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INSTITUTO DE OCEANOGRAFIA
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BIOLÓGICA**

**VARIABILIDADE DE LONGO-PRAZO NA ESTRUTURA
TRÓFICA EM ZONAS RASAS ESTUARINAS E SUAS
RELAÇÕES COM FATORES AMBIENTAIS E EVENTOS
*EL NIÑO***

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Dissertação ou Tese apresentada ao Programa de Pós-graduação em Oceanografia Biológica da Universidade Federal do Rio Grande - FURG, como requisito parcial à obtenção do título de DOUTORA.

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RIO GRANDE

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Aos meus pais Salete e Pedro Ivo,
Guerreiros e companheiros de batalhas...

“Education is the most powerful weapon which you can use to change the world.”

“Educação é a arma mais poderosa a qual você pode usar para mudar o mundo”

- Nelson Mandela

“O mar é tudo. Ele cobre sete décimos do globo terrestre. Sua respiração é pura e saudável. É um imenso deserto onde o homem nunca está só, pois ele pulsa vida por todos os lados.”

- Jules Verne

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PREFÁCIO

Esta tese é o trabalho final de quatro anos de doutorado, e está estruturada conforme as normas do Programa de Pós-Graduação em Oceanografia Biológica da Universidade Federal do Rio Grande (FURG). Está organizada em diversas seções, sendo os produtos publicáveis apresentados ao fim do documento na forma de manuscritos (Anexos 1, 2 e 3). No corpo da tese, uma síntese de cada um dos manuscritos é apresentada na língua portuguesa, os quais nomeei de Capítulos I, II e III.

Na primeira seção, apresento uma introdução geral sobre o tema principal da tese, introduzindo os assuntos de uma forma mais resumida e buscando melhor entendimento pelos leitores. Após a introdução, apresento o local estudado e sigo explicando em subtópicos separados e de modo sucinto, os métodos utilizados em cada capítulo. A apresentação dos resultados segue o mesmo padrão. Por fim, há uma discussão geral unindo todos os capítulos, bem como uma conclusão.

Os produtos principais da tese estão nos anexos 1, 2 e 3. O primeiro anexo refere-se ao manuscrito do artigo publicado na *Estuarine and Coastal Shelf Science* em 2018, no qual avaliamos como os efeitos abióticos induzidos pelo *El Niño* afetam a composição e abundância de guildas tróficas de peixes no estuário. Já o anexo dois é um manuscrito que está em processo de primeira revisão na *Estuaries and Coasts*, o qual desenvolvi durante meu doutorado sanduíche na *University of North Texas*. Nesse trabalho avaliamos se a variação dos fatores ambientais associados ao *El Niño* aumentava a assimilação de recursos de água doce pelos peixes no estuário. Já o terceiro anexo está formatado de acordo com as normas da revista *Ecosystems*. Nesse último, avaliamos se fatores abióticos induzidos por eventos *El Niño* causam alteração no comprimento da cadeia alimentar em zonas rasas estuarinas. Por fim, vários outros anexos estão ao fim do documento, e correspondem aos materiais suplementares dos manuscritos.

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RESUMO

Comunidades biológicas que vivem em ambientes naturalmente dinâmicos, como os estuários, estão sujeitas aos efeitos da variabilidade nos fatores abióticos que ocorrem regularmente em diferentes escalas de tempo (p. ex., ciclos circadianos e sazonais) . Porém, eventos extremos ou ocasionais podem desestabilizar a organização da comunidade, alterando atributos como a dominância de espécies, o fluxo energético, o comprimento da cadeia alimentar, entre outros. A estabilidade de um ecossistema é fundamental, pois comunidades estáveis sobrevivem mais que as instáveis. A estabilidade em uma comunidade é dependente da sua estrutura trófica, e alterações nessa estrutura podem afetar, por exemplo, taxas de produção primária e secundária, dinâmicas populacionais de presas e predadores e funções ecossistêmicas. O *El Niño* é um fenômeno climático natural que ocorre com frequência irregular que causa impactos em estuários, alterando a sua hidrologia e regimes de salinidade, e, portanto, com potencial de alteração da organização trófica das suas comunidades biológicas. A partir de um banco de 19 anos de dados mensais de abundância de guildas tróficas de peixes e outro com 8 anos de dados isotópicos da comunidade estuarina em zonas rasas do estuário da Lagoa dos Patos avaliamos se as alterações hidrológicas geradas pelo *El Niño* afetaram (i) a composição e dominância de guildas tróficas de peixes, (ii) a assimilação de recursos alóctones por peixes estuarinos e (iii) o comprimento da cadeia alimentar. Maiores descargas de água doce foram observadas no estuário durante eventos *El Niño*, aumentando a riqueza de espécies e a riqueza de guildas tróficas. Organismos vindos de ambientes de água doce contribuíram para esse aumento nas riquezas, porém não houve aumento na assimilação desses recursos dulcícolas pelos consumidores estuarinos. Contrariamente, a utilização de recursos marinhos aumentou nesses períodos, sugerindo que os consumidores estuarinos buscam zonas mais salinas, como a entrada do estuário, para forragear durante esses eventos. Apesar do aumento na riqueza e na modificação na assimilação de recursos, o comprimento da cadeia alimentar não foi alterado pelos fatores abióticos, sugerindo que essa comunidade de águas rasas é estável. Essa comunidade demonstrou estabilidade e resiliência no atributo comprimento de cadeia frente aos distúrbios ocasionados pelo *El Niño*, e esses são características desejáveis uma vez que o estuário é um ambiente bastante dinâmico. A compreensão da dinâmica trófica e os fatores que influenciam suas características nos possibilitam prever perda de biodiversidade e extinções locais frente às mudanças climáticas. As informações obtidas podem contribuir para o melhor entendimento da vulnerabilidade de comunidades estuarinas frente ao impacto de eventos climáticos extremos como o *El Niño*, que podem se tornar mais frequentes e intensos num cenário de aquecimento global.

Palavras-chave: *El Niño*, estabilidade, guildas tróficas, isótopos estáveis, PELD, posição trófica, resiliência, comprimento de cadeia alimentar.

ABSTRACT

Communities which live in naturally dynamics environments, like estuaries, are face up to the effects of abiotic factors variability that occurs regularly in different time scales (e.g. circadian and seasonal cycles). However, extreme or occasional events may unstabilize the community organization by varying attributes as the species dominance, energy flow, food-chain length, and others. The stability of an ecosystem is fundamental, once stable communities survive more than unstable ones. The stability in a community depends on their trophic structure, and modifying this structure can affect primary and secondary production rates, the dynamic of prey and predators and ecosystemic functions. *El Niño* is a natural climatic event which occurs with irregular frequency and impacts estuaries, modifying the hydrology and salinity regimes. Therefore, it has the potential to change the estuarine biological community and its trophic structure. Counting with 19 years monthly database of trophic guilds abundance and with 8-years isotopic data of the shallow-waters' community in Patos Lagoon estuary, we analyzed if hydrologic variation caused by *El Niño* in the estuary affected: (i) the trophic guilds composition and dominance, (ii) the assimilation of allochthonous resources by estuarine fishes, and (iii) the food-chain length. The higher freshwater discharge was observed during *El Niño* periods in the estuary, and the species richness and trophic richness increased as well. Freshwater organisms contributed to this increase in richness, but there was not increasing in freshwater resources assimilation by estuarine consumers. Contrary, during *El Niño* events the assimilation of marine resources increased, suggesting that estuarine consumers use more saline areas (as the estuarine mouth) to forage during these events. Although the increase in richness and variation in resources assimilation, the food-chain length did not change by abiotic factors, suggesting that this estuarine shallow-water community is stable. This community showed stability and resilience in the food-chain length attribute against *El Niño*-induced disturbances, and these features are welcome in a very dynamic environment as the estuary. Comprehension of trophic dynamic and the factors which influence its features provide us to prevent biodiversity losses and local extinction in reason of climate changes. The information obtained here may contribute to a better understanding of the estuarine communities vulnerability against the extreme climate events as *El Niño*, which can be more frequent and intense in a scenario of global warming.

Key-words: *El Niño*, food-chain length, LTER, resilience, stability, stable isotopes, trophic guilds, trophic position.

INTRODUÇÃO GERAL

A estrutura trófica de um grupo de organismos é compreendida pelo conjunto das relações alimentares que interconectam as presas e seus consumidores num determinado habitat ou ecossistema (Pimm 1982). Alterações na estrutura trófica e nas cadeias alimentares podem afetar as características estruturais da comunidade e suas funções ecossistêmicas, bem como a composição e/ou biomassa dos seus predadores de topo (Post *et al.* 2000). Ainda, a estabilidade das comunidades biológicas é diretamente dependente da estrutura das cadeias alimentares (MacArthur 1955), sendo que sua estabilidade, muitas vezes, implica na própria sobrevivência da comunidade biológica (Elton 1927; MacArthur 1955). Desse modo, compreender a dinâmica trófica de um sistema e os fatores que influenciam suas características é fundamental para entender o funcionamento das comunidades biológicas e prever desastres ambientais, como por exemplo, perda de biodiversidade e extinções locais.

Visando compreender o funcionamento dos sistemas biológicos, alguns métodos vêm sendo aplicados para o estudo das relações alimentares e estrutura trófica de comunidades biológicas. Usualmente a estrutura trófica é representada por meio de modelos ou diagramas de redes alimentares conectando espécies, mas pode também ser avaliada por uma abordagem mais simplificada, a partir do uso de guildas tróficas (Buchheister e Latour 2015; Konan *et al.* 2015). Guildas reúnem grupos de espécies que exploram o mesmo tipo de recurso ambiental de modo semelhante (Root 1967). Portanto, guildas tróficas são utilizadas para agrupar organismos com dietas similares (Elliott *et al.* 2007). Geralmente, a classificação de peixes em guildas tróficas é resultado da análise de conteúdos gástricos. A utilização de guildas tróficas nos permite sintetizar a complexidade comumente observada nas teias alimentares (Elliott *et al.* 2007; Buchheister e Latour 2015; Garcia *et al.* 2019a), simplificando as análises dentro das comunidades biológicas. Ainda, a utilização de guildas tróficas permite a comparação de comunidades biológicas de diferentes áreas biogeográficas, demonstrando as características estruturais de cada comunidade (Figura 1) (Sazima 1986; Elliot *et al.* 2007).

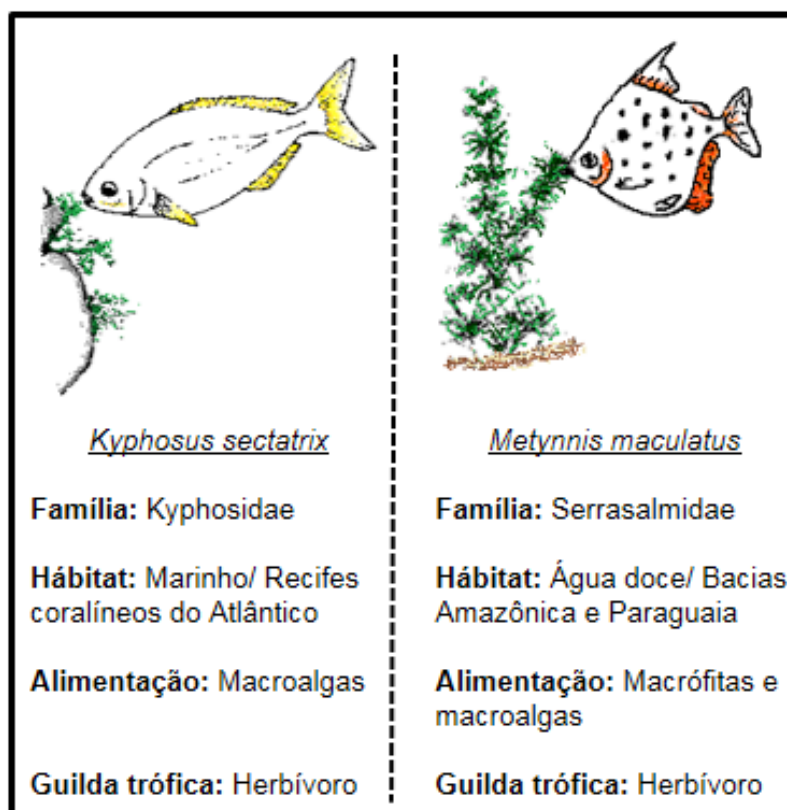


Figura 1. Exemplo de classificação de espécies de diferentes sistemas em guildas tróficas. Adaptado de Sazima (1986).

As guildas tróficas nos permitem avaliar a proporção de indivíduos em cada compartimento trófico, porém não conseguimos avaliar fluxos de energia. A análise de isótopos estáveis, por outro lado, nos possibilita avaliar os fluxos energéticos e de matéria dentro e entre os ecossistemas, fornecendo ainda mais informações sobre a estrutura trófica. Diferentemente da análise de conteúdos gástricos, a análise de isótopos estáveis permite avaliar o alimento que, de fato, foi assimilado nos tecidos do consumidor. Além disso, integra uma determinada janela temporal de assimilação do recurso (que varia em extensão de acordo com a taxa metabólica do tecido analisado) e não apenas uma medida instantânea dos alimentos encontrados no trato digestório do consumidor (Phillips *et al.* 2014). Essa janela temporal pode ser de dois a três meses no caso de músculo de pequenos peixes (Mont'Alverne *et al.* 2016; Oliveira *et al.* 2017), o que nos permite utilizar amostragens sazonais da comunidade de peixes para responder às questões de fluxos energéticos e conectividade trófica de ambientes.

Isótopos estáveis são átomos de um mesmo elemento químico (mesmo número de prótons) que possuem diferentes números de nêutrons. Deste modo, chamamos aquele átomo com um nêutron a mais de “isótopo pesado” e o outro de “isótopo leve” (Figura 2). São chamados “estáveis” porque não se deterioram, ou seja, não sofrem decaimento radioativo. Assim, esses isótopos são encontrados naturalmente na natureza, em proporções já conhecidas (Wada 2009), sendo o isótopo leve geralmente o mais comum (Caxito e Silva 2015). Cada organismo varia a sua composição isotópica dependendo do ambiente, das reações metabólicas, das condições físicas e químicas, e, portanto, cada material biogênico possui um “perfil isotópico” (Fry, 2006; Wada 2009). Para estudos de ecologia trófica, a premissa fundamental da técnica de isótopos estáveis é que os consumidores refletem isotopicamente os alimentos que consomem (Fry 2006). Comumente, utilizamos os isótopos de carbono e de nitrogênio para avaliarmos fluxos de energia ao longo da cadeia alimentar. Os isótopos de carbono nos permitem avaliar a origem da matéria orgânica na cadeia alimentar, enquanto que os isótopos de nitrogênio nos permite verificar o nível trófico dos organismos (Fry 2006). Isso ocorre porque a cada passo da cadeia alimentar existe uma retenção preferencial do isótopo leve de cada elemento, uma vez que é mais facilitada sua entrada nas reações metabólicas dos organismos (Figura 2) (Fry 2006). A esse fenômeno damos o nome de “fracionamento isotópico” ou “discriminação isotópica”, o qual pode variar de elemento para elemento. Usualmente, o fracionamento isotópico do carbono é $< 1\%$, enquanto o nitrogênio tende a ser maior (geralmente entre 2 e 4%) (Vander-Zanden e Rasmussen 2001; Vanderklift e Ponsard 2003). Como o fracionamento do nitrogênio é bem mais marcado que o carbono ele é utilizado como parâmetro no cálculo do nível trófico (p.ex. descontando-se esse valor a cada passo da cadeia alimentar que leva a determinado consumidor). Entretanto, a estimativa de posições tróficas a partir de isótopos estáveis depende da linha de base (baseline), a qual determina um valor referência de $\delta^{15}\text{N}$ para o ambiente em questão e todas as estimativas de posição trófica são realizadas a partir desse valor. Desse modo, as estimativas são bastante sensíveis à baseline utilizada, e a mesma varia no tempo e no espaço (Post 2002). Portanto, para estudos de posição trófica deve-se sempre avaliar a melhor *baseline* e considerar essas variações. Os valores isotópicos são expressos pela notação delta (δ), a qual significa a razão entre o isótopo leve e pesado comparado à razão isotópica de um padrão

internacional, conforme a equação: $\delta X = [\text{Razão}_{\text{amostra}}/\text{Razão}_{\text{padrão}} - 1] * 1000$. Essa padronização da amostra com a razão isotópica de um material de referência internacional permite a comparação entre os valores isotópicos de organismos em locais distintos, bem como o controle do método entre instrumentos de análises isotópicas.



Figura 2. Diferenças na quantidade de nêutrons podem fazer a diferença em algumas reações; ter nêutrons extras geralmente torna as reações mais lentas. Essas diferenças nas reações é o que chamamos “fracionamento”. Traduzido de Fry (2006).

Estudos utilizando os diferentes métodos acima descritos têm gerado informações a respeito da estruturação trófica e variação na dominância de guildas tróficas frente a perturbações ambientais em estuários (Livingston *et al.* 1997; Buchheister e Latour 2015; Possamai *et al.* 2018), fluxo de carbono dentro do sistema estuarino (Hoeinghaus *et al.* 2011; Claudino *et al.* 2013; Claudino *et al.* 2015; Garcia *et al.* 2016; Domingos e Lana 2017), contribuição de material alóctone para consumidores estuarinos (França *et al.* 2015; Nelson *et al.* 2015; Garcia *et al.* 2017; Morais *et al.* 2017; Garcia *et al.* 2019b), variabilidade nas posições tróficas de consumidores e no comprimento das cadeias alimentares (Hoeinghaus *et al.* 2008; Saigo *et al.* 2015; Ishikawa *et al.* 2017; Ruiz-Cooley *et al.* 2017). Essas informações são essenciais para entendermos os sistemas biológicos, pois o fluxo de matéria e energia é um dos principais processos nos ecossistemas, uma vez que todas as atividades biológicas de uma comunidade dependem do suprimento de energia (Elton 1927; Pimm 1982; Begon *et al.* 2006). Fatores ambientais podem influenciar na estruturação trófica e no funcionamento das relações alimentares através de modificações nas abundâncias das espécies (Garcia *et al.* 2004; Alegre *et al.* 2015), padrões de dominância (Livingston *et*

al. 1997), relações interespecíficas (Livingston *et al.* 1997; Winemiller e Layman 2005; Begon *et al.* 2006; Nelson *et al.* 2015; Possamai *et al.* 2018, Garcia *et al.* 2019), entre outros atributos das comunidades biológicas.

Ambientes dinâmicos possuem forte variação em seus fatores ambientais, sendo os estuários um bom exemplo desse tipo de ambiente. Estuários podem ser definidos como "*um corpo de água costeiro semi-fechado, permanentemente ou periodicamente conectado ao mar, diferindo a sua salinidade da do oceano adjacente devido às descargas de água-doce provindas dos rios, incluindo também uma biota característica*" (Elliott e Whitfield 2011). Portanto, são ambientes naturalmente dinâmicos que possuem marcada variabilidade nos seus perfis de temperatura, salinidade, pH em diferentes escalas de tempo (dias, meses, anos) (McLusky 1990; McLusky e Elliott 2004). As adaptações da biota frente a esses ambientes dinâmicos resulta em distintas paisagens dentro do ecossistema estuarino, como manguezais, marismas, ambientes límnicos, canais salinos, bem como a conexão com o oceano adjacente (Day *et al.* 2012). Eventos extremos como, por exemplo, perturbações climáticas, podem intensificar essa variabilidade ambiental comumente encontrada nos estuários, podendo afetar a estruturação trófica das comunidades biológicas.

O *El Niño* Oscilação Sul (ENOS) é um evento global com forte influência no Atlântico Sul Ocidental. O ENOS é caracterizado por interações oceano-atmosfera que provocam anomalias de temperatura da água na região equatorial do Oceano Pacífico, influenciando o clima globalmente (Trenberth 1997). A fase quente do ENOS é conhecida como *El Niño* e a fase fria como *La Niña*, e ambas podem acarretar anomalias positivas e negativas de chuvas no sul do Brasil, respectivamente (Grimm *et al.* 1998). Essas anomalias de chuva intensificam a vazão de água doce para os estuários, influenciando desde a base até os consumidores finais da cadeia trófica estuarina (Haraguchi *et al.* 2015; Teixeira-Amaral *et al.* 2017; Possamai *et al.* 2018; Garcia *et al.* 2019b). O fitoplâncton responde às mudanças hidrológicas alterando a sua composição específica e biomassa, muito em função da tolerância às diferentes salinidades (Abreu *et al.* 2010; Hall *et al.* 2013; Nursuhayati *et al.* 2013; Haraguchi *et al.* 2015; Nche-Fambo *et al.* 2015; Mendes *et al.* 2016). Da mesma forma, as macroalgas e gramas marinhas reduzem suas abundâncias durante períodos de maior aporte de água doce (Lanari e Copertino 2016) e o zooplâncton responde às alterações

hidrológicas induzidas pelo *El Niño* alterando a composição e biomassa (Vignatti *et al.* 2012; Teixeira-Amaral *et al.* 2017). A ictiofauna estuarina pode ter sua riqueza de espécies aumentada devido à entrada de espécies de água doce no estuário durante esses eventos (Garcia *et al.* 2003; Garcia *et al.* 2004), o que pode modificar a estrutura trófica estuarina (Possamai *et al.* 2018; Garcia *et al.* 2019b). Existe uma crescente necessidade de se entender como os fatores ambientais afetam a disponibilidade de fontes alimentares, bem como seus subsequentes efeitos na estrutura trófica de ambientes aquáticos (Layman *et al.* 2015). Sendo assim, estudos investigando como fenômenos globais afetam atributos ecossistêmicos (como disponibilidade de recursos, transferência de energia, comprimento de cadeia alimentar) são essenciais para o manejo de comunidades biológicas e a previsão de perda de biodiversidade frente a mudanças climáticas globais.

No contexto teórico de mudanças ambientais moderadas, nos deparamos com a “Teoria do Distúrbio Intermediário”, a qual prevê que a ocorrência frequente de distúrbios moderados no ambiente torna a comunidade mais diversa e rica (Grime 1973; Connell 1978; Wilkinson 1999), pois há uma mistura de espécies pioneiras/colonizadoras, espécies sucessionais intermediárias e espécies climáticas na comunidade (Begon *et al.* 2006) (Figura 3). Em baixas taxas de ocorrência de distúrbios, as espécies mais competitivas monopolizam os recursos. De fato, estudos anteriores observaram que eventos *El Niño* aumentam a descarga de água doce no estuário e esse efeito torna-o mais rico em espécies de peixes, devido ao incremento daquelas provindas dos ambientes dulcícolas (Garcia *et al.* 2003; 2004). A riqueza trófica também aumenta com esses eventos moderados de perturbação, com a entrada de guildas tróficas (herbívoros, insetívoros, piscívoros), as quais são usualmente ausentes no estuário (Possamai *et al.* 2018). Ainda, com novas espécies provindas de água doce, é plausível imaginar que novos recursos estejam disponíveis para os consumidores estuarinos, e que esses possam utilizar esses recursos alóctones durante os períodos de perturbação do ecossistema (Garcia *et al.* 2019b). Esses fatos mostram como alterações nos fatores ambientais podem causar efeitos na estruturação trófica estuarina, bem como em atributos ecossistêmicos (e.g. comprimento da cadeia alimentar, taxas de produção primária, entre outros).

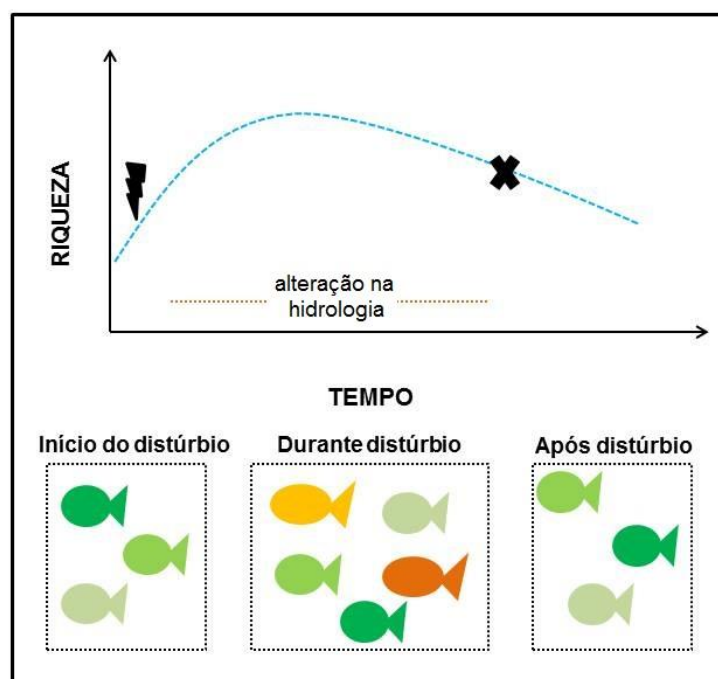


Figura 3. Exemplo hipotético de sucessão em um estuário. Durante a perturbação a riqueza é máxima, pois diferentes espécies com estratégias distintas de colonização habitam o ambiente. Os símbolos 'raio' e 'x' indicam o início e o fim da perturbação, respectivamente. Adaptado de Begon *et al.* (2006).

Aliado aos efeitos em nível ecossistêmico, efeitos em nível de população também podem ocorrer em função da variabilidade ambiental, e assim refletir na comunidade biológica. A plasticidade alimentar dos consumidores é uma característica que acarreta em variabilidade trófica na comunidade (Gerking 1994). É comum observarmos tal plasticidade em organismos onívoros, os quais possuem a capacidade de se alimentar com recursos tanto de origem vegetal como animal. Dessa forma, respondem mais favoravelmente às alterações na disponibilidade de recursos alimentares e na assimilação dos nutrientes de diferentes fontes de alimentos (Zandonà *et al.* 2015). Por exemplo, Zandonà *et al.* (2015) mostraram que o peixe *Poecilia reticulata* consome uma maior quantidade de invertebrados na estação úmida provavelmente devido à maior disponibilidade desse recurso. Porém, com a queda na disponibilidade de invertebrados na seca, a espécie passa a se alimentar mais de detritos, demonstrando plasticidade alimentar frente às variações sazonais na disponibilidade de recursos. Espécies detritívoras também demonstram variação sazonal na dieta em decorrência de mudanças na disponibilidade de detritos. Cardona (2001) observou que

quando os detritos orgânicos foram mais escassos no sedimento, as espécies detritívoras analisadas pelo autor passaram a consumir maior quantidade de diatomáceas e algas filamentosas. Isso demonstra que distúrbios nos fatores ambientais causam efeitos nas mais diferentes escalas de organização trófica, desde indivíduos até atributos ecossistêmicos.

O comprimento da cadeia alimentar é um dos mais importantes atributos dos ecossistemas, pois definem a estabilidade das comunidades (MacArthur 1955) e o funcionamento dos ecossistemas (Pimm 1982). Esse atributo representa o número de trocas unidirecionais de matéria e energia que ocorre via alimentação. Diversas são as discussões a respeito do que influencia os diferentes comprimentos de cadeia alimentar dentro dos ambientes, e dentre as razões mais discutidas estão a produtividade (Elton 1927; Lalli e Parsons 1996; Post *et al.* 2000), o tamanho do hábitat (Hoeinghaus *et al.* 2008; Saigo *et al.* 2015), a riqueza de espécies (Paine 1966; Ishkawa *et al.* 2017), e a dinâmica do ambiente (Saigo *et al.* 2015; Ruiz-Cooley *et al.* 2017). Como mencionado anteriormente o *El Niño* é um fenômeno global que resulta em perturbações climáticas em diferentes partes do mundo (Kim e Kang 2000; Lehman 2000; Escribano *et al.* 2004; Odebrecht *et al.* 2017), incluindo efeitos na riqueza de espécies e dinâmica do ambiente em estuários. Nesse contexto, é importante avançar no entendimento de como esse fenômeno e seus efeitos ambientais podem afetar a estrutura trófica de comunidades estuarinas, incluindo (i) efeitos sobre a composição e abundância de guildas tróficas, (ii) alteração na assimilação de recursos alóctones e (iii) possíveis variações em atributos do ecossistema como o comprimento de cadeia alimentar. Sendo um fenômeno de abrangência global que pode se tornar mais frequente e intenso num cenário de aquecimento global (Timmermann *et al.* 1999; Yeh *et al.* 2009), gerar informações dos efeitos do *El Niño* na biota pode auxiliar numa melhor avaliação de impactos de mudanças climáticas nos ecossistemas estuarinos a nível global.

Hipóteses foram formuladas para investigação na presente tese, as quais são explicitadas a seguir de acordo com os capítulos.

1.1. Hipóteses

Capítulo I

- I) H₁: Haverá um aumento na diversidade trófica estuarina em decorrência da modificação das variáveis ambientais em períodos de *El Niño*.
- II) H₁: Haverá um aumento na abundância de peixes onívoros em anos de *El Niño*, uma vez que sua maior plasticidade alimentar permite maior adaptabilidade às flutuações ambientais ocasionadas por esse evento.
- III) H₁: A guilda trófica de detritívoros aumentará sua abundância nos períodos de *El Niño* devido à maior disponibilidade de detritos carregados para o estuário nos períodos mais chuvosos.

Capítulo II

- IV) H₁: Mudanças sazonais e interanuais nas razões de carbono de consumidores estuarinos são associadas com variáveis locais (salinidade, temperatura) e regionais (pluviosidade, vazão).
- V) H₁: Haverá um aumento na assimilação de fontes alimentares alóctones provindas de água-doce pelos consumidores estuarinos durante episódios de alta pluviosidade e vazão dos rios.

Capítulo III

- VI) H₁: A posição trófica dos consumidores estuarinos irá ser alterada em razão dos distúrbios hidrológicos no estuário.
- VII) H₁: Durante períodos de *El Niño*, com o aumento do aporte de água doce no estuário e consequente aumento na riqueza de espécies, o comprimento da cadeia alimentar da comunidade aumentará.

1.2. Objetivos

- Investigar as relações entre a variabilidade interanual das guildas tróficas de peixes de áreas rasas estuarinas com eventos *El Niño* e fatores abióticos locais (p.ex. temperatura e salinidade) e regionais (p.ex. descarga continental);
- Descrever possíveis variações interanuais na intensidade dos elos tróficos de peixes estuarinos dominantes nas áreas rasas estuarinas com fontes de carbono no estuário e nos sistemas adjacentes;
- Analisar possíveis variações na intensidade dos elos tróficos de peixes de áreas rasas com os efeitos ambientais induzidos por eventos *El Niño*;
- Identificar o melhor método para a definição de linha de base isotópica (*isotopic baseline*) em estimativas de posição trófica;
- Averiguar possíveis variações interanuais na posição trófica de peixes dominantes nas zonas rasas do estuário e suas relações com flutuações abióticas locais (p.ex. salinidade) induzidas por eventos *El Niño*;
- Identificar alterações no comprimento da cadeia alimentar da comunidade de águas rasas estuarina e relacioná-las às variações hidrológicas e efeitos abióticos relacionados ao fenômeno *El Niño*.

2. MATERIAIS E MÉTODOS

2.1. Área de estudo

A Lagoa dos Patos (Fig. 4) possui uma área de 10.227 km², estendendo-se por cerca de 270 km de norte a sul, onde encontra-se com o Oceano Atlântico, formando uma porção estuarina. O estuário da Lagoa dos Patos (ELP) compreende uma área de 971 km² (Fig. 4), entre as latitudes 30°30'S e 32°12'S, no sul do Brasil. O encontro com o oceano se dá por um canal de 20 km de comprimento, que varia de 3 km de largura até 0,5 km na extremidade mais próxima ao oceano (Asmus 1998). Assim, devido ao formato afunilado, a água marinha tem sua entrada reduzida e a influência da água doce é maior no estuário, recebendo de 700 a 3000 m³/s de descarga da porção norte (Garcia 1998). A maré tem pouca influência no ELP (~0,5 m), sendo a circulação estuarina ditada pelo regime de ventos, especialmente dos quadrantes NE-SW. O ELP sofre influência dos eventos ENOS, sendo que a fase quente (*El Niño*) contribui para o

aumento na pluviosidade e consequente aumento na descarga de água doce, alterando a circulação e os processos de salinização estuarinos (Möller e Fernandes 2010).

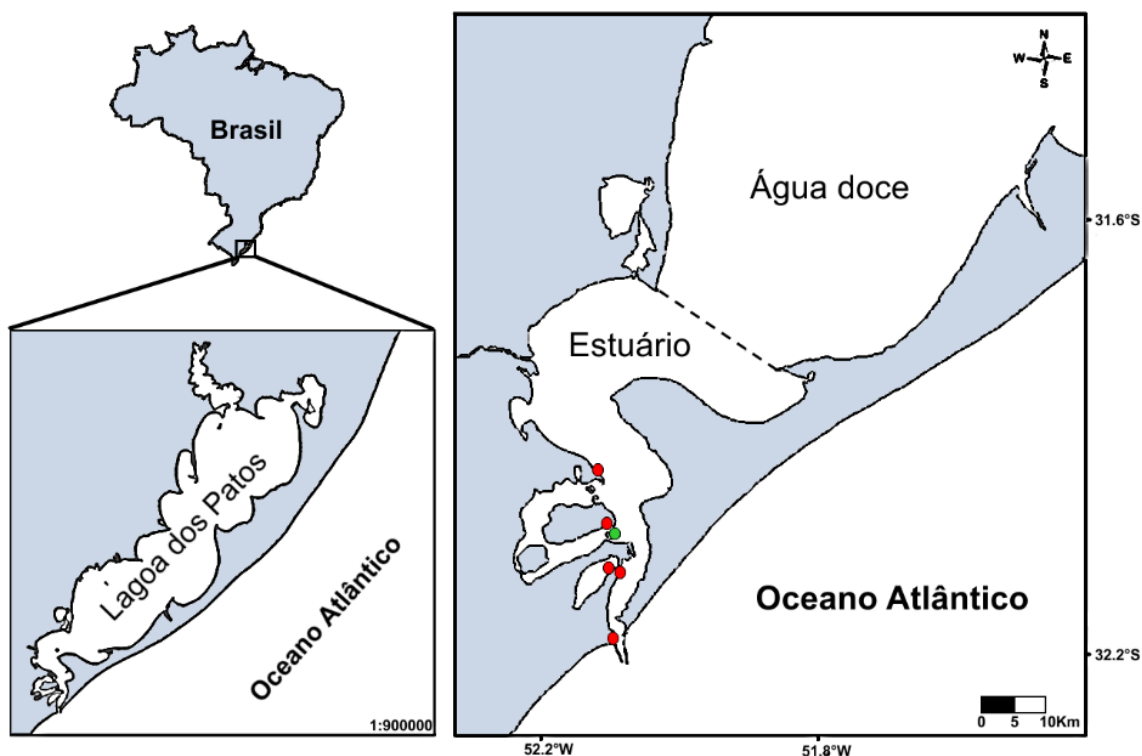


Figura 4. Mapa da área de estudo. À direita está a localização da Lagoa dos Patos dentro do território brasileiro; à esquerda o mapa do estuário da Lagoa dos Patos com os respectivos pontos amostrais (em vermelho os pontos amostrais do capítulo I e em verde o ponto amostral estuarino dos capítulos II e III).

As margens do estuário são ocupadas por marismas com cerca de 70 espécies de plantas, sendo as mais comuns *Spartina alterniflora*, *S. densiflora*, *Scirpus olneyi*, *S. maritimus* e *Juncus effusus* (Costa 1998). A flora submersa é composta por microalgas, fanerógamas submersas e macroalgas de deriva, enquanto a fitoplânctônica é dominada por maior biomassa de diatomáceas, cianobactérias e dinoflagelados, sendo essas principalmente células de origem marinha (Odebrecht *et al.* 2010). Em estações chuvosas com consequente diminuição da salinidade, as diatomáceas e cianobactérias de água doce e salobra passam a dominar a comunidade fitoplânctônica do estuário (Odebrecht *et al.* 2010). Com influência do *El Niño*, a descarga de água doce e o aporte de nutrientes aumentam, elevando o consumo desses nutrientes pelas diatomáceas. Assim, ocorre maior crescimento de cianobactérias fixadoras de N_2 , aumentando suas densidades. Já em regiões do estuário com maior influência de águas marinhas e maior

transparência da água, a produção das microalgas bentônicas aumenta (Odebrecht *et al.* 2010). As fanerógamas e macroalgas são dependentes do gradiente salino e da luminosidade do estuário. A espécie mais abundante de fanerógama é a *Ruppia maritima*, enquanto as de macroalgas são várias espécies de *Ulva* (*U. clathrata*, *U. intestinalis*, *U. ramulosa*, *U. flexuosa* e *U. micrococa*), além de *Cladophora* sp. e *Rhizoclonium riparium* (Copertino e Seeliger 2010). Com o aumento de macroalgas, a cobertura de fanerógamas decresce devido à diminuição da luminosidade que alcança o substrato. Isso pode refletir em diversos compartimentos da fauna, pois os fundos vegetados aumentam a complexidade estrutural de habitats de áreas rasas (Copertino e Seeliger 2010) gerando microhabitats para diversas espécies de peixes e invertebrados. Os macroinvertebrados bentônicos mais representativos do ELP são os crustáceos decápodes *Neohelice granulata*, *Callinectes sapidus* e *Palaemonetes argentinus* (Bemvenuti e Colling 2010). A ictiofauna nas zonas rasas do ELP é composta por cerca de 100 espécies (Vieira *et al.* 1998), sendo dominantes as estuarino residentes *Atherinella brasiliensis*, *Jenynsia multidentata* e juvenis de espécies marinhas, especialmente *Mugil liza*, *M. curema* e *Brevoortia pectinata* (Vieira *et al.* 2010). A fauna e a flora do ELP sofrem grandes influências dependendo do regime hidrológico, alterando a dominância e composição de espécies em todos os compartimentos da comunidade (Odebrecht *et al.* 2010).

2.2. Amostragens

A tese está inserida no programa Pesquisas Ecológicas de Longa Duração (PELD), sítio 8 – Estuário da Lagoa dos Patos e costa marinha adjacente (mais informações: peld.furg.br). Dentro desse projeto, diversas linhas de pesquisas são executadas, sendo a tese inserida em duas vertentes: pesquisas de monitoramento da ictiofauna de águas rasas (desde 1997 até o presente), e pesquisas com isótopos estáveis (desde 2010 até o presente) visando caracterizar relações tróficas nas zonas rasas do ELP, bem como a conectividade trófica com ambientes adjacentes. A seguir, as amostragens e os métodos específicos de cada capítulo serão explicitados.

2.2.1. *Capítulo I: Variabilidade temporal de guildas tróficas de peixes e suas relações com eventos El Niño em um estuário subtropical.*

A ictiofauna de águas rasas (< 1,5 m) foi amostrada mensalmente nos cinco pontos do estuário (Fig. 4) entre 1997 e 2015, com arrastos de praia (rede picaré de 9 m, malha entrenós adjacentes 13 mm nas asas e 5 mm no centro). As espécies foram identificadas, contabilizadas e categorizadas em guildas tróficas (adaptadas de Elliott *et al.* 2007) a partir de revisão bibliográfica (Anexo 4 – Tabela S1 do Anexo 1). As guildas tróficas utilizadas foram: Detritívoros (DTV), Herbívoros de Fitoplâncton (HVF), Herbívoros de Macroalgas e Macrófitas (HVM), Insetívoros (ISV), Onívoros (OMN), Piscívoros (PSV), Zoobentívoros (ZBV) e Zooplactívoros (ZPL).

Concomitantemente com as amostragens biológicas, dados de salinidade, transparência (m) e temperatura da água (°C) foram amostrados. A pluviosidade (mm) e vazão (m³/s) foram cedidos pela Agência Nacional de Águas (ANA) (80 estações pluviométricas e seis estações para vazão na bacia de drenagem da Lagoa dos Patos), e os valores do Oceanic Niño Index (ONI) foram retirados do banco de dados *online* da *National Oceanic and Atmospheric Administration* (NOAA). O ONI é uma medida da anomalia trimestral na temperatura da superfície do mar na região Niño 3.4 (5°N-5°S, 120°-170°W), sendo que valores acima de 0,5 caracterizam a ocorrência de *El Niño*. No período estudado ocorreram dois eventos de *El Niño* classificados como "muito forte" (1997-1998 e 2015-2016), com valores de ONI ≥ 2 e dois classificados como "moderado" (2002-2003 e 2009-2010), com valores de ONI entre 1 e 1,4 em pelo menos três meses consecutivos (Golden Gate Weather Services 2017).

Para apresentação gráfica das variáveis abióticas foram calculadas as anomalias mensais das mesmas. Para tanto, foram computadas médias mensais para cada variável (usando os dados de 1997 a 2015) e, posteriormente, subtraídas do valor observado para cada mês. Uma Análise de Correspondência Canônica (CCA) foi realizada relacionando a matriz de abundância de guildas tróficas com a matriz de variáveis abióticas. A vazão não foi utilizada para essa análise por não terem sido obtidos os dados dessa variável no ano de 2015. Uma Análise de Variância permutacional (ANOVA) foi realizada a fim de verificar a significância de cada eixo da CCA e o eixo de maior explicabilidade foi escolhido. Desse eixo, foram retirados os *scores constrained*, os quais sumarizavam as

variáveis abióticas, e a partir de correlações de Spearman, a abundância das guildas tróficas foi relacionada com os *scores*. Todas as análises estatísticas foram realizadas no software R 3.3.1 com $\alpha=0,05$ (R Core Team 2018).

2.2.2. *Capítulo II: Variabilidade nos fluxos de água doce afetam a importância relativa de fontes alóctones assimiladas por peixes estuarinos.*

Fontes primárias de carbono e consumidores estuarinos foram amostrados em uma enseada rasa (Fig. 4) sazonalmente desde 2010 até 2016. As fontes estuarinas utilizadas como “fontes autóctones” foram matéria orgânica particulada em suspensão (MOP) e plantas de marisma (*Juncus acutus*, *Spartina alterniflora* e *S. densiflora*). Somente plantas C_4 foram avaliadas pois estudos anteriores têm demonstrado que a contribuição de plantas C_3 para esses consumidores estuarinos é negligenciável (Claudino *et al.* 2013; Lanari *et al.* 2018). O MOP foi obtido filtrando-se 0,25 a 1 litro de água em filtros de fibra de vidro Whatman (1,2 μm). As plantas de marisma foram coletadas manualmente com auxílio de tesoura e os peixes por arrasto de praia (malhas de 13 mm entrenós adjacentes nas asas e 5 mm no centro). As fontes alóctones foram obtidas por amostragens de MOP na região dulcícola da Lagoa dos Patos (primavera de 2009, verão de 2010 e inverno de 2011) e na região marinha adjacente (primavera de 2014).

Em laboratório, as amostras foram lavadas em água destilada e secas em estufa a 60° por 48 h. Após secagem, as amostras foram maceradas, pesadas (10^{-3}g), armazenadas em cápsulas de estanho ultra-puras (Costech, Valencia, CA) e enviadas ao *Analytical Chemistry Laboratory, University of Georgia* nos EUA, para determinação das razões isotópicas de carbono e nitrogênio. O padrão de carbono utilizado foi *PeeDee Belemnite* e do nitrogênio o ar atmosférico. O desvio padrão das referências internas do laboratório para carbono foram de 0,07‰ e 0,06‰ para amostras animal e vegetal respectivamente, e para nitrogênio de 0,15‰ e 0,37‰ para amostras animal e vegetal, respectivamente.

Ainda no programa PELD, contamos com amostragens mensais de fitoplâncton e clorofila- α no mesmo sítio amostral realizadas pelo Laboratório de Fitoplâncton e Microorganismos Marinhos da FURG. A abundância e composição do fitoplâncton foram analisadas pelo método de sedimentação de Utermöhl (Utermöhl 1958) seguindo

as recomendações de Hasle (1978) (detalhes em Haraguchi *et al.* 2015). A clorofila- α foi extraída com acetona 90% v/v no escuro e à baixa temperatura (-12°C), e sua concentração determinada fluorometricamente com um Turner TD700 calibrado (maiores detalhes ver Abreu *et al.* 2010). Transparência da água (cm) foi obtida por Disco de Secchi e a salinidade e temperatura ($^{\circ}\text{C}$) por um termosalinômetro. Médias mensais de pluviosidade (mm) foram obtidas do Instituto Nacional de Meteorologia (INMET) em quatro estações nas margens da Lagoa dos Patos e as médias mensais de vazão dos rios foram fornecidas pela Agência Nacional de Águas (ANA) em nove estações da bacia de drenagem do ELP.

Todos os parâmetros mensais foram agrupados em médias sazonais (Verão: dezembro a fevereiro; Outono: março a maio; Inverno: junho a agosto; Primavera: setembro a novembro). Os peixes foram classificados nas guildas zooplancívoros ou detritívoros seguindo classificação do capítulo I (Possamai *et al.* 2018). Dados ambientais foram sumarizados através de estações e anos por Análise de Componentes Principais (PCA), padronizando-se os dados (z-transformados) e avaliando-se a sua multicolinearidade pelo Fator de Inflação da Variância. O método *Broken-Stick* foi utilizado para seleção dos eixos mais relevantes. Modelos lineares de efeitos mistos foram calculados para cada guilda trófica separadamente para identificar quais variáveis predizem a variação do $\delta^{13}\text{C}$. Devido ao sistema ser caracterizado por forte sazonalidade e variabilidade interanual, ‘ano’ e ‘estação’ (aninhada em ‘ano’) foram incluídas como fatores aleatórios para controlar a variação temporal nas variáveis ambientais. A seleção do melhor modelo foi realizada pelo critério de AIC.

Para avaliar a contribuição de fontes alóctones e autóctones aos consumidores estuarinos utilizamos o modelo Bayesiano de mistura isotópica contido no pacote MixSIAR (Bayesian Mixing Models in R) (Stock e Semmens 2016), a partir de um modelo hierárquico com dois fatores (‘estação’ e ‘ano’), sendo que ‘estação’ foi aninhada em ‘ano’. A mediana de 50% e o intervalo de 95% de credibilidade Bayesiano foram utilizados para sumarizar a contribuição das fontes para cada guilda trófica. O fracionamento isotópico difere entre herbívoros/detritívoros e carnívoros (Bastos *et al.* 2017), portanto utilizamos $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$ de $0,54 \pm 0,53$ e $4,78 \pm 1,30$ para detritívoros, e $1,08 \pm 0,53$ e $6,04 \pm 1,00$ para zooplancívoros, respectivamente (adaptado de Bastos *et al.* 2017). A mediana da contribuição alóctone foi correlacionada com as variáveis

ambientais por correlações de Spearman. Aplicamos simulações no Polígono de Mistura Isotópica para determinar se as fontes alimentares selecionadas poderiam explicar com confiabilidade (95%) a variabilidade isotópica ($\delta^{13}\text{C}$ - $\delta^{15}\text{N}$) dos consumidores (Smith *et al.* 2013) (Anexos 6 e 7 – Figuras S1 e S2 do Anexo 2). Todas as análises foram realizadas com $\alpha=0,05$ utilizando-se o software R versão 3.5.1 (R Core Team 2018).

2.2.3. Capítulo III: Fatores ambientais induzindo a variabilidade interanual do comprimento da cadeia alimentar estuarina de águas rasas.

O banco de dados utilizados para esse trabalho é uma extensão (2010 a 2018) do banco de dados do capítulo anterior. Portanto, os métodos amostrais e de processamento laboratorial para análise isotópica foram os mesmos descritos anteriormente. Além de MOP e plantas de marismas C_4 (*Spartina alterniflora* e *S. densiflora*), macroalgas (*Rizochlonium* sp. e *Ulva* sp.) e grama marinha (*Ruppia maritima*) foram coletadas manualmente com auxílio de tesoura. A matéria orgânica no sedimento (MOS) foi amostrada coletando-se os primeiros dois centímetros do sedimento com um *corer* (10 cm de diâmetro). Com a rede de arrasto foram amostrados peixes, siris e camarões, enquanto que os caranguejos terrestres foram coletados próximos às marismas manualmente (lista de espécies no Anexo 15 – Tabela S5 do Anexo 3).

Para estimar a posição trófica, testamos quatro métodos para selecionar a linha de base isotópica (*isotopic baseline*) que melhor representasse a comunidade estuarina em cada ocasião: (i) média global ($\pm$ desvio padrão) dos valores de $\delta^{15}\text{N}$ de todas as fontes coletadas entre 2010 e 2018 (método ‘Global’); (ii) média ($\pm$ desvio padrão) dos valores de $\delta^{15}\text{N}$ das fontes coletadas simultaneamente com os consumidores (método ‘Concomitante’); (iii) média ($\pm$ desvio padrão) dos valores de $\delta^{15}\text{N}$ das fontes coletadas na estação anterior à coleta dos consumidores (método ‘Atrasado’) e; (iv) média ($\pm$ desvio padrão) dos valores de $\delta^{15}\text{N}$ das fontes coletadas na mesma estação e na estação anterior aos consumidores (método ‘=Interpolação’). Para cada método (exceto o ‘Global’), testamos as diferenças entre os valores de $\delta^{15}\text{N}$ das fontes pelo teste de Tukey HSD para cada estação (Anexos 11 e 12 – Tabelas S1 e S2 do Anexo 3). Quando o teste distinguiu dois grupos, ambos foram utilizados como *baselines* distintas para as

estimativas de posição trófica. As fontes e *baselines* utilizadas para cada método são encontradas nos anexos 11, 12 e 13 (Tabelas S1, S2 e S3 do Anexo 3).

Para decidir o método de *baseline* mais apropriado, classificamos primeiramente as espécies em guildas tróficas (Possamai *et al.* 2018 para peixes; Collins 1999, Oliveira *et al.* 2006, Abreu *et al.* 2007 e Bemvenuti e Colling 2010 para crustáceos). As guildas utilizadas foram detritívoros (DTV), herbívoros (HBV), onívoros (OMN), zooplancívoros (ZPL), zoobentívoros (ZBV) e piscívoros (PSV). Após classificação, ordenamos as guildas de acordo com o aumento esperado de nível trófico desde a base até o topo da cadeia alimentar, seguindo os dados de nível trófico das espécies encontrados no FishBase (Froese e Pauly 2019) (Anexo 14 – Tabela S4 do Anexo 3). A partir desses dados, criamos um modelo linear: $NT = 0,426x + 1,534$; em que NT é o nível trófico e x é a posição da guilda trófica (1 para DTV, 2 para HBV, 3 para OMN, 4 para ZPL, 5 para ZBV e 6 para PSV). Conceitualmente, esse modelo visa representar o aumento esperado no nível trófico de guildas posicionadas desde a base da cadeia (DTV) em direção ao topo (PSV). Finalmente, escolhemos dentre os quatro métodos de *baseline* aquele que melhor se ajustou ao modelo linear esperado.

As estimativas de posição trófica foram realizadas separadamente para cada estação utilizando o pacote *tRophicPosition* (Quezada-Romegialli *et al.* 2018) do *software* R. Esse método permite o uso de duas *baselines*, as quais incluem também seus valores isotópicos de carbono no modelo para tornar as estimativas de posição trófica mais acuradas. Considerando às diferenças no fracionamento isotópico de acordo com taxas metabólicas e posição na cadeia trófica (Olive *et al.* 2003), os fracionamentos utilizados foram $\delta^{13}C = 0,54 \pm 0,53$ e $\delta^{15}N = 3,02 \pm 0,47$ para onívoros, zooplancívoros e zoobentívoros; $\delta^{13}C = 0,54 \pm 0,53$ e $\delta^{15}N = 4,78 \pm 1,30$ para herbívoros e detritívoros e; $\delta^{13}C = 0,82 \pm 0,47$ e $\delta^{15}N = 1,77 \pm 0,30$ para piscívoros (detalhes em Bastos *et al.* 2017).

As possíveis relações entre os atributos ecológicos riqueza de espécies (= número de espécies), riqueza trófica (número de guildas tróficas) e posição trófica (posição trófica de cada espécie) e os fatores ambientais foram avaliados através de correlações de Spearman. A posição trófica máxima de cada estação foi utilizada como comprimento de cadeia para as análises (ver Post *et al.* 2000), e um modelo linear foi realizado para avaliar a resposta das variáveis ambientais à variação no comprimento de cadeia. As variáveis explicativas foram transformadas em unidades de variância. Um

modelo linear generalizado (GLM) foi utilizado (distribuição Gamma com função de ligação inversa) para avaliar as variáveis ambientais que melhor explicam as variações nas posições tróficas dos consumidores. A variável resposta foi logaritimizada para melhor ajuste na distribuição Gamma. Todas as análises foram efetuadas com $\alpha=0,05$ no *software* R versão 3.5.3 (R Core Team 2019).

3. RESULTADOS E DISCUSSÃO

3.1. Capítulo I: Variabilidade temporal de guildas tróficas de peixes e suas relações com eventos *El Niño* em um estuário subtropical.

Os eventos *El Niño* foram associados às maiores chuvas e descargas fluviais, bem como menores salinidades e transparência da água no estuário. A CCA demonstrou que as guildas de DTV, OMN e ZPL foram associadas aos períodos sem a ocorrência de eventos *El Niño*, enquanto que as guildas HVM, HVF, ISV e PSV foram associadas com os períodos de *El Niño*. Esses resultados corroboraram a hipótese I. O aumento de herbívoros (macroalgas, macrófitas e fitoplâncton) durante os eventos *El Niño* pode dever-se ao fato de que com aumento da descarga fluvial, as vegetações que compõem o sistema dulcícola puderam ser carregadas para o estuário aumentando suas abundâncias (Copertino e Seeliger 2010; Garcia *et al.* 2017). Juntamente, os peixes herbívoros do ambiente límnic também teriam sido carregados e, esse cenário de aumento de vegetação e diminuição de salinidade do estuário propicia a permanência dessa guilda trófica durante os eventos *El Niño*. Insetívoros não são comuns em estuários, porém com o aumento da vazão fluvial, o estuário aumenta sua área lateral, alagando banhados e conectando-se com esses ambientes dulcícolas onde a disponibilidade de insetos tende a ser maior e favorece a ocorrência de peixes da guilda ISV.

Contrariamente às hipóteses II e III, OMN e DTV diminuíram suas abundâncias em decorrências dos fatores induzidos pelo *El Niño*. Gramas marinhas são importantes fontes alimentares para os onívoros (Garcia *et al.* 2016), porém durante períodos de alta descarga de água doce, a cobertura de gramas marinhas tende a diminuir drasticamente no estuário (Copertino e Seeliger 2010), e isso pode ter influenciado na abundância e recrutamento das espécies onívoras. Já em relação aos DTV, parece mais provável que

as forçantes ambientais hidrodinâmicas, como o excesso de chuvas e a elevada descarga fluvial, influenciaram mais que a disponibilidade de alimento. As principais espécies compondo essa guilda trófica foram as tainhas (*M. curema* e *M. liza*), que são organismos marinhos que se utilizam do estuário como berçário (Lemos *et al.* 2014). O aumento da vazão nos períodos de atuação do *El Niño* dificulta a entrada dos juvenis de tainha do mar para o estuário, principalmente devido ao formato afunilado do mesmo, que favorece a descarga de água doce (Garcia *et al.* 2004). Portanto, o recrutamento dessas espécies detritívoras no interior do estuário foi menor ou mesmo atrasado durante os eventos de *El Niño*.

Concluindo, foi possível verificar a influência de eventos *El Niño* com intensidades mais fortes nas variáveis ambientais no ELP, sendo que o fenômeno aumenta a pluviosidade da região, gerando aumento da descarga continental no estuário, diminuição da sua salinidade e transparência da água. Essa modificação ambiental acaba refletindo nos padrões de composição e dominância das guildas tróficas da ictiofauna das áreas rasas, transportando espécies de água doce para as áreas estuarinas e assim aumentando a diversidade trófica do estuário. As guildas alimentares dominantes no estuário são detritívoros, onívoros e zooplancívoros, de forma que os efeitos indiretos do *El Niño* diminuem a abundância dessas guildas e aumentam a abundância de insetívoros, piscívoros e herbívoros. Os efeitos dessas modificações na teia alimentar estuarina (controles do tipo *bottom-up* e *top-down*), bem como nas relações de competição e predação não puderam ser analisados, mas as hipóteses formuladas para os padrões observados poderiam ser avaliadas em estudos futuros, preferencialmente combinando monitoramento em campo e manipulação de variáveis em experimentos. O monitoramento de longo prazo desses componentes tróficos por meio de banco de dados de conteúdo estomacal e isótopos estáveis são cruciais para começarmos a entender a amplitude das modificações na estrutura trófica estuarina ocasionadas por fenômenos climáticos.

3.2. Capítulo II: Variabilidade nos fluxos de água doce afetam a importância relativa de fontes alóctones assimiladas por peixes estuarinos.

Os resultados corroboraram a hipótese IV, uma vez que a abundância de diatomáceas, cianobactérias e a salinidade foram importantes para o decréscimo do $\delta^{13}\text{C}$ dos detritívoros. Já para os zooplactívoros, o aumento na vazão fluvial e na abundância de cianobactérias relacionadas às baixas salinidades ($\rho = -0,21$, $p\text{-valor} = 0,012$) diminuíram os valores de $\delta^{13}\text{C}$. No sistema estudado, a descarga de água doce geralmente aumenta durante o inverno e a primavera e, em decorrência, maiores abundâncias de fitoplâncton são observadas (Abreu *et al.* 2010; Haraguchi *et al.* 2015). Portanto, esses resultados sugerem que pulsos sazonais na abundância fitoplanctônica são importantes preditores nas mudanças isotópicas de carbono em consumidores estuarinos. A variação nos valores de $\delta^{13}\text{C}$ das fontes e consumidores ao longo das estações/anos pode ser observado nos anexos 8 e 9 (Tabelas S1 e S2 do Anexo 2).

Com relação à origem das fontes de carbono assimiladas pelos consumidores estuarinos, as fontes autóctones foram as principais contribuintes para as espécies ao longo dos anos e estações. Porém, para os detritívoros, a contribuição de material marinho foi bastante variável ao longo dos anos (Anexo 10 - Tabela S3 do Anexo 2). Em alguns períodos, principalmente durante a primavera de 2014 e de 2016, a contribuição de material marinho excedeu (mais de 75%) a autóctone.

Surpreendentemente, durante períodos de maiores chuvas a contribuição marinha aumentou para esses consumidores. Esse padrão discorda da hipótese V, pois a assimilação de material de água doce foi a menor em todos os períodos. Esses detritívoros analisados (juvenis de *Mugil liza*) provavelmente adentram o estuário durante os períodos de inverno e primavera (Lemos *et al.* 2014). Durante sua estadia no mar, esses consumidores se alimentam de plâncton na coluna da água, e ao atingirem comprimentos entre 2 e 3 cm, adentram o estuário e começam a se alimentar de microfitobentos (Blaber e Whitfield 1977; Vieira *et al.* 2008; Cardona 2016; Garcia *et al.* 2018; Oliveira *et al.* 2018). Eventos de grande descarga de água doce podem atrasar a entrada desses juvenis no estuário (Garcia *et al.* 2001; Vieira *et al.* 2008; Possamai *et al.* 2018). Portanto, esses indivíduos podem estar refletindo uma assimilação de matéria marinha relacionada a sua fase de permanência na região oceânica, pois seriam recém-

chegados no estuário e possivelmente não houve tempo para o *turnover* isotópico, que é de 68 e 60 dias para o carbono e nitrogênio, respectivamente (Oliveira *et al.* 2017).

O mesmo padrão foi encontrado para os zooplancívoros. Porém, esses consumidores são estuarino-residentes e não espécies que se reproduzem no oceano e recrutam para o interior do estuário (Bemvenuti 1987; Fávaro *et al.* 2003). Esses consumidores podem estar se afastando para zonas mais salinas perto da boca do estuário durante períodos de grande descarga de água doce, a fim de evitar as baixas salinidades (Garcia *et al.* 2001; Possamai *et al.* 2018). Desse modo, estariam refletindo assinaturas isotópicas de plâncton marinho ao invés do estuarino. Desse modo, a hipótese V também foi refutada para o caso dos zooplancívoros. Concluímos que os peixes estuarinos utilizam recursos alóctones, e que a hidrologia poderia influenciar a ocorrência e a intensidade dessa assimilação. Fatores não relacionados diretamente com a alta descarga fluvial, como a movimentação dos consumidores (do mar para o estuário e entre diferentes regiões do estuário), tempo de residência da água dentro do sistema, entre outros, devem ser considerados em estudos futuros.

3.3. Capítulo III: Fatores ambientais induzindo a variabilidade interanual do comprimento da cadeia alimentar estuarina de águas rasas.

O método de *baseline* que obteve o melhor ajuste ao modelo esperado ($r^2 = 0,99$) foi o ‘Atrasado’, sugerindo que os diferentes organismos possuem diferentes tempos de assimilação isotópica de seus recursos (Fry e Arnold 1982). Para peixes, o tempo de *turnover* no músculo é entre 2 e 3 meses (Mont’Alverne *et al.* 2016; Oliveira *et al.* 2017), correspondendo à janela temporal do método selecionado. Portanto, encorajamos a amostragem sazonal de fontes e consumidores, considerando as diferenças entre os tempos de assimilação isotópica de cada grupo de organismo para melhor estimativa das posições tróficas de consumidores.

A riqueza de espécies aumentou durante períodos de maior pluviosidade, bem como a riqueza trófica, concordando com trabalhos anteriores (Garcia *et al.* 2003; Garcia *et al.* 2004; Possamai *et al.* 2018). As posições tróficas dos consumidores variaram em função da pluviosidade, salinidade e descarga de rios, corroborando a hipótese VI. Entretanto, contrariando a hipótese VII, o comprimento da cadeia alimentar não aumentou em períodos mais chuvosos, mas sim mostrou um leve decréscimo. Isto contraria a hipótese

da produtividade (Post *et al.* 2000; Nelson *et al.* 2015), uma vez que a pluviosidade favorece o aumento da produtividade primária estuarina (Abreu *et al.* 2010; Haraguchi *et al.* 2015). Por outro lado, Lalli e Parsons (1996) demonstram que em ambientes marinhos a cadeia alimentar tende a ser mais curta em habitats mais produtivos. Predar em níveis baixos da cadeia trófica é mais vantajoso, uma vez que há mais recursos disponíveis (Pimm e Lawton 1977). Assim, nesses períodos de maior pluviosidade e provavelmente, maior disponibilidade de recursos, a competição é menor favorecendo menores comprimentos de cadeia alimentar (Elton 1927; Hoeinghaus *et al.* 2008; Ishikawa *et al.* 2017).

No geral, a comunidade de águas rasas mostrou-se estável, pois o comprimento da cadeia alimentar não sofreu grandes alterações com os distúrbios ambientais decorrentes do *El Niño*. Concluímos que as cadeias alimentares são variáveis, e que alterações moderadas nos fatores abióticos podem alterar levemente o comprimento da cadeia. Entretanto, como as comunidades buscam manter-se estáveis (MacArthur 1955), as alterações nas cadeias alimentares estuarinas são rapidamente contornadas para o estado anterior de estabilidade, demonstrando alta resiliência (ou seja, alta capacidade de retornar rapidamente ao estado original após perturbações) na estruturação trófica dessa comunidade.

DISCUSSÃO GERAL

Esta tese tratou da influência de variações nos fatores ambientais provenientes do fenômeno *El Niño* na estrutura trófica estuarina de águas rasas. No primeiro capítulo (vide versão completa no Anexo 1) tratamos da influência dessas variações ambientais na riqueza trófica estuarina, utilizando a classificação das espécies de peixes em guildas tróficas para avaliar tais efeitos. Já no segundo capítulo (vide versão completa no Anexo 2), verificamos se essas variações ambientais alteravam a razão entre contribuição de recursos autóctones e alóctones para esses consumidores estuarinos. No terceiro e último capítulo (vide versão completa no Anexo 3) verificamos se tais flutuações ambientais alteraram atributos ecossistêmicos, como o comprimento da cadeia alimentar.

As séries de dados analisados forneceram oportunidades distintas de avaliação dos efeitos de eventos *El Niño* nos fatores ambientais estuarinos. O capítulo I contou com 19 anos de dados mensais (1997 a 2015), contemplando quatro eventos *El Niños* em que dois são classificados como ‘muito forte’ (1997-1998 e 2015-2016) e dois como ‘moderados’ (2002-2003 e 2009-2010). Já os capítulos II (dados de 2010 a 2016) e III (dados de 2010 a 2018) contaram com um banco de dados que contemplou dois eventos: os *El Niños* de 2009-2010 e 2015-2016. Todas as análises corroboraram com resultados já descritos na literatura (Garcia e Vieira 2001; Garcia *et al.* 2004), em que durante esses eventos há aumento de chuva e consequente vazão dos rios que desaguam no estuário, tornando-o menos salino. Essas variáveis foram, portanto, os *proxies* utilizados na tese para avaliar os efeitos do *El Niño* na estrutura trófica estuarina. Entretanto vale ressaltar que houveram períodos de chuvas e vazão acima das médias sem a ocorrência de *El Niño* (p. ex. entre 2000 e 2001, durante 2014). Isso sugere futuras investigações para avaliar quais outros fenômenos climáticos desencadeiam essas alterações hidrológicas, e como podem estar afetando a biota.

No geral, analisando dois bancos de dados distintos podemos verificar que os efeitos induzidos pelo *El Niño* aumentaram a riqueza de espécies e a riqueza trófica. Garcia e Vieira (2001) e Garcia *et al.* (2004) já haviam descrito o aumento na riqueza de espécies em função dos efeitos do *El Niño* no estuário em decorrência da entrada de espécies dulcícolas. Essas mesmas espécies acabaram sendo as responsáveis pelo aumento da riqueza trófica, trazendo guildas tróficas novas para o estuário (como insetívoros), bem como aumentando a abundância de guildas mais raras no ambiente estuarino, como os herbívoros e piscívoros (Possamai *et al.* 2018). Em estuários, a fonte de alimento preponderante para os peixes são os organismos bentônicos, em especial nas latitudes médias, onde há menor ocorrência e diversidade de vegetação aquática submersa e, consequentemente, de peixes herbívoros quando comparado aos trópicos (Buchheister e Latour 2015; Copertino *et al.* 2016). Em contrapartida, no ambiente límnico há maior abundância de produtores (como macrófitas, fitobentos, perifíton), oferecendo maior nicho para herbívoros nesses ambientes. Devido ao aumento da descarga fluvial, as vegetações que compõem o sistema dulcícola provavelmente foram carregadas para o estuário e assim aumentaram suas abundâncias (Copertino e Seeliger 2010; Garcia *et al.* 2017). Esse carreamento da vegetação dulcícola no sistema estuarino propicia o uso do

mesmo pelas espécies herbívoras no período de *El Niño* e, teoricamente, essa energia provinda do ambiente de água doce entraria na cadeia alimentar estuarina. Entretanto, não foi o que detectamos pelos nossos modelos. Em eventos de maior pluviosidade, a contribuição de fontes marinhas aumentou para os consumidores estuarinos ao invés das fontes dulcícolas.

O zooplancívoro utilizado como modelo (o peixe-rei *Atherinella brasiliensis*) é uma espécie estuarino-residente (Bemvenuti 1987; Fávaro *et al.* 2003) e, portanto, podemos supor que esses indivíduos refletem uma assimilação de recursos no estuário e não nas áreas adjacentes. Contrariamente à hipótese V, esses consumidores estuarinos não assimilaram material proveniente da água doce durante os episódios de maior vazão dos rios para o estuário, mas sim houve um incremento na contribuição de material marinho. Durante os eventos *El Niño*, essa guilda alimentar diminuiu suas abundâncias no estuário (Possamai *et al.* 2018), o que pode ter ocorrido em decorrência das baixas salinidades. Garcia *et al.* (2001) identificaram um aumento de *A. brasiliensis* nas áreas mais salinas do estuário em períodos de *El Niño*. Esses consumidores podem estar habitando áreas na boca do estuário a fim de evitar as baixas salinidades impostas pelo aumento da vazão fluvial e assim, assimilam material estuarino-marinho, refletindo essa assimilação isotopicamente e, assim, contrariando nossa hipótese V inicial.

Em relação ao modelo detritívoro, as espécies utilizadas foram estuarino-dependentes (predominantemente a tainha *Mugil liza*), sendo que os adultos reproduzem no mar próximo à costa (Lemos *et al.* 2014) e os alevinos habitam as zonas costeiras alimentando-se de plâncton até atingirem entre 2 e 3 cm, quando adentram os estuários e passam a se alimentar de fitobentos e detritos (Blaber e Whitfield 1977; Vieira *et al.* 2008; Cardona 2016; Oliveira *et al.* 2018). Os detritívoros sofreram uma queda em suas abundâncias nos períodos de *El Niño* (Possamai *et al.* 2018), mostrando que apesar do maior aporte de detritos durante os eventos de maior descarga de água doce (Rozas Odum 1988; Netto e Lana 1999; Walker *et al.* 2013; Copertino *et al.* 2016; Campanyà-Llovet *et al.* 2017), esses consumidores não aumentam suas abundâncias no estuário e, aqueles que no estuário estão, não assimilam material de origem dulcícola. Deste modo, concluímos que para os detritívoros as forçantes ambientais hidrodinâmicas influenciam mais que a disponibilidade de alimento. Com o aumento da vazão nos períodos de atuação do *El Niño*, ocorre o impedimento da entrada dos juvenis de tainha para o

estuário, principalmente devido ao formato afunilado do mesmo, que aumenta a descarga de água doce (Garcia *et al.* 2004). Desse modo, os juvenis atrasam sua entrada no estuário, pois não contam com o auxílio da cunha salina (Garcia *et al.* 2001; Vieira *et al.* 2008) e, provavelmente, os indivíduos coletados eram recém-chegados que ainda estavam refletindo a assinatura isotópica marinha. Aliado a esse possível atraso na entrada dos juvenis ao estuário, há o aquecimento das águas costeiras do Atlântico Sul Ocidental durante o fenômeno de *El Niño* (Grimm *et al.* 2000). Os adultos de tainhas habitam a região marinha costeira, tendo seu pico reprodutivo com a queda das temperaturas das águas (Lemos *et al.* 2014). Assim, pode ser que em decorrência do evento *El Niño* as temperaturas não tenham diminuído o suficiente no período habitual, não oferecendo o gatilho ambiental necessário para a desova dos animais e também sendo outro motivo para o atraso da entrada de juvenis ao estuário.

Contrapondo a hipótese VII, o comprimento da cadeia alimentar não aumentou com os efeitos da maior descarga de água doce no estuário. Durante esses eventos a comunidade tornou-se mais rica em termos de identidade taxonômica e também troficamente (maior número de guildas tróficas distintas), e essas características propunham que o comprimento da cadeia iria aumentar (Paine 1966). Porém, essas variáveis não foram diretamente relacionadas, pois a adição de predadores pode não induzir diretamente ao aumento de cadeia, mas sim a um aumento de trocas energéticas (Hoeinghaus *et al.* 2008). O aumento na riqueza trófica deveu-se principalmente a organismos de base de cadeia, como herbívoros (Possamai *et al.* 2018), e isso levou a uma diminuição nas posições tróficas dos consumidores. Desse modo, o comprimento da cadeia alimentar, embora tenha variado entre 2,45 e 4,60 ao longo dos 9 anos analisados, permaneceu de modo geral estável (média $\pm$ desvio padrão = 3,34 $\pm$ 0,67).

A guilda trófica com maior número de espécies foi a de zoobentívoros, compondo varias espécies dulcícolas, estuarinas e marinhas. Os estuários são conhecidos por prover uma abundância de organismos bentônicos e, portanto, é natural que essa guilda seja importante no contexto estuarino (McLusky 1990; Bemvenuti e Colling 2010; Buchheister e Latour 2015). De fato, os zoobentívoros foram os responsáveis pelo aumento da posição trófica da comunidade. Como em geral os zoobentívoros são organismos generalistas, mudanças na posição trófica são prováveis de ocorrer devido à variabilidade da dieta desses consumidores (Nelson *et al.* 2015).

Dentro da comunidade avaliada, esses organismos foram, de fato, os predadores de topo, uma vez que a abundância de piscívoros foi bastante baixa (Possamai *et al.* 2018). Apenas durante eventos de forte descarga de água doce, alguns poucos indivíduos piscívoros são trazidos do ambiente dulcícola (Possamai *et al.* 2018) e poderiam aumentar o comprimento da cadeia alimentar. Desse modo, o comprimento da cadeia alimentar foi mensurado pelos zoobentívoros, os quais são mais generalistas. A habilidade de se alimentar de diversas presas é uma vantagem quando a abundância das presas diminui (Elton 1927), e essa característica pode também causar decréscimo no comprimento das cadeias alimentares (Lindeman 1942). Porém, o que detectamos foi uma comunidade estável, que não alterou o comprimento médio da cadeia alimentar frente às perturbações hidrológicas. Estabilidade é um atributo desejável às comunidades biológicas, pois a resistência frente às perturbações pode significar sobrevivência (Elton 1927; MacArthur 1955; Begon *et al.* 2006), principalmente em ambientes tão dinâmicos quanto os estuários.

Por fim, uma ressalva deve ser feita quanto à extrapolação dos achados. Avaliamos apenas um atributo da comunidade (comprimento de cadeia) e ele se mostrou bastante estável e resiliente frente às mudanças hidrológicas ocasionadas pelo *El Niño*. Outros trabalhos já haviam sugerido a alta resiliência da fauna de peixes do estuário, com um retorno ao estado anterior de composição específica e dominância em aproximadamente 3 meses após os distúrbios causados pelo *El Niño* (Garcia *et al.* 2003, Garcia *et al.* 2004). Ainda, estuários são ambientes dinâmicos e portanto a biota desses sistemas está adaptada à enfrentar variabilidade nos fatores abióticos (McLusky 1990). Entretanto, devemos ter cautela com a generalização desses resultados para todo o ecossistema estuarino estudado, pois não avaliamos a produtividade primária, bem como perturbações crônicas, como poluição e perda de habitats. Mais atributos ecossistêmicos devem ser estudados para que possamos definir a estabilidade e resiliência dessa comunidade estuarina, bem como quais as perturbações que os alteram.

4. CONCLUSÃO

Em suma, foi possível verificar a influência de eventos *El Niño* nas variáveis ambientais no estuário da Lagoa dos Patos, sendo que o fenômeno aumenta a

pluviosidade da região, gerando aumento da descarga continental no estuário e diminuição da sua salinidade. Essa modificação ambiental aumenta a riqueza de espécies e reflete nos padrões de composição e dominância das guildas tróficas das áreas rasas estuarinas. Espécies de água doce são transportadas para o estuário aumentando a sua diversidade trófica. Ainda, esse aumento de água doce influencia na assimilação de recursos alóctones e na posição trófica dos consumidores estuarinos.

As guildas alimentares dominantes no estuário são detritívoros, onívoros e zooplancívoros, de forma que os efeitos indiretos do *El Niño* diminuem a abundância dessas guildas e aumentam a abundância de insetívoros, piscívoros e herbívoros. Esse aumento de herbívoros demonstra que as relações diretas que são comumente realizadas entre aumento de riqueza resultando em aumento de cadeia alimentar não são verdadeiras. O aumento da riqueza pode dever-se a espécies de base de cadeia alimentar e, portanto, tendo pouca ou nenhuma influência no comprimento da cadeia, mas possivelmente apenas nas trocas de energia.

Os eventos *El Niño* trouxeram espécies de peixes de água doce e, portanto, fontes de carbono dulcícolas para o estuário. Entretanto, ao contrário do esperado a assimilação de material alóctone de água doce não foi maior durante os períodos de maior descarga fluvial. Surpreendentemente, a assimilação de material marinho aumentou, mostrando que relações diretas não explicam a conectividade trófica entre o ambiente dulcícola e estuarino no sistema ecológico estudado. Os resultados demonstraram que o aumento de um atributo não reflete diretamente no aumento de outro, e muitos fatores devem ser levados em consideração ao tentar prever a resposta de espécies a determinados eventos. As comunidades biológicas buscam a estabilidade, então eventos que não sejam extremos ou que perdurem por determinado período tendem a não causar grandes efeitos negativos. A estrutura trófica e seus atributos são espelhos da estabilidade e funcionamento das comunidades biológicas e ecossistemas, e, portanto, podemos avaliar que essa comunidade estuarina de águas rasas é bastante resiliente aos distúrbios ocasionados pelo *El Niño*.

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ANEXO 1 (REFERENTE AO CAPÍTULO I)

Temporal variability (1997-2015) of trophic fish guilds and its relationships with *El Niño* events in a subtropical estuary

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ABSTRACT

Global climatic phenomena like *El Niño* events are known to alter hydrological cycles and local abiotic conditions leading to changes in structure and dynamics of terrestrial and aquatic biological communities worldwide. Based on a long-term (19 years) standardized sampling of shallow water estuarine fishes, this study investigated the temporal variability in composition and dominance patterns of trophic guilds in a subtropical estuary (Patos Lagoon estuary, Southern Brazil) and their relationship with local and regional driving forces associated with moderate (2002-2003 and 2009-2010) and very strong (1997-1998 and 2015-2016) *El Niño* events. Fish species were classified into eight trophic guilds (DTV detritivore, HVP herbivore-phytoplankton, HVM macroalgae herbivore, ISV insectivore, OMN omnivore, PSV piscivore, ZBV zoobenthivore and ZPL zooplanktivore) and their abundances were correlated with environmental factors. Canonical correspondence analysis revealed that less dominant (those comprising < 10% of total abundance) trophic guilds, such as HVP, HVM, ISV, PSV, increased their relative abundance in the estuary during higher rainfall and lower salinity conditions associated with moderate and very strong *El Niño* events. An opposite pattern was observed for the dominant trophic fish guilds like OMN and, at lesser extent, DTV and ZPL, which had greater association with higher values of salinity and water transparency occurring mostly during non-*El Niño* conditions. In contrast, ZBV's abundance was not correlated with contrasting environmental conditions, but rather, had higher association with samples characterized by intermediate environmental values. Overall, these findings show that moderate and very strong *El Niño* events did not substantially disrupt the dominance patterns among trophic fish guilds in the estuary. Rather, they increased trophic estuarine diversity by flushing freshwater fishes with distinct feeding habits into the estuary.

Keywords: ENSO, estuarine fish assemblage, feeding guilds, Long-Term Ecological Research (LTER), trophic structure, Patos Lagoon Estuary.

1. Introduction

The trophic structure of a group of organisms is defined as the all the feeding relationships that interconnect food and its consumers in a particular habitat or ecosystem (Pimm 1982). The trophic structure is usually represented by models or diagrams of food networks connecting species, but it can also be assessed by a more simplified approach based on the use of trophic guilds (Buchheister and Latour 2015; Konan *et al.* 2015). Trophic guilds aggregate species that utilize similar food resources, allowing synthesis of the complexity commonly observed in food webs, and a better understanding of trophic structure and dynamics in biological communities (Elliott *et al.* 1997; Winemiller and Layman 2005).

The trophic relationships in a community can be influenced by variations in environmental factors. For instance, short and long term changes in the freshwater inflow in estuaries can cause variation in the trophic organisation, either by the entrance of trophic subsidies (Nelson *et al.* 2015; Garcia *et al.* 2017) or by variations in the dominance (i.e., when the numerical abundance or biomass of a species or trophic guild is higher than others) patterns of the consumers (Livingston *et al.* 1997). One of these changes can be the addition of flooded areas on the estuary banks due to increased river flow, which provides terrestrial prey (such as insects) to the aquatic community. Such higher availability and accessibility of allochthonous prey may increase the trophic position of consumers (Wainwright *et al.* 1993; Nelson *et al.* 2015). Dominance shifts from carnivorous to herbivorous fishes in estuaries have been observed with the decrease of freshwater discharge, probably because the subsequently increase in salinity and water transparency favoured the growth of seagrasses and algae (Livingston *et al.* 1997).

The food plasticity exhibited by consumers, especially fishes, can also affect trophic relationships in biological communities (Gerking 1994). This plasticity is commonly observed in omnivores, which feed on both plant and animal resources and, theoretically, are expected to respond more favourably to changes in the availability of food resources (Zandonà *et al.* 2015). For instance, the omnivorous fish *Poecilia reticulata* changes its diet seasonally according to the availability of resources, shifting from a diet based on invertebrates in the wet season to a diet with predominance of detritus in the dry season (Zandonà *et al.* 2015). Detritivorous species can also change their diet seasonally according to changes in resource availability. When detritus in sediment are scarce, some detritivorous species may consume higher amounts of diatoms and filamentous algae (Cardona 2001).

Although the effects of environmental variability on the trophic structure of estuarine fish assemblages have been investigated, most works are based on short-term sampling focusing mainly on local factors (e.g., temperature, turbidity and salinity) (Konan *et al.* 2015; Nelson *et al.* 2015). However, many ecological processes and environmental phenomena occur on a long-term time scale or are cyclical processes, requiring several years or even decades of continuous monitoring and investigation to understand their influences on the biota (Franklin 1989; Lindenmayer *et al.* 2012).

The coupled ocean-atmosphere *El Niño* Southern Oscillation (ENSO) is a cyclical phenomenon with a strong impact on the global climate and biota (Glantz 2001; Fisher *et al.* 2015; Glynn *et al.* 2017). In terms of temperature anomalies of the sea surface in the central and eastern equatorial Pacific, ENSO is characterized by a warmer (*El Niño*) and a colder phase (*La Niña*). The 2015-2016 *El Niño* was one of the most intense on record, comparable to the 1997-1998 event, and even surpassing it in terms of temperature and pressure anomalies in some regions (Climate Prediction

Center/NOAA 2017). *El Niño* episodes, in particular, strongly influences climate and hydrology in southeast of South America (Grimm *et al.* 1998; Grimm *et al.* 2000). It can lead to positive rainfall anomalies that increase river discharges in Uruguay, Argentina and southern Brazil (Robertson and Mechoso 1997; Grimm 2009). Such higher river discharges triggered by *El Niño* events can increase freshwater inflow into estuaries and, subsequently, the input of continental detritus (Netto and Lana 1999). Relationships between higher freshwater discharge triggered by *El Niño* events and changes in diversity and abundance patterns of estuarine fish fauna has been already studied in southern Brazil (Garcia and Vieira 2001; Garcia *et al.* 2004). During these events, freshwater species are carried out into the estuary and the recruitment of marine and estuarine-dependent species declines, resulting in marked changes in relative abundance and diversity (Garcia and Vieira 2001; Garcia *et al.* 2004). However, no studies have investigated the effects of *El Niño*-induced hydrological changes on the trophic structure of estuarine fish assemblages.

Based on a long-term sampling (1997-2015) of the ichthyofauna and environmental variables in a subtropical estuary in Brazil, this study investigated relationships between indirect effects of moderate and strong *El Niño* events with the composition and dominance patterns of trophic fish guilds. The general hypothesis that guides this work is that estuarine trophic diversity will increase due to the change of environmental variables during *El Niño* periods. Additionally, we investigated two other hypotheses: (1) there will be an increase in the dominance of omnivorous fish in *El Niño* years, since their higher food plasticity allows greater adaptability to the environmental fluctuations caused by this event; (2) abundance of the detritivore guild will increase in *El Niño* periods due to the greater availability of detritus carried into the estuary by higher freshwater discharge.

2. Materials and Methods

2.1. Study area

Patos Lagoon (Fig. 1) covers an area of 10,227 km², extending over approximately 270 km from north to south, where it falls into the Atlantic Ocean, forming an estuarine portion. The Patos Lagoon estuary (PLE) comprises an area of 971km² (Fig. 1), located between latitudes 30°30'S and 32°12'S ° in southern Brazil. The connection to the ocean is provided by a 20-km long channel, ranging from 3 km to 0.5 km wide at the end nearest to the ocean (Asmus 1998). Thus, due to the funnel-shaped of this estuary, the entrance of sea water is reduced and the entrance of continental freshwater, which ranges from 700 to 3,000 m³ at the lagoon's northern portion, tends to be favoured (Garcia 1998). The tide has little influence on the PLE (~ 0.5 m), with an estuarine circulation dictated mainly by winds, especially NE-SW quadrants and hydrology patterns. The ENSO events influence the PLE, and the warm phase (*El Niño*) contributes to a rainfall increase in the basin and, consequently, to an increase in the freshwater discharge, changing the circulation and the estuarine salinization (Möller and Fernandes 2010).

2.2. Sampling and data analyses

Since 1997, we have been monthly sampling the ichthyofauna in shallow areas (<1.5 m) and measured environmental parameters (water temperature, transparency and salinity) in five sampling stations (Fig. 1) of Patos Lagoon Estuary (PLE). This long-term monitoring is conducted by the Brazilian Long-Term Ecological Research (LTER) program, which has been conducting long-term studies on its biota and the main driving forces influencing its structure and dynamics (see Odebrecht *et al.* 2017 for an update review on these researchers). Fish are sampled with a 9 m beach seine with size meshes

(adjacent internodes) of 13 mm in the wings and 5 mm in the centre. At each one of the five sampling sites, we conducted five beach seine hauls each month. The seining hauls were conducted in open estuarine beaches with sand as the dominant fraction in the sediment (Calliari *et al.* 1977; Moura *et al.* 2012) and over unobstructed bottoms (i.e., without seagrasses meadows, calcareous algae, oyster banks or other hard substrates). All sites are characterized by adjacent patches of saltmarshes and marsh creeks vegetated mainly by dense flowered cordgrass *Spartina densiflora* and alkali bulrush *Scirpus maritimus* (Costa and Marangoni 2010; Claudino *et al.* 2013).

Fish species were identified, counted and have their total length (in mm) measured. The database of abundances from January 1997 to December 2015 was used for analysis in the present study. In the subsequent data analyses, fish abundance at each month was obtained by summing up the total catch per species from all seine hauls carried out in the five sampling stations (i.e., 25 samples or hauls per month). The species were categorised into feeding guilds (adapted from Elliott *et al.* 2007) and their abundances were summed. Categorisation of feeding guilds was performed based on a literature review of fish feeding habits (Table S1), always prioritising work done with the stomach contents at the PLE or at areas geographically nearby. When this was not possible, works performed at similar aquatic systems were used. Additionally, diet changes due to ontogeny were considered for each analysed fish species, and whenever needed diet information on juvenile was chosen. This because the long-term abundance dataset is based on beach seine samplings performed in shallow areas (<1.5 m) of PLE, where predominates juvenile individuals for most of fish species occurring in the estuary (Chao *et al.* 1985; Vieira and Castello 1996). The trophic guilds used were: Detritivores (DTV), herbivore-phytoplankton (HVP), herbivore-macroalgae/macrophytes (HVM), Insectivores (ISV), Omnivores (OMN), piscivores (PSV),

zoobenthivores (ZBV) and zooplanktivores (ZPL). When one species fed on different sources, it was established that the dominance of the main food item should be greater than 40%. When plant and animal materials had the same dominance proportion, the species was categorised as omnivorous.

Regarding the use of trophic guilds to describe trophic structure, it is worth noting that every simplification has disadvantages and risks. The guild approach may be subjective because many species present trophic plasticity, i.e., environmental conditions can alter the diet (Gerking 1994). Additionally, in competition conditions, species can change the exploited resources. In the present study, we tried to minimise most of these errors, searching at least three works (when available) for each species and selecting the one that was most appropriate to the conditions of the present study (e.g., using juvenile diet information when necessary to control for ontogenetic diet shifts and focusing on diet data available for the current study estuary or other subtropical estuarine system).

With regard to the environmental data, the database contained the following variables: salinity, transparency (measured using a Secchi disk) and water temperature (°C), which were used as monthly averages of sampling stations. Rainfall data (mm) and river flow (m^3/s) were provided by the Brazilian National Water Agency (ANA) (80 rainfall stations and six stations for water flow in the drainage basin of the Patos Lagoon, respectively). Values for Oceanic *Niño* Index (ONI) were obtained from the online database of the National Oceanic and Atmospheric Administration (NOAA). 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), and values above 0.5 characterise the occurrence of *El Niño*. To calculate a single value for each month, we used the average of the ONI values for each quarter in which the month was included. During the study period, there were

two *El Niño* events classified as "very strong" (1997-1998 and 2015-2016), with ONI values ≥ 2 , and two classified as "moderate" (2002-2003 and 2009-2010), with ONI values between 1 and 1.4 in at least three consecutive months (Golden Gate Weather Services 2017).

Monthly anomalies of abiotic variables were calculated using the mean values for each variable based on the historical dataset (from 1997 to 2015) and then subtracted from the observed value for each month. The average number of distinct trophic guilds per year between *El Niño* and non-*El Niño* periods were compared with a Wilcoxon-test. A Canonical Correspondence Analysis (CCA) was performed using matrices of abundance of the trophic guilds and environmental variables. CCA was performed based on covariance/correlation matrices. Abundance data were standardized by log (x+1), whereas the environmental variables were standardized using the 'decostand' function with 'method =standardize' of the vegan package of R. The water flow was not used for this analysis because there was no data available in 2015. An Analysis of Variance (ANOVA) like permutation test was performed to assess the significance of each constrained axis of the CCA and the axis with greater explanation was chosen. The constrained scores were removed from this axis and summarised the abiotic variables. The abundance of trophic guilds was related to the scores of each axis using Spearman correlations. All statistical analyses were performed using the R 3.3.1 software with $\alpha = 0.05$.

3. Results

El Niño events were associated with higher rainfall and river flows in the drainage basin of the lagoon and lower salinity and water transparency in its estuarine area (Fig. 2). In contrast, the temperature showed marked seasonality and little relation to the

oscillation of the ONI index between 1997 and 2015. Mean rainfall in the drainage basin was 133.49 mm, and the more intense rainfall peaks occurred in *El Niño* periods, reaching 342.90, 361.80 and 303.70 mm in October 1997, November 2009 and October 2015, respectively. Water flow followed a similar pattern, with its historical mean of 247.77 m³/s and records of 1,206.00 m³/s in November 1997 and 887.80 m³/s in September 2009. Salinity and water transparency showed an inverse pattern, with both showing zero values for the same period. The *El Niño* events did not occur at the same temperature conditions. The episodes of 1997-98 and 2015-16 occurred in periods colder than those of 2002-03 and 2009-10 (Fig. 2).

There was a marked interannual variation in average total abundance (CPUE) of fishes in the estuary between 1997 and 2015 (Fig. 3). The trophic guilds DTV, ZPL OMN, and ZBV comprised more than 90% of total catches each year and remained as the most dominant guilds across the entire studied period (Fig. 3). In contrast, the relative abundance (%) of the trophic guilds HVP, HVM, ISV and PSV comprised less than 10% of total abundance and were also less frequent in the estuary along the entire study period. Overall, these five less dominant trophic guilds occurred concomitantly in the estuary only during the studied *El Niño* events (Fig. 3). The average number of distinct trophic fish guilds per year was higher during *El Niño* (7.86) compared with non-*El Niño* periods (7.00) ($W = 65$, $p\text{-value} < 0.05$).

The first two axes of the CCA together accounted to over 97% of the explained variability. The first axis (CCA1) alone explained 82.91%, and the variables that contributed most to this axis were ONI (0.53) and rainfall (0.38) (positively related) and salinity (-0.82) and water transparency (-0.69) (negatively related) (Fig. 4). Temperature was most associated with the second axis (CCA2) (0.97). A differentiation can be observed in CCA in samples taken during the occurrence of *El Niño* events in

comparison to samples taken in years without the occurrence of this event (Fig. 4). *El Niño* samples were distributed throughout the positive side of CCA1, i.e., associated with higher values of precipitation and ONI, but with lower values of salinity and water transparency. The opposite pattern was found for most of the samples taken during years with no occurrence of *El Niño* events.

Concerning the distribution of trophic guilds, HVM, HVP, ISV and PSV were positively associated with CCA1, whereas DTV, OMN and ZPL were negatively associated with this axis (Fig. 4). Considering that CCA1 includes positive contributions to higher values of ONI and rainfall and lower values of salinity and water transparency, the guilds HVP, HVM, ISV and PSV could be associated to *El Niño* events. This pattern was corroborated by the positive correlation between abundance of these guilds and scores of CCA1 (Table 1). On the other hand, DTV, OMN and ZPL were related to the higher mean values of salinity and water transparency, which occurred mostly in periods without *El Niño* events (Fig. 4, Table 1). The ZBV was the only trophic guild showing no statistically significant ($p > 0.05$) correlation with the environmental variables analysed (Table 1).

When analysing the trophic guilds more associated with *El Niño* events (HVP, HVM, ISV and PSV) in relation to their habitat use, we observed that nearly all of them ($\geq 97\%$) were composed of fish species originated from freshwater habitats (Fig. 5). The only exception was the guild HVP that was composed of estuarine residents. In contrast, the trophic fish guilds oppositely related to *El Niño* events (DTV, OMN and ZPL) corresponded to organisms that live year-round within the estuary or were estuarine-dependent fish species (Fig. 5). The guild ZBV were equally divided between estuarine and marine-related fish species (49.18% and 49.9%, respectively).

4. Discussion

Our findings corroborated our general hypothesis that hydrological effects triggered by moderate and very strong *El Niño* events are correlated with higher trophic diversity in a subtropical estuary. The above than average rainfall in the drainage basin during the studied *El Niño* events led to higher freshwater discharge that promoted the displacement of herbivores, insectivores and piscivorous, commonly found in the freshwater reaches of the lagoon, into the estuarine zone. These trophic fish guilds were much less abundant than the ones that dominate (> 90% of the total abundance) the fish assemblage's trophic structure in the estuary, such as detritivorous, omnivorous, zoobenthivorous and zooplanktivorous. These trophic guilds remained dominant irrespective of moderate or very strong *El Niño* events, whilst *El Niño* significantly increased the abundance of distinct trophic fish guilds transported from the freshwater into the estuarine compartments of the lagoon. This process led to a significant increase in the number of trophic fish guilds occurring simultaneously in the estuary during periods under influence of moderate and very strong *El Niño* events.

The increase in herbivorous fishes feeding on macroalgae and macrophytes (HVM) and phytoplankton (HVP) during *El Niño* events is due these herbivorous were primary freshwater fishes (i.e., no physiological tolerance to salinity, sensu Myers 1938), mainly characins of the genus *Astyanax* and *Hyphessobrycon*. They explore the great biomass of primary producers (e.g., floating and rooted macrophytes) usually found in the freshwater reaches of the lagoon (Garcia *et al.* 2007; Mont'Alverne *et al.* 2016). A potential mechanism favouring this transport of herbivorous fishes into the estuary could be animal dispersal associated with floating and algal rafting (Highsmith *et al.* 1985; Luzzatto *et al.* 2013). Considering that floating macrophytes can be used by fishes as preferential sites for feeding and refuge (Vandendriessche *et al.* 2007) and

prior records of freshwater vegetation transport into the estuary during periods of high freshwater discharge (Garcia *et al.* 2003a,b, Costa and Marangoni 2010, Moura *et al.* 2012), it seems plausible to hypothesize that this can be one of the mechanisms favouring the dispersion of herbivorous fishes in the studied estuary during moderate to very strong *El Niño* events.

Insectivores were also more abundant during *El Niño* events. This guild was exclusively composed of primary freshwater fishes, especially characins of the genus *Astyanax*. Although there are no true insectivores in the studied estuary, some opportunistic species feed on insects, such as the estuarine-resident silverside *Atherinella brasiliensis* (Bemvenuti 1990). The margins of the studied estuary are mostly covered by extensive marshes, which provide a great diversity and abundance of insects (Bolico *et al.* 2012; Gantes *et al.* 2013; Carrasco *et al.* 2014). During the longer periods of low to nearly zero salinities in the estuary associated with moderate to very strong *El Niño* events these newly arrived characin insectivores could be using the salt marshes for refuge and foraging areas, altogether with estuarine-residents who also feed opportunistically on insects. Therefore, these *El Niño* events may have positive effects on this particularly trophic guild as they may promote expansion of the lateral portion of the estuary and flooding of inland areas, increasing the foraging area for these insectivorous species. It can be also hypothesized that the addition of insectivorous in the estuary would facilitate potential its trophic links with adjacent terrestrial environments. The trophic role of insects as an input of allochthonous energy is well known for freshwater environments (e.g. rivers and streams; Nelson *et al.* 2015), but their trophic role in estuaries is still little known. Future studies should evaluate if the increase in insectivorous fish during *El Niño* events can favour the assimilation of

allochthonous material (insects), increasing terrestrial-derived energy supply into the estuarine food web.

Piscivorous estuarine fish species may be rare, especially at juvenile stages, in some temperate estuaries (Buchheister and Latour 2015). In the studied subtropical estuary, piscivorous are restricted to adult forms that temporally enter the estuary with salinity intrusion during summer months (Chao *et al.* 1985, Vieira and Castello 1996). Therefore, the occurrence of piscivorous (both juvenile and adult forms) during episodes of high rainfall and freshwater discharge triggered by moderate and very strong *El Niño* events represents an unexpected addition of top predators in the shallow waters of the studied estuary. These piscivores entering the estuary were mainly the primary freshwater characins *Oligosarcus jeninsii* and *O. robustus*, which are known to feed on macroinvertebrates, but mostly on juvenile fishes (Nunes and Hartz 2006). This can have potential ecological implications considering these shallow waters are considered as nursery grounds for juvenile forms of several marine estuarine-related and estuarine-resident fishes (Muelbert and Weiss 1991; Lemos *et al.* 2014). Future studies are needed to evaluate the potential effects of these piscivorous fishes on the fish prey biomass in shallow waters of the estuary.

Contrary to our initial hypothesis that detritivorous fishes would be favoured by the increase amounts of detritus carried by higher freshwater discharge during *El Niño* events, there was a tendency of lower abundance of this trophic guild during these climatic episodes. This pattern can be explained by the negative effects of *El Niño* phenomenon on the abundance of the fish species comprising this trophic guild (Garcia *et al.* 2004). The detritivore guild was composed mainly by mullet species (*Mugil brevirostris*, *M. curema* and *M. liza*), which are marine-spawning fishes that use estuaries as nursery grounds (Lemos *et al.* 2014). The entrance of marine-originated

mullet juveniles into the studied estuary decreases during high freshwater discharges triggered by *El Niño* events, as has been observed during the very strong 1982-1983 and 1997-1998 episodes (Garcia *et al.* 2004). On the other hand, it seems plausible to speculate that those mullet juveniles who succeed entering the estuary during *El Niño* years probably would find favourable trophic condition. This because they would have higher abundance of detritus carried by higher continental freshwater input (Netto and Lana 1999) and potentially lesser intraspecific food resource competition. These conditions could result in higher growth rates, with positive consequences for the cohorts that will comprise mullet stocks in the following years. Future studies evaluating body condition and growth rates of mullet juveniles in and out of the estuary during *El Niño* periods would be necessary to evaluate this hypothesis.

The zooplanktivores followed the same pattern as the detritivores, showing decreased abundance during the studied *El Niño* events. The estuarine zooplankton is dominated by marine copepods, which are also influenced by *El Niño* and are replaced by Cladocera and other freshwater organisms during such climatic events in this estuary (Muelbert *et al.* 2010). The prey shift towards cladocerans, that are smaller than copepods (Brooks and Dodson 1965), may have decrease the feeding efficiency of zooplanktivorous fishes. Additionally, higher freshwater inputs in the studied estuary can increase biomass of cyanobacteria and decrease of chlorophytes (Haraguchi *et al.* 2015), which, in turn, could affect the availability of zooplankton preys. These potential bottom-up effects could have led to nutritional deficiency in zooplanktivorous fishes with negative effects on their reproduction and, consequently, on their abundance. A non-food related mechanism could also explain the decreasing abundance of zooplanktivorous fishes in the estuary during the studied *El Niño* events. The silverside *A. brasiliensis* was one of the most representative species comprising the

zooplanktivore guild and prior studies have shown its displacement toward the marine adjacent area during high freshwater discharges (Garcia *et al.* 2001).

The response of omnivores did not corroborate our initial hypothesis that the dominance of this guild would increase during *El Niño* years, because their higher food plasticity will render greater adaptability to the environmental fluctuations associated with this climatic phenomenon. The one-sided livebearer *Jenynsia multidentata* was the species that most contributed to the omnivore abundance. This species feeds on benthic invertebrates, insects, benthic algae and seagrass (Mai *et al.* 2006). During *El Niño* events, meadows of the widgeon grass *Ruppia maritima* decrease their biomass or even disappear from the studied estuary and are replaced by drift macroalgae (Copertino and Seeliger 2010). Thus, the lower abundance of omnivores during moderate and very strong *El Niño* events could be associated with lower biomass of seagrass meadows in the estuary. The trophic linkage between *J. multidentata* and the widgeon grass *R. maritima* in this estuary has been recently demonstrated by food ecology studies using stable isotopes (Garcia *et al.* 2016). Aside the trophic aspect, a prior study has also pointed out the importance of seagrass meadows as preferential estuarine habitats for *J. multidentata* (Garcia and Vieira 1997). Therefore, the decrease of these submerged aquatic vegetated habitats during higher freshwater discharges and, consequently, the loss of their ecological role as food source and refuge for this omnivorous fish may explain the decreasing pattern in abundance of this trophic guild during moderate and very strong *El Niño* events.

It is worth noting that although our findings corroborated the hypothesis of higher trophic diversity associated with moderate and very strong *El Niño* events, there were some mismatches in the appearance and abundance of freshwater-related trophic fish guilds (HVM, HVP, PSV, ISV) and the timing of ONI index and rainfall and

salinity anomalies. In some cases, these guilds increased their abundance in periods that were not associated with studied *El Niño* events. This might have occurred due to delayed responses of fishes' abundance to the abiotic and hydrological triggered by *El Niño* events in the studied estuary, which can take from 1 to 2 months according to the species ecological attributes and the magnitude of the hydrological effects (Moraes *et al.* 2012). Another alternative explanation for potential mismatches between occurrence of freshwater-related trophic fish guilds and *El Niño* events could be associated with other climatic driving forces unrelated to ENSO (e.g., cold fronts and sea surface temperatures off the coast of southern Brazil) leading to positive rainfall anomalies (Diaz *et al.* 1998, Kane 1999). For instance, higher rainfall and lower salinity in 2000 and 2001 was probably caused by local and regional climatic drivers, since no *El Niño* event was in course during these years in the equatorial Pacific Ocean.

In conclusion, our findings revealed that abiotic and hydrologic changes triggered by moderate and very strong *El Niño* events led to a significant increase in the trophic diversity of an estuarine fish assemblage. This occurred due to the transport of trophic fish guilds more commonly found in the freshwater reaches of the lagoon (herbivores, insectivores and piscivorous) into the estuarine zone. Importantly, the addition of these freshwater-originated trophic guilds did not substantially alter the dominance pattern of the trophic fish guilds usually found in the estuary (detritivorous, omnivorous, zoobenthivorous and zooplanktivorous), which account for more than 90% of the total abundance regardless of the occurrence of the *El Niño* phenomenon. Despite their relative lower numbers, the trophic fish guilds transported into the estuary during these climatic episodes represent the addition of consumers with distinct feeding habits, which could have important ecological roles in structuring the estuarine food web (e.g., top-down effects of piscivorous and herbivorous, and assimilation of terrestrial-derived

sources by insectivorous). These hypotheses should be evaluate in future experimental studies (e.g., mesocosm approaches to evaluate top-down effect; Williams *et al.* 2002) coupled with the continuing monitoring of composition and dominance of trophic guilds and environmental variations (e.g., as conducted by Long Term Ecological Research programs; Lindenmayer *et al.* 2012). The investigation of *El Niño* effects on trophic organization, as reported for this subtropical estuary, should be evaluated in other estuaries located in latitudes under the influence of the ENSO phenomenon, especially when considering some predictions that such climatic events are becoming more frequent and intense in a global warming scenario (Yeh *et al.* 2009). We are aware that each estuary has its particularities regarding hydrogeomorphology and biota, which could render different results after interacting with abiotic changes induced by *El Niño* events. Despite these constraints, our work provides a study case (based on a standardized long-term experimental time series) of how hydrological disturbances triggered by *El Niño* may influence aspects of trophic organizations in estuaries under the influence of this global phenomenon and may be useful for future cross-system studies.

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Table 1. Spearman correlations between temporal abundance (1997-2015) of trophic fish guilds and scores of the CCA axis 1, which showed positive correlations with the *Oceanic Niño Index* ONI and rainfall and negative correlations with salinity and water transparency. Codes for trophic fish guilds are: ZPL: zooplanktivore, ZBV: zoobenthivore, OMN: omnivore, DTV: detritivore, PSV: piscivore, ISV: insectivore, HVM: herbivore-macroalgae/macrophytes, and HVP: herbivore-phytoplankton. ρ = correlation coefficient, $\alpha=0.05$ = level of significance.

Guild	ρ	p-value	Correlation
ISV	0.642	>0.001	positively related with <i>El Niño</i>
PSV	0.541	>0.001	
HVP	0.487	>0.001	
HVM	0.392	>0.001	
DTV	-0.425	>0.001	negatively related with <i>El Niño</i>
OMN	-0.398	>0.001	
ZPL	-0.305	>0.001	
ZBV	0.030	0.646	No relation

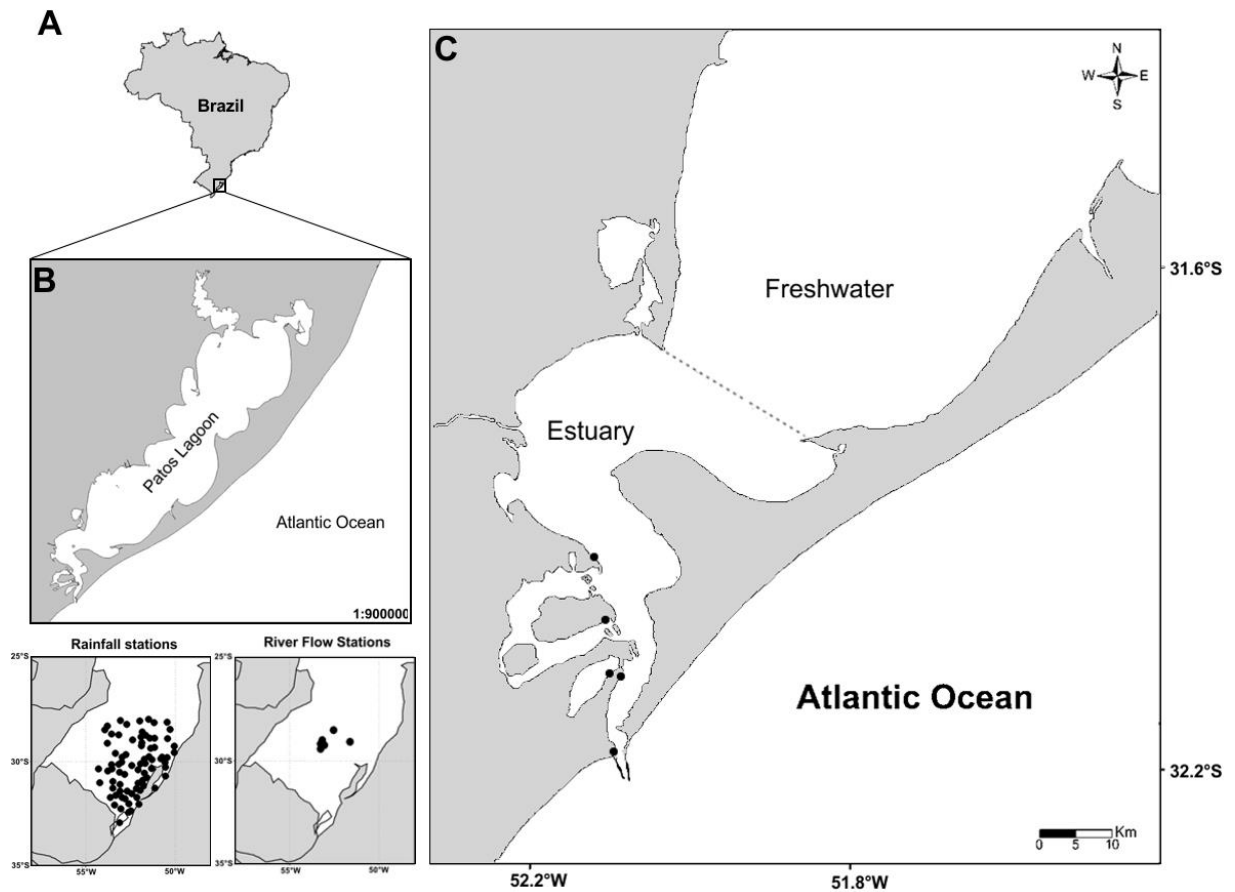


Figure 1. Map of Brazil (A) and the location of Patos Lagoon in Rio Grande do Sul (RS) state (B) with its freshwater and estuarine zones showing the position of the five sampling stations (filled circles) (C). The smaller panels (bottom left) show the locations of the meteorological station where rainfall and river flow data were obtained in the drainage basin of Patos Lagoon (RS e Brazil).

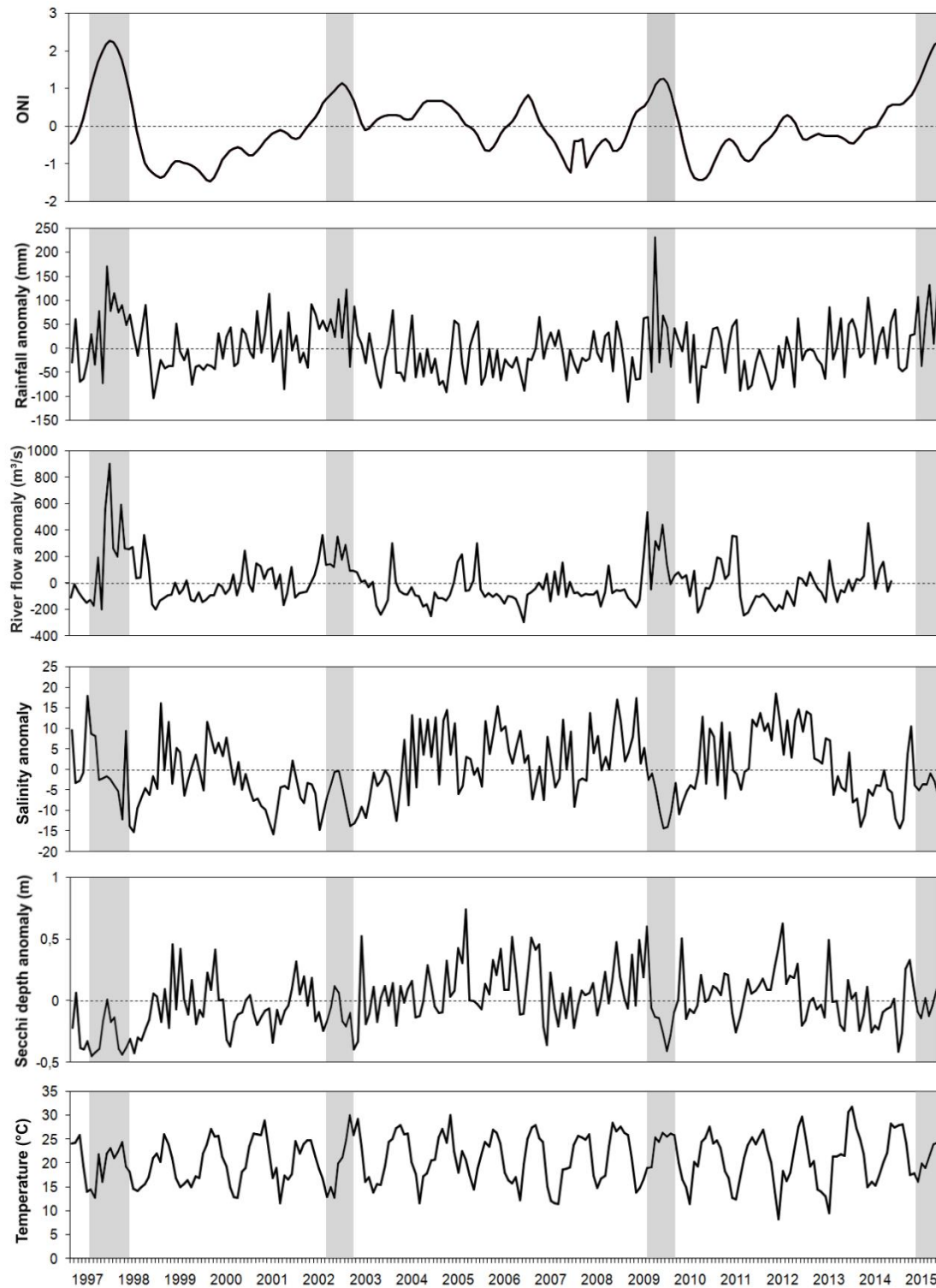


Figure 2. Temporal variation (1997-2015) in monthly values of the Oceanic Niño Index (ONI) corresponding to sea surface temperature anomalies in the Niño 3.4 region (5°N-5°S, 120°-170°W) in the Pacific Ocean, anomalies of rainfall (mm) and river water flow (m³/s) in the drainage basin of Patos Lagoon, and anomalies of salinity and water transparency (m), and average values of water temperature (°C) in the estuarine zone of the lagoon. Shaded areas denote moderate (2002-2003 and 2009-2010) and very strong (1997-1998 and 2015-2016) *El Niño* events.

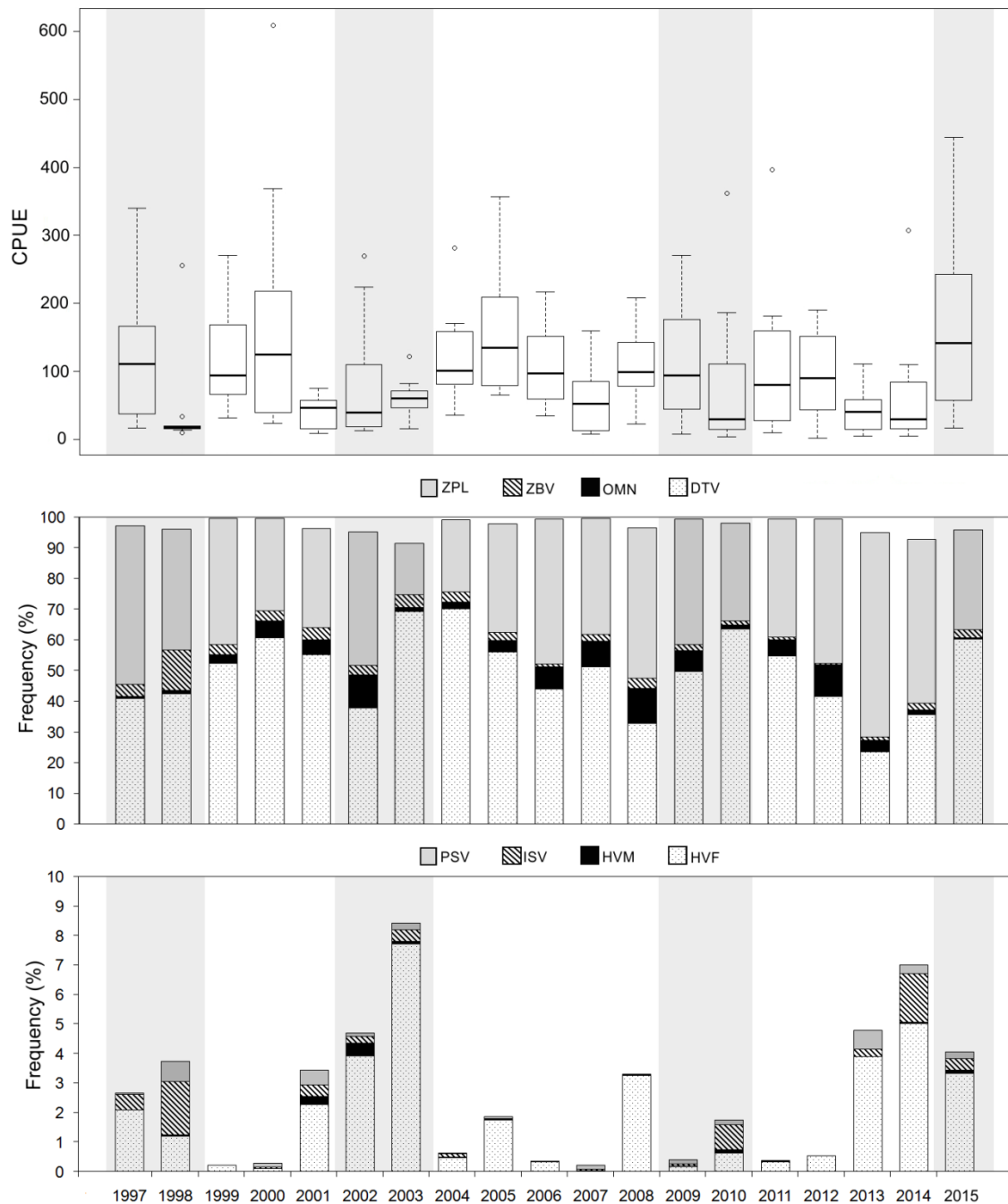


Figure 3. Temporal variation (1997-2015) in total fish abundance (CPUE, catch per unit of effort) (upper panel) the relative abundance (%CPUE) of the dominant (middle panel) and lesser abundant (lower panel) trophic fish guilds sampled in the estuarine zone of Patos Lagoon. Codes for trophic guilds are: ZPL: zooplanktivore, ZBV: zoobenthivore, OMN: omnivore, DTV: detritivore, PSV: piscivore, ISV: insectivore, HVM: herbivore-macroalgae/macrophytes, and HVP: herbivore-phytoplankton. Shaded-grey denote moderate (2002- 2003 and 2009-2010) and very strong (1997-1998 and 2015-2016) *El Niño* events.

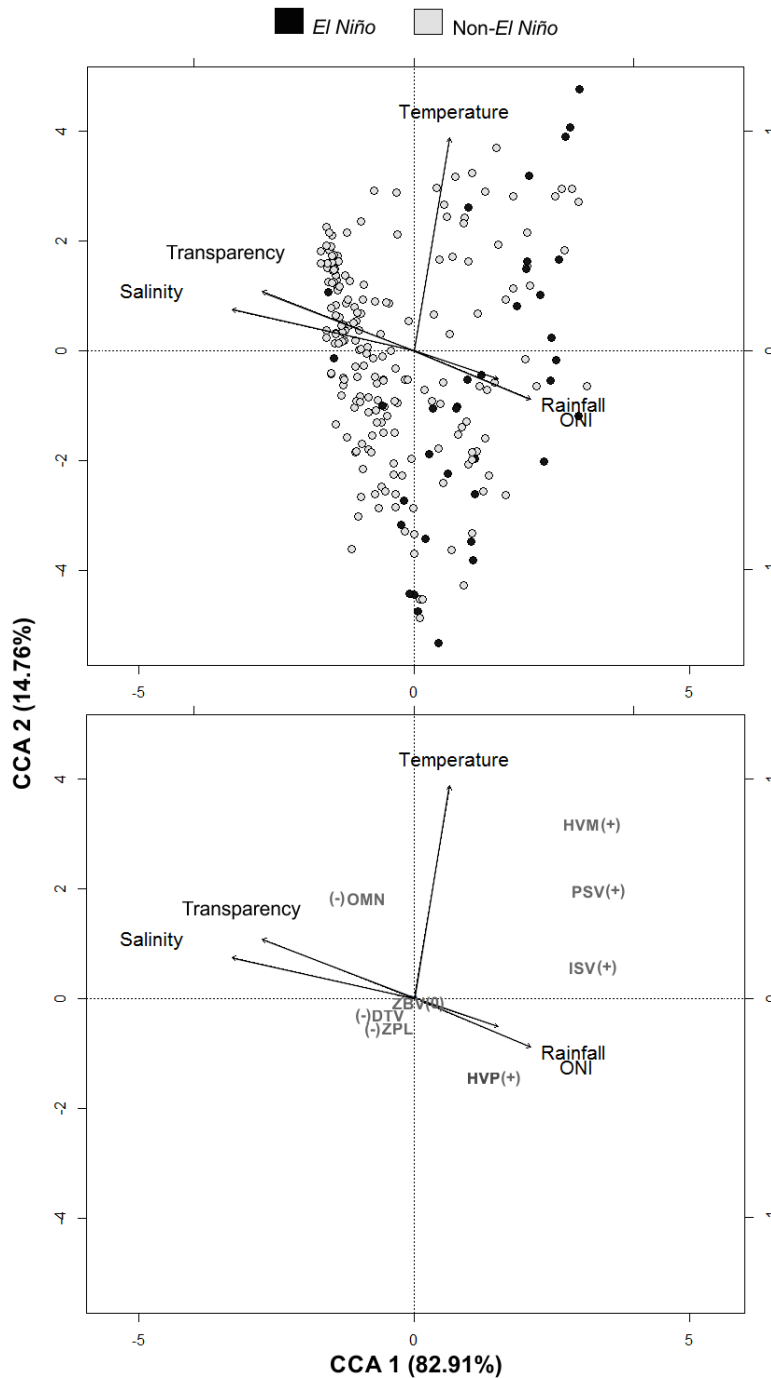


Figure 4. CCA ordination plots. Upper panel showing sample sites (closed and open circles denote *El Niño* and non-*El Niño* conditions) and environmental variables (arrows). Each point represents a single sample station and date (five beach seine hauls at the location for a given month). Environmental variables are salinity, water transparency (m), rainfall (mm), Oceanic Niño Index (ONI, °C) and water temperature (°C). Lower panel where association of fish species with each axis is represented by its score (plotted) and correlation between environmental variables and axes are represented by the length and angle of arrows. The trophic fish groups were labeled as ZPL: zooplanktivore, ZBV: zoobenthivore, OMN: omnivore, DTV: detritivore, PSV: piscivore, ISV: insectivore, HVM: herbivore-macroalgae/macrophytes, and HVP: herbivore-phytoplankton. Symbols (+) and (-) denote positive and negative association with *El Niño* events respectively and the symbol (0) represents neutrality.

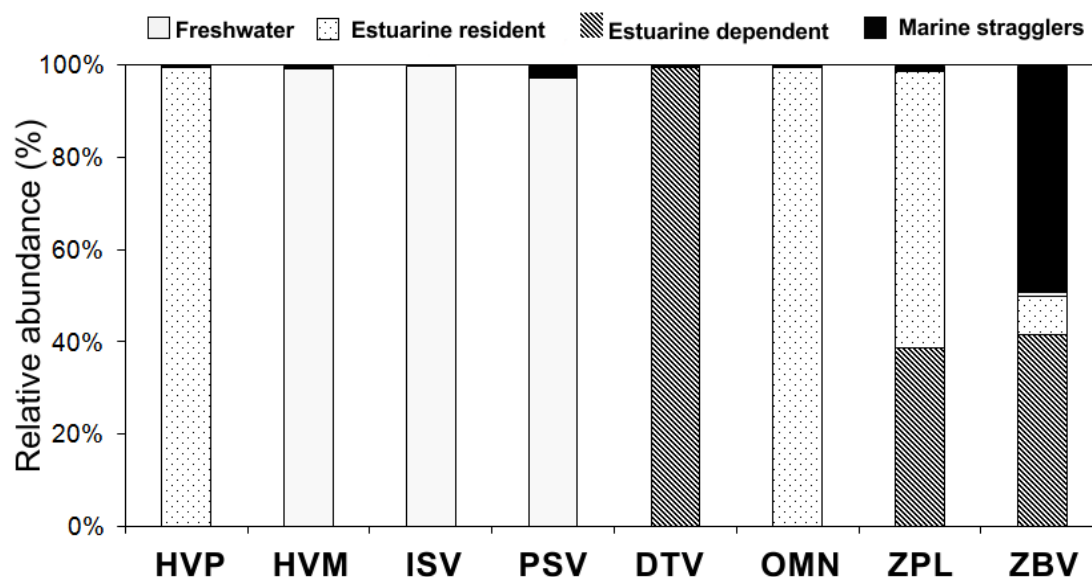


Figure 5. Relative abundance (%) of fishes classified in four habitat use guilds (freshwater, estuarine resident, estuarine dependent, marine stragglers) for each one the trophic fish guilds at the estuarine zone of Patos Lagoon. Codes for trophic fish guilds are: ZPL: zooplanktivore, ZBV: zoobenthivore, OMN: omnivore, DTV: detritivore, PSV: piscivore, ISV: insectivore, HVM: herbivore-macroalgae/macrophytes, and HVP: herbivore-phytoplankton.

ANEXO 2 (REFERENTE AO CAPÍTULO II)

**Freshwater inflow variability affects the relative importance of allochthonous
sources assimilated by estuarine fishes**

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ABSTRACT

Estuaries are influenced by seasonal and inter-annual variability in marine and freshwater intrusion. This variability affects the abundance and distribution of resources and consumers and may drive estuarine food web dynamics. This study tests for relationships among environmental variables and estuarine consumer $\delta^{13}\text{C}$, and assesses whether estuarine consumers assimilate greater proportions of freshwater-derived sources during high freshwater inflow events. Data were collected seasonally from 2010 through 2016 for Patos Lagoon, Brazil, and included rainfall and river discharge in the drainage basin, and salinity, chlorophyll- α , and phytoplankton abundance in the estuarine zone of the lagoon. Similarly, stable isotope data ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) were collected seasonally for autochthonous sources and consumers (detritivorous and zooplanktivorous fishes) in the estuarine zone, and allochthonous sources of freshwater and marine origin. Mixed effects models assessed relationships among consumer $\delta^{13}\text{C}$ and environmental variables, and Bayesian mixing models (MixSIAR) estimated the relative importance of autochthonous and allochthonous sources for estuarine consumers. Hydrology affected consumer $\delta^{13}\text{C}$, corresponding to greater assimilation of marine-derived organic material during periods with high freshwater inflow, especially for detritivorous juvenile mullet. This unexpected finding is likely due to high freshwater inflows delaying recruitment of juvenile mullet from coastal areas into the estuarine zone such that data from seasonal sampling reflected marine rather than local feeding and active transport of marine-derived sources into the estuarine zone. In addition to transporting freshwater-derived organic material, high freshwater inflow events may have other important indirect effects on the dynamics of trophic subsidies to estuarine food webs which require further study.

Keywords (4-6): freshwater inflows, hydrologic dynamics, long-term ecological research, primary production, trophic guilds, trophic subsidies

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INTRODUCTION

Estuaries are transitional environments with natural dynamics in environmental conditions, such as temperature, salinity, and pH profiles, that are variable across time scales from days, to months, to years (McLusky 1990; McLusky and Elliott 2004). These systems receive matter and energy from connected freshwater and marine environments, and also export matter and energy produced within the estuarine system to those environments. In general, the predominant hydrologic regime determines the relative influx of nutrients and allochthonous organic matter provided by continental versus marine waters (McLusky and Elliott 2004; Day et al. 2012). For example, Garcia et al. (2017) demonstrated that during a flood episode, freshwater-derived organic matter is passively transported into the estuary, where it is assimilated by resident fishes. In the opposite direction, Abreu et al. (1995) demonstrated that nutrient-rich salt water entering the estuarine region positively affected phytoplankton production.

There is an urgent need to understand how environmental drivers may affect availability of food sources and their subsequent effects on the trophic structure of aquatic food webs (Layman et al. 2015). Depending on the relative availability and biomass of autotrophs, different primary carbon sources may sustain estuarine fishes across seasons and years (Garcia et al. 2016). The occurrence and intensity of these trophic links can change across shorter (e.g. hours, days or months) to longer (e.g. years) time-scales (Campanyà-Llovet et al. 2017). Phytoplankton exhibit direct responses to hydrologic changes (e.g. rainfall) in time scales ranging from days, months to years (Odebrecht et al. 2015). River flow, in particular, plays an important role as a driving force influencing species composition and biomass in estuarine phytoplankton assemblages, especially by influencing the salinity regime (Hall et al. 2013; Haraguchi et al. 2015). Moreover, saltwater intrusion increases

chlorophyll-*a* in estuaries due to the resuspension or advection of benthic algae (Abreu et al. 1995; Abreu et al. 2010; Odebrecht et al. 2015). These events change the availability of microalgae to consumers across different time scales.

Stable isotopes are useful tracers of the relative importance of autochthonous and allochthonous sources in estuarine food webs (Garcia et al. 2007, 2017). Estuarine primary producers tend to have more enriched values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than either freshwater or marine producers, which are also distinguished by carbon and nitrogen isotope values (Garcia et al. 2007, Bergamino et al. 2011). Therefore, analyses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ enable distinction of relative inputs of allochthonous sources into estuarine food webs or movement of matter and organisms among freshwater, estuarine and marine systems. Carbon isotope ratios in particular are considered better proxies to track food assimilation by consumers due to lower isotopic fractionation compared with nitrogen isotope ratios (Fry 2006). For example, Samways et al. (2015) and Tonra et al. (2015) used stable isotope analyses to quantify the importance of marine subsidies to freshwater food webs. Comparatively, much less information is available on the assimilation of allochthonous matter derived from marine and freshwater ecosystems by estuarine consumers. This is probably due to the fact that estuaries are very productive systems and are thus usually considered as sources (and not receivers) of trophic subsidies to adjacent freshwater and marine systems (McLusky 1990, Bruno et al. 2017).

Variation in the estuarine hydrologic regime is expected to affect the relative importance of allochthonous organic material of continental versus marine waters assimilated by estuarine consumers. In this study, we used stable isotope values of sources and consumers to evaluate the following questions: (1) Are seasonal and inter-annual changes in carbon isotope ratios of estuarine consumers associated with local conditions (salinity, phytoplankton abundance) and regional (rainfall, river flow) variables?; (2) Does variation in carbon isotope

ratios of estuarine consumers reflect increased assimilation of freshwater sources by estuarine consumers during high rainfall and river flow episodes? In order to answer these questions, we analyzed temporal changes in precipitation, freshwater inflows, phytoplankton composition and abundance, and isotopic composition of primary food sources and detritivorous and zooplanktivorous fishes from 2010 through 2016 in a subtropical estuary in southern Brazil. We focused on detritivore and zooplanktivore functional guilds to allow us to investigate effects on estuarine consumers positioned at basal and intermediate levels of the estuarine food web.

MATERIALS AND METHODS

Study area

Patos Lagoon (Fig. 1) is the largest choked lagoon in the world (*sensu* Kjerfve 1986), covering 10,227 km² and extending 270 km along its main south-north axis. The estuarine area (971 km²) is located in the southern portion of the lagoon and connects with the Atlantic Ocean throughout a single and narrow inlet about 4 km long and 740m wide at the mouth (Asmus 1998). This funnel-shaped geomorphology reduces the entrance of marine water and favors the influence of freshwater discharge in this estuary (Asmus 1998). Fluvial discharge and wind patterns (predominantly in the NE-SW direction) are the main drivers controlling circulation, salinity distribution, and water levels at Patos Lagoon Estuary (PLE). Freshwater discharges vary considerably among years (700-3,000 m³s⁻¹), with high discharge in late winter and early spring followed by low to moderate discharges through summer and autumn (Möller et al. 2010). However, runoff greatly exceeds average values during *El Niño* events and estuarine regions may be dominated by freshwater for several months (Garcia et al. 2001;

Garcia et al. 2004). The influence of tidal regime is minimal with the tidal range within the estuary limited to low diurnal amplitude (mean = 0.47 m) (Costa et al. 1988).

The PLE's fauna and flora are influenced by the hydrological regime that constitutes one of the main physical drivers controlling species composition and abundance patterns (Odebrecht et al. 2017). Substrates in shallow waters (<1.5 m) may be covered by submerged aquatic vegetation, such as meadows of Widgeon seagrass (*Ruppia maritima*) and free-floating macroalgae (*Ulva* sp., *Cladophora* sp., *Rhizoclonium* sp.) (Copertino and Seelinger 2010). However, sediment resuspension limits light penetration during high rainfall and river discharge periods, which decreases the biomass of submerged aquatic vegetation (Lanari and Copertino 2016). Moreover, high rainfall and freshwater discharge associated with *El Niño* events, in particular, promote the entrance of freshwater species into the estuary and the subsequent decrease of marine species of several groups of organisms, including phytoplankton (Odebrecht et al. 2010), zooplankton (Teixeira-Amaral et al. 2017), and fishes (Garcia et al. 2003; Possamai et al. 2018).

Data collection and laboratory processing

Representative food web components (basal sources and consumers) were sampled seasonally in a shallow embayment (< 1.5 m) of PLE since 2010 by the Brazilian Long-Term Ecological Research (B-LTER) program (Fig. 1). The primary food sources sampled in the estuary are particulate organic matter in suspension (POM) and C₄ saltmarsh plants (*Spartina alterniflora* and *S. densiflora*) as proxies of autochthonous sources. Prior studies at PLE have shown very low assimilation of C₃ saltmarsh plants by estuarine consumers (Claudino et al. 2013; Lanari et al. 2018), and because of this, just C₄ plants were considered in this study. POM was obtained by filtering 0.25–1.00 liters of water onto a pre-combusted (450°C for 4 h) Whatman glassfiber filter (0.75 µm). Macrophytes were collected by hand using scissors and

fishes were collected using beach seines (13 mm mesh size in the wings and 5 mm in the center of the net). We used POM as a proxy for the isotopic composition of phytoplankton and organic matter in the water column (Kopp et al. 2013; Garcia et al. 2017) due to the difficulty to determine the isotopic composition of planktonic microalgae species separately (Fry 2006). Allochthonous sources (POM) were also collected from marine and freshwater regions adjacent to the PLE (Fig. 1) to be used as end-members in isotope mixing models (see next subsection). Marine POM was obtained in November 2014 and December 2014 in the coastal area (6 samples) outside the influence of the estuarine plume, and freshwater POM was collected during spring 2009, summer 2010 and winter 2011 in freshwater reaches of Patos Lagoon (15 samples) (Fig. 1). Although we did not collect allochthonous sources in all seasons, the use of these data is justified by the lower variation in isotopic values of marine and freshwater POM compared to estuarine POM (Bouillon et al. 2011; Rosli et al. 2017). Field collections were conducted at the end of each season when possible under the assumption that the isotopic compositions of consumer muscle tissues were derived from food assimilated during the preceding months corresponding to each season (Phillips et al. 2014). Based on experimental studies on carbon and nitrogen isotopic turnover of species representative of PLE (Mont'Alverne et al. 2016; Oliveira et al. 2017), isotopic composition of fish muscle is expected to reflect food assimilated during the previous 2 to 3 months. All samples were stored in plastic bags, packed in ice for transportation, and stored in a freezer in the laboratory until processing.

In the laboratory, thawed samples were rinsed with distilled water, placed in sterile Petri dishes and dried in an oven at 60°C for 48 h or until constant weight. Dried samples were ground to a fine powder with a mortar and pestle and stored in clean 2 ml plastic tubes. Subsamples were weighed to 10^{-3} g and pressed into Ultra-Pure tin capsules (Costech, Valencia, CA) and sent to the Analytical Chemistry Laboratory of the Institute of Ecology,

University of Georgia, for analysis of carbon and nitrogen isotope ratios. Stable isotopes results are reported as parts per thousand (‰) differences from a corresponding standard: $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where $R = {}^{15}\text{N}/{}^{14}\text{N}$ or ${}^{13}\text{C}/{}^{12}\text{C}$. Standards were carbon in the PeeDee Belemnite and nitrogen in air. Internal standards (animal and vegetal) of known carbon and nitrogen composition were analyzed with each sequence in order to estimate instrument precision. Standard deviations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of these replicate analyses were 0.07‰ and 0.06‰ and 0.15‰ and 0.37‰ for animal and vegetal samples, respectively.

Monthly phytoplankton samples were collected in the same estuarine site by the B-LTER program (Fig. 1). Phytoplankton abundance and composition were analyzed using the Utermöhl sedimentation method (Utermöhl 1958) and following recommendations by Hasle (1978) (for more details see Haraguchi et al. 2015). Chlorophyll-*a* was extracted in the dark and cold (-12°C) using 90% v/v acetone, and its concentration was determined fluorometrically using a calibrated Turner TD700. For more details of chlorophyll-*a* collecting and determinations see Abreu et al. (2010). Monthly values of salinity were obtained using thermo-salinometer. Average monthly rainfall (mm) was provided by the Brazilian National Institute of Meteorology (INMET) for four stations located around the margins of Patos Lagoon, and monthly river flow values (m^3/s) were provided by the Brazilian National Water Agency (ANA) in nine stations distributed in the PLE drainage basin (Fig. 1).

Data analyses

Monthly values of abiotic parameters, chlorophyll-*a* and phytoplankton abundances (diatoms and cyanobacteria, separately) were averaged for each season (Summer: December through February; Autumn: March through May; Winter: June through August; Spring: September through November). The anomalies of these parameters were calculated as the

difference between the seasonal average and the historic mean (global average of the variables from 2009 through 2016). Fish species were classified as detritivores or zooplanktivores following Possamai et al. (2018) (Table S1), with other species not included in this study.

To address our first research question, we fit linear mixed-effects models to identify environmental factors predicting changes in $\delta^{13}\text{C}$ values for each fish trophic guild. We chose $\delta^{13}\text{C}$ as the response variable because it is a suitable proxy to track allochthonous and autochthonous source assimilation by consumers in this system (e.g. Garcia et al. 2007, 2016, 2017), and because $\delta^{13}\text{C}$ is generally a more suitable tracer of source contributions than $\delta^{15}\text{N}$ due to lower trophic fractionation (Fry 2006). Because this system is characterized by strong seasonal and inter-annual variation, ‘year’ and ‘season’ (nested within ‘year’) were included as random factors to control for temporal variation in values of environmental variables (Zuur et al. 2009; Harrison et al. 2018). Environmental variables were z-transformed and multicollinearity was assessed by pairwise Pearson’s correlations (Fig. S1). Residuals were assessed to evaluate model reliability and to validate assumptions (Fig. S2 and S3). Model-selection was performed by comparison of variance using the AIC criteria. Spearman correlations were performed to describe the directions of observed interactions. These analyses were performed using the R packages ‘vegan’, ‘faraway’, ‘GGally’ and ‘nlme’.

To address our second research question, Bayesian isotope mixing models were used to estimate relative contributions of autochthonous (i.e. estuarine) and allochthonous (i.e. marine and/or freshwater) sources supporting detritivorous and zooplanktivorous fish guilds across years (2010-2016) (see details in Table S2). Mixing models were constructed using the following end-members: C_4 plants and $\text{POM}_{\text{estuarine}}$ as autochthonous sources, and $\text{POM}_{\text{freshwater}}$ and $\text{POM}_{\text{marine}}$ as allochthonous sources. Trophic discrimination of carbon is typically $<1\text{‰}$, whereas the trophic discrimination for nitrogen is between 2 and 4‰ (Vander-Zanden and Rasmussen 2001; Vanderkluft and Ponsard 2003). These trophic discrimination

factors (TDF) also may differ between herbivores/detritivores and carnivores (Bastos et al. 2017), therefore we used different TDF values to correct for fractionation of isotopic values for our studied fish guilds. We used the average ($\pm$ standard deviation) TDF values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of 0.54 ± 0.53 and 4.78 ± 1.30 for detritivores, and 1.08 ± 0.53 and 6.04 ± 1.00 for zooplanktivores, respectively (adapted from Bastos et al. 2017). We ran mixing models using the ‘MixSIAR’ package (Bayesian Mixing Models in R; Stock and Semmens 2016) with a nested-structure (‘seasons’ nested in ‘years’). Each model was fit using the Markov chain Monte Carlo (MCMC) method, running 100,000 simulations for each model and discarding the first 50,000 to choose the best fit. We used the median (50%) and 95% Bayesian credibility intervals to summarize the contribution of each carbon source to consumer guilds. Bayesian analyses were performed using JAGS 4.3.0. Allochthonous source contributions (median percent contribution estimated by MixSIAR) were correlated with seasonal averages of environmental variables using Spearman correlation.

In order to test the basic assumption that isotopic variability of consumers (after accounting for fractionation) is contained within the variability of assimilated food sources (Phillips et al. 2014), we applied isotope mixing polygon simulations to determine boundaries of possible source values in the $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ biplot space that can explain the isotope mixture (Smith et al. 2013). Individual consumers located outside the 95% mixing polygon region were excluded from the isotope mixing model analysis (Smith et al. 2013; Phillips et al. 2014) (see Fig. S4 and Fig. S5). We carried out this analysis using the R packages ‘sp’ (Pebesma and Bivand 2005; Bivand et al. 2013) and ‘splancs’ (Rowlingson and Diggle 2017). All statistical tests were performed using R software version 3.5.1 (R Core Team 2018).

RESULTS

Variation in environmental factors and consumer isotope values

Seasonal values of several environmental factors varied widely throughout the 2010-2016 study period (rainfall: 5.00-372.40mm; river flow: 9.97- 397.16 m³/s; salinity: 0.00-29.76; chlorophyll- α : 0.17-36.98 μ g/L; diatom density: 1-20,458,480 cell/L; cyanobacteria density: 100-71,567,250 cell/L). Rainfall and river flow were above average during Spring 2013 through Spring 2015, and consequently the salinity was below average during those seasons (Fig. 2). The seasonal and annual variation of environmental variables can be observed in figure 2.

For detritivores, the overall $\delta^{13}\text{C}$ average was -16.19 ± 2.72 , but there were variations among seasons and years (see Tables S2 and S3 for source and consumer data). For example, during the spring of 2014, average $\delta^{13}\text{C}$ decreased from -15.10 ± 1.64 to -21.65 ± 2.03 (Fig. 3). The overall $\delta^{15}\text{N}$ average was 9.88 ± 1.35 and its temporal variation followed the general pattern observed for carbon isotope ratios. For zooplanktivores, changes in average $\delta^{13}\text{C}$ also occurred among seasons (Fig. 4). Their overall $\delta^{13}\text{C}$ average was -15.94 ± 1.65 , but between summer of 2013 and summer of 2014 there was a drop of approximately 3‰ in average $\delta^{13}\text{C}$ values. In contrast, seasonal changes in $\delta^{15}\text{N}$ were less pronounced from 2010 to 2016, with no marked drop in average values during 2013-2014 as observed for average carbon isotope ratios (Fig. 4).

Environmental correlates of consumer $\delta^{13}\text{C}$ values

The linear mixed-effects models showed that dynamics of environmental factors were important in explaining variation in consumer $\delta^{13}\text{C}$ values. The best-fit model for the detritivore guild included 'year' as a random factor, and higher values of diatom abundance

and river flow were related to more depleted consumer $\delta^{13}\text{C}$ values (Table 1). Salinity and Diatom were negatively related ($\rho = -0.32$, $p\text{-value} < 0.001$), and this interaction also contributed to a decrease in the $\delta^{13}\text{C}$ of detritivores (Table 1). No correlation was found between Cyanobacteria and Diatoms ($\rho = 0.07$, $p\text{-value} = 0.392$), however, the interaction of these groups was weakly related to more depleted detritivore $\delta^{13}\text{C}$ values.

For zooplanktivores, the model with the smallest AIC value did not meet the model assumptions (Fig. S3), so the model including 'year' was considered the single most acceptable model for interpretation (Table 2). River flow and the interaction between salinity and Cyanobacteria ($\rho = -0.21$, $p\text{-value} = 0.012$) were related with decreasing consumer $\delta^{13}\text{C}$ values (Table 2).

Relative contributions of autochthonous and allochthonous sources

Isotope mixing models revealed seasonal changes from 2010 to 2016 in the relative contribution of primary food sources for both detritivores and zooplanktivores (Fig. 5, Table S4). In general, the contribution of allochthonous sources was more variable for detritivores (coefficient of variation = 0.6) than zooplanktivores (0.4). When compared between marine vs. freshwater sources, there was higher variability in the contribution of freshwater than marine allochthonous sources for both detritivores (1.2 vs. 0.9, respectively) and zooplanktivores (1.0 vs. 0.5).

For detritivores, autochthonous matter was the most assimilated (> 50%) primary food source in several seasons, but its contribution varied markedly across years, with median values ranging from 7.4% to 84.7% (Fig. 5, Table S4). We observed substantial increases in allochthonous contribution of marine-derived POM during spring and winter of some years, with median values ranging from 69.1% in winter 2011 to 90.6% in spring 2016. There was prevalence (above 75%) of assimilation of marine allochthonous matter by detritivores

especially from spring of 2014 to spring of 2016 (Fig. 5; Table S4). For zooplanktivores, autochthonous sources were also the most assimilated ($> 50\%$) in most seasons. However, in contrast with detritivores, the assimilation of autochthonous matter by zooplanktivores was comparatively less variable across years, with median values ranging from 38.7% to 83.0%. Higher contributions of marine and freshwater-derived POM (Fig. 5, Table S4) were associated with more depleted $\delta^{13}\text{C}$ values observed from Autumn 2013 through Winter 2015 (Fig. 4). There was no correlation ($p > 0.05$) between assimilation of freshwater allochthonous matter and the environmental variables for either trophic guild (Table 3). In contrast, we found positive correlations between assimilation of marine allochthonous matter by detritivores with rainfall and river flow, but no correlations for zooplanktivores fishes (Table 3).

DISCUSSION

Temporal variability in carbon isotope ratios and environmental correlates

Our findings indicate that temporal changes in carbon isotope values of estuarine consumers are associated with dynamics of environmental factors (i.e. dynamics of estuarine hydrological conditions and productivity, including as affected by rainfall and river discharge). More depleted $\delta^{13}\text{C}$ values for detritivores and zooplanktivores were related to increased freshwater inflow to the estuary. A similar pattern of freshwater discharge driving $\delta^{13}\text{C}$ variability of estuarine consumers was reported for estuaries at temperate latitudes (e.g. Plum Island Estuary; Nelson et al. 2015). In our study system, freshwater discharge usually increases during winter and spring and higher phytoplankton abundance is commonly observed in spring and summer (Abreu et al. 2010; Haraguchi et al. 2015). Our findings

suggest, therefore, that seasonal pulses of phytoplankton abundance seem to be an important predictor of changes in carbon isotope ratios of estuarine fishes in subtropical estuaries.

Moreover, we observed similar relationships between $\delta^{13}\text{C}$ dynamics and environmental factors for detritivores and zooplanktivores. For detritivores, the best fit linear mixed-effects model revealed that higher values of river flow and diatom abundance were associated with depleted $\delta^{13}\text{C}$ values. Considering that the diatom interaction with salinity was also important to decrease the $\delta^{13}\text{C}$ of detritivorous fishes, and that marine diatoms had more depleted $\delta^{13}\text{C}$ than estuarine diatoms (Bergamino et al. 2011; Bergamino et al. 2016; Bruno et al. 2017), we speculate that the mechanism behind the observed correlations is immigration of juvenile mullets from the ocean to the estuary (see further details in the next section). Similarly, the best fit model for zooplanktivores indicated that more depleted consumer $\delta^{13}\text{C}$ values are related to higher values of river flow and cyanobacteria abundance interacting with salinity. Indeed, prior studies have reported the dominance of cyanobacteria in estuaries as a consequence of higher freshwater discharge (Abreu et al. 2010; Carstensen et al. 2015; Haraguchi et al. 2015; Mendes et al. 2016; Abreu et al. 2017), and we could observe this in the negative correlation found between salinity and cyanobacteria. This negative interaction was related with depleted $\delta^{13}\text{C}$ values of zooplanktivores, and this agrees with previous observations in our study system and elsewhere that freshwater-derived organic matter (Garcia et al. 2007; França et al. 2015; Morais et al. 2017) and cyanobacteria (Lesutienè et al. 2014; Lehman et al. 2015) have more depleted $\delta^{13}\text{C}$ values than other potential sources. Therefore, these results could be viewed as supporting the expectation that transport of depleted freshwater-derived organic matter into the estuary due to higher river flow drives a subsequent decrease in carbon isotope values of organisms at the base of the food web and of zooplanktivorous fishes feeding upon them.

Relative importance of allochthonous matter for estuarine fish guilds

Contrary to the previously stated expectations, mixing model results indicated that freshwater-derived allochthonous organic material was not assimilated by estuarine fishes in greater proportions during episodes of higher rainfall and river flow. Interestingly, we found that detritivores assimilated higher amounts of marine than freshwater allochthonous material, especially during the winter and spring seasons. We speculate that the relatively large contributions of marine organic material indicated by the mixing model for the detritivore guild is associated with assimilation of marine POM in the ocean prior to juvenile fishes recruiting into the estuary. The detritivore guild was comprised by juvenile mullets (mainly *Mugil liza*), that are marine estuarine-dependent species (i.e. adults reproduce in the ocean and their recruits use estuaries as nurseries) (Lemos et al. 2016; Mai et al. 2019). Adults reproduce in the ocean during winter (Lemos et al. 2014), and larvae feed on plankton in the water column (Cardona 2016; Oliveira et al. 2018). After reaching between 20 to 30 mm TL (see Table S3), they enter into estuaries and begin feeding on benthic microalgae and detritus (Blaber and Whitfield 1977; Vieira et al. 2008; Cardona 2016). Recruitment of juvenile mullets into the estuary thus represents active rather than passive transport of allochthonous sources into the estuarine food web and explains why the marine-derived contribution increases in importance against the predominant hydrologic gradient. Similarly, a prior study in coastal streams near our study system found that juvenile mullets may act as biological vectors promoting the transfer of marine-derived nutrients to freshwater piscivores (Oliveira et al. 2014). Future studies are needed to evaluate if juvenile mullets carry out a similar ecological role for higher-order consumers in subtropical estuaries.

Increased phytoplankton biomass in the estuary is typically observed in the spring, principally due to higher precipitation in this period that increases nutrient runoff and cyanobacteria biomass in the estuary (Abreu et al. 2010; Haraguchi et al. 2015; Mendes et al.

2016; Abreu et al. 2017). Higher phytoplankton biomass could be favorable to juvenile mullets foraging in the estuary. During episodes of higher freshwater inflow, chlorophyll- α increases in response to greater nutrient inputs and estuarine fish larvae have higher body condition (Salvador and Muelbert 2019). However, in the spring of 2014 and winter and spring of 2015, the modeled importance of marine-derived sources was higher than in other years, reaching more than 70% of all source contributions. In 2015, Patos Lagoon Estuary was under the influence of a strong *El Niño* event that caused changes in the trophic structure of the fish assemblage. In particular, several freshwater fish species entered the estuary presumably as a consequence of reduced salinity due to higher rainfall and river flow (Possamai et al. 2018). In the other direction, the same *El Niño* event may have delayed the recruitment of juvenile mullets into the estuary because elevated river discharge restricts the salinity intrusion and the consequent entrance of marine mullet recruits (Garcia et al. 2001; Vieira et al. 2008). Therefore, we consider that the episodes of greatest contribution of marine-derived sources to estuarine detritivores in 2014 and 2015 reflect the recent arrival of mullets in the estuary prior to being collected. That is, high discharge delayed recruitment and thus the feeding time in the estuary for tissues to turnover to reflect the isotopic values of the new estuarine diet.

Prior studies in Patos Lagoon Estuary and elsewhere found that heavy rainfall and high freshwater discharge, in some cases associated with *El Niño* events, are related with marked changes in species composition and relative abundances of several estuarine organisms, including phytoplankton (Nursuhayati et al. 2013; Nche-Fambo et al. 2015; Mendes et al. 2016) and zooplankton (Vignatti et al. 2012; Teixeira-Amaral et al. 2017). For example, estuarine and marine copepods may be replaced by cladocerans and other freshwater zooplankton (Teixeira-Amaral et al. 2017). Despite the higher input of freshwater-derived material into the estuary, our mixing models indicated that zooplanktivorous fishes did not

incorporate greater proportions of freshwater allochthonous resources during episodes of heavy rainfall and high freshwater discharge. One explanation could be a mismatch in the isotopic turnover rates among freshwater microalgae, zooplankton and the studied tissues (muscle) of the zooplanktivorous fishes. The isotopic turnover of the former are usually faster (days to weeks) than the latter (weeks to months) (Glober et al. 2005; Samways et al. 2015; Mont'Alverne et al. 2016). Experimental studies on isotope turnover in fish muscle tissues of Patos Lagoon Estuary suggest that turnover would take two to three months to reflect a new diet with distinct isotope composition (Mont'Alverne et al. 2016; Oliveira et al. 2017). This suggests that the assimilation of freshwater organic material by zooplanktivorous fishes should have occurred for at least two to three months prior to fish sampling to reflect isotopic differences in their muscle tissue samples.

Another explanation for the limited importance of freshwater-derived sources during high inflow events is displacement of zooplanktivores within the estuary to avoid freshwater intrusion. The zooplanktivore guild was comprised by the Brazilian silverside *Atherinella brasiliensis*, and this species is an estuarine-resident (Bemvenuti 1987; Fávoro et al. 2003; da Silva Cortinhas et al. 2016). Prior research in this estuary has shown that this zooplanktivorous species may be pushed towards the estuarine mouth and adjacent marine area during episodes of high freshwater discharge triggered by strong *El Niño* events (Garcia et al. 2001). Moreover, a recent study showed that higher river discharge and rainfall during *El Niño* events decreases the abundance of zooplanktivorous fishes in the estuary (Possamai et al. 2018). Hence, it seems reasonable to hypothesize that zooplanktivores could be moving closer to the estuary mouth to avoid lower salinities, where they would probably assimilate estuarine and marine sources in greater proportion than freshwater-derived organic material, which would preclude the expected shift in assimilation of freshwater-derived sources.

An important caveat is that our mixing models include more sources than tracers, which can inhibit resolution of source contributions and subsequent interpretations (Fry 2013). For the detritivores mixing model, our diagnostics plots did not show a high correlation between the isotopic values of sources (Fig. S6). However, for the zooplanktivore model, POM_{marine} and $POM_{\text{freshwater}}$ showed a high correlation, and thus it is difficult for the model to distinguish between these two sources (Fig. S6). In this case, both sources are allochthonous, so we can still robustly assess our main question regarding dynamics in the relative importance of autochthonous versus allochthonous sources. However, the correlation between values of marine and freshwater POM limits our ability to distinguish specifically which allochthonous source is driving the observed dynamics for the zooplanktivore model. In this case, we feel that our ecological interpretations supported by prior research in this system can serve as valid working hypotheses for subsequent research that incorporates additional tracers, greater temporal resolution, or other complementary approaches.

Conclusions

We found that intra- and inter-annual changes in carbon isotope ratios of estuarine trophic guilds (detritivorous and zooplanktivorous fishes) are correlated with environmental factors. The effect of rainfall and freshwater discharge on local estuarine salinity seemed to be an important driver of isotopic variability in these consumer guilds. However, our results did not support our expectations of greater assimilation of freshwater-derived allochthonous sources by estuarine fishes during high rainfall and river flow episodes. Instead, we found substantial contributions of marine-derived allochthonous material to the studied estuarine consumers, especially for the detritivore fish guild which was comprised by juvenile mullets. We speculate that juvenile mullets assimilated marine organic material during their initial development in the ocean and, delayed by high freshwater discharge, actively transported the

marine source contribution into the estuary shortly before being collected. Future studies are needed to evaluate the environmental factors that drive variability in the magnitude and timing of this potential subsidy as well as the ecological implications for consumers at intermediate and higher trophic levels in estuarine food webs. This is particularly important for estuaries along the southwestern Atlantic coast harboring mullet populations that have been increasingly exploited by fisheries and which are experiencing changing rainfall regimes due to climate change (Haimovici and Cardoso 2016). In addition to their well-known economic and social relevance for artisanal and industrial fisheries (Reis and D'incao 2000; Santana et al. 2017), our study suggests that mullet populations may have crucial roles as biological vectors linking marine and coastal systems.

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Table 1 Mixed-effects model results for detritivorous fish $\delta^{13}\text{C}$. Environmental parameters are direct factors and ‘Season’ and ‘Year’ are random factors. (n°) is the model number; (*) denote interactions among factors; (/) indicates nested factors. Values in bold are significant at $\alpha=0.05$.

n°	Model	AIC	
1	Salinity*Flow*Cyanobacteria*Diatom, random = ~1 Year	583.85	
2	Salinity*Flow*Cyanobacteria*Diatom, random = ~1 Season	586.05	
3	Salinity*Flow*Cyanobacteria*Diatom, random = ~1 Year/Season	585.85	
4	Salinity*Flow*Cyanobacteria*Diatom, random = ~1+Season Year	601.85	
5	Salinity*Flow*Cyanobacteria*Diatom, random = ~1+Season Year/Season	621.85	
Results model 1 – residual standard deviation = 2.340			
Variable	estimate	ChiSq	p-value
(Intercept)	-17.571		
Salinity	-2.638	0.014	0.902
River flow	-29.032	7.621	0.005
Cyanobacteria	1.157	0.479	0.488
Diatom	-8.061	47.591	< 0.001
Salinity*River flow	-26.913	0.603	0.437
Salinity*Cyanobacteria	-14.521	0.106	0.744
River flow*Cyanobacteria	-44.801	1.414	0.234
Salinity*Diatom	-14.669	14.898	< 0.001
River flow*Diatom	-62.493	0.608	0.435
Cyanobacteria*Diatom	-0.172	5.656	0.017
Salinity*River flow*Cyanobacteria	-44.346	0.875	0.349
Salinity*River flow*Diatom	-62.380	0.015	0.900
Salinity*Cyanobacteria*Diatom	-51.777	2.090	0.148
River flow*Cyanobacteria*Diatom	-102.714	0.091	0.762
Salinity*River flow*Cyanobacteria*Diatom	-103.031	0.564	0.452

Table 2 Mixed-effects model results for zooplanktivorous fish $\delta^{13}\text{C}$. Environmental parameters are direct factors and ‘Season’ and ‘Year’ are random factors. (n°) is the model number; (*) denote interactions among factors; (/) indicates nested factors. Values in bold are significant at $\alpha=0.05$

n°	Model	AIC		
1	Salinity*Flow*Cyanobacteria*Diatom, random = ~1 Year	396.53		
2	Salinity*Flow*Cyanobacteria*Diatom, random = ~1 Season	395.38		
3	Salinity*Flow*Cyanobacteria*Diatom, random = ~1 Year/Season	398.40		
Results model 1 – residual standard deviation = 0.883				
	Variable	estimate	ChiSq	p-value
	(Intercept)	-30.19		0.295
	Salinity	-4.82	0.295	0.478
	River flow	-25.90	0.478	0.031
	Cyanobacteria	27.420	0.031	0.107
	Diatom	-26.45	0.107	0.227
	Salinity*River flow	8.56	0.227	0.966
	Salinity*Cyanobacteria	-14.17	0.966	0.035
	River flow*Cyanobacteria	-50.19	0.035	0.818
	Salinity*Diatom	23.12	0.818	0.979
	River flow*Diatom	1.25	0.979	0.404
	Cyanobacteria*Diatom	17.12	0.404	0.483
	Salinity*River flow*Cyanobacteria	-42.10	0.483	0.363
	Salinity*River flow*Diatom	-42.10	0.363	0.089
	Salinity*Cyanobacteria*Diatom	-22.51	0.895	0.643
	River flow*Cyanobacteria*Diatom	-0.76	0.643	0.680
	Salinity*River flow*Cyanobacteria*Diatom	-35.31	0.680	0.295

Table 3 Spearman correlation results ($\alpha=0.05$) between environmental variables and assimilation of allochthonous (freshwater and marine) material by detritivorous and zooplanktivorous fishes

	Freshwater		Marine	
	ρ	p-value	ρ	p-value
Detritivores				
Rainfall	-0.62	0.996	0.63	0.002
River flow	-0.48	0.970	0.49	0.019
Salinity	0.36	0.933	-0.42	0.024
Chlorophyll- <i>a</i>	-0.37	0.121	0.34	0.158
Zooplanktivores				
Rainfall	0.30	0.109	-0.11	0.679
River flow	-0.11	0.674	0.22	0.180
Salinity	-0.02	0.464	-0.24	0.160
Chlorophyll- <i>a</i>	0.17	0.475	-0.21	0.380

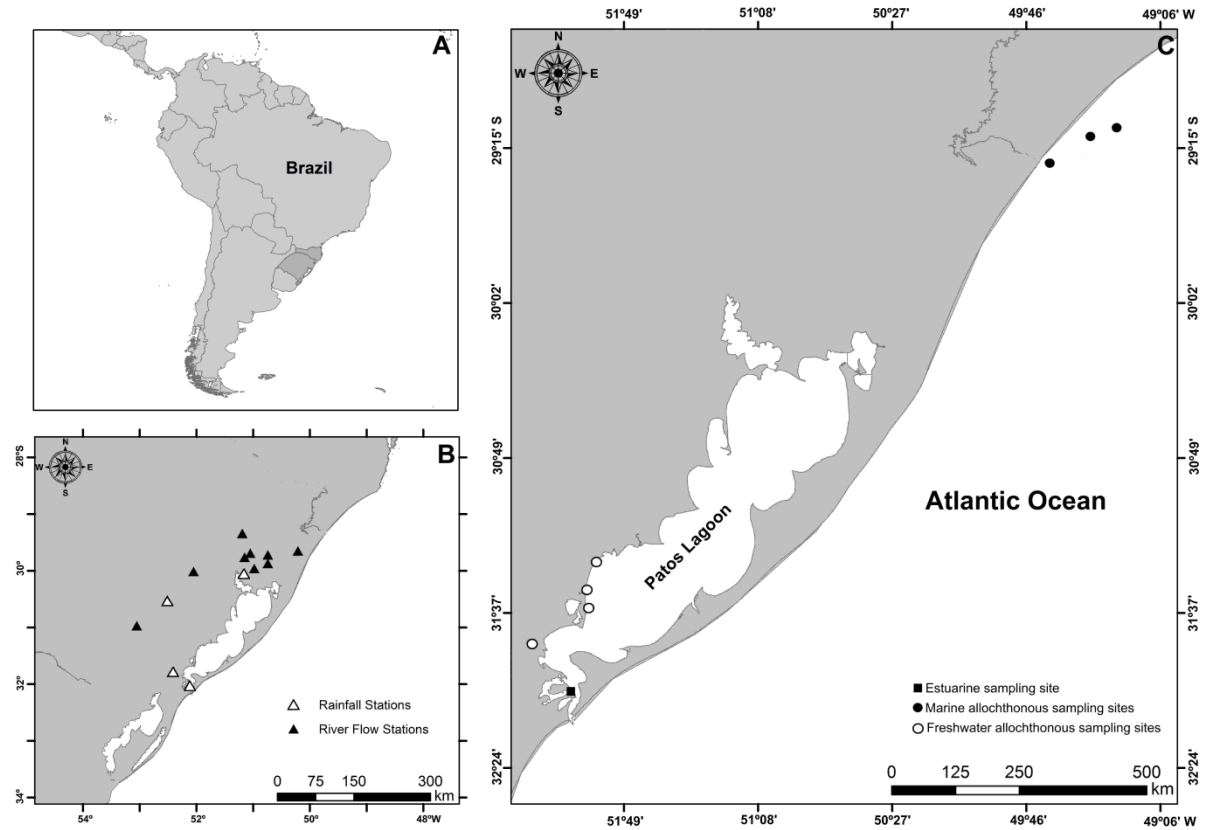


Fig. 1 Map of South America (A) showing the locations of the meteorological stations in the drainage basin of Patos Lagoon (B), where we obtained rainfall (filled triangles) and river flow (open triangles) data. Location of the long-term ecological research site in the estuary (filled square), where we sampled autochthonous sources and consumers (detritivorous and zooplanktivorous fishes) from 2010 through 2016, and locations at the northern portion of the lagoon and in the adjacent marine area (circles) where we obtained samples of freshwater (open circles) and marine (filled circles) allochthonous sources, respectively (C)

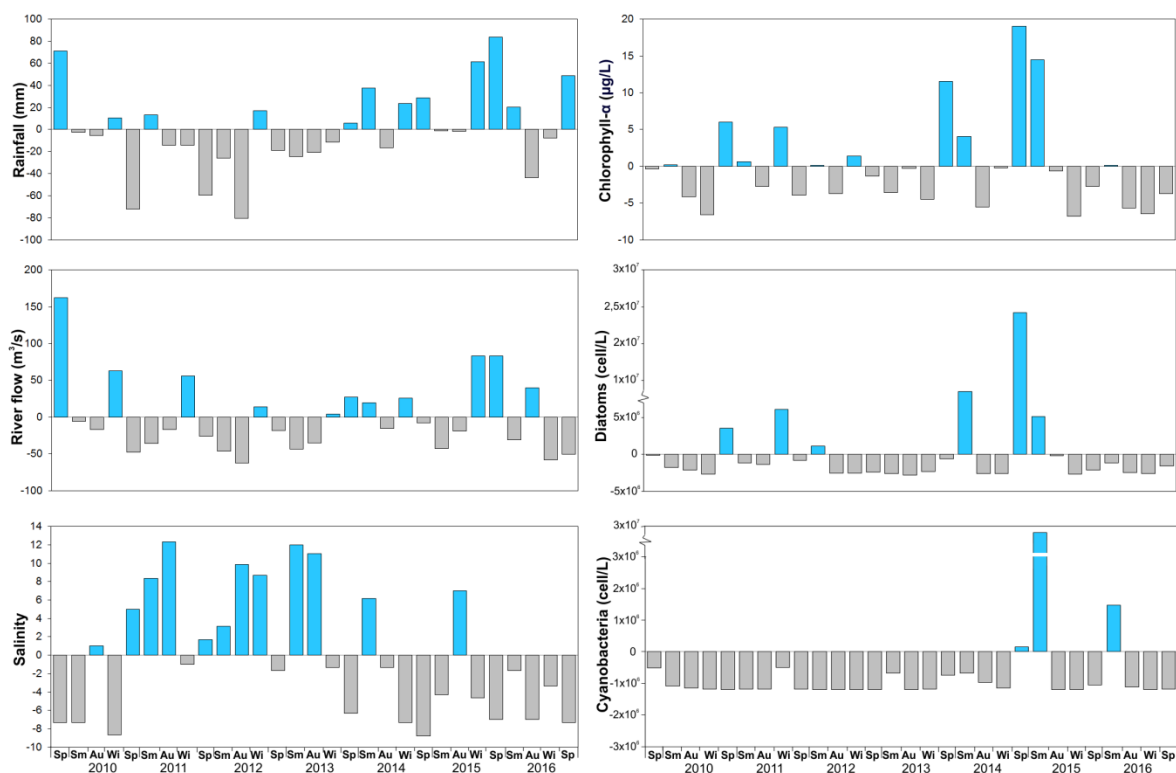


Fig. 2 Seasonal variation (2009-2016) in rainfall (mm) and river water flow (m^3/s) anomalies in the drainage basin of Patos Lagoon, and in salinity, chlorophyll- α ($\mu\text{g/L}$) and phytoplankton abundance (cells/L) anomalies in the estuarine zone of the lagoon

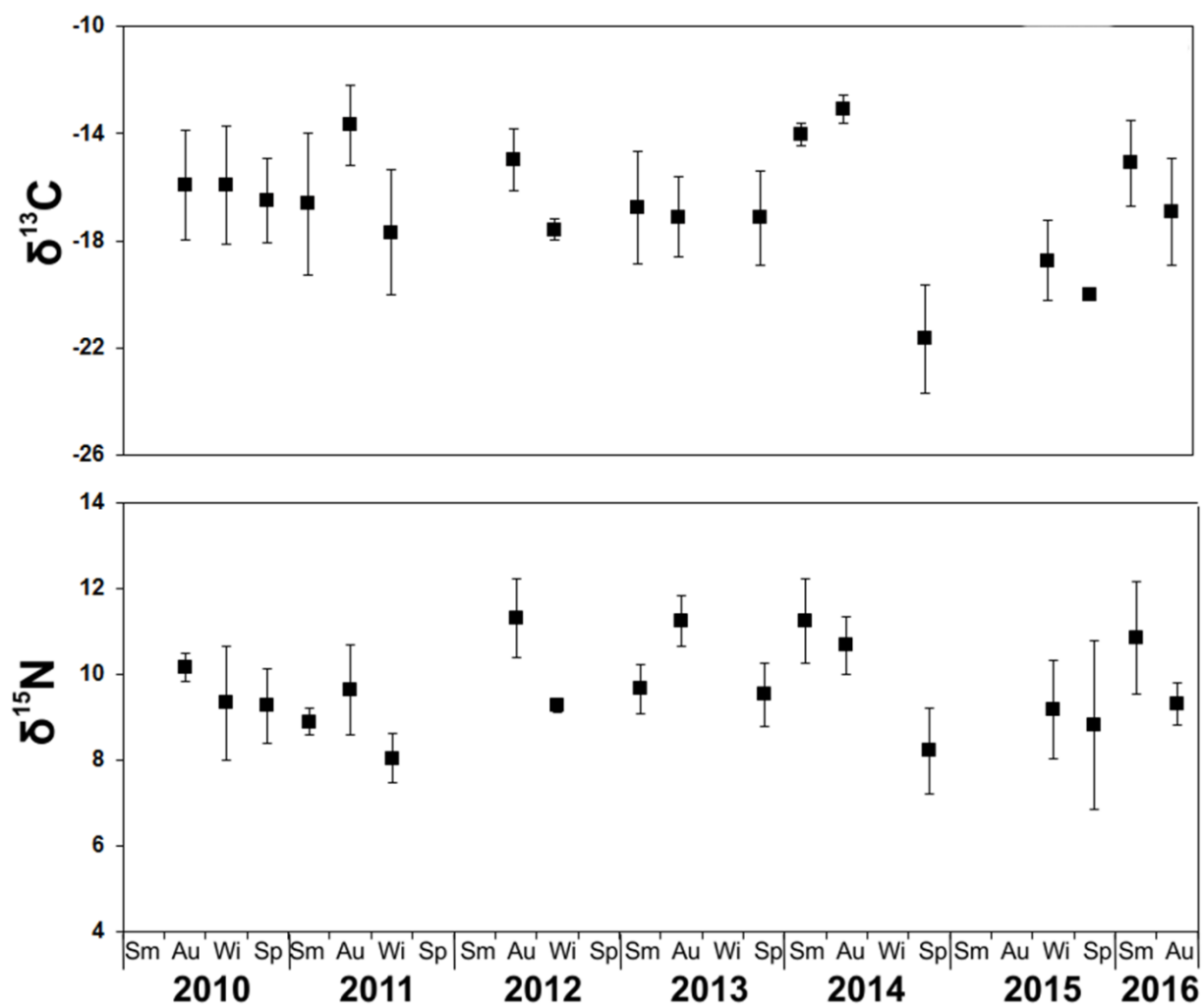


Fig. 3 Seasonal changes in average ($\pm$ SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of detritivorous fishes sampled from 2010 through 2016 at Patos Lagoon Estuary. Sm: Summer, Au: Autumn, Wi: Winter and Sp: Spring

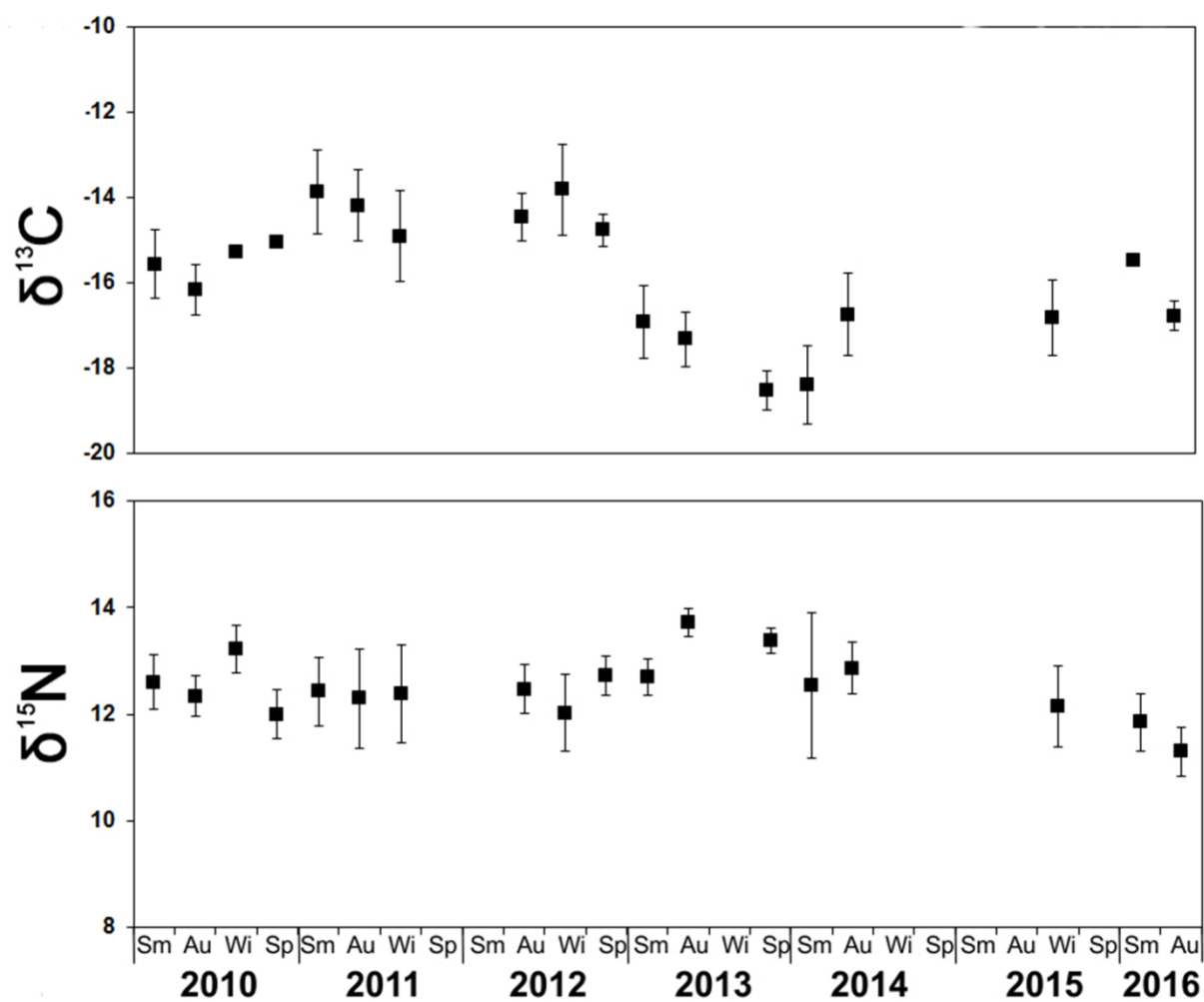


Fig. 4 Seasonal changes in average ($\pm$ SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of zooplanktivorous fishes sampled from 2010 through 2016 at Patos Lagoon Estuary. Sm: Summer, Au: Autumn, Wi: Winter and Sp: Spring

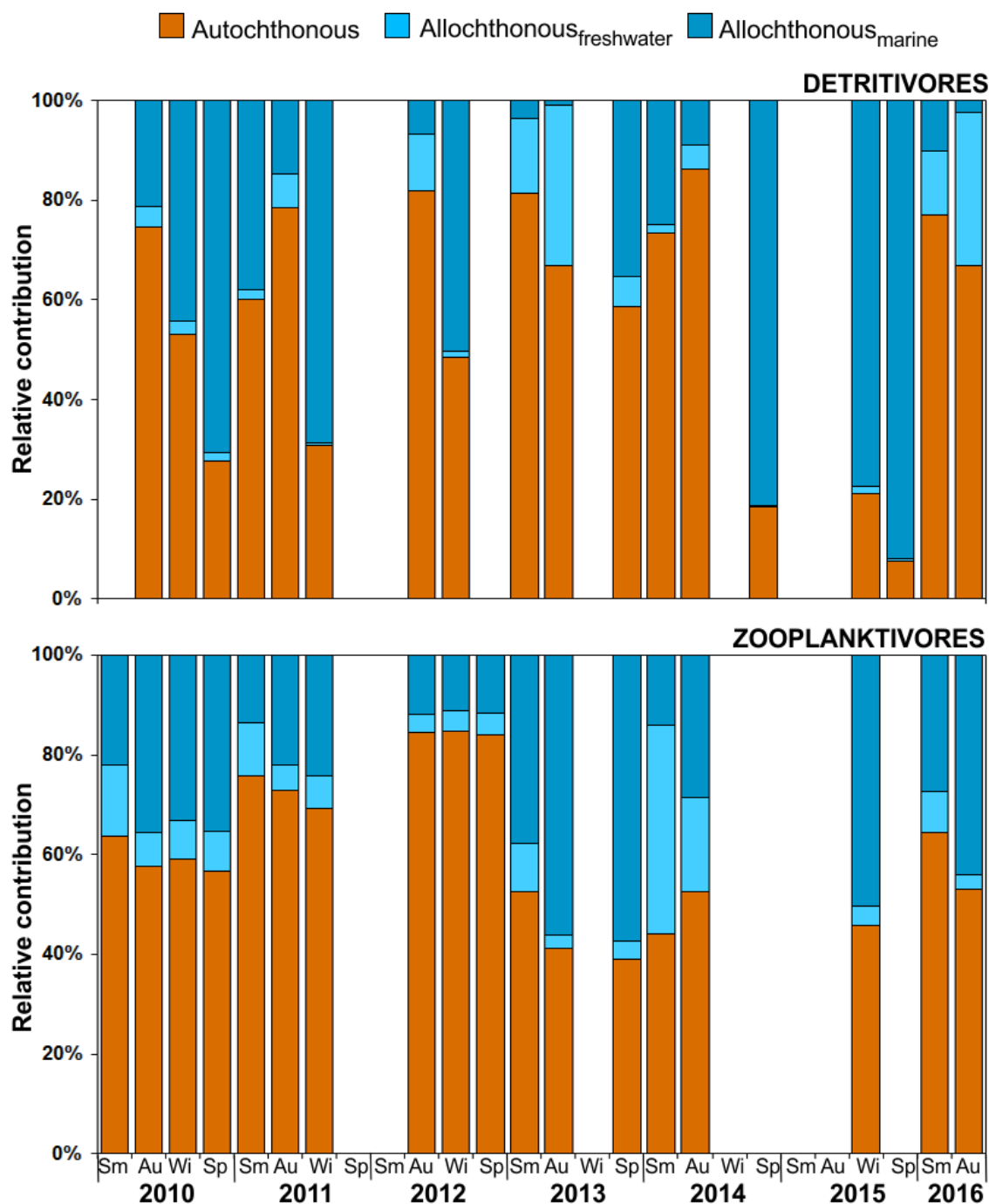


Fig. 5 Median (50%) percent contribution of carbon sources to detritivorous and zooplanktivorous fishes in Patos Lagoon Estuary from 2010 through 2016. Sm: Summer, Au: Autumn, Wi: Winter and Sp: Spring. See Table S3 for 95% Bayesian credibility intervals for each median value

ANEXO 3 (REFERENTE AO CAPÍTULO III)

**Environmental Factors Driving Interannual Variability in a Shallow-Water Estuarine
Food-Chain Length**

*Refere-se ao terceiro capítulo da tese, em preparação
para submissão na Ecosystems.*

Formatado nas normas da Ecosystems.

ABSTRACT

Biological communities are strongly affected by environmental variability. Estuarine biota, in particular, faces local (salinity) and regional (rainfall, river flow) abiotic factors varying at distinct spatial and time scales. The *El Niño* is a coupled ocean–atmosphere phenomenon occurring at irregular time scales that affects biological communities worldwide, including changes in species composition and diversity patterns. One of the most important attributes in community ecology is the food-chain length because determines the ecosystem functioning. Prior theories predict food-chain length increases with productivity and species richness. Based on the ‘intermediate disturbance’ theory and prior evidences showing effects of *El Niño* on animal diversity, we hypothesized this phenomenon will increase richness (both at species and trophic guilds levels) and food-chain length in a shallow-water estuarine community. To evaluate our hypothesis, we analyzed a 9-yr dataset of carbon and nitrogen stable isotope ratios of food web components in a shallow embayment of a subtropical estuary. We estimated consumer’s trophic positions (TP) using a recent Bayesian approach that incorporates individual variability and sampling error in isotope fractionation, isotopic baselines and consumers. We also designed four approaches to generate baselines and to reveal the sensitivity of TP estimations to different methods of computation. As expected, both species and trophic guilds richness were higher during *El Niño* events. Food-chain length ranged from 2.45 to 4.60, but against our prediction showed a slight decreased during higher rainfall periods. These finding may suggest that food-chain length in estuaries is resilient to environmental variability and natural climatic perturbations.

Keywords: Baseline, community, ecosystem attributes, *El Niño*, intermediate disturbance theory, resilience, stability, stable isotopes, trophic chain, trophic position.

1. INTRODUCTION

Biological communities are dynamic in space and time (Begon and others 2006; Day and others 2012) and environmental variability is constantly influencing their attributes. This influence can occur by changes on abundance (Garcia and others 2004; Alegre and others 2015), dominance (Livingston and others 1997), interspecific relationships (Winemiller and Layman 2005; Begon and others 2006), trophic interactions (Livingston and others 1997; Nelson and others 2015; Possamai and others 2018, Garcia and others 2019), among other attributes.

Estuaries are examples of environments where abiotic factors fluctuate almost constantly. It is a very dynamic environment, changing its profiles of temperature, salinity, pH, at different time scales (days, months, years) (McLusky 1990; McLusky and Elliott 2004). This dynamic system is mostly driven by hydrology, which has a strong influence on community attributes. For example, in dry periods with low freshwater inflow, estuaries could be hypersaline and the decrease in nutrient supply does not support high levels of primary production (Bishop and others 2017). Additionally, differences in hydrogeomorphology in estuaries influence the assimilation of food resources by consumers (Livingston and others 1997; Hoeinghaus and others 2011, Garcia and others 2017; Garcia and others 2019), and might even affect the food-chain length of these ecosystems, as observed in other aquatic environments (Saigo and others 2015). In some cases, estuarine hydrology are indirectly influenced by large-scale climatic events that alter the input of freshwater discharge (Robertson and Mechoso 1997; Fernandes and others 2002).

El Niño is a large-scale climatic phenomenon affecting abiotic parameters in estuaries and, consequently, their biological community attributes (Barber and Chavez 1983; Fisher and others 2015). In South America, the major consequence of these events is higher rainfall and freshwater discharge into estuaries of the Western Atlantic Ocean (Robertson and

Mechoso 1997; Grimm and others 1998; Garcia and others 2003; Grimm 2009; Possamai and others 2018). Due to these hydrological effects triggered by *El Niño* events, estuarine communities in South Western Atlantic change some of their biological attributes. For instance, Garcia and others (2003; 2004) observed an increase of freshwater species in the estuary during *El Niño* events concomitantly with a decrease of estuarine-resident fish species. Possamai and others (2018) found higher trophic diversity of fish assemblages, with insectivores, herbivores, and piscivores species increasing their abundances in the estuary during *El Niño* events. Furthermore, Garcia and others (2019) showed that allochthonous matter derived from freshwater systems can be assimilated by estuarine consumers during *El Niño* events. In fact, climatic effects of this phenomenon are noticed in different parts of the world, leading to changes in biological community attributes in different ways. In the Korean coast, the anchovy abundances increase (Kim and Kang 2000), whereas in Chilean coast this species decrease its abundance during *El Niño* events (Escribano and others 2004). In San Francisco Bay, United States, the planktonic community change in biomass and composition during these events (Lehman 2000).

El Niño effects on estuarine communities could be considered as example of disturbance in the context of the ‘intermediate disturbance theory’ (Grime 1973; Connell 1978; see Wilkinson 1999). According to this theory, there will be high diversity at the peak of the disturbance, and after that, the community will spring back to the previous condition. Prior works on *El Niño* effects on estuarine biota already provided evidences showing that community attributes like species richness (Garcia and others 2003; 2004) and trophic guild diversity (Livingston and others 1997; Possamai and others 2018) are significantly affected by this climatic disturbance, but they return to previous conditions soon after the event. However, it remains largely unknown what will be the effects of this climatic disturbance on

ecosystem attributes as consumers' trophic positions and the food-chain length of estuarine communities.

Food-chain length is the number of unidirectional exchange of matter and energy *via* feeding. The transfer of energy into an ecosystem is the main process in ecological systems, once it determines the ecosystem functioning (Pimm 1982). There are many hypothesis about what is the reason for different food-chain length found in different ecosystems. One of the first hypothesis was described by Elton (1927), who argued that prey abundance determines the food-chain length, which would be no greater than five levels. He predicted that in more productive habitats, the availability of food allows larger animals to survive and the food-chain length would be longer than in less productive habitats (Post and others 2000). Likewise, Paine (1966) analyzing a variety of systems observed that the food-chain length in biological communities is higher when diversity increases, especially because top-predators control the abundance of dominant intermediate species. It is worth noting, however, that species addition in a community will not lead necessarily to longer food-chains, but could result simply in a greater number of trophic links within the food web (Hoeinghaus and others 2008). Hence, despite of the considerable theoretical development on the physical and biological driving forces controlling food-chain length, there is still need to increase our empirical evidences mechanisms affecting this attribute in aquatic ecosystems (Winemiller and Layman 2005; Lotze and others 2006). Considering the predictions that *El Niño* events may become more frequent and intense in a global warming scenario (Timmermann and others 1999; Yeh and others 2009), it is important to advance our current knowledge on its effects on ecosystem's attributes to provide empirical evidences to evaluate theoretical predictive models (Winemiller and Layman 2005).

Based on the 'intermediate disturbance' theory and prior evidences showing effects of *El Niño* on animal diversity, we hypothesized this phenomenon will increase trophic richness

and food-chain length in a shallow-water estuarine community. To evaluate our hypothesis, we analyzed a 9-yr dataset of carbon and nitrogen stable isotope ratios of food web components in a coastal lagoon in South America, which is under influence of *El Niño*. Nitrogen stable isotope ratios (N^{15}/N^{14}) have been used to estimate consumers' trophic positions (TP) due to its marked isotopic fractionation at each food chain level (Post 2002; Vanderklift and Ponsard 2003). However, this method is very sensitive to the chosen isotopic baseline, which may vary in time and space (Post 2002). Thus, to improve our TP estimations, we designed four approaches to generate isotopic baselines that revealed the sensitivity of TP estimations to different methods of isotopic baseline computation.

2. MATERIALS AND METHODS

2.1. Studied estuary

Patos Lagoon (Fig. 1) is the largest choked lagoon in the world (*sensu* Kjerfve 1986) and is located in South Brazil. Its southern portion has estuarine conditions (around 10% of the total size of the lagoon) and it is connected to the Atlantic Ocean throughout a single and narrow inlet (Fig. 1). This funnel-shaped geomorphology disfavors the entrance of marine waters into the lagoon and allows a marked influence of freshwater coming from the limnetic area of the lagoon located in its northern portion. Hence, freshwater discharge and NE and SW winds (driving seaward and landward flows, respectively) are the main drivers controlling the hydrodynamic and circulation of Patos Lagoon Estuary (hereafter 'PLE'). The mean annual freshwater discharge in the lagoon is $2000 \text{ m}^3\text{s}^{-1}$, but peaks of 8,000 and 12,000 m^3/s can occur during *El Niño* events (Moller and others 1996; Fernandes and others 2002). During these events, PLE may remain fresh by several months (Garcia and others 2001; Garcia and others 2004).

The hydrological regime drives species composition and abundance patterns at PLE (Odebrecht and others 2017). For instance, during strong *El Niño* events the increase freshwater inflow carries out freshwater fish species (Garcia and others 2003; Possamai and others 2018), zooplankton (Teixeira-Amaral and others 2017) and phytoplankton (Odebrecht and others 2010) into the estuary.

2.2. Sampling and laboratory processing

Primary food sources (i.e. primary producers and particulate organic matter) and consumers have been sampled for stable isotopes analysis seasonally in a shallow embayment (< 1.5 m) of PLE since 2010 by the Brazilian Long-Term Ecological Research (B-LTER) program. The representative primary food sources sampled are saltmarshes (C_3 and C_4 plants), macroalgae, seagrass, particulate organic matter in suspension (POM) and in sediments (SOM). Prior studies at PLE had showed that the assimilation of saltmarsh plants with C_3 photosynthetic pathway (e.g. *Scirpus maritimus*) is negligible (Claudino and others 2013; Lanari and others 2018). Hence, we considered only saltmarsh plants with C_4 photosynthetic pathways in our isotopic analysis (see next section). Saltmarsh plants (*Spartina alterniflora* and *S. densiflora*), macroalgae (*Rizochlonium* sp. and *Ulva* sp.) and seagrass (*Ruppia maritima*) were collected by hand using scissors. POM was obtained by filtering 0.25–1.00 liters of water onto a pre-combusted (450°C for 4 h) Whatman glassfibre filter (0.75 μ m), whereas SOM was obtained by removing the top 2 cm from the surface of the sediment using a plastic core (10 cm diameter). Fishes, blue crabs, and shrimps were collected using beach seine hauls (13 mm mesh size in the wings and 5 mm in the center of the net), whereas terrestrial crabs near the edge of the saltmarsh were collected by hand (see Table S5 for species list). All samples were stored in plastic bags, preserved on ice for transportation, and stored in a freezer in the laboratory until processing. Field collections were carried at the end

of each season because experimental studies with fish species of PLE showed that their carbon and nitrogen isotopic turnovers in muscle tissues reflect food assimilated during the previous 2 to 3 months (Mont'Alverne and others 2016; Oliveira and others 2017).

In laboratory, thawed samples were rinsed with distilled water, placed in sterile Petri dishes and dried in an oven at 60°C for 48 h. Dried samples were ground to a fine powder with a mortar and pestle and stored in clean 2 ml plastic tubes. Subsamples were weighed to 10^{-3} g and pressed into Ultra-Pure tin capsules (Costech, Valencia, CA) and sent to the specialized laboratory for analysis of carbon and nitrogen isotope ratios. Stable isotopes results are reported as parts per thousand (‰) differences from a corresponding standard: $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where $R = {}^{15}\text{N}/{}^{14}\text{N}$ or ${}^{13}\text{C}/{}^{12}\text{C}$. Standards were carbon in the PeeDee Belemnite and nitrogen in air. Internal standards (animal and vegetal) of known carbon and nitrogen composition were analyzed with each sequence in order to estimate instrument precision. For animal samples the standard deviation were $\delta^{13}\text{C} = 0.07\text{‰}$ and $\delta^{15}\text{N} = 0.06\text{‰}$; for vegetal were $\delta^{13}\text{C} = 0.15\text{‰}$ and $\delta^{15}\text{N} = 0.37\text{‰}$. As we utilized two different laboratories along the years (2010-2015: Analytical Chemistry Laboratory, University of Georgia and 2016-2018: Limnology Laboratory, University of North Texas), we run a subset of random samples in both laboratories to check for consistency in their isotopic determination. The test revealed low between-laboratory standard deviation and no statistically significant differences both for nitrogen (SD= 0.08‰; $t=0.004$, d.f.= 7.988, p-value=0.996) and carbon (SD=0.03‰, $t=0.008$, d.f.=8, p-value=0.993) isotope ratios.

Monthly values of water temperature and salinity were obtained using a thermometer and refractometer respectively. Monthly rainfall (mm) and river discharge (m^3/s) was provided by meteorological stations of the Brazilian National Water Agency (ANA – Agência Nacional de Águas) located at drainage basin around the margins of Patos Lagoon (Fig. 1). Monthly values were averaged across seasons for subsequent data analysis, as follow:

Summer (December, January, February), Autumn (March, April, May), Winter (June, July, August) and Spring (September, October, November).

2.3. Data analysis

2.3.1. Evaluating different isotopic baseline computations

A key step before estimating trophic position (TP) of a consumer using stable isotope analysis is the establishment of an appropriate isotopic baseline. For instance, an appropriate isotopic baseline should incorporate all temporal isotopic variability occurring in the base of the food web sustaining the consumer (Vander Zanden and Rasmussen 2001; Post 2002), but there is no consensus on the different methods for generating an isotopic baseline (Phillips and others 2014). In order to evaluate the sensitive of our TP estimations to different ways to generate isotopic baselines, we employed four distinct methods of computation: (i) an overall average (+SD) of the $\delta^{15}\text{N}$ values of all primary food sources collected between 2010 and 2018 period (denoted as ‘global’), (ii) average (+SD) $\delta^{15}\text{N}$ values of primary food sources collected concomitantly with consumers in the same season (denoted as ‘concomitantly’), (iii) average (+SD) $\delta^{15}\text{N}$ values of primary food sources (producers) collected in the prior season before the collection of consumers (denoted as ‘delayed’), and (iv) average (+SD) $\delta^{15}\text{N}$ values of primary food sources collected in the prior and the same season where consumers were sampled (denoted as ‘interpolation’). These approaches allowed us to evaluate isotopic baselines encompassing distinct time windows of isotopic variability in the primary food sources (e.g. from years to different season intervals). For each method (excluding “global”), we evaluated the differences in average values of $\delta^{15}\text{N}$ among primary food sources in each season using Tukey HSD test (Table S1 and S2). When the test distinguished two different groups with distinct average $\delta^{15}\text{N}$ values, we used these groups as two different isotopic

baselines in our models to estimate TPs. The primary food sources for each season and the isotopic baselines chosen for each method can be found in tables S1, S2 and S3.

We used the following criteria to choose the best method to generate the isotopic baseline. Firstly, we classified the species in the following trophic guilds based on stomach content data obtained in the literature (Possamai and others 2018 for fishes; Collins 1999, Oliveira and others 2006, Abreu and others 2007 and Bemvenuti and Colling 2010 for crustaceans): detritivores (DTV), herbivores (HBV), omnivores (OMN), zooplanktivores (ZPL), zoobenthivores (ZBV) and piscivores (PSV). Secondly, we ordered these trophic guilds according to the expected increasing trophic levels from the base to the top of the food chain, following FishBase data (Froese and Pauly 2019) (Table S4). Finally, we chose the isotopic baseline generated by the method that better matched the expected model ($TL = 0.426x + 1.534$; where TL is the trophic level and x is the number of the trophic guild – 1 for DTV, 2 for HBV, 3 for OMN, 4 for ZPL, 5 for ZBV and, 6 for PSV).

2.3.2. Trophic position estimation

The TP estimation was performed separately in each season using the ‘tRophicPosition’ package in R (Quezada-Romegialli and others 2018). This new method constitutes a better tool to estimate consumer’s TP than prior methods because uses a Bayesian approach that incorporates individual variability and propagating sampling error of trophic discrimination, isotopic baselines and consumers and posterior estimates of parameters. Moreover, this method allows estimation of TP based on one or two isotopic baselines, including the carbon values (Quezada-Romegialli and others 2018). The TP of each species was modeled using the Markov chain Monte Carlo (MCMC) method with 10,000 interactions and 10,000 adaptive samples in JAGS 4.3.0 for both models of one or two baselines. The trophic discrimination factors used were $\delta^{13}C = 0.54 \pm 0.53$ and $\delta^{15}N =$

3.02 ± 0.47 for omnivores, zoopanktivores and zoobenthivores; $\delta^{13}\text{C} = 0.54 \pm 0.53$ and $\delta^{15}\text{N} = 4.78 \pm 1.30$ for herbivores and detritivores and $\delta^{13}\text{C} = 0.82 \pm 0.47$ and $\delta^{15}\text{N} = 1.77 \pm 0.30$ for piscivores (for details see Bastos and others 2017).

2.3.3. Relationships between trophic position and environmental factors

We used Spearman correlations to evaluate potential relationships between the ecological attributes of species richness (i.e. number of species sampled at each season), trophic richness (i.e. number of trophic guilds sampled in each season) and TP with environmental factors. Maximum TP of each season was used as food-chain length (see Post and others 2000). A linear model was performed to evaluate the response of the abiotic factors to the food-chain length. Explaining variables were transformed by units of variance (`decostand(x, method="standardize")`) using the ‘vegan’ package of R software. We employed a Generalized Linear Model (GLM) with Gamma distribution [$Y \sim \text{Ga}(\alpha, \beta)$] and link function “inverse” to evaluate which environmental variable better explained variation in consumer’s TP (‘stats’ package). The response variable was log-transformed for the best fit in Gamma distribution. As the link of Gamma is the inverse matrix, we need to interpret inversely the estimators. All statistical tests were performed with $\alpha=0.05$ and using R software version 3.5.3 (R Core Team 2019).

3. RESULTS

3.1. Environmental variability

There were relationships between some of environmental factors along the studied period (2010-2018) (Fig. 2). Rainfall was positively correlated with river flow ($\rho = 0.54$, p-value < 0.001) and both were negatively correlated with salinity ($\rho = -0.28$, p-value < 0.001 and $\rho = -0.59$, p-value < 0.001 ; respectively) (Fig. 2). During the 2009-2010 and 2015-2016

El Niño events, rainfall increased substantially and was followed by seasons with lower river discharges and salinity values. Water temperature variations between 2010 and 2018 were characterized by clear annual cycles with lower and higher average values during winter ($15.88 \pm 3.48^\circ\text{C}$) and summer ($25.23 \pm 2.24^\circ\text{C}$), respectively, with no statistically significant correlations between temperature and the other environmental factors (Fig. 2).

3.2. Isotopic baseline selection

The application of the four methods to generate isotopic baselines resulted in different values of average trophic positions for the analyzed trophic guilds. The range of food-chain length was from 3.44 to 4.61 for “Global” method; from 2.30 to 4.32 for “Concomitantly” method; from 2.45 to 4.60 for “Delayed” method and; from 2.40 to 4.22 for “Interpolation” method. The pattern obtained with the ‘delayed’ method matched more closely the expected model of increasing trophic levels according to their trophic guilds: primary consumers (DTV, HBV), secondary or tertiary consumers (OMN, ZPL, ZBV) and tertiary consumers (PSV) (Fig. 3). Hence, we considered the “delayed” method as the best fit model and used its output data in the subsequent analysis of correlation with environmental factors.

3.3. Trophic position and environmental correlations

The overall trophic position was 2.85 ± 0.59 for this shallow waters community, and the TP for each species can be found in Table S4. We observed a slightly tendency for lower TP values during periods of high rainfall ($\rho = -0.15$, p-value = 0.033) and higher salinities ($\rho = -0.14$, p-value = 0.038) (Fig. 4). In contrast, river flow was positively related with variation in overall TP ($\rho = 0.23$, p-value < 0.001). There was no relationship between trophic position and temperature ($\rho = -0.05$, p-value = 0.457) and richness ($\rho = 0.14$, p-value = 0.452). The GLM models employed to further investigated these relationships also showed a negative

relationship with rainfall and a positive relationship with river flow, but not with salinity (Table 1).

3.4. Temporal variation in food-chain length and trophic richness

Food-chain lengths ranged from 2.45 (autumn of 2014) to 4.60 (spring of 2013), with values decreasing during higher rainfall periods (Table 2). The food-chain lengths were not affected by temperature, salinity and discharge. Although the food-chain length seems to decrease with the increasing of trophic richness (Fig. 5), the linear model did not revealed a relationship between these variables (Table 2).

The species richness was correlated with trophic guilds richness ($\rho = 0.61$, $p\text{-value} < 0.001$) (Fig. 5). A relationship was observed between trophic richness and rainfall ($\rho = 0.38$, $p\text{-value} = 0.042$), with higher richness values found in rainy periods. Salinity ($\rho = -0.23$, $p\text{-value} = 0.216$), river discharge ($\rho = 0.17$, $p\text{-value} = 0.383$) and temperature ($\rho = -0.16$, $p\text{-value} = 0.388$) were not related with the trophic richness.

The GLM showed a positive effect of zooplanktivores and zoobenthivores in increasing the overall TP compared to the detritivores (Table 3). The influence of piscivores was statistically marginally significant, which could be partially explained by their lower number of samples (Table 3).

4. DISCUSSION

The food-chain length is one of the most important attributes of biological communities (Elton 1927) because its strong influence in the functioning of the ecosystems (e.g. energy flow and productivity). Here, we evaluated the variability of food-chain length in an estuarine shallow water community, observing how biological communities are influenced

in different ways by abiotic factors. Moreover, we evaluated different methods to choose a baseline and proposed the more reliable one for trophic position estimation.

Two *El Niño* events (2009-2010 and 2015-2016) occurred and influenced hydrological conditions in the studied estuary during our analyzed isotopic time-series (2010-2018). Both *El Niño* events were associated with rainfall and river flow above the average (Fig. 2). Aside these events, some seasons like the Spring 2013 and Summer 2014 also showed rainfall above the average. During these hydrological effects, the species richness increased in the estuary, as well as the trophic richness, which allowed the investigation of our hypothesis of increasing of the food-chain length during this phenomenon. Indeed, the hydrology affected the trophic position of the consumers, increasing it in higher freshwater flow periods. Hereafter, firstly we discussed our methodological approach to choose the isotopic baseline in a separated topic, followed by topics discussing the ecological meanings of variation in food-chain length.

4.1. Isotopic baseline choice

The baseline choice is one of the most critical issues when computing trophic position using stable isotope analysis (Post 2002). This because nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) stable isotope ratios of an organism may vary among ecosystems and through time. Moreover, long-lived migrating organisms, like some fishes, can incorporate the isotopic variation of producers and detritus in different ecosystems (Post 2002) during the different phases of its life cycle, which difficult the definition of a unique baseline for these consumers.

Besides the isotopic variation issues concerning spatial and time variations, the isotopic turnover differences between the lifetime of consumers and producers always must be taken into the account in the isotopic analysis (Vander Zanden and Rasmussen 2001). In our study, consumers (especially fishes) have a longer lifetime than some of the analyzed

primary producers. This means they have differences in organism growth rates, which influences the isotopic assimilation (Fry and Arnold 1982) and the baseline choice must consider this isotopic mismatch. Smaller organisms with fast growth rates show quicker isotopic incorporation of food in their tissues than slower growth organisms (Fry and Arnold 1982). This fact could explain why the "delayed" method shown to be the best choice for a baseline. The time lag used in this method was 3 months, which matched approximately with the isotopic turnover time expected of food assimilation and incorporation in the tissues of fishes (Mont'Alverne and others 2016; Oliveira and others 2017). In contrast, the isotopic baseline based on concomitantly sampling of consumers and food sources, which are commonly used in many work (e.g. Hoeinghaus and others 2008; Saigo and others 2015; Mazumder and others 2017), did not render good fits probably because failed to isotopically match the isotopic turnover between consumers and their assimilated food. Therefore, our findings reinforce the need to have an appropriate sample design of basal food sources to match the isotopic composition of the the consumers, before computing their trophic positions (Post 2002; Phillips and others 2014). It is important to evaluate the lifespan and isotopic turnover of the consumers before deciding on the frequency and necessary delay of the food sources. We encourage seasonal sampling of sources and consumers to enable the use of different isotopic time-lags in order to improve the trophic position estimations.

4.2. Trophic position

Overall, as predicted by our hypothesis, trophic position (TP) variation was related to the *El Niño* induced effects. However, the direction and strength of these relationships revealed a scenario more complex than we initially expected. The increase in rainfall decreased the overall TP, which was contradictory to the positive relationship found between river flow and TP and the negative relationship between salinity and TP. One factor that could

be influencing these relationships is the time-lags among the analyzed abiotic variables (Moraes and others 2012). For instance, the river discharge was measured in the north portion of the studied lagoon (Fig. 1B) and some time is needed to water flows until the south portion of the lagoon and reaches its estuarine zone. In order to taken this effect into account, we analyzed our data in a seasonal basis, which incorporated three months of variability in these variables. Our findings showed that rainfall and river flow variables were positively correlated ($\rho = 0.54$) and both negatively related to the salinity, with a good fit. Therefore, time-lag between rainfall and river flow probably was not the reason why these variables respond differently than expected in relation to TP.

The increase in rainfall and, consequently, in river discharge can provide a lateral extension of the estuary, giving access to different marginal habitats to the shallow water community. In the marshes, there is more availability of invertebrates to be used as prey by carnivorous fishes (Nelson and others 2015), as well as, of primary producers and detritus to be explored by herbivores and detritivores (Rozas and Odum 1988). Moreover, habitat expansion during flooding events tends to decrease the intraspecific competition for food (Nelson and others 2015) and, with less competition and higher availability of resources, consumers trophic' levels may decrease (Elton 1927; Paine 1966; Hoeninghaus and others 2008; Ishikawa and others 2017). These mechanisms could explained the decreasing in consumer's TP in the studied estuarine shallow water community during events of higher rainfall.

The contradictory patterns found between rainfall and river discharge demonstrated the unexpected complex relationships of these factors with temporal changes in TP. The estimates of the model were very low, indicating weak relationships among these variables. Moreover, the trophic position is an attribute of an individual specimen or species (Post and others 2000) and, therefore, each species could be responding differently to environmental

changes. Different trophic guilds were analyzed together, which could create noise in the analyzes once the trophic guilds represent different trophic levels. In this way, to analyze in an ecosystem level, the food-chain length could be a choice to make conclusions.

4.3. Variation in food-chain length

The food-chain length is one of the most important topics in ecosystems ecology. The structure and dynamics of a biological community are dependent of food supply (Elton 1927; Begon and others 2006), and changes in food-chain structure may alter the community dynamics, ecosystem functioning and primary and secondary productivity (Post and others 2000). MacArthur (1955) describes the stability of communities as totally dependent on the food-chain structure. More stable communities can survive more than less stable ones, and more trophic links among species would lead to more stability to the community (MacArthur 1955). Thereby, to know the food-chain length dynamic and what factors can influence its features is fundamental to understand biological communities and prevent environmental disasters.

The food-chain length was very variable from 2010 to 2018 in the studied estuarine shallow-water community. Against our initial hypothesis, the food-chain length decreased in more rainy periods. Overall, during periods of high rainfall and river discharge, estuaries receive higher terrestrial/freshwater contribution and increase their primary productivity (Abreu and others 2010; Haraguchi and others 2015), which theoretically, would allow a greater number of trophic levels in the food chain (Post and others 2000; Nelson and others 2015). However, Pimm (1982) and Briand and Cohen (1987) did not find evidence to this affirmation in the literature. Accordingly our study did not provided empirical evidence supporting the hypothesis. In fact, our results showed that the the food-chain length decreased during periods of higher rainfall and, supposedly, higher nutrients supply.

Therefore, the ‘productivity hypothesis’ was not corroborated by our empirical evidences based on our 9-yrs study of food-chain length of an estuarine shallow-water community. The ‘productivity hypothesis’ may be more suitable for less productive ecosystems (Begon and others 2006), which is not the case of estuaries, that are among the more productive marine systems (Lalli and Parsons 1996). The pattern reported in our study seems to be more in consonance with the one described by Lalli and Parsons (1996), with shorter food-chain in more productive systems. Feeding low in the food-chain is an advantageous adaptation, because more food resources are available on the basis of the food web (Pimm and Lawton 1977). In highly productivity ecosystems as estuaries, the competition for resources would be lower as well as their food-chain length (Elton 1927; Hoeinghaus and others 2008; Ishikawa and others 2017).

Our findings seem to give empirical support to the ‘intermediate disturbance’ theory, since we observed higher species richness and trophic guilds richness during periods of hydrological changes associated with the *El Niño* phenomenon. In contrast, our findings seem to be contrary to the idea that in diverse communities the food-chain length is longer because top predators control the abundances of intermediate consumers (Paine 1966). Increasing the number of species in the top of the chain does not necessarily imply the increasing of the food chain length. Rather, it can lead simply a greater number of energy exchanges (food links) in the food web (Hoeinghaus and others 2008). Adding apex predators at the community it may make the omnivory decreases, and this will increase the consumers' trophic positions (Post and others 2000). However, increasing the richness of a community not necessarily occurs by adding top predators; it can also be by addition of more basal consumers (as herbivores and detritivores). In fact, during *El Niño* periods, Possamai and others (2018) found an increase of trophic richness in the studied estuary, especially due

to the entrance in the ecosystem of herbivores (phytoplankton feeders and macrophytes/macroalgae feeders).

Our analysis of the temporal changes in trophic guilds composition showed that zooplanktivores and zoobenthivores were responsible for the increase in overall trophic position of the community. These are trophic guilds with more generalist consumers, so the variability of their diets favor the detection of changes in TP (Nelson and others 2015). Top-predators tend to be more generalists than their prey (Lindeman 1942), which is a good feature when the abundance of prey decrease (Elton 1927). This ability to feed on different prey possibly shortens the food-chain (Lindeman 1942). Piscivores are the apex consumers in this analyzed shallow-water community, and their abundance increased during rainy periods associated with *El Niño* events (Possamai and others 2018). However, their influence was not detected by the model, probably because of low number of piscivores in the samples (only 3), which was much lower than the other guilds (average of 39). Therefore, in the majority of the analyzed periods, zoobenthivores were the top-predators, and its influence in determining the food-chain length was higher.

It seems reasonable to argue that the high variability in local abiotic factors and in climatic perturbations associated with *El Niño* phenomenon observed in the studied ecosystem could contribute to shorter food-chain length in its shallow-water communities. Prior work comparing food-chains between high-gradient rivers and reservoirs had shown higher food-chain lengths in the more stable than in more dynamic ecosystem (Hoeinghaus and others 2008). Similarly, during *El Niño* periods, the water temperature increased in the California Current and this decreased the food-chain length of this system compared to non-*El Niño* periods (Ruiz-Cooley and others 2017). These studies suggest that ecosystems with high variability in their abiotic factors tend to have shorter food-chains. However, other studies comparing ecosystems have found that space/size and dimensionality of the habitat are more

important to define the food-chain length than productivity, dynamic or other abiotic factors (Briand and Cohen 1987; Post and others 2000; Begon and others 2006; Hoeinghaus and others 2008). These contradictory findings demonstrated that generalizations regarding the driving forces controlling food-chain length still depends of further theoretical and field work, especially in highly dynamic ecosystems as estuaries.

In summary, our results showed that both species richness (number os species) and trophic richness (number of trophic guilds) were higher during *El Niño* events. The average trophic level in the community ranged from 2.45 to 4.60 along the 9-yrs studied period. This variability can be due to the dynamism of the estuarine system, as well as the seasonality of estuary use by different species, which causes high variability in species composition reflecting in the trophic level of the community. However, food-chain length in our studied aquatic community seemed to be resilient to climatic perturbations associated with the El Niño phenomenon. This important ecosystem attribute was weakly or not statistically significantly related with abiotic factors like rainfall, river discharge, salinity and temperature. Although our findings suggest food-chain length in the studied estuary is resilient to environmental variability and natural climatic perturbations, future studies are needed on other subtropical estuaries to better understand how these productive ecosystem would cope with increasing man-made and climatic perturbations.

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Table 1. Generalized Linear Model results for the community trophic position (TP). Data from 2010 through 2018 in a shallow-water community of Patos Lagoon estuary, Brazil.

Model (Gamma, link = inverse)				AIC
Model 1 = $\log(\text{TP}) \sim \text{Temperature} + \text{Salinity} + \text{Rainfall} + \text{River flow} + e$				-138.34
Model 2 = $\log(\text{TP}) \sim \text{Temperature} + \text{Rainfall} + \text{River flow} + e$				-141.22
<i>Best choice (Chisq test) = Model 2</i>				
	Estimate	t	p-value	AIC
Intercept	0.908	25.862	<0.001	
Rainfall	0.002	6.459	<0.001	-100.82
River Flow	-0.000	-6.185	<0.001	-105.14
Residual deviance: 5.4259 on 197 degrees of freedom				

Table 2. Linear model between food-chain length and environmental variables. Data from 2010 through 2018 of Patos Lagoon estuary, Brazil.

	Estimate	t	p-value
Intercept	5.118	5.252	<0.001
River flow	0.001	1.997	0.057
Rainfall	-0.009	-2.647	0.014
Temperature	-0.028	-0.94	0.357
Salinity	-0.014	-0.73	0.472
Trophic richness	-0.13	-1.044	0.307
Residual standard error: 0.603 on 23 degrees of freedom			
$R^2 = 0.35$		p-value = 0.058	

Table 3. Generalized Linear Model for overall trophic position and trophic guilds. Data from 2010 through 2018 in a shallow-water community of Patos Lagoon estuary, Brazil.

	n	Estimate	t	p-value
Intercept (DTV)	33	1.058	30.638	< 0.001
Herbivores	23	-0.044	-0.840	0.402
Omnivores	38	-0.071	-1.561	0.120
Zooplanktivores	36	-0.108	-2.383	0.018
Zoobenthivores	67	-0.123	-3.045	0.002
Piscivores	3	-0.172	-1.696	0.091
Residual deviance: 6.4607 on 194 degrees of freedom				

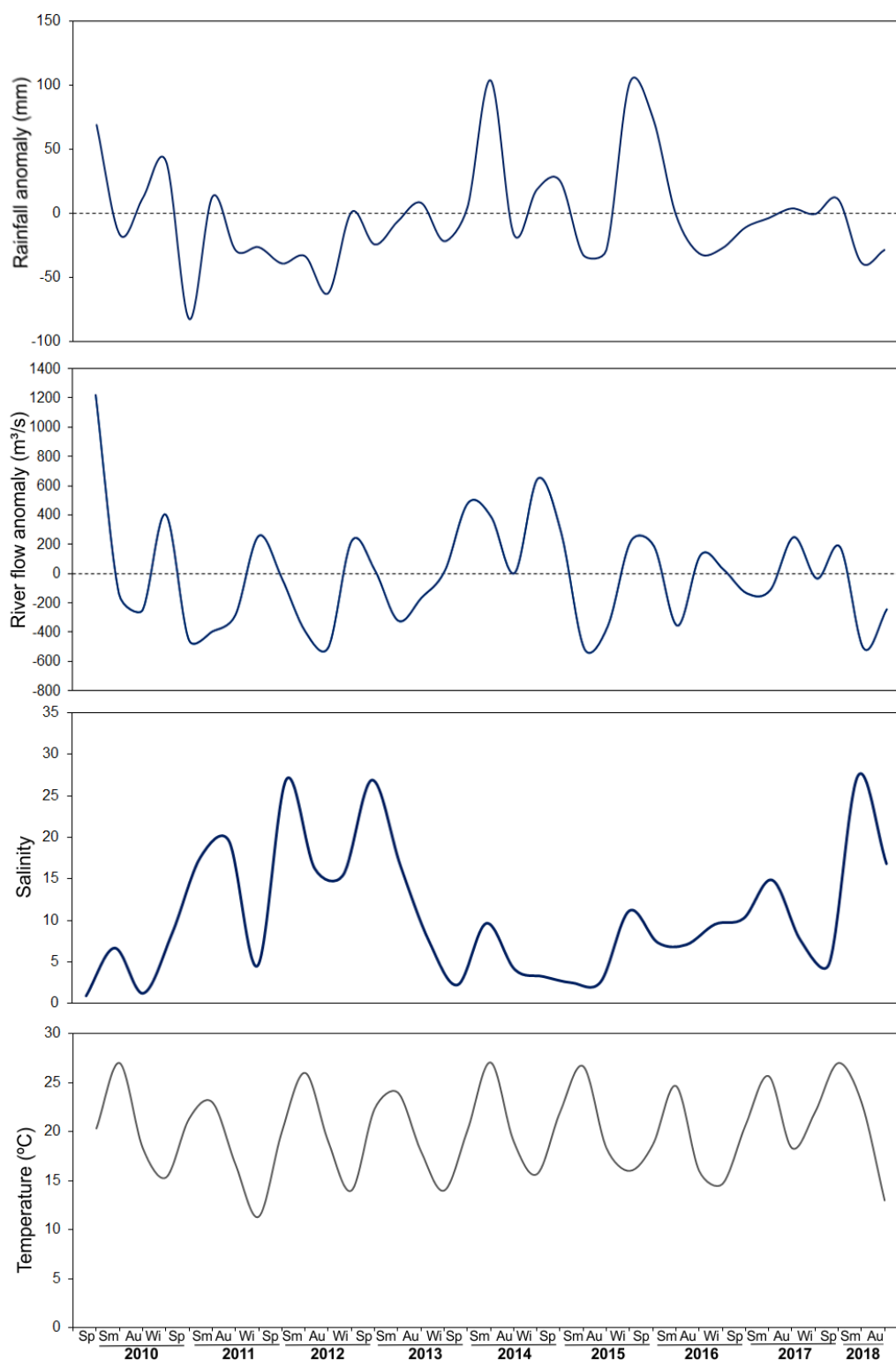


Figure 2. Seasonal variation (2010-2016) in rainfall (mm) and river water flow (m^3/s) anomalies in the drainage basin of Patos Lagoon, and in salinity and water temperature ($^{\circ}\text{C}$) in the estuarine zone of the lagoon. (Sm) Summer; (Au) Autumn; (Wi) Winter; and (Sp) Spring.

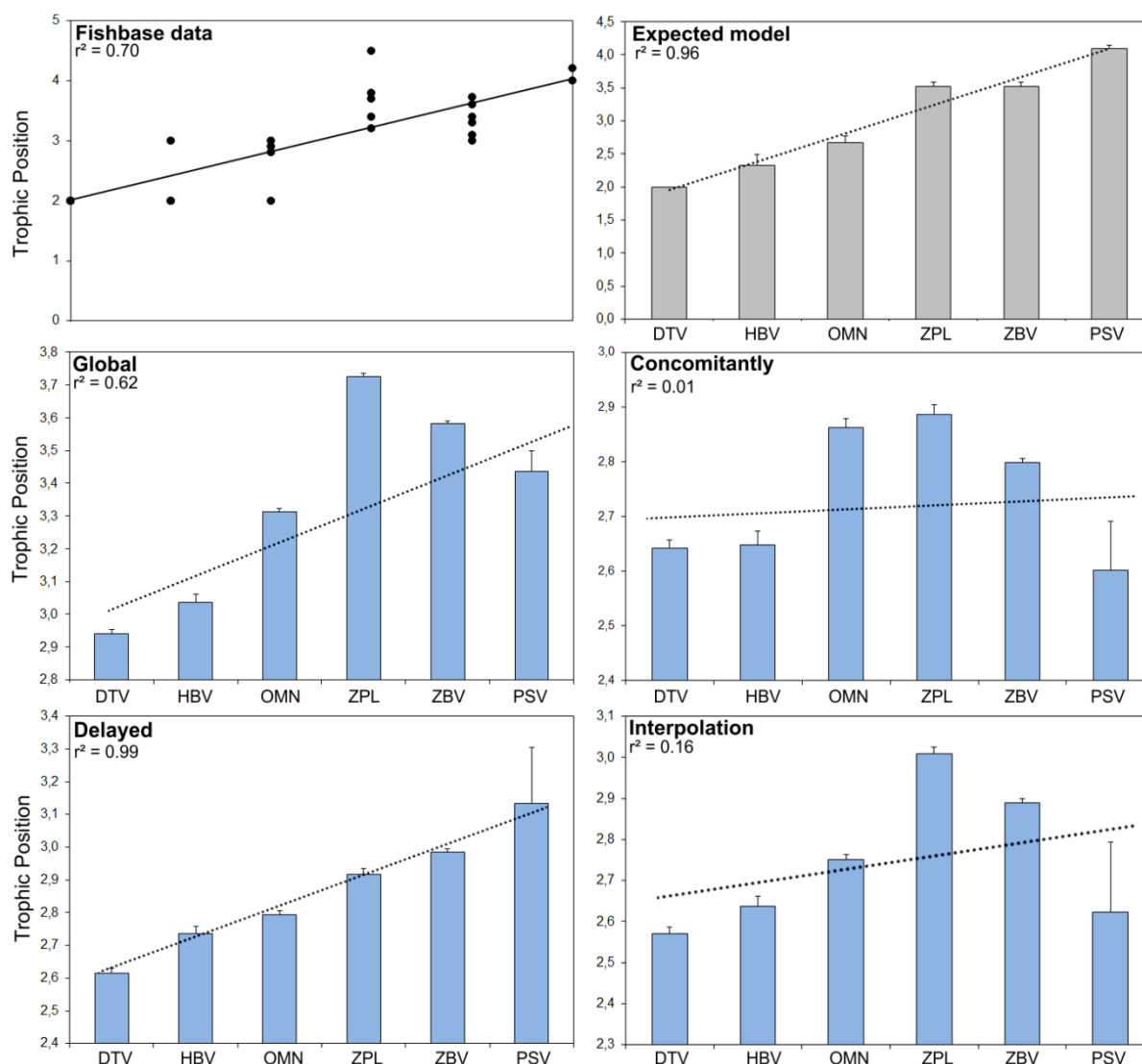


Figure 3. Expected model for trophic position of trophic guilds (grey bars) based in FishBase consumers' data. Trophic position estimates ($\pm$ sd error) for different trophic guilds by four different baseline methods (blue bars). Dashed lines represent the fitted linear model for each estimate. Details of each method in the 'Materials and Methods section'. DTV – detritivores, HBV – herbivores, OMN – omnivores, ZPL – zooplanktivores, ZBV – zoobenthivores and, PSV – piscivores. Data from Patos Lagoon estuary between 2010 and 2018.

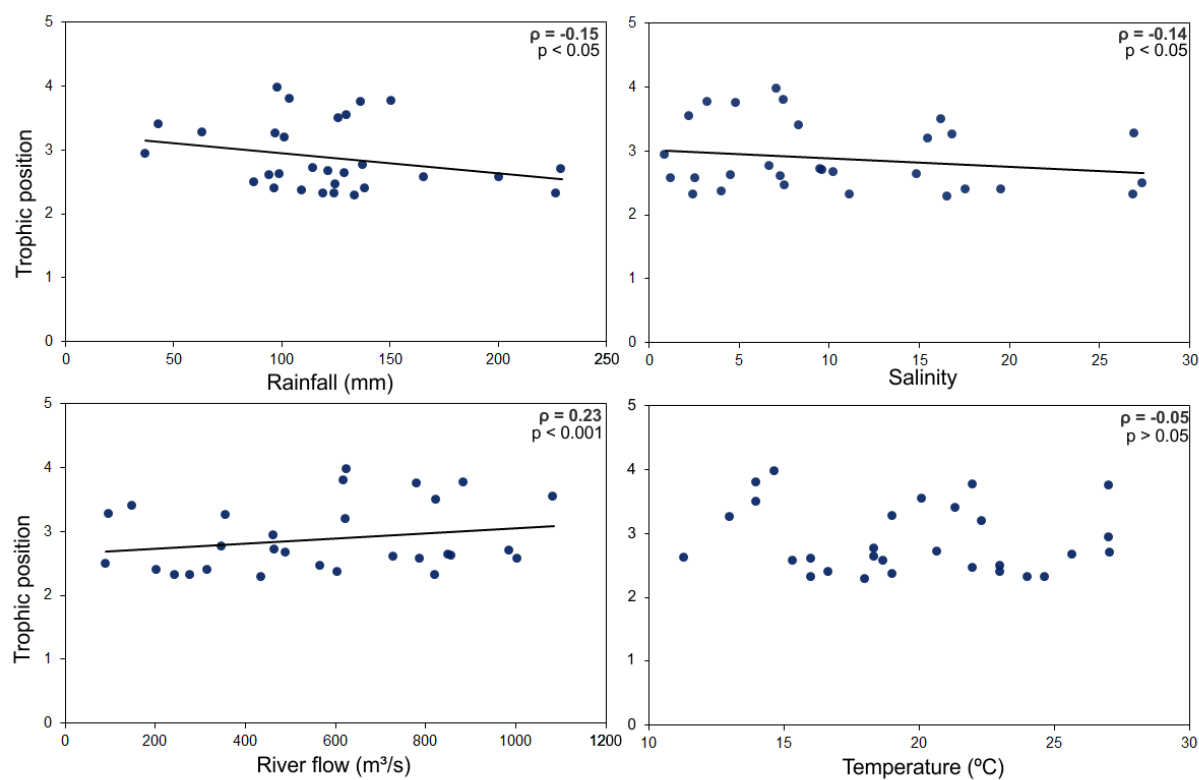


Figure 4. Relationship between trophic position estimation and abiotic factors. The ρ of Spearman correlation and the significance is expressed on the top right of each panel. Data from 2010 to 2018 of the Patos Lagoon estuary, Brazil.

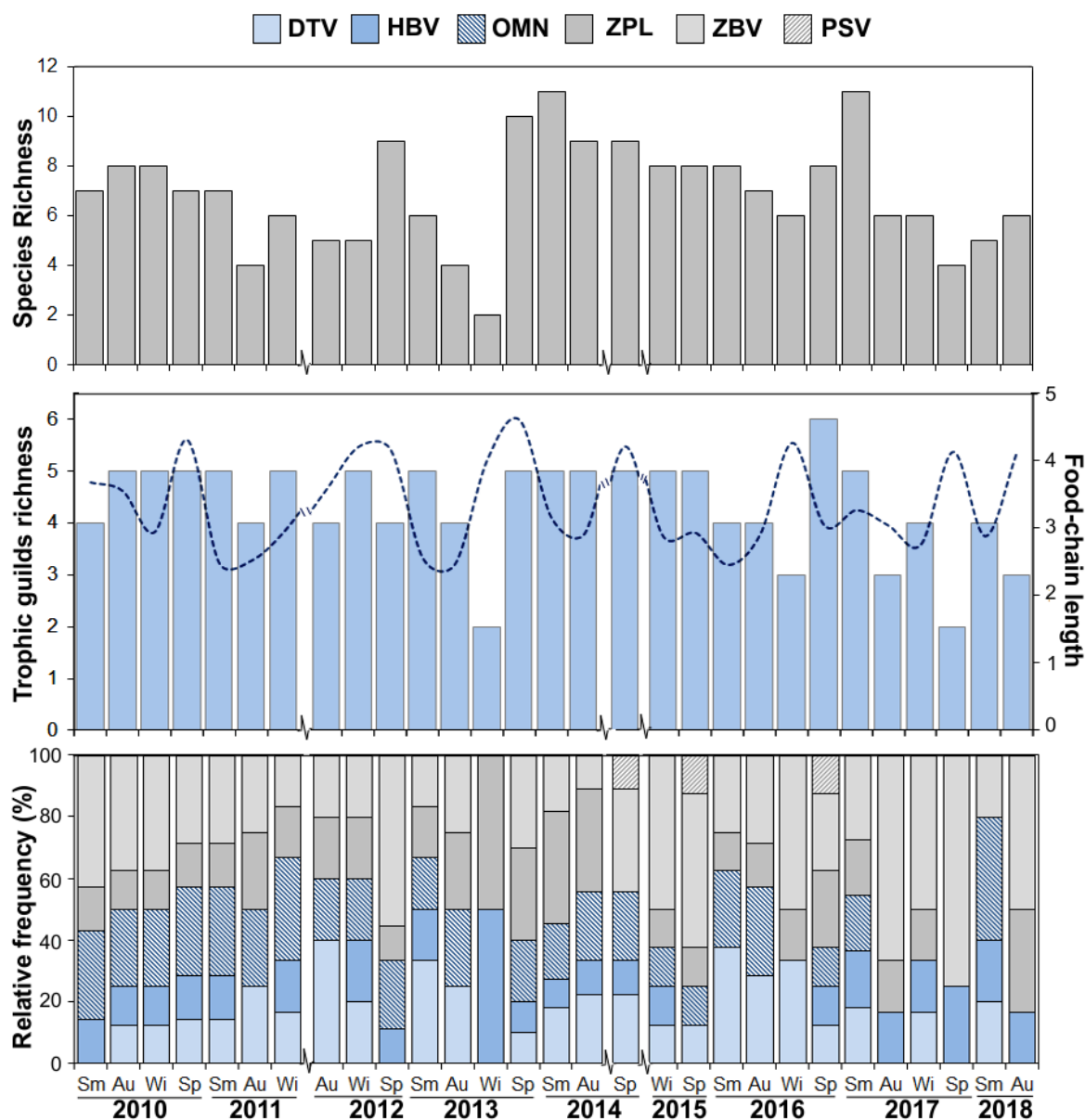


Figure 5. Temporal variation in the community attributes in Patos Lagoon estuary between 2010 and 2018. In the second panel, blue columns show the trophic richness and the dashed line shows food-chain length. The relative richness in each trophic guild is shown in the third panel. DTV – detritivores, HBV – herbivores, OMN – omnivores, ZPL – zooplanktivores, ZBV – zoobenthivores and, PSV – piscivores. (Sm) Summer; (Au) Autumn; (Wi) Winter; and (Sp) Spring.

ANEXO 4 Tabela suplementar (Table S1) do Anexo 1.

Table S1. List of scientific works and location where they were conducted that were used for classification of the shallow-water fish assemblage of the estuarine zone of Patos Lagoon (southern Brazil) in the following trophic guilds: detritivore (DTV), herbivore-phytoplankton (HVP), herbivore-macroalgae/macrophytes (HVM), insectivore (ISV), omnivore (OMN), piscivore (PSV), zoobenthivore (ZBV) and zooplanktivore (ZPL).

<i>Specie</i>	<i>Diet/Items</i>	<i>Guild</i>	<i>Reference</i>	<i>Locality</i>
<i>Albula vulpes</i>	Crustaceans, Diptera larvae, mollusks	ZBV	Layman and Silliman 2002	Tidal rivers in Bahamas
<i>Anchoa marinii</i>	Zooplankton	ZPL	Fischer <i>et al.</i> 2011	
<i>Astyanax eigenmanniorum</i>	Aquatic insects and vegetal fragments	ISV	Fernandéz <i>et al.</i> 2012	Manantiales River - Argentina
<i>Astyanax fasciatus</i>	Plants and insects	ISV	Vilella <i>et al.</i> 2002	North of RS state - Brazil
<i>Astyanax lacustres</i>	Plants and insects	HVM	Corrêa <i>et al.</i> 2010	Lagoa do Peixe - RS - Brazil
<i>Atherinella brasiliensis</i>	Copepods, cipris larvae	ZPL	Bemvenuti 1990	Patos Lagoon estuary - RS - Brazil
<i>Bathygobius soporator</i>	Brachyura, fishes and algae	ZBV	Lopes and Oliveira-Silva 1997	Mangroves of Itaparica - BA - Brazil
<i>Brevoortia pectinata</i>	Copepods, Ostracods	ZPL	Own data	Patos Lagoon estuary - RS - Brazil
<i>Bryconamericus iheringii</i>	Algae, insect larvae, Cladocera, Copepoda, Ostracoda and vegetal	ZPL	Fernandéz <i>et al.</i> 2012	Manantiales River - Argentina
<i>Caranx hippos</i>	Small fishes, Caridea, <i>Macrobrachium</i> spp., Penaeidea, <i>Peneus duorarum</i>	PSV	Kwei 1978	Coast of Ghana
<i>Caranx latus</i>	Fishes and Decapoda	PSV	Höfling <i>et al.</i> 1998	Cananéia - SP - Brazil
<i>Catathyridium garmani</i>	<i>Monokalliapseuds shubartii</i> , Polychaeta	ZBV	Pereyra <i>et al.</i> 2017	Patos Lagoon estuary - RS - Brazil
<i>Charax stenopterus</i>	Insects larvae and vegetal fragments	ISV	Abilhôa <i>et al.</i> 2009	Passaúna Reservoir - PR - Brazil
<i>Cheirodon ibicuiensis</i>	Bacillariophyta, Chlorophyta, Microcrustaceans, Diptera	OMN	Dias 2007	Lagoa Negra - RS - Brazil
<i>Cheirodon interruptus</i>	Cladocera, Amphipoda, filamentous algae	OMN	Borba 2016	Taim - RS - Brazil
<i>Chloroscombrus chrysurus</i>	Copepoda, plants, Ostracoda, fishes and Polychaeta	ZPL	Chaves and Umbria 2003	Guaratuba - PR - Brazil
<i>Cichlasoma portalegreense</i>	Crustaceans, fish, insect, plants	ZBV	Corrêa <i>et al.</i> 2010	Lagoa do Peixe - RS - Brazil
<i>Citharichthys spilopterus</i>	Gastropoda, Amphipoda, Mysidacea, Sergestidae and Isopoda	ZBV	Zanlorenzi 2016	Guaratuba Bay - PR - Brazil
<i>Cnesterodon decemmaculatus</i>	Phytoplankton and Peryphyton	HVP	Quintanas <i>et al.</i> 2009	Shallow lakes in Uruguay
<i>Corydoras paleatus</i>	Insects larvae, Ostracods, Algae and vegetal fragments	ZBV	Fernandéz <i>et al.</i> 2012	Manantiales River - Argentina
<i>Crenicichla lepidota</i>	Insects, crustaceans, fishes	ISV	Corrêa <i>et al.</i> 2010	Lagoa do Peixe - RS - Brazil
<i>Ctenogobius shufeldti</i>	Ostracods, Tanaidacea	ZBV	Zanlorenzi and Chaves 2011	Guaratuba Bay - PR - Brazil
<i>Cynoscion leiarchus</i>	Fishes, plant, Polychaeta, Isopoda, Ascidiacea	ZBV	Chaves and Umbria 2003	Guaratuba - PR - Brazil
<i>Cynoscion striatus</i>	Mysids, Decapods and fishes	ZBV	Carzola 1996	Blanca Bay - Argentina
<i>Cyphocharax saladensis</i>	Debris	DTV	Froese and Pauly 2017 - FishBase	Rivers in Amazon - AM - Brazil

<i>Cyphocharax voga</i>	Algae, vegetal fragments, debris	DTV	Fernandéz <i>et al.</i> 2012	Manantiales River - Argentina
<i>Diapoma alburnus</i>	Colembola, Amphipoda and Diptera	ZBV	Vilella <i>et al.</i> 2002	North of RS state - Brazil
<i>Diapterus rhombeus</i>	Foraminifera, Ostracods and Tanaidacea	ZBV	Aguirre-León and Díaz-Ruiz 2004	An estuary in Mexico
<i>Elops saurus</i>	Fishes, shrimp	PSV	Santos-Martínez and Arboleda 1993	Colombian Caribbean
<i>Engraulis anchoita</i>	Calanoid and cyclopoid copepods, Phytoplankton, Amphipods, Mollusks	ZPL	Schwingel 1998	Southeast-South coast of Brazil
<i>Epinephelus</i> sp.	Fishes and crustaceans	ZBV	Condini <i>et al.</i> 2015	Rio Grande - RS - Brazil
<i>Eucinostomus argenteus</i>	Polychaetes, Calanoida copepods, shrimps, microalgae, sand	ZBV	Branco <i>et al.</i> 1997	Coastal lagoons in RJ state - Brazil
<i>Eucinostomus gula</i>	Hymenoptera, Amphipoda	ZBV	Zahorcsak <i>et al.</i> 2000	Anchieta Island - SP - Brazil
<i>Eucinostomus melanopterus</i>	Bivalvia, Amphipods, <i>Corophium</i> sp., Cirripedia, Brachyura	ZBV	Zanlorenzi 2016	Guaratuba Bay - PR - Brazil
<i>Genidens barbatus</i>	Polychaetes, <i>Monokalliapseudis</i> sp. and Amphipods	ZBV	Araújo 1984	Patos Lagoon estuary - RS - Brazil
<i>Genidens genidens</i>	Copepoda, Amphipoda, Polychaeta, <i>Monokalliapseudis</i> sp. and <i>Callinectes</i> sp.	ZBV	Araújo 1984	Patos Lagoon estuary - RS - Brazil
<i>Genidens planifrons</i>	Amphipods, <i>Monokalliapseudis</i> sp. and soybean	ZBV	Araújo 1984	Patos Lagoon estuary - RS - Brazil
<i>Geophagus brasiliensis</i>	Amphipods, gastropods and vascular plants	ZBV	Bastos <i>et al.</i> 2011	Patos-Mirim Complex- RS - Brazil
<i>Gobionellus oceanicus</i>	Benthic diatoms	DTV	Zanlorenzi 2008	Guaratuba Bay - PR - Brazil
<i>Gobiesox strumosus</i>	Polychaetes, Isopods and Amphipods	ZBV	Chesapeake Bay Program.	Chesapeake Bay - United States
<i>Gymnogeophagus gymnogenys</i>	Insects, copepods, Ostracods, Cladocera.	ZPL	Longoni 2009	Jacuí Delta - RS - Brazil
<i>Harengula clupeiola</i>	Zooplankton	ZPL	Stefanoni 2008	Paranaguá Bay- PR - Brazil
<i>Hemiramphus brasiliensis</i>	81% benthic algae	HVM	Froese and Pauly 2017 - FishBase	Puerto Rico
<i>Hoplias malabaricus</i>	Fishes (principally of Characidae family)	PSV	Correa <i>et al.</i> 2012	Lagoa do Peixe - RS - Brazil
<i>Hyphessobrycon anisitsi</i>	Terrestrial insects and Chironomidae	ISV	Soneira <i>et al.</i> 2006	Iberá System - Argentina
<i>Hyphessobrycon bifasciatus</i>	Macrophytes and Algae fragments	HVM	Coutinho <i>et al.</i> 2000	Lagoon in Macaé - RJ - Brazil
<i>Hyphessobrycon boulengeri</i>	Crustaceans, plants and insects	OMN	Corrêa <i>et al.</i> 2010	Lagoa do Peixe - RS - Brazil
<i>Hyphessobrycon igneus</i>	Cladocera, Chironomidae	ZPL	Soneira <i>et al.</i> 2006	Iberá System - Argentina
<i>Hyphessobrycon luetkenii</i>	Vegetal fragments and Algae	HVM	Correa 2011	Lagoa do Peixe - RS - Brazil
<i>Hyphessobrycon meridionalis</i>	Debris, Algae	DTV	Soneira <i>et al.</i> 2006	Iberá System - Argentina
<i>Hyphessobrycon reticulatus</i>	Terrestrial Arthropoda, Bivalvia, Gastropoda, Hymenoptera	ZBV	Costa 1987	Mato Grosso River - RJ - Brazil
<i>Hyporhamphus unifasciatus</i>	51% benthic algae	HVM	Froese and Pauly 2017 - FishBase	Crystal River Estuary - FL - United States
<i>Jenynsia multidentata</i>	Chlorophyceae, <i>M. shubartii</i> , Polychaetes	OMN	Mai <i>et al.</i> 2006	Patos Lagoon estuary - RS - Brazil
<i>Lagocephalus laevigatus</i>	Polychaetes	ZBV	Amaral and Migotto 1980	Ubatuba - SP - Brazil
<i>Leptocephalus larvae</i>	Suspended organic matter	ZPL	Mochioka <i>et al.</i> 1993	Laboratory experiment

<i>Loricariichthys anus</i>	Debris, vegetal fragments and Copepoda	DTV	Albrecht and Silveira 2001	Lagoa Marcelino - RS - Brazil
<i>Lycengraulis grossidens</i>	Fishes and Cladocera	ZPL	Bortoluzzi <i>et al.</i> 2006	Uruguai Medio River - RS - Brazil
<i>Macrodon ancylodon</i>	Shrimps and fishes	ZBV	Juras and Yamaguchi 1985	Southeast-South coast of Brazil
<i>Macropsobrycon uruguayanae</i>	Diptera, microcrustaceans and Chlorophyta	ZPL	Dias 2007	Ibicuí River - RS - Brazil
<i>Menticirrhus americanus</i>	Amphipods, Mysidacea	ZBV	Rodrigues and Vieira 2010	Cassino Beach - RS - Brazil
<i>Menticirrhus littoralis</i>	Amphipods, Polychaetes, Anomura	ZBV	Rodrigues and Vieira 2010	Cassino Beach - RS - Brazil
<i>Micropogonias furnieri</i>	<i>M. schubartii</i> , Polychaeta, <i>Ruppia maritima</i>	ZBV	Mendoza-Carranza and Vieira 2008	Patos Lagoon estuary - RS - Brazil
<i>Mugil brevisrostris</i>	Sand, Diatoms, Cyanophyceae	DTV	Vieira 1985	Patos Lagoon estuary - RS - Brazil
<i>Mugil curema</i>	Sand and diatoms	DTV	Vieira 1985	Patos Lagoon estuary - RS - Brazil
<i>Mugil liza</i>	Sand, diatoms, Cyanophyceae	DTV	Vieira 1985	Patos Lagoon estuary - RS - Brazil
<i>Odontesthes argentinensis</i>	Insects, Copepods, Cirripedia, diatoms	ZPL	Bemvenuti 1990	Patos Lagoon estuary - RS - Brazil
<i>Odontesthes bonariensis</i>	Copepods	ZPL	Bemvenuti 1984	RS state - Brazil
<i>Odontesthes perugiae</i>	No information about itens	ZBV	Fialho and Verani 1994	Lagoa Emboaba- RS - Brazil
<i>Oligosarcus jenynsii</i>	Fishes, decapods	PSV	Nunes and Hartz 2006	Lagoa Fortaleza - RS - Brazil
<i>Oligosarcus robustus</i>	Fishes	PSV	Nunes and Hartz 2006	Lagoa Fortaleza - RS - Brazil
<i>Oligoplites saliens</i>	Mysidacea, Polychaeta, Zooplankton	ZBV	Stefanoni 2008	Paranaguá Bay- PR - Brazil
<i>Oligoplites saurus</i>	Fishscales, decapods	ZBV	Höfling <i>et al.</i> 1998	Cananéia - SP - Brazil
<i>Oncopeltus darwinii</i>	Herpaticoida copepods, Ostracods, Mysidacea and eggs of crustaceans	ZBV	Prisco <i>et al.</i> 2001	Coastal lagoon in Argentina
<i>Opisthonema oglinum</i>	Cumacea, Macrophytes, fishes	OMN	Vega-Candejas <i>et al.</i> 1997	Campeche Bank - Mexico
<i>Orthopristis ruber</i>	Amphipods, Isopods	ZBV	Aguiar and Filomeno 1995	Lagoa da Conceição - SC - Brazil
<i>Parapimelodus nigribarbis</i>	No information about itens	ZPL	Lucena <i>et al.</i> 1992	Lagoa dos Patos - RS - Brazil
<i>Paralichthys orbignyanus</i>	Myisidacea, Ostracoda	ZBV	Prisco <i>et al.</i> 2001	Coastal lagoons in RJ state - Brazil
<i>Phalloceros caudimaculatus</i>	Cladocera, Amphippoda, vegetal fragments	ZPL	Corrêa <i>et al.</i> 2009	Lagoa do Peixe - RS - Brazil
<i>Phalloptychus iheringii</i>	Microalgae and debris	DTV	Moraes and Andreato 1994	Laguna de Jacarepaguá - RJ - Brazil
<i>Phalloptychus januarius</i>	Microalgae and debris	DTV	Moraes and Andreato 1994	Laguna de Jacarepaguá - RJ - Brazil
<i>Pimelodella australis</i>	Diptera (<i>Chaoborus</i> sp., Chironomidae larvae and pupae), Hirudinea and vegetal fragments	ISV	Mello <i>et al.</i> 2007	Laguna Blanca - Uruguay
<i>Pimelodus maculatus</i>	Aquatic insects, plants, fishes	ISV	Lima-Júnior and Goitien 2004	Piracicaba River - SP -Brazil
<i>Platanichthys platana</i>	Microalgae, crustaceans larvae	HVP	Corrêa 2016	Lagoa do Peixe - RS - Brazil
<i>Poecilia vivipara</i>	Microalgae and debris	DTV	Moraes and Andreato 1994	Laguna de Jacarepaguá - RJ - Brazil
<i>Pogonias cromis</i>	Bivalvia, Brachyura	ZBV	Blasina <i>et al.</i> 2010	Mar Chiquita Estuary - Argentina
<i>Pomadasys corvinaeformis</i>	Amphipoda, Mysidacea, Zooplankton	ZBV	Stefanoni 2008	Paranaguá Bay- PR - Brazil
<i>Pomatomus saltatrix</i>	Mysidacea, fishes	ZBV	Stefanoni 2008	Paranaguá Bay- PR - Brazil

<i>Porichthys porosissimus</i>	Crustaceans and fishes	ZBV	Corneta 2008	Ubatuba - SP - Brazil
<i>Prionotus punctatus</i>	Brachyura, Panopeidae, fishes	ZBV	Teixeira and Haimovici 1989	RS state - Brazil
<i>Ramnogaster arcuata</i>	Copepoda [<i>Acartia tonsa</i>], Mysidacea	ZPL	Lopez-Carzola <i>et al.</i> 2011	Blanca Bay - Argentina
<i>Rhamdia quelen</i>	Macrocrustaceans, insects larvae and aquatic insects	ZBV	Fernández <i>et al.</i> 2012	Manantiales River - Argentina
<i>Sardinella brasiliensis</i>	<i>Coscinodiscos</i> sp. , copepods	HVP	Schneider and Schwingel 1999	Coast from PR to RJ - Brazil
<i>Selene vomer</i>	Tanaidacea, Bivalvia	ZBV	Höfling <i>et al.</i> 1998	Cananéia - SP - Brazil
<i>Stellifer brasiliensis</i>	Polychaeta, fish, crustaceans	ZBV	Frehse 2009	South coast of PR to North coast SC - Brazil
<i>Stephanolepis hispidus</i>	Invertebrates, algae, fish scale	ZBV	Sazima <i>et al.</i> 1986	Ubatuba - SP - Brazil
<i>Stellifer rastrifer</i>	Fishes, sand, Polychaeta, Portunidae	ZBV	Frehse 2009	South coast of PR to North coast SC - Brazil
<i>Stephanolepis setifer</i>	Bryozoans, coral	ZBV	McKinney <i>et al.</i> 2003	NC - United States
<i>Symphurus jenynsii</i>	Mysidacea, Amphipods, Decapoda larvae	ZBV	Wakabara <i>et al.</i> 1993	Cananéia Estuary - SP - Brazil
<i>Syngnatus folletti</i>	Isopoda, Amphipods, copepods	ZBV	Garcia <i>et al.</i> 2005	Patos Lagoon estuary - RS - Brazil
<i>Trachinotus carolinus</i>	Amphipoda, Polychaeta	ZBV	Stefanoni 2008	Paranaguá Bay- PR - Brazil
<i>Trachinotus falcatus</i>	Mysidacea, Zooplankton	ZBV	Stefanoni 2008	Paranaguá Bay- PR - Brazil
<i>Trachinotus marginatus</i>	<i>Emerita brasiliensis</i> , Copepoda, Mysidacea, Polychaeta	ZBV	Monteiro-Neto and Cunha 1990	Cassino Beach - RS - Brazil
<i>Trichiurus lepturus</i>	Copepoda, Mysidacea, fishes	ZBV	Martins <i>et al.</i> 2005	Coast of RS - Brazil
<i>Ulaema lefroyi</i>	Polychaeta, Cyclopoida Copepoda	ZBV	Carvalho 2013	Rio Tubarão Estuary - RN - Brazil
<i>Umbrina canosai</i>	Amphipods, Mysidacea, Polychaetes	ZBV	Haimovici <i>et al.</i> 1989	Coast of RS - Brazil

ANEXO 5 Tabela suplementar (Table S2) do Anexo 1.

Table S2. Temporal variation (1997-2015) in total numerical abundance (Abund.) and frequency of occurrence (Freq.) of shallow-water fishes collected at the estuarine zone of Patos Lagoon in southern Brazil. Shaded areas denote moderate (2002-2003 and 2009-2010) and very strong (1997-1998 and 2015-2016) *El Niño* periods. Codes for trophic fish guilds are: DTV: detritivore, ZPL: zooplanktivore, OMN: omnivore, ZBV: zoobenthivore, HVP: herbivore-phytoplankton. ISV: insectivore, PSV: piscivore and HVM: herbivore-macroalgae/macrophytes.

Guilds	Metric	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
DTV	Abund.	9367	3648	11975	29248	6419	8736	12455	25484	27308	14097	8558	10735	15650	15234	16583	11366	2849	6944	29548
	Freq.	69.1	50.0	86.7	86.9	64.0	66.6	72.5	93.2	89.2	89.5	76.3	81.5	79.5	68.1	79.9	78.1	60.1	67.1	80.6
ZPL	Abund.	11836	3379	9414	14462	3750	10028	3012	8590	17290	15133	6302	15962	12837	7613	11682	12856	8013	10369	15916
	Freq.	92.6	77.5	90.3	88.3	83.7	79.7	68.3	82.4	90.2	91.5	81.2	87.3	47.3	73.7	81.7	86.4	91.5	88.5	89.9
OMN	Abund.	155	79	651	2686	547	2515	252	814	1901	2254	1405	3683	2104	272	1608	2827	444	292	264
	Freq.	12.5	7.4	42.8	39.6	26.9	34.7	15.1	22.9	28.2	34.0	34.3	47.1	39.2	17.6	30.1	0.3	24.1	15.2	14.5
ZBV	Abund.	896	1132	763	1571	476	699	732	1156	1256	303	392	1117	644	354	259	116	128	433	1272
	Freq.	64.9	66.8	49.5	47.3	46.7	52.1	53.6	49.3	47.4	22.9	33.6	45.0	39.5	34.4	21.8	15.0	21.1	31.4	47.7
HVP	Abund.	479	104	53	51	266	907	1387	170	865	111	6	1073	62	155	112	150	469	976	1630
	Freq.	25.1	22.9	9.6	7.4	19.0	26.2	32.3	9.8	14.4	6.4	1.0	27.6	3.8	19.8	9.6	6.7	12.2	31.1	45.3
ISV	Abund.	120	154		22	43	54	70	40	7	3	11		16	201	4		31	317	188
	Freq.	6.2	14.9		4.9	6.2	7.8	9.9	2.0	1.6	0.6	2.7		3.5	14.9	1.4		4.6	11.7	11.7
PSV	Abund.	15	58		73	58	25	41	13	43	3	22	3	42	40			76	57	117
	Freq.	6.2	15.8		8.8	5.8	3.9	6.1	3.3	8.4	1.0	4.1	1.0	5.6	9.3			9.9	5.8	12.4
HVM	Abund.		4			32	100	17	11	2			1	6	26	1			10	61
	Freq.		0.9			5.2	9.2	4.1	1.7	0.6			0.3	1.0	4.8	0.3			2.7	5.8

ANEXO 6 Figura suplementar (Fig. S1) do Anexo 2.

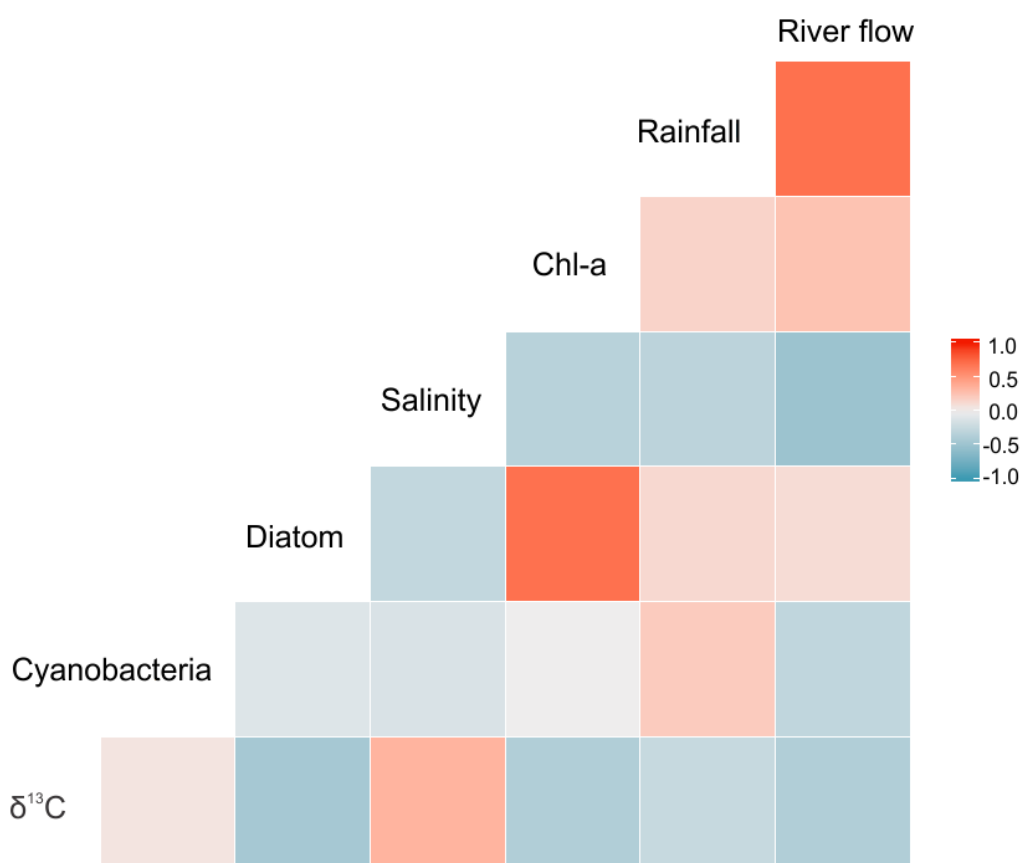


Fig S1. Pairwise Pearson's Correlation between the environmental variables used in the mixed-effects models. Data from Patos Lagoon estuary, Brazil. Chl-a is Chlorophyll-a.

ANEXO 7 Figura suplementar (Fig. S2) do Anexo 2.

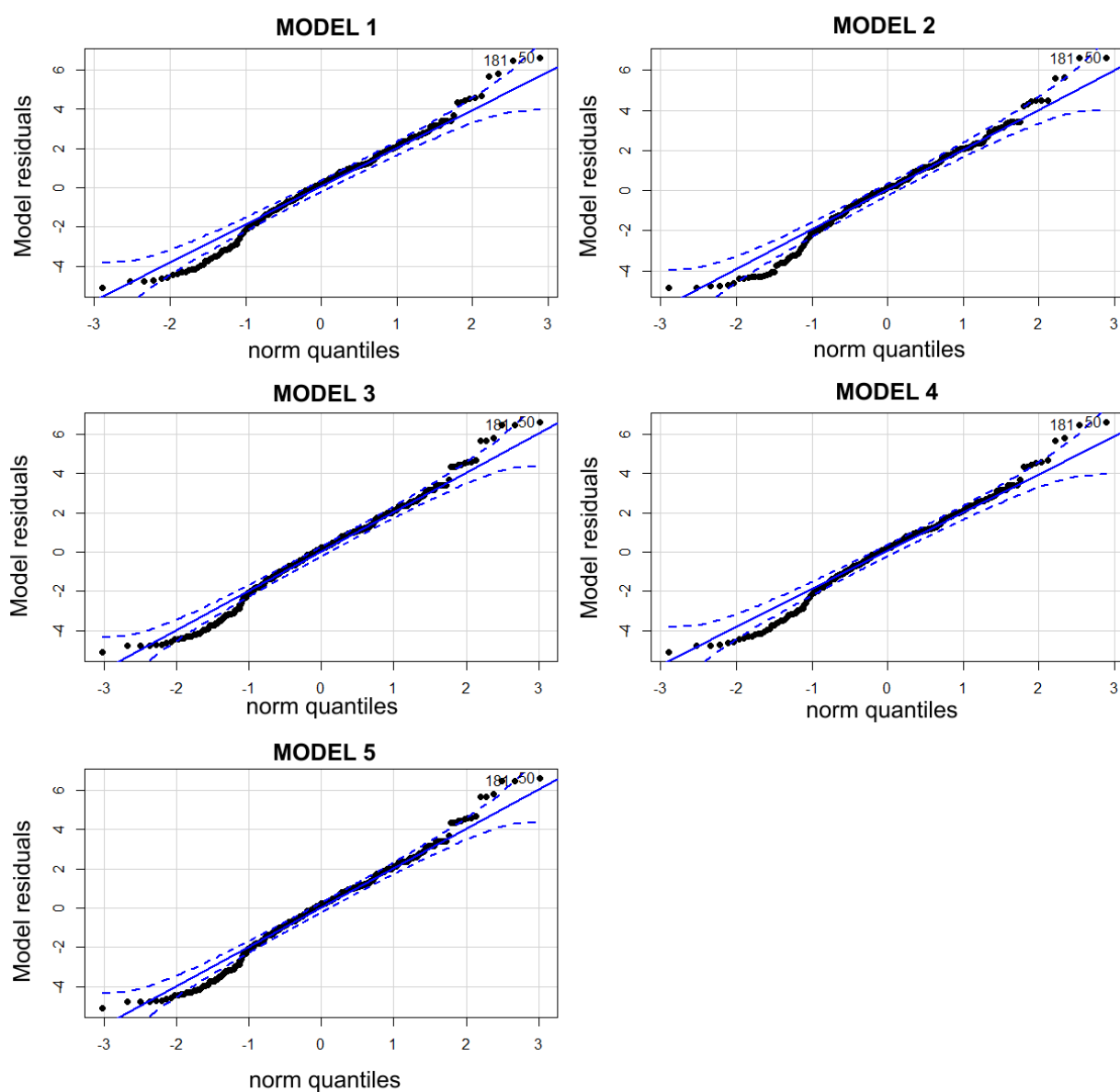


Fig S2. Fitted residuals of the mixed effects models for detritivorous fishes of Patos Lagoon estuary. Referred to the models of Table 1.

ANEXO 8 Figura suplementar (Fig. S3) do Anexo 2.

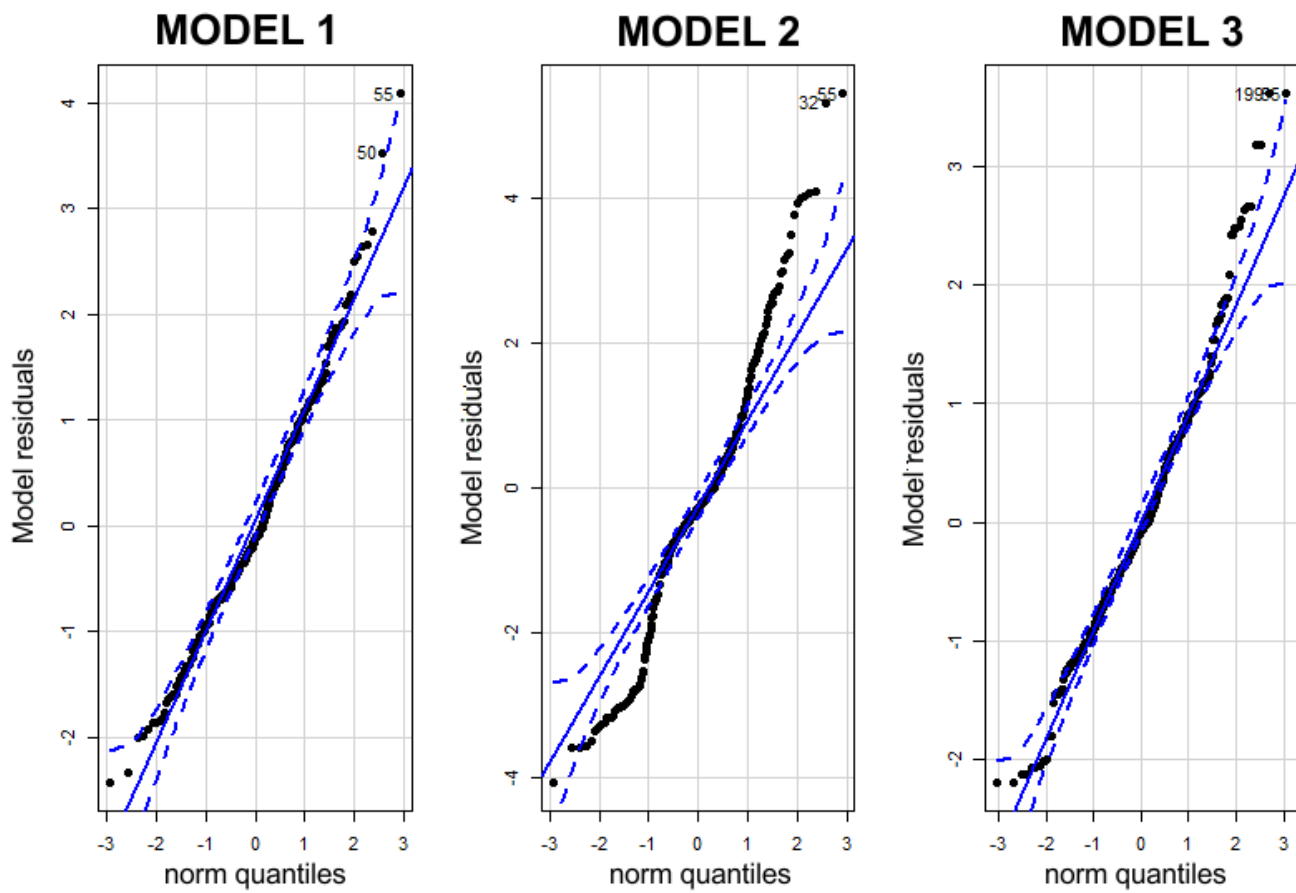


Fig S3. Fitted residuals of the mixed effects models for zooplanktivorous fishes of Patos Lagoon estuary. Referred to the models of Table 2.

ANEXO 9 Figura suplementar (Fig. S4) do Anexo 2.

DETRITIVORES FISHES

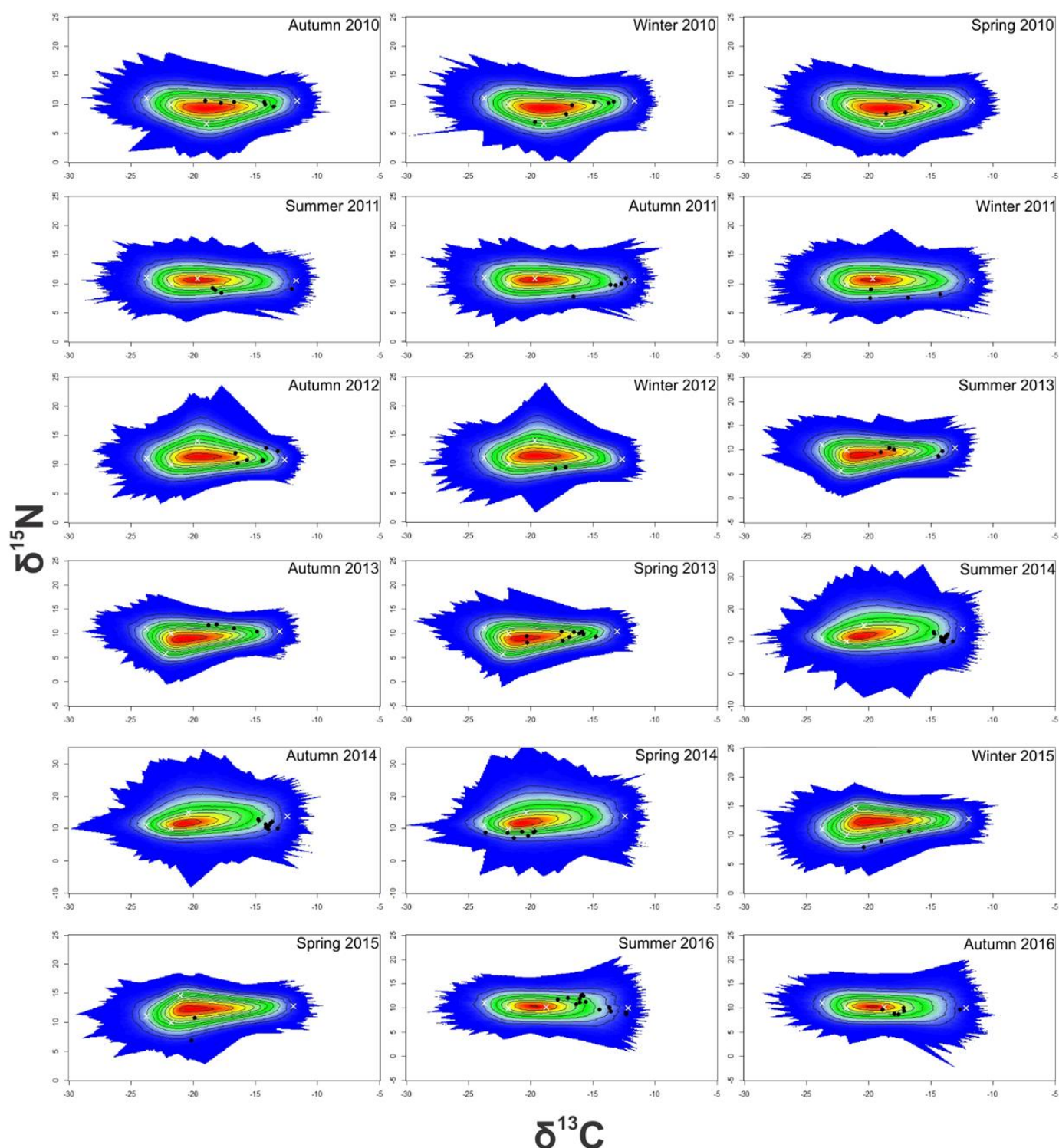


Fig. S4 Biplots of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios with simulated mixing polygons for each season for detritivorous fishes. Filled circles denote consumers and white crosses denote average values for primary carbon sources. The color gradient 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 explained by the mixing model

ANEXO 10 Figura suplementar (Fig. S5) do Anexo 2.

ZOOPLANKTIVOROUS

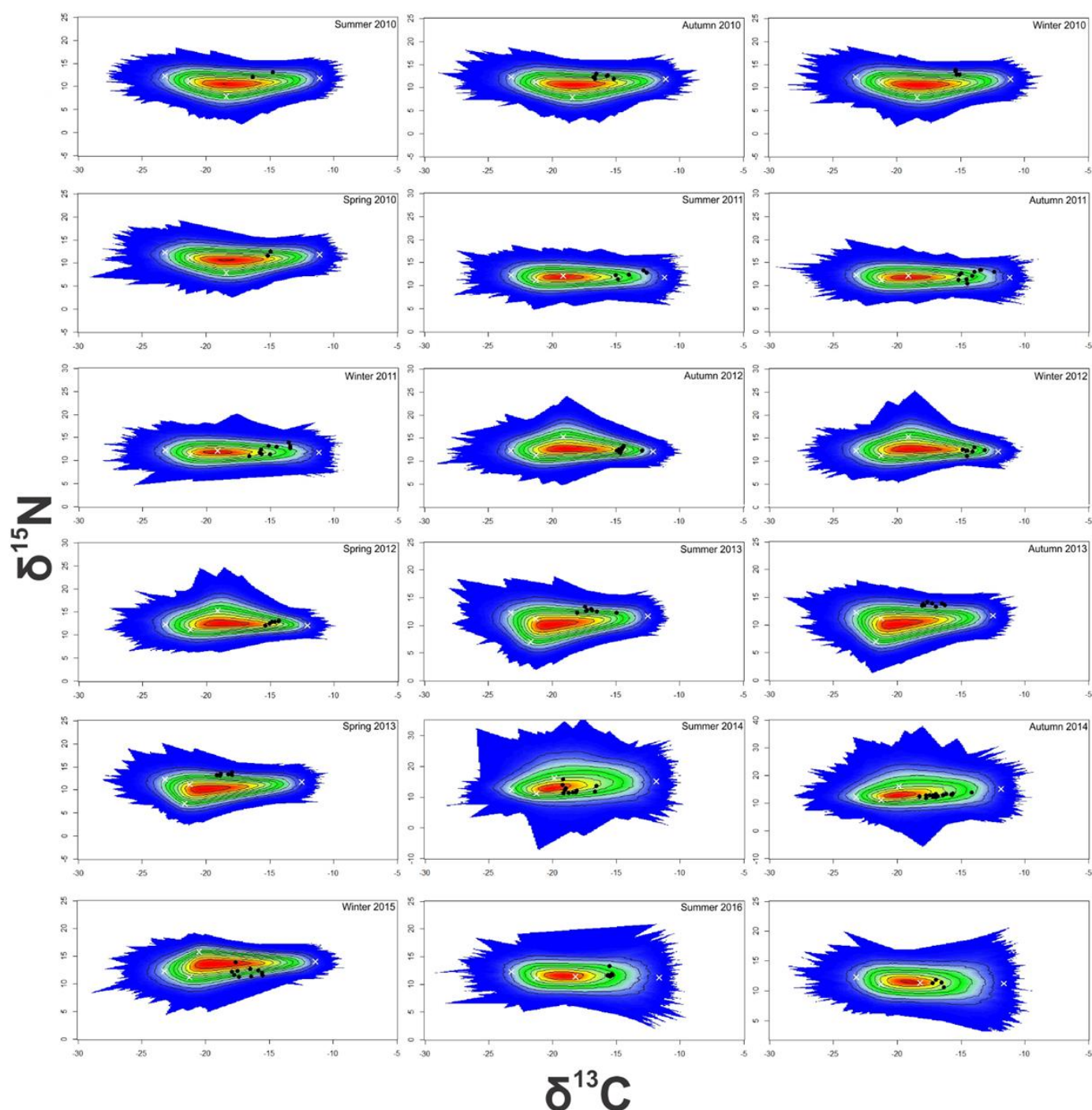


Fig. S5 Biplots of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios with simulated mixing polygons for each season for zooplanktivorous fishes. Filled circles denote consumers and white crosses denote average values for primary carbon sources. The color gradient 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 explained by the mixing model

ANEXO 11 Figura suplementar (Fig. S6) do Anexo 2.

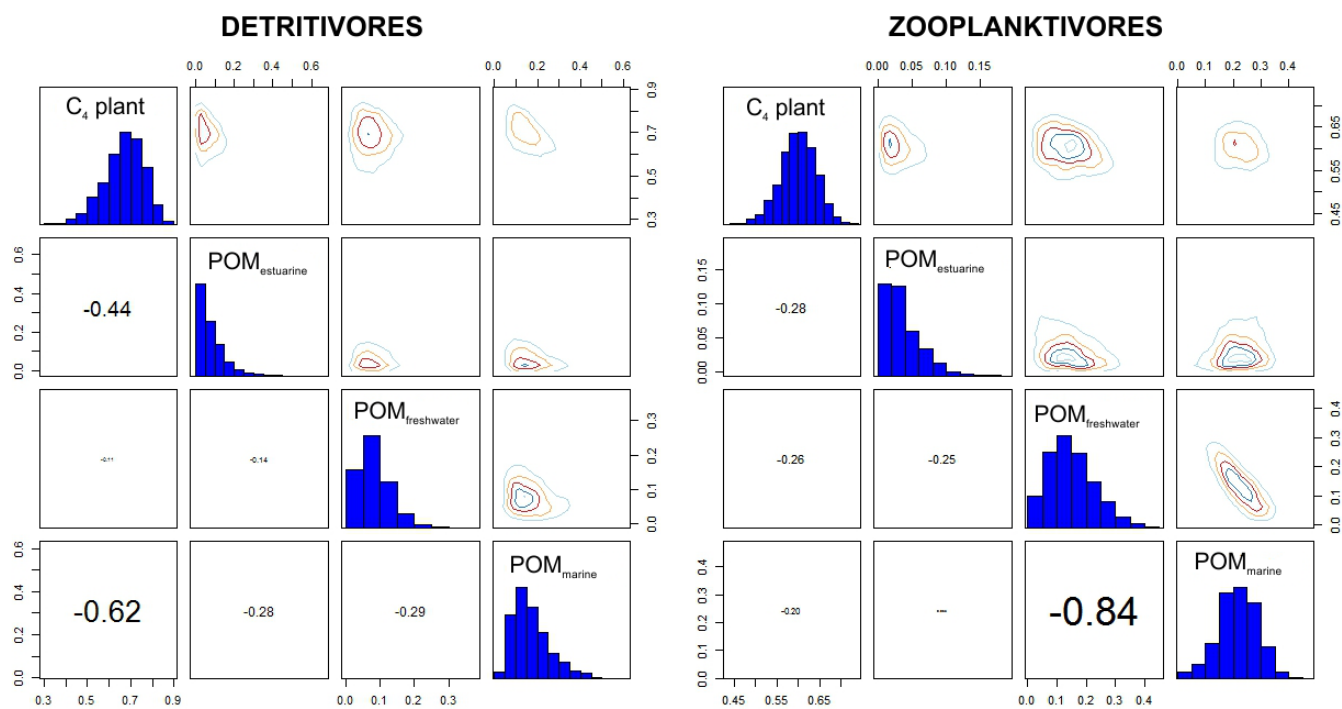


Fig. S6 Correlation plots between the sources used in each MixSIAR model. Histograms show the proportion of the contribution of the source to the diet and the lower-diagonal shows the correlation between sources.

ANEXO 12 Tabela suplementar (Table S1) do Anexo 2.

Table S1. Trophic guilds classification of the shallow-water fish species in Patos Lagoon estuary (southern Brazil). Classification is following Possamai et al. 2018

Species	Food items	Reference	Local
DETRITIVORES			
<i>Mugil brevirostris</i>	Sand, Diatoms, Cyanophyceae	Vieira 1985	Patos Lagoon estuary - RS - Brazil
<i>Mugil curema</i>	Sand and diatoms	Vieira 1985	Patos Lagoon estuary - RS - Brazil
<i>Mugil liza</i>	Sand, diatoms, Cyanophyceae	Vieira 1985	Patos Lagoon estuary - RS - Brazil
ZOOPLANKTIVORES			
<i>Atherinella brasiliensis</i>	Copepods, cipris larvae	Bemvenuti 1990	Patos Lagoon estuary - RS - Brazil

ANEXO 13 Tabela suplementar (Table S2) do Anexo 2.

Table S2. Average ($\pm$ SD) values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and number of samples (n) of primary food sources collected seasonally from 2010 to 2016 at Patos Lagoon Estuary (PLE). Allochthonous sources averages were: POM_{marine} (n=6, $\delta^{13}\text{C}$ = -22.3 ± 0.6 and $\delta^{15}\text{N}$ = 5.1 ± 1.4), and POM_{freshwater} (n=15, $\delta^{13}\text{C}$ = -24.3 ± 1.5 , $\delta^{15}\text{N}$ = 6.2 ± 1.7). Sm: Summer, Au: Autumn, Wi: Winter and Sp: Spring

Year/Season		C ₄ Plants									POM		
		<i>Spartina</i> sp.			<i>Spartina alterniflora</i>			<i>Spartina densiflora</i>					
		n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
2010	Summer							2	-12.0 ± 0.0	6.8 ± 0.1	2	-21.0 ± 0.4	1.2 ± 0.3
	Autumn							5	-12.2 ± 0.3	4.9 ± 0.9	4	-18.7 ± 0.6	2.1 ± 1.7
	Winter							6	-12.3 ± 0.5	5.5 ± 1.0			
	Spring							6	-12.0 ± 0.5	6.3 ± 0.5			
2011	Summer							4	-12.0 ± 0.1	6.4 ± 0.4	3	-18.4 ± 0.3	6.6 ± 0.2
	Autumn	3	-12.8 ± 0.0	5.4 ± 0.7							1	-20.0 ± 0.0	3.8 ± 0.0
	Winter							6	-12.1 ± 0.3	5.4 ± 1.8	4	-21.6 ± 0.7	6.2 ± 2.2
2012	Autumn										2	-19.9 ± 0.0	10.2 ± 3.6
	Winter										3	-20.3 ± 0.5	8.6 ± 1.6
	Spring				3	-13.4 ± 0.3	6.0 ± 0.4	3	-12.9 ± 0.1	6.0 ± 0.1			
2013	Summer							3	-13.3 ± 0.1	5.5 ± 0.1			
	Autumn				3	-13.3 ± 0.0	4.8 ± 0.4						
	Winter							3	-13.9 ± 0.0	6.6 ± 0.5			
	Spring							3	-13.7 ± 0.0	5.6 ± 1.2	3	-22.7 ± 0.6	1.0 ± 1.1
2014	Summer	3	-12.8 ± 0.1	13.1 ± 1.7				3	-13.2 ± 0.3	13.8 ± 1.1	3	-20.1 ± 0.3	18.2 ± 1.7
	Autumn	3	-13.0 ± 0.1	4.2 ± 0.6				3	-13.3 ± 1.1	4.9 ± 0.1	3	-18.9 ± 0.5	3.9 ± 0.8
	Spring							2	-12.0 ± 0.2	9.2 ± 0.3	3	-23.8 ± 0.1	8.3 ± 0.3
2015	Winter							3	-12.7 ± 0.3	8.0 ± 0.3	3	-21.6 ± 0.2	9.8 ± 0.3
	Spring							3	-12.1 ± 0.0	7.9 ± 0.1			
2016	Summer							3	-12.4 ± 0.1	7.5 ± 0.1	2	-18.3 ± 0.2	4.1 ± 0.6
	Autumn							3	-13.0 ± 0.1	2.8 ± 1.9	3	-19.9 ± 0.3	6.1 ± 0.9

ANEXO 14 Tabela suplementar (Table S3) do Anexo 2.

Table S3. Average ($\pm$ SD) values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and number of samples (n) and total length (TL) average ($\pm$ SD) (mm) of detritivores and zooplanktivores fishes collected seasonally from 2010 to 2016 at in Patos Lagoon Estuary (PLE).

Year/Season		Detritivores												Zooplanktivores			
		<i>Mugil brevisrostris</i>				<i>Mugil curema</i>				<i>Mugil liza</i>				<i>Atherinella brasiliensis</i>			
		n	TL	δ ¹³ C	δ ¹⁵ N	n	TL	δ ¹³ C	δ ¹⁵ N	n	TL	δ ¹³ C	δ ¹⁵ N	n	δ ¹³ C	δ ¹⁵ N	
2010	Summer													2	107.5±0.5	-15.5±0.8	12.6±0.5
	Autumn								6	35.3±6.6	-15.9±2.0	10.2±0.3	7	34.1±6.7	-16.2±0.6	12.3±0.4	
	Winter								6	35.5±6.0	-15.9±2.2	9.3±1.3	4	81.5±39.6	-15.3±0.1	13.2±0.4	
	Spring								4	32.5±5.0	-16.5±1.5	9.3±0.8	2	36.5±0.5	-15.0±0.1	12.0±0.4	
2011	Summer								4	33.8±17.5	-16.6±2.6	8.9±0.3	6	57.1±13.5	-13.7±0.9	12.7±0.8	
	Autumn								5	149.6±130.0	-13.7±1.5	9.6±1.0	10	62.5±18.0	-14.2±0.8	12.2±0.9	
	Winter								4	36.0±9.7	-17.7±2.3	8.1±0.6	10	70.7±18.7	-14.5±1.3	12.2±1.2	
2012	Autumn				3	67.7±2.1	-14.6±1.4	12.3±0.3	4	34.5±3.0	-15.2±0.9	10.6±0.2	9	77.8±10.7	-14.4±0.5	12.5±0.5	
	Winter								2	31.5±0.5	-17.6±0.4	9.3±0.1	9	80.7±13.6	-13.8±1.0	12.0±0.7	
	Spring												6	96.8±4.5	-14.7±0.4	12.7±0.4	
2013	Summer				1	39.0±0.0	-14.4±0.0	12.3±0.0	4	31.8±6.6	-17.3±1.9	9.8±0.3	8	49.4±9.1	-16.9±0.9	12.7±0.3	
	Autumn								4	32.3±2.5	-17.1±1.5	11.2±0.6	8	63.9±13.9	-17.3±0.6	13.8±0.3	
	Winter										-14.7±0.0	9.4±0.0					
	Spring								10	43.9±21.3	-17.1±1.8	9.5±0.7	6	57.5±2.2	-18.5±0.4	13.4±0.2	
2014	Summer				2	50.0±5.0	-13.7±0.5	10.2±0.1	8	50.9±4.0	-14.1±0.4	11.5±0.9	11	47.3±20.9	-18.4±0.9	12.5±1.3	
	Autumn				5	49.8±10.4	-13.2±0.5	10.3±0.7	4	60.0±3.7	-12.9±0.5	11.1±0.3	17	55.6±13.5	-16.7±0.9	12.8±0.5	
	Spring								7	60.1±11.5	-21.0±1.3	8.5±0.7					
2015	Winter								3	48.3±9.3	-18.7±1.5	9.2±1.1	10	59.3±15.8	-16.8±0.9	12.1±0.7	
	Spring								2	26.0±0.0	-20.0±0.1	8.8±1.9					
2016	Summer	6	42.8±6.8	-15.2±1.1	10.9±0.8	4	39.3±7.7	-15.3±1.8	11.3±1.6	5	42.4±7.2	-14.8±1.9	10.4±1.4	8	55.0±23.4	-15.5±0.1	11.8±0.5
	Autumn	1	100.0±0.0	-12.6±0.0	9.6±0.0					5	32.8±2.8	-17.7±0.6	9.2±0.5	4	59.8±16.6	-16.7±0.3	11.3±0.4

ANEXO 15 Tabela suplementar (Table S4) do Anexo 2.

Table S4. Contribution (%) of each source to consumers (detritivores and zooplanktivores fishes) in Patos Lagoon estuary simulated by MixSIAR package in R software and the total explained by the model. Quantiles of 50% (2.5%-97.5%)

	Season	C ₄ Plant	POM estuarine	POM freshwater	POM marine	Explained
DETRITIVORES						
2010	Autumn	68.1 (54.5 - 81.2)	3.8 (0.1 - 19.2)	20.5 (5.5 - 34.5)	4.7 (0.7 - 22.0)	96.2%
	Winter	49.7 (15.6 - 66.5)	2.2 (0.1 - 22.0)	2.6 (0.3 - 11.6)	43.2 (21.5 - 69.0)	97.7%
	Spring	24.3 (6.0 - 46.3)	2.8 (0.1 - 19.0)	1.6 (0.2 - 8.3)	69.1 (41.1 - 92.1)	97.8%
2011	Summer	55.9 (37.0 - 73.4)	2.5 (0.1 - 33.7)	1.8 (0.2 - 9.3)	36.9 (9.3 - 57.5)	97.1%
	Autumn	73.3 (57.6 - 85.8)	2.3 (0.1 - 24.0)	6.7 (0.9 - 27.0)	14.1 (2.6 - 39.0)	96.4%
	Winter	29.6 (10.8 - 49.9)	0.7 (0.0 - 18.6)	0.5 (0.0 - 4.0)	67.6 (38.8 - 88.1)	98.4%
2012	Autumn	74.8 (60.1 - 87.4)	4.0 (0.1 - 23.0)	10.7 (1.6 - 26.1)	6.6 (0.9 - 19.9)	96.1%
	Winter	45.4 (22.8 - 69.9)	1.9 (0.0 - 20.0)	1.1 (0.1 - 8.4)	49.2 (20.0 - 73.6)	97.6%
2013	Summer	71.7 (57.2 - 86.3)	6.7 (0.1 - 26.7)	14.5 (2.3 - 37.0)	3.5 (0.6 - 15.1)	96.4%
	Autumn	62.7 (42.3 - 85.0)	3.4 (0.1 - 15.0)	31.7 (8.4 - 53.1)	0.9 (0.1 - 6.2)	98.7%
	Spring	50.1 (32.5 - 62.4)	7.2 (0.1 - 25.1)	6.0 (0.6 - 23.1)	34.5 (9.5 - 61.2)	97.8%
2014	Summer	71.1 (60.5 - 84.0)	1.6 (0.1 - 9.3)	1.5 (0.2 - 5.7)	24.7 (14.1 - 36.1)	98.9%
	Autumn	83.5 (71.5 - 92.0)	1.2 (0.0 - 7.8)	4.8 (0.8 - 13.8)	8.8 (1.9 - 22.5)	98.3%
	Spring	17.9 (8.8 - 31.0)	0.6 (0.0 - 4.2)	0.2 (0.0 - 1.7)	80.8 (68.1 - 93.0)	99.5%
2015	Winter	19.5 (3.5 - 38.2)	1.1 (0.0 - 14.0)	1.4 (0.1 - 12.9)	75.7 (54.3 - 94.2)	97.7%
	Spring	6.4 (0.8 - 21.7)	1.0 (0.0 - 9.6)	0.6 (0.0 - 8.1)	90.6 (70.7 - 98.4)	98.6%
2016	Summer	67.8 (53.6 - 78.0)	5.9 (0.1 - 34.7)	12.1 (1.6 - 25.4)	9.8 (1.2 - 25.8)	95.6%
	Autumn	61.3 (41.6 - 74.0)	2.8 (0.1 - 45.8)	29.3 (3.2 - 45.5)	2.4 (0.2 - 16.9)	95.8%
ZOOPLANKTIVORES						
2010	Summer	60.1(51.3 - 67.7)	2.8 (0.5 - 10.1)	14.4 (3.0 - 32.3)	21.7 (5.7 - 34.6)	98.7%
	Autumn	55.0 (47.7 - 62.5)	2.5 (0.2 - 9.5)	6.6 (0.5 - 36.2)	35.4 (2.4 - 47.5)	99.5%
	Winter	57.9 (48.9 - 66.7)	0.6 (0.1 - 3.0)	7.6 (0.5 - 37.3)	32.8 (1.9 - 48.6)	98.9%
	Spring	51.8 (42.6 - 62.6)	4.2 (0.3 - 14.3)	7.8 (0.8 - 35.4)	34.8 (2.8 - 49.4)	98.6%
2011	Summer	73.0 (65.6 - 80.0)	1.5 (0.1 - 11.3)	10.5 (0.8 - 25.4)	13.4 (1.6 - 26.7)	98.4%
	Autumn	70.3 (63.2 - 77.2)	1.3 (0.1 - 11.4)	4.9 (0.2 - 26.6)	21.7 (10. - 33.8)	98.2%
	Winter	68.0 (61.3 - 74.6)	0.3 (0.0 - 4.0)	6.3 (0.1 - 30.6)	23.8 (0.4 - 37.2)	98.4%
2012	Autumn	74.4 (67.2 - 81.5)	7.7 (0.1 - 20.4)	3.5 (0.1 - 19.4)	11.6 (0.7 - 24.6)	97.2%
	Winter	81.4 (15.4 - 87.4)	1.6 (0.1 - 7.3)	4.0 (0.2 - 16.8)	10.8 (0.8 - 21.1)	97.8%
	Spring	69.4 (61.6 - 78.8)	12.8 (0.2 - 26.4)	4.2 (0.3 - 20.8)	11.4 (0.8 - 26.2)	97.8%
2013	Summer	51.6 (44.1 - 60.7)	0.9 (0.1 - 5.4)	9.7 (0.85 - 39.6)	37.6 (2.1 - 51.5)	99.8%
	Autumn	40.4 (31.4 - 53.3)	0.6 (0.0 - 2.8)	2.8 (0.2 - 53.1)	55.8 (0.8 - 66.7)	99.6%
	Spring	37.6 (28.9 - 50.0)	1.1 (0.1 - 5.3)	3.7 (0.2 - 55.1)	56.9 (0.6 - 68.5)	99.3%
2014	Summer	40.5 (30.1 - 49.0)	2.0 (0.1 - 24.0)	40.1 (1.1 - 55.0)	13.5 (2.1 - 65.5)	96.1%
	Autumn	49.1 (40.9 - 55.8)	2.3 (0.1 - 16.5)	18.2 (2.1 - 39.9)	27.9 (5.3 - 51.1)	97.5%
2015	Winter	44.9 (37.8 - 51.8)	0.4 (0.0 - 3.6)	3.9 (0.1 - 48.1)	49.9 (1.5 - 59.7)	99.1%
2016	Summer	58.6 (49.1 - 65.7)	3.9 (0.2 - 26.8)	8.0 (0.9 - 28.1)	26.4 (4.7 - 39.4)	96.9%
	Autumn	48.7 (37.2 - 61.2)	2.6 (0.1 - 37.5)	2.8 (0.1 - 40.1)	42.4 (1.3 - 55.1)	96.5%

ANEXO 16 Tabela suplementar (Table S1) do Anexo 3.

Table S1. Sources utilized as a baseline for trophic position estimates in “Concomitantly” and in “Delayed” methods. The p-value is a result of Tukey HSD comparisons between $\delta^{15}\text{N}$ of these sources in each period. (b1) and (b2) demonstrate the sources combined as a baseline when $p > 0.05$. Differences between sources are in bold.

SEASON	Sources	p-value	b1	b2	$\delta^{15}\text{N}$ b1	$\delta^{15}\text{N}$ b2
2010 Summer	Plankton-Saltmarsh	> 0.001	plankton	saltmarsh	3.06±2.57	6.98±0.00
2010 Autumn	Biofilm-Macroalgae	0.668	biofilm + macroalgae + saltmarsh + seagrass	plankton	6.08±1.29	2.09±1.76
	Biofilm-Plankton	> 0.001				
	Biofilm-Saltmarsh	0.350				
	Biofilm-Seagrass	0.963				
	Macroalgae-Plankton	0.001				
	Macroalgae-Saltmarsh	0.103				
	Macroalgae-Seagrass	0.503				
	Plankton-Saltmarsh	0.033				
	Plankton-Seagrass	0.034				
2010 Winter	Saltmarsh-Seagrass	0.938	all	-	6.45±1.04	-
	Biofilm-Macroalgae	0.379				
	Biofilm-Saltmarsh	0.029				
	Biofilm-Seagrass	0.822				
	Macroalgae-Saltmarsh	0.600				
	Macroalgae-Seagrass	0.794				
2010 Spring	Saltmarsh-Seagrass	0.112	biofilm + saltmarsh	seagrass	6.85±0.84	8.98±0.98
	Biofilm-Saltmarsh	0.132				
	Biofilm-Seagrass	0.019				
2011 Summer	Saltmarsh-Seagrass	> 0.001	biofilm	macroalgae + plankton + saltmarsh + seagrass	5.37±0.71	6.86±0.68
	Biofilm-Macroalgae	0.023				
	Biofilm-Plankton	0.198				
	Biofilm-Saltmarsh	0.261				
	Biofilm-Seagrass	0.040				
	Macroalgae-Plankton	0.560				
	Macroalgae-Saltmarsh	0.355				
	Macroalgae-Seagrass	0.954				
	Plankton-Saltmarsh	0.996				
2011 Autumn	Plankton-Seagrass	0.873	biofilm	plankton	8.47±1.03	3.84±0.00
	Saltmarsh-Seagrass	0.660				
2011 Winter	Biofilm-Plankton	-	all	-	6.16±1.94	-
	Biofilm-Saltmarsh	0.331				
	Plankton-Saltmarsh	0.080				
2012 Autumn	Biofilm-Plankton	0.739	both	-	13.09±6.75	-
2012 Winter	Biofilm-Saltmarsh	0.572				
2012 Spring	Biofilm-Plankton	0.968	biofilm	saltmarsh	9.51±0.70	6.02±0.29
2013 Summer	Biofilm-Saltmarsh	> 0.001				
	Biofilm-Macroalgae	> 0.001	biofilm	macroalgae + saltmarsh	11.13±0.37	6.52±0.94
	Macroalgae-Saltmarsh	0.001				
2013 Autumn	Biofilm-Saltmarsh	0.212	all	-	5.17±0.60	-
2013 Winter	Biofilm-Macroalgae	0.894				
	Biofilm-Saltmarsh	0.882				
	Macroalgae-Saltmarsh	0.582				

2013 Spring	Biofilm-Plankton	0.433	biofilm + saltmarsh + seagrass	plankton	5.33±1.87	1.00±1.12
	Biofilm-Saltmarsh	0.359				
	Biofilm-Seagrass	0.149				
	Plankton-Saltmarsh	0.033				
	Plankton-Seagrass	0.177				
	Saltmarsh-Seagrass	0.762				
2014 Summer	Macroalgae-Plankton	0.003	biofilm + saltmarsh + seagrass	plankton	14.52±4.18	18.25±1.73
	Macroalgae-Saltmarsh	0.269				
	Plankton-Saltmarsh	0.014				
2014 Autumn	Biofilm-Macroalgae	0.612	all	-	5.24±2.02	-
	Biofilm-Plankton	0.121				
	Biofilm-Saltmarsh	0.148				
	Macroalgae-Plankton	0.530				
	Macroalgae-Saltmarsh	0.689				
	Plankton-Saltmarsh	0.958				
2014 Spring	Biofilm-Plankton	0.029	biofilm	plankton + saltmarsh	13.01±1.94	8.66±0.56
	Biofilm-Saltmarsh	0.091				
	Plankton-Saltmarsh	0.799				
2015 Winter	Biofilm-Macroalgae	> 0.001	biofilm + plankton + saltmarsh	macroalgae + seagrass	9.07±0.98	4.70±0.34
	Biofilm-Plankton	0.982				
	Biofilm-Saltmarsh	0.018				
	Biofilm-Seagrass	> 0.001				
	Macroalgae-Plankton	> 0.001				
	Macroalgae-Saltmarsh	> 0.001				
	Macroalgae-Seagrass	0.943				
	Plankton-Saltmarsh	0.005				
	Plankton-Seagrass	> 0.001				
	Saltmarsh-Seagrass	> 0.001				
2015 Spring	Macroalgae-Saltmarsh	0.044	macroalgae + seagrass	saltmarsh	6.30±0.27	7.98±0.16
	Macroalgae-Seagrass	0.326				
	Saltmarsh-Seagrass	0.023				
2016 Summer	Biofilm-Macroalgae	0.021	biofilm + saltmarsh	macroalgae + plankton	6.97±0.66	3.84±0.84
	Biofilm-Plankton	0.057				
	Biofilm-Saltmarsh	0.377				
	Macroalgae-Plankton	0.848				
	Macroalgae-Saltmarsh	0.004				
	Plankton-Saltmarsh	0.010				
2016 Autumn	Biofilm-Macroalgae	0.859	all	-	4.70±1.88	-
	Biofilm-Plankton	0.666				
	Biofilm-Saltmarsh	0.673				
	Macroalgae-Plankton	0.993				
	Macroalgae-Saltmarsh	0.342				
	Plankton-Saltmarsh	0.183				
2016 Winter	Biofilm-Macroalgae	> 0.001	biofilm	macroalgae + plankton + saltmarsh	3.32±0.64	7.17±0.64
	Biofilm-Plankton	> 0.001				
	Biofilm-Saltmarsh	> 0.001				
	Macroalgae-Plankton	0.040				
	Macroalgae-Saltmarsh	0.806				
	Plankton-Saltmarsh	0.079				
2016 Spring	Biofilm-Plankton	0.131	plankton	saltmarsh	4.27±0.21	7.36±0.65

	Biofilm-Saltmarsh	0.109				
	Plankton-Saltmarsh	0.002				
2017 Summer	Biofilm-Plankton	0.297				
	Biofilm-Saltmarsh	0.185	plankton	saltmarsh	5.45±0.62	7.97±0.89
	Plankton-Saltmarsh	0.011				
2017 Autumn	Biofilm-Plankton	0.089				
	Biofilm-Saltmarsh	0.009	biofilm	plankton + saltmarsh	6.25±1.27	9.44±0.83
	Plankton-Saltmarsh	0.214				
2017 Winter	Biofilm-Plankton	0.909				
	Biofilm-Saltmarsh	0.727	all	-	8.29±0.92	-
	Plankton-Saltmarsh	0.954				
2017 Spring	Biofilm-Macroalgae	> 0.001				
	Biofilm-Plankton	> 0.001				
	Biofilm-Saltmarsh	> 0.001	biofilm	macroalgae + plankton + saltmarsh	12.52±0.56	6.26±0.64
	Macroalgae-Plankton	0.501				
	Macroalgae-Saltmarsh	0.292				
	Plankton-Saltmarsh	0.981				
2018 Summer	Biofilm-Macroalgae	0.022				
	Biofilm-Plankton	0.343				
	Biofilm-Saltmarsh	0.100	all	-	9.09±4.66	-
	Macroalgae-Plankton	0.428				
	Macroalgae-Saltmarsh	0.918				
	Plankton-Saltmarsh	0.830				
2018 Autumn	Biofilm-Macroalgae	0.888				
	Biofilm-Plankton	0.158				
	Biofilm-Saltmarsh	0.635	biofilm + macroalgae + saltmarsh	plankton	7.82±1.14	12.68±2.89
	Macroalgae-Plankton	0.053				
	Macroalgae-Saltmarsh	0.956				
	Plankton-Saltmarsh	0.028				

ANEXO 17 Tabela suplementar (Table S2) do Anexo 3.

Table S2. Sources utilized as a baseline for trophic position estimates in “Intercalation” method. The p-value is a result of Tukey HSD comparisons between $\delta^{15}\text{N}$ of these sources in each period. (b1) and (b2) demonstrate the sources combined as a baseline when $p > 0.05$. Differences between sources are in bold.

SEASON	Sources	p-value	b1	b2	$\delta^{15}\text{N}$ b1	$\delta^{15}\text{N}$ b2
2010 Summer	Plankton-Saltmarsh	> 0.001	plankton	saltmarsh	3.06±2.57	6.98±0.00
2010 Summer / Autumn	SOM-Macroalgae	0.659	SOM + macroalgae + saltmarsh + seagrass	plankton	6.17±1.23	1.81±1.50
	SOM-Plankton	0.000				
	SOM-Saltmarsh	0.652				
	SOM-Seagrass	0.963				
	Macroalgae-Plankton	0.000				
	Macroalgae-Saltmarsh	0.178				
	Macroalgae-Seagrass	0.489				
	Plankton-Saltmarsh	0.001				
	Plankton-Seagrass	0.010				
	Saltmarsh-Seagrass	0.998				
2010 Autumn / Winter	SOM-Macroalgae	1.000	SOM + macroalgae + saltmarsh + seagrass	plankton	6.13±1.15	2.08±1.75
	SOM-Plankton	0.000				
	SOM-Saltmarsh	0.017				
	SOM-Seagrass	0.976				
	Macroalgae-Plankton	0.000				
	Macroalgae-Saltmarsh	0.083				
	Macroalgae-Seagrass	0.998				
	Plankton-Saltmarsh	0.000				
	Plankton-Seagrass	0.000				
	Saltmarsh-Seagrass	0.096				
2010 Winter / Spring	SOM-Macroalgae	0.313	SOM + macroalgae + seagrass	saltmarsh	7.24±1.19	5.94±0.93
	SOM-Saltmarsh	0.026				
	SOM-Seagrass	0.880				
	Macroalgae-Saltmarsh	0.955				
	Macroalgae-Seagrass	0.108				
	Saltmarsh-Seagrass	0.003				
2010 Spring / 2011 Summer	SOM-Macroalgae	0.717	SOM + macroalgae + plankton+ saltmarsh	seagrass	6.58±0.95	8.30±1.19
	SOM-Plankton	1.000				
	SOM-Saltmarsh	0.995				
	SOM-Seagrass	0.014				
	Macroalgae-Plankton	0.847				
	Macroalgae-Saltmarsh	0.578				
	Macroalgae-Seagrass	0.919				
	Plankton-Saltmarsh	0.997				
	Plankton-Seagrass	0.164				
2011 Summer / Autumn	Saltmarsh-Seagrass	0.006				
	SOM-Macroalgae	0.814	all sources	-	6.55±1.28	-
	SOM-Plankton	0.982				
	SOM-Saltmarsh	1.000				
	SOM-Seagrass	0.930				
	Macroalgae-Plankton	0.627				

	Macroalgae-Saltmarsh	0.856				
	Macroalgae-Seagrass	0.996				
	Plankton-Saltmarsh	0.983				
	Plankton-Seagrass	0.760				
	Saltmarsh-Seagrass	0.954				
2011 Autumn / Winter	SOM-Plankton	0.778				
	SOM-Saltmarsh	0.218	all sources	-	6.27±2.03	-
	Plankton-Saltmarsh	0.939				
2011 Winter / 2012 Autumn	SOM-Plankton	0.300				
	SOM-Saltmarsh	0.047	all sources	-	7.54±4.45	-
	Plankton-Saltmarsh	0.593				
2012 Autumn / Winter	SOM-Plankton	0.331	all sources	-	10.39±4.79	-
2012 Winter / Spring	SOM-Plankton	0.921				
	SOM-Saltmarsh	0.145	all sources	-	7.80±1.96	-
	Plankton-Saltmarsh	0.211				
2012 Spring / 2013 Summer	SOM-Macroalgae	0.000				
	SOM-Saltmarsh	0.000	SOM	saltmarsh	10.32±0.98	5.84±0.36
	Macroalgae-Saltmarsh	0.004				
2013 Summer / Autumn	SOM-Macroalgae	0.649				
	SOM-Saltmarsh	0.034	SOM	saltmarsh	8.39±2.76	5.16±0.46
	Macroalgae-Saltmarsh	0.174				
2013 Autumn / Winter	SOM-Macroalgae	0.857				
	SOM-Saltmarsh	0.999	all sources	-	5.62±1.25	-
	Macroalgae-Saltmarsh	0.859				
2013 Winter / Spring	SOM-Macroalgae	0.984				
	SOM-Plankton	0.091				
	SOM-Saltmarsh	0.591				
	SOM-Seagrass	0.471				
	Macroalgae-Plankton	0.054	SOM + macroalgae + saltmarsh + seagrass	plankton	5.64±1.68	0.99±1.11
	Macroalgae-Saltmarsh	0.929				
	Macroalgae-Seagrass	0.766				
	Plankton-Saltmarsh	0.006				
	Plankton-Seagrass	0.012				
	Saltmarsh-Seagrass	0.968				
2013 Spring / 2014 Summer	SOM-Macroalgae	0.998				
	SOM-Plankton	0.955				
	SOM-Saltmarsh	0.990				
	SOM-Seagrass	0.878				
	Macroalgae-Plankton	0.997	all sources	-	10.61±6.59	-
	Macroalgae-Saltmarsh	1.000				
	Macroalgae-Seagrass	0.965				
	Plankton-Saltmarsh	0.997				
	Plankton-Seagrass	0.991				
	Saltmarsh-Seagrass	0.957				
2014 Summer / Autumn	SOM-Macroalgae	0.337				
	SOM-Plankton	0.705				
	SOM-Saltmarsh	0.295	all sources	-	10.27±5.98	-
	Macroalgae-Plankton	0.891				
	Macroalgae-Saltmarsh	0.999				
	Plankton-Saltmarsh	0.899				

2014 Autumn / Spring	SOM-Macroalgae	0.090	SOM	macroalgae + plankton + saltmarsh	11.02±3.60	5.91±2.03
	SOM-Plankton	0.038				
	SOM-Saltmarsh	0.016				
	Macroalgae-Plankton	1.000				
	Macroalgae-Saltmarsh	0.999				
	Plankton-Saltmarsh	0.993				
2014 Spring / 2015 Winter	SOM-Macroalgae	0.000	macroalgae + seagrass	plankton + saltmarsh	4.70±0.34	8.79±0.82
	SOM-Plankton	0.008				
	SOM-Saltmarsh	0.003				
	SOM-Seagrass	0.000				
	Macroalgae-Plankton	0.002				
	Macroalgae-Saltmarsh	0.008				
	Macroalgae-Seagrass	0.999				
	Plankton-Saltmarsh	0.943				
	Plankton-Seagrass	0.001				
2015 Winter / Spring	Saltmarsh-Seagrass	0.004				
	SOM-Macroalgae	0.000	SOM + plankton + saltmarsh	macroalgae + seagrass	8.74±0.96	5.10±0.76
	SOM-Plankton	0.997				
	SOM-Saltmarsh	0.068				
	SOM-Seagrass	0.000				
	Macroalgae-Plankton	0.000				
	Macroalgae-Saltmarsh	0.000				
	Macroalgae-Seagrass	0.950				
	Plankton-Saltmarsh	0.011				
	Plankton-Seagrass	0.000				
	Saltmarsh-Seagrass	0.000				
2015 Spring / 2016 Summer	SOM-Macroalgae	0.216	SOM + macroalgae + plankton + seagrass	saltmarsh	5.25±1.42	7.74±0.27
	SOM-Plankton	0.162				
	SOM-Saltmarsh	0.393				
	SOM-Seagrass	0.996				
	Macroalgae-Plankton	0.991				
	Macroalgae-Saltmarsh	0.007				
	Macroalgae-Seagrass	0.704				
	Plankton-Saltmarsh	0.009				
	Plankton-Seagrass	0.559				
	Saltmarsh-Seagrass	0.532				
2016 Summer / Autumn	SOM-Macroalgae	0.922	all sources	-	5.18±1.86	-
	SOM-Plankton	1.000				
	SOM-Saltmarsh	0.996				
	Macroalgae-Plankton	0.956				
	Macroalgae-Saltmarsh	0.974				
	Plankton-Saltmarsh	0.999				
2016 Autumn / Winter	SOM-Macroalgae	0.052	all sources	-	5.68±1.94	-
	SOM-Plankton	0.142				
	SOM-Saltmarsh	0.194				
	Macroalgae-Plankton	0.926				
	Macroalgae-Saltmarsh	0.729				
	Plankton-Saltmarsh	0.979				
2016 Winter / Spring	SOM-Macroalgae	0.003	SOM +	macroalgae +	4.95±1.44	7.41±0.49

	SOM-Plankton	0.680	plankton	saltmarsh		
	SOM-Saltmarsh	0.000				
	Macroalgae-Plankton	0.024				
	Macroalgae-Saltmarsh	0.967				
	Plankton-Saltmarsh	0.005				
2016 Spring / 2017 Summer	SOM-Plankton	0.047	SOM + plankton	saltmarsh	5.57±1.20	7.66±0.83
	SOM-Saltmarsh	0.025				
	Plankton-Saltmarsh	0.000				
2017 Summer / Autumn	SOM-Plankton	0.148	all sources	-	7.14±1.60	-
	SOM-Saltmarsh	0.494				
	Plankton-Saltmarsh	0.584				
2017 Autumn / Winter	SOM-Plankton	0.561	all sources	-	7.87±1.49	-
	SOM-Saltmarsh	0.295				
	Plankton-Saltmarsh	0.951				
2017 Winter / Spring	SOM-Macroalgae	0.007	SOM	macroalgae + plankton + saltmarsh	10.22±2.63	7.18±1.18
	SOM-Plankton	0.023				
	SOM-Saltmarsh	0.036				
	Macroalgae-Plankton	0.955				
	Macroalgae-Saltmarsh	0.710				
	Plankton-Saltmarsh	0.957				
2017 Spring / 2018 Summer	SOM-Macroalgae	0.000	SOM	macroalgae + plankton + saltmarsh	13.85±3.48	6.81±2.22
	SOM-Plankton	0.004				
	SOM-Saltmarsh	0.000				
	Macroalgae-Plankton	0.577				
	Macroalgae-Saltmarsh	0.991				
	Plankton-Saltmarsh	0.815				
2018 Summer / Autumn	SOM-Macroalgae	0.026	SOM + plankton + saltmarsh	macroalgae	10.18±3.71	6.53±3.00
	SOM-Plankton	0.984				
	SOM-Saltmarsh	0.082				
	Macroalgae-Plankton	0.059				
	Macroalgae-Saltmarsh	0.982				
	Plankton-Saltmarsh	0.161				

ANEXO 18 Tabela suplementar (Table S3) do Anexo 3.

Table S3. Sources utilized as a baseline for trophic position estimates in “Global” method.

Sources	$\delta^{15}\text{N}$	baseline
SOM	8.38	
Macroalgae	6.67	
Plankton	6.99	7.13±0.63
Saltmarsh	6.76	
Seagrass	6.89	

ANEXO 19 Tabela suplementar (Table S4) do Anexo 3.

Table S4. Trophic position (TP) estimates of the FishBase (Froese and Pauly 2019) used as an expected model to define the best baseline method. All the species chosen were sampled in our study (Patos Lagoon estuary between 2010 and 2018). Trophic guilds (TG): DTV – detritivores; HBV – herbivores; OMN – omnivores; ZPL – zooplanktivores; ZBV – zoobenthivores and; PSV – piscivores.

TG	Species	Descriptor	TP	Reference
DTV	<i>Cyphocharax voga</i>	(Hensel, 1869)	2,00	Froese & Pauly 2019
DTV	<i>Mugil brevirostris</i>	(Ribeiro, 1915)	2,00	Froese & Pauly 2019
DTV	<i>Mugil curema</i>	Valenciennes, 1836	2,00	Froese & Pauly 2019
DTV	<i>Mugil liza</i>	Valenciennes, 1836	2,00	Froese & Pauly 2019
DTV	<i>Mugil</i> sp.	Linnaeus, 1758	2,00	Froese & Pauly 2019
HBV	Clupeidae		2,00	Froese & Pauly 2019
HBV	<i>Neohelice granulata</i>	(Dana, 1851)	2,00	Bemvenuti & Colling 2010
HBV	<i>Platanichthys platana</i>	(Regan, 1917)	3,00	Froese & Pauly 2019
OMN	<i>Palaemon argentinus</i>	(Nobili, 1901)	2,00	Rodrigues et al. 2013
OMN	<i>Astyanax eigenmannianorum</i>	Cope, 1894	2,80	Froese & Pauly 2019
OMN	<i>Jenynsia multidentata</i>	Jenyns, 1842	2,90	Froese & Pauly 2019
OMN	<i>Penaeus paulensis</i>	(Pérez Farfante, 1967)	3,00	Bemvenuti & Colling 2010
ZPL	<i>Atherinella brasiliensis</i>	(Quoy & Gaimard, 1824)	3,20	Froese & Pauly 2019
ZPL	<i>Brevoortia pectinata</i>	(Jenyns, 1842)	3,40	Froese & Pauly 2019
ZPL	<i>Lycengraulis grossidens</i>	(Spix & Agassiz, 1829)	3,70	Froese & Pauly 2019
ZPL	<i>Odontesthes argentinensis</i>	(Valenciennes, 1835)	3,80	Froese & Pauly 2019
ZPL	<i>Pomatomus saltatrix</i>	(Linnaeus, 1766)	4,50	Froese & Pauly 2019
ZBV	<i>Cyrtograpsus angulatus</i>	Dana, 1851	3,00	Bemvenuti & Colling 2010
ZBV	<i>Micropogonias furnieri</i>	(Desmarest, 1823)	3,10	Froese & Pauly 2019
ZBV	<i>Syngnathus folletti</i>	Herald, 1942	3,30	Froese & Pauly 2019
ZBV	<i>Ctenogobius shufeldti</i>	(Jordan & Eigenmann, 1887)	3,40	Froese & Pauly 2019
ZBV	<i>Citharichthys spilopterus</i>	(Günther 1862)	3,60	Froese & Pauly 2019
ZBV	<i>Callinectes sapidus</i>	Rathbun, 1896	3,73	Mancinelli et al. 2016
PSV	<i>Oligosarcus jenynsii</i>	(Günther, 1864)	4,00	Froese & Pauly 2019
PSV	<i>Oligosarcus robustus</i>	(Menezes, 1969)	4,20	Froese & Pauly 2019

ANEXO 20 Tabela suplementar (Table S5) do Anexo 3.

Table S5. Trophic position (TP) estimates based on $\delta^{15}\text{N}$, and total length $\pm$ standard deviation (TL $\pm$ sd) (mm) of species collected in Patos Lagoon estuary between 2010 and 2018. (n) is the number of individuals sampled. Trophic guilds (TG): DTV – detritivores; HBV – herbivores; OMN – omnivores; ZPL – zooplanktivores; ZBV – zoobenthivores and; PSV – piscivores.

Period	Family	Species	Group	TG	n	TP	TL ($\pm$ sd)
2010 Summer	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	2	2.60	31.00 $\pm$ 0.00
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	6	2.60	67.08 $\pm$ 29.56
	Penaeidae	<i>Penaeus paulensis</i>	Shrimp	OMN	2	2.75	88.00 $\pm$ 37.00
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	2	2.79	107.5 $\pm$ 0.50
	Gobiidae	<i>Ctenogobius shufeldti</i>	Fish	ZBV	2	2.86	34.00 $\pm$ 1.00
	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	6	3.28	56.83 $\pm$ 29.17
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	3	3.68	45.33 $\pm$ 15.11
2010 Autumn	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	7	2.31	40.43 $\pm$ 13.92
	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	6	2.42	30.83 $\pm$ 4.26
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	6	2.42	35.33 $\pm$ 6.65
	Gobiidae	<i>Ctenogobius shufeldti</i>	Fish	ZBV	5	2.62	46.80 $\pm$ 6.27
	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	7	2.77	83.28 $\pm$ 33.52
	Penaeidae	<i>Penaeus paulensis</i>	Shrimp	OMN	6	2.82	68.33 $\pm$ 14.25
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	7	3.13	45.71 $\pm$ 14.93
2010 Winter	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	7	3.55	34.14 $\pm$ 6.69
	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	4	2.31	32.50 $\pm$ 2.50
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	6	2.35	35.50 $\pm$ 6.02
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	4	2.37	52.25 $\pm$ 15.35
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	10	2.51	48.30 $\pm$ 22.49
	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	5	2.60	110.00 $\pm$ 15.80
	Gobiidae	<i>Ctenogobius shufeldti</i>	Fish	ZBV	2	2.64	55.50 $\pm$ 8.50
	Penaeidae	<i>Penaeus paulensis</i>	Shrimp	OMN	3	2.77	35.83 $\pm$ 2.90
2010 Spring	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	4	2.94	81.50 $\pm$ 39.60
	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	4	2.59	30.50 $\pm$ 2.29
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	4	2.88	32.50 $\pm$ 5.02
	Penaeidae	<i>Penaeus paulensis</i>	Shrimp	OMN	5	3.31	34.00 $\pm$ 1.79
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	5	3.32	66.00 $\pm$ 37.64
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	6	3.61	48.83 $\pm$ 16.24
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	2	3.71	36.50 $\pm$ 0.50
2011 Summer	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	1	4.31	123.00 $\pm$ 0.00
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	6	2.20	42.67 $\pm$ 19.44
	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	2	2.23	29.50 $\pm$ 1.50
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	5	2.42	66.60 $\pm$ 29.20
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZBV	6	2.45	57.17 $\pm$ 13.56
	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZPL	4	2.45	96.50 $\pm$ 43.18
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	9	2.46	46.22 $\pm$ 14.14
2011 Autumn	Penaeidae	<i>Penaeus paulensis</i>	Shrimp	OMN	7	2.48	63.71 $\pm$ 17.68
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	9	2.33	59.89 $\pm$ 17.12
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	11	2.34	140.27 $\pm$ 114.17
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	3	2.37	60.67 $\pm$ 4.92

	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	6	2.51	50.00±19.96
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	5	2.29	38.80±10.30
	Penaeidae	<i>Penaeus paulensis</i>	Shrimp	OMN	3	2.54	31.67±4.50
2011	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	6	2.59	38.17±10.75
Winter	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	3	2.60	53.00±5.35
	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	6	2.71	30.83±2.91
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	12	2.94	73.75±18.44
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	7	2.94	46.80±8.16
2012	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	4	3.13	34.00±2.94
Autumn	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	5	3.21	34.60±2.42
	Mugilidae	<i>Mugil curema</i>	Fish	DTV	3	3.49	67.67±2.05
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	12	3.57	75.33±10.93
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	9	2.90	80.67±13.60
2012	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	3	3.04	31.33±9.10
Winter	Mugilidae	<i>Mugil liza</i>	Fish	DTV	4	3.17	44.00±0.00
	Clupeidae	<i>Platanichthys platana</i>	Fish	HBV	1	4.10	33.00±0.00
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	1	4.20	26.00±0.00
	Penaeidae	<i>Penaeus paulensis</i>	Shrimp	OMN	7	2.52	54.00±27.40
	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	6	2.59	30.50±2.75
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	9	2.60	61.44±29.44
2012	Varunidae	<i>Cyrtograpsus angulatus</i>	Crab	ZBV	6	2.71	23.00±3.92
Spring	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	6	2.91	59.17±10.95
	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	4	3.48	38.25±7.76
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	6	3.53	96.83±4.52
	Gobiidae	<i>Ctenogobius shufeldti</i>	Fish	ZBV	1	4.15	42.00±0.00
	Syngnathidae	<i>Syngnathus folletti</i>	Fish	ZBV	1	4.16	78.00±0.00
	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	3	2.14	28.33±2.05
2013	Mugilidae	<i>Mugil curema</i>	Fish	DTV	4	2.18	43.75±3.11
Summer	Mugilidae	<i>Mugil liza</i>	Fish	DTV	6	2.20	40.67±14.77
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	6	2.25	39.17±12.56
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	9	2.54	45.11±7.84
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	9	2.55	51.33±10.22
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	4	2.17	32.25±2.49
2013	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	6	2.23	27.33±6.97
Autumn	Penaeidae	<i>Penaeus paulensis</i>	Shrimp	OMN	7	2.25	47.71±20.71
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Crab	ZPL	10	2.46	67.30±14.35
2013	Clupeidae	n.i.	Fish	HBV	2	3.58	27.00±1.00
Winter	Atherinopsidae	<i>Odontesthes argentinensis</i>	Fish	ZPL	3	4.01	27.66±2.05
	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	7	2.95	31.00±5.18
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	10	3.05	43.90±21.27
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	6	3.21	45.33±7.48
2013	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	6	3.30	57.50±2.22
Spring	Clupeidae	<i>Brevoortia pectinata</i>	Fish	ZPL	8	3.41	39.75±4.29
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	9	3.54	47.11±8.75
	Palaemonidae	<i>Palaemon argentinus</i>	Shrimp	OMN	3	3.66	35.33±0.47
	Atherinopsidae	<i>Odontesthes argentinensis</i>	Fish	ZPL	3	3.67	40.33±3.68
	Gobiidae	<i>Ctenogobius shufeldti</i>	Fish	ZBV	3	3.98	41.00±4.97
	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	15	4.60	59.20±15.02

2014 Summer	Penaeidae	<i>Penaeus paulensis</i>	Shrimp	OMN	11	2.36	62.36±17.81
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	7	2.47	53.29±12.56
	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	3	2.51	30.00±0.82
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	8	2.55	50.88±3.95
	Mugilidae	<i>Mugil curema</i>	Fish	DTV	2	2.61	50.00±5.00
	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	16	2.61	57.56±27.13
	Engraulidae	<i>Lycengraulis grossidens</i>	Fish	ZPL	18	2.65	57.72±18.49
	Clupeidae	<i>Brevoortia pectinata</i>	Fish	ZPL	11	2.69	43.82±9.64
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	11	2.86	47.27±20.86
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	1	3.09	51.00±0.00
	Atherinopsidae	<i>Odontesthes argentinensis</i>	Fish	ZPL	1	3.15	168.00±0.00
2014 Autumn	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	17	2.12	55.59±13.48
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	2	2.24	39.00±1.00
	Clupeidae	<i>Brevoortia pectinata</i>	Fish	DTV	3	2.26	38.33±1.25
	Mugilidae	<i>Mugil curema</i>	Fish	ZPL	5	2.26	49.80±10.40
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	6	2.28	27.50±6.10
	Penaeidae	<i>Penaeus paulensis</i>	Shrimp	OMN	3	2.30	63.33±10.50
	Atherinopsidae	<i>Odontesthes argentinensis</i>	Fish	ZPL	2	2.34	40.50±3.50
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	4	2.47	60.00±3.67
	Clupeidae	<i>Platanichthys platana</i>	Fish	HBV	1	2.90	34.00±0.00
2014 Spring	Mugilidae	<i>Mugil liza</i>	Fish	DTV	7	2.97	60.14±11.53
	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	2	3.21	28.50±4.50
	Palaemonidae	<i>Palaemon argentinus</i>	Shrimp	OMN	5	3.73	36.00±3.03
	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	6	3.83	87.33±28.14
	Characidae	<i>Oligosarcus jenynsii</i>	Fish	PSV	3	3.85	61.67±2.36
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	6	3.87	57.33±23.49
	Curimatidae	<i>Cyphocharax voga</i>	Fish	DTV	1	3.98	62.00±0.00
	Characidae	<i>Astyanax eigenmanniorum</i>	Fish	OMN	1	4.18	67.00±0.00
	Gobiidae	<i>Ctenogobius shufeldti</i>	Fish	ZBV	7	4.21	46.14±10.47
2015 Winter	Mugilidae	<i>Mugil liza</i>	Fish	DTV	3	2.12	48.33±9.29
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	8	2.14	43.00±13.14
	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	3	2.22	26.67±2.49
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	10	2.24	59.30±15.82
	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	5	2.27	52.40±11.77
	Paralichthyidae	<i>Citharichthys spilopterus</i>	Fish	ZBV	3	2.28	70.00±7.79
	Gobiidae	<i>Ctenogobius shufeldti</i>	Fish	ZBV	9	2.29	43.44±13.01
	Penaeidae	<i>Penaeus paulensis</i>	Shrimp	OMN	1	2.87	35.00±0.00
2015 Spring	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	7	2.16	59.57±36.48
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	2	2.35	26.00±0.00
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	2	2.36	35.50±0.50
	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	7	2.45	89.71±22.74
	Atherinopsidae	<i>Odontesthes argentinensis</i>	Fish	ZPL	3	2.57	109.33±95.93
	Characidae	<i>Oligosarcus robustus</i>	Fish	PSV	2	2.79	61.00±2.00
	Varunidae	<i>Cyrtograpsus angulatus</i>	Crab	ZBV	1	2.88	27.00±0.00
	Gobiidae	<i>Ctenogobius shufeldti</i>	Fish	ZBV	1	2.93	34.00±0.00
2016 Summer	Penaeidae	<i>Penaeus paulensis</i>	Shrimp	OMN	9	2.14	60.11±10.24
	Gobiidae	<i>Ctenogobius shufeldti</i>	Fish	ZBV	5	2.26	39.40±5.57
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	6	2.28	45.17±9.03

	Mugilidae	<i>Mugil curema</i>	Fish	DTV	5	2.30	42.60±9.58
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	6	2.31	36.83±4.37
	Mugilidae	<i>Mugil brevisrostris</i>	Fish	DTV	6	2.34	42.83±6.84
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	8	2.36	55.00±23.41
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	9	2.45	51.11±11.35
2016 Autumn	Mugilidae	<i>Mugil liza</i>	Fish	DTV	5	2.34	32.80±2.79
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	10	2.35	40.10±16.08
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	4	2.39	59.75±16.62
	Gobiidae	<i>Ctenogobius shufeldti</i>	Fish	ZBV	8	2.59	39.25±11.58
	Palaemonidae	<i>Palaemon argentinus</i>	Shrimp	OMN	6	2.69	28.67±2.29
	Mugilidae	<i>Mugil brevisrostris</i>	Fish	DTV	1	2.85	100.00±0.00
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	1	2.90	41.00±0.00
2016 Winter	Mugilidae	<i>Mugil</i> sp.	Fish	DTV	2	3.42	45.00±5.00
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	5	3.60	55.60±23.27
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	1	4.09	350.00±0.00
	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	2	4.19	137.50±27.50
	Varunidae	<i>Cyrtograpsus angulatus</i>	Crab	ZBV	1	4.21	30.00±0.00
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	1	4.26	66.00±0.00
2016 Spring	Mugilidae	<i>Mugil liza</i>	Fish	DTV	5	2.26	60.80±9.11
	Palaemonidae	<i>Palaemon argentinus</i>	Shrimp	OMN	5	2.45	31.80±2.86
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	2	2.61	53.00±21.00
	Engraulidae	<i>Lycengraulis grossidens</i>	Fish	ZPL	2	2.68	41.00±0.00
	Characidae	<i>Oligosarcus jenynsii</i>	Fish	PSV	2	2.74	81.00±28.00
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	5	2.89	44.60±31.33
	Clupeidae	<i>Platanichthys platana</i>	Fish	HBV	1	2.95	33.00±0.00
	Gobiidae	<i>Ctenogobius shufeldti</i>	Fish	ZBV	1	3.04	41.00±0.00
2017 Summer	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	5	2.22	45.60±13.54
	Mugilidae	<i>Mugil curema</i>	Fish	DTV	5	2.33	51.00±11.30
	Penaeidae	<i>Penaeus paulensis</i>	Shrimp	OMN	3	2.44	31.00±3.27
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	5	2.45	44.60±17.33
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	5	2.48	55.40±21.80
	Mugilidae	<i>Mugil liza</i>	Fish	DTV	5	2.51	35.80±5.78
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	5	2.57	28.60±8.11
	Clupeidae	<i>Platanichthys platana</i>	Fish	HBV	1	2.92	-
	Clupeidae	<i>Brevoortia pectinata</i>	Fish	ZPL	5	2.98	40.40±11.22
	Gobiidae	<i>Ctenogobius shufeldti</i>	Fish	ZBV	1	3.06	45.00±0.00
	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	5	3.26	55.20±15.37
2017 Autumn	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	6	2.39	29.17±8.91
	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	5	2.40	31.00±2.83
	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	5	2.48	44.00±10.81
	Varunidae	<i>Cyrtograpsus angulatus</i>	Crab	ZBV	2	2.56	17.50±2.50
	Atherinopsidae	<i>Atherinella brasiliensis</i>	Fish	ZPL	1	2.88	37.00±0.00
	Gobiidae	<i>Ctenogobius shufeldti</i>	Fish	ZBV	1	3.03	29.00±0.00
2017 Winter	Mugilidae	<i>Mugil liza</i>	Fish	DTV	2	2.30	32.00±0.00
	Varunidae	<i>Cyrtograpsus angulatus</i>	Crab	ZBV	4	2.31	31.00±4.64
	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	3	2.33	24.33±0.47
	Engraulidae	<i>Lycengraulis grossidens</i>	Fish	ZPL	3	2.40	82.00±6.48
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	2	2.57	55.00±30.00

	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	4	2.75	142.00±59.66
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	3	3.28	65.00±11.86
2017	Pomatomidae	<i>Pomatomus saltatrix</i>	Fish	ZBV	2	3.61	66.50±3.50
Spring	Sciaenidae	<i>Micropogonias furnieri</i>	Fish	ZBV	3	3.95	98.33±20.95
	Clupeidae	<i>Platanichthys platana</i>	Fish	HBV	1	4.13	82.00±0.00
	Mugilidae	<i>Mugil</i> sp.	Fish	DTV	1	2.17	40.00±0.00
2018	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	5	2.19	47.00±5.18
Summer	Penaeidae	<i>Penaeus paulensis</i>	Shrimp	OMN	3	2.32	30.67±2.49
	Anablepidae	<i>Jenynsia multidentata</i>	Fish	OMN	4	2.83	47.75±8.55
	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	1	2.88	50.00±0.00
	Varunidae	<i>Neohelice granulata</i>	Crab	HBV	4	2.51	30.25±1.48
	Clupeidae	<i>Brevoortia pectinata</i>	Fish	ZPL	5	2.76	30.80±1.47
2018	Portunidae	<i>Callinectes sapidus</i>	Crab	ZBV	4	2.86	16.50±2.06
Autumn	Gobiidae	<i>Ctenogobius shufeldti</i>	Fish	ZBV	3	3.21	30.33±4.99
	Varunidae	<i>Cyrtograpsus angulatus</i>	Crab	ZBV	1	4.04	12.00±0.00
	Atherinopsidae	<i>Odontesthes argentinensis</i>	Fish	ZPL	1	4.14	68.00±0.00

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