ZOOBEN	ZOOPLA	MICALG	MACALG	MATVEG
IN	49	-	94.81	-	0.02	-	3.79	1.38	0.01
PV	6	-	-	100.00	-	-	-	-	-
ZB-EI	133	0.31	0.01	0.12	91.56	6.00	0.01	1.70	0.28
ZB-H	68	69.75	0.02	22.62	4.17	3.43	-	-	-
ZP	127	0.25	0.04	0.01	4.26	95.41	0.03	-	-
OV	258	0.81	0.13	19.85	37.18	2.32	-	1.32	38.39
HV-P	48	-	-	-	0.16	1.24	98.45	0.14	-
HV-M	8	4.44	-	7.01	0.01	-	0.01	86.21	2.33
DV**									

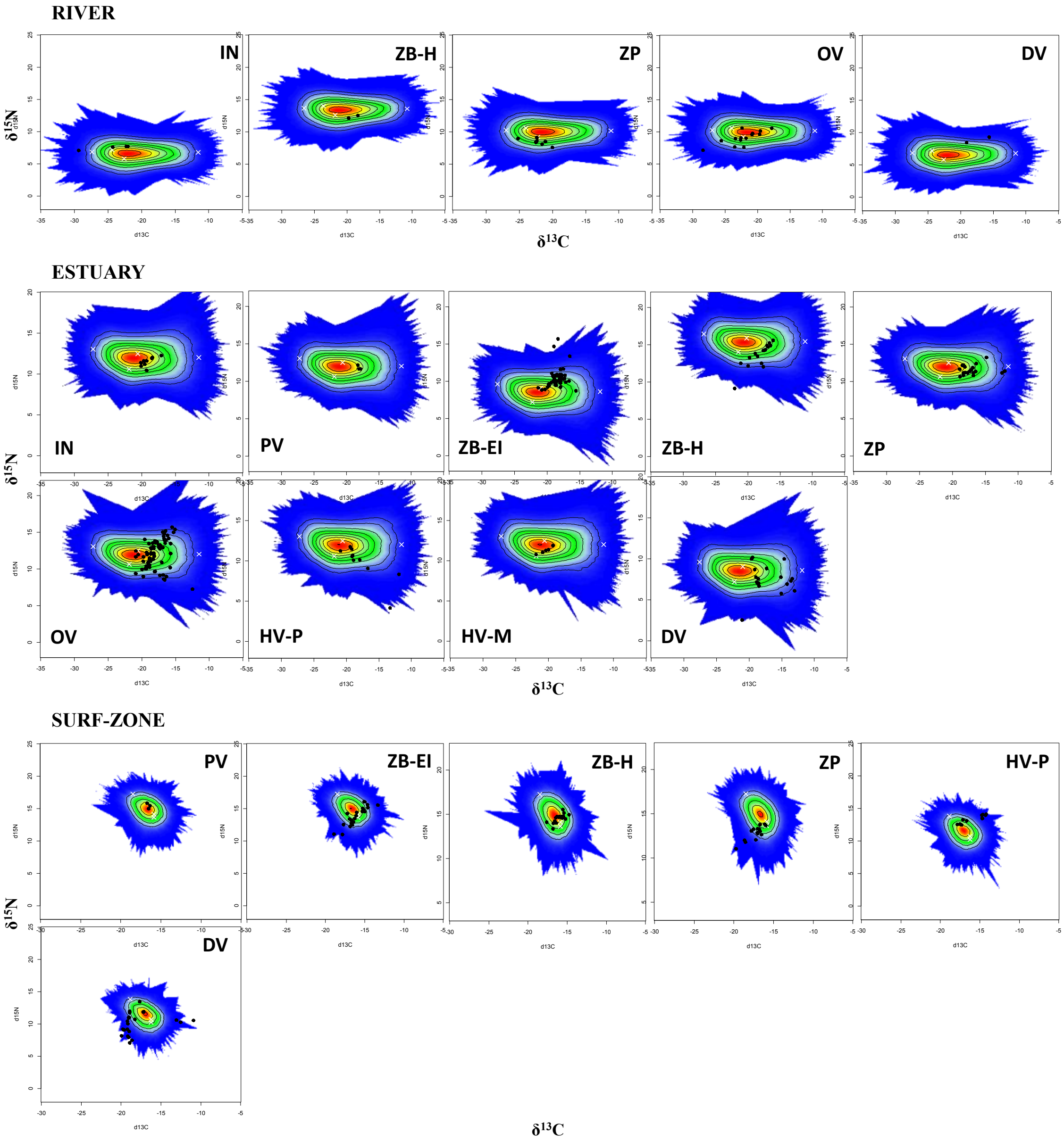

Fig. Supplementary 1. Biplots of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios with simulated mixing polygons, where filled circles represent consumers within each trophic guild and white crosses average autotrophic sources values. Color gradient represents probability contours which indicate how often a mixing polygon encloses an area. The outermost contour represents the 5% likelihood fit of a mixing model. The isotopic composition of those consumers situated outside the 95% mixing region (the outermost contour) cannot be adequately explain by the mixing model. Codes for trophic guilds are piscivore (PV), zoobenthivores of epifauna-infauna (ZB-EI), zoobenthivores of hyperbenthos (ZB-H), zooplanktivore (ZP), omnivore (OV), herbivore of phytoplankton (HV-P); herbivore of macroalgae/macrophytes (HV-M), detritivore (DV) and insectivore (IN).

AUTHOR INSTRUCTIONS

Presentation

The work should be presented in clear and concise English. All text should be in Times New Roman, 12 point font, with double or 1.5-line spacing throughout, and with a margin of at least 3 cm on the left-hand side. Every line of each page must be consecutively numbered in the left-hand margin, starting from 1 to the highest numbers needed as this greatly assists the referees. All pages of the manuscript must be numbered consecutively, including those carrying references, tables and captions to illustrations, all of which are to be placed after the text. Follow the form of headings, tables and illustrations exemplified in recent issues of the Journal.

Supplementary material which is not essential in the printed paper (e.g. large raw data files) but that may be useful to other workers can be lodged with the Editor if submitted with the manuscript for inspection by the referees. Such material will be published online as Supplementary Material in association with the published paper and made available free to all users.

Format

Papers should usually be in the form Title, Abstract, Additional keywords, Introduction, Materials and methods, Results, Discussion, Acknowledgements, Conflicts of Interest and References. If authors choose to combine the Results and Discussion sections, they must also include a Conclusion to summarise their key findings. Consider using subheadings to organize material.

The title should be concise and appropriately informative and should contain all keywords necessary to facilitate retrieval by online search engines. The abstract (< 200 words) should open with a clear statement of the broad relevance of the work, briefly summarise the aims and research approach, give the principal findings, and conclude by specifying the main implications of the results to aquatic science. Additional keywords not already in the title or abstract should be listed beneath the abstract. A running head (< 50 letter spaces) should be supplied for use at the top of the printed page.

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Supplementary material

In an effort to make best use of printed journal space, Marine and Freshwater Research strongly encourages authors to place supporting files such as additional tables, figures and raw data in ′Supplementary Material′, which is linked online to the paper when it is published electronically. Such material is not crucial to the paper‟s interpretation but would bolster claims, illustrate specific aspects of interest, or expand on a point in the text. There is no special format for Supplementary Material and it should be cited in the main text as ′..available as Supplementary Material...′ or ′(see Supplementary Material)′.

CAPÍTULO 2

Manuscrito em revisão na revista *Estuaries and Coasts*.

ASSIMILATION OF ALLOCHTHONOUS MATTER BY ESTUARINE CONSUMERS DURING THE 2015 EL NIÑO EVENT

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Abstract

The *El Niño* phenomenon refers to a warming of the tropical Pacific basin whose meteorological effects influence the dynamics of aquatic ecosystems around the world. Prior studies have shown that strong *El Niño* events are highly correlated with high rainfall episodes and high freshwater discharge into subtropical estuaries, with subsequent changes in species composition, abundance and diversity of their biota. In this work, we evaluated the hypothesis that riverine allochthonous matter associated with the strong 2015 *El Niño* event is assimilated by macroinvertebrates and fishes of a southwestern Atlantic estuary. In order to investigate this hypothesis, we analyzed carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of primary food sources and consumers in the estuary and also of riverine allochthonous matter. Our findings showed that most estuarine consumers had lower average $\delta^{13}C$ values during the wet period associated with the 2015 *El Niño*. Such decreasing in $\delta^{13}C$ values seemed to reflect the assimilation of ^{13}C depleted riverine matter, which according with Bayesian isotope mixing models ranged from 16% to 62% during the 2015 *El Niño*. Further studies are needed to evaluate the role of *El Niño* events on structuring food web organization in estuaries under the influence of this climatic phenomenon, which may become more frequent and intense in a global warming scenario.

Keywords: fishes, macroinvertebrates, trophic guilds, stable isotopes, allochthonous assimilation, estuarine consumers

INTRODUCTION

Estuaries are characterized by abundant basal food sources (i.e. primary producers and particulate organic matter sources) sustaining high secondary production, which have been exploited by humans over millennia (Day et al. 2012). A key step in order to manage properly the exploitation of these natural resources is to understand which factors drive temporal changes in the occurrence and intensity of food linkages between basal food sources and estuarine consumers (Winemiller et al. 2007; Hoffman et al. 2008; Le Pape et al. 2013; Bergamino and Richoux 2015; Garcia et al. 2017a).

One of the major driving forces influencing trophic structure in estuaries is the hydrological pulse associated with temporal changes in rainfall over coastal drainage basins and, consequently, the increase in freshwater inflow (Day et al. 2012). The discharge in large amounts of freshwater into estuaries affects not only salinity and nutrient profiles (Bishop et al. 2017), but also the input of continental-derived organic matter, preys, and predators

(Livingston et al. 1997; Mooney and McClelland 2012; Garcia et al. 2017b). Sudden increase in freshwater discharge and continental allochthonous sources may change population dynamics and community structure in estuaries (Garcia et al. 2004; Abrantes et al. 2013; Odebrecht et al. 2017), including effects on food web organization (Livingston et al. 1997; Possamai et al. 2018). Differences in estuarine hydrogeomorphology, size of riverine and basin systems emptying into estuaries, and the strength of the hydrological pulse account for the variability in the responses of estuarine organisms to the input of allochthonous matter (Livingston et al. 1997; Hoeinghaus et al. 2011; Abrantes et al. 2013; Garcia et al. 2017b).

The effect of inputs of allochthonous matter due to increased rainfall levels on aquatic consumers (mainly fishes) is better known in large rivers and flood plains of tropical latitudes (Lowe-McConnell 1987). For example, studies carried out in Amazon rivers (e.g. Winemiller 1990) and in flood plains of the Paraná river basin (e.g. Luz-Agostinho et al. 2008) revealed that hydrological changes associated with dry and wet seasons affects the trophic structure of fish assemblages. However, the effects of hydrological changes on the trophic structure of estuarine biota have been poorly investigated (Livingston et al 1997; Abrantes et al. 2013), especially in subtropical latitudes (Possamai et al. 2018).

Southwestern Atlantic estuaries under influence of the phenomenon *El Niño* are good models to investigate the effects of hydrological changes on trophic organization of estuarine communities (Odebrecht et al. 2017; Garcia et al. 2017b). *El Niño* is the warm phase of a global climatic phenomenon (*El Niño* Southern Oscillation, ENSO) that is highly correlated with positive rainfall anomalies and, consequently, high freshwater discharge into estuaries located in subtropical latitudes of South America (Robertson and Mechoso 1998; Grimm et al. 2000; Sun et al. 2015). One of the strongest *El Niño* events ever recorded was the 2015 *El Niño*, which was associated with episodes of excessive rainfall in several regions of the globe, including South America (Zhai et al. 2016; Paek et al. 2017). The excessive rainfall over the drainage basin of subtropical estuaries in South America has been associated with marked changes in the estuarine biota (Odebrecht et al. 2017). At Patos Lagoon estuary (32°S), for instance, high freshwater inflow associated with moderate to strong *El Niño* events are associated with changes in species composition, abundance and diversity patterns (Garcia et al. 2001; Garcia et al. 2004) and trophic organization of fish assemblages (Possamai et al. 2018). However, the potential effects of hydrological changes associated with *El Niño* events on the occurrence and intensity of food linkages between basal food sources and estuarine consumers remain largely unknown.

One tool to study trophic links between food sources and consumers is stable isotope analysis (SIA) (Pasquaud et al. 2008; Claudino et al. 2013; Carassou et al. 2016). This technique relies on the fundamental assumption that consumers reflect the ratios of lighter (e.g. ^{12}C, ^{14}N) and heavier (e.g. ^{13}C, ^{15}N) stable isotopes of their assimilated food sources, after accounting the effects of isotopic fractionation (Fry 2006). Carbon and nitrogen stable isotope ratios (δ^{13}C and δ^{15}N, respectively) are the most widely used natural markers in SIA to reconstruct consumer‟s diet and to infer trophic links in food webs (Layman et al. 2012). Moreover, in contrast with traditional methods such as stomach content analysis, SIA allows the estimation of the relative contribution of primary food sources in sustaining not only primary consumers (e.g., herbivores and detritivores), but also consumers positioned at higher trophic levels (e.g. omnivores, carnivores) (Fry 2006; Condini et al. 2015; Garcia et al. 2017a).

In this work, we used SIA to investigate temporal changes in the relative contribution of autochthonous and allochthonous basal food sources to estuarine of the Tramandaí-Armazém estuarine complex in south Brazil, a southwestern Atlantic estuary under influence of the ENSO phenomenon. Our hypothesis is that the increase in water surplus associated with the 2015 *El Niño* enhanced the transport of riverine allochthonous matter into the estuary, where it is assimilated by macroinvertebrates and fishes. Our findings are discussed in the light of hydrological impacts of other strong *El Niño* events (1982-1983 and 1997-1998) on fish assemblages of southwestern estuaries (Garcia et al. 2001; Garcia et al. 2004; Possamai et al. 2018).

MATERIAL AND METHODS

Study area

Sampling of representative food web components occurred in the Tramandaí-Armazém estuarine complex (Fig. 1). The estuarine zone has an area of 30 km² and a drainage basin of 2.697 km^2 and is connected with the sea by a permanent channel (1.5 km long and 0.3 km wide). Depths inside the estuary range from 1.5 m in the shallow embayment to 5 m in the main channel (Schwarzbold and Schäfer 1984). The estuary is under influence of a micro-tidal regime (Loitzenbauer and Mendes 2012). The most representative macro-primary producers found along the margins and shallow embayment are marsh plants (e.g., *Juncus acutus*, *Rhynchospora gigantea*, *Scirpus olneyi*), floating macrophytes (e.g., *Eichhornia crassipes*), submerged beds of the widgeon grass *Ruppia maritima* and terrestrial grasses (Poacea) in the margins (AFSG, personal observation).

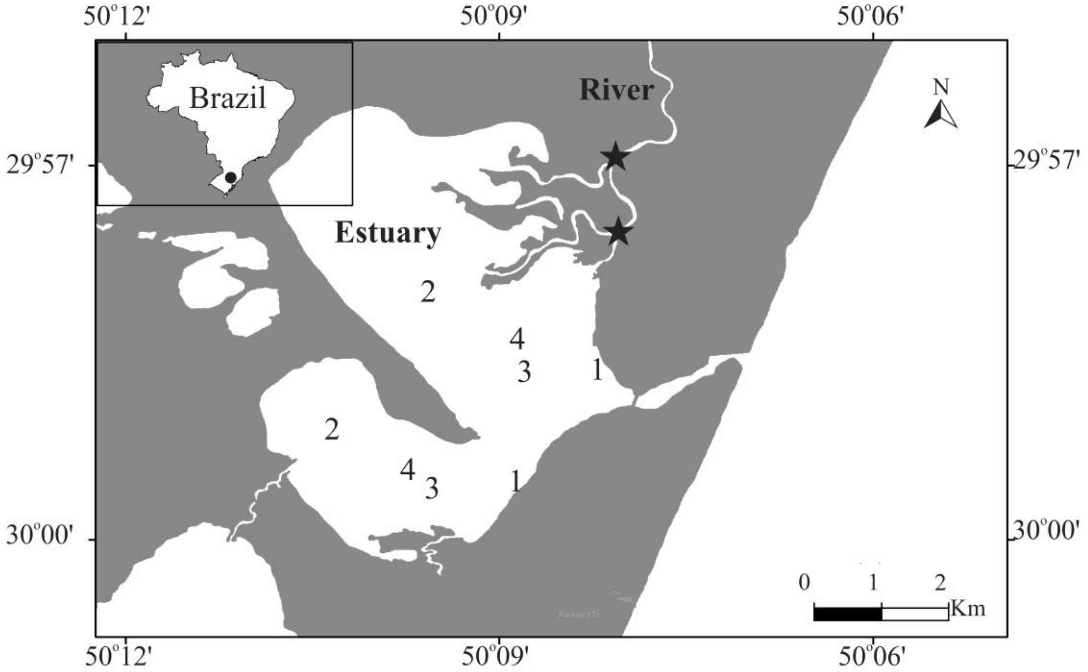

Fig. 1 Tramandaí-Armazém estuarine complex in southern Brazil showing the locations where the fishing gears (1.beach seine, 2.bottom trawling, 3.casting net, 4.gillnets) were used to sample macroinvertebrates and fishes. The location of bottom trawling (2) denotes the approximately start position of each haul, which follows the main channel towards the estuarine mouth. Dark stars denote the locations in the river where we sampled allochthonous continental matter (i.e. particulate organic matter in suspension (POM) and in sediment (SOM)).

Field collections and sample processing

We collected representative food web components of basal food sources and consumers in sampling stations in the estuary on March and October of 2015 (Fig. 1). These months corresponded to the end of the dry/warm and wet/cold season, respectively (see more details in the next subsection). We carried out sampling under field collection permit nº 47567-1 provided by the Brazilian National Environmental Authority (ICMBio). Marsh plants, macrophytes, seagrasses and terrestrial grasses were collected by hand with the help of scissors along the margins and shallow waters (< 2 m). We obtained particulate organic matter in suspension (POM) by filtering approximately 1.5 liters of water into a pre-combusted (450°C for 4 h) Whatman glass-fiber filter (0.75 μm), and obtained particulate organic matter in the sediment (SOM) by removing the top 2 cm from the surface of the sediment using a plastic core (10 cm diameter). Pelagic and demersal macroinvertebrates and fishes were sampled using beach seine hauls, bottom trawling, casting net and gillnets (see Garcia et al. 2006 for gears" dimensions and operation procedures). Whenever possible, we sampled each consumer and basal food source from three to up 15 individuals at each field trip. In order to have proxies for riverine-derived allochthonous matter that could be carried out into the estuary by the water surplus associated with the 2015 *El Niño*, we obtained samples of particulate organic matter (both POM and SOM) in the main river flowing into the estuary (Fig. 1).

Biological samples were kept frozen until later processing in laboratory, where each food web component was identified to the lowest taxonomic level possible. Macroinvertebrates and fishes were dissected to obtain approximately 5 g of muscular tissues per sample, with exception of polychaetes that were processed whole due to their lower body size (< 3 cm). For primary producers we used only their leaves. We rinsed each sample with distilled water, placed in a sterile Petri dish, and dried in an oven at 60°C to a constant weight (minimum of 48 h). Dried samples were ground to a fine powder with a mortar and pestle and stored in Eppendorf tubes. We weighed subsamples (~1 mg) and pressed them into ultra-pure tin capsules (Costech Analytical Technologies) and sent to the Analytical Chemistry Laboratory of the Institute of Ecology, University of Georgia, for analysis of carbon and nitrogen isotope ratios. The carbon standard was Pee Dee Belemnite limestone, and the nitrogen standard was atmospheric nitrogen. We expressed results in delta notation (parts per thousand deviation from a standard material): $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard})-1]*1000$, where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. We applied mathematical normalization on $\delta^{13}C$ values to control for potential effects of lipid contents (DeNiro and Epstein 1977) using the equation proposed by Post and collaborators (2007): $\Delta\delta^{13}C = -3.32 + 0.99 * C{:}N$. We applied this correction for those samples with C:N higher than 3.5 (Post et al. 2007).

Data analyses

To evaluate the potential influence of the 2015 *El Niño* on hydrological conditions during the two months preceding each field collection, we obtained daily values of rainfall, evapotranspiration, and temperature in a meteorological station (29°21"S, 49°43"W) approximately 90 km up north of the study area. This station was the closest one of our study site with meteorological data available for the studied years (INMET, 2018). The average meteorological conditions in the two months prior the field collection carried out on March 2015 corresponded to a warmer/drier period, with higher temperature (27.8 °C) and lower water surplus (i.e. the net balance between rainfall and evapotranspiration) (3.2 mm) (Fig. supplementary 1). In contrast, conditions preceding the sampling on October 2015 corresponded to a colder/wetter period, with lower temperature (21.4 °C) and higher water surplus (6.8 mm). The cold/wet period coincided with the onset of the strong 2015 *El Niño* event, which occurrence was evaluated using the Oceanic *Niño* Index (ONI) (Fig. supplementary 1). The ONI is a measure of the quarterly anomaly of the sea surface temperature in the region Niño 3.4 (5°N-5°S, 120°-170°W), with values above 0.5 indicating the occurrence of *El Niño* and values between 1.5 to 1.9 of „strong" events (Golden Gate Weather Services 2018).

Each macroinvertebrate and fish species were assigned to ecological guilds based on their life cycle and habitat use patterns (Chao et al. 1985; Rupert and Barnes 1994; Vieira and Castello 1997; Garcia et al. 2001; Day et al. 2012). Hence, we pooled macroinvertebrates into estuarine resident polychaetes (P-RES), estuarine resident crustaceans (C-RES) and estuarine dependent crustaceans (C-DEP) (Rupert and Barnes 1994; Day et al. 2012). We classified fishes into estuarine resident (F-RES), estuarine dependent (F-DEP), marine (F-MAR) and freshwater (F-FRE). FRE was further subdivided in those freshwater fishes with tolerance to brackish waters (secondary division, F-FRE_S) and no tolerance to salinity (primary division, F-FRE_P) (Chao et al. 1985; Vieira and Castello 1997; Garcia et al. 2001). We also classified fishes according with their body sizes (total length, TL, mm). We dissected individuals and inspected visually the development stages of their gonads (early development, ripe, spent) to evaluate sexual maturity (Rocha et al. 2008). Whenever necessary, we consulted the FishBase catalog for complementary information on reproduction (e.g. L_{50}) and developmental stages (Froese and Pauly 2018). After taking into account body size and sexual maturity, we subdivided the guild F-DEP into those containing juveniles (F-DEP_J) and adults (F-DEP_A). We made this distinction to take into account the contrasting degree of mobility and residence time of juveniles and adults of F-DEP inside the estuary. We assumed that juveniles are more likely to remain year-round inside estuary than adults and, therefore, juveniles would be better proxies to evaluate estuarine food assimilation (see implications for „isotope mixing models" in the next paragraph). This assumption is corroborated by prior works showing that juveniles use estuaries as nursery grounds for feeding and growing site (Vieira and Castello 1997; Wootton 1999; Day et al. 2012). We did not classify the other fish guilds (F-RES, F-MAR, F-FRE_S, F-FRE_P) into juvenile/adult groups because the great majority (> 95%) of their individuals were juveniles.

We used $\delta^{13}C$-$\delta^{15}N$ biplots to reveal patterns in isotopic compositions of macroinvertebrates and fishes guilds and basal food sources (Fry 2006). We used one-way ANOVA to test for differences in average values of carbon stable isotope ratios of consumers" guilds between dry and wet periods. We applied isotope Bayesian mixing models to estimate contributions of food sources to consumers (Parnell et al. 2013). We estimated assimilation of allochthonous sources only for those guilds occurring year-round (residents) or predictably occurring for several months inside the estuary (e.g. juveniles of estuarine dependent species). We excluded from this analysis highly mobile forms (e.g., adults of estuarine dependent species) based on the premise they would not reside long enough in the estuary to assimilate allochthonous matter transported during the wet period. For instance, based on a prior isotopic turnover experiment carried out the estuarine dependent *Micropogonias furnieri*, it would take 103.3 and 73.0 days to reflect food assimilation in their $\delta^{15}N$ and $\delta^{13}C$ at estuarine conditions, respectively (Mont'alverne et al. 2016). Hence, the guilds chosen as response variable to test our hypothesis of assimilation of riverine allochthonous matter were F-RES, F-DEP_J, F-FRE_S, C-DEP, P-RES and C-RES in the estuary.

We considered as end-members in the mixing models the following basal food sources: C_3 and C_4 plants, POM, SOM and riverine-derived allochthonous matter (referred as $ALLOC_{riv}$). As proxy for $ALLOC_{riv}$, we used average values of SOM sampled in the upstream river emptying into the estuary, because only organic matter in the sediment (and not in suspension, POM) showed statistically significant differences in both average $\delta^{13}C$ and $\delta^{15}N$ values across river and estuary (Fig. supplementary 2).

We ran the isotope Bayesian mixing models with the SIMMR (Stable Isotope Mixing Models in R) package, which employs Gaussian likelihood and fits the model to the data via Markov chain Monte Carlo (MCMC) (Parnell 2016). One of the benefits of this model is that it takes into account uncertainty and variation in consumers, sources, and trophic enrichment factors (TEF) (Parnell et al. 2013). For each mixing model, we run 500,000 iterations, followed by a burn-in and thinning of 50,000 and 5,000, respectively (Parnell 2016). We applied fractionation corrections according to the overall trophic position occupied by the trophic guilds (e.g., one correction for herbivorous and two to three for carnivorous guilds) (Feng et al. 2014; Phillips et al. 2014). We used average (±standard deviation) TEF values of 0.39 (1.3) and 3.4 (0.98) for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios (Post 2002).

Considering that fractionation values can be markedly different for herbivorous, we applied distinct TEF for the mullets (*Mugil liza*, *M. curema*, *M. brevirostris*), which fed mainly on microalgae (Garcia et al. 2018a). The TEF values were 3.6 (0.7) and 4.3 (0.4) for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios (Oliveira et al. 2017).

A fundamental assumption of isotope mixing models is that the isotopic variability of consumers, after accounting for fractionation corrections, are contained within the variability of the assimilated food sources (Phillips et al. 2014). We tested this assumption using isotope mixing polygon simulations that quantitatively determine boundaries of possible source values in the $\delta^{13}C$-$\delta^{15}N$ biplot space that can explain the isotope mixture (Smith et al. 2013). We run this analysis using the packages *sp* (Pebesma and Bivand 2005; Bivand et al. 2013) and *splancs* (Rowlingson and Diggle 2017) in R (R Core Team 2017). Individual consumers located outside the 95% mixing polygon region, which indicate they could not be confidently explained by the food sources (Phillips et al. 2014), were omitted from the final mixing models of which the results are presented in the main text (see Fig. supplementary 3).

RESULTS

Changes in isotopic composition between dry and wet periods

A total of 99 samples of macroinvertebrates, 366 of fishes and 81 of basal food sources had their isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) (Table 1 and 2). The number of species of fishes and macroinvertebrates sampled was 27 and 6, respectively, which were classified into 6 and 3 functional guilds of fishes and macroinvertebrates. There was an increase in the estuary in the number of species comprising the guild of primary freshwater fishes (F-FRE_P) from 2 to 4 species during the wet period associated with the 2015 *El Niño* event. In contrast, there was a concomitantly decrease in the number of species comprising the marine and estuarine dependent guilds (both juvenile and adults) in the same period (Table 1). Regarding size structure, most fishes had average total length (TL) lower than 150 mm, with exception of F-DEP_A that had the largest average body sizes (e.g., 274.0 mm for the sea catfish *Genidens barbus*) (Table supplementary 1).

Table 1 Species composition of each guild of fishes and macroinvertebrates, number of individuals (N), average (±SD) values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of each fish species sampled during dry and wet periods in the Tramandaí-Armazém estuarine complex.

	DRY							WET						
		$\delta^{13}C$			$\delta^{15}N$				$\delta^{13}C$			$\delta^{15}N$		
	N	Mean		SD	Mean		SD	N	Mean		SD	Mean		SD
FISHES														
Freshwater primary (F-FRE_P)														
Astyanax fasciatus	6	-20.4	±	0.9	11.2	±	0.4	5	-24.9	±	1.3	9.0	±	0.9
Oligosarcus jenynsii	3	-18.0	±	0.2	11.8	±	0.3	3	-27.5	±	1.2	8.4	±	0.8
O. robustus								5	-27.9	±	3.7	8.4	±	0.8
Pimelodella australis								5	-23.1	±	3.0	9.1	±	1.6
Freshwater secondary (F-FRE_S)														
Geophagus brasiliensis	14	-17.4	±	2.2	9.3	±	1.0	9	-17.8	±	1.0	9.2	±	0.5
Jenynsia multidentata	3	-14.0	±	2.5	7.2	±	2.6	3	-19.4	±	5.1	10.1	±	0.5
Estuarine Resident (F-RES)														
Atherinella brasiliensis	10	-19.2	±	0.9	11.5	±	0.5	2	-19.7	±	0.4	12.4	±	0.2
Ctenogobius shufeldti								13	-20.9	±	3.7	9.9	±	0.7
Estuarine Dependent Juvenile (F-DEP_J)														
Anchoa marinii	7	-19.2	±	0.9	10.9	±	0.7							
Brevoortia pectinata	5	-18.2	±	1.9	10.5	±	0.8							
Diapterus rhomeus	9	-19.0	±	0.9	11.3	±	2.3	7	-18.6	±	1.1	10.5	±	0.7
Eucinostomus argenteus	12	-17.7	±	0.5	10.4	±	0.3							
E. melanopterus	15	-17.2	±	2.1	11.4	±	0.5	5	-20.8	±	0.3	10.7	±	0.2
Genidens barbus	15	-17.6	±	1.7	15.5	±	2.5	10	-18.7	±	1.4	12.4	±	1.9
G. genidens	14	-18.7	±	0.8	12.7	±	0.7	7	-18.3	±	0.8	11.4	±	0.3
Lycengraulis grossidens	5	-19.3	±	2.0	12.6	±	2.1	3	-20.1	±	0.2	11.0	±	0.7
Micropogonia furnieri	14	-18.6	±	0.8	11.0	±	1.0	15	-17.3	±	1.1	11.9	±	1.3
Mugil brevirostris	5	-16.0	±	2.3	10.6	±	1.0							
M. curema	14	-18.6	±	0.9	8.5	±	1.4							
M. liza	6	-14.9	±	1.4	7.6	±	1.6	8	-18.7	±	3.9	7.3	±	2.1
Platanichthys platana								2	-24.1	±	0.3	10.1	±	0.1
Estuarine Dependent Adults (F-DEP_A)														
Brevoortia pectinata	5	-16.4	±	0.9	12.0	±	0.9							
Diapterus rhomeus	2	-19.1	±	0.3	10.9	±	0.2							
Eucinostomus argenteus	4	-18.0	±	0.4	11.1	±	0.4							
E. melanopterus								3	-17.8	±	0.4	12.0	±	1.6
Genidens barbus	4	-15.7	±	0.7	14.9	±	0.5							
G. genidens	11	-17.5	±	1.3	13.3	±	0.5	13	-16.7	±	1.1	12.0	±	0.8

Lycengraulis grossidens	10	-17.7 ± 1.5	14.0 ± 1.3	1	-21.1 ± 0.0	9.1 ± 0.0
Micropogonia furnieri	3	-17.7 ± 1.9	13.4 ± 2.4			
Mugil liza	3	-16.0 ± 4.4	5.8 ± 2.8			
Marine (F-MAR)						
Centropomus parallelus				5	-20.2 ± 0.5	10.8 ± 1.0
C. undecimalis				5	-19.9 ± 0.5	11.2 ± 0.3
Citharichthys spilopterus	13	-19.1 ± 1.0	9.6 ± 0.5	15	-19.5 ± 1.1	9.6 ± 0.9
Gobionellus oceanicus	2	-17.2 ± 4.3	5.5 ± 0.8			
Symphurus tesselatus	2	-19.6 ± 0.3	9.7 ± 0.2			
Trachinotus carolinus	6	-17.3 ± 1.4	12.3 ± 1.4			
MACROINVERTEBRATES						
Estuarine Resident						
Polychaeta (P-RES)						
Heteromastus similis	6	-18.2 ± 0.5	8.5 ± 0.6			
Polychaeta	5	-17.7 ± 1.2	8.4 ± 0.8	4	-18.9 ± 2.3	11.7 ± 1.8
Crustacea (C-RES)						
Palaemonetes argentinus	3	-15.6 ± 0.9	6.8 ± 0.5	5	-16.7 ± 1.6	8.4 ± 1.3
Estuarine Dependent						
Crustacea (C-DEP)						
Callinectes danae	12	-19.7 ± 5.1	9.6 ± 0.7			
Callinectes sapidus	13	-16.4 ± 2.2	8.5 ± 1.7	29	-18.5 ± 2.5	8.6 ± 1.3
Farfantepenaeus paulensis	22	-16.9 ± 1.9	8.0 ± 0.9			

Table 2 Number of samples (N), average (±SD) values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of each basal food source obtained during dry and wet periods in the Tramandaí-Armazém estuarine complex.

		DRY					WET				
			$\delta^{13}C$		$\delta^{15}N$			$\delta^{13}C$		$\delta^{15}N$	
SPECIES		N	Mean	SD	Mean	SD	N	Mean	SD	Mean	SD
ESTUARY											
Eichornia crassipes	C_3	6	-28.3	± 0.3	5.8	± 0.5	3	-29.9	± 0.5	5.3	± 0.3
Salvinia herzogii	C_3						3	-29.1	± 0.7	2.9	± 0.5
Juncus acutus	C_3	3	-26.6	± 0.4	5.6	± 1.1	3	-26.7	± 0.4	6.0	± 0.8
Rhynchospora gigantea	C_3	3	-27.9	± 0.2	8.2	± 0.3					
Scirpus californicus	C_3						3	-27.8	± 0.2	9.2	± 0.3
Scirpus olneyi	C_3	6	-28.5	± 0.7	6.0	± 1.6	6	-27.3	± 0.6	6.9	± 0.7
Ruppia maritima	C_4	3	-12.1	± 0.5	3.1	± 0.5	3	-16.9	± 0.5	5.3	± 0.2
Poacea morphotype 1	C_4						3	-11.2	± 0.4	5.7	± 1.0
Poacea morphotype 2	C_4	3	-12.5	± 0.0	7.3	± 0.3					
Poacea morphotype 3	C_4						3	-12.6	± 0.1	4.0	± 0.5
Poacea morphotype 4	C_4						3	-13.1	± 0.2	8.9	± 0.5
Organic matter in suspension	POM	6	-22.6	± 0.1	3.8	± 1.1	6	-25.1	± 0.8	6.0	± 1.0
Organic matter in the sediment	SOM	6	-21.4	± 1.1	5.7	± 0.4	9	-23.2	± 0.8	7.7	± 2.6

There was higher amplitude in the average $\delta^{13}C$ values of consumers during the wet period associated with the 2015 *El Niño* event (from -25.85 to -16.7) compared with the dry period (from -19.2 to -15.6) (Fig. 2). All guilds showed lower average $\delta^{13}C$ values during the wet period, with the exception of F-DEP_A (Table 1). However, only three guilds (F-FRE_P, F-DEP_J and F-MAR) showed statistically significant ($p < 0.05$) differences in their average $\delta^{13}C$ values between periods (Table 3). Among basal food sources, only POM and SOM showed statistically significant differences in average $\delta^{13}C$ values between periods, with both basal sources decreasing during the wet period (Table 3).

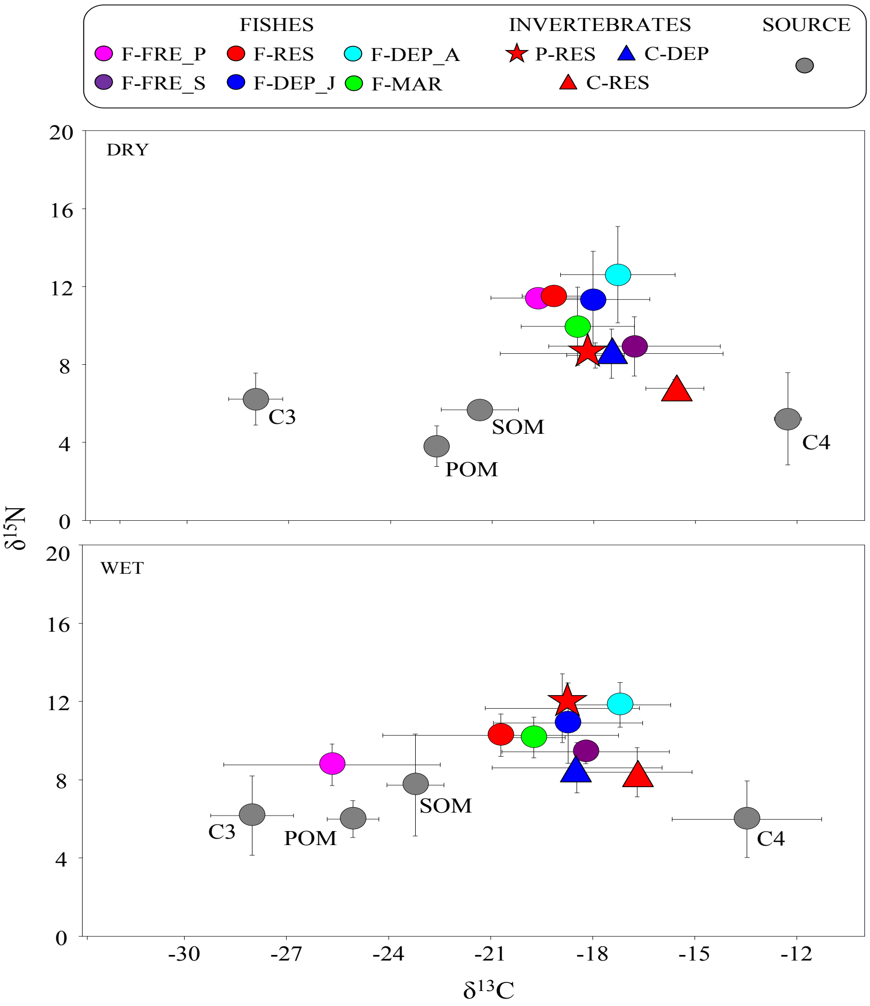

Fig. 2 Average (±SD) values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of macroinvertebrates (triangle, star and squares), fishes (colored circles) and basal food sources (gray circles) sampled during dry and wet periods in the Tramandaí-Armazém estuarine complex. Codes for fishes‟ guilds are freshwater primary (F-FRE_P), freshwater secondary (F-FRE_S), estuarine resident (F-RES), estuarine dependent juvenile (F-DEP_J), estuarine dependent adults (F-DEP_A) and marine (F-MAR) and for macroinvertebrates‟ guilds are estuarine resident polychaeta (P-RES), estuarine resident crustacea (C-RES), and estuarine dependent crustacea (C-DEP).

Table 3 Results of the one-way ANOVA of carbon stable isotope ratio ($\delta^{13}C$) of consumers‟ guild and basal food sources that co-occur in both dry and wet periods in the Tramandaí-Armazém estuarine complex. Codes for fishes‟ guilds are freshwater primary (F-FRE_P), freshwater secondary (F-FRE_S), estuarine resident (F-RES), estuarine dependent juvenile (F-DEP_J), estuarine dependent adults (F-DEP_A) and marine (F-MAR) and for macroinvertebrates‟ guilds are estuarine resident polychaeta (P-RES), estuarine resident crustacea (C-RES), and estuarine dependent crustacea (C-DEP).

	df	SS	F	*P*	
	ESTUARY				
Guilds					
F-FRE_P	1	213.20	28.11	0.000	Dry > Wet
F-FRE_S	1	14.27	2.12	0.157	
F-RES	1	14.05	1.89	0.183	
F-DEP_J	1	21.46	6.30	0.013	Dry > Wet
F-DEP_A	1	0.02	0.01	0.931	
F-MAR	1	18.00	10.72	0.002	Dry > Wet
P-RES	1	1.74	0.92	0.356	
C-RES	1	2.36	1.20	0.315	
C-DEP	1	16.62	1.84	0.179	
Basal food sources					
POM	1	13.85	54.08	0.000	Dry > Wet
SOM	1	12.33	13.24	0.003	Dry > Wet
C_3	1	0.01	0.01	0.938	
C_4	1	5.62	1.66	0.215	

Assimilation of allochthonous matter by estuarine consumers

Overall, the relative assimilation of autochthonous food sources in the estuary (POM, SOM, C_3, C_4) varied among consumers‟ guilds, being C_4 plants, POM, and SOM the most assimilated ones. C_3 plants, in contrast, showed substantial assimilation only by secondary freshwater fishes (F-FRE_S) during the dry period (Fig. 3). The assimilation of riverine-derived allochthonous matter ($ALLOC_{riv}$) by estuarine consumers increased in the wet period, being the only exception the F-FRE_S guild (Fig. 3 and 4). Such assimilation was higher in fishes (median values: 62% for F-RES, 40% for F-DEP_J) than in macroinvertebrates (24% for C-DEP, 18% for P-RES, 16% for C-RES) (Fig. 4).

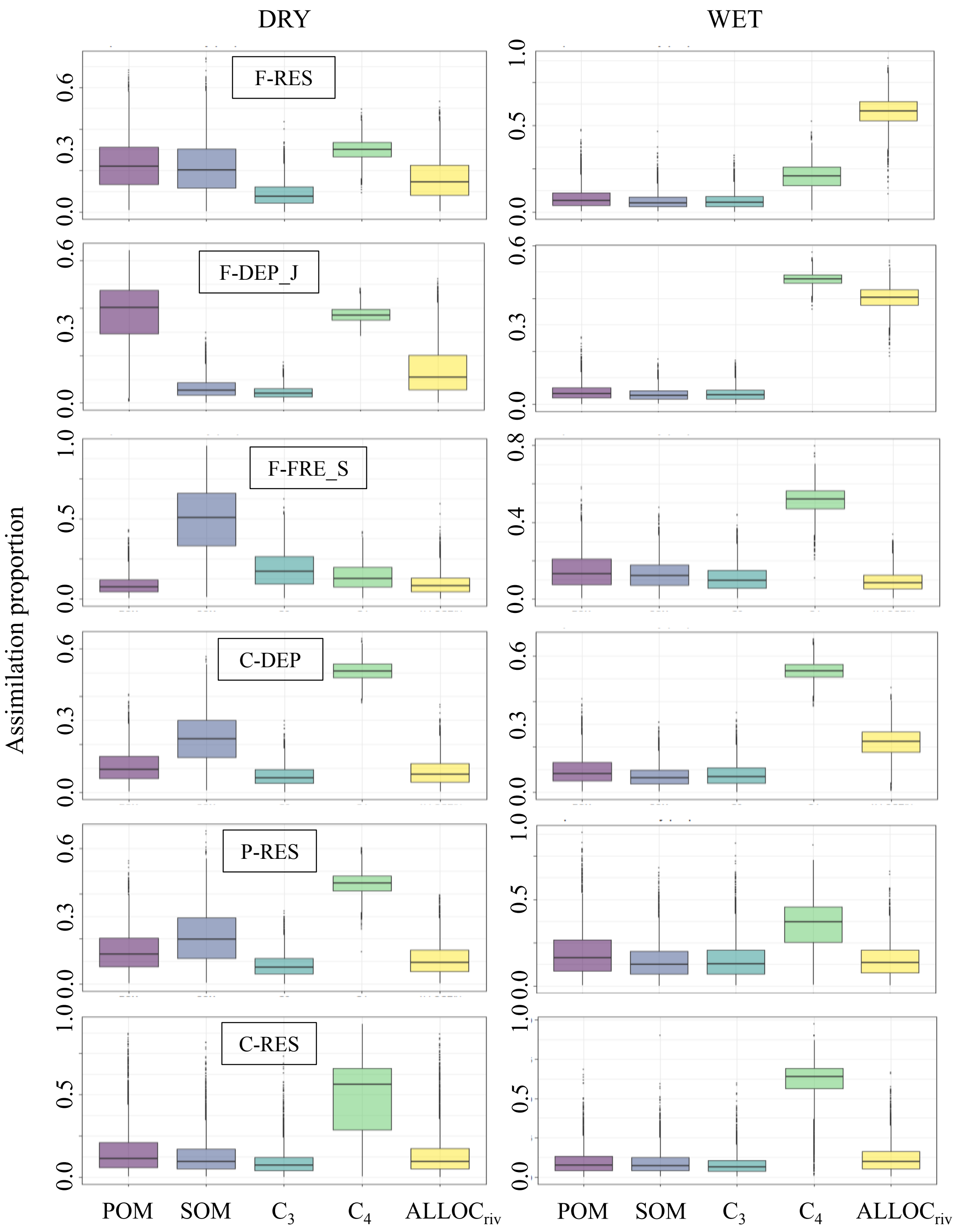

Fig. 3 Relative contributions of basal food sources (POM, SOM, $ALLOC_{riv}$, C_3 and C_4 plants) to macroinvertebrates and fishes sampled in the Tramandaí-Armazém estuarine complex. Each assimilation plot shows 50 (gray), 75 (dark gray) and 95% (light grey) Bayesian credibility intervals. Codes for fishes‶ guilds are estuarine resident (F-RES), estuarine dependent juvenile (F-DEP_J), secondary freshwater (F-FRE_S) and for macroinvertebrates‶ guilds are estuarine dependent crustacean (C-DEP), estuarine resident polychaeta (P-RES) and estuarine resident crustacean (C-RES).

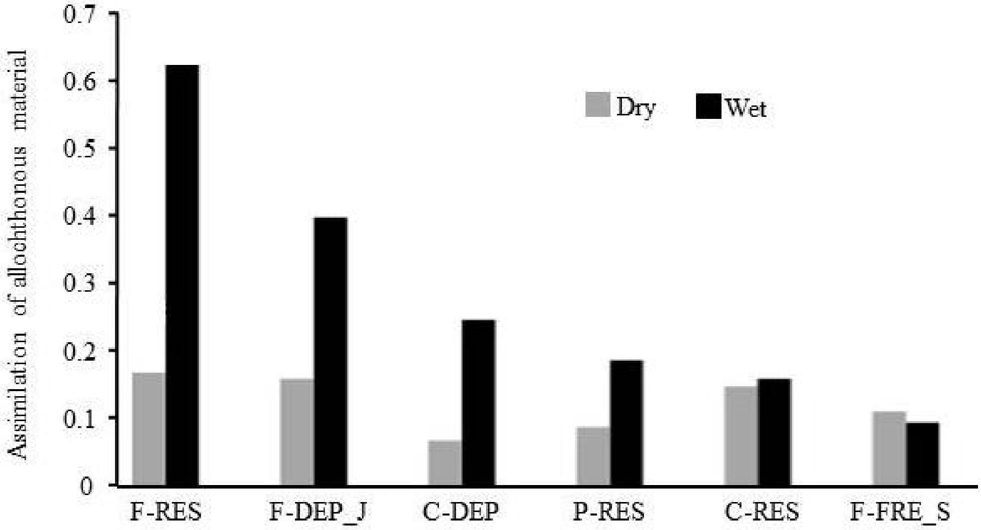

Fig. 4 Median values of assimilation of riverine-derived allochthonous matter ($ALLOC_{riv}$) by consumers‟ guilds during dry and wet periods in the Tramandaí-Armazém estuarine complex. Codes for fishes‟ guilds are freshwater secondary (F-FRE_S), estuarine resident (F-RES) and estuarine dependent juvenile (F-DEP_J) and for macroinvertebrates‟ guilds are estuarine resident polychaeta (P-RES), estuarine resident crustacea (C-RES), and estuarine dependent crustacea (C-DEP).

DISCUSSION

Our findings corroborated our initial hypothesis of assimilation of riverine allochthonous matter by estuarine organisms during the elevated rainfall period associated with the strong 2015 *El Niño* event. The water surplus and higher freshwater discharge usually associated with this climatological phenomenon in southeast of South America (Grimm et al. 2000; Robertson and Mechoso 1998) promoted the transport of riverine-derived particulate organic matter with lower carbon isotope ratios into the estuary. The input of riverine allochthonous matter into the estuary coincided with the decrease in average carbon isotope ratios values of several food web components (e.g., macroinvertebrates and fishes), when compared with the dry period preceding the 2015 *El Niño* event. In fact, all consumers‟ guilds showed lower average $\delta^{13}C$ values during the wet period associated with the 2015 *El Niño* event. It seems reasonable to assume that such decreasing in the carbon isotope ratios of estuarine consumers reflected the assimilation of isotopically depleted riverine-derived organic matter carried into the estuary during the high rainfall period. This assumption was reinforced by Bayesian isotope mixing models showing that assimilation of riverine allochthonous matter ranged from 16% to 62% during the 2015 *El Niño*. The only guild showing no decrease in its average carbon isotope ratios values in the estuary during the 2015 *El Niño* was adults of estuarine dependent fishes (e.g., mullet *Mugil liza*, whitemouth croaker *Micropogonias furnieri*). These euryhaline species are highly mobile and adults usually move in and out of estuaries in search of feeding opportunities or for reproductive purposes (Chao et al. 1985; Vieira and Castello 1997; Lemos et al. 2016, Mont'Alverne et al. 2016b).

Non-isotopic related evidences also corroborated our assumption of transport of riverine matter into the studied estuary during the heavy rainfall associated with the 2015 *El Niño* event. For example, there was an increase in the number of freshwater fish species (from 2 to 4) into the estuary. Concomitantly, there was a decrease during the wet period in the number of species comprising the marine (4 to 3) and estuarine dependent guilds, both for juvenile (12 to 8) and adults (8 to 3). Similar patterns had been observed during strong *El Niño* events in other Southern Brazilian estuary (Patos Lagoon, 32°S) (Garcia et al. 2001; Garcia et al. 2004; Vieira et al. 2008), which is located in the same coastal plain of the present study (Tramandai-Armazém, 29°S). For instance, Garcia and collaborators (2004) showed an increase in species richness and abundance of freshwater fish species (especially characins and freshwater catfishes) and a concomitant decrease in marine estuarine-related species during the 1982-1983 and 1997-1998 *El Niño* events at Patos Lagoon Estuary. Such similar changes in species composition in response to *El Niño* events in both estuaries (only 3° latitude apart of each other) suggest predictable effects of this climatic phenomenon on their fish assemblages (i.e. increase and decrease of freshwater and marine-related fishes in the estuary, respectively).

There was a marked variability in the proportion of riverine allochthonous matter assimilated by each consumer guild, which were probably associated with differences in feeding habits, microhabitat use, and residence time inside the estuary. Overall, those fish guilds predictably using the estuary year-round (estuarine residents) or as nursery grounds during part of their life cycle (juvenile stages of estuarine dependents) showed the highest assimilation of riverine allochthonous matter during the wet period associated with the 2015 *El Niño*. The feeding habits of species comprising these guilds were zoobenthivorous (e.g., whitemouth croaker *M. furnieri*, American freshwater goby *Ctenogobius shufeldti*), zooplanktivorous (e.g. Brazilian silverside *Atherinella brasiliensis*, Argentine menhaden *Brevoortia pectinata*) and detritivorous (e.g., mullets *M. liza*, *M. curema*) (Elliot et al. 2007; Possamai et al. 2018; Garcia et al. 2018b). Hence, trophic links channeling riverine organic matter to these consumers are likely integrating both pelagic and benthic pathways (Rooney et al. 2006) and probably occurred through the consumption of microcrustaceans (e.g., copepods, cladocerans), polychaetes, and benthic microalgae (this study, Garcia et al. 2018b). For instance, the estuarine consumers with highest assimilation of riverine allochthonous matter were year-round estuarine residents (F-RES). This guild comprised only the species *A. brasiliensis* and *C. shufeldti*. The inspection of their stomach content during the wet period revealed the presence of microcrustaceans (e.g., isopods, amphipods) and adult insects, for *A. brasiliensis*, and microcrustaceans (e.g., copepods, ostracods, amphipods, tanaidaceans), chironomidae larvae and filamentous algae, for *C. shufeldti* (AFSG, unpublished data). Further studies on the feeding ecology of these species using stomach content and stable isotopes would be needed to unravel the trophic pathways linking allochthonous organic matter sources and the studied estuarine consumers.

The assimilation of allochthonous organic matter by fishes and its relationships with environmental conditions (e.g., hydrology, habitat structure) is well described in freshwater systems (Lowe-McConnell 1987; Winemiller 1990; Hicks 1997; Winemiller and Jepsen 2004; Baxter et al. 2005; Luz-Agostinho et al. 2008), but it is still poorly investigated in estuarine systems (Abrantes et al. 2013; Garcia et al. 2017b). For example, higher water levels in coastal lagoons had been associated with higher hydrologic connectivity between freshwater and estuarine compartments. Such conditions promote assimilation of freshwater-derived organic matter by estuarine consumers, which ceases following the drop in estuarine water levels and the subsequent hydrologic disconnection between lagoon‟s compartments (Garcia et al. 2017b). Up to our knowledge, our study is the first to show that hydrological alterations during a strong *El Niño* event may promote the assimilation of riverine allochthonous matter by estuarine organisms. The potential role of *El Niño* events promoting assimilation of continental organic matter by estuarine consumers should be investigated in estuaries under the influence of this climatic phenomenon, especially considering it may become more frequent and intense in a global warming scenario (Yeh et al. 2009).

Finally, it is worth noting that we used as proxy for the riverine allochthonous matter in our isotope mixing models only the particulate organic matter sampled in the sediment (SOM), and not the one in suspension in the water column (POM). This was because only SOM showed statistically significant differences in $\delta^{13}C$ between the riverine-estuarine transect and, therefore, could be a useful tracer to infer assimilation of riverine-derived organic matter to estuarine consumers. We are aware that the transport and subsequent assimilation of riverine allochthonous matter could also occur via POM as demonstrated by studies elsewhere showing assimilation of continental-derived POM by consumers in coastal systems (Heymarns and McLachlan 1996; Bergamino et al. 2011; Savage et al. 2012; Hoffman et al. 2015). Further investigation in our study site using additional isotope tracers (e.g., sulfur, hydrogen) are needed to investigate the trophic relevance of riverine-derived

POM as primary carbon source to consumers feeding downstream in the estuary (e.g. Whitney et al. 2018).

Our findings revealed that heavy rainfall associated with the 2015 *El Niño* led to higher assimilation of riverine allochthonous matter by estuarine consumers (both macroinvertebrates and fishes). Understanding the remote effects of ENSO-induced climatic changes on the energy fluxes of coastal food webs are of paramount importance to safeguard relationships between biodiversity and ecosystem functioning in natural communities (Barnes et al. 2018) and to subsidize restoration actions to maintain connectivity among ecosystems (Howe and Simenstad 2015). Prior work have shown that man-made changes in natural hydrological conditions in riverine systems may affect the composition and organization of their food webs downstream via additions or deletions of taxa and variation in the strength and occurrence of food linkages (Mor et al. 2018). Here, we highlighted that natural climatic phenomenon like *El Niño* and its global impacts on climatic and hydrologic conditions may affect not only the composition (Garcia et al. 2004) and food web organization (Possamai et al. 2018) in southwestern Atlantic estuaries, but also the assimilation of riverine allochthonous matter by estuarine organisms. Future studies should be carried out in other estuaries under the influence of this climatic phenomenon to evaluate its role as a potential driving force affecting food web organization, especially considering predictions this phenomenon may become more frequent and intense in a global warming scenario (Timmermann et al. 1999; Yeh et al. 2009).

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SUPLEMENTARY MATERIAL

Table supplementary 1 Total range (minimum and maximum) and mean (±SD) values of total length (TL, mm) of fishes and macroinvertebrates sampled during the dry and wet periods at the Tramandaí-Armazém estuarine complex. n.m.: not measured

	DRY						WET					
	TL (mm)						TL (mm)					
	N	Min	Max	Mean		SD	N	Min	Max	Mean		SD
FISHES												
Freshwater primary (F-FRE_P)												
Astyanax fasciatus	6	61	154	134.2	±	36.2	5	38	65	53.0	±	9.9
Oligosarcus jenynsii	3	139	163	152.7	±	12.3	3	83	96	87.7	±	7.2
O. robustus							5	60	84	70.0	±	9.0
Pimelodella australis							5	52	65	58.2	±	5.0
Freshwater secondary (F-FRE_S)												
Geophagus brasiliensis	14	32	185	92.1	±	57.6	9	41	90	62.2	±	17.8
Jenynsia multidentata	3	25	33	29.3	±	4.0	3	51	76	60.0	±	13.9
Estuarine Resident (F-RES)												
Atherinella brasiliensis	10	52	147	80.0	±	30.2	2	79	87	83.0	±	5.7
Ctenogobius shufeldti							13	30	65	46.4	±	10.9
Estuarine Dependent Juveniles (F-DEP_J)												
Anchoa marinii	7	49	65	56.9	±	6.6						
Brevoortia pectinata	5	42	51	47.0	±	4.2						
Diapterus rhomeus	9	37	159	88.2	±	53.5	7	55	122	92.7	±	27.3
Eucinostomus argenteus	12	53	95	73.8	±	16.5						
E. melanopterus	15	41	85	65.9	±	13.2	5	44	66	51.6	±	8.6
Genidens barbus	15	72	92	84.1	±	5.6	10	87	130	99.9	±	15.0
G. genidens	14	80	119	104.4	±	15.0	7	87	93	90.3	±	2.4
Lycengraulis grossidens	5	98	119	111.0	±	9.1	3	60	85	68.7	±	14.2
Micropogonia furnieri	14	52	124	90.9	±	20.9	15	52	182	109.4	±	31.5
Mugil brevirostris	5	66	96	75.0	±	12.3						
M. curema	14	29	41	34.3	±	3.5						
M. liza	6	57	251	132.0	±	83.1	8	26	297	120.5	±	127.3
Platanichthys platana							2	46	50	48.0	±	2.8
Estuarine Dependent Adults (F-DEP_A)												
Brevoortia pectinata	5	218	240	230.0	±	8.2						
Diapterus rhomeus	2	170	180	175.0	±	7.1						
Eucinostomus argenteus	4	100	109	102.8	±	4.2						
E. melanopterus							3	103	152	125.0	±	24.9
Genidens barbus	4	252	316	274.0	±	30.2						
G. genidens	11	120	390	229.9	±	94.2	13	128	283	194.0	±	57.2
Lycengraulis grossidens	10	128	172	146.3	±	18.0	1	153	153	153.0	±	0.0
Micropogonia furnieri	3	240	261	251.0	±	10.5						
Mugil liza	3	313	341	327.0	±	14.0						
Marine (F-MAR)												
Centropomus parallelus							5	27	48	37.4	±	10.1
C. undecimalis							5	30	52	39.4	±	9.3
Citharichthys spilopterus	13	28	96	63.6	±	22.9	15	62	92	74.3	±	10.7

Gobionellus oceanicus	2	52	113	82.5	±	43.1						
Symphurus tesselatus	2	75	95	85.0	±	14.1						
Trachinotus carolinus	6	85	193	163.7	±	39.4						
MACROINVERTEBRATES												
Estuarine Resident												
Polychaeta (P-RES)												
Heteromastus similis	6	n.m.	n.m.	n.m.		n.m.						
Crustacea (C-RES)												
Palaemonetes argentinus	3	27	29	28.0	±	1.0	5	31	41	38.5	±	4.2
Estuarine Dependent												
Crustacea (C-DEP)												
Callinectes danae	12	36	106	64.9	±	22.3						
Callinectes sapidus	13	12	155	77.9	±	38.7	29	21	148	72.0	±	41.5
Farfantepenaeus paulensis	22	35	113	77.8	±	26.5						

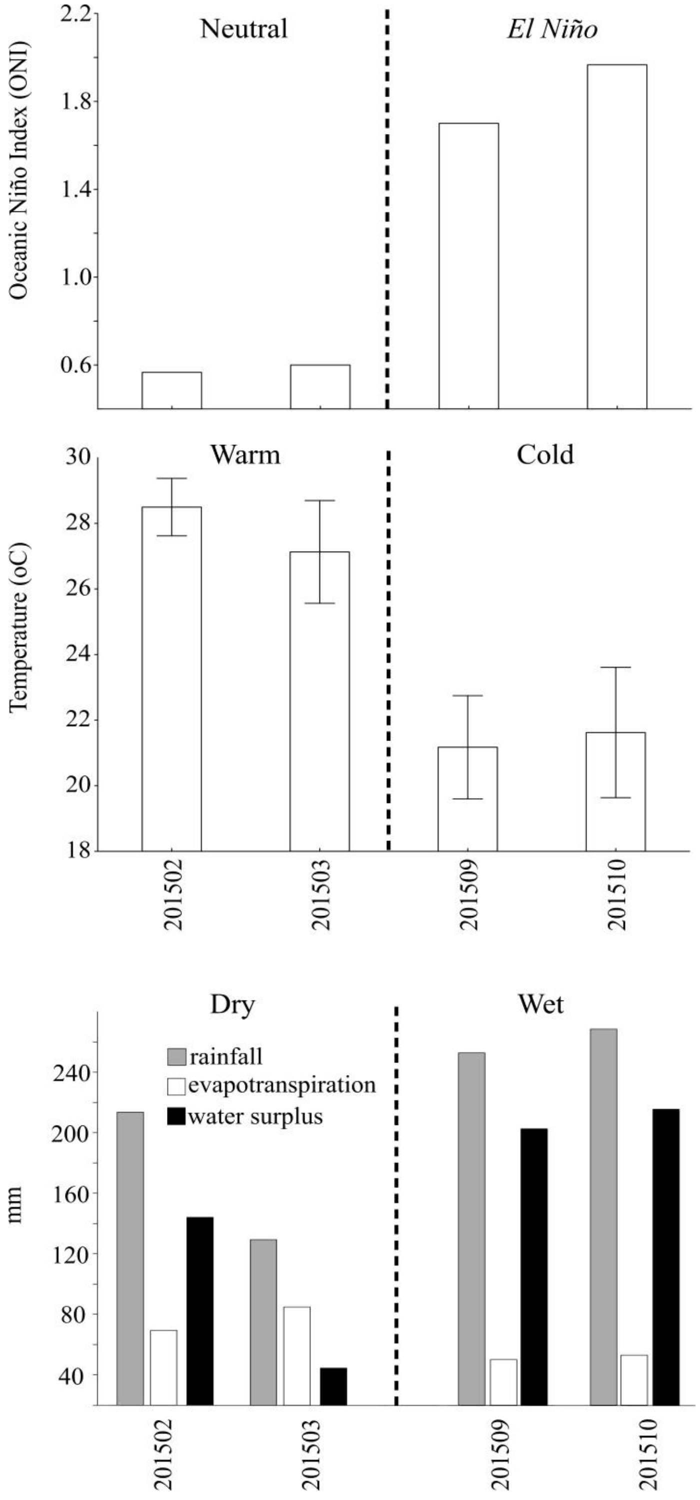

Fig. supplementary 1 Temporal variations in the Oceanic Niño Index (ONI), air temperature (^{o}C) (superior and middle panels), and rainfall, evapotranspiration and water surplus (bottom panel) in the months preceding each field collection.

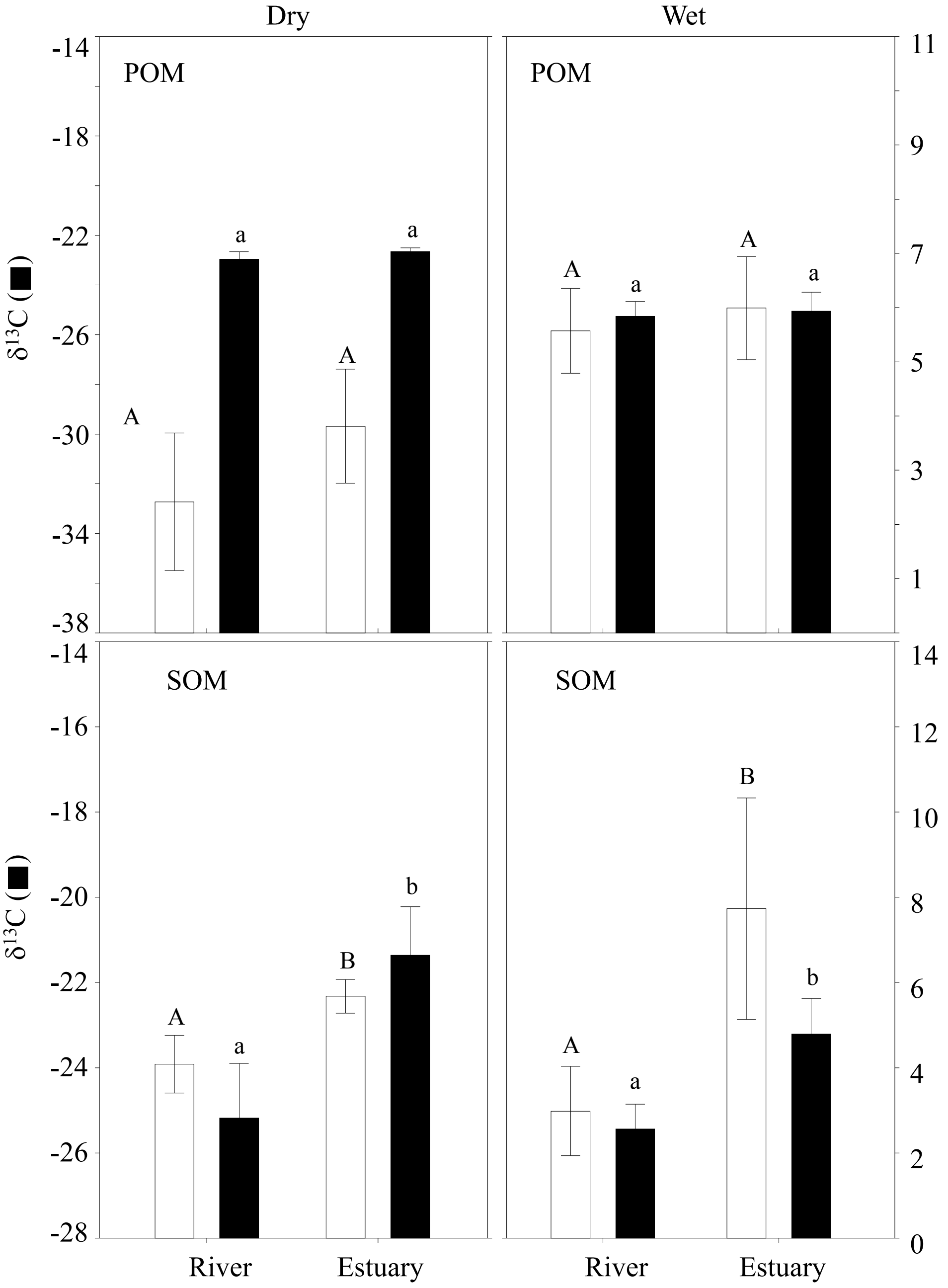

Fig. suplementary 2 Average values of carbon ($\delta^{13}C$) (filled columns) and nitrogen ($\delta^{15}N$) (open columns) stable isotope ratios of particulate organic matter in suspension (POM) and in sediment (SOM) sampled in the river emptying into the estuary during the dry and wet periods. Letters denote statistically significant differences (Tukey pos-hoc test, α=5%), with uppper case letters indicating differences in $\delta^{15}N$ and lower case letters differences in $\delta^{13}C$ average values.

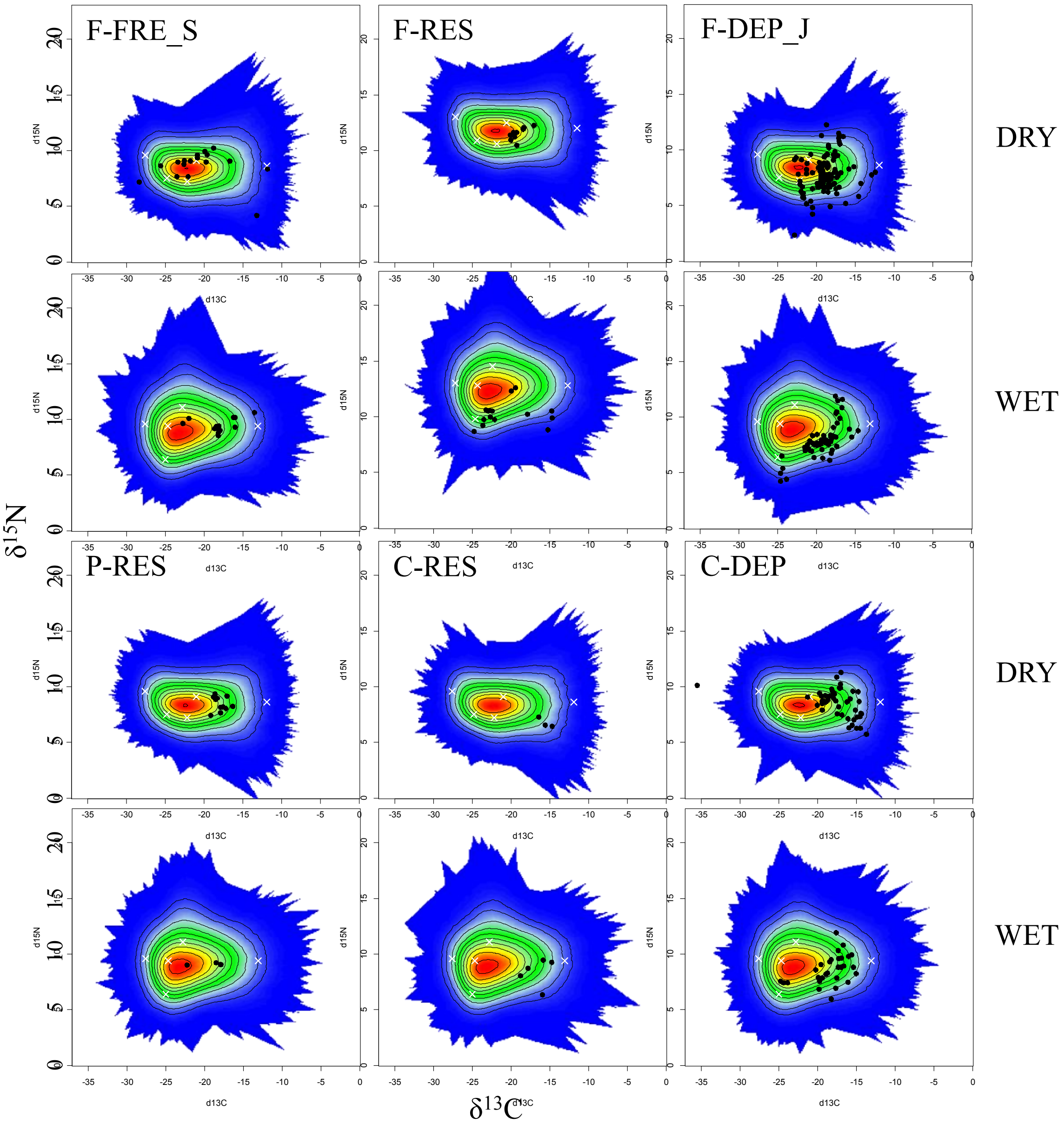

Fig. suplementary 3 Biplots of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios with simulated mixing polygons, where filled circles represent consumers within each trophic guild and white crosses average autotrophic sources values. Color gradient represents probability contours which indicate how often a mixing polygon encloses an area. The outermost contour represents the 5% likelihood fit of a mixing model. The isotopic composition of those consumers situated outside the 95% mixing region (the outermost contour) cannot be adequately explain by the mixing model. See tables 1 and 2 for codes of consumer‟s guilds.

INSTRUCTIONS TO AUTHORS

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- This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1999).

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- Book

Cameron, Deborah. 1985. Feminism and linguistic theory. New York: St. Martin‟s Press.

- Book chapter

Cameron, Deborah. 1997. Theoretical debates in feminist linguistics: Questions of sex and gender. In Gender and discourse, ed. Ruth Wodak, 99-119. London: Sage Publications.

- Online document

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CAPÍTULO 3

Manuscrito aceito para publicação na revista *Community Ecology.*

SPATIAL DIET OVERLAP AND FOOD RESOURCE IN TWO CONGENERIC MULLET SPECIES REVEALED BY STABLE ISOTOPES AND STOMACH CONTENT ANALYSES

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Short-title: Food niche comparisons in mullets

Keywords: trophic ecology; isotopic niche; food niche; Mugilidae; *Mugil curema; Mugil liza;* surf-zone diatoms; marine surf-zone; estuary

Abstract: Food partitioning among coexisting species in different habitats remains an important research topic in trophic ecology. In this work, we combined carbon and nitrogen stable isotope ratios and stomach content analyses to investigate differences in diet and niche overlap of two congeneric juvenile mullet species (*Mugil curema* and *Mugil liza*) coexisting in a marine surf-zone and an estuarine zone in southern Brazil (29°S). These habitats have contrasting levels of food availability, especially in terms of prey diversity, with higher microalgae diversity in the estuary than in the marine surf-zone. In these contrasting conditions, we predicted that both mullet species will have (a) higher niche overlap and smaller niche breadth at the marine surf-zone due to the common exploration of highly abundant surf-zone diatoms and (b) lower niche overlap and higher niche breadth inside the estuary due to selective feeding on more diverse food resources. Isotope niche areas (measured as standard ellipse areas) were higher in the estuary (6.10 and 6.18) than in the marine surf-zone (3.68 and 3.37) for both *M. curema* and *M. liza*, respectively. We observed an overlap of 52% in isotopic niches of both species in the marine surf-zone and none in the estuary. We also found contrasting patterns in the diet composition between species according to the habitat. At the marine surf-zone, diatoms of the classes Bacillariophyceae and Coscinodiscophyceae dominated (> 99%) the food content of both mullet species. In contrast, green algae, cyanobacteria, dinoflagellates and flagellates comprised the diet of both species in the estuary. These results could be explained by spatial differences in food availability (especially regarding diversity of microalgae) between both habitats. At the marine site, both species explored the most abundant microalgae available (mostly the surf-zone diatom *Asterionellopsis* cf. *guyunusae* and fragments of *Coscinodiscus*), whereas in the estuary both species shifted their diets to explore the greater diverse of microalgae resources. Overall, our findings revealed that niche partitioning theory could not fully predict changes in breadth and overlap of food niches of estuarine dependent fish species with complex life cycles encompassing marine to estuarine systems with contrasting food availabilities.

Abbreviations: ICMBio – Brazilian National Environmental authority; SCA – Stomach content analysis; SEA$_B$ – Bayesian standard ellipse areas; SEA$_C$ – Small sample size-corrected standard ellipse areas; SIA – Stable isotope analysis; SIBER – Stable Isotope Bayesian Ellipses in R; UPGMA – unweighted pair group method with arithmetic mean; TL – Total length.

Introduction

Species coexistence and food partition among species across different habitats have been investigated since the pioneer work of Elton (1927), but still remain as a main research topic in trophic ecology (e.g. Winemiller et al. 2015, Dupke et al. 2016, Fitzgerald et al. 2017). Several theories are used to explain the main mechanisms and controlling driving forces of these interactions (Pianka 1976, Winemiller and Layman 2005). Niche partitioning theory, for instance, predicts that stable coexistence of competing species will occur through niche differentiation that reduces overlap among competitors. Hence, consumers are expected to adjust feeding to reduce niche overlap (e.g. narrowing their diet breadth by selective feeding) with competitors in scenarios of relatively food scarcity (Pianka 1976). Accordingly, field studies have shown that fishes of tropical streams may change from small and distinct food niches during periods of lower resource availability (dry season) to broader overlapping food niches in more productive periods (wet season) (e.g. Correa and Winemiller 2014).

However, predictions of niche partitioning theory are difficult to be observed in nature because variability in food resources in space or time can influence the feeding behavior of consumers (Gerking 1994, Bastos et al. 2017). Hence, a species may explore a restrict number of food resources (specialist diet) in a habitat simply because these items are the most abundant or because there are no alternative resources available. In contrast, this same species may have a broad food niche (generalist diet) in a habitat that has a greater variety of food resources available. This spatial (or temporal) effect of resource availability on species feeding strategy is not always taken into account when investigating the use of food resources by consumers (Fox and Morrow 1981).

Comparison of food habits and diet overlap of coexisting species among habitats with contrasting levels of food availability in terms of abundance and prey diversity are useful to provide empirical evidence to better understand the mechanisms controlling diet variation and food consumption across ecosystems (Winemiller and Layman 2005). One challenge of comparative feeding studies among habitats is to measure the width and overlap of food niches in a standardized and meaningful manner (Bearhop et al. 2004). A traditional approach to describe diet and determine food niche breadth has been stomach content analysis (SCA) of consumers (Nielsen et al. 2017). Although it may achieve high taxonomic resolution, this method can be biased due to the difficulty to determine the origin of partially digested food items and the impossibility to evaluate the true assimilation of nutrients derived from ingested food. In the past decades, stable isotope analysis (SIA) has been considered useful natural markers to reconstruct consumers' diet and food assimilation patterns (Layman et al. 2012). More recently, isotopic niche metrics have been proposed as useful proxies to evaluate the breadth and overlap of food niches (Bearhop et al. 2004, Newsome et al. 2007, Jackson et al. 2011). It is currently recognized that the combination of both techniques (SCA and SIA) provides more accurate representations of consumers' diet and food resource use (Layman et al. 2012, Condini et al. 2015).

Fishes have been considered an adequate model to investigate spatial changes in food resources use and diet overlap due to their diversity and high abundance across virtually all aquatic systems (Gerking 1994). Many fishes have complex life histories encompassing the use of contrasting aquatic systems with marked changes in food availability along their life cycle (Wootton 1999). Mullets are good examples of fishes occurring from tropical to temperate zones that explore contrasting habitats along their life history. They usually spawn in the sea and their juveniles seek shallow protected waters in estuaries and coastal lagoons, where they feed upon highly abundant resources. Most mullet species have diets comprised by large amounts of detritus, benthic invertebrates, green filamentous macroalgae and microalgae (Cardona 2015). SCA has been traditionally used to describe their diet composition, a technique that can be highly time-consuming in mullet species feeding mostly on microalgae (Vieira 1991, Cardona 2001). A few studies have applied SIA to investigate trophic ecology of mullets (Lebreton et al. 2011, Le Loc'h et al. 2015), but only a handful of studies combined SCA and SIA techniques (e.g. Carassou et al. 2017). For instance, Carassou and collaborators (2017) employed SIA and SCA (and other natural markers) to investigate spatiotemporal changes in the diet of the freshwater mullet *Myxus capensis* in a river-estuary interface in South Africa. The authors highlighted the importance of a multiple technique approach to describe the diet of mullets because different species within the family Mugilidae may rely on different food sources within the microphytobenthos or organic detrital matter pools (Le Loc'h et al. 2015, Carassou et al. 2017).

In the present work, we combined SIA and SCA techniques to investigate differences in diet and niche overlap of two congeneric juvenile mullet species (*Mugil curema* and *Mugil liza*) in a marine surf-zone and after their recruitment into an estuarine zone in southern Brazil (29^{o}S). These habitats have contrasting levels of food availability, especially in terms of prey diversity. Estuaries along this region are characterized by high biomass of submerged aquatic vegetation (mainly seagrass and floating macroalgae), great diversity of benthic invertebrates and microalgae and large amounts of detritus (Odebrecht et al. 2010a, 2014, Hoeinghaus et al. 2011). In contrast, the marine surf-zone along this sandy coastline is characterized by high *in situ* phytoplankton production and microalgae blooms, dominated by frequent and dense accumulations of diatoms (Odebrecht et al. 2010b, 2014). In these contrasting scenarios of food availability, we predicted that both mullet species will have higher niche overlap and smaller niche breadth at the marine surf-zone due to the common exploration of the highly abundant surf-zone diatoms, whereas they will have lower niche overlap and higher niche breadth inside the estuary due to selective feeding on more diverse food resources.

Material and Methods

Study area

We carried out the study at the Tramandaí-Armazém estuarine complex and its adjacent marine surf-zone located in southern Brazil (29°58'S; 50°08'W) (Fig. 1). The estuary has an area of approximately 30 km^2 and is comprised of two embayments and a main channel, from which the salinity intrusion enters the estuarine zone, especially when south winds predominate. The estuary has depths ranging from 1 to 5m and is under the influence of a micro-tidal regime (Loitzenbauer and Mendes 2012). The most representative vegetation are marsh plants (e.g. *Juncus acutus*, *Rhynchospora gigantea*, *Scirpus olneyi*), the widgeon grass *Ruppia maritima*, floating macrophytes (e.g. *Eichhornia crassipes*) and terrestrial grasses along the estuarine margins (Hoeinghaus et al. 2011). The marine surf-zone has characteristics of a dissipative beach being directly exposed to waves with medium to high energy and may receive freshwater discharge from coastal streams draining freshwater wetlands. In contrast with the estuary, as described in a more southern (32°S) location (Odebrecht et al. 2010a), the surf zone is characterized by the dominance of phytoplankton, being absent other primary producers as aquatic macrophytes, macroalgae beds and seagrasses (AFSG, personal observation). Regarding anthropogenic impacts, the study area faces increasing environmental pressures from furniture industry, Pinus afforestation, rice irrigation, cattle rising, sand extraction and unplanned human occupation of the coastal zone (Malabarba et al. 2013).

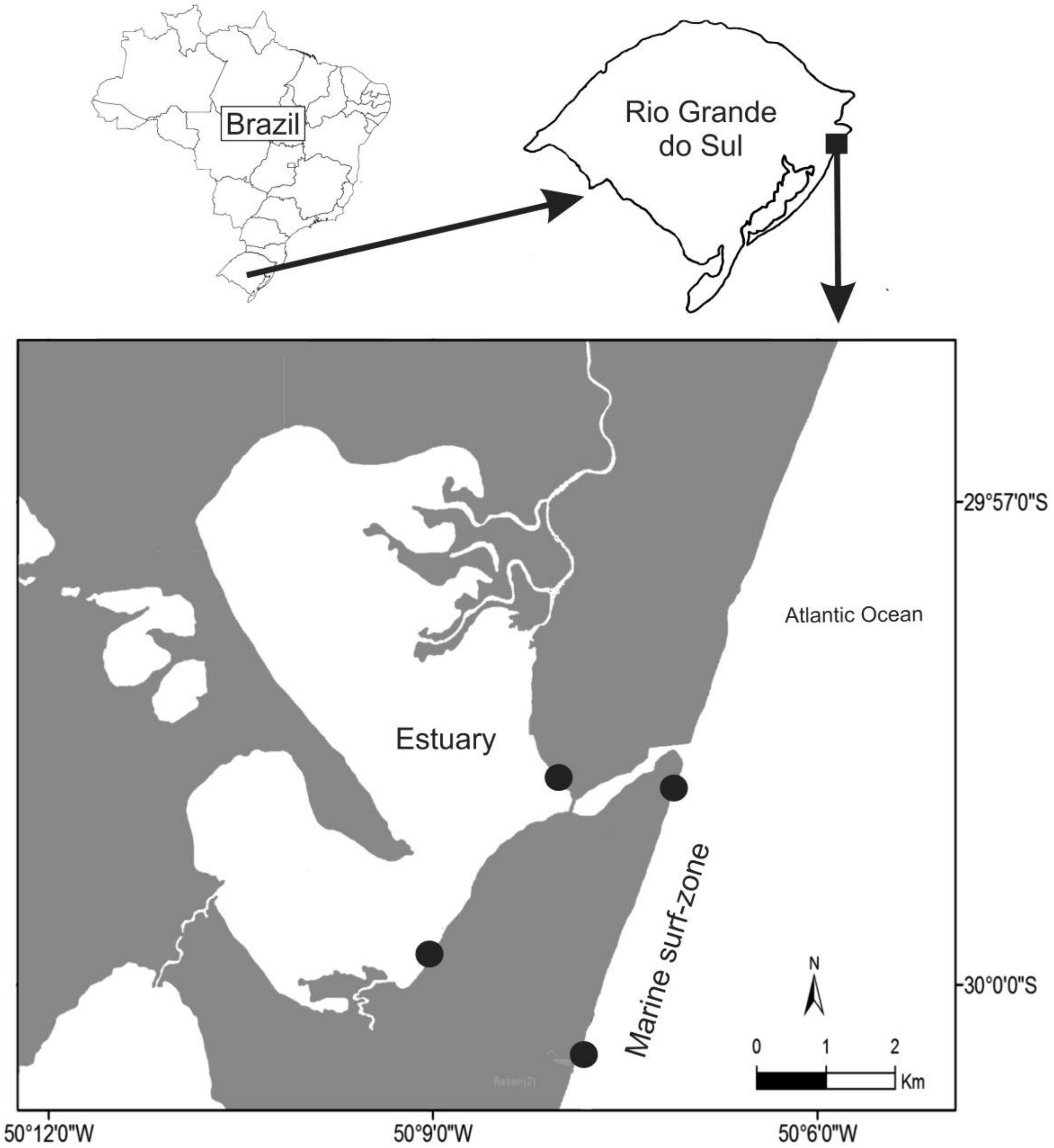

Figure 1. Map of Brazil and its southernmost state (Rio Grande do Sul) where is located the Tramandaí-Armazém estuarine complex. Filled circles (lower panel) denote the sampling stations at the marine surf-zone and within the estuary where mullet individuals were sampled.

Field collections and sampling processing

We sampled mullet species on March 2015 using beach seine hauls, casting net and gillnets (see Garcia et al. 2006 for gears' dimensions and operation procedures) under field collection permit nº 47567-1 provided by the Brazilian National Environmental authority (ICMBio).

Individuals for isotopic analysis were kept frozen until later processing in laboratory, where they were dissected to obtain approximately 5 g of anterodorsal muscular tissues per sample. We rinsed each sample with distilled water, placed in a sterile Petri dish, and dried in an oven at 60°C to a constant weight (minimum of 48 h). Dried samples were ground to a fine powder with a mortar, and pestle and stored in Eppendorf tubes. We weighed subsamples (~1 mg) and pressed them into ultra-pure tin capsules (Costech Analytical Technologies) and sent to the Analytical Chemistry Laboratory of the Institute of Ecology, University of Georgia, for analysis of carbon and nitrogen isotope ratios. The carbon standard was Pee Dee Belemnite limestone, and the nitrogen standard was atmospheric nitrogen. We expressed results in delta notation (parts per thousand deviation from a standard material): $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard})-1]*1000$, where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. We applied mathematical normalization on $\delta^{13}C$ values to control for potential effects of lipid contents (DeNiro and Epstein 1977) using the equation proposed by Post and collaborators (2007): $\Delta\delta^{13}C = -3.32 + 0.99 * C{:}N$. We applied this correction for those samples with C:N higher than 3.5 (Post et al. 2007).

We preserved individuals for stomach content analysis in 10% formaldehyde and later in 70% alcohol. In laboratory, we dissected each individual and removed its stomach. The food content of each stomach was analyzed following the guidelines suggested by Cardona (2015). It was not practical to employ gravimetric or volumetric prey measurements due to the presence of detritus and sand in most of the stomachs. Therefore, we opted to evaluate resource exploitation patterns using the frequency of occurrence of prey species (Cardona 2015). The food content of one to three specimens of each species at each region was combined into a unique sample to enable its quantitative analysis, summing up four samples of each species in each region, with the exception of *M. liza* who had five samples analyzed in the marine surf-zone. Table appendix 1 shows the total number of specimens (n=30) used in each sample and location and their individual total length (TL) in millimeters. Each food content sample was mixed with ethanol and stirred. One aliquot was immediately collected with a micropipette and microalgae were counted at ×400 magnification under a light microscope using a Fuchs Rosenthal chamber (Cardona 2015). The counts were carried out until reaching at least 100 individuals (cells, colonies or filaments) in each sample. Cell fragments of *Coscinodiscus* were counted separately. Microalgae were identified to the lowest practical taxonomic level based on specialized literature. Additional subsamples were acid cleaned and mounted on glass slides using Naphrax™ (Brunel Microscopes Ltd., Chippenham, UK) in toluene and examined at ×1000 under a light microscope (Biggs and Kilroy 2000) to identify diatoms.

Our goal was to provide an overall comparison of food intake for each species in each region to contrast with isotope evidences and not to provide a full and detailed dietary study of each species. Therefore, we believe our analysis of only four samples (summing up 30 individuals) of each species in each region was sufficient to provide such comparison (Table appendix 1). The great majority (77%) of analyzed specimens were juveniles (total length, TL, less than 50 mm). Regardless of a few larger individuals, we polled all specimens together for analysis because diet is very similar for specimens >50 mm (Drake et al. 1984).

Data analyses

As a measure of the isotope niche (Newsome et al. 2007), we used small sample size-corrected standard ellipse areas (SEA_C) containing ca. 40% of all data to measure and compare isotopic niche between species in each region (Jackson et al. 2011). We also computed Bayesian standard ellipse areas (SEA_B) ($n = 200$ iterations) to produce a range of probable values (95%, 75% and 50%) for the calculated standard ellipses (Jackson et al. 2011). Overlap in SEA_C between mullet species in each region was used as a measure of isotopic niche partitioning. Isotopic niche overlap was expressed as a proportion of the area of overlap between two SEA_C and its own SEA_C (Catry et al. 2016). All analyzes were carried out using the SIBER package v 2.1.3 in R (Jackson et al. 2011).

We employed a cluster analysis to evaluate the similarity in diet composition of *M. curema* and *M. liza* separately for each region based on Euclidian distances and the agglomeration UPGMA method on a matrix comprised of dietary information ($\log_{10}$(abundance+1)) of each sample. We carried out a correlation between the original distance matrix and the cophenetic matrix to evaluate how well the dendrogram preserved the pairwise distances between the original unmodeled data points (Rohlf and Fisher 1968). We run these analyses using the vegan package in R (Oksanen et al. 2017). We also analyzed the niche overlap based on diet composition between the two mullet species at each region using the Morisita-Horn index following the formula: $C_H=2*(\sum^n_i p_{ij}*p_{ik})\ (\sum^n_i p^2_{ij}+\sum^n_i p^2_{ik})^{-1}$, where C_H is the diet niche overlap between species *j* and *k*, p_{ij} is the proportion of resource *i* from the total resources used by species *j*, p_{ik} is the proportion of resource *i* from the total resources used by species *k*, *n* is the total number of resources (Krebs 1999, Cardona 2001).

Results

Differences in isotopic composition and isotope niches

All (44) individuals sampled in the marine surf-zone (*M. curema* = 10, *M. liza* =10) and estuary (*M. curema* = 16, *M. liza* = 8) had their carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios analyzed. All specimens were juveniles with total length (TL, mm) lower than 100, with exception of *M. liza* that ranged from 57 to 341 within the estuary. The comparison of average $\delta^{13}C$ and $\delta^{15}N$ values between species revealed statistically significant differences only for $\delta^{13}C$ at the estuary (KW-H (1;20) = 7; $p < 0.008$), with *M. liza* showing higher average values than *M. curema* (-15.43 vs. -18.27, respectively).

Standard ellipse areas (SEA_C) of both *M. curema* and *M. liza* had higher values in the estuary (6.10 and 6.18, respectively) than in the marine surf-zone (3.68 and 3.37) (Fig. 2). Bayesian estimation of these ellipses areas (SEA_B) corroborated this pattern of greater isotope ellipses in the estuary at 75% and 50% levels of credibility, but also revealed greater variability at 95% credibility intervals mainly for *M. liza* (Fig. 3). Overlap in isotopic niches between species based on SEA_C changed markedly between regions, showing an overlap of 52% in the marine surf-zone and no overlap in the estuary (Fig. 2).

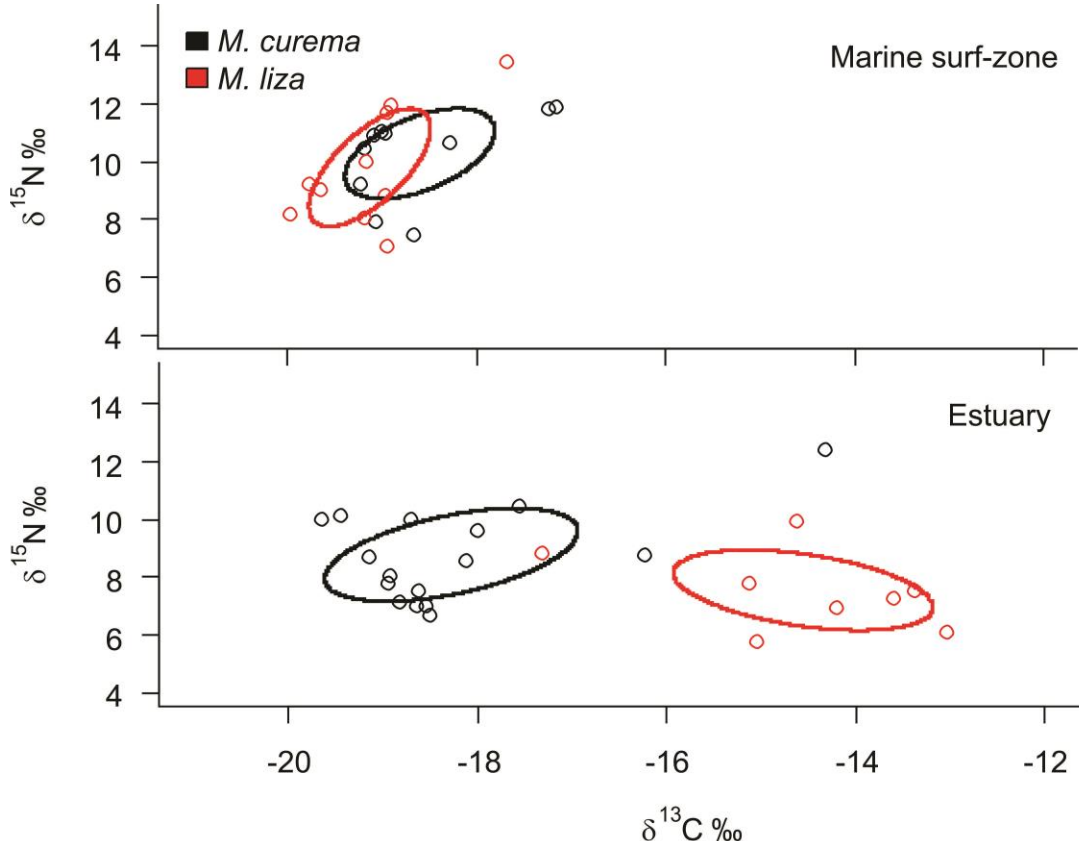

Figure 2. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios and standard ellipse area (SEA) for *Mugil curema* (black circles) and *Mugil liza* (red circles) individuals sampled in the marine surf-zone and in the estuary of Tramandaí-Armazém estuarine complex.

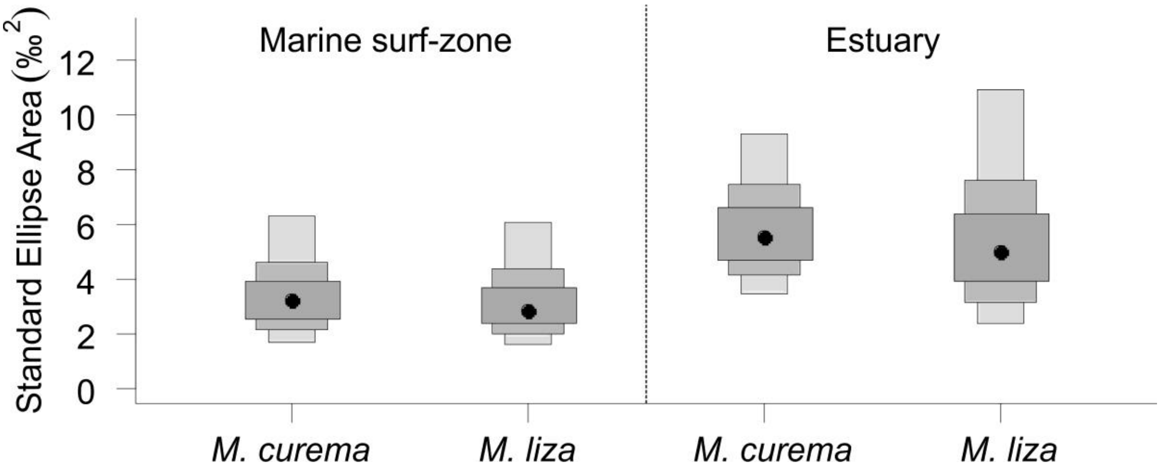

Figure 3. Bayesian standard ellipse areas for *Mugil curema* and *M. liza* individuals sampled in the marine surf-zone and in the estuary of Tramandaí-Armazém estuarine complex. Black dots represent the mode and boxes present the 50%, 75% and 95% credible intervals.

Interspecific and between-habitat differences in food stomach contents

The analysis of food contents of both species revealed contrasting patterns in diet composition and niche overlap (C_H) between species according to the region. At the marine surf-zone, diatoms of the classes Bacillariophyceae and Coscinodiscophyceae dominated (> 99%) the food content of *M. curema* (60.1% and 39.2%, respectively) and *M. liza* (77.5% and 22.1%). In contrast, a greater variety of taxonomic groups, such as Chlorophyceae, Cyanobacteria and Dinophyceae, in addition to non-identified flagellates, contributed in different proportions to the diet of *M. curema* and *M. liza* in the estuary (Figure appendix 1). Accordingly, the niche overlap (C_H) was more than three-folds higher at the marine surf-zone (0.95) than in the estuary (0.27).

Cluster analysis revealed that *M. curema* and *M. liza* showed more dissimilar diets in the estuary than in the marine surf-zone (Fig. 4). At the marine surf-zone, *M. curema* and *M. liza* exhibited a diet comprised of a few distinct food items (five and six items, respectively) with a marked predominance of the surf-zone diatom *Asterionellopsis* cf. *guyunusae* (50.9% and 57.3%, respectively) in their stomach contents. In contrast, the diets of *M. curema* and *M. liza* were much richer and with no predominance of particular food items in the estuary (Fig. 5; Table appendix 2). Moreover, the number of food items in the diet of *M. liza* was more than two-folds higher than of *M. curema* (71 vs. 30 items) and there were also differences in the most consumed items by each species (Fig. 5; Table appendix 2).

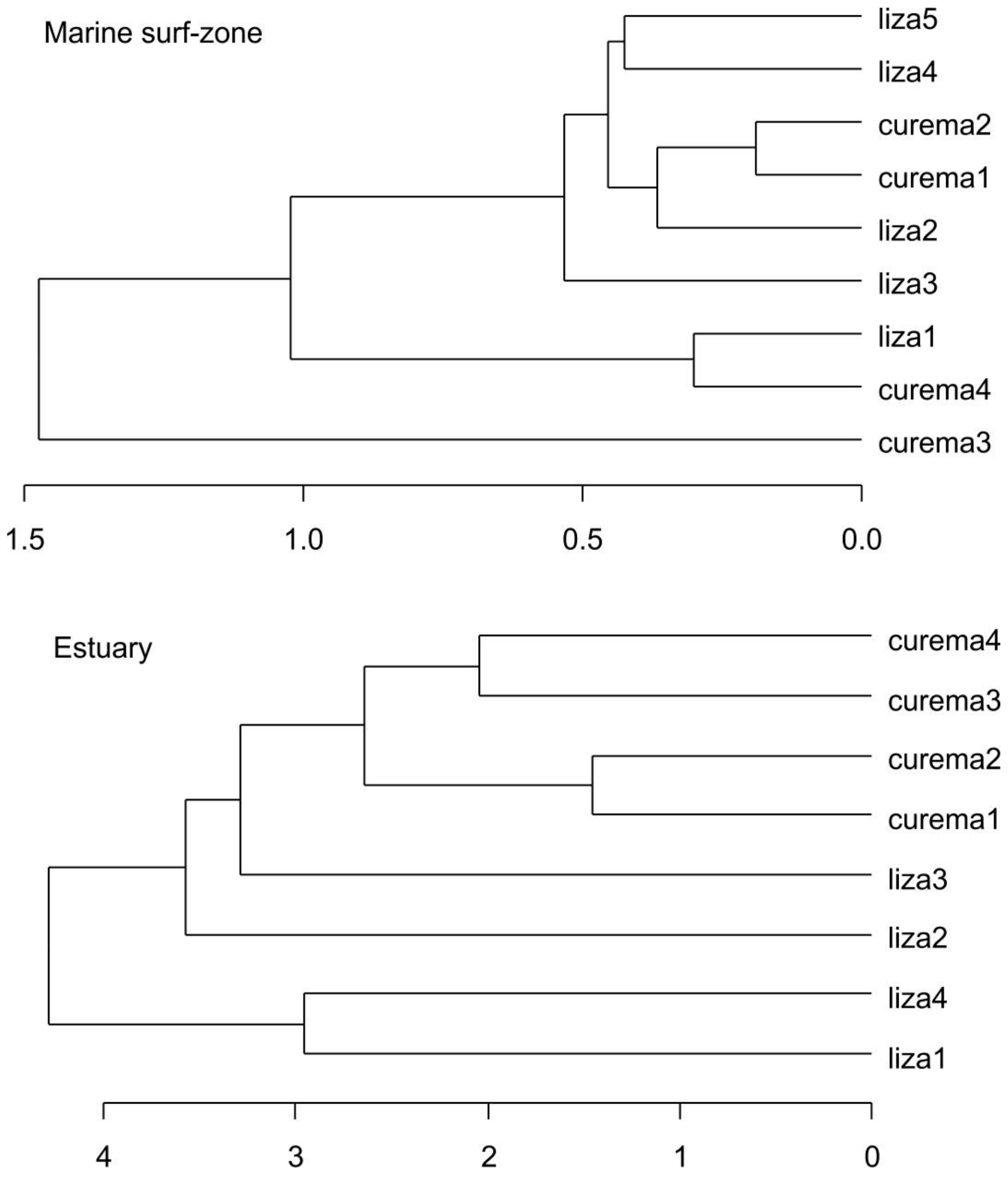

Figure 4. Results of the cluster analysis showing the classification of *Mugil curema* and *M. liza* based on food items found in each sample (numbered from 1 to 4; one to three individuals from each species in each region were combined in composite samples, see Table appendix 1) at the marine surf-zone and estuary of Tramandaí-Armazém estuarine complex. See figure 5 for the diet composition of each species in each location.

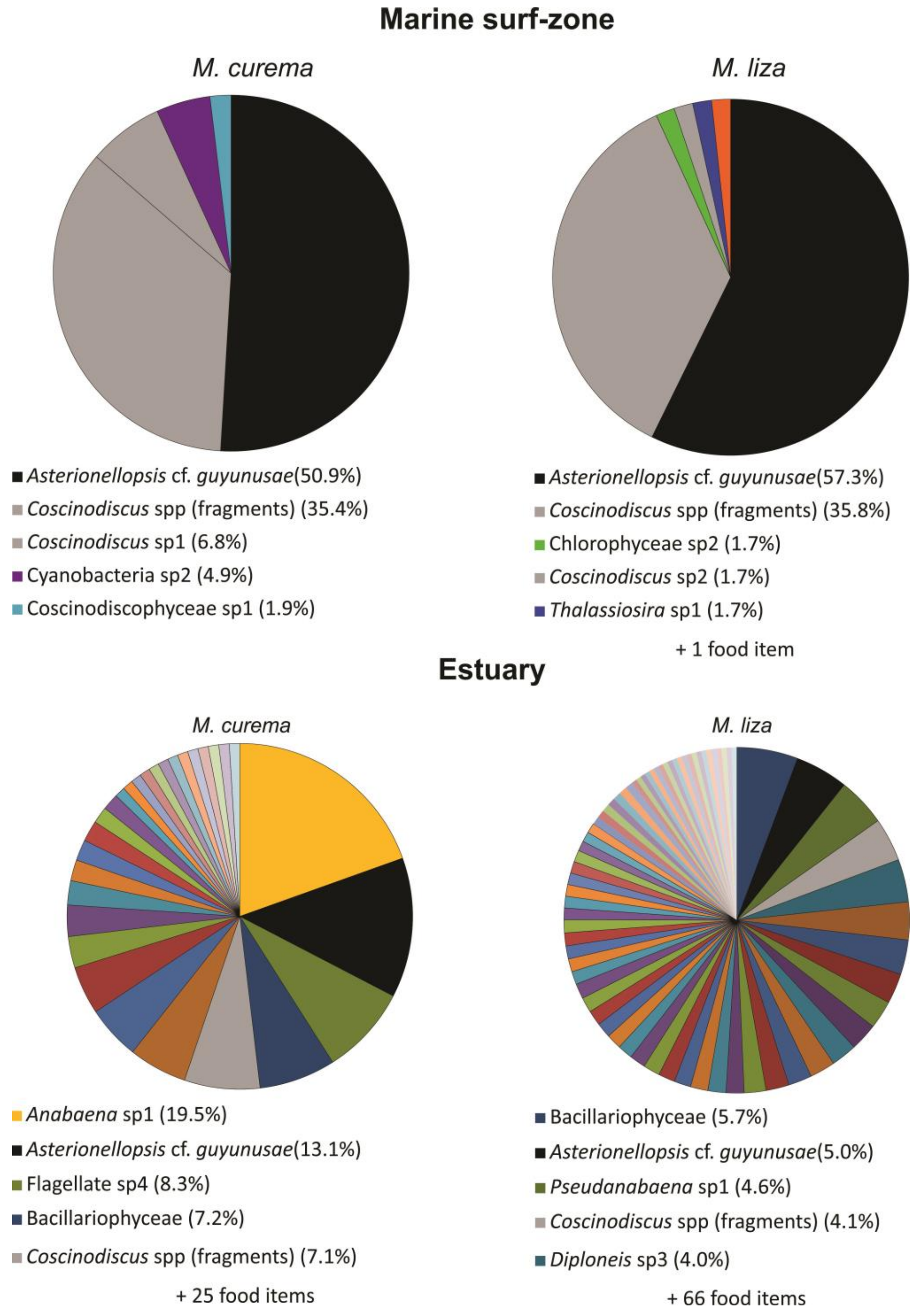

Figure 5. Diet composition and relative frequency of occurrence (%) of food items found in the stomach content of individuals of *Mugil curema* and *M. liza* sampled in the marine surf-zone and in the estuary of Tramandaí-Armazem estuarine complex. Legends below each chart show only the five most abundant (%) food items in all analyzed stomachs for each species and location, followed by the remaining number of distinct food items recorded in the stomachs. See Table appendix 1 for the complete list of food items.

Discussion

Our findings corroborated our predictions of spatial changes in niche breadth and overlap of congeneric mullet species occurring in a marine surf-zone and an estuarine region. We observed lower isotope niche area for both species at the marine surf-zone compared with the estuary, which coincided with a less rich diet for both species. Conversely, we observed a two-fold increase in isotope niches inside the estuary for both species and a six- to nearly twelve-fold increase in diet richness for *M. curema* and *M. liza*, respectively. These between-site changes in niche breadths seemed to reflect marked differences in food resources availability between the marine surf-zone and the estuary and also reflect the Estuarine Marine Migrant behavior (*sensu* Elliott *et al.*, 2007) of these closely related species (Vieira, 1991). Prior studies along the studied coastline revealed comparatively lower diversity of microalgae and the prevalence of blooms of the surf-zone diatom *Asterionellopsis* cf. *guyunusae* (Odebrecht et al 2010b, 2014). Accordingly, we found that this surf-zone diatom dominated the food content of both mullet species at the marine surf-zone, followed by fragments of the diatom *Coscinodiscus* spp. This diatom-based diet resulted in lower isotope niches for both consumers, suggesting that a restricted number of primary carbon sources sustain these juvenile mullets at the marine surf-zone.

However, in contradiction with the niche partitioning theory (Pianka 1976), we did not observe niche segregation between *M. curema* and *M. liza* in the habitat with comparatively lower food diversity (i.e. the marine surf-zone). This apparent discrepancy could be explained by different factors. Firstly, although the availability of food resources at the marine surf-zone may be comparatively lower than in the estuary, there is still high abundance of food items such that probably there was no food limitation for both consumers. Although dominated by a few species, the *in situ* phytoplankton production is high (1.647 mg m^{-3}) and characterized by dense blooms (10^9 cells l^{-1}) of surf-zone diatoms in this marine coastline (Odebrecht and Garcia 1997, Odebrecht et al. 2010b). Hence, it seems plausible to assume that there were enough food resources to be exploited by both mullet species. In such condition, as predicted, we did not observe the effects of niche segregation (Pianka 1976). A high degree of overlap in food niches has also been observed in other marine areas with high *in situ* productivity (e.g. upwelling ecosystems), where food resources may not be a limiting factor and inter-specific competition apparently is not an important factor in structuring fish assemblages (Abdellaoui et al. 2017). Secondly, even in a situation of limited food resources, other factors could allow non-competitive coexistence despite of the observed high food niche overlap between mullets in the marine surf-zone. For instance, Cardona (2001) observed that food niche overlap among coexisting mullet species remained similar along the year despite seasonal changes in food availability, including seasons with food limitation. According to this author, an alternative explanation may be related with the reproductive biology of mullets, as they spawn off-shore and their young-of-the-year are dispersed over a wide geographic area. In these Estuarine Marine Migrant species with a complex life cycle encompassing marine to estuarine systems, recruitment at a particular habitat (e.g. surf-zone, estuary) may be decoupled from growth performance of the species in the area and, hence, competitive interaction and niche segregation do not operate even when food supply is limited (Wootton 1999, Cardona 2001).

As predicted, niche breadths increase substantially inside the estuary for both mullet species. Their diet shifted from almost exclusively diatoms (Bacillariophyceae and Coscinodiscophyceae) to a higher variety of food items such as green algae, cyanobacteria and flagellates. Hence, the diet of both species seemed to shift from a more specialist in the marine surf-zone to a more generalist diet inside the estuary. Such change in feeding behavior may be explained by the abundance and great diversity of microalgae commonly found in estuaries (Day et al. 2012). For instance, prior studies on microalgae diversity in Patos Lagoon estuary (situated in the same coastal plain and ~375 Km apart of our study area) revealed the presence of several groups of freshwater and marine species of diatoms, cyanobacteria, chlorophytes, dinoflagellates, and other flagellates in the water column and high biomass of epiphytic microalgae (mainly pennate diatoms *Cocconeis*, *Synedra*, *Amphora*) on the leaves and shoots of seagrasses (Seeliger 1997, Odebrecht et al. 2010b). Our evidences obtained from stable isotope and stomach content analyses highlighted the trophic plasticity of the two studied mullet species, which apparently shift their diet in response to the more diverse microalgae availability inside the estuary.

In contrast with our initial expectation, the increase in niche breadth inside the estuary did not occur in similar magnitude for both mullet species. In fact, we observed that *M. liza* had a diet two-folds richer than *M. curema* in the estuary. A possible hypothesis to explain such between-species differences in diet richness could be related with the exploitation of distinct microhabitats within the estuary by each species. These microhabitats could be associated with differences in depths and/or particle size preferences (Cardona 2015). Prior studies in Colombia, for example, revealed distinct preference for mean particle size in the substrate associated with foraging activity, with lower particle sizes for *M. curema* (163 μm) and higher for *M. liza* (401 μm) (Osorio-Dualiby 1988). Such particle size preferences could reduce interspecific competition between congeneric mullet species in estuaries (Marais 1980, Cardona 2015). Further, differences in the composition of microalgae in the stomach contents may indicate that the mullet species are foraging in distinct depths or at least in different regions of the estuary that have sediments with distinct properties and, thus, distinct compositions of microalgae. For instance, the microalgal community of intertidal sandy areas is composed of few species than the community that develops on fine sediments (i.e. epipelon) of deeper areas (Round 1984) and is dominated by mats of cyanobacteria (Stal 2001), as we found for *M. curema*. Moreover, some typical epipelic diatoms, such as *Nitzschia*, *Navicula*, *Tryblionella* and *Terpsinoe*, predominant in the sediments of another estuary in the same coastal plain (Bergesch et al. 1995, Silva et al. 2010), were not found in the stomach content of *M. curema*. This species also consumed a low diversity of flagellates, commonly found in the epipelon (Round 1984), in comparison to *M. liza*. Other explanation may be related with differences in zoogeographical distribution and residence time inside the estuary. In contrast to *M. curema* that has a more tropical distribution and peak in abundance in the studied subtropical estuary (29^{o}C) only during warmer periods, *M. liza* occurs year-round in subtropical estuaries of the Southwestern Atlantic (Vieira 1991). It is possible, therefore, that for subtropical estuaries *M. liza* is better adapted than *M. curema* to explore different microhabitats and a great variety of food resources. Further field studies comparing microhabitat uses and foraging activity rates in subtropical vs. tropical estuaries are needed to evaluate these hypotheses.

Overall, dietary analyses based on stomach content analysis have often revealed a high similarity in the diet of sympatric mullet species in both their planktophagous fry stage and the juvenile/adult sedimentivorous stage (Cardona 2015). However, recently work based on stable isotope evidences has suggested food niche segregation among congeneric mullet species in estuaries (Le Loc'h et al. 2015), which agrees with our stable isotope results. Although we did not found differences in average nitrogen isotope ratios between species, we observed differences in carbon isotope ratios suggesting they assimilated distinct carbon primary sources inside the estuary, which could be associated with feeding on distinct groups of microalgae. In fact, our stomach content results showed that *M. liza* explores a wider range of microalgae in the estuary.

In summary, our evidences based on stable isotope and stomach content analyses revealed marked changes in the breadth and overlap of food niches of congeneric mullets between a marine surf-zone and an estuary. This pattern could be explained by spatial differences in food availability (especially regarding diversity of microalgae) between both habitats. At the marine surf-zone, both species explored the most abundant microalgae available, mostly the surf-zone diatom *Asterionellopsis* cf. *guyunusae*. However, after recruiting into the estuary, their diets became richer and their isotope niches segregated, probably due to the exploration of different estuarine microhabitats.

Acknowledgments

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SUPLEMENTARY MATERIAL

Table appendix 1. Number of specimens and total length (TL, mm) of *Mugil curema* and *Mugil liza* used in each sample (#) of stomach content analyzed at the marine surf-zone and estuary of Tramandaí-Armazém estuarine complex. The number of specimens comprising each sample ranged from one to three.

Mugil curema				*Mugil liza*			
Marine surf-zone		Estuary		Marine surf-zone		Estuary	
Sample #	Individual (TL, mm)	Sample #	Individual (TL, mm)	Sample #	Individual (TL, mm)	Sample #	Individual (TL, mm)
1	32	1	31	1	24	1	217
1	33	1	32	1	25	2	341
2	32	1	34	1	25	3	251
2	33	2	31	2	24	4	313
2	35	2	33	2	25		
3	30	3	34	2	25		
3	34	4	40	3	400		
4	31			4	410		
4	32			5	373		
4	33						

Table appendix 2. Average numerical abundance of microalgae found in the stomach contents of *Mugil curema* and *Mugil liza* at the marine surf-zone and estuary of Tramandaí-Armazém estuarine complex. NI: not identified. N = number of samples analyzed. n = total number of specimens analyzed. Each sample was comprised of one to three specimens (see Table appendix 1).

	Marine surf-zone		Estuary	
	M. curema (N=4, n=10)	*M. liza* (N=5, n=9)	*M. curema* (N=4, n=7)	*M. liza* (N=4, n=4)
CYANOBACTERIA				
Anabaena sp.	0	0	50.5	0.5
Chroococcus sp. 1	0	0	0	6
Chroococcus sp. 2	0	0	0.25	1
Chroococcus sp. 3	0	0	0	1
Cyanobacteria sp. 1	0	0	0	1
Cyanobacteria sp. 2	1.25	0	1	1
Cyanobacteria sp. 3	0	0	0	6.25
Cyanobacteria sp. 4	0	0	0.5	0
Gomphosphaeria sp.	0	0	0	6.75
Johannesbaptistia pellucida	0	0	0	0.25
Merismopedia sp.	0	0	0.25	0
Oscillatoriales sp.	0	0	0	0.25
Pseudanabaena sp.	0	0	0	7.5

Spirulina sp.	0	0	0	1
BACILLARIOPHYCEAE				
Achnanthidium sp.	0	0	0.25	0
Amphora sp. 1	0	0	1	1
Amphora sp. 2	0	0	0	1
Amphora sp. 3	0	0	0.25	0
Asterionellopsis cf. *guyunusae*	102	101.5	24.75	23.75
Bacillariophyceae sp. 1	0	0	0.5	0.25
Bacillariophyceae sp. 2	0	0	0	0.25
Bacillariophyceae spp.	0	0	3.25	8.25
Diploneis ovalis	0	0	0	1
Diploneis sp. 1	0	0	0	2.5
Diploneis sp. 2	0	0	0	3.5
Diploneis sp. 3	0	0	0.25	5
Diploneis sp. 4	0	0	0.5	3
Encyonema sp. 1	0	0	0.25	0.75
Encyonema sp. 2	0	0	0	0.25
Eolimna sp.	0	0	0.25	0.75
Eunotia sp.	0	0	0	0.5
Fragilariales spp.	0	0	2	1
Gomphonema sp. 1	0	0	0	1
Halamphora sp.	0	0	0	1.5
Hantzschia sp.	0	0	0	0.75
Navicula sp. 1	0	0	0	0.5
Navicula sp. 2	0	0	0	0.5
Nitzschia sp. 1	0	0	0	1.5
Nitzschia sp. 2	0	0	0	0.25
Nitzschia sp. 3	0	0	0	0.25
Nitzschia sp. 4	0	0	0	1
Planothidium sp.	0	0	0.75	0.5
Rhopalodia cf. *brebissonii*	0	0	0	0.75
Tryblionella sp. 1	0	0.25	0	0.25
Tryblionella sp. 2	0	0	0	1
COSCINODISCOPHYCEAE				
Coscinodiscophyceae sp.	0.25	0	1	1.5
Coscinodiscus cf. *asteromphalos*	0	0	0	1.25
Coscinodiscus sp. 1	1	0	0	3
Coscinodiscus sp. 2	0	0.25	0	0.25
Coscinodiscus sp. 3	0	0	0	0.25
Melosira sp. 1	0	0	0	1.25
Melosira sp. 2	0	0	0.25	0
Coscinodiscus spp. (cell fragments)	65.25	28.75	2.75	15.5
MEDIOPHYCEAE				
Terpsinoe sp.	0	0	0	0.25
Thalassiosira sp.	0	0.25	0	0.25
CHLOROPHYCEAE				
Chlorophyceae sp. 1	0	0	1.75	1.5
Chlorophyceae sp. 2	0	0.25	0	0
Crucigenia sp.	0	0	0.25	0
Desmodesmus sp. 1	0	0	0	0.25

Desmodesmus sp. 2	0	0	0.25	0
Scenedesmus sp.	0	0	0	0.25
ZYGNEMAPHYCEAE				
Cosmarium sp. 1	0	0	0.25	0
Cosmarium sp. 2	0	0	0.25	0
Gonatozygon sp.	0	0	0	0.25
Staurastrum sp.	0	0	0	0.25
DINOPHYCEAE				
Dinophyceae sp. 1	0	0	0	0.5
Dinophyceae sp. 2	0	0	0	0.5
FLAGELLATES				
Flagellate sp. 1	0	0	0	0.25
Flagellate sp. 2	0	0	0	0.5
Flagellate sp. 3	0	0	0	3
Flagellate sp. 4	0	0	16.5	0
Flagellate sp. 5	0	0	0	0.25
Flagellate sp. 6	0	0	1.75	1.25
Flagellate sp. 7	0	0	0	1.25
Flagellate sp. 8	0	0	0	0.5
Flagellate sp. 9	0	0	0	0.25
Flagellate sp. 10	0	0	0	1
NOT IDENTIFIED				
NI1	0	0	0	0.25
NI2	0	0	0.5	1.25
NI3	0	0	0	2.5
NI4	0	0	0	0.25
NI5	0	0	0	0.25
NI6	0	0	0.25	0

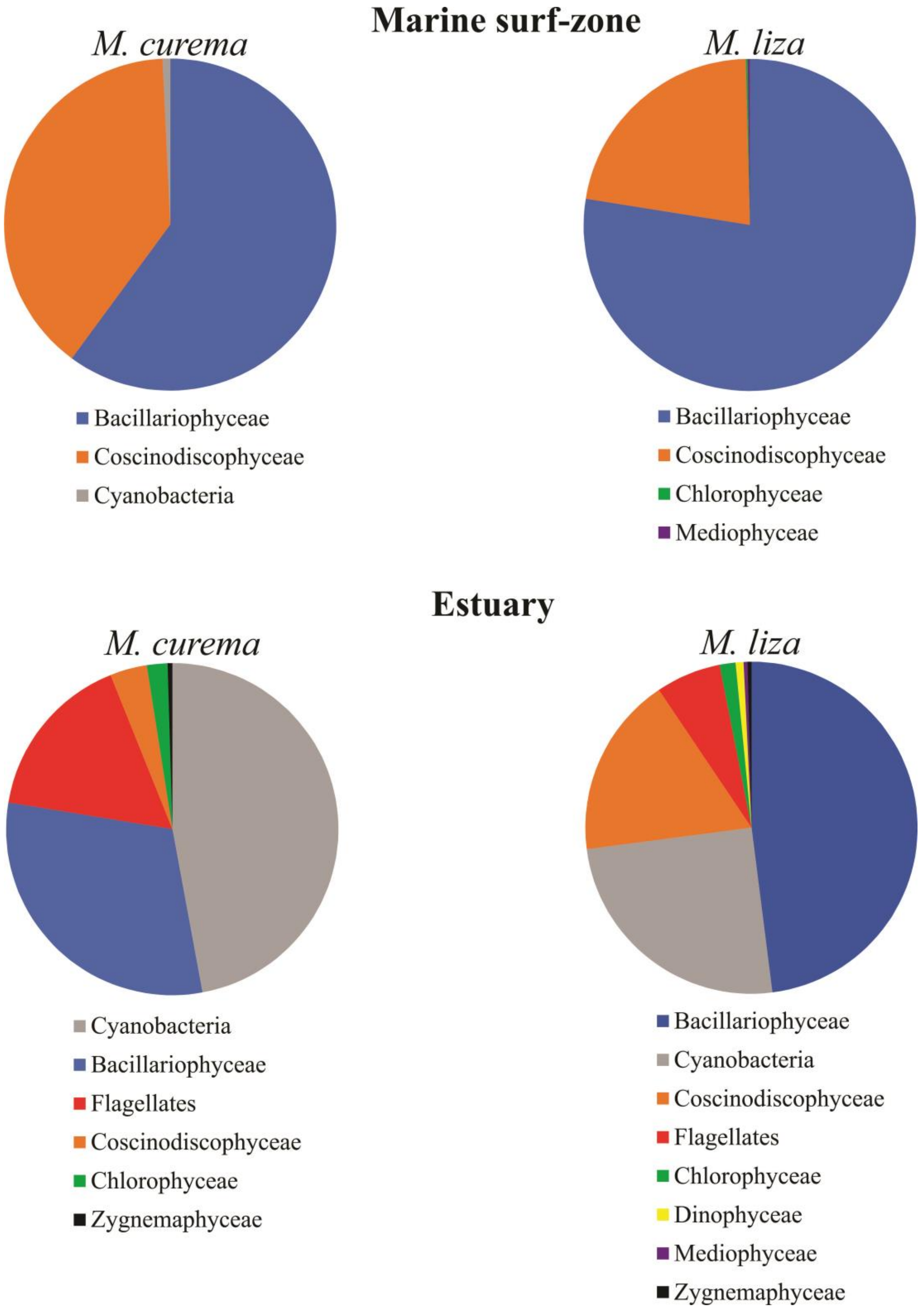

Figure appendix 1. Diet composition and relative frequency of occurrence (%) of classes of microalgae (in addition to non-identified flagellates) observed in the stomach content of individuals of *Mugil curema* and *M. liza* sampled in the marine surf-zone and in the estuary of Tramandaí-Armazém estuarine complex.

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CONSIDERAÇÕES FINAIS E PERSPECTIVAS

Conclusões

As principais conclusões obtidas, a partir do estudo das relações alimentares da fauna de peixes no Complexo Estuarino Tramandaí-Armazém e seus sistemas (fluvial e marinho) adjacentes, foram:

- A importância relativa das fontes autotróficas que sustentam as assembleias de peixes muda notadamente ao longo do transecto rio-estuário-mar, com maior variedade de fontes contribuindo no sistema continental (especialmente no rio) do que no marinho.
- Um maior número de fontes alimentares primárias associadas às vias tróficas pelágica (POM) e bentônica (plantas C_3, SOM e, em menor extensão, plantas C_4), sustentou a assembleia de peixes no rio, quando comparado com o estuário (principalmente POM, seguida de plantas C_4) e a zona de arrebentação marinha (POM).
- A maior riqueza de guildas e presas de peixes observados no ambiente estuarino resultaram em uma rede alimentar (do tipo bipartida presa-consumidor) mais complexa neste ambiente, quando comparado com os sistemas adjacentes (rio e zona de arrebentação marinha). Em contrapartida, a assembleia de peixes amostrada na região do rio apresentou uma rede bipartida mais simplificada, com menor número de guildas e presas e, consequetemnete, da densidade de ligações tróficas.
- A elevada precipitação associada ao *El Niño* de 2015 acarretou uma maior assimilação da material alóctone continental de origem fluvial por consumidores estuarinos (macroinvertebrados e peixes) quando comparado ao período com menor precipitação e sem a influência desse fenômeno climático.
- Espécies congenéricas de tainhas apresentaram mudanças marcantes na amplitude e na sobreposição de nichos alimentares entre a zona de arrebentação marinha e o estuário.
- Na zona de arrebentação marinha, ambas as espécies exploraram as microalgas mais abundantes disponíveis, principalmente a diatomácea Asterionellopsis cf. guyunusae. No entanto, após o recrutamento para o estuário, suas dietas tornaram-se mais ricas e seus nichos isotópicos foram segregados, provavelmente devido à exploração de microalgas em diferentes microhabitats estuarinos.

Perspectivas futuras

A vulnerabilidade das comunidades biológicas aos impactos naturais e provocados pelo homem está intimamente relacionada com a sua organização trófica. Por exemplo, vários trabalhos têm demonstrado que as redes alimentares mais simplificadas são mais vulneráveis a perturbações do que as redes tróficas mais complexas (PIMM, 1982; WINEMILLER; LAYMAN, 2005; PERALTA-MARAVER et al., 2017). Portanto, as ações de manejo e conservação com o objetivo de mitigar os impactos antrópicos crescentes nos sistemas costeiros (SMITH, 2003; LOTZE et al., 2006) devem levar em consideração possíveis mudanças espaciais e temporais na estrutura tróficas das comunidades biológicas a fim de preservar sua diversidade e serviços ecossistêmicos. Os resultados obtidos no âmbito dessa Tese contribuem com essa questão ao demonstrar com estudos de campo que pode ocorrer variações importantes na organização trófica de assembleia de peixes ao longo do transecto salino (rio-estuário-mar) de um sistema costeiro subtropical. Além de contribuir regionalmente no preenchimento de lacunas de conhecimento sobre esse tema no Complexo Estuarino Tramandaí-Armazém (RS), esses resultados contribuem aos atuais esforços dos cientistas na compreensão da estrutura e dinâmica das teias alimentares em ambientes aquáticos (MOORE et al., 2017). Isso porque a marcada variabilidade nos sistemas costeiros exige estudos empíricos em diferentes sistemas localizados em distintas latitudes para permitir previsões mais acuradas sobre possíveis mudanças nas teias alimentares frente aos impactos antrópicos decorrentes da crescente urbanização e dos efeitos do aquecimento global nas zonas costeiras (ULLAH et al., 2018; TOFT et al., 2018).

Assim, compreender os efeitos remotos das mudanças climáticas induzidas, por exemplo, pelo fenômeno *El Niño* nos fluxos de energia das redes alimentares costeiras é de suma importância para salvaguardar as relações entre a biodiversidade e o funcionamento dos ecossistemas nas comunidades naturais (BARNES et al., 2018) e subsidiar ações de restauração para manter a conectividade entre os ecossistemas (HOWE; SIMENSTAD, 2015). Pesquisas anteriores já mostraram que as alterações nas condições hidrológicas provocadas por eventos *El Niño* podem afetar não apenas a diversidade (GARCIA et al., 2004) e a composição de guildas tróficas de peixes em estuários (POSSAMAI et al., 2018) mas, como demonstrado nessa Tese, a assimilação de matéria alóctone continental por organismos estuarinos. Estudos futuros devem ser realizados em outros estuários sob influência deste fenômeno climático para avaliar sua influência na organização da teia alimentar e na conectividade trófica com ambientes adjacentes, especialmente considerando as previsões de que esse fenômeno pode se tornar mais frequente e intenso em um cenário de aquecimento global (TIMMERMANN et al., 1999;YEH et al., 2009).

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