

UNIVERSIDADE FEDERAL DO RIO GRANDE - FURG
PÓS-GRADUAÇÃO EM OCEANOGRAFIA BIOLÓGICA

**USO DE ISÓTOPOS ESTÁVEIS (C, N, S) NO
ESTUDO DE RELAÇÕES TRÓFICAS DE
PEIXES: VARIAÇÕES AO LONGO DE
GRADIENTES ESPACIAIS E SALINOS**

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“Any attempt to understand broad ecological patterns will be challenged by the complexity of nature.”

Nelson Hairston Jr. and Nelson Hairston Sr.

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RESUMO GERAL

As análises de isótopos estáveis são ferramentas úteis para o estudo dos ciclos de matéria e energia no ambiente. Marcadas mudanças nas razões de isótopos estáveis comumente ocorrem em gradientes salinos e tais mudanças são registradas através das relações tróficas. Contudo, a ausência de validação experimental de alguns pressupostos pode limitar o uso desta ferramenta, inclusive levando a interpretações equivocadas. Nesta tese, o uso e a assimilação de recursos tróficos por peixes foram investigados ao longo de um amplo gradiente salino, englobando as regiões límnic, estuarina e marinha adjacente à desembocadura da Lagoa dos Patos, empregando-se isótopos estáveis. Além de examinar possíveis interações tróficas entre os ambientes rasos e profundos nas regiões dentro da lagoa costeira, posteriormente incluindo o isótopo de enxofre (S) em combinação aos tradicionais carbono (C) e nitrogênio (N), procurou-se avançar o conhecimento sobre aspectos analíticos da ferramenta, como a determinação experimental em diferentes salinidades dos parâmetros taxa de renovação e discriminação isotópica. Os resultados obtidos permitiram responder a questões essenciais para o entendimento desta importante lagoa costeira, demonstrando por exemplo a existência de gradientes laterais de profundidade e que isótopos em tecido muscular de uma espécie eurihalina apresentam maiores estimativas de meia-vida em água-doce, quando comparados a ambientes de maiores salinidades, aprofundando assim o atual conhecimento sobre as relações tróficas e interações com componentes físicos e espaciais do ecossistema.

Palavras-chave: relações tróficas, gradientes, salinidade, profundidade, taxa de renovação isotópica, discriminação isotópica, enxofre.

GENERAL ABSTRACT

Stable isotopes analyses are useful tools in the study of cycles of energy and organic matter in the environment, reflecting the history of physical and metabolic processes in ecosystems. Marked changes in stable isotope ratios commonly occur along salinity gradients and those are recorded through trophic relationships. However, the lack of experimental validation of certain assumptions can limit the applicability of this analysis. In this thesis, the use and assimilation of food resources by fishes were investigated along a salinity gradient, encompassing freshwater, estuarine and marine regions, using stable isotopes analysis. In addition to examining possible trophic interactions between shallow and deep areas inside the coastal lagoon, and later to include the sulfur isotope (S) in combination with traditional carbon (C) and nitrogen (N), we aimed to attend a call for more studies on analytical aspects of this tool, such as determining turnover rates and trophic discrimination factors under different salinities. Results allowed us to answer key questions for the understanding of this important coastal lagoon, demonstrating for example the importance of considering the existence of lateral gradients and that carbon and nitrogen half-life estimates in muscle tissue of a euryhaline fish take longer in freshwater compared to environments with higher salinity values, therefore enhancing the current knowledge on trophic relationships and their interactions with physical and spatial components of the ecosystem.

Keywords: trophic relationships, gradients, salinity, depth, turnover rate, trophic discrimination factors, Sulphur.

INTRODUÇÃO GERAL

Lagunas costeiras podem ser definidas como ecossistemas aquáticos continentais, geralmente orientadas paralelas à costa, separadas do oceano por uma barreira mas conectadas a este por ao menos uma entrada, podendo ser permanentemente abertas ou intermitentemente fechadas (Kjerfve, 1994). Estes ecossistemas ocupam 13% das zonas costeiras em todo o mundo e experimentam os mais diversos tipos de forçantes como, por exemplo, deságue de rios, marés, equilíbrio nas variações da precipitação/evaporação, estresses eólicos e mudanças induzidas pelo homem, o que os tornam sistemas únicos em comparação com outros ambientes aquáticos (Kjerfve, 1994, Esteves et al. 2008). Todas estas características fazem das lagunas costeiras ambientes altamente produtivos e fortemente valorizados aos olhos das sociedades, portanto, objetos de profundo interesse econômico e científico (Esteves et al. 2008, Anthony et al. 2009, Seeliger & Odebrecht 2010).

A heterogeneidade fisiográfica observada em lagunas costeiras pode ser responsável pela criação de uma vasta gama de gradientes ecológicos e micro-habitats, os quais são cruciais para o suporte da biodiversidade local (Belgrano et al. 2006, Esteves et al. 2008). Dentre estes gradientes, o mais conhecido é o gerado pela variação de salinidade (Deegan & Garritt 1997, Fry 2002, Prado et al. 2014), mas um conhecimento incompleto destes processos contribui para agravar incertezas resultantes da complexidade inerente em ecossistemas aquáticos (Day Jr. et al. 2013). A salinidade pode influenciar a fisiologia e a distribuição de organismos dentre e entre habitats, com efeitos contexto-dependentes sobre a biodiversidade, tanto positivos quanto negativos (Kozlowsky-Suzuki & Bozelli 2004, Sosa-López et al. 2007, Moura et al. 2012). Consequentemente, fica claro que ecossistemas lagunares costeiros são

compostos por muitas interações de componentes bióticos e abióticos (e.g., nutrientes, organismos), das quais o funcionamento não é sempre óbvio e de fácil entendimento a partir de observações.

A biota de uma lagoa costeira pode ser classificada de diversas maneiras, seja pela taxonomia (e.g., algas, crustáceos, peixes), seja pela função trófica (e.g., produtores primários, decompositores), ou pelo hábitat e/ou nicho (e.g., bentônicos, pelágicos), e cabe ao estudo das tramas tróficas examinar o arranjo e a interdependência desses componentes (Polis et al. 1997, Day Jr. et al. 2013). Um dos princípios básicos da ecologia é que os componentes biológicos estão intimamente interligados entre si e com o ambiente físico e, portanto, compreender a estrutura e dinâmica de vias tróficas é crucial para a avaliação do funcionamento e da qualidade do hábitat (Elton 1927). Algumas abordagens sugerem que a heterogeneidade espacial do ambiente pode criar módulos ou compartimentos tróficos, onde as relações alimentares seriam mais intensas/frequentes dentro de um mesmo módulo, sentidas principalmente em níveis tróficos inferiores, e que organismos nos níveis tróficos superiores poderiam agir como "integradores" dessa variabilidade no espaço (de Ruiter et al. 2005). No caso particular dos peixes, a grande disponibilidade de informações sobre o ciclo de vida destes animais, quando comparados a outros grupos taxonômicos, e a diversidade trófica existente na ictiofauna são características que fornecem uma visão integrada do sistema analisado (Pasquaud et al. 2010, Harrison & Whitfield 2012). Estudos realizados em sistemas aquáticos fechados considerando esta variação espacial principalmente em relação à profundidade (ambientes rasos vs. ambiente profundos) demonstraram haver mudanças nos padrões de alocação de recursos e a existência destes compartimentos tróficos (Schindler & Scheuerell 2002,

Bertolo et al. 2005, Sierszen et al. 2014), mas por algum motivo este tipo de investigação não tem tido a mesma importância em sistemas abertos, como lagunas costeiras (Stasko et al. 2016).

Uma vasta gama de conceitos, perspectivas de pesquisa e ferramentas metodológicas podem ser relacionadas ao estudo da ecologia alimentar, a qual pode fornecer informações sobre quase todas as áreas de pesquisas ecológicas, desde a ciclagem de nutrientes através dos ecossistemas até a dinâmica de populações (Pimm 2002, Layman et al. 2015). Três etapas podem ser identificadas no desenvolvimento histórico do estudo de relações alimentares: uma fase inicial caracterizada por observações e reconhecimento de padrões, uma fase intermediária visando analisar e comparar propriedades para compreender sistemas ecológicos, e uma fase contemporânea com abordagens aprimoradas (Dunne 2012, Layman et al. 2015).

O uso de isótopos estáveis é um exemplo de abordagem analítica aprimorada que tem atraído o interesse de ecólogos aquáticos nas últimas décadas, porque na maioria das vezes o meio aquático impede a observação direta do comportamento do organismo e sua interação com o ambiente. Isótopos são átomos de um mesmo elemento que possuem diferente número de nêutrons, por exemplo, carbono 12 (mais leve e abundante) e carbono 13 (mais pesado e menos abundante). Muitos estudos ecológicos expressam a composição isotópica em termos de δ (delta), que representam partes por mil (‰) de diferença do isótopo da amostra em relação ao padrão: $\delta X = [(R_{amostra}/R_{padrão}) - 1] \times 10^3$, onde R é a razão correspondente às formas pesadas e leves. Talvez um dos atributos mais atraentes dessa técnica seja o fato de que a abundância relativa dos isótopos estáveis na natureza pode ser empregada como

marcador natural de fluxos de matéria e energia entre os organismos e ecossistemas (Michener & Lajtha 2007). Atualmente, os isótopos estáveis vêm sendo utilizados para encontrar padrões através de uma gama de escalas, desde o nível molecular até a caracterização de cadeias alimentares inteiras, podendo reconstruir paleoambientes, traçar fluxos de nutrientes entre os ecossistemas e identificar subsídios tróficos entre ecossistemas, a origem ou movimento de organismos, bem como para detectar respostas a alterações ambientais (Michener & Lajtha 2007, Pasquad et al. 2010, Maier et al. 2011).

Os isótopos estáveis mais utilizados nas investigações e estudos tróficos são os de carbono ($^{13}\text{C}/^{12}\text{C}$) e nitrogênio ($^{15}\text{N}/^{14}\text{N}$) (Grey 2006). Por causa de sua baixa discriminação isotópica (usualmente entre 0,5 e 1 permil), o carbono é o que melhor correlaciona as fontes alimentares e os consumidores, ao passo que o nitrogênio, que possui uma discriminação mais acentuada (usualmente entre 2 e 4 permil) a cada nível trófico, permite estimar o nível trófico do consumidor (Vanderklift & Ponsard 2003). Ainda que a pesquisa ecológica usando isótopos estáveis tenha progredido rapidamente durante os últimos 20 anos (Boecklen et al. 2011, Layman et al. 2012), uma limitação deste método é a possível sobreposição nos valores das razões isotópicas das fontes autotróficas e dos consumidores, que usualmente ocorre quando se utilizam apenas dois isótopos estáveis (por exemplo, C e N) (Connolly et al. 2004, Herzka 2005). Neste sentido, a aplicação de isótopos estáveis de enxofre (S) em uso simultâneo com os isótopos de carbono e nitrogênio pode esclarecer ainda mais as relações tróficas em sistemas aquáticos, gerando caracterizações mais detalhadas dos fluxos de energia/matéria e do movimento dos organismos entre ecossistemas adjacentes (Hesslein et al. 1991, Nehlich et al. 2011).

A fim de ser incorporado nos principais compostos orgânicos dentro das células, a saber os aminoácidos essenciais metionina e cisteína, o sulfato deve ser assimilado e reduzido a sulfeto (Canfield 2001). O processo de redução do sulfato (SR) é especialmente ativo em ambientes aquáticos, ocorrendo principalmente em sedimentos do fundo, mas também podendo ocorrer na coluna d'água em condições anaeróbicas pronunciadas, sendo assim úteis na diferenciação de cadeias tróficas baseadas em recursos pelágicos e associados ao substrato bentônico (Croisetière et al. 2009). Os íons sulfato estão entre os grandes contribuintes para a salinidade da água e, por este motivo, os isótopos de enxofre apresentam uma íntima relação com esta variável, reforçando o uso deste elemento em estudos de residência em ambientes aquáticos e de movimento entre habitats e ecossistemas aquáticos (Couillard et al. 2011).

Um aspecto fundamental em estudos de ecologia trófica que utilizam a técnica de isótopos estáveis, que muitas vezes é negligenciado, é a validação experimental de pressupostos (Martínez del Rio 2009). Por exemplo, a relação entre a dieta e as razões isotópicas em tecidos de consumidores nem sempre é direta e pode estar sujeita a variabilidade por diferenças de assimilação de nutrientes, discriminação isotópica e taxas de renovação de tecidos (Suring & Wing 2009), levando a uma interpretação inacurada/errônea dos dados (Dalerum & Angerbjorn 2005). Idealmente, esses pressupostos deveriam ser validados em laboratório, através de experimentos controlados (Layman et al. 2012). As inferências que podemos fazer sobre o uso de recursos por animais a partir de dados isotópicos é limitada pela rapidez com a qual os tecidos refletem diferenças na sua dieta (Martínez del Rio & Anderson-Sprecher 2008). Cada tecido pode apresentar taxa de renovação isotópica (TRI) própria em função do conteúdo isotópico da alimentação e da taxa de renovação bioquímica. Entende-se TRI

como sendo a renovação contínua dos elementos químicos e, conseqüentemente, de seus isótopos que compõem o tecido corporal ou o organismo como um todo. A TRI pode ocorrer por meio de renovação tecidual resultante do processo de síntese e degradação em tecidos adultos e/ou pelo próprio crescimento nos tecidos em formação (diluição isotópica). Desta maneira, os principais fatores que controlam e influenciam a taxa de renovação isotópica em tecidos são o crescimento do indivíduo e o metabolismo (Ducatti 2007). Porém, são escassos os trabalhos que determinaram a TRI de espécies, considerando fatores endógenos (e.g., metabolismo, taxa de crescimento) e exógenos (e.g., temperatura, salinidade), os quais podem afetar diretamente o metabolismo (Hesslein et al. 1993, Zuanon et al. 2006, German & Miles 2010).

Outro parâmetro muito utilizado em estudos isotópicos, mas para o qual existem poucas validações experimentais, é o fator de discriminação isotópica (*Trophic Discrimination Factor* - TDF) (Vanderklift & Ponsard 2003). Ele se refere ao processo no qual as razões entre os isótopos estáveis se alteram entre os valores da presa e do consumidor durante o processamento metabólico do material consumido (Fry 2006). A maioria dos valores de TDF disponíveis na bibliografia são oriundos de experimentos com insetos terrestres, mamíferos, aves e animais de água doce, havendo pouca informação disponível para animais marinhos costeiros e estuarinos (Yokoyama et al. 2005, Caut 2009). O uso de TDFs inexatos pode introduzir erros substanciais nas estimativas da contribuição relativa dos produtores primários ou das presas para os consumidores. Portanto, é crucial ter medidas válidas e robustas de discriminação para os tecidos e espécies de interesse.

A Lagoa dos Patos, localizada na planície costeira do Rio Grande do Sul, possui uma área total superior a 10.000 km², sendo a maior laguna costeira do tipo estrangulado do mundo (Kjerfve 1994). Suas águas vem sendo objeto de estudos ecológicos nos mais diversos campos - físicos, químicos, geológicos e biológicos - buscando avançar o conhecimento de processos indispensáveis para um manejo ambiental integrado (Seeliger & Odebrecht 2010). A configuração batimétrica do corpo principal lagunar é caracterizada tanto por canais naturais quanto artificiais, extensas áreas adjacentes aos mesmos e enseadas marginais rasas (Calliari 1998). A área estuarina ocupa 10% deste ecossistema e representa um ambiente de transição, assumindo funções críticas de interface entre a água doce e a plataforma continental, sendo essencial para o ciclo de vida de diversas espécies e importante também economicamente para a região na qual está inserida (Kalikoski & Vasconcellos 2012, Oliveira et al. 2013). Nesta região predominam áreas largas e rasas (1 – 5 m) e a principal troca de água com o oceano adjacente ocorre através de um canal estreito e profundo (~ 18 m), o qual é frequentemente dragado para propósitos de navegação. A hidrodinâmica deste estuário é determinada principalmente pelo regime de ventos predominantes e pelo aporte de água doce oriundo da grande bacia de drenagem que compõe o sistema lagunar Patos-Mirim, sendo portanto altamente dependente do regime de chuvas da região (Möller et al. 2001, Seeliger & Odebrecht 2010). O limite superior da região estuarina é usualmente considerado 80 km ao norte da boca da laguna, mas seu alcance pode se estender a 200 km durante severos períodos de seca (Hartmann & Schettini 1991). Fatores como vento, chuva e evaporação também são responsáveis por regular a variabilidade a curto prazo de organismos fitoplanctônicos, ao passo que variações interanuais estão mais relacionadas a fenômenos climáticos

remotos, tal qual o El Niño - Oscilação Sul (Fujita & Odebrecht 2007, Haraguchi et al. 2015, Abreu et al. 2016). O estuário da Lagoa dos Patos se caracteriza como uma zona de berçário para diversas espécies de peixes e crustáceos, e os altos níveis de produção primária ali encontrados sustentam energeticamente vários organismos aquáticos de extrema importância econômica para as atividades pesqueiras, seja no estuário e/ou na região costeira adjacente. Um exemplo é a corvina, *Micropogonias furnieri* (Desmarest, 1823), uma espécie pelágica-demersal, estuarina-dependente, amplamente distribuída ao longo do Atlântico Oeste (Vasconcellos & Haimovici 2006, Costa et al. 2014). Em estuários e lagoas costeiras, indivíduos juvenis usualmente ocupam habitats de baixa salinidade, a partir dos quais eles se movem em direção a áreas de maior salinidade e ali permanecem até o momento em que atingem maturidade, então migrando para o oceano para se reproduzirem (Costa & Araújo 2003, Jaureguizar et al. 2003). Entender a ocupação destes ambientes e movimento entre eles é de extrema importância, pois estudos já demonstraram existir diferença entre os tamanhos de indivíduos de mesma idade capturados na região marinha costeira e no estuário, podendo esta diferença ser atribuída a estresses ambientais experimentados por aqueles que permanecem na região estuarina, uma área marcada por flutuações nos valores de salinidade (Cavole & Haimovici 2015).

Ainda que estudos utilizando a ferramenta de isótopos estáveis para se investigar relações tróficas de peixes já tenham sido conduzidos na região, estes foram principalmente restritos ao estuário e limitados às áreas rasas, não considerando áreas adjacentes (Abreu et al. 2006, Garcia et al. 2007, Hoeinghaus et al. 2011, Claudino et al. 2013). Nesse contexto, esta tese teve como objetivo geral empregar o uso de isótopos estáveis no estudo de relações tróficas de peixes ao longo de um gradientes

espaciais e salinos, englobando a região límnic, estuarina e marinha adjacente à desembocadura da Lagoa dos Patos. Além de investigar possíveis interações tróficas entre os ambientes rasos e profundos ao longo do gradiente límnic-estuarino (capítulo 1), a tese procurou avançar o conhecimento sobre aspectos analíticos da ferramenta isotópica como a determinação experimental dos parâmetros TRI e TDF em diferentes salinidades (capítulo 2), e a inclusão do isótopo de enxofre (S) em combinação com os tradicionais de carbono (C) e nitrogênio (N) (capítulo 3).

OBJETIVOS

Os principais objetivos desta tese foram:

- i) investigar potenciais diferenças na organização trófica das assembleias de peixes ao longo de gradientes laterais de profundidade (áreas rasas vs. áreas profundas) em uma lagoa costeira subtropical;
- ii) determinar em laboratório a taxa de renovação (TRI) e os fatores de discriminação (TDF) dos isótopos estáveis dos elementos carbono e nitrogênio no tecido muscular da corvina (*Micropogonias furnieri*) sob diferentes salinidades;
- iii) comparar o uso de dois (C, N) e três (C, N, S) isótopos estáveis no estudo da ecologia trófica da corvina ao longo de um gradiente límnic-estuarino-marinho em uma área subtropical.

HIPÓTESES

Considerando o contexto e as informações acima expostas, as hipóteses de trabalho foram:

Hipótese 1: As assembleias de peixes que ocorrem em áreas rasas e canais profundos de lagunas costeiras estão estruturadas em módulos tróficos parcialmente segregados entre si.

Hipótese 2: As taxas de renovação e discriminação tróficas ocorrem mais lentamente quando espécies eurihalinas estão em ambientes de água doce, como resultado de um maior custo energético devido à atividade osmorregulatória.

Hipótese 3: a incorporação do isótopo de enxofre a estudos ecológicos com os elementos carbono e nitrogênio permite diferenciar com maior exatidão a importância relativa das fontes alimentares basais (i.e., produtores primários e outras fontes orgânicas na base da cadeia alimentar) que sustentam uma espécie generalista ao longo de um gradiente salino.

RESULTADOS

Os resultados serão apresentados na forma de capítulos. O primeiro e segundo capítulos são apresentados de forma sintética no corpo principal, enquanto suas versões na íntegra estão na forma de artigos já publicados em língua estrangeira e disponibilizadas na sessão Apêndices. Já o terceiro capítulo é apresentado em sua versão integral e em português no corpo principal da tese.

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CAPÍTULO 1

SEGREGAÇÃO TRÓFICA DE UMA ASSEMBLEIA DE PEIXES AO LONGO DE GRADIENTES LATERAIS DE PROFUNDIDADE EM UMA LAGOA COSTEIRA SUBTROPICAL, REVELADA PELA ANÁLISE DE ISÓTOPOS ESTÁVEIS.

MONT'ALVERNE, R, PER PEREYRA & AM GARCIA. 2016. Trophic segregation of a fish assemblage along lateral depth gradients in a subtropical coastal lagoon revealed by stable isotope analyses. *Journal of Fish Biology*, doi:10.1111/jfb.12903.

Versão completa acessível no **APÊNDICE I**

RESUMO DO ARTIGO

Embora investigações sobre conectividade entre habitats localizados em um gradiente de profundidade, usando peixes como indicadores, tenham sido realizadas para sistemas aquáticos fechados (Schindler & Scheuerell 2002, Sierszen et al. 2014), a mesma atenção não foi dada a sistemas abertos, tais como rios (Roach et al. 2009) e estuários (Grimaldo et al. 2009, Neves et al. 2013). A análise de isótopos estáveis é uma das abordagens mais utilizadas para avaliar as vias tróficas e a organização de teias alimentares em ecossistemas aquáticos (Fry 2006, Reynolds 2008, Middelburg 2013), fornecendo uma maneira conveniente e informativa para integrar o fluxo de

energia através de escalas espaciais e detectar compartimentalização (Quevedo et al. 2009). Pela aplicação desta técnica, este estudo teve como objetivo fornecer informações sobre a organização trófica e as fontes alimentares basais que sustentam a ictiofauna em áreas rasas e profundas, situadas ao longo de um gradiente água doce-estuário, em uma das maiores lagoas costeiras do mundo. A hipótese é que as assembleias de peixes que ocorrem em áreas rasas e canais profundos estão estruturadas em módulos tróficos distintos e parcialmente segregados, com os consumidores apresentando distintos graus de assimilação de fontes alimentares bentônicas e pelágicas.

Para investigar tal hipótese, componentes da cadeia alimentar foram amostrados em áreas rasas (<1,5 m) e profundas (~ 5m) em três regiões: I) Água doce, II) Estuário superior e III) Estuário inferior. As fontes alimentares basais amostradas incluíram macroalgas de deriva, plantas de marismas (macrófitas C3 e C4), matéria orgânica em suspensão (POM) e matéria orgânica particulada no sedimento (SOM). Os consumidores amostrados, por sua vez, incluíram peixes e suas possíveis presas, como o zooplâncton, infauna bentônica e macrocrustáceos decápodes. As espécies de peixes capturadas foram classificadas em seis grupos funcionais, de acordo com o modo de alimentação, a saber: (1) zooplantívoros (ZP), (2) detritívoros (D), (3) herbívoros (H), (4) onívoros (OM), (5) piscívoros (P) e (6) zoobentívoros (ZB). Um diagrama conceitual foi construído para resumir os resultados dos modelos de mistura isotópicos, facilitando a comparação das relações tróficas ecológicas entre as áreas rasas e profundas ao longo deste gradiente água doce-estuário.

Análises estatísticas revelaram um significativo efeito da lateralidade (ambientes rasos vs. profundos) nos valores de $\delta^{13}\text{C}$ para fontes alimentares basais e para a ictiofauna, em quase todas as regiões amostradas. A tendência no enriquecimento isotópico de ^{13}C da água doce para o oceano observada para as fontes de alimento basais, embora não tão acentuada, também pôde ser vista para os consumidores, o que demonstra que os padrões de composição isotópica exibidos por fontes primárias tendem a serem refletidos de maneira similar ao longo da cadeia alimentar.

Os resultados dos modelos de mistura isotópica revelaram um forte subsídio trófico de origem bentônica, o qual seria responsável por explicar não somente uma maior diversidade de peixes encontrados em áreas rasas, mas também altas densidades em áreas mais profundas, especialmente para o grupo funcional ZB. Os diagramas conceituais destacam o maior uso de múltiplas fontes de alimento por diferentes grupos funcionais em áreas rasas, em oposição ao predomínio da fonte basal SOM em áreas profundas.

Em conclusão, ainda que peixes desempenhem importantes papéis no acoplamento de habitats, os resultados suportam a hipótese que a organização trófica em áreas rasas e profundas é parcialmente segregada, e que estes ambientes devem ser considerados como diferentes módulos ou subsistemas tróficos, tanto na água doce quanto no estuário. Este estudo indica a importância de se considerar a existência de gradientes laterais de profundidade em estudos ecológicos em lagoas costeiras, oferecendo uma perspectiva complementar sobre ecologia espacial em ecossistemas aquáticos.

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CAPÍTULO 2

**TAXAS DE RENOVAÇÃO E DISCRIMINAÇÃO ISOTÓPICAS DOS ELEMENTOS CARBONO E
NITROGÊNIO EM UMA ESPÉCIE EURIHALINA DE PEIXE CRIADA EM DIFERENTES SALINIDADES:
IMPLICAÇÕES PARA ESTUDOS DE MOVIMENTO.**

MONT'ALVERNE, R, TD JARDINE, PER PEREYRA, MCLM OLIVEIRA, RS MEDEIROS, LA SAMPAIO, MB TESSER & AM GARCIA. 2016. Elemental turnover rates and isotopic discrimination in a euryhaline fish reared under different salinities: Implications for movement studies. *Journal of Experimental Marine Biology and Ecology*, 480: 36–44.

Versão completa acessível no **APÊNDICE II**

RESUMO DO ARTIGO

A análise de isótopos estáveis é uma ferramenta útil para estudos de movimento e residência de espécies entre/em ambientes aquáticos. No entanto, ainda existem muitas dúvidas sobre a sua utilização, sobretudo relacionadas a pressupostos que deveriam ser validados experimentalmente, tais como taxas de renovação (TRI) e fatores de discriminação trófica (TDF). Mesmo sabendo que diferenças em valores de salinidade representam um fator chave para determinar a distribuição de peixes e uso

do hábitat em estuários e lagoas costeiras, a influência desta variável sobre os parâmetros isotópicos acima mencionados ainda não foi investigada em peixes estuarinos dependentes. Desta forma, foi realizado um experimento de alimentação controlada para determinar tanto as taxas de renovação (TRI) quanto TDFs dos isótopos estáveis de carbono ($\delta^{13}\text{C}$) e nitrogênio ($\delta^{15}\text{N}$) em tecido muscular de uma espécie eurihalina de peixe, popularmente conhecida como corvina (*Micropogonias furnieri*). Neste estudo, procurou-se verificar a hipótese de que a taxa de renovação no ambiente de água doce seria mais lenta, como resultado de um maior custo energético devido à atividade osmorregulatória.

Os exemplares de corvina foram capturados em seu ambiente natural no estuário da Lagoa dos Patos, transportados para uma estação de aquicultura, selecionados (massa entre 8 e 10 g) e distribuídos aleatoriamente entre três tratamentos de diferentes salinidades, nas densidades de 30 peixes/tanque (90 peixes por tratamento). Inicialmente, para se evitar choque osmótico, a salinidade foi mantida constante e similar àquelas que as corvinas experimentavam em campo (~20). Durante a primeira semana este valor foi gradualmente ajustado até que os tratamentos atingissem valores simulando os ambientes de água doce, estuarinos e ambiente marinho (respectivamente salinidades de 3, 16 e 27). Os peixes foram alimentados com uma dieta experimental isotopicamente distinta dos valores de carbono e de nitrogênio comumente encontrados para esta região. O experimento durou 90 dias e, uma vez por semana, três indivíduos de cada tratamento (um por tanque) foram letalmente amostrados para obter amostras de músculo para a análise isotópica. Dois dias após o encerramento do experimento (dia 92), todos os peixes remanescentes foram também

letalmente amostrados, a fim de se medir a variação nos fatores de discriminação tróficos uma vez que os animais alcançassem um estado de equilíbrio com a nova dieta. Lípidios não foram removidos das nossas amostras antes das análises, mas, uma vez que níveis elevados destas moléculas podem reduzir os valores de carbono (Logan et al. 2008), $\delta^{13}\text{C}$ de amostras da alimentação fornecida e de amostras de tecido muscular das corvinas foram corrigidas matematicamente ($\delta^{13}\text{C}_{\text{corr}}$).

Estimativas indicam que na água doce os isótopos de carbono demoram quase um mês a mais para atingir a renovação quase completa no tecido muscular ($^{13}\text{C } T_{95} = 110,9$ dias; $^{13}\text{C}_{\text{corr}} T_{95} = 93,6$ dias), quando comparados ao comportamento do mesmo elemento na salinidade estuarina ($^{13}\text{C } T_{95} = 80,9$ dias; $^{13}\text{C}_{\text{corr}} T_{95} = 73,0$ dias) e na salinidade marinha ($^{13}\text{C } T_{95} = 76,8$ dias; $^{13}\text{C}_{\text{corr}} T_{95} = 69,6$ dias). Análises dos valores de nitrogênio indicam que este elemento na água doce também leva mais tempo para renovar ($^{15}\text{N } T_{95} = 149,7$ dias), em comparação àqueles na salinidade estuarina ($^{15}\text{N } T_{95} = 103,3$ dias) ou na salinidade do mar ($^{15}\text{N } T_{95} = 96,6$ dias). Uma vez que apenas alguns dos tratamentos alcançaram o equilíbrio entre o tecido do consumidor e da sua dieta, a discriminação trófica é apresentada em duas formas, uma calculada a partir de animais amostrados após o final do experimento (dia 92; $\text{TDF}_{\text{final}}$), e outra a partir de assíntotas resultantes de equações matemáticas ($\text{TDF}_{\text{asympt}}$). Os valores de $\text{TDF}_{\text{final}}$ foram mais consistentes entre os tratamentos para $\delta^{15}\text{N}$, variando de 3,42 a 3,89‰, ao passo que para o carbono os valores foram semelhantes entre a água doce ($\delta^{13}\text{C} = 0,82 \pm 0,54$; $\delta^{13}\text{C}_{\text{corr}} = -0,41 \pm 0,28$) e a salinidade estuarina ($\delta^{13}\text{C} = 0,88 \pm 0,58$; $\delta^{13}\text{C}_{\text{corr}} = -0,32 \pm 0,30$), mas superior na salinidade marinha ($\delta^{13}\text{C} = 1,44 \pm 0,66$; $\delta^{13}\text{C}_{\text{corr}} = 0,02 \pm 0,37$). Como esperado, os resultados $\text{TDF}_{\text{asympt}}$ foram menores do que $\text{TDF}_{\text{final}}$ (em

aproximadamente 0,5‰), mas exibiram o mesmo padrão para todos os isótopos, com valores mais baixos sendo encontrado em água doce, intermediário em águas estuarinas e mais altos no tratamento marinho.

Em conclusão, este estudo forneceu evidências experimentais dos efeitos da salinidade sobre taxas de renovação e fatores de discriminação isotópicos, demonstrando que estes diferem não somente entre isótopos, mas também entre os ambientes. Os resultados obtidos apoiam parcialmente a hipótese inicial, com isótopos nos tecidos musculares de fato levando mais tempo para renovarem em água doce. Contudo, uma vez que a incorporação isotópica dos elementos presentes na dieta não foi mais rápida na salinidade isosmótica para esta espécie (tratamento estuarino) e as taxas metabólicas não foram medidas diretamente, essas diferenças nas taxas de renovação entre tratamentos não pode ser exclusivamente atribuída à osmorregulação. Esse foi o primeiro trabalho a demonstrar experimentalmente que esses parâmetros são influenciados pela salinidade. Desta maneira, as informações aqui obtidas contribuem para refinar o uso de isótopos estáveis em estudos de transporte de material dentro e entre cadeias alimentares, sendo essenciais para inferir padrões de uso do hábitat, residência e de migração de espécies eurihalinas.

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CAPÍTULO 3

Comparação do uso de dois (C-N) e três (C-N-S) isótopos estáveis no estudo da ecologia trófica de *Micropogonias furnieri* ao longo de um gradiente límnic – estuarino – marinho.

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RESUMO

A corvina (*Micropogonias furnieri*) é uma espécie generalista que transita ao longo de um gradiente límnic-estuarino-marinho durante seu ciclo de vida. Modelos de mistura isotópica bayesianos duplos (C-N) e triplos (C-N-S) foram usados na investigação das fontes alimentares basais e presas assimiladas por esta espécie em diferentes salinidades. De modo geral não foram observadas diferenças significativas entre as classes de tamanho dos peixes com relação a cada isótopo, dentro de uma

mesma área, sugerindo uma constância no padrão geral das relações alimentares ao longo do ciclo de vida. A adição do isótopo de enxofre mostrou-se especialmente importante para a discriminação da importância da matéria orgânica particulada no sedimento e representou um ganho marcante na acurácia para a determinação dos elos tróficos basais que sustentam a corvina no estuário.

Palavras chave: enxofre, isótopos estáveis, relações tróficas, corvina, salinidade

INTRODUÇÃO

Uma compreensão mais profunda de ecossistemas ao longo de gradientes ambientais é um grande desafio para ecólogos e exige a consideração das múltiplas relações tróficas nestes ambientes. Vários estudos científicos enfatizam o papel central destas relações para o conhecimento do funcionamento de sistemas aquáticos (Belgrano et al. 2006, Pasquaud et al. 2007), sendo um dos desafios identificar os mecanismos por trás de padrões observados, especialmente em estuários e zonas costeiras, onde a gama de recursos alimentares é bastante ampla e variável (Day Jr. et al. 2013, Magnone et al. 2015).

Desvendar um pouco da complexidade inerente à ecologia de ecossistemas exige constantemente o desenvolvimento e aplicação de novas abordagens. Neste sentido, isótopos estáveis vêm sendo cada vez mais utilizados para responder a diferentes tipos de questões ecológicas, permitindo avanços consideráveis na descrição/reconstrução de dietas, determinação de fluxos de matéria e energia (elos

tróficos), uso de hábitat e padrões de migração de organismos (Hobson 1999, Boecklen et al. 2011). Enquanto há certamente muita informação que pode ser elucidada através da análise de isótopos estáveis, sendo o carbono ($^{13}\text{C}/^{12}\text{C}$) e o nitrogênio ($^{15}\text{N}/^{14}\text{N}$) os principais elementos utilizados, possíveis sobreposições nos valores das razões isotópicas limitam o poder de discriminação da importância relativa das fontes alimentares para os consumidores, principalmente para predadores generalistas (Neil & Cornwell 1992). Em tais casos, a incorporação de elementos adicionais é recomendada e pode ajudar a superar essa limitação, sendo o enxofre ($^{34}\text{S}/^{32}\text{S}$) um dos principais elementos utilizados.

Isótopos de enxofre estão presentes na composição de dois aminoácidos essenciais e, por isto, são bastante conservativos (Hesslein et al. 1991, Vander Zanden et al. 2015). A íntima relação deste elemento com os íons de sulfato (SO_4^{2-}) faz com que seus valores isotópicos e variações tendam a refletir gradientes de salinidade (Krouse & Grinenko 1991, MacAvoy et al. 2015), além de se mostrar útil na diferenciação de cadeias tróficas baseadas em recursos pelágicos e associados ao substrato bentônico (Croisetière et al. 2009). Estas características fazem do enxofre um excelente traçador de movimento e fluxos de energia, com grande potencial para esclarecer as relações tróficas existentes em diferentes sistemas aquáticos, em especial os estuarinos. Por exemplo, Couillard e colaboradores (2011) demonstraram que os valores de $\delta^{34}\text{S}$ para o tomcod (*Microgadus tomcod*), um peixe da família do bacalhau, tendiam a refletir a localização das amostras no gradiente de salinidade da região, mas que estes nem sempre estavam em conformidade com as assinaturas de

cada local, como revelado pela comparação com os valores de $\delta^{34}\text{S}$ de invertebrados bentônicos coletados nas mesmas salinidades.

A corvina, *Micropogonias furnieri*, é uma espécie pelágica-demersal amplamente distribuída ao longo do Atlântico Oeste, desde o México até a Argentina, e de grande importância para a pesca brasileira. Por isso essa espécie tem sido alvo de estudos nos mais diversos campos, sejam ecológicos, morfométricos-merísticos, genéticos e/ou econômicos (Vazzoler 1971, Isaac 1988, Reis 1992, Puchnick-Legat & Levy 2006, Velloso & Pereira Jr). Um estudo conduzido no estuário da Lagoa dos Patos por Gonçalves (1997) identificou os principais grupos que compõem a dieta de *Micropogonias furnieri* ao longo das fases do seu desenvolvimento ontogenético. Demonstrou-se que a dieta de corvinas entre 30 mm e 90 mm de comprimento total (CT) é composta principalmente por organismos zooplanctônicos e organismos epifaunais (< 2,5 mm³). Por sua vez, indivíduos entre 90 mm CT e 160 mm CT se alimentam principalmente de organismos infaunais (< 2,5 mm³, entre 5 e 10 mm³, e entre 20 e 50 mm³) e epifaunais (entre 2,5 e 5 mm³, e entre 20 e 50 mm³), ainda com ocorrência de organismos zooplanctônicos (destaque para os copépodes). A dieta de corvinas entre 160 mm CT e 705 mm CT é dominada, em termos de frequência e abundância numérica, por organismos infaunais (ente 5 e 10 mm³). Os resultados encontrados corroboram outras literaturas (Issac 1988, Vazzoler et al. 1991) e, portanto, esta espécie pode ser classificada, ao longo de todas as fases de seu ciclo de vida, como uma espécie de comportamento alimentar generalista-oportunista, sendo o generalismo caracterizado pela riqueza de itens alimentares ingeridos, e o oportunismo vinculado a ingestão de presas pouco frequentes mas com elevada dominância

numérica ou volumétrica. A plasticidade alimentar desta espécie (Giberto et al. 2007) e sua capacidade de transitar ao longo de gradientes salinos (Albuquerque et al. 2012, Costa et al. 2014) são atributos que a caracterizam como um modelo satisfatório para avaliar variações espaciais no uso de recursos alimentares. Neste sentido, este trabalho teve por objetivo comparar o uso de dois (C, N) e três isótopos estáveis (C, N, S) no estudo da ecologia trófica da corvina ao longo de um gradiente límnico-estuarino-marinho em uma área subtropical. A hipótese investigada é que os isótopos do elemento enxofre permitirão diferenciar com maior exatidão a importância relativa das fontes alimentares basais que sustentam a espécie ao longo de um gradiente salino, elucidando principalmente a importância de vias pelágicas ou bentônicas.

MATERIAIS E MÉTODOS

As amostragens de dados bióticos e abióticos foram realizadas ao longo do eixo longitudinal da Lagoa dos Patos, a maior lagoa do tipo estrangulado do mundo (Kjerfve 1994). O formato desta laguna é de extrema relevância na hidrodinâmica, transporte e dispersão de materiais. Seu afunilamento natural, do estuário em direção ao mar, intensificado devido à construção de dois molhes, é decisivo na circulação por intensificar as correntes de vazante (Seeliger & Odebrecht 2010). Em contrapartida, a maior entrada de água marinha se dá junto ao fundo, na forma de uma cunha salina, sendo sua extensão principalmente controlada por efeitos combinados da alta variação da descarga de água doce e ventos (Schwochow & Zanboni 2007).

Os locais de coleta, dez ao total, foram distribuídos de modo a abranger tanto a porção límnic quanto estuarina da lagoa, bem como a porção marinha adjacente à

desembocadura desta (Fig. 1). Os pontos amostrados situados dentro da lagoa foram localizados distantes das margens, em profundidades de aproximadamente 5 m, ao passo que os pontos na região marinha estavam próximos às isóbatas de 15 m e 20 m. O período no qual as coletas foram feitas, de 12 a 16 de março de 2012, correspondeu a uma época de salinidades acima da média, devido à menor vazão dos principais rios tributários à Lagoa dos Patos (Vaz et al. 2006). Em todos os locais foram coletadas amostras de água para mensurar a temperatura e a salinidade por meio de um termômetro de mercúrio e refratômetro portátil, respectivamente.

Em cada local foram coletadas amostras da corvina *Micropogonias furnieri*, presas potenciais (invertebrados bentônicos e zooplânctônicos) e fontes alimentares basais (produtores primários e matéria orgânica particulada) para análise da composição isotópica (C, N, S). As fontes alimentares basais (i.e., produtores primários e outras fontes orgânicas na base da cadeia) amostradas incluíram macroalgas, a fanerógama marinha *Ruppia maritima*, matéria orgânica particulada em suspensão (POM) e no sedimento (SOM). POM pode ser composto por materiais de diferentes origens, incluindo fitoplâncton, detritos de plantas vasculares e outras fontes de matéria orgânica em ressuspensão (Deegan & Garritt 1997; Sato et al. 2006), enquanto SOM pode consistir principalmente em agrupamentos de microalgas, matéria orgânica e detritos de origem bentônica (Day Jr. et al. 2013). POM foi obtido com a filtragem de 0,25 a 1 L de água superficial recolhida em cada local em filtros de fibra de vidro (0,75 μ m). A fim de obter uma amostra de SOM, uma camada de cerca de 2 cm de sedimento superficial foi removida utilizando amostras coletadas com o auxílio do pegador de fundo tipo van Veen. A coleta de zooplâncton ocorreu através de arrastos

oblíquos utilizando uma rede cônica de aro de 30 cm de diâmetro e malha de 200 µm. Um arrasto adicional para amostragem de zooplâncton foi realizado em cada local e o material obtido foi fixado em solução de formaldeído 4% neutralizado com bórax para posterior identificação dos organismos e caracterização da composição taxonômica da amostra. Esse procedimento foi adotado para auxiliar na interpretação da composição isotópica das amostras de zooplâncton, as quais não puderam ser analisadas isotopicamente em grupos taxonômicos distintos (vide próximo parágrafo). Os organismos bentônicos foram obtidos peneirando em uma malha de 500 µm o restante da amostra coletada com o pegador de fundo tipo de van Veen para obtenção do SOM. Os peixes foram coletados com o uso de redes de arrasto de fundo. Quando possível, estes consumidores foram coletados em diferentes classes de tamanho, para avaliar possíveis diferenças nas razões isotópicas devido a variações ontogenéticas. Macroalgas, fanerógamas marinhas e macrocrustáceos coletados nos arrastos de fundo durante amostragem da ictiofauna também foram utilizados para complementar a caracterização isotópica das fontes alimentares basais e presas em cada local. Amostras de fontes alimentares basais e consumidores foram coletadas duas vezes, sequencialmente, em cada local e preservadas em gelo.

Em laboratório, as amostras de zooplâncton foram acondicionadas em frascos apropriados, mantendo-se a proporção de 9 partes de solução fixadora para uma de plâncton. A composição de cada amostra foi determinada através da contagem de organismos presentes em sub-amostras, tomadas da amostra original mediante a utilização do sub-amostrador colher sueca, utilizando microscópios estereoscópicos. Todos os organismos foram identificados ao nível de grandes grupos taxonômicos,

utilizando bibliografia especializada (Montú & Gloeden 1986, Boltovskoy 1999). Para a análise de isótopos estáveis, as amostras foram descongeladas e processadas seguindo o protocolo básico de separação do tecido, desidratação, moagem e pesagem de subamostras (Jardine et al. 2003; Garcia et al. 2007). Antes da extração do tecido, cada amostra animal e vegetal foi cuidadosamente inspecionada para remover epífitas nos filamentos de algas e fanerógamas, ossos ou escamas em tecidos de peixes, e qualquer outro material aderido à amostra. Dos indivíduos da ictiofauna foram tomadas as seguintes medidas de cada exemplar: o comprimento total em milímetros (CT-mm) e massa total em gramas (PT-g), utilizando ictiômetro e balança analítica de precisão, respectivamente. Posteriormente, foi extraído o tecido alvo de cada produtor primário, presa e consumidor: folhas e filamentos de fanerógamas e macroalgas, respectivamente, e tecido muscular antero-dorsal e abdominal (aproximadamente 5 g de cada amostra) de peixes e macrocrustáceos decápodes, respectivamente. Organismos da infauna e zooplâncton foram processados inteiros devido à dificuldade em extrair apenas o tecido alvo (e.g., músculo) de organismos muito pequenos (<1 mm). Cada amostra foi lavada em água destilada e colocada individualmente em uma placa Petri (previamente esterilizada com banho de ácido clorídrico por 24 h) e levada à estufa (60°C) por 48 h. Após, estas foram maceradas, com o auxílio de pistilo e grau, e duas sub-amostras foram pesadas em cápsulas de estanho ultra-puras (Costech, Valencia, CA), sendo uma para a análise das razões dos isótopos estáveis de carbono ($\delta^{13}\text{C}$) e nitrogênio ($\delta^{15}\text{N}$), e outra para obtenção da razão dos isótopos estáveis de enxofre ($\delta^{34}\text{S}$). Para as sub-amostras que teriam a composição isotópica de enxofre analisada foi adicionado, no momento da pesagem, o

produto pentóxido de vanádio, um catalisador e doador de oxigênio na reação de combustão que ocorre dentro do espectrômetro de massa de razão isotópica (Krouse & Grinenko 1991). As amostras foram enviadas ao *Stable Isotope/Soil Biology Laboratory, University of Georgia*, EUA, para determinação da razão isotópica dos elementos carbono e nitrogênio ($\delta^{13}\text{C}$ e $\delta^{15}\text{N}$), e ao *Iso-Analytical Laboratory*, UK, para determinação da razão isotópica do elemento enxofre ($\delta^{34}\text{S}$). Os resultados foram expressos em notação delta (δ) conforme a fórmula: $\delta^{13}\text{C}$ ou $\delta^{15}\text{N}$ ou $\delta^{34}\text{S} = [(R_{\text{amostra}}/R_{\text{padrão}}) - 1] * 1000$, onde $R = {}^{13}\text{C}/{}^{12}\text{C}$ ou ${}^{15}\text{N}/{}^{14}\text{N}$ ou ${}^{34}\text{S}/{}^{32}\text{S}$. O material padrão utilizado para o carbono foi um fóssil marinho da formação Pee Dee, para o nitrogênio o gás N_2 atmosférico, e para o enxofre o mineral troilita do meteorito “Canyon Diablo”.

Amostras provenientes de locais com valores de salinidade similares foram agrupadas em três regiões (água doce, estuarina e marinha) e utilizadas como réplicas durante as análises dos dados. Originalmente, amostras da fanerógama marinha *R. maritima* foram registradas na região de água doce junto à rede de arrasto de fundo durante a coleta dos peixes. Contudo, pelo fato dessa espécie de fanerógama ter sua distribuição restrita ao estuário (Seeliger & Odebrecht 2010), estas amostras foram consideradas como pertencentes à região estuarina. Além disso, informações provenientes do programa ecológico de longa duração (PELD - Sítio 8, dados não publicados, Margareth Copertino) mostram que a fanerógama *R. maritima* ocupava a região do estuário na mesma época das coletas. Estes resultados nos deram indicativos adicionais de que os fragmentos de *R. maritima* obtidos nos arrastos de água doce eram realmente originários da região estuarina, tendo sido provavelmente transportados pelo movimento da cunha salina.

Para ilustrar a composição de tamanhos dos exemplares de corvina amostrados foram elaborados histogramas de tamanho (CT, mm) dos indivíduos coletados em cada uma das três regiões do gradiente salino. A relação entre o tamanho (CT, mm) de cada exemplar de corvina e os isótopos de carbono ($\delta^{13}\text{C}$), nitrogênio ($\delta^{15}\text{N}$) e enxofre ($\delta^{34}\text{S}$) em cada uma das regiões foi avaliada a partir de análises de regressão. As variações espaciais nas razões isotópicas das fontes alimentares basais e consumidores foram investigadas por meio de representações gráficas (diagramas triplots C-N-S). A similaridade dos grupos taxonômicos de zooplâncton entre regiões ao longo do gradiente salino foi verificada através de uma análise de agrupamento, utilizando o índice de dissimilaridade de Bray-Curtis, a métrica de encadeamento simples e os dados de composição das amostras transformados [$\log_{10}(x+1)$].

Modelos isotópicos bayesianos de mistura (pacote SIAR, versão 3.0.1, plataforma R) foram utilizados para estimar a contribuição de recursos alimentares disponíveis para a composição isotópica final das corvinas nas regiões amostradas (Parnell et al. 2010). Visando comparar a eficácia de modelos de mistura com e sem a inclusão do enxofre (S) como um terceiro elemento na estimativa dos padrões de assimilação de recursos pela corvina, foram usados modelos isotópicos duplos (C-N) e triplos (C-N-S). Em ambos os modelos de mistura, os valores isotópicos dos peixes foram utilizados individualmente, enquanto que para as fontes alimentares basais e presas (macroinvertebrados bentônicos, macrocrustáceos decápodes, zooplâncton) foram utilizadas suas médias e desvios padrões (Parnell et al. 2010). Os dados das concentrações elementares de carbono (%C) e nitrogênio (%N) de cada recurso alimentar foram incorporadas como uma matriz adicional (modelo de concentração)

durante a modelagem isotópica (Phillips et al. 2014). Os valores de discriminação isotópica (TDF: *trophic discrimination factor*) utilizados para o carbono e nitrogênio foram obtidos experimentalmente para a espécie (Capítulo 2), enquanto os valores para o enxofre foram obtidos da bibliografia (Barnes & Jennings 2007). Nos modelos de mistura estimando a importância relativa das fontes alimentares basais e das presas para as corvinas foram considerados dois (2) e um (1) níveis tróficos, respectivamente. Ou seja, os valores de TDF nos modelos com fontes alimentares basais foram multiplicados por dois (TDF x 2) e no de presas por um (TDF x 1).

RESULTADOS

A temperatura da água superficial variou entre 26 e 28 °C para todos os locais amostrados, enquanto os valores médios (\pm desvio padrão) de salinidade para as regiões de água doce, estuarina e marinha foram respectivamente 4,7 (\pm 0,6), 15,2 (\pm 4,4) e 36 (\pm 1,0).

Ao todo foram analisadas 71 amostras de corvinas, 161 de presas e 64 de fontes alimentares basais (Tabela 1). Além da corvina e das amostras de zooplâncton, apenas duas espécies de macrocrustáceos decápodes, o siri-azul *Callinectes sapidus* e o camarão-rosa *Farfantepenaeus paulensis*, foram obtidas ao longo de todo o gradiente salino. Em relação às fontes alimentares basais, além das amostras de POM e SOM, também foi observada a presença da macroalga *Ulva* spp. nas três regiões ao longo do gradiente salino.

As análises de regressão entre composição isotópica e tamanho (CT, mm) das corvinas revelaram correlações estatisticamente significativas para a razão isotópica do

nitrogênio ($\delta^{15}\text{N}$) na região estuarina ($R^2 = 0,11$; $p = 0,023$) e para a razão isotópica do enxofre ($\delta^{34}\text{S}$) na região de água doce ($R^2 = 0,36$; $p = 0,009$). A análise da composição de tamanhos das corvinas coletadas mostrou uma maior amplitude e número de classes de tamanhos na região estuarina (20 classes de tamanho entre 40 e 240 mm), quando comparado à região marinha (sete classes de tamanho entre 140 e 250 mm) e à região de água doce (11 classes de tamanho entre 40 e 230 mm, sendo dez destas inferiores a 170 mm). O número de indivíduos amostrados também variou entre as regiões marinha (7), estuarina (46) e de água doce (18) (Fig. 1b).

A análise de agrupamentos indicou a presença de um grupo composto pelo zooplâncton das regiões de água doce e do estuário (84% de similaridade). Contudo, embora estas regiões compartilhassem seis entre sete das classificações taxonômicas identificadas, estas diferiram principalmente em relação à quantidade de organismos presentes em cada ambiente (Tabela II). Apesar dessas variações na composição e abundância dos grupos, os valores isotópicos foram semelhantes entre as amostras e as principais diferenças encontradas, por exemplo em relação ao $\delta^{13}\text{C}$, podem ser mais relacionadas aos locais do que com a composição em si, sugerindo que os organismos do zooplâncton em uma mesma área pudessem ser analisados de modo agrupado (Tabelas I e II).

De modo geral, espécies ou grupos tróficos coletados na região de água doce apresentaram menores amplitudes dos valores isotópicos de carbono, nitrogênio e enxofre quando comparado àqueles das regiões estuarinas e marinhas (Fig. 2). O SOM exibiu a maior variação tanto em relação aos componentes amostrados em todas as regiões (Tabela I) quanto entre amostras coletadas em uma mesma região (Fig. 2).

Os resultados dos modelos de mistura isotópicos para a corvina na água doce se mostraram bastante semelhantes quando considerando somente os isótopos de carbono e nitrogênio (C-N) ou quando foi incluído os valores de enxofre (C-N-S) (Fig. 3). Já na estimativa da contribuição das fontes alimentares basais para a corvina na região do estuário e do ambiente marinho, a adição do terceiro isótopo resultou em melhoras na resolução dos modelos com a diminuição nas incertezas nas estimativas de assimilação dos alimentos (especialmente os basais) pelo consumidor (Fig. 3a). No estuário, em particular, foi observado um aumento marcante na acurácia na determinação dos elos tróficos, com estimativas de assimilação dos alimentos mais precisas, ou seja, com intervalos de credibilidade (IC) menores (e.g., 95% IC C-N = $0,31 < \text{POM} < 0,71$; 95% IC C-N-S = $0,36 < \text{POM} < 0,51$). A adição do isótopo de enxofre se mostrou especialmente importante para a discriminação da importância do SOM, visto que esta fonte de matéria orgânica exibiu uma contribuição maior, quando comparada àquela resultante do modelo com apenas dois isótopos tanto na região estuarina (e.g., 95% IC C-N = $0 < \text{SOM} < 0,52$; 95% IC C-N-S = $0,26 < \text{SOM} < 0,46$) quanto na região marinha (e.g., 95% IC C-N = $0 < \text{SOM} < 0,55$; 95% IC C-N-S = $0,16 < \text{SOM} < 0,75$). Em relação aos modelos de assimilação das presas, não houve diferenças nos resultados obtidos usando modelos isotópicos duplos (C-N) e triplos (C-N-S). Ambos os modelos mostraram uma maior assimilação de macrocrustáceos decápodes na região de água doce e uma tendência de uma assimilação mais equitativa das presas na região estuarina e marinha (Fig. 3b).

DISCUSSÃO

A combinação de vários isótopos estáveis (por exemplo, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$) fornecem um meio útil de distinguir entre ambientes dos quais esses elementos foram derivados, tendo por isto ampla aplicabilidade em estudos de relações tróficas. Diversos trabalhos já demonstraram que isótopos de carbono e enxofre tendem a ser mais deplecionados em ambientes de água doce quando comparados a áreas marinhas (Canfield 2001, Fry 2006, Fry & Chumchal 2011). Apesar dos resultados do presente estudo seguirem este padrão para o carbono, tais diferenças esperadas para o isótopo de enxofre ao longo do gradiente salino não foram confirmadas. Os valores encontrados para o $\delta^{34}\text{S}$ na região de água doce foram maiores do que os comumente esperados para uma região límnic (MacAvoy et al. 1998) e alguns organismos amostrados nesta área chegaram inclusive a apresentar valores maiores do que aqueles encontrados para exemplares da mesma espécie nas regiões estuarina e marinha, como por exemplo a corvina *M. furnieri* e os macrocrustáceos decápodes *C. sapidus* e *F. paulensis* (Tabela I). Ainda que sejam possíveis valores de $\delta^{34}\text{S}$ acima de +10‰ em ambientes dulcícolas, estes são bastante raros, podendo ocorrer em locais específicos devido à geologia do local, aporte de material terrígeno ou a processos de oxidação e redução deste elemento, realizados por bactérias anaeróbicas (Krouse & Grinenko 1991, Sayle et al. 2013). A hipótese mais plausível para os valores altos de $\delta^{34}\text{S}$ na região de água doce encontrados em nosso estudo é de que estes seriam resultados de uma mistura promovida pela entrada de água marinha, na forma de uma cunha salina, que estava ocorrendo durante a coleta das amostras. As marcadas diferenças nos valores de salinidade encontrados entre as áreas amostradas são

condizentes com o esperado para a época do verão, quando foram realizadas as amostragens, as quais são caracterizadas por menor descarga continental e maior penetração da cunha salina (Barros et al. 2014, Sinque & Muelbert 1997). Importante ressaltar que os locais amostrados situam-se próximos a um profundo canal (aproximadamente 16 m), o qual funcionaria como um corredor facilitando a intrusão de água salgada em períodos de baixa descarga de água doce, possivelmente desempenhando um papel crítico na dispersão e transporte de recursos (Seiler et al. 2015), e suportando esta hipótese.

Já no ambiente estuarino a incorporação do enxofre ($\delta^{34}\text{S}$) como terceiro elemento em combinação com carbono ($\delta^{13}\text{C}$) e nitrogênio ($\delta^{15}\text{N}$) nos modelos de mistura isotópicos diminuiu consideravelmente as incertezas na estimativa da assimilação de nutrientes oriundos de fontes alimentares basais pelo consumidor estudado. Embora em menor grau, modelos de mistura com três isótopos (C-N-S) ao invés de dois (C-N) também resultaram em modelos com menor grau de incertezas nas estimativas de assimilação de fontes alimentares no ambiente marinho, com maior convergência em direção à contribuição de nutrientes oriundos da via bentônica para o consumidor. Em ambos os casos, estas vantagens foram percebidas principalmente para o SOM, o qual corresponde a uma combinação de microrganismos e matéria orgânica particulada acumulada sobre o sedimento oriunda da decomposição de animais e plantas. Diversos estudos já demonstraram a importância da via bentônica em ambientes aquáticos (Vadeboncoeur et al. 2002, Ofukani et al. 2014, Mont'Alverne et al. 2016 - 1º capítulo), mas a composição muitas vezes heterogênea do SOM colabora para que haja sobreposição de seus valores isotópicos com outras fontes

alimentares basais (Claudino et al. 2013). Portanto, os resultados obtidos nesse trabalho sugerem que estudos conduzidos principalmente em áreas mais rasas de estuários, onde nota-se maior diversidade de fontes alimentares basais, como por exemplo macrófitas, macroalgas, fanerógamas marinhas, epífitas, biofilme, além do próprio SOM (Currin et al. 1995), seriam significativamente beneficiados se empregassem modelos de mistura isotópicos que incluíssem o isótopo de enxofre.

Em contrapartida, os modelos de mistura com três isótopos (C, N, S) não resultaram num aumento na acurácia nas estimativas de assimilação das presas pelo consumidor estudado. As possíveis explicações para esse fato podem estar relacionadas ao hábito alimentar generalista e oportunista da corvina (Gonçalves 1997, Giberto et al. 2007) que poderia resultar em complexas interações presas-predador. Assim, à medida que a matéria e a energia transitam entre os elos intermediários da cadeia (i.e., as presas) até a corvina, as diferenças isotópicas na base da cadeia discriminadas pela abordagem tripla (C, N, S) poderiam ser mascaradas ou diluídas. Nesse cenário, uma abordagem dual (C, N) ou tripla (C, N, S) seriam igualmente insuficientes para essa mistura isotópica. Uma explicação alternativa e não mutuamente exclusiva pode estar relacionada simplesmente à resolução taxonômica utilizada para classificar as presas (Costas & Pardo 2015, Nehlich 2015). Futuros estudos com maior detalhamento na amostragem e análise dos elos tróficos intermediários envolvendo agrupamento de presas em distintas classes taxonômicas ou unidades tróficas seriam necessários para avaliar a validade dessas hipóteses e avançar nosso atual entendimento sobre a eficácia de modelos de mistura multi-isotópicos.

Para o nosso conhecimento, esta é a primeira vez que este tipo de abordagem com três isótopos é utilizada para estimar as relações tróficas de um consumidor generalista em ambientes aquáticos ao longo de um gradiente salino na América do Sul. Nosso trabalho destaca a utilidade de se incluir um terceiro isótopo para melhorar a resolução e distinção nas contribuições de fontes alimentares basais, principalmente em estudos de relações tróficas em áreas estuarinas, onde há grande variabilidade e mistura de recursos alimentares devido a influências de regiões adjacentes. Contudo, no que concerne à identificação da dieta baseada em presas, ainda que os resultados obtidos no presente estudo estejam de acordo com informações já publicadas, a alta sobreposição dos valores de contribuição de cada grupo, com exceção para as análises realizadas em água doce, e os discretos ganhos representados pela inclusão do isótopo de enxofre, nos levam a afirmar que estudos fazendo uso de uma abordagem mais tradicional como, por exemplo, análises de conteúdo estomacal, se mostram mais satisfatórios em seus resultados, e seriam, até o momento, mais indicados do que a ferramenta de isótopos estáveis.

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Tabela I: Número de amostras e valores médios (\pm DP) das razões isotópicas de carbono ($\delta^{13}\text{C}$), nitrogênio ($\delta^{15}\text{N}$) e enxofre ($\delta^{34}\text{S}$) para a corvina *Micropogonias furnieri*, suas principais presas e fontes alimentares basais amostradas ao longo de um gradiente salino na Lagoa dos Patos e região marinha adjacente.

	ÁGUA DOCE				ESTUÁRIO				MARINHO			
	N	$\delta^{13}\text{C} \pm \text{DP}$	$\delta^{15}\text{N} \pm \text{DP}$	$\delta^{34}\text{S} \pm \text{DP}$	N	$\delta^{13}\text{C} \pm \text{DP}$	$\delta^{15}\text{N} \pm \text{DP}$	$\delta^{34}\text{S} \pm \text{DP}$	N	$\delta^{13}\text{C} \pm \text{DP}$	$\delta^{15}\text{N} \pm \text{DP}$	$\delta^{34}\text{S} \pm \text{DP}$
Corvinas												
<i>Micropogonias furnieri</i>	18	-16,30 \pm 1,02	13,68 \pm 1,16	17,18 \pm 0,93	46	-15,76 \pm 1,51	13,53 \pm 1,17	15,74 \pm 1,70	7	-15,38 \pm 0,79	14,27 \pm 0,66	15,78 \pm 0,99
Presas												
<u>Macrocrustáceos decápodes</u>												
<i>Artemesia longinaris</i>									6	-15,79 \pm 0,53	12,57 \pm 0,39	17,27 \pm 0,56
<i>Callinectes danae</i>									1	-13,29	12,79	16,80
<i>Callinectes ornatus</i>									8	-15,48 \pm 0,34	12,96 \pm 0,54	17,17 \pm 0,67
<i>Callinectes sapidus</i>	8	-16,34 \pm 0,66	10,88 \pm 0,36	16,63 \pm 0,74	21	-14,22 \pm 1,54	11,49 \pm 1,32	15,93 \pm 1,35	1	-13,78	11,90	14,08
<i>Farfantepenaeus paulensis</i>	19	-15,94 \pm 1,79	11,37 \pm 0,41	18,22 \pm 0,67	21	-13,43 \pm 2,02	10,96 \pm 1,16	16,42 \pm 1,36	4	-13,72 \pm 1,11	10,81 \pm 2,39	14,91 \pm 2,07
<i>Hepatus pudibundus</i>									1	-15,08	14,51	17,91
<i>Loxopagurus loxochelis</i>									3	-19,37 \pm 1,90	10,98 \pm 2,06	17,21 \pm 0,82
<i>Pleoticus muelleri</i>									5	-15,78 \pm 0,60	12,75 \pm 0,15	16,69 \pm 0,57
<u>Microinvertebrados bentônicos</u>												
<i>Erodona mactroides</i>	20	-19,30 \pm 0,34	8,79 \pm 0,20	17,49 \pm 0,75	2	-19,52 \pm 0,38	8,25 \pm 0,23	16,67 \pm ,10				
Gastrópode não-identificado									1	-15,15	13,39	13,57
<i>Heleobia australis</i>					2	-13,87 \pm 2,61	9,92 \pm 0,20	14,38 \pm 0,32				
<i>Kalliapseudes schubartii</i>					5	-17,69 \pm 0,73	7,87 \pm 0,28	15,81 \pm 0,57				
<i>Kinberyonuphis</i> sp.									1	-18,42	11,21	16,75
<i>Limnoperna fortunei</i>	1	-20,17	8,69	17,16								
<i>Mactra isabelleana</i>									4	-15,89 \pm 0,15	9,02 \pm 0,34	15,87 \pm 1,14

Ofiuróide									1	-6,77	11,55	21,11
<i>Parandalia tricuspes</i>									1	-17,10	13,05	11,39
<i>Pherusa capitata</i>									1	-18,47	10,23	8,14
Poliplacóforo									1	-14,75	13,28	17,23
Zooplâncton												
Amostras compostas	2	-21,29±0,56	10,58±1,07	17,13±1,90	12	-17,98±1,98	9,23±0,71	17,65±1,44	4	-16,82±0,81	8,20±0,88	20,02±1,28
<i>Loligo</i> sp.									5	-16,24±0,35	14,63±0,27	17,75±0,28
Fontes alimentares basais												
Matéria orgânica particulada em suspensão (POM)	5	-23,75±1,13	7,72±1,12	17,35±1,20	13	-22,87±0,65	6,09±1,17	18,81±2,65	4	-18,95±4,71	5,54±0,75	18,65±1,16
Matéria orgânica particulada no sedimento (SOM)	2	-17,80±2,62	8,21±1,30	13,53±7,78	10	-16,21±2,77	5,22±3,51	9,61±3,60	5	-18,07±3,75	4,04±3,77	5,95±3,72
Macroalga não-identificada					3	-12,94±1,00	6,31±1,28	17,19±0,13				
Macroalga não-identificada (em decomposição)					4	-12,34±0,53	2,77±0,43	17,21±0,59				
<i>Ruppia maritima</i>					4	-12,56±0,78	7,46±0,83	16,34±2,12				
<i>Ulva</i> spp.	3	-16,56±0,27	7,29±0,39	16,89±0,89	7	-11,13±2,36	5,82±1,14	18,06±0,58	4	-16,62±0,92	6,25±0,75	18,45±0,23

Tabela II: Número de exemplares do zooplâncton por grandes categorias taxonômicas em amostras coletadas ao longo do eixo longitudinal da Lagoa dos Patos, desde o ambiente de água doce até o marinho.

		ÁGUA DOCE	ESTUÁRIO	MARINHO
ARTHROPODA	Cirripedia	1.200	47.440	
	Cladocera	8.640	280	
	Copepoda	88.920	89.360	10.080
	Decapoda	120	2.040	31.080
CHORDATA	Appendicularia			1.120
	Ictioplâncton	120		
CNIDARIA	Medusozoa	22.920	190.720	
MOLLUSCA	Mollusca	4.680	9.920	280
TOTAL		126.600	339.760	42.560

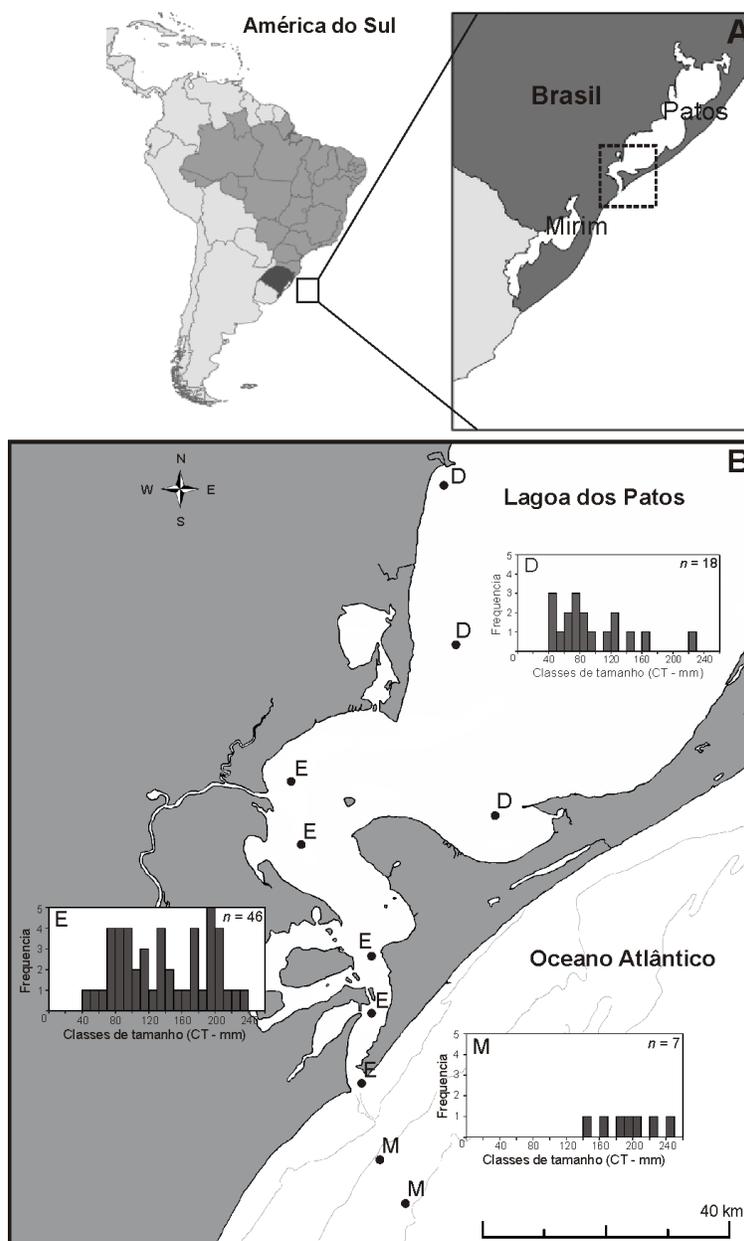


Figura 1: (A) Complexo lagunar Patos-Mirim, localizado no sul do Brasil, e (B) histogramas por regiões ecológicas exibindo o número de indivíduos coletados por classe de tamanho (comprimento total em mm) de *Micropogonias furnieri* (AD = ambiente de água doce ; E = estuarino ; M = marinho).

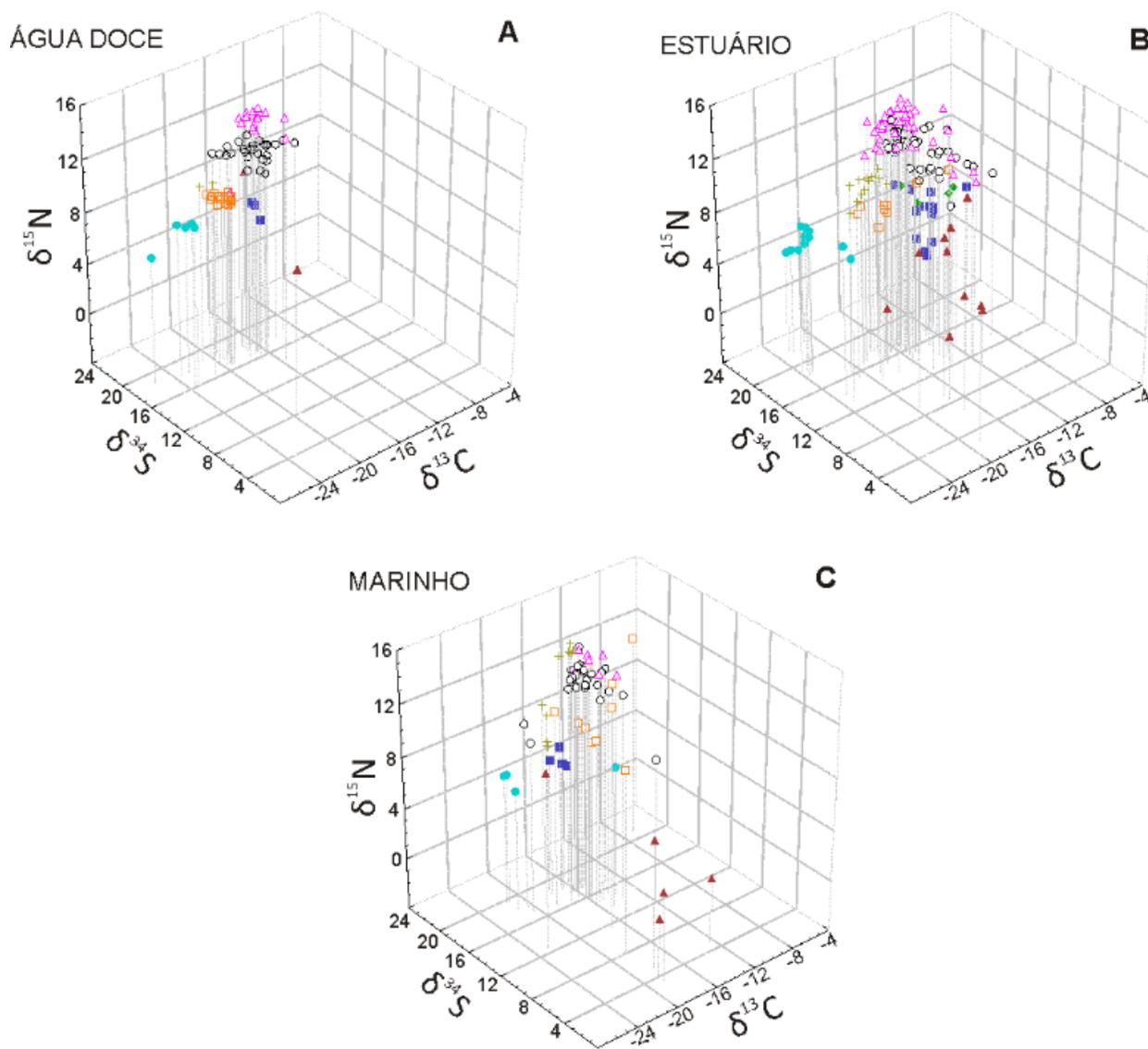


Figura 2: Triplots de valores absolutos das razões dos isótopos estáveis de carbono ($\delta^{13}\text{C}$), nitrogênio ($\delta^{15}\text{N}$) e enxofre ($\delta^{34}\text{S}$) de fonte basais (símbolos cheios) e consumidores (símbolos vazados) coletados em três grandes regiões ecológicas (Água doce, Estuário e Marinho) ao longo de um gradiente de salinidade na Lagoa dos Patos e região marinha adjacente.

FONTES BASAIS: ● Matéria orgânica particulada em suspensão (POM); ▲ Matéria orgânica no sedimento (SOM); ■ Macroalgas flutuantes; ◆ Grama marinha *Ruppia maritima*

CONSUMIDORES: + Zooplâncton; □ Microinvertebrados bentônicos; ○ Macrocrustáceos decápodes; △ Peixes da espécie *Micropogonias furnieri*.

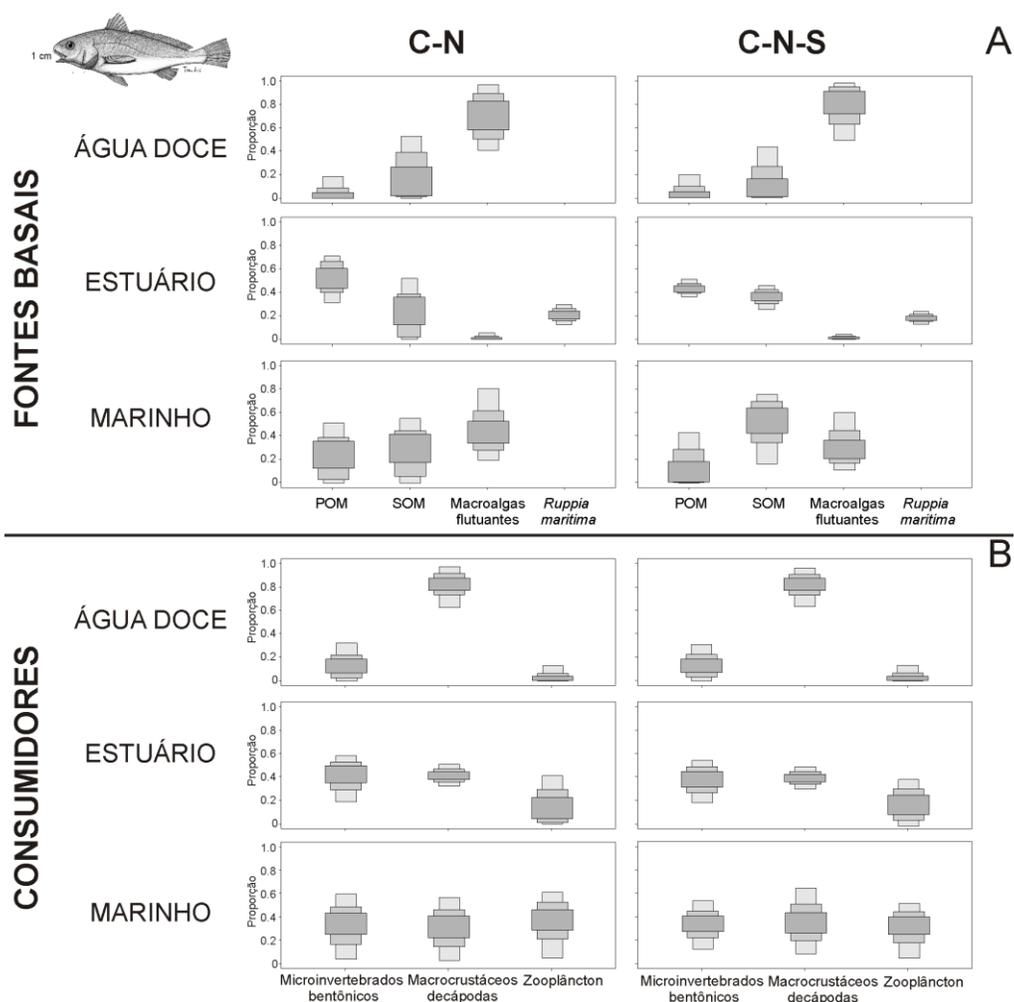


Figura 3: Contribuições relativas de (A) fontes alimentares basais (POM, SOM, macroalgas flutuantes e a grama marinha *Ruppia maritima*) e (B) principais grupos de consumidores presas de uma espécie representante da ictiofauna, *Micropogonias furnieri*, em três ambientes de diferentes salinidades (Água doce, Estuário e Marinho). Estas contribuições foram analisadas a partir de duas abordagens, uma utilizando os resultados da análises de dois isótopos (carbono e nitrogênio - coluna da esquerda) e outra três isótopos (carbono, nitrogênio e enxofre - coluna da direita). Intervalos de credibilidade Bayesianos das possíveis contribuições de cada grupo para o grupo selecionado: 50 (cinza escuro), 75 (cinza médio) e 95% (cinza claro).

CONCLUSÕES GERAIS DA TESE

Maior compreensão da estrutura de determinadas relações alimentares e de conceitos organizacionais permite aos cientistas e gestores entender e gerenciar com maior precisão os ecossistemas naturais. Neste sentido, as principais contribuições desta tese são:

- áreas rasas e profundas de lagoas costeiras devem ser consideradas, a princípio, como diferentes módulos tróficos, demonstrando a existência de gradientes laterais de profundidade também em sistemas abertos;

- investigações baseadas na tríade C-N-S propiciam estimativas mais acuradas e confiáveis dos elos tróficos entre produtores primários e consumidores no estuário da Lagoa dos Patos;

- atendendo a uma chamada internacional de mais estudos experimentais, demonstrou-se que diferentes valores nos fatores de discriminação dos isótopos de carbono e nitrogênio, assim como para as taxas de renovação de ambos os elementos, devem ser utilizados em estudos ecológicos de consumidores aquáticos conduzidos em regiões de diferentes salinidades.

APÊNDICE I

**Trophic segregation of a fish assemblage along lateral depth gradients in a
subtropical coastal lagoon revealed by stable isotope analyses.**

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Trophic segregation of a fish assemblage along lateral depth gradients in a subtropical coastal lagoon revealed by stable isotope analyses

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Stable isotopes were used to evaluate the hypothesis that fish assemblages occurring in shallow and deep areas of a large coastal lagoon are structured in partially segregated trophic modules with consumers showing contrasting reliance on benthic or pelagic food sources. The results revealed that fishes in deep areas were mainly dependent on particulate organic matter in the sediment (SOM), whereas emergent macrophytes were as important as SOM to fish consumers in shallow areas. Conceptual trophic diagrams depicting relationships among basal food sources and consumers in different regions of the lagoon highlighted the greater use of multiple basal food sources by more feeding mode functional guilds in shallow water compared with the use of predominantly benthic resources (SOM) in deep areas. The findings appear to corroborate the initial hypothesis and offer complementary perspectives in understanding the role of spatial ecology in structuring coastal ecosystem function and productivity.

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Key words: benthic subsidy; depth compartmentalization; Patos Lagoon estuary; trophic modules.

INTRODUCTION

Ecosystem models are used to simulate the flow of energy and materials among different abiotic and biotic compartments of a system (Wolfe & Kjerfve, 1986; Schindler & Lubetkin, 2004), but their ability to make accurate prediction on the functioning of real ecosystems are strongly dependent on empirical food web studies (Winemiller & Layman, 2005). Many empirical studies describe food web organization through aggregate measures such as connectivity or compartmentalization (Pimm & Lawton, 1980; Krause *et al.*, 2003; Rezende *et al.*, 2009), focusing on smaller sub-webs, known as trophic modules (Winemiller & Layman, 2005; Stouffer & Bascompte, 2010; Gjata *et al.*, 2012). In these modules, trophic pathways can be strongly linked to spatial attributes, such as proximity, connectivity and the shape of habitats (Deegan & Garritt, 1997; Connolly *et al.*, 2005; Williams *et al.*, 2005; Nordström *et al.*, 2010). In lakes, coupling among various habitats (pelagic, benthic and littoral) is critical for maintaining processes that control overall ecosystem structure and function

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(Schindler & Scheuerell (2002). For example, depth-based changes in the abundance and distribution of invertebrate and fish species suggest that there may be concomitant changes in patterns of resource allocation (Sierszen *et al.*, 2014). The phytoplankton and zooplankton horizontal (*i.e.* shallow *v.* deep areas) distribution may also exhibit distinct local patches along gradients, having important consequences for understanding and predicting the food resources available for larval and juvenile fishes (Lucas *et al.*, 1999; Zhang *et al.*, 2006; Seebens *et al.*, 2013). In Patos Lagoon estuary (32° S), for example, wind-induced resuspension associated with high productivity in shallow areas results in a higher concentration of microalgal biomass compared with adjacent deep channels, which are characterized by a marked horizontal variability in chlorophyll *a* (Odebrecht & Abreu, 1998). Consequently, habitat coupling can have important consequences for nutrient cycling, predator–prey interactions and food web structure and stability (Schindler & Scheuerell, 2002). Although depth connectivity and biological linkages among habitats using fishes as proxies have recently been studied for small and large aquatic closed systems (Schindler & Scheuerell, 2002; Kato *et al.*, 2010; Sierszen *et al.*, 2014), they have been neglected for open systems, such as rivers (Roach *et al.*, 2009) and estuaries (Grimaldo *et al.*, 2009; Neves *et al.*, 2013).

Stable isotope analyses are one of the most widely used approaches to evaluate trophic pathways and food web organization in aquatic ecosystems (Fry, 2006; Reynolds, 2008; Middelburg, 2013). Carbon stable isotope ratios ($^{13}\text{C}:^{12}\text{C}$) are mainly used to identify basal food sources sustaining a consumer, whereas nitrogen stable isotope ratios ($^{15}\text{N}:^{14}\text{N}$) are employed to estimate the trophic position of consumers, due to greater isotopic fractionations (usually between 2 and 5‰) between consumers and their food sources (Peterson & Fry, 1987; Bouillon *et al.*, 2011). In aquatic systems, differences in isotopic composition among food sources allow quantification of their nutritional contributions to consumers and identification of trophic pathways (*e.g.* benthic *v.* planktonic sources), providing a convenient and informative way to integrate energy flow across spatial scales and detect compartmentalization (Quevedo *et al.*, 2009). Although application of this technique can be limited in complex systems (*e.g.* inability to distinguish between food sources with similar isotopic composition), stable isotopes remain important tools in unravelling the functioning of estuaries and improving the understanding of ecological processes (Schindler & Lubetkin, 2004; Bouillon *et al.*, 2011).

In this study, potential differences in trophic organization of fish assemblages occurring along lateral gradients (shallow *v.* deep areas) in a large subtropical lagoon were investigated. The hypothesis was that fish assemblages occurring in shallow areas and deep channels are structured in distinct, partially segregated, trophic modules with consumers showing contrasting reliance on benthic and pelagic food sources. In order to evaluate this hypothesis, carbon and nitrogen stable isotope ratios of fishes and their main primary food sources in shallow and deep areas along different sectors of Patos Lagoon estuary [32° S; the largest coastal lagoon in the world (Kjerfve, 1986)] were analysed. The findings are summarized in conceptual models depicting the main trophic pathways sustaining estuarine fish assemblages along shallow–deep lateral gradients.

MATERIALS AND METHODS

STUDY AREA

Patos Lagoon (30°–32° S; 50°–52° W) is located in the coastal plain of the southernmost region of Brazil [Fig. 1(a)]. The lagoon is funnel-shaped, connected to the sea through a single inlet (5 km long, 800 m wide at its narrowest section and 18 m deep) bordered by two rocky jetties. The bathymetric configuration of the main lagoon body is characterized by both natural and artificial channels, with depths ranging between 8 and 9 m, wide adjacent areas (>5 m deep) and a large embayment with marginal shallow areas (<2 m) (Calliari, 1998). Large and shallow banks (1–5 m) predominate in this estuary, as well as sandy spurs typical of shallow embayments (<1 m) (Calliari *et al.*, 2010). Over 50% of the estuarine margins are covered by irregular flooded salt marshes, with the predominance of C₄ (Hatch–Slack) photosynthetic pathways plants at the lower estuary and an increase in the occurrence of C₃ (Calvin cycle) plants along the longitudinal axis of the lagoon, towards regions with lower salinity values (Marangoni & Costa, 2009). Shallower areas provide optimal conditions for the growth of benthic macroalgae, which can be separated into distinct groups of species according to water salinity distribution and soil stability (Seeliger, 1997). In deeper waters, where silt-clay sediments are predominant, macroalgae usually occur as floating masses, as a result of the transport from shallow areas by estuarine currents and local wind patterns.

The lagoon choked-type morphology (Kjerfve, 1986) plays an important role in the estuarine circulation as it is responsible for intensifying ebb currents. The estuary is micro-tidal, with mean tidal amplitude of 0.47 m (Möller *et al.*, 2001), and salinity distribution mainly results from wind action and freshwater runoff (Fernandes *et al.*, 2005). According to different combinations of these two forces, Patos Lagoon estuarine region can be classified into four different vertical salinity models proposed by Cameron & Pritchard (1963): salt wedge, strongly stratified, weakly stratified or vertically mixed. Vertical salinity profiles, however, can change drastically (in a matter of hours) due to meteorological conditions. The upper limit of the estuarine region is usually considered 80 km north from the estuary mouth, but it can reach up to 200 km during severe drought periods (Hartmann & Schettini, 1991). Salinity in the estuary follows a seasonal pattern, with average values in late spring and summer (>15) and higher values in the months of April and May (*c.* 25), due to low freshwater inflow and the dominance of the south-west winds, forcing salt water into the estuary. In contrast, during the winter, ebb streams derived from high rainfall and increased river discharge contribute to a significant decrease in salinity, with values ranging from 0 to 8 (Möller & Fernandes, 2010; Pereira *et al.*, 2011). The increased flux of freshwater discharge in the system could result in a lower release of nutrients in the sediment, reducing drastically the concentration of dissolved inorganic nutrients for the primary producers (Barros *et al.*, 2014).

FIELD COLLECTION AND SAMPLE PROCESSING

Food web components were sampled in late austral winter (September 2011), during a 1 week expedition, at 15 sampling stations at shallow (depth <1.5 m) and deep (depth *c.* 5 m) areas in regions with distinct salinity characteristics along the longitudinal axis of Patos Lagoon: fresh water, upper estuary and lower estuary [Fig. 1(b)]. During this time of the year, the estuarine region may be classified as vertically mixed and a great portion of river-borne material is deposited along the lagoon body. The basal food sources sampled included drift macroalgae (unattached), salt marsh plants (C₃ and C₄ macrophytes), suspended particulate organic matter (POM) and particulate organic matter in the sediment (SOM) (Table I). POM comprises several components of different origins, including phytoplankton, higher plant organic detritus and resuspended organic matter (Deegan & Garritt, 1997; Sato *et al.*, 2006), whereas SOM consists of pools of microalgae, organic matter and benthic origin organic detritus (Day *et al.*, 1989). POM was obtained by filtering *c.* 0.25–1.00 l of water collected at each sampling location into fibreglass filters (0.75 µm) and SOM by removing *c.* 2 cm of surface sediment. Sampled consumers included fishes and their possible prey, such as zooplankton, benthic infauna and decapod macrocrustaceans (Table I). All groups of basal food sources and consumers were sampled twice at each station. Samples were preserved on ice until transported to the laboratory and later

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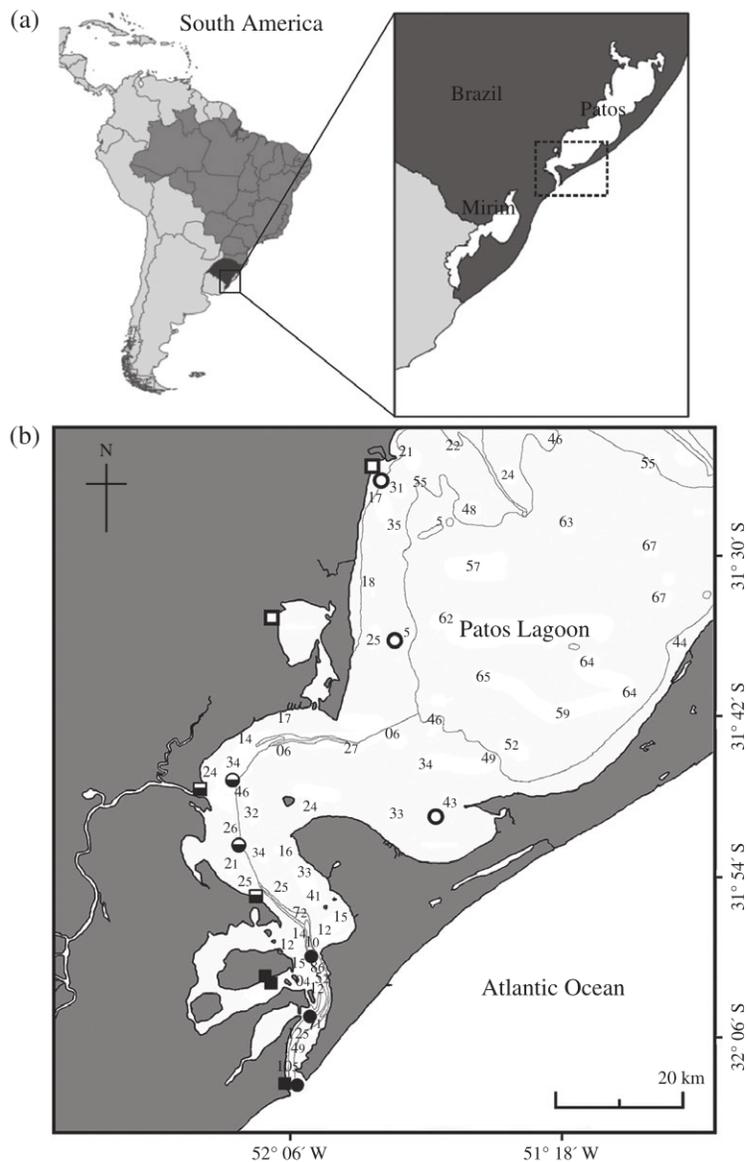
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FIG. 1. (a) Patos-Mirim Lagoon complex in southern Brazil and (b) the study area in southern portion of Patos Lagoon showing the sampling sites at three major ecological regions: fresh water (□ and ○), upper estuary (▣ and ●) and lower estuary (■ and ●) in shallow (squares) and deep (circles) areas of the lagoon.

processed for stable isotope analyses, following Garcia *et al.* (2007). Briefly, all basal food sources and animal muscle samples were oven dried at 60° C for 48 h. Dried samples were ground to a fine powder with a mortar and pestle and stored in clean Eppendorf tubes. Sub-samples were weighed (1 mg for animal tissues, 25–30 mg for SOM and 3 mg for other basal sources), pressed into Ultra-Pure tin capsules (Costech Analytical; <http://costechanalytical.com/>) and sent to the Analytical Chemistry Laboratory of the Institute of Ecology, University of Georgia, for analysis of carbon and nitrogen isotopic composition.

Carbon and nitrogen isotope ratios were expressed in the typical delta notation ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$), in units of ‰, and normalized relative to Vienna Pee Dee Belemnite (PDB) limestone standard and atmospheric N_2 , respectively.

DATA ANALYSIS

Lateral spatial patterns (shallow *v.* deep areas) in the carbon and nitrogen stable isotope ratios of basal food sources and consumers were initially investigated using biplot diagrams for the three sampled regions (fresh water, upper estuary and lower estuary). Samples from stations placed inside the same salinity region were pooled together and used as replicates for data analyses. The assumptions of normality and homoscedasticity were assessed through Shapiro–Wilk and Levene tests, respectively. Although data fitted a normal distribution, they had unequal variance. Therefore, Kruskal–Wallis tests were used to compare between-depth differences in carbon and nitrogen isotope values of basal sources, overall invertebrate consumers (zooplankton, benthos and decapod macrocrustaceans) and only the fish fauna among the three sampled regions of the lagoon. The level of statistical significance considered was 5% (Zar, 2010).

A bibliographic review of the studied lagoon and similar systems provided information on prey trophic ecology for sampled fish species and allowed their classification into six feeding mode functional groups (FMFGs) according to the categories proposed by Elliott *et al.* (2007). The FMFGs were: (1) zooplanktivore (ZP), feeding predominantly on zooplankton; (2) detritivore (D), feeding predominantly on detritus; (3) herbivore (H), grazing predominantly on living macroalgal and macrophyte material or phytoplankton; (4) omnivore (OM), feeding on a mix of filamentous algae, macrophytes, periphyton, epifauna and infauna; (5) piscivore (P), feeding predominantly on fishes and (6) zoobenthivore (ZB), feeding predominantly on invertebrates associated with the substratum. Spatial patterns in isotopic composition of fish feeding guilds were observed using biplot diagrams.

Food linkages between basal food sources of shallow and deep areas and fish consumers were established using the Bayesian stable isotope mixing model in R (SIAR, version 3.0.1; www.r-project.org) (Parnell *et al.*, 2010). In the mixing model, input data employed were carbon and nitrogen means and s.d. for basal food sources and single values for consumers from each FMFG, as well as two-level discrimination data obtained from literature (Vander Zanden & Rasmussen, 2001; Vanderklift & Ponsard, 2003). A conceptual diagram was built to summarize and to compare trophic ecological relationships between shallow and deep areas along different regions of the estuary. The relative importance of each food linkage was defined based on the 50% Bayesian credibility interval (C.I.) estimated for the contribution of each basal food source to consumers. When the 50% C.I. estimated contribution of a food source to FMFG was below 20%, the source was considered to have a non-substantial contribution to those consumers in that trophic guild and, therefore, the food linkage between them was assumed negligible and not depicted in the diagram. When the majority or the entire contribution of the 50% C.I. was between 21 and 70%, it was assumed that these sources had sufficient contribution to the consumers to establish a food linkage between them. Contributions to consumers >70% were considered to be stronger food linkages and are denoted with thicker lines in the diagram. Each fish drawn in the diagram corresponds to a maximum of 10 fish that had their isotopic composition analysed in this study. FMFG trophic levels were not considered in the diagram.

RESULTS

A total of 108 samples of five basal food sources (POM = 23, SOM = 27, macroalgae = 6, C_3 plants = 36 and C_4 plants = 16) and 723 samples of consumers (zooplankton = 15, benthic infauna = 67, decapod macrocrustaceans = 162 and fishes = 479) had their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values analysed (Table I, and Appendices I and II). Macrophytes were found only in shallow areas and they showed an increasing trend from depleted to more enriched $\delta^{13}\text{C}$ values from fresh water to the lower estuary. This trend was related to a predominance of C_3 plants in northern regions of the lagoon (fresh

TABLE I. Number of samples and mean \pm s.d. values of stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) of basal food sources, main invertebrates and fish feeding mode feeding groups (FMFGs) sampled along lateral depth gradients (shallow–deep areas) in three major salinity regions of Patos Lagoon

	Lateral areas					
	Shallow Salinity regions			Deep Salinity regions		
	Fresh water	Upper estuary	Lower estuary	Fresh water	Upper estuary	Lower estuary
Basal food sources						
Macroalgae						
$\delta^{13}\text{C}$						-10.28 ± 0.73
$\delta^{15}\text{N}$						8.64 ± 0.66
Macrophytes (C ₃)	24	10	6			
$\delta^{13}\text{C}$	-26.94 ± 1.65	-27.58 ± 1.92	-25.03 ± 0.79			
$\delta^{15}\text{N}$	7.01 ± 2.18	7.81 ± 2.02	4.52 ± 0.37			
Macrophytes (C ₄)	1	5	10			
$\delta^{13}\text{C}$	-11.99	-12.13 ± 0.83	-12.09 ± 0.29			
$\delta^{15}\text{N}$	11.49	8.05 ± 1.50	6.90 ± 2.46			
Particulate organic matter	4	4	6			
$\delta^{13}\text{C}$	-22.44 ± 0.50	-22.74 ± 1.95	-22.21 ± 1.06			
$\delta^{15}\text{N}$	4.85 ± 0.66	4.64 ± 0.84	5.58 ± 2.55			
Sedimentary organic matter	4	1	6			
$\delta^{13}\text{C}$	-21.12 ± 1.70	-26.08	-18.34 ± 2.31			
$\delta^{15}\text{N}$	5.10 ± 0.85	0.12	7.26 ± 1.21			
Consumers						
Zooplankton						
$\delta^{13}\text{C}$						-20.38 ± 1.35
$\delta^{15}\text{N}$						7.99 ± 2.70

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TABLE I. Continued

	Lateral areas					
	Shallow Salinity regions			Deep Salinity regions		
	Fresh water	Upper estuary	Lower estuary	Fresh water	Upper estuary	Lower estuary
Benthic infauna			12	26	8	22
$\delta^{13}\text{C}$			-15.48 ± 2.35	-19.34 ± 1.41	-18.43 ± 0.26	-17.43 ± 1.18
$\delta^{15}\text{N}$			10.68 ± 2.21	8.79 ± 1.46	9.88 ± 3.15	10.07 ± 2.52
Macrocrustaceans		9	23	53	33	35
$\delta^{13}\text{C}$	-17.73 ± 3.26	-16.96 ± 2.61	-15.29 ± 3.01	-16.86 ± 1.77	-14.91 ± 2.51	-12.69 ± 1.71
$\delta^{15}\text{N}$	10.41 ± 1.61	10.42 ± 1.79	9.56 ± 1.48	11.11 ± 1.01	11.19 ± 0.95	10.46 ± 1.66
Detritivorous fishes		9	17	1		
$\delta^{13}\text{C}$	-32.94 ± 1.10	-18.39 ± 5.54	-15.15 ± 3.07	-14.00		
$\delta^{15}\text{N}$	8.45 ± 0.73	9.38 ± 1.92	10.51 ± 1.61	9.13		
Herbivorous fishes		7				
$\delta^{13}\text{C}$	-19.81 ± 1.64					
$\delta^{15}\text{N}$	13.70 ± 2.79					
Omnivorous fishes		12	25	24	13	1
$\delta^{13}\text{C}$	-21.53 ± 6.29	-21.42 ± 3.54	-15.32 ± 2.54	-19.00 ± 1.61	-20.57 ± 2.16	-18.77
$\delta^{15}\text{N}$	12.52 ± 3.05	9.78 ± 2.08	12.35 ± 1.48	14.12 ± 1.59	12.59 ± 1.14	13.90
Piscivorous fishes		3		1		
$\delta^{13}\text{C}$		-18.78 ± 1.37		-17.85		
$\delta^{15}\text{N}$		15.08 ± 1.24		13.63		
Zoobenthivorous fishes		21	6	95	70	102
$\delta^{13}\text{C}$	-19.06 ± 1.26	-17.44 ± 2.88	-16.83 ± 1.40	-17.52 ± 2.21	-17.29 ± 2.19	-16.70 ± 2.27
$\delta^{15}\text{N}$	13.63 ± 0.79	12.89 ± 1.94	13.47 ± 0.88	14.28 ± 1.30	14.20 ± 0.82	13.94 ± 0.97
Zooplanktivorous fishes		11		15	2	4
$\delta^{13}\text{C}$	-19.78 ± 1.33	-20.62 ± 3.18		-18.55 ± 1.65	-18.04 ± 0.70	-17.62 ± 1.05
$\delta^{15}\text{N}$	13.39 ± 0.74	12.43 ± 1.21		13.42 ± 1.07	13.55 ± 2.38	14.66 ± 0.69

water and upper estuary) when compared with the southern portion (lower estuary), where an abundance of C₄ plants prevailed.

The variance in $\delta^{13}\text{C}$ values of basal food sources was greater for shallow areas compared with deep areas (Fig. 2). This variation was especially clear for the freshwater region, in which $\delta^{13}\text{C}$ values ranged from -26.9 to -12.0‰ in shallow areas, due to the occurrence of C₃ and C₄ macrophytes. In contrast, deeper areas, which did not have the presence of rooted macrophytes, only particulate organic matter (POM and SOM), showed comparatively lower variance in $\delta^{13}\text{C}$ values, ranging from -23.5 to -18.9‰ . Kruskal–Wallis tests revealed a significant lateral effect (shallow *v.* deep) on $\delta^{13}\text{C}$ values for basal food sources and fishes, for nearly all sampled regions. The trend in isotopic enrichment for ¹³C observed for basal food sources from fresh water towards the ocean, although not as marked, was also observed for consumers, demonstrating that the isotopic composition patterns exhibited by basal food sources cascaded up the food chain. There were no clear patterns in the variance of $\delta^{15}\text{N}$ between depth gradients and regions.

In total, 479 fishes were collected, representing 27 species, with higher species richness ($S = 20$) and lower number of individuals ($n = 151$) in shallower areas, compared with deeper areas (14 species and 328 individuals) (Appendix II). All FMFGs occurred in both shallow and deep locations, except herbivores that were found only in the shallow fresh water. For both shallow and deep areas, the diversity of FMFG decreased from fresh water towards the ocean, with the lower estuary region exhibiting only three FMFGs in each of the lateral depth gradient compartments. On the whole, fishes in deep areas were grouped into fewer FMFGs, with a predominance of zoobenthivores (81%) and omnivores (11%), whereas in shallow areas individuals were more evenly distributed among FMFGs, but with a slight predominance of omnivores (36%) and zoobenthivores (22%) (Fig. 3 and Table I). In shallow areas, fish variation along the $\delta^{13}\text{C}$ axis of the isotopic biplot appeared to reflect the overall choked-type shape of Patos Lagoon: greater distance between extreme carbon values in the freshwater region (20.2‰) followed by a gradual reduction of the differences between these values towards the ocean (17.7‰ in the upper estuary and 11.9‰ in the lower estuary) (Fig. 3). Variation in $\delta^{15}\text{N}$, in contrast to $\delta^{13}\text{C}$, was more pronounced along the lateral axis, with lower values in shallow compared with deep areas.

Stable isotope mixing model results indicated a more equal contribution of basal food sources for fish FMFG in the shallow areas of all the three major salinity regions, whereas for deep areas there was a higher contribution of SOM as a carbon source, mainly in the freshwater region and for the zoobenthivores FMFG (Fig. 4). The conceptual trophic diagram depicting relationships among basal food sources and consumers better illustrates the results of the mixing models and highlights the greater use of multiple food sources by different FMFGs in shallow areas as opposed to the predominance of benthic resources in deep areas (Fig. 5).

DISCUSSION

Fishes play important ecological roles as habitat couplers because of their high mobility and flexible foraging (Schindler & Scheuerell, 2002), but the present findings broadly support the hypothesis that the food web of the studied lagoon may be seen as two compartments or trophic modules, one for shallow areas and another for deep

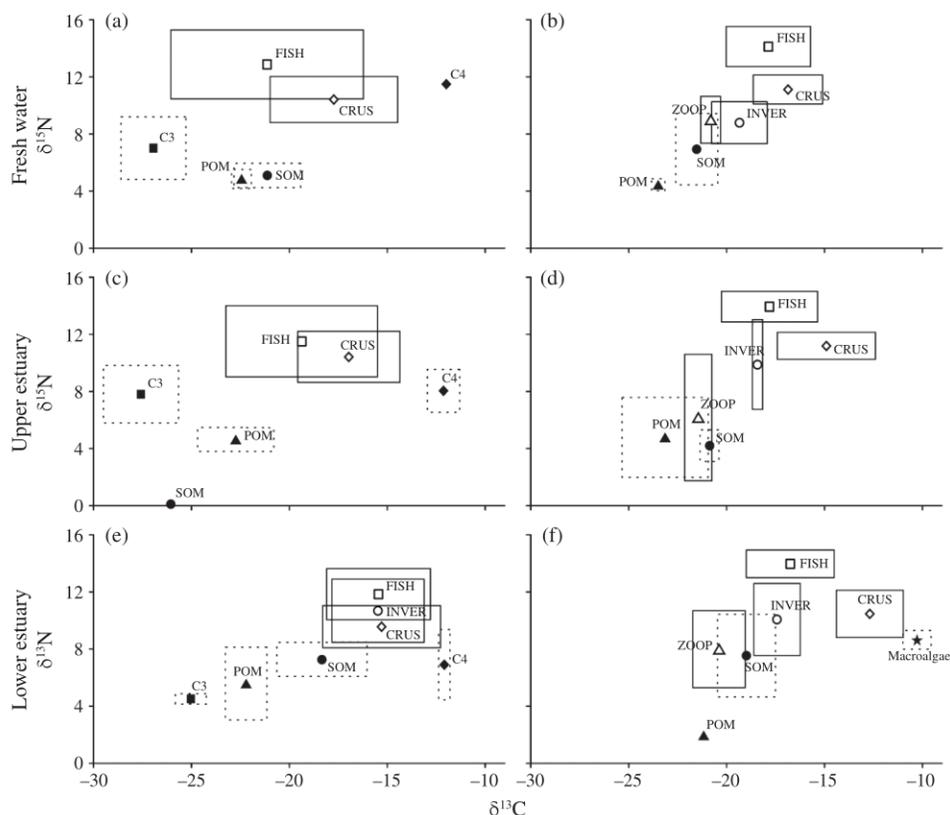


FIG. 2. Biplots of average values of stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) of basal food sources (.....) and consumers (—) collected at (a, c, e) shallow and (b, d, f) deep sites along a salinity gradient: (a, b) fresh water, (c, d) upper estuary and (e, f) lower estuary of the Patos Lagoon. Boxes denote s.d. around each mean value: POM, particulate organic matter in suspension; SOM, particulate organic matter in sediment; C3 and C4, macrophytes with C₃ and C₄ photosynthetic pathways; ZOOP, zooplankton; INVER, benthic invertebrates; CRUS, crustaceans; FISH, fishes.

areas. Obviously, such lateral trophic segregation is not complete and may be broken by those fish species moving from shallow to deep habitats of the lagoon. At Patos Lagoon, the whitemouth croaker *Micropogonias furnieri* (Desmarest 1823), which was the only species caught in both shallow and deep habitats along the salinity gradient, is an example of a fish moving between different habitats during its life cycle. This species spawns at sea and its recruits (20–90 mm total length, L_T) are abundant in shallow waters of the estuary, but after increasing in size, they move to deeper waters of the lagoon (Vieira *et al.*, 2010). Other fish species (*e.g.* Atherinopsidae and Mugilidae) are commonly found in both shallow and deep habitats and may also promote trophic integration of these habitats. Many fish species at Patos Lagoon, however, have a discrete lateral distribution, with the dominant fish fauna in deep areas being clearly distinct from shallow ones. For instance, Chao *et al.* (1985) demonstrated that seven Sciaenidae species, two Ariidae and the Atlantic midshipman *Porichthys porosissimus* (Cuvier 1829) constitute 90% of total numerical abundance in

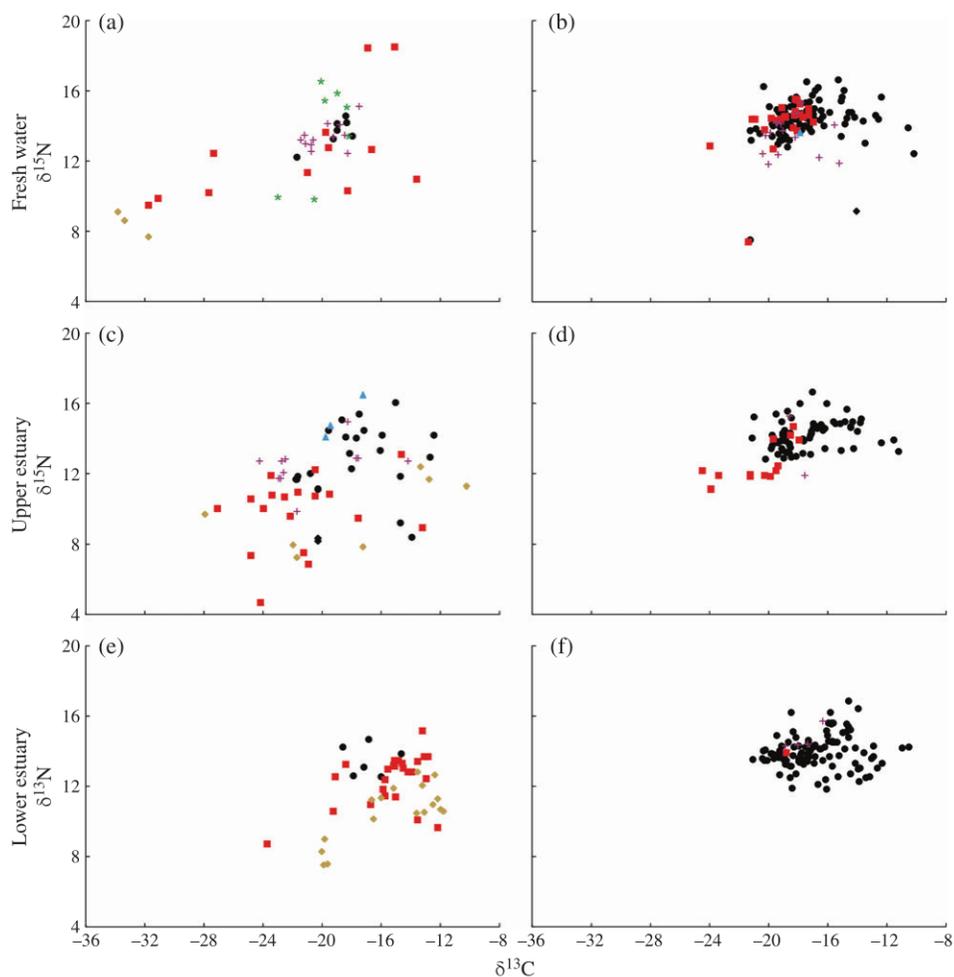


FIG. 3. Scatterplots of stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) values of fish individuals collected at (a, c, e) shallow and (b, d, f) deep sites along a salinity gradient: (a, b) fresh water, (c, d) upper estuary and (e, f) lower estuary of the Patos Lagoon. Fishes were classified into six functional feeding mode groups: zoobenthivore (●), herbivore (*), detritivore (◆), omnivore (■), zooplanktivore (+) and piscivore (▲).

deep areas of Patos Lagoon estuary, while three species of Mugilidae, two atherinids, three juvenile Clupeidae, one Anablepidae and juveniles of *M. furnieri* comprise 95% of total numerical abundance in shallow areas. Therefore, even though it is known that small to modest amounts of energy and material may flow between adjacent estuarine habitats (Grimaldo *et al.*, 2009; Neves *et al.*, 2013), the present results suggest that trophic structure in shallow and deep habitats is partially segregated and the compartments should be considered as different sub-systems, both in freshwater and estuarine reaches of this coastal lagoon.

In Patos Lagoon estuary, the species composition of some taxa (*e.g.* zooplankton) is directly related to local hydro-geomorphological conditions (Montú *et al.*, 1998) and

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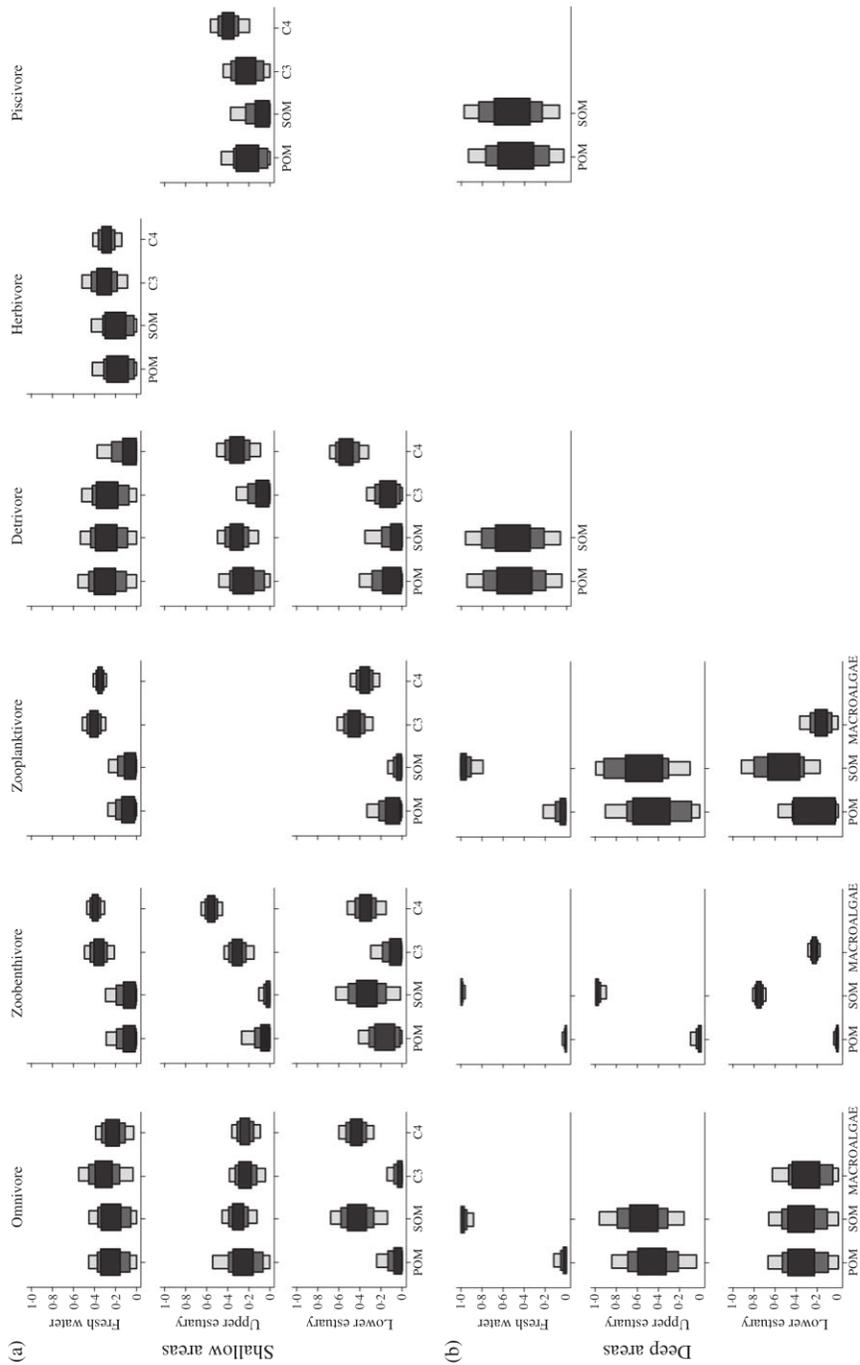


FIG. 4. Legend on next page.

the distances between the opposite margins of the lagoon, which decrease markedly towards the ocean, evidenced by the choked shape (Kjerfve, 1986). The distance between shallow and deep areas, greater for the freshwater region and less for the lower estuary, however, did not appear to have much influence on the ichthyofauna. Although fish FMFG occurrence was almost the same between shallow and deep areas for each salinity region and it would be expected that closer areas presented higher similarity, the species composition was very different within this depth gradient, with shallow and deep areas in the freshwater and lower estuary regions sharing only two and one fish species, respectively. Isotope values better reflected this funnel-shaped morphology of the lagoon, but even though the isotopic composition of primary sources and consumers in shallow and deep areas was more similar for the lower estuary than for the freshwater region, those areas within the depth gradient were still isotopically distinct, suggesting different web compartments.

Greater variation in carbon isotope values observed in the lagoon's margins, together with higher faunal diversity, may be explained by the higher habitat complexity commonly found in shallow environments (Gratwicke & Speight, 2005; Teixeira-de-Mello *et al.*, 2009). This complex structure is mainly driven by greater availability of primary producers, such as the presence of rooted aquatic plants that exert multiple effects on ecosystem structure and functioning. The presence of vegetation can modify biotic and abiotic conditions (*e.g.* benthic fauna composition and sediment penetrability), having significant effects on habitat use by fishes (Crinall & Hindell, 2004; Valinãs *et al.*, 2012).

Food webs are constructed in such a way that energy channels based on different basal food resources are coupled by higher trophic categories (Rooney *et al.*, 2006). Although consumers in aquatic food webs typically rely on multiple resource pathways and functional groups may differ in their resource use (Alfaro *et al.*, 2006), predictions may be made regarding dominant energy sources supporting consumers at the level of the entire assemblage (Hoeinghaus *et al.*, 2011). The present lateral gradient conceptual diagrams for fish basal food source use in different salinity regions confirmed that consumer reliance on multiple resource pathways is more marked in shallow areas. In the deep areas of the coastal lagoon, SOM was an important carbon source for fishes, especially the zoobenthivores, whereas in shallow locations its importance as a primary food source for fishes was mainly shared with or even substituted by macrophytes. The high proportion of lower elevation littoral and larger mixohaline zones in Patos Lagoon estuary, when compared with adjacent subtropical estuaries, favours the presence of typical salt marsh vegetation, such as the genus *Spartina* (Costa, 1997; Hoeinghaus *et al.*, 2011). Besides playing an ecological role as a refuge from predators, emergent and submerged macrophytes also represent a richer feeding habitat (Halpin, 2000; Teixeira-de-Mello *et al.*, 2009; Whitley & Bollens, 2014). Herbivory is an important structuring force for communities in aquatic systems, but the taxonomic and functional

FIG. 4. Relative contributions of basal food sources (POM, particulate organic matter in suspension; SOM, particulate organic matter in sediment; C3 and C4, macrophytes with C₃ and C₄ photosynthetic pathways; macroalgae) to fish consumers distributed into feeding mode functional groups (FMFGs): omnivore, zoobenthivore, zooplanktivore, detritivore, herbivore and piscivore. Each contribution was investigated for shallow and deep areas of the three major ecological regions of a coastal lagoon, fresh water, upper estuary and lower estuary. Bayesian credible intervals of the feasible contributions of each basal food source to the selected group: 50% (■), 75% (■) and 95% (■).

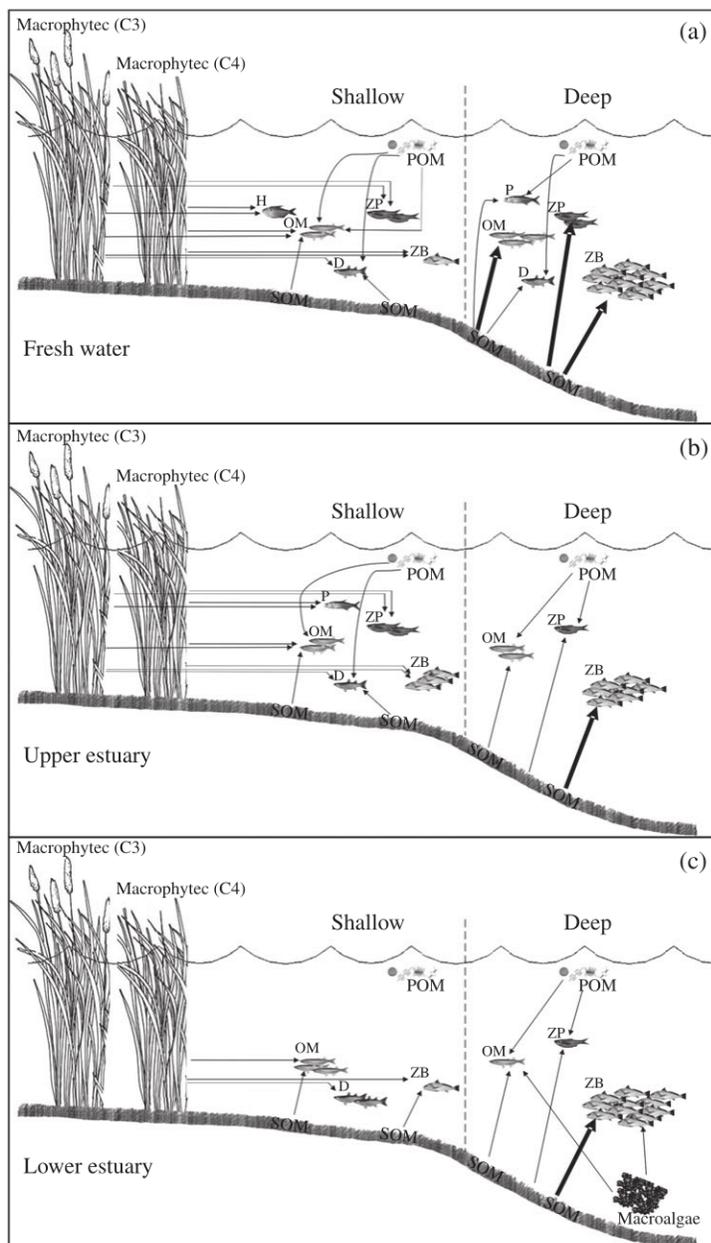


FIG. 5. Conceptual diagrams of basal sources and fish feeding mode functional groups (FMFGs) trophic relationships in a lateral depth gradient (shallow to deep) for three major ecological regions of a coastal lagoon: (a) fresh water, (b) upper estuary and (c) lower estuary. Basal food sources probable contributions to each functional feeding group are indicated by arrow width, where thinner arrows denote the existence of lower source contributions to the consumer (between 20 and $\leq 70\%$), whereas broader arrows indicate larger source contributions ($>70\%$). D, detritivores; H, herbivores; OM, omnivores; P, piscivores; ZB, zoobenthivores; ZP, zooplanktivores; POM, particulate organic matter in suspension; SOM, particulate organic matter in the sediment; C3 and C4, macrophytes with C_3 and C_4 photosynthetic pathways.

diversity of herbivorous fishes in subtropical and temperate systems is low when compared with tropical systems (Meekan & Choat, 1997; Shurin *et al.*, 2002; Vergés *et al.*, 2014). Some studies applying different techniques, either stomach contents or stable isotope analyses, have highlighted the importance of detritus derived primarily from aquatic macrophytes as a food source for consumers (Winemiller, 1990; Botto *et al.*, 2005). In this study, doubt remains whether fish assimilated carbon from these plants by direct consumption and by an indirect pathway, for instance, from plant-derived carbon stored in the SOM, which in some cases, can represent up to 60% of total carbon (Aichner *et al.*, 2010; Rodrigues *et al.*, 2014). Considering these subtropical systems follow the general trend of a decreasing proportion of herbivorous fishes with increasing latitude (Vieira *et al.*, 1998; Floeter *et al.*, 2005), it is likely that indirect consumption of macrophyte-derived carbon (*i.e. via* organic matter stored in the sediment) is a more likely pathway supporting fish consumers in the shallow habitats of Patos Lagoon.

Although other studies have shown the importance of phytoplankton to estuarine trophic webs (Deegan & Garritt, 1997; Grimaldo *et al.*, 2009), the present mixing model outputs revealed dominant flows of energy originating from benthic systems. It has been shown that the benthic pathway is particularly important in nutrient-poor lakes, where low phytoplankton biomass and high transparency favour benthic production (Vadeboncoeur *et al.*, 2002). Moreover, if phytoplankton biomass is relatively low, there can be a dilution of the plankton isotopic signature and the heavier detrital isotopic signature may appear in primary consumers, even when particulate organic matter (POM) dominates their diets (*e.g.* zooplankton that normally feed on phytoplankton) (del Giorgio & France, 1996). In Patos Lagoon estuary, phytoplankton exhibits great temporal variability in density, bio-volume and chlorophyll *a*, with maximum primary production occurring during spring, summer and autumn, whereas during winter net production is negative, even in shallow areas (Odebrecht *et al.*, 2010a). The relationship of phytoplankton biomass with flow in an estuary exhibits a parabolic behaviour, decreasing for both higher and lower river flow, which may be explained by lower residence time and lower nutrient and biomass inputs, respectively (Azevedo *et al.*, 2014). Because the present isotopic data were obtained in September, late austral winter and a period of high freshwater inflow, it is possible that the higher proportion of benthic contributions to fishes and the limited POM contribution could be a function of seasonality. Therefore, further examination and replication of this study in different seasons are warranted.

Carbon influx into aquatic food webs occurs through multiple primary consumers, but is not symmetrical (Rooney *et al.*, 2006). Both fast (*via* phytoplankton) and slow (*via* organic detritus) pathways contribute to food webs, but these contributions vary spatially and temporally. The relative importance of these basal food resources to estuarine consumers may be influenced by many factors, such as hydro-geomorphologic features that affect the relative size of the estuarine zone, freshwater inflow patterns, types of connection with the sea and slope and extent of the littoral area relative to open water (Hoeinghaus *et al.*, 2011). Also, accumulation of organic detritus in large amounts in estuaries plays an important role as a food source for fishes and may explain their attraction to these coastal systems (Whitfield, 1999). Apart from being consumed directly by fishes, organic detritus in estuaries also provides food for filter- and deposit-feeding invertebrate prey (de Villiers *et al.*, 1999). Some studies on estuaries found that benthic animals were the predominant prey, supporting the relevance of detritus-based food webs for estuarine nekton and the central role of invertebrates within these systems

(Elliott & Dewailly, 1995; Franco *et al.*, 2008). Hence, the strong ‘benthic subsidy’ (Vander Zanden *et al.*, 2005; Teixeira-de-Mello *et al.*, 2009) appears to explain not only the higher fish diversity found in shallow areas, but also high overall fish densities, such as observed for the zoobenthivore FMFG in deep areas of the present coastal lagoon. In lakes, widespread omnivory within subtropical fish communities is well documented (Vander Zanden *et al.*, 2005; Teixeira-de-Mello *et al.*, 2009), whereas in estuaries the relative contribution of omnivores to total fish diversity is highest in small closed estuaries and lower in moderate-to-large closed systems and permanently open estuaries (Harrison & Whitfield, 2012). For these reasons, it appears plausible to conclude that the presence of omnivores is related to the amount of margin as a proportion of total area, with higher values favouring fish species from this FMFG. This could explain why omnivores were slightly dominant in shallow areas and less abundant in deep areas, when compared with zoobenthivores.

In conclusion, this study indicates the importance of considering the existence of lateral (shallow *v.* deep) gradients in coastal lagoon ecological studies. While in lakes foraging by fishes in the littoral zone is considered an important inflow to the adjacent pelagic ecosystem (Schindler *et al.*, 1996), the present results suggest that shallow and deep areas in estuaries should be seen as sub-systems or trophic modules with distinct trophic organization. The uncoupling of shallow and deep areas in estuarine food webs [see Bertolo *et al.* (2005) for lake systems] offers a complementary perspective on spatial ecology in aquatic ecosystems. The present findings reinforce the importance of benthic pathways to fishes at the ecosystem level and highlight the different roles of food resources in this food linkage. A significant contribution of macrophytes, probably *via* indirect detrital pathways, as food sources sustaining a diverse group of fishes in the shallow zone was also revealed. This is especially significant considering that the increasing habitat disturbance associated with human activities (*e.g.* harbour operations, urbanization and untreated sewage outflow) has contributed to a reduction of salt marsh areas (Marangoni & Costa, 2009; Odebrecht *et al.*, 2010*b*) that could lead to dramatic changes in the biota due to reduction and loss of key elements of habitat.

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APPENDIX II

Mean \pm S.D. values of stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) of fish species collected at shallow and deep sites along a freshwater–estuarine gradient in three different zones (fresh water, upper estuary and lower estuary) of Patos Lagoon. Each species have been designated to a feeding mode functional group (FMFG)

	FMFG	Fresh water		Upper estuary		Lower estuary				
		n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Shallow areas										
<i>Astyanax jacuiensis</i>	Herbivore	7	-19.81 \pm 1.64	13.70 \pm 2.79						
<i>Charax stenopterus</i>	Omnivore	3	-30.14 \pm 2.21	9.84 \pm 0.38						
<i>Geophagus brasiliensis</i>	Omnivore	2	-18.89 \pm 0.95	11.50 \pm 1.76						
<i>Astyanax fasciatus</i>	Omnivore	4	-19.61 \pm 5.96	11.82 \pm 0.83	6	-22.42 \pm 2.68	10.66 \pm 0.32			
<i>Cyphocharax voga</i>	Detritivore	3	-32.94 \pm 1.10	8.45 \pm 0.73	2	-21.82 \pm 0.18	7.58 \pm 0.47			
<i>Jenynsia multidentata</i>	Omnivore	3	-17.22 \pm 2.36	16.83 \pm 2.78	1	-13.19	8.90	6	-15.44 \pm 2.65	13.40 \pm 0.93
<i>Lycengraulis grossidens</i>	Zooplanktivore	6	-18.62 \pm 0.74	13.75 \pm 0.90	4	-16.89 \pm 1.85	13.34 \pm 1.06			
<i>Odonthestes perugiae</i>	Zoobenthivore	1	-17.90	13.42	4	-21.32 \pm 0.73	11.55 \pm 0.32			
<i>Micropogonias furnieri</i>	Zoobenthivore	6	-19.25 \pm 1.26	13.67 \pm 0.86	10	-17.71 \pm 1.55	14.04 \pm 1.30	6	-16.83 \pm 1.40	13.47 \pm 0.88
<i>Platanichthys platana</i>	Zooplanktivore	6	-20.94 \pm 0.33	13.03 \pm 0.31	7	-22.75 \pm 0.76	11.91 \pm 1.01			
<i>Hypostomus commersoni</i>	Detritivore				1	-27.88	9.68			
<i>Odonthestes humensis</i>	Zoobenthivore				7	-14.84 \pm 2.42	12.02 \pm 2.39			
<i>Oligosarcus jenynsii</i>	Piscivore				1	-17.21	16.47			
<i>Oligosarcus robustus</i>	Piscivore				2	-19.56 \pm 0.22	14.39 \pm 0.44			
<i>Pimelodus pintado</i>	Omnivore				3	-24.05 \pm 0.70	10.81 \pm 0.95			
<i>Rhamdia quellen</i>	Omnivore				2	-21.68 \pm 0.67	8.54 \pm 1.45			
<i>Astyanax eigenmanniorum</i>	Omnivore				4	-21.83 \pm 3.34	7.08 \pm 1.98	1	-23.71	8.67
<i>Atherinella brasiliensis</i>	Omnivore				1	-14.60	13.08	17	-14.56 \pm 1.24	12.31 \pm 1.24
<i>Mugil liza</i>	Detritivore				6	-15.67 \pm 4.19	9.93 \pm 2.05	17	-15.15 \pm 3.07	10.51 \pm 1.61
<i>Odonthestes argentinensis</i>	Omnivore				1	-20.48	12.19	1	-19.21	10.55

APPENDIX II

Continued

	Fresh water			Upper estuary			Lower estuary			
	FMFG	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Deep areas										
<i>Mugil liza</i>		1	-14.00	9.13						
<i>Oligosarcus jenynsii</i>		1	-17.85	13.63						
<i>Parapimelodus nigribarbis</i>		4	-16.83 ± 2.21	12.49 ± 1.07						
<i>Platanichthys platana</i>		2	-19.24 ± 0.29	13.96 ± 0.31	1	-17.55	11.87			
<i>Odontheistes argentinensis</i>		1	-19.68	12.67	3	-20.02 ± 1.05	12.17 ± 0.25			
<i>Catathyridium garmani</i>		33	-15.82 ± 2.49	14.64 ± 1.55	16	-14.22 ± 1.58	14.48 ± 0.66	2	-12.17 ± 1.75	13.31 ± 1.19
<i>Genidens barbatus</i>		10	-17.66 ± 0.72	14.41 ± 0.64	12	-16.75 ± 0.51	14.74 ± 0.59	6	-15.43 ± 1.30	15.60 ± 1.09
<i>Genidens genidens</i>		6	-20.39 ± 0.93	13.73 ± 0.34	14	-18.81 ± 1.29	13.53 ± 0.38	29	-18.34 ± 1.70	13.72 ± 0.31
<i>Lycengraulis grossidens</i>		9	-19.16 ± 0.95	13.72 ± 0.98	1	-18.54	15.23	4	-17.62 ± 1.05	14.66 ± 0.69
<i>Microponogonias furnieri</i>		46	-18.32 ± 1.32	14.07 ± 1.25	27	-18.65 ± 1.03	14.16 ± 0.92	49	-16.69 ± 2.03	14.12 ± 0.92
<i>Pimelodus pintado</i>		23	-18.97 ± 1.64	14.18 ± 1.60	10	-20.74 ± 2.42	12.72 ± 1.28	1	-18.77	13.90
<i>Paralichthys orbignyanus</i>					1	-15.04	13.28	1	-16.11	12.35
<i>Pogonias cromis</i>								10	-13.92 ± 1.36	12.86 ± 0.47
<i>Symphurus jenynsii</i>								5	-16.24 ± 1.24	14.17 ± 1.45

 n , sample size.

APÊNDICE II**Elemental turnover rates and isotopic discrimination in a euryhaline fish reared under different salinities: Implications for movement studies.**

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Elemental turnover rates and isotopic discrimination in a euryhaline fish reared under different salinities: Implications for movement studies



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ABSTRACT

Stable isotopes are useful tools for studying species residency and movement in aquatic environments. Yet, many questions about their use still remain, mostly related to assumptions that should be experimentally validated, such as turnover rates and discrimination factors. Salinity is a key environmental variable that may influence turnover and discrimination factors, but its effects have not been tested. A controlled diet-shift experiment was conducted with whitemouth croakers (*Micropogonias furnieri*), a euryhaline species, to determine the turnover rates and diet-tissue discrimination of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes in muscle tissue. Fish captured in Patos Lagoon, Brazil were distributed into three independent recirculating aquaculture systems with salinities adjusted to represent freshwater, estuarine and marine environments, and fed for 90 days on an isotopically distinct diet. Half-life estimates were longer for both isotopes in the freshwater group ($\delta^{13}\text{C} = 25.6$ days, and $\delta^{15}\text{N} = 34.6$ days) compared to the estuarine ($\delta^{13}\text{C} = 18.7$ days, and $\delta^{15}\text{N} = 23.9$ days) and marine treatments ($\delta^{13}\text{C} = 17.7$ days, and $\delta^{15}\text{N} = 22.3$ days). Overall, carbon isotopic turnover was mainly driven by catabolism, whereas growth was the main factor responsible for nitrogen turnover. Trophic discrimination factors (TDFs) for carbon isotope were similar between fresh and estuarine treatments ($\text{TDF}_{\text{final}} = 0.82 \pm 0.54\%$ and $0.88 \pm 0.58\%$, respectively), but higher in marine ($\text{TDF}_{\text{final}} = 1.44 \pm 0.66\%$). Hence, in addition to providing species-specific isotopic parameters of a widespread sciaenid in the western Atlantic Ocean crucial to elucidate residence time along salinity gradients, these findings provide lab-based evidence of salinity effects on diet-tissue discrimination of a euryhaline species.

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1. Introduction

Understanding fish dependence on estuaries and establishing their patterns of migration or movement therein is fundamental for comprehension of the ecology, life history and behavior of these animals, in addition to being prerequisites for their effective conservation and sustainable exploitation (Bennett et al., 2015; Guelinckx et al., 2008; Litvin and Weinstein, 2004). The abundance and stability of fish populations over a year vary as a result of the continued ability of its individuals to move to, from and within bays and estuaries for important foraging and reproductive activities (Kennish and Paerl, 2010; Kerr et al., 2010).

Natural biogeochemical tracers (e.g., stable isotopes) are particularly useful for studying animal movements because they do not require capture-mark-recapture or other tracking methods, and provide spatial and time-integrated information (Rubenstein and Hobson, 2004).

Marked differences in stable isotope values can be found along salinity gradients, from freshwater to the sea, and these are incorporated into organisms and their food webs (Deegan, 1993; Fry and Chumchal, 2011; Oliveira et al., 2014). The basic premise underlining the use of stable isotopes in dietary studies is that tissues of consumers will isotopically resemble what is consumed; however, the relationship between isotopic ratios in the diet and that in the tissues is not always straightforward. The dynamics of elemental incorporation depends on a variety of factors, such as nutrient composition of the diet and differences in assimilation (Gaye-Siessegger et al., 2003; Zuanon et al., 2007), tissue turnover rate (Heady and Moore, 2012; Vander Zanden et al., 2015) and isotope trophic discrimination (Buchheister and Latour, 2010; Caut et al., 2013).

Isotopic changes in tissues, in response to dietary or habitat switches, may occur through the dilution of existing mass by new mass (addition) and/or by the synthesis and degradation of existing tissues using material from the new diet (replacement). As a result, the main factors controlling the rate at which an organism's tissues reflect the isotopic signature of its food are the growth of an individual and

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its metabolic activity (Buchheister and Latour, 2010; Hesslein et al., 1993; MacAvoy et al., 2001). Proper consideration of and knowledge about specific turnover rates may determine the length of time that an immigrant to a given habitat will be distinguishable from a longtime resident that has reached isotopic equilibrium, thus being essential to the inference of movement patterns (Guelinckx et al., 2008; Herzka, 2005). Once at equilibrium, trophic discrimination refers to the process by which ratios of stable isotopes change between prey and consumer during metabolic processing of consumed material (Mill et al., 2007; Tieszen et al., 1983). Although it is known that the employment of inaccurate discrimination values may introduce substantial errors in the estimation of contributions by food sources to consumer tissues, many values used in isotopic studies for estuarine species originate from studies conducted with mammals, birds and freshwater animals (Vander Zanden and Rasmussen, 2001; Vanderklift and Ponsard, 2003). This creates a demand for more studies on euryhaline species.

Ideally, both isotopic turnover and trophic discrimination should be validated through controlled experiments, therefore serving as models for field-based results and providing accurate knowledge for the interpretation of the isotopic data gathered in the field (Martínez del Río et al., 2009). This has not been the case, as the number of observational field studies that apply stable isotopes to ecological problems far surpasses the number of experimental studies designed to clarify the mechanisms behind the patterns found by isotopic ecologists (Wolf et al., 2009). While studies on turnover rates and discrimination factors for fish species have increased over time (Herzka, 2005; Vander Zanden et al., 2015), many questions about their use still remain. For example, abiotic variables (e.g., temperature and salinity) are known to affect metabolic rate through different mechanisms (Claireaux and Lagardère, 1999; Gillooly et al., 2001; Swanson, 1998) and could, therefore, affect turnover rates. The effects of temperature on both isotopic turnover and discrimination have been considered by some studies (Barnes and Jennings, 2007; Bosley et al., 2002; Weidel et al., 2011), but results have been equivocal. Unlike temperature, the influence of salinity on these factors has not yet been tested, even though it is known that differences in salinity values are a key factor determining habitat use and fish distribution in estuaries and coastal lagoons (Jaureguizar et al., 2003; Moura et al., 2012) and may increase the energetic cost of an organism either due to osmoregulation or related physiological processes (Swanson, 1998).

In this study, a controlled diet-shift experiment was conducted with the whitemouth croaker *Micropogonias furnieri* (Desmarest, 1823), an important euryhaline, estuarine-dependent sciaenid widely distributed along the western Atlantic coast from Mexico (20°N) to Argentina (41°S) (Isaac, 1988). This species is an important component of commercial and recreational fisheries in Brazil (where fish landings exceed 40,000 metric tons, Chao et al., 2015), Uruguay and Argentina (Vasconcellos and Haimovici, 2006). Nowadays, stocks are considerably depleted, either due to overexploitation (Cergole et al., 2005; Seeliger and Odebrecht, 2010) and/or climatic events (e.g. ENSO), which may exert a great influence on the distribution of early life stages and recruitment success (García et al., 2001). In estuaries and coastal lagoons, young-of-the-year usually occupy low salinity habitats, from which they move towards more saline areas downstream until reaching maturity, then migrate to sea for reproduction (Costa and Araújo, 2003; Jaureguizar et al., 2003). A previous isotopic study in Patos Lagoon estuary, the largest nursery area for this species in southern Brazil, provided first evidence that whitemouth croaker sub-adults stay in freshwater long enough to have their carbon isotope ratios reflect local autochthonous freshwater food sources (García et al., 2007). A lack of lab-determined values for isotope turnover rates and trophic discrimination, however, hinders a precise inference of residence time periods in estuarine and freshwater conditions experienced by the whitemouth croaker, and other species, as they move along salinity gradients.

Hence, the objectives of this study were to determine the turnover rates and trophic discrimination factors of carbon ($^{13}\text{C}/^{12}\text{C}$) and

nitrogen ($^{15}\text{N}/^{14}\text{N}$) stable isotopes in muscle tissue of the whitemouth croaker reared under different salinities. The hypothesis was that isotopic turnover would take longer in the freshwater environment as a result of an enhanced energetic cost due to osmoregulatory activity (Laiz-Carrión et al., 2005). These results will elucidate the temporal utilization of the estuary region and adjacent areas by croakers, and their applicability can be extended to other fish species with the same behavior, helping to better understand life-history patterns, eventually leading to more efficient management and conservation of these species.

2. Material and methods

2.1. Experimental design and fish facilities

Whitemouth croakers were captured from their natural environment in Patos Lagoon estuary, in a site approximately 5 m deep, dominated by silty-clay sediments and where the presence of floating macroalgae depends on estuarine currents and local winds (32°04'0" S, 52°05'00" W). The temperature at time of capture was 26 °C and salinity, measured using the Practical Salinity Scale (UNESCO, 1985), was 18. Fish were captured by 5 min hauls using a bottom trawl (15 m head rope, 5 m opening mouth, 13 mm mesh size on the end, and a pair of 10 kg weighted otter doors) towed by a small boat (SISBIO collecting permit no. 33369-1). Immediately after capture, fishes were kept inside a plastic container (170 L) equipped with aeration. Later, they were transferred to a transport unit box (1000 L) where dissolved oxygen concentration was maintained above saturation with air stones and bottled oxygen and, then, transported by truck (25 km) to the Marine Aquaculture Station of the Federal University of Rio Grande (EMAFURG) (Rio Grande City, Brazil), where the experiment was conducted. Upon arrival, fish were stocked in three 300 L static tanks and, after a 12 h period of acclimation, they were measured to the nearest mm (total length – TL) and weighed (g). Twenty-three individuals were randomly selected, measured and euthanized to determine the initial isotopic composition (δx_i) prior to the diet switch. Fish rearing and euthanasia procedures followed all Brazilian Ethics Committee Guidelines (Proc. 23116.001894/2013-86).

Fish with initial weight between 8 and 9.9 g were selected and randomly distributed into three salinity treatments, at densities of 30 fish/tank (90 fish per treatment). Each treatment consisted of an independent recirculating aquaculture system (RAS), comprised of three tanks (300 L each), a reservoir (sump – 300 L) with bioballs, a skimmer and a sand filter. Initially, to avoid osmotic shock, salinity was held constant in each treatment (~20), approximately the same as the croakers were experiencing in the field. That value was gradually adjusted, either by adding sea water to the system or by diluting the existing water with dechlorinated tap water, until the treatments reached values simulating freshwater, estuarine and marine environment. Salinity was changed at rates of approximately 1.5 (on the practical salinity scale) per day for the freshwater system and 0.5 per day for the estuarine and marine system, reaching values of 3, 16 and 27, respectively, which remained fixed until the end of the experiment. Each tank was equipped with an air diffuser to maintain dissolved oxygen close to saturation. Temperature (thermometer INCOTERM Hg), salinity (refractometer Atago, S/Mill-E), pH (digital pHmeter Mettler Toledo, FiveEasy FE20), alkalinity (titrimetry – APHA, 1998), total ammonia-nitrogen (TAN – Grasshoff et al., 1999) and nitrite (Aminot and Chaussepied, 1983) were monitored daily. The photoperiod was fixed at 14 L:10 D, simulating summer conditions, and flow rate in the RAS was held constant at 60 L h⁻¹.

Because the initial isotope value (δx_i) for $\delta^{13}\text{C}$ was $-17.54 \pm 0.95\%$, consistent with a combination of organic matter in suspension, organic matter in the sediment and C₄ plant-derived diet for croakers in Patos Lagoon estuary (García et al., 2007), the experimental diet was formulated using fish meal combined with C₃ plants (rice and soybean) (Table 1). This resulted in a large difference between initial and expected equilibrium values (δx_{eq}), important for adequately

Table 1

Food ingredients, stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and proximal composition of the experimental diet.

Ingredient (%)	Experimental diet
Soybean meal	45.00
Rice meal	34.00
Dry fish muscle	17.00
Fish oil	2.00
Premix ^a	2.00
Total	100.00
$\delta^{13}\text{C}$, measured (‰)	-26.90 ± 0.79
$\delta^{15}\text{N}$, measured (‰)	3.71 ± 0.28
C:N ratio (‰)	9.45 ± 1.06
Proximal composition (g % dry matter)	Experimental diet
Dry matter	89.52
Crude protein	40.87
Crude fiber	5.99
Ashes	6.71
Ether extract	11.40
Non-nitrogenous extracts	30.27

^a Premix M. Cassab, SP – Brazil (Vit. A (500,000 UI kg⁻¹), Vit. D3 (250,000 UI kg⁻¹), Vit. E (5000 mg kg⁻¹), Vit. K3 (500 mg kg⁻¹), Vit. B1 (1000 mg kg⁻¹), Vit. B2 (1000 mg kg⁻¹), Vit. B6 (1000 mg kg⁻¹), Vit. B12 (2000 µg kg⁻¹), niacin (2500 mg kg⁻¹), calcium pantothenate (4000 mg kg⁻¹), folic acid (500 mg kg⁻¹), biotin (10 mg kg⁻¹), Vit. C (10,000 mg kg⁻¹), colin (100,000 mg kg⁻¹), inositol (1000 mg kg⁻¹). Trace elements: selenium (30 mg kg⁻¹), iron (5000 mg kg⁻¹), copper (5000 mg kg⁻¹), manganese (5000 mg kg⁻¹), zinc (9000 mg kg⁻¹), cobalt (50 mg kg⁻¹), iodine (200 mg kg⁻¹).

characterizing elemental turnover with stable isotopes. Similarly, δX_i for $\delta^{15}\text{N}$ was $13.54 \pm 0.95\%$ and, because the main ingredients used to formulate the experimental diet were primary producers, a large difference between δX_i and δX_{eq} was also achieved for $\delta^{15}\text{N}$ (Table 1). Fish were hand-fed three times per day until apparent satiation at 9:00, 13:00 and 17:00 h.

Fish muscle used in diet formulation was obtained by filleting wild characin (*Cyphocharax voga*) from Mirim Lagoon (32°50' S, 52°69' W), southern Brazil, and drying it in an oven at 60 °C for 48 h. This option was used instead of industrial marine fish meal because characin in that particular lagoon exhibit carbon isotope values of $\delta^{13}\text{C} = -24.64 \pm 1.15\%$ (Silva-Costa and Bugoni, 2013), whereas the commercial product, usually composed of estuarine and marine forage fishes, was likely to have a similar value to that of our wild croakers. All dry ingredients were homogenized, and oil and distilled water were subsequently added until achieving a consistent texture that enabled pelleting in a meat grinder with 2 mm diameter. Next, the pellets were dried in a convection oven for 7 h at 60 °C. After drying, diets were stored frozen (-20 °C) in hermetically sealed plastic bags until they were used. Proximate analyses of the experimental diet were conducted by a specialized laboratory in the Food Technology and Science Department, Federal University of Santa Maria (UFSM, RS – Brazil).

Before and after each feeding event, rations were weighed on a precision analytical scale (0.01 g) to monitor feed consumption rates. During the experiment, rations and pellet size were adapted to the increasing fish biomass. After the first feeding each day, feces and uneaten food in the tanks and the systems sumps were siphoned away. The sumps were then refilled with chlorinated water previously filtered through bag filters (5 µm mesh size) and treated with sodium thiosulfate for chlorine neutralization, with salinity values adjusted to correspond to each treatment. The experiment lasted for 90 days and, once a week, three individuals from each salinity treatment (one per tank) were lethally sampled to obtain muscle samples for isotope analysis. Two days after the end of the experiment (day 92), all remaining fish were also lethally sampled in order to gauge variation in trophic discrimination after reaching a steady state with the new diet. For all sampling, fish were euthanized with an overdose of benzocaine (500 ppm), measured and weighed to obtain the following growth indexes:

1. Weight gain (g): final weight – initial weight;
2. Specific growth rate (% day⁻¹): $[(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{experiment days}] \times 100$; and
3. Apparent feed conversion ratio: diet supplied/weight gain.

2.2. Stable isotope analyses

For stable isotope analyses, samples were processed following Garcia et al. (2007). Briefly, muscle and feed samples were dried for 48 h at 60 °C to constant weight and ground with a mortar and pestle. Subsamples of 1 mg were placed into Ultra-Pure tin capsules (Costech Analytical, Valencia, California), and sent to the Analytical Chemistry Laboratory of the Institute of Ecology, University of Georgia, for analysis of carbon and nitrogen isotope ratios. Results were expressed in delta notation (parts per thousand deviation from a standard material): $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$, where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. The standard material for carbon was Pee Dee Belemnite (PDB) limestone, and the nitrogen standard was atmospheric nitrogen. Organic laboratory reference materials were run after every twelve samples and they were both accurate and precise (Spinach standard: $\delta^{13}\text{C}$ SD = 0.14‰ and $\delta^{15}\text{N}$ SD = 0.50‰; Bovine standard: $\delta^{13}\text{C}$ SD = 0.03‰ and $\delta^{15}\text{N}$ SD = 0.09‰).

Lipids were not removed from our samples prior to analysis, but because high lipid levels can drive carbon values in a negative direction (Logan et al., 2008), $\delta^{13}\text{C}$ of both the diet and croaker muscle was lipid-normalized (henceforth $\delta^{13}\text{C}_{\text{corr}}$). The C:N ratio is a proxy for lipid content, so we used the following equation in Logan et al. (2008): $\delta^{13}\text{C}_{\text{corr}} = \delta^{13}\text{C} - ((-2.976 * \ln(\text{C:N})) + 3.093)$.

2.3. Data analyses

Measures of fish productive performance (weight gain, apparent feed conversion ratio and specific growth rate) in each treatment were compared with an analysis of variance (ANOVA) and muscle lipid content (C:N ratio) with repeated measures ANOVA. Following the ANOVAs, if significant differences were detected, the Tukey test was applied to identify which treatments differed. In all cases, the significance level was fixed at 5% (Zar, 2010).

In order to model the isotopic change observed in muscle following the diet switch, a three parameter (3P) time-based exponential model was used to fit the isotope data ($\delta^{13}\text{C}$, $\delta^{13}\text{C}_{\text{corr}}$ and $\delta^{15}\text{N}$), following Hesslein et al. (1993) and Suzuki et al. (2005):

$$\delta X_t = \delta X_{\text{eq}} + (\delta X_i - \delta X_{\text{eq}}) \cdot e^{(\lambda \cdot t)}$$

where δX_t is the stable isotope ratio of fish muscle tissue t days after the start of the experiment, δX_i and δX_{eq} are the initial and asymptotic stable isotope ratios, respectively, and λ is the fractional rate of isotopic incorporation. This particular model accounts for both tissue addition and tissue replacement, because $|\lambda|$ is the sum of growth (k) and catabolism (c) (Warne et al. 2010). The values of λ and δX_{eq} with approximate standard errors were estimated for each treatment using the software JMP version 11. Treatment-specific growth rate constants (k) were determined by fitting exponential growth models to the available data ($W_f = W_i e^{k \cdot t}$), where W_i is initial weight and W_f is the final weight when sampled on day t . Any isotopic turnover in excess of what was attributable to growth was metabolic tissue replacement (c) according to $c = |\lambda| - k$.

In order to estimate the length of time required to reach the point at which 50% (half-life) and 95% (near complete) turnover of the tissue occurred, the following equation from Tieszen et al. (1983) was solved:

$$T = \ln(1 - (\alpha/100)) / \lambda$$

where T is the time in days and α is percent turnover. The trophic discrimination factor (TDF) was calculated for $\delta^{13}\text{C}$, $\delta^{13}\text{C}_{\text{corr}}$ and $\delta^{15}\text{N}$ in

two different ways: 1) TDF_{final} representing the difference between mean values for croakers at the end of the experiment (sampled on day 92) and the value of the diet, and 2) TDF_{asympt} was estimated by subtracting the value of the diet from the δX_{eq} value of the exponential model.

3. Results

The physical and chemical parameters of water during the experiment were maintained within the tolerance range for most teleost species used in aquaculture. Water conditions were similar among all treatments, with the exception of salinity and alkalinity (Table 2). Some individuals died during the experiment, but that did not affect sample sizes, since mortality rates were similar among treatments (16, 13 and 20 individuals in the freshwater, estuarine and marine treatment, respectively). Fish increased in weight during the 92 days of the experiment (Fig. 1A), with those reared under a salinity corresponding to an estuarine environment exhibiting the highest weight gain (58.14 ± 1.39 g), followed by the marine group (48.59 ± 3.56 g) and the freshwater group (33.11 ± 3.16 g), respectively ($F_{2,152} = 31.1$; $p < 0.01$) (Table 2). The same trend, with the estuarine treatment exhibiting highest values and the freshwater treatment the lowest, was observed for the specific growth rate (Table 2) and for the growth constant (k), this last one fitted with an exponential model (Fig. 1B). Croakers reared under freshwater salinities consumed more food in order to gain weight when compared to other treatments, as indicated by a higher feed conversion ratio (Table 2).

During the experiment there was an increase in muscle lipid content, indicated by the C:N ratio (day 0 C:N = 3.84 ± 0.05 ; day 92 C:N = 6.19 ± 0.73), but no significant difference was found among treatments ($p > 0.05$) (Fig. 2). Considering that at the end of the experiment croakers had a lipid concentration high enough to alter their isotope values, results are shown for both non- and lipid-corrected $\delta^{13}C$.

Isotopic turnover was clearly evident as muscle tissue of croakers in all salinity treatments reached an apparent steady state with the new diet after 92 days (Fig. 3). Half-life estimates were longer for both isotopes in the freshwater group ($\delta^{13}C = 25.6$ days, $\delta^{15}N_{corr} = 21.6$ days and $\delta^{15}N = 34.6$ days) compared to the estuarine ($T_{50}^{13}C = 18.7$ days, $T_{50}^{15}C_{corr} = 16.9$ days, $T_{50}^{15}N = 23.9$ days) and the marine treatments ($T_{50}^{13}C = 17.7$ days, $T_{50}^{15}C_{corr} = 16.1$ days, $T_{50}^{15}N = 22.3$ days) (Table 3). When considering the near complete turnover (T_{95}) values, croakers in freshwater require almost a month longer for carbon ($T_{95}^{13}C = 110.9$, $T_{95}^{15}C_{corr} = 93.6$ days) and nearly 50 days longer for nitrogen ($T_{95}^{15}N = 149.7$ days) to reach equilibrium, as opposed to those in estuarine ($T_{95}^{13}C = 80.9$ days, $T_{95}^{15}C_{corr} = 73.0$ days, $T_{95}^{15}N = 103.3$ days) or marine conditions ($T_{95}^{13}C = 76.8$ days, $T_{95}^{15}C_{corr} = 69.6$ days, $T_{95}^{15}N = 96.6$ days)

Table 2

Water quality parameters and growth performance (mean \pm SD) of whitemouth croakers (*Microptogonias furnieri*) reared under three different salinities. Significant differences among treatments in fish parameters are indicated by different superscripts (ANOVA, $p < 0.05$).

Parameter	Treatments		
	Freshwater	Estuarine	Marine
<i>Water quality</i>			
Temperature (°C)	26.64 \pm 0.91	26.76 \pm 0.98	26.90 \pm 0.92
Salinity	2.32 \pm 1.16	15.20 \pm 1.06	27.55 \pm 1.79
pH	7.70 \pm 0.29	7.98 \pm 0.16	8.09 \pm 0.11
Alkalinity (mg CaCO ₃ mL ⁻¹)	43.15 \pm 8.23	105.06 \pm 14.13	157.71 \pm 9.91
TAN (mg·L ⁻¹)	0.06 \pm 0.03	0.06 \pm 0.03	0.08 \pm 0.03
Nitrite (NO ₂ -N mg·L ⁻¹)	0.13 \pm 0.30	0.02 \pm 0.01	0.06 \pm 0.02
<i>Growth performance</i>			
Final average weight (g)	42.86 \pm 7.83	67.65 \pm 12.67	57.95 \pm 14.65
Weight gain (g)	33.11 \pm 3.16 ^a	58.14 \pm 1.39 ^b	48.59 \pm 3.56 ^c
Specific growth rate (% day ⁻¹)	1.67 \pm 0.08 ^b	2.19 \pm 0.07 ^a	2.01 \pm 0.10 ^a
Feed conversion rate	2.14 \pm 0.31 ^b	1.49 \pm 0.13 ^a	1.79 \pm 0.30 ^{ab}

(Table 3). In all three treatments, nitrogen turnover appeared to be slower than carbon turnover (half-lives of 22 to 35 days for nitrogen vs. 16 to 26 days for carbon).

Both growth and catabolism contributed to isotopic change. Overall, muscle carbon turnover (λ) was mainly driven by catabolism (c) as $c > k$ in all cases except for the non-corrected values in freshwater (Table 3). Conversely, growth was the main factor accounting for nitrogen turnover in all three salinity treatments ($k > c$, Table 3).

Trophic discrimination factors (TDFs) calculated from individuals sampled at the end of the experiment (TDF_{final}) were most consistent among treatments for $\delta^{15}N$, ranging from 3.42 to 3.89‰, whereas for both $\delta^{13}C$ (from 0.82 to 1.44‰) and $\delta^{13}C_{corr}$ (from -0.41 to 0.02‰) the TDF_{final} was similar between fresh and estuarine waters, but higher in marine (Table 3). Discrimination factors calculated from δX_{eq} (TDF_{asympt}) differed from TDF_{final} , but exhibited the same pattern for all isotopes, with lowest values being found in freshwater, intermediate in estuarine waters and highest in the marine treatment (Table 3).

4. Discussion

The unique feature of this study was the determination of isotopic turnover rates and discrimination factors for a species raised in different salinity conditions. Usually, these parameters are calculated by rearing the species of interest in similar environmental conditions, hence corresponding to only one type of aquatic environment, even when the species is known to migrate throughout its life cycle (e.g. Buchheister and Latour, 2010). This study demonstrated that differences in salinity affected both growth and metabolic turnover of the euryhaline whitemouth croaker and led to different half-life estimates in muscle. Turnover rates for carbon and nitrogen were similar between estuarine and marine environments, but considerably slower in freshwater. This finding has implications for ecologists and fisheries scientists who have been using stable isotopes to determine residence time, movement patterns and the role of fishes as biological vectors transporting nutrients between freshwater and estuarine ecosystems (Jardine et al., 2012; Kline et al., 1990; MacAvoy et al., 2000; Naiman et al., 2002; Oliveira et al., 2014), without taking into account the salinity effect in isotope turnover rates of these migrant animals.

Salinity is one of the most important abiotic factors in aquatic environments and its optimal levels are species-specific for survival and production efficiency, with euryhaline species almost always exhibiting highest growth rates in intermediate salinity conditions (estuarine waters) (Boeuf and Payan, 2001; De Silva and Perera, 1976; Fazio et al., 2013). Results from the current study corroborate this observation, as croakers reared under estuarine waters exhibited higher specific growth rates than those in other salinities. Whitemouth croakers can move through a wide range of salinities and acclimation to these new environmental conditions is often associated with an increase in energy demands, but there is not much agreement concerning the magnitude of these costs and some experiments indicate that the true energetic demands of osmoregulatory processes may constitute less than 10% of the total energy budget (Boeuf and Payan, 2001; Kidder et al., 2006; Morgan and Iwama, 1991). Because the rate of isotopic turnover depends on both the growth rate and the degree of metabolic turnover, it was expected that this rate would differ among environments, being greatest in isosmotic waters. While it was found that muscle turnover was indeed faster in the estuarine treatment compared with freshwater, turnover was surprisingly fast in the marine salinity treatment, slightly exceeding that of the estuarine treatment.

Given these points, the results support the hypothesis that muscle isotopes in freshwater indeed take longer to turn over. Since this isotopic incorporation rate was not fastest in the isosmotic salinity (estuarine treatment) and metabolic rates were not directly measured, these turnover differences among treatments may not be exclusively associated with osmoregulation. This is not the first study in which salinity close to the isosmotic zone did not provide metabolic advantages (Morgan

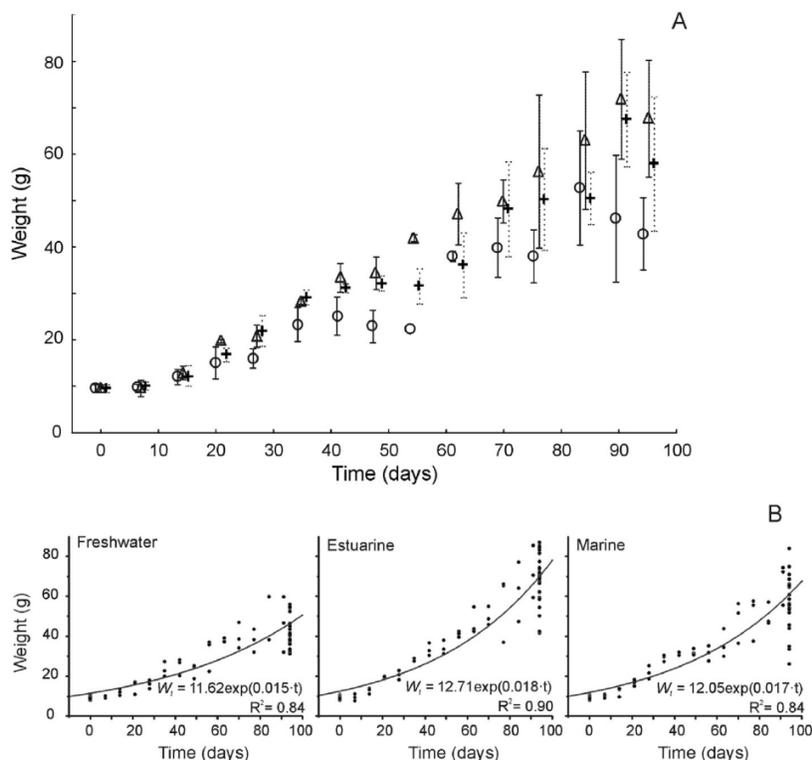


Fig. 1. A) Average (\pm SD) change in whitemouth croakers' (*Micropogonias furnieri*) weight over time (days) in captivity. Circles = freshwater group; triangles = estuarine group; and black cross = marine group; B) exponential growth curves represent the growth of each treatment (gray line) with best-fit equations and coefficients of determination (R^2).

and Iwama, 1991), despite the belief it would give the lowest energetic demands for maintaining ion balance. Hence, modifications in food intake and hormonal changes, among other possibilities induced by environmental salinity, must play a role along with osmoregulation (Laiz-Carrión et al., 2005; Vargas-Chacoff et al., 2011).

To date, most diet switch studies with fishes have concluded that growth was primarily responsible for isotopic changes in different tissues (Bosley et al., 2002; Carleton and Martínez del Río, 2010; Herzka and Holt, 2000; Hesslein et al., 1993; Maruyama et al., 2001; Perga

and Gerdeux 2005) and few have demonstrated a strong role for catabolism (Tarboush et al., 2006; Xia et al., 2013). Growth is expected to dominate turnover in small, young and active animals, characteristics present in the majority of turnover experiment models. Though the experimental croakers also had these features, results suggested that catabolism contributed substantially to carbon turnover in muscle, while growth was the main cause of nitrogen turnover. Some may attribute higher growth rates and better food conversion ratios to uncontrolled factors, such as spontaneous activity and swimming performance rather than to changes in metabolic rate (Boeuf and Payan, 2001). Although not measured quantitatively, visual observations indicated similar behavior of croakers among tanks, suggesting that differences in turnover rates among environments may indeed be a consequence of changes in metabolic rates following salinity adaptation demands. Overall, while metabolic rate does not dictate growth, it is coadjusted with it (Glazier, 2015) and these different patterns of growth and catabolism for a single species in different environmental salinities could confound interpretation of field isotopic data.

As previously stated, turnover rates not only differed between environments but also between isotopes within treatments, with carbon always exhibiting faster turnover than nitrogen. Other studies have also documented distinct turnover of isotopes in some tissues, highlighting that muscle N turnover times are usually slower than C to track changes in diet (Buchheister and Latour, 2010; Guelinckx et al., 2007; MacNeil et al., 2006). Many reasons may be responsible for this disparity, and these may not be mutually exclusive. Carbon and nitrogen have different metabolic routing, hence being allocated differentially to specific tissues and tissue components (Boecklen et al., 2011; Guelinckx et al., 2007). While N is used only to build proteins, carbon present in the diet is used in proteins, lipids and carbohydrates. Therefore, the higher demand for carbon could cause more rapid turnover for this element,

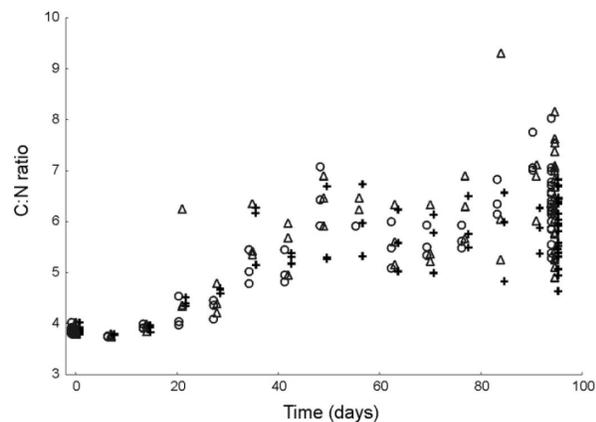


Fig. 2. Muscle C:N ratio versus time in a controlled diet-shift experiment with whitemouth croakers (*Micropogonias furnieri*). Circles = freshwater group; triangles = estuarine group; and black cross = marine group.

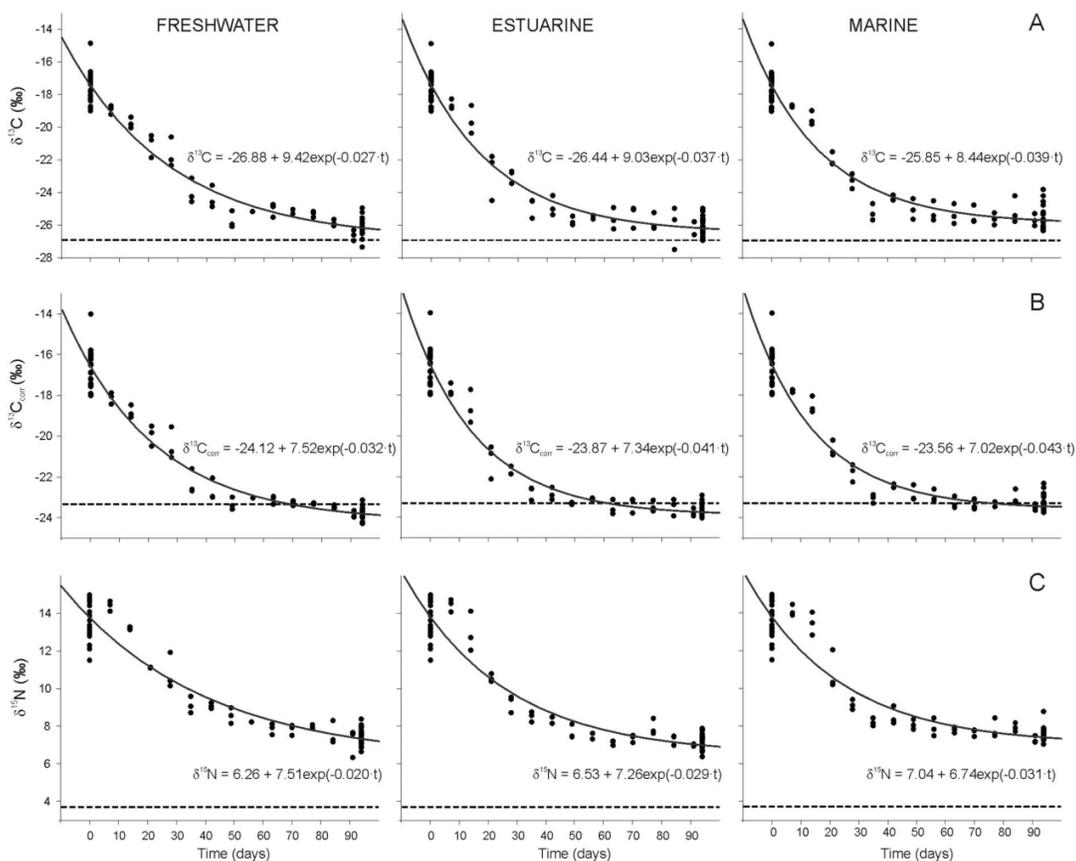


Fig. 3. Isotopic change in muscle tissue of whitemouth croakers (*Micropogonias furnieri*). $\delta^{13}\text{C}$ (A), $\delta^{13}\text{C}_{\text{corr}}$ (B) and $\delta^{15}\text{N}$ (C) are shown as a function of time (days) after the beginning of isotopically distinct diet switch. Solid lines correspond to exponential model fits as a function of growth and metabolic turnover together, for fish in a freshwater (left column), estuarine (center column) and saltwater (right column) systems. The horizontal broken line indicates the mean isotope value of diet supplied during the experiment.

when compared to nitrogen. Turnover differences between elements may also be a function of food quality (e.g. amount of protein vs. fiber) and availability (e.g. ad libitum vs. starvation) (Braun et al., 2013; Mirón et al., 2006). Altogether, the interaction between metabolism, growth and processes such as routing must be better understood to clarify how incorporation and elimination phases differ among elements and tissues (MacNeil et al., 2006).

Biological incorporation and elimination are generally unidirectional, consisting of a number of steps with potential for discrimination, where kinetic reactions usually dominate over isotope exchange effects (Kendall and Caldwell, 1998; Michener and Lajtha, 2007). This is one of the reasons why discrimination factors are usually determined after reactions reach equilibrium, because until this point lighter isotopes may be preferentially concentrated in the products (White, 2013). Since biological processes are examples of open-systems, a perfect steady-state is never achieved and TDFs may also be calculated in the absence of marked changes in dietary isotope ratios or when those changes are too small (MacNeil et al., 2006; Schoeller, 1999). Because only some of the treatments achieved equilibrium between consumer tissue and their diet, trophic discrimination is presented in two ways, one calculated from animals sampled at the end of the experiment ($\text{TDF}_{\text{final}}$) and the other from asymptotes derived from turnover equations ($\text{TDF}_{\text{asympt}}$). As expected, $\text{TDF}_{\text{asympt}}$ results were all lower than $\text{TDF}_{\text{final}}$ (by approximately 0.5‰), and they differed even for treatments that had reached a practical steady-state (T_{95}), such as the marine system. Despite this discrepancy, by either method, TDFs calculated from this study were consistent with previous review studies. When considering

the $\text{TDF}_{\text{final}}$ values for both carbon and nitrogen isotopes in all salinity treatments fell within the range proposed by Post (2002) [$\Delta^{13}\text{C} = 0.4\text{‰}$ (1SD = 1.3‰) and $\Delta^{15}\text{N} = 3.4\text{‰}$ (1SD = 1‰)]. As for $\text{TDF}_{\text{asympt}}$ values, $\Delta^{13}\text{C}$ in all treatments and $\Delta^{15}\text{N}$ in the marine treatment were still in this range (Post, 2002), whereas $\Delta^{15}\text{N}$ in the freshwater and estuarine treatment were more similar to lower values summarized by McCutchan et al. (2003) and Vanderklift and Ponsard (2003).

Potential effects of environment factors on isotopic discrimination, such as the differences observed in TDF values among fresh, estuarine and marine waters, often have not been taken into account. One review that considered 66 TDF studies in different groups of animals identified a significant environment effect on carbon discrimination, with organisms inhabiting freshwater presenting higher averages ($\Delta^{13}\text{C} = 1.33\text{‰}$, SE = 0.07) when compared to saltwater ones ($\Delta^{13}\text{C} = 0.96\text{‰}$, SE = 0.18) (Caut et al., 2009). Yet when analyzing fishes separately in that review, there was no significant effect of the environment on carbon or nitrogen TDFs. Although TDFs in the present study are the opposite of those identified by Caut et al. (2009), with values for croakers in marine waters being higher compared with estuarine and freshwater, it can be suggested that they are more accurate, as results were calculated from a single experiment with only one species and not obtained through the grouping of previous studies with different animals that likely have different salinity optima. The magnitude of this isotope discrimination can be affected by many factors, including nutritional stress, different digestibility of diet components or its protein content (Codron et al., 2011; Gaye-Siessegger et al., 2004; Hobson et al., 1993; Mill et al., 2007). Even though the experimental diet was formulated to

Table 3
Parameter estimates (95% confidence intervals) for time-based exponential fit models, stable isotopes ($\delta^{13}\text{C}$, $\delta^{13}\text{C}_{\text{corr}}$, $\delta^{15}\text{N}$) half-lives (T_{50}), near complete turnover (T_{95}) and trophic discrimination factor (TDF) for whitemouth croakers (*Micropogonias furnieri*) muscle reared under different salinities.

Parameters	$\delta^{13}\text{C}$ (‰)				$\delta^{13}\text{C}_{\text{corr}}$ (‰)				$\delta^{15}\text{N}$ (‰)			
	Freshwater	Estuarine	Marine	Marine	Freshwater	Estuarine	Marine	Marine	Freshwater	Estuarine	Marine	Marine
a	-26.88 (-27.45, -26.30)	-26.44 (-26.83, -26.04)	-25.85 (-26.22, -25.50)	-23.87 (-24.14, -23.61)	-24.12 (-24.49, -23.75)	-23.87 (-24.14, -23.61)	-23.56 (-23.82, -23.29)	-23.56 (-23.82, -23.29)	6.76 (5.26, 7.26)	6.53 (5.98, 7.08)	7.04 (6.53, 7.55)	7.04 (6.53, 7.55)
b	9.42 (8.83, 10.01)	9.03 (8.56, 9.50)	8.44 (8.00, 8.90)	7.34 (7.00, 7.69)	7.52 (7.13, 7.95)	7.34 (7.00, 7.69)	7.02 (6.67, 7.37)	7.02 (6.67, 7.37)	7.51 (6.55, 8.48)	7.26 (6.68, 7.84)	6.74 (6.19, 7.30)	6.74 (6.19, 7.30)
λ	-0.027 (-0.032, -0.022)	-0.037 (-0.044, -0.030)	-0.039 (-0.046, -0.032)	-0.041 (-0.048, -0.034)	-0.032 (-0.037, -0.026)	-0.041 (-0.048, -0.034)	-0.043 (-0.050, -0.035)	-0.043 (-0.050, -0.035)	-0.020 (-0.027, -0.014)	-0.029 (-0.036, -0.021)	-0.031 (-0.039, -0.023)	-0.031 (-0.039, -0.023)
r^2	0.96	0.95	0.95	0.96	0.96	0.96	0.95	0.95	0.93	0.93	0.91	0.91
k	0.015	0.018	0.017	0.018	0.015	0.018	0.017	0.017	0.015	0.018	0.017	0.017
k^2	0.84	0.90	0.84	0.90	0.84	0.90	0.84	0.84	0.84	0.90	0.84	0.84
c	0.012	0.019	0.022	0.023	0.017	0.023	0.026	0.026	0.005	0.011	0.014	0.014
T_{50} (days)	25.6	18.7	17.7	16.9	21.6	16.9	16.1	16.1	34.6	23.9	22.3	22.3
T_{95} (days)	110.9	80.9	76.8	73.0	93.6	73.0	69.6	69.6	149.7	103.3	96.6	96.6
TDF _{final}	0.82 ± 0.54	0.88 ± 0.58	1.44 ± 0.66	-0.32 ± 0.30	-0.41 ± 0.28	-0.32 ± 0.30	0.02 ± 0.37	0.02 ± 0.37	3.81 ± 0.45	3.42 ± 0.42	3.89 ± 0.33	3.89 ± 0.33
TDF _{asympt}	0.02	0.46	1.05	-0.56	-0.81	-0.56	-0.25	-0.25	2.55	2.82	3.33	3.33

specifically meet the nutritional requirements of croakers and also to be attractive, palatable and isotopically depleted, it still likely had a different biochemical composition from the croaker's natural diet. Therefore, there may exist a slight difference between TDFs from the experiment and values under natural conditions. All this emphasizes that the discrimination factor is not a constant and the use of standard values may bias ecological results, because dietary mixing models, one of the most common tools to analyze data and infer diet composition, are highly sensitive to TDF variation (Bond and Diamond, 2011).

In conclusion, this study demonstrated that for a single fish species, both turnover rates and isotopic discrimination factors differ not only between isotopes, but also among environments of distinct salinities. When considering ecological studies using the isotope approach and fishes as proxies, species-specific values are a better alternative to TDF values from published reviews, as these are rarely determined from the organism in question or available for closely related species, and usually have very large error. Information obtained in this study improves applications of stable isotopes to quantify material transport in and between food webs, essential for inferring habitat use, residency and migration patterns in euryhaline species. The authors reinforce Martínez del Rio et al. (2009) call for more laboratory experiments, also focusing on physiology and behavior, which may provide a more thorough understanding of the main processes driving isotopic changes in different species and environments and, thereby, facilitating ecological inferences.

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