

**PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE BIOCÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA
DOUTORADO**

RODRIGO FERREIRA BASTOS

**USO DE ISÓTOPOS ESTÁVEIS DE CARBONO (^{12}C , ^{13}C) E
NITROGÊNIO (^{14}N , ^{15}N) COMO INDICADORES DO FLUXO
DE MATÉRIA E ESTRUTURA TRÓFICA EM AMBIENTES
AQUÁTICOS: PROPOSTAS METODOLÓGICAS PARA A
SUPERAÇÃO DE PRESSUPOSTOS E LIMITAÇÕES DE
TÉCNICAS ANALÍTICAS.**

Prof. Dr. Nelson Ferreira Fontoura

Orientador

Porto Alegre

2016

**PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE BIOCÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA**

**USO DE ISÓTOPOS ESTÁVEIS DE CARBONO (^{12}C , ^{13}C) E NITROGÊNIO (^{14}N , ^{15}N)
COMO INDICADORES DO FLUXO DE MATÉRIA E ESTRUTURA TRÓFICA EM
AMBIENTES AQUÁTICOS: PROPOSTAS METODOLÓGICAS PARA A
SUPERAÇÃO DE PRESSUPOSTOS E LIMITAÇÕES DAS TÉCNICAS
ANALÍTICAS.**

Autor: Rodrigo Ferreira Bastos

Orientador: Dr. Nelson Ferreira Fontoura

Co-orientadores: Dr. Alexandre Miranda Garcia

Dr. Kirk O Winemiller

**TESE DE DOUTORADO
PORTO ALEGRE – RS – BRASIL**

2016

SUMÁRIO

DEDICATÓRIA.....	iv
AGRADECIMENTOS	v
RESUMO	vii
ABSTRACT	viii
APRESENTAÇÃO.....	9
CAPÍTULO 1	24
SUMMARY	26
INTRODUCTION.....	28
METHODS.....	31
STUDY AREA AND SAMPLING DESIGN	31
DATA ANALYSIS	35
RESULTS.....	39
DISCUSSION.....	57
ACKNOWLEDGMENTS	65
REFERENCES	66
CAPÍTULO 2	73

SUMMARY	75
INTRODUCTION	77
MATERIALS AND METHODS	79
FIELD COLLECTIONS AND SAMPLE PROCESSING	79
ESTIMATES OF INGESTED RESOURCES	81
EFFECTS OF TDFS ON ESTIMATES OF FOOD ASSIMILATION AND TROPHIC POSITION	82
RESULTS	86
INGESTED RESOURCES	86
SPATIAL VARIATION OF $\delta^{15}\text{N}$	90
EFFECT OF TDFS ON MIXING MODEL ESTIMATES	92
EFFECTS OF TDFS ON TROPHIC POSITION ESTIMATES	94
DISCUSSION.....	97
ACKNOWLEDGEMENTS	100
DATA ACCESSIBILITY	100
REFERENCES	101
CONCLUSÕES	106
REFERÊNCIAS BIBLIOGRÁFICAS	109

*Dedicado às minhas avós: Dona Alaídes e Dona Nita
Sempre zelosas, de onde quer que estejam*

AGRADECIMENTOS

Aos meus pais, Jorge e Madalena, e madrasta, Adriana, sou grato por me incentivarem, pelo exemplo, desde cedo aos estudos, por acreditarem e apoiarem as minhas escolhas profissionais e, acima de tudo, pelo amor incondicional;

À minha ficante, namorada, namorada e esposa Raquel, pela presença, suporte, companheirismo e muito amor;

À minha sogra, Rosana, pelo exemplo e incentivo ao enobrecimento pela leitura, e claro pelo amor maternal.

Ao meu orientador, professor Dr. Nelson Ferreira Fontoura pela parceria iniciada com o doutorado, pelo exemplo de professor, pesquisador e de como encher a formação de um Doutor, dando liberdade para desenvolvimento intelectual do aluno, mas atencioso nos momentos adequados;

Ao meu co-orientador, orientador durante a iniciação científica e o mestrado, e amigo, professor Dr. Alexandre Miranda Garcia, pelo exemplo de professor e pesquisador, sempre atento ao desenvolvimento desta tese e por proporcionar a oportunidade de escrever e submeter os projetos de financiamento, por manter as portas abertas do laboratório de Ictiologia da Universidade Federal do Rio Grande para o desenvolvimento destes e outros trabalhos, por incentivar e bater na porta para o período sanduíche na Texas A&M.

Ao meu co-orientador durante o período sanduíche, professor PhD. Kirk O. Winemiller, pelo exemplo de professor e pesquisador e pelo pronto atendimento sempre que preciso durante o sanduíche.

Ao professor Dr. João Paes Vieira Sobrinho pela parceria ao longo da última década, pela preocupação e por também influenciar na minha formação;

Aos professores Dr. Júlio Cesar Bicca-Marques, Dr. Roberto Esser dos Reis e Dra. Sandra Maria Hartz pela colaboração no desenvolvimento da qualificação;

À professora Dra. Sandra Maria Hartz pela colaboração no desenvolvimento da qualificação e pela oportunidade da tentativa de continuidade das pesquisas no pós doc;

Aos amigos do Laboratório de Ecologia Aquática, Gian, Zé, Zi, Thaís e Cibele, pelas indaiadas cortando o lago Guaíba a bordo do caveirão e pela companhia e troca de experiências no laboratório;

Aos velhos e novos, antigos e recentes, amigos do laboratório de Ictiologia da FURG, MVC, Mauro, Lameiro, Fabiano, Mauricio, Renatinha, Malu, Sônia, Valéria, Débora, Ana e

Burns, pelos inúmeros momentos de ócio produtivo, triagens que não acabavam, coletas fantasmas e discussões pertinentes acerca de qualquer coisa e Paula, Cindy, Gabriel e Kerolen pelas ajudas nas coletas e triagens, além da oportunidade de (s) orienta-los;

Aos órgãos financiadores, CNPq, pela bolsa de doutorado (140474/2012-9) e pelo financiamento de parte do capítulo 1 e 2, International foundation for Science pelo financiamento de parte do capítulo 2, Fundação o Boticário de proteção a Natureza, pelo financiamento de parte do capítulo 1; CAPES pela bolsa de doutorado sanduíche (BEX 9936/14-9)

As equipes da Estação Ecológica do Taim – ICMBio, por sempre estarem dispostos a ajudar, e a equipe do Parque Nacional da lagoa do peixe.

RESUMO

Uma questão central no entendimento da ecologia aquática é a origem e o fluxo de matéria e energia nas teias alimentares. Diversas hipóteses e teorias tentam explicar (1) os diferentes níveis de contribuição da produção nos ambientes terrestres (2) os diferentes comprimentos de cadeia trófica entre diferentes porções e diferentes tipos de ambientes aquáticos. Há cerca de 60 anos, as razões isotópicas dos componentes das tramas tróficas, principalmente carbono ($\delta^{13}\text{C}$) e nitrogênio ($\delta^{15}\text{N}$), tem sido empregadas para identificar e quantificar as relações entre eles. O carbono proporciona a identificação das fontes de matéria, pois sua razão isotópica muda pouco entre o consumidor e o recurso. Já o nitrogênio propicia a oportunidade de mensuração das posições tróficas dos consumidores, uma vez que sua razão isotópica muda de maneira similar entre um nível trófico e o subsequente. Desde então, diversas técnicas analíticas emergiram para a superação dos pressupostos e limitações no uso dos isótopos estáveis. Pelo menos dois fatores chave são identificados para estimar posições tróficas, traçar a assimilação de recursos e comparar estes em diferentes situações: (1) a consideração dos valores isotópicos na base das cadeias, ou *baselines*, e (2) os fatores de discriminação trófica (FDT), ou seja, a mudança que ocorre nas razões isotópicas entre uma fonte de matéria e o organismo que consome esta. No primeiro capítulo a estrutura trófica de uma lagoa e um banhado na Estação Ecológica do TAIM foi acompanhada durante o período seco (verão) e o de inundação (inverno). A hipótese de que a lagoa, por ser um ambiente maior e mais estável que o banhado apresentaria uma (1) complexidade trófica maior, e que o banhado, por ser menor e mais instável (2) apresentaria maior relação com material terrestre, principalmente no inverno foi trabalhada. Além disso, é feita a proposição do uso da posição trófica (PT) em substituição ao $\delta^{15}\text{N}$ para as medidas verticais das teias alimentares, pois assim é considerado o *baseline* de cada situação comparada. A estrutura trófica do banhado foi mais simples do que a da lagoa, e foi mais ligada ao material terrestre durante o inverno, período em que os comprimentos das cadeias tróficas foram mais elevados, corroborando hipóteses relacionadas ao tamanho, pulso hidrológico e estabilidade dos ambientes. No segundo capítulo, populações do barrigudinho *Jenynsia multidentata*, um consumidor omnívoro, foram estudadas ao longo de um gradiente estuarino para avaliar o efeito do uso de diferentes valores de FDT, incluindo os associados com dietas específicas (carnívora e herbívora) sobre as estimativas de assimilação e PT. Ademais, um novo método para estimativa de PT de consumidores sujeitos a diferentes fontes de variação nos FDT, foi proposto. O novo método (TP_{PA}) considera a proporção de assimilação de cada fonte alimentar e o FDT associado a esta, sendo o valor proporcional de assimilação usado como peso do FDT de cada fonte para o FDT final. Os resultados mostraram que grande parte do material vegetal consumido não foi assimilado e assim, mesmo quando animais foram menos ingeridos, estes foram o recurso mais assimilado. Além disso, o método proposto foi o mais adequado para traçar a assimilação e a PT revelando que o uso de médias amplamente usadas podem não ser adequadas quando o consumidor apresenta diferentes tipos de dieta. Os resultados aqui obtidos, além de contribuir para um maior conhecimento acerca da estrutura e funcionamento de áreas de preservação globalmente importantes e presentes no estado do Rio Grande do Sul, colaboram para a melhoria das técnicas analíticas envolvendo isótopos estáveis como traçadores de matéria ao longo das tramas tróficas.

Palavras-chave: assimilação, fracionamento, pulso hidrológico, trama trófica, SIAR, SIBER, nicho trófico.

ABSTRACT

One of the key stones of aquatic ecology field is the understanding on the origin and flow of energy and matter along food webs. Several hypotheses and theories try to explain (1) the differential contribution levels of production in terrestrial habitats and (2) the differential trophic chain lengths between different parts and types of aquatic habitats. About 60 years ago, the isotopic ratios of the components of trophic webs, mainly carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), has been used to identify and quantify the relationships between them. Carbon provides the identification of sources of matter because its isotope ratio almost no change between the consumer and the resource. On the other hand, the nitrogen provides the opportunity to measure the trophic position of consumers, since their isotopic ratio changes in a similar way between subsequent trophic levels. Since then, various analytical techniques have been emerged to overcome the assumptions and limitations on the use of stable isotopes. At least two key factors are identified for estimating trophic positions, to trace the assimilation of resources and to compare these in different situations: (1) the consideration of isotopic values on the baseline of the food webs, and (2) the trophic discrimination factors (TDF), or the change which occurs in the isotope ratios between a source and the organism that consumes it. In the first chapter, the trophic structure of a lake and a wetland at Taim Ecological Station was evaluated during the dry (summer) and wet (winter) seasons. The hypothesis are that the lake, a larger and more stable habitat, will present (1) a larger trophic complexity whereas the wetland, being smaller and more instable will present (2) greater relationship with terrestrial sources, especially during flood period. In addition, the proposition of the use of trophic position (PT) replacing $\delta^{15}\text{N}$ as proxy for vertical dimension on the isotopic space metrics, was made in order to consider differences in baselines between situations. The trophic structure of the wetland was simpler than the lake, and was more related to terrestrial material during flood, as well as the trophic length was higher during flood, confirming those hypotheses related to the size, hydrological pulse and stable environments. In the second chapter, populations of livebearer *Jenynsia multidentata*, an omnivore consumer, were studied over a estuarine gradient to evaluate the effect of using different values of TDF, including those associated with specific diets (carnivore and herbivore), on the assimilation and PT estimates. Furthermore, a new method for TP estimation of consumers subject to different sources of variation in the FDT was proposed. The new method (TP_{PA}) takes the proportion of food assimilation estimates for each source and its associated TDF, with this being the proportional value used to give weight for TDFs of each source to the final TDS. The results indicated that most of the plant material consumed was not assimilated, and thus even when animals were less ingested, these were the most assimilated resource. Furthermore, the proposed method was suitable to trace the assimilation and reveal that the use of widely used means of TDFs may not be suitable when the consumer presents different types of diet. The results obtained, contribute to improve the knowledge about the structure and functioning of globally important conservation areas present in the Rio Grande do Sul state and also seem to contribute to the development of analytical techniques involving stable isotopes as tracers along the food webs.

Keywords: assimilation, fractionation, flood pulse, food web, SIAR, SIBER, trophic niche

APRESENTAÇÃO

Um pré-requisito fundamental para a compreensão da estrutura e dinâmica de comunidades biológicas é o estudo dos efeitos diretos e indiretos das interações entre espécies na trama trófica (Polis & Winemiller 1996). As teias tróficas representam as relações e interações entre as comunidades, proporcionando uma descrição holística e detalhada dos ecossistemas (Winemiller & Polis 1996). Considerando isso, o conhecimento da dinâmica trófica é uma abordagem consistente para avaliar os processos interativos das comunidades aquáticas (Winemiller 1989).

Diferenças na estruturação espacial e temporal de ecossistemas, na morfologia de indivíduos ao longo do desenvolvimento ontogenético, nos processos fisiológicos de assimilação, assim como em aspectos comportamentais relacionados à seleção de presa, podem trazer elementos adicionais de complexidade para a análise da dinâmica trófica. Hábitos alimentares distintos dentro de uma mesma espécie são frequentemente encontrados conforme os estágios de desenvolvimento dos indivíduos (unidades tróficas ontogenéticas), decorrentes das diferenças na demanda energética e nas limitações morfológicas, implicando em dietas diferenciadas durante o desenvolvimento destas (Winemiller 1989; Hahn, Pavanelli & Okada 2000; Abelha, Agostinho & Goulart 2001; Fontoura *et al.* 2015). Cada um dos grandes grupos tróficos de peixes (herbívoros, detritívoros, omnívoros, invertívoros e piscívoros) forma um compartimento importante das teias tróficas aquáticas. A estimativa do nível trófico de cada espécie depende de um balanço entre a quantidade e o nível trófico dos recursos consumidos. Por exemplo, entre os omnívoros podem existir grandes diferenças de nível trófico, dependendo do

quanto de cada diferente recurso (vegetal/animal) é consumido e posteriormente assimilado.

A distribuição dos organismos, recursos e processos biológicos alteram-se ao longo dos gradientes ambientais que ocorrem naturalmente nos ambientes aquáticos. Em ambientes lóticos, tais como rios, a mudança ocorre normalmente ao longo do gradiente longitudinal e altitudinal, enquanto que nos ambientes lênticos, o gradiente mais comum, é o de profundidade. A produção de qualquer sistema biológico tem como componente biológico primário os produtores que sustentam todos os níveis tróficos posteriores (consumidores). Diferentes produtores podem sustentar cada uma destas componentes da trama trófica. Se os recursos alimentares utilizados são diferentes, as vias de entrada de energia podem ser diferentes. A identificação e generalização de padrões gerais é um desafio, visto que a interação com processos em larga escala (e.g. clima, hidrologia, geomorfologia), bem como em escala local (e.g. microhabitats, heterogeneidade ambiental, interações biológicas) interferem nos padrões potencialmente identificáveis.

A identificação e proposição de padrões gerais para rios foi introduzida através do “River Continuum Concept” (RCC) ou a teoria do rio contínuo (Vannote *et al.* 1980). A teoria propõe um modelo geral de mudanças nas comunidades biológicas aquáticas e fontes de matéria orgânica ao longo do gradiente longitudinal de rios, com base nas mudanças físicas e químicas e das suas relações com o ambiente terrestre. De modo geral, o conceito prediz que em pequenos riachos, tipicamente em áreas de cabeceira, que são sombreados pela mata ciliar, a entrada de matéria orgânica se dá principalmente pelo folhiço terrestre (Material Orgânico Particulado Grosseiro - MOPG) que excede em muito a produção primária local, limitada pelo sombreamento,

sendo a heterotrofia dominante, ou seja, a respiração é maior do que a produção ($P/R < 1$). Movendo-se a jusante, os rios aumentam em largura e descarga, e a importância da vegetação ciliar (MOPG e efeito de sombreamento) diminui, levando ao domínio da autotrofia ($P/R > 1$). Em trechos de maior porte (profundidade e largura), há um aumento na turbidez devido às partículas finas provenientes de trechos de montante, o que limita a produção local pela pouca quantidade de luz e o sistema reverte para predominantemente heterotrófico ($P/R < 1$), já que a matéria orgânica proveniente de trechos rio acima é abundante e disponível (Vannote *et al.* 1980).

Entretanto, apesar da “Teoria do Rio Contínuo” ser amplamente citada e aceita, existem algumas críticas a respeito da sua generalização. O RCC foi basicamente centrado em estudos de rios de baixa ordem (pequenos e médios) de regiões temperadas, com densa cobertura de mata ciliar nas cabeceiras e com forte regulação (barragens) e ocupação de terra. Ocorre que os rios de maior ordem, principalmente aqueles não regulados, possuem variabilidade de nível e possuem planícies adjacentes que frequentemente sofrem inundação e conectam os ambientes terrestres com o corpo de água principal, muitas vezes formando lagoas marginais ou aumentando o nível e tamanho de lagoas já existentes. Assim, durante o primeiro “Large River Symposium” ocorrido em 1986 em Toronto, Canadá, as discussões sobre a aplicabilidade do RCC para sistemas com planície de inundação levaram a formulação do “Flood Pulse Concept” (FPC) ou “Teoria do Pulso de Inundação” (Junk, Bayley & Sparks 1989). O FPC se baseia na conectividade existente entre o ambiente terrestre e aquático durante os períodos ou locais em que o rio inunda as planícies adjacentes, acrescentando um fator lateral de fluxo de matéria (Junk *et al.* 1989) ao modelo longitudinal proposto no RCC, onde a entrada lateral ocorre apenas nas cabeceiras (Vannote *et al.* 1980). Além

desses dois conceitos, um terceiro modelo de fluxo de matéria e energia foi ainda proposto. O “Riverine Productivity Model” (RPM) (Thorp & DeLong 1994) prediz que a produção autóctone (fitoplâncton) no canal do rio é a principal fonte de carbono orgânico usado pelos animais em trechos à jusante.

De maneira geral, as teorias de fluxo de matéria propostas e difundidas para rios podem ser aplicadas para ambientes lênticos, considerando suas particularidades. Por exemplo, na teoria do rio contínuo (Vannote *et al.* 1980) as mudanças previstas basicamente propõem uma relação inversa entre a largura do rio e a entrada de material terrestre. Extrapolando o conceito para ambientes lênticos, tais como lagoas e banhados, espera-se também que quanto maior a razão entre a área de contato (perímetro) e o volume do corpo de água, maior pode ser a importância de material terrestre. A teoria do pulso de inundação (Junk *et al.* 1989) é também claramente aplicável à ambientes lênticos, desde que estes estejam sujeitos a variações de nível de água e estas impliquem em variações na conectividade com ambientes adjacentes. Da mesma maneira, O “Riverine Productivity Model” (Thorp & DeLong 1994) prediria que a produção autóctone (fitoplâncton) de uma lagoa é a principal fonte de carbono orgânico usado pelos animais na região pelágica, distante das fontes do litoral. Evidentemente, o aumento da conectividade com ambientes terrestres não garante a importância destes para os ambientes aquáticos. A disponibilidade e, principalmente, a qualidade das fontes terrestres, além da capacidade específica dos consumidores em explorar estas novas fontes, são fatores que vão determinar se a conectividade física se traduz em conectividade trófica (Hoeinghaus *et al.* 2011; Abrantes *et al.* 2013).

Dessa maneira, ambientes costeiros rasos, tais como lagoas e banhados, nos quais a luz pode facilmente chegar ao fundo, podem apresentar a predominância tanto

de macrófitas quanto de algas, dependendo de fatores locais condicionantes e promotores da abundância de um ou de outro tipo de produção (Mao *et al.* 2011; Rodrigues, Motta-Marques & Fontoura 2015). Portanto, a produção primária em lagoas rasas e banhados, a qual é efetivamente transferida para os níveis tróficos subsequentes, depende não apenas de qual produtor é a fonte dominante na região, sejam as macrófitas ou o fitoplâncton, mas também de todas as interações que permitem a transferência de matéria e energia entre níveis tróficos. Além disso, durante os eventos de alagamento em que os banhados, lagoas e campos marginais permanecem interconectados, a produção de um sistema pode ser incorporada no outro, em magnitude muitas vezes difícil de ser mensurada.

Além das hipóteses e teorias preditivas (RCC, FPC e RPM) para a identificação das fontes de produção primária (estrutura horizontal) que sustentam os demais componentes das teias tróficas em ambientes aquáticos, existem algumas generalizações acerca do comprimento das cadeias tróficas (estrutura vertical). Considerando que o tamanho de lagoas e banhados e a variabilidade sazonal na conectividade com ambientes terrestres podem alterar as fontes de sustentação e, conseqüentemente, os recursos secundários que dela dependem, tais fatores podem afetar a estrutura vertical das teias tróficas, alterando o comprimento destas. Em relação à estrutura vertical, duas principais hipóteses têm sido exploradas como preditoras de comprimento de cadeia trófica: a hipótese da produtividade e a hipótese do tamanho do ecossistema, além do debate acerca do papel da estabilidade, ou instabilidade, do ecossistema sobre o comprimento da teia trófica. (Post, Pace & Hairston 2000; Sabo, Finlay & Post 2009).

Existem evidências de que os forçantes de produtividade, tamanho e estabilidade estão positivamente relacionados com o comprimento das cadeias tróficas, embora

atuando de modo distinto (Pimm & Lawton 1977; Post *et al.* 2000; Sabo *et al.* 2010). Em um estudo recente que utilizou meta-análise (Takimoto & Post 2013) envolvendo diversos tipos de ecossistemas aquáticos, incluindo sistemas lóticos e lênticos, um forçante significativo foi encontrado para o tamanho do ecossistema sobre o comprimento da teia trófica. Ao mesmo tempo, não houve efeito da estabilidade, assim como um efeito fraco, embora consistente, relacionado à disponibilidade de recursos (indicativo de produtividade) foi encontrado (Takimoto & Post 2013).

Em suma, ambas as teorias e hipóteses que se propõem a explicar a origem e a importância das fontes de sustentação (dimensão horizontal), ou comprimento (dimensão vertical) das teias tróficas, parecem convergir a determinados aspectos. O tamanho e a produtividade, ou disponibilidade de recursos, estão diretamente relacionados com o papel das fontes externas para a estrutura trófica aquática. Já a instabilidade (pulso de inundação) promove a conexão e consequente desestabilização das relações tróficas.

O estudo da dieta de peixes, por meio da Análise de Conteúdo Estomacal (ACE) revela os recursos consumidos pelas espécies recentemente (Winemiller, Akin & Zeug 2007). Além disso, este método (ACE) dificilmente consegue responder quais produtores primários são a via de entrada de nutrientes para as espécies. Um problema que se soma a isso, é que muitos alimentos ingeridos podem não ser assimilados, devido a sua baixa digestibilidade e/ou características fisiológicas específicas da espécie (Winemiller *et al.* 2007; Caut, Angulo & Courchamp 2009), levando a interpretações errôneas da importância de determinados recursos alimentares.

Uma alternativa para a resolução deste problema é o uso de isótopos estáveis de Carbono (^{12}C , ^{13}C) e de Nitrogênio (^{14}N , ^{15}N). Estes elementos são abundantes no ambiente físico e químico e são componentes fundamentais na estrutura dos tecidos vivos, na forma de carboidratos, lipídeos, proteínas, aminoácidos e ácidos nucleicos. A utilização destes, têm se revelado uma importante metodologia para estimar vias e relações tróficas (Garcia *et al.* 2006b; Winemiller *et al.* 2007; Rodrigues, Fontoura & Motta-Marques 2012, 2014)

A abundância da forma isotópica mais pesada e mais rara (^{13}C e ^{15}N), em relação à forma isotópica mais leve e mais comum na natureza (^{12}C e ^{14}N), pode ser medida nos tecidos dos organismos com grande precisão com o auxílio de um espectrômetro de massas de razões isotópicas - IRMS (DeNiro & Epstein 1981; Vander Zanden, Cabana & Rasmussen 1997; Post 2002; Fry 2006). As razões isotópicas (quantidade do isótopo mais pesado em relação ao mais leve) do carbono são bastante conservativas ao longo da cadeia trófica, sofrendo um fracionamento (mudança na razão entre os isótopos pesados e leves) de cerca de 1‰ (uma parte por mil) mais enriquecido que a fonte de matéria orgânica assimilada a cada transferência na cadeia alimentar (nível trófico) (DeNiro & Epstein 1978; Fry 2006). Já a razão do Nitrogênio, geralmente, sofre um enriquecimento de cerca de 3‰ a cada nível trófico (DeNiro & Epstein 1981; Vander Zanden *et al.* 1997; Pinnegar & Polunin 2000; Post 2002; Vanderklift & Ponsard 2003; Caut *et al.* 2009). Para as medidas de isótopo de carbono, a diferença entre ^{13}C e ^{12}C é comparada com o padrão “marine limestone fossil”, Pee Dee Belemnite (PDB), enquanto que para nitrogênio (^{15}N e ^{14}N) é utilizado o ar atmosférico como padrão. Assim, os valores utilizados nas análises são uma razão entre a quantidade de isótopos

pesados presentes na amostra e presentes no padrão, sendo expressos pela notação “ δ ” (Garcia *et al.* 2006b; Winemiller *et al.* 2007, 2011a).

De modo geral, através da razão isotópica do carbono ($\delta^{13}\text{C}$) é possível investigar quais os produtores primários e outras fontes orgânicas formam a base de sustentação (através da transferência do carbono orgânico assimilado pelas plantas) para um determinado consumidor na cadeia trófica (seja ele herbívoro ou não), já que a composição isotópica de carbono dos produtores é assimilada pelos consumidores que por sua vez vão possuir razões isotópicas muito similares (mudança de cerca de 1‰) aquelas dos produtores que os sustentam. Mesmo consumidores em níveis tróficos mais superiores, que não se alimentam diretamente de uma planta ou alga vão refletir a composição destes, com um pequeno aumento na diferença entre os $\delta^{13}\text{C}$. Dessa maneira, consumidores primários apresentarão os valores de $\delta^{13}\text{C}$ dos produtores assimilados +1‰ de enriquecimento, consumidores secundários +2‰, consumidores terciários +3‰, quaternários +4‰ e assim sucessivamente.

Posto isso, o uso de isótopos estáveis se mostra muito interessante quando os diferentes produtores de um sistema apresentam valores de $\delta^{13}\text{C}$ suficientemente distintos, possibilitando a separação destes, do contrário a técnica pode ser ineficaz quanto a identificação das fontes primárias. Estudos mostram que as razões isotópicas de fontes de produção primária com metabolismo diferenciado, tais como algas, plantas C3 e plantas C4 (Fry 2006), produtores em diferentes regiões de lagoas (zona pelágica/zona litorânea)(Paterson & Whitfield 1997) e diferentes condições hidrológicas (seca/alagamento) em banhados (Wantzen *et al.* 2002), apresentam valores de $\delta^{13}\text{C}$ suficientemente diferentes para a identificação das fontes primárias que estão efetivamente sustentando as comunidades.

Por outro lado, por meio da razão isotópica do nitrogênio ($\delta^{15}\text{N}$) é possível estimar a posição trófica de um consumidor na cadeia alimentar (Vander Zanden *et al.* 1997; Post 2002) e assim estimar o comprimento das cadeias tróficas dos ecossistemas estudados. Entretanto, embora a mudança nos valores de $\delta^{15}\text{N}$ entre um nível trófico e o seguinte seja normalmente de maior magnitude, 3‰ em média, este valor pode ser muito variável. Especial atenção tem sido dada para esta mudança, também chamada de fator de discriminação trófica de nitrogênio (FDT_N) e frequentemente representada pela letra delta, assim como as razões isotópicas ($\delta^{15}\text{N}$), mas com a letra delta maiúscula ($\Delta^{15}\text{N}$) (Caut *et al.* 2009; Bond & Hobson 2012).

A estimativa de posições tróficas e conseqüentemente de comprimento de cadeias tróficas, dependem dos valores de FDT utilizados, bem como dos valores de $\delta^{15}\text{N}$ na base das cadeias alimentares (Post 2002). Sendo assim, o FDT é um parâmetro crítico para a estimativa de assimilação de matéria orgânica e principalmente de posições tróficas. Cerca de 20 anos após um chamado para mais experimentos em laboratórios a respeito dos FDT (Gannes, O'Brien & Martínez Del Rio 1997; Wolf, Carleton & Martínez Del Rio 2009), estudos tem revelado múltiplos fatores influenciando os valores de FDT, incluindo qualidade dos alimentos ingeridos (e.g., conteúdo proteico, perfil de aminoácidos), estado metabólico (e.g., anabolismo vs. catabolismo), taxa de ingestão alimentar, estágio de desenvolvimento, massa corpórea, sexo e até mesmo fatores controversos, tais como a composição isotópica dos alimentos (Robbins, Felicetti & Sponheimer 2005; Gaye-Siessegger *et al.* 2007; Caut *et al.* 2009; Kelly & Martínez del Rio 2010; Wessels & Hahn 2010; McMahon *et al.* 2010; Florin, Felicetti & Robbins 2011; Newsome *et al.* 2011; Codron *et al.* 2011; Poupin *et al.* 2011).

Diversos trabalhos de revisão dos FDT mostraram resultados inconsistentes quando compararam consumidores herbívoros com consumidores carnívoros (Vander Zanden & Rasmussen 2001; Post 2002; Vanderklift & Ponsard 2003; Caut *et al.* 2009). Especificamente para peixes, diferentes FDT_N para herbívoros e carnívoros foram observados, sendo que estes últimos geralmente apresentam menores valores (Varela, Larrañaga & Medina 2011; Madigan *et al.* 2012) enquanto herbívoros mostraram valores maiores de FDT_N (Keegan & DeNiro 1988; Jennings *et al.* 1997; Pinnegar & Polunin 2000; Mill, Pinnegar & Polunin 2007). De fato, estudos recentes têm demonstrado que os fatores de discriminação para Nitrogênio podem variar consideravelmente entre os níveis tróficos (Hussey *et al.* 2014). Para os níveis tróficos inferiores, tais como os consumidores primários, que usualmente se alimentam de material de menor qualidade e com maiores taxas de ingestão e conseqüentemente de excreção, valores maiores são observados (Keegan & DeNiro 1988; Jennings *et al.* 1997; Pinnegar & Polunin 2000; Mill *et al.* 2007), enquanto que para consumidores de topo, que se alimentam de material de alta qualidade e com menores taxas de ingestão e excreção, valores menores vem sendo reportados (Varela *et al.* 2011; Madigan *et al.* 2012).

Em ecossistemas naturais, diversos produtores primários e recursos secundários estão usualmente disponíveis, e sendo assim, diversos tipos de produção/recursos podem sustentar as comunidades. Além disso, diferenças no tipo de alimentação de diferentes espécies ou guildas alimentares podem implicar em distintas fontes primárias de sustentação. A utilização da técnica de isótopos estáveis em estudos ecológicos foi proporcionada pelo estudo pioneiro de Craig (1953), onde este reportou valores isotópicos distintos para diferentes tipos de plantas. Mais tarde, O'Leary (1988) mostrou

que as diferenças nas composições isotópicas de plantas, estavam relacionadas ao tipo de metabolismo (C_3 e C_4) dos produtores primários.

Desde então, as diferenças isotópicas entre os produtores têm sido usadas como “assinaturas” ao longo das cadeias tróficas, e diferentes modelos de mistura vem sendo empregados para a identificação da contribuição relativa destes para os consumidores. Um dos primeiros modelos amplamente usado foi proposto por (Phillips & Gregg 2003), um modelo linear de balanço de massas em que o número de fontes que podem ser usadas é igual ao número de isótopos utilizados mais um, o que normalmente limitava o número de fontes no modelo a 3 (e.g. C e N +1 = 3). Além disso, os modelos não geravam medidas de erro e intervalo de confiança, limitando os resultados a um intervalo de valores possíveis de contribuição de cada fonte. O mesmo grupo seguiu aprimorando a técnica sugerindo o agrupamento de fontes isotopicamente e ecologicamente similares, incorporando medidas de erro nos modelos gerados (Phillips, Newsome & Gregg 2005). Ainda assim, estes modelos deixavam de fora certas variações, tais como a variabilidade dos FDT's.

Meia década após a publicação do IsoSource, Parnell *et al.* (2008) propuseram um modelo de mistura com abordagem estatística bayesiana, disponível no pacote SIAR para R, que possibilitou a inclusão de diversas fontes e as variações nos valores de discriminação trófica, inclusive atribuir valores diferentes para cada uma das fontes (Parnell *et al.* 2010). Com isso, o emprego destes modelos de mistura vem crescendo gradativamente, sendo que em 2013 o número de citações já ultrapassava 1500 (Parnell *et al.* 2013).

Considerando que as razões isotópicas de Carbono revelam as fontes alimentares e de Nitrogênio as posições tróficas, o espaço bivariado de carbono e nitrogênio representa, ao menos, parte do espaço trófico de consumidores. Dessa maneira, o espaço trófico de espécies ou grupos representaria, em sentido amplo, parte dos nichos tróficos destes (Newsome *et al.* 2007). Diversas métricas utilizando isótopos estáveis foram propostas para extrapolações deste tipo (Layman *et al.* 2007), incluindo a área total ou *convex hull area*, uma medida da área total ocupada pelo polígono formado por todos os valores de uma espécie, grupos funcionais ou mesmo assembleias, que serviria como medida de nicho trófico destes. Assim, grandes áreas representam uma amplitude trófica maior, ou seja, mais recursos são consumidos e assimilados. Entretanto, existem críticas a respeito dessa extrapolação. Hoeninghaus & Zeug (2008) argumentam que o uso de métricas isotópicas para extrapolar questões acerca da teoria do nicho (Hutchinson 1957) é frequentemente inadequada, pois o consumo de poucas espécies e recursos com valores isotópicos distantes aumentaria a medida de área isotópica, enquanto que na verdade o nicho deveria ser estreito. Além disso, se os FDT_N forem diferentes entre os grupos comparados, os valores brutos de $\delta^{15}N$ não seriam o *proxy* ideal para representar a estrutura vertical (número de níveis tróficos) de um ecossistema.

Mais recentemente, uma proposta de métrica de área isotópica baseada em estatística bayesiana, disponível no pacote SIBER para R, foi feita pelo mesmo grupo que propôs os modelos de mistura bayesianos (Jackson *et al.* 2011). A técnica utiliza como medida de área isotópica, a área de uma elipse padrão (SEA). A elipse padrão é comparada ao desvio padrão, contendo cerca de 40 % dos dados, e dessa maneira inclui uma medida de desvio para a área isotópica que passa a considerar a grande

variabilidade existente e se torna mais comparável entre gradientes temporais e espaciais, já que os *outliers* passam a não ter grande efeito sobre a medida. Além disso, as SEA's não são afetadas por erros associados ao número de amostras, permitindo comparações entre grupos com diferentes tamanhos (Jackson *et al.* 2011). Dessa maneira, medidas de sobreposição de SEA's são medidas de sobreposição de espaço isotópico, e conseqüentemente, *proxy* para sobreposição de nicho entre os grupos comparados (Jackson *et al.* 2011). Entretanto, diferentes grupos funcionais podem estar sujeitos a diferentes recursos, o que modifica o *baseline* de valores isotópicos disponíveis para aquele grupo. Apesar do avanço dessa métrica em relação a outras, as críticas ainda são aplicáveis a esta, mesmo que em menor importância.

A planície costeira do estado do Rio Grande do Sul (Brasil) é composta por uma série de estuários, lagoas costeiras e áreas alagadas (banhados), onde a feição dominante é o complexo lagunar Patos-Mirim, com uma área aproximada de 14.000 Km², que se estende do centro-oeste até o extremo sul do estado do Rio Grande do Sul (RS) (Buchmann, Barbosa & Villwock. 1998). As lagoas dos Patos e Mirim se unem por meio do canal natural de São Gonçalo.

A partir dos anos 1960, os governos do Brasil e Uruguai incentivam o desenvolvimento econômico regional na área industrial e de agronegócio (Burns *et al.* 2006b). Desde então, diversas atividades antrópicas têm sido conduzidas, as quais representam um risco para a biota e os ecossistemas. Um exemplo foi a construção da rodovia BR- 471 na região entre a Lagoa Mirim e Lagoa Mangueira. O crescimento da rizicultura e silvicultura também tem causado, ao longo de vários anos, alterações no balanço hídrico da região (Villanueva, Motta-Marques & Tucci 2000; Seeliger & Costa 2003).

Com o objetivo de preservar parte dessa importante área e seus ecossistemas, em 1986 foram criadas duas importantes áreas de preservação do ecossistema costeiro no Rio Grande do Sul: (1) A Estação Ecológica do Taim (ESEC-TAIM - dec. Nº 92963) e (2) o Parque Nacional da Lagoa do Peixe (PNLP).

Originalmente a ESEC-TAIM possuía 315 Km², que em 2003 foram ampliados para 1100 Km². Entretanto, diversas propriedades particulares usadas para a agricultura, especialmente produtoras de arroz, pecuária e até mesmo florestamento de espécies exóticas (e.g., *Pinus* spp.) estão presentes dentro dos limites da estação, pois sua situação latifundiária ainda não foi resolvida. Além disso, espécies aquáticas exóticas como o bagre porruado (*Trachelyopterus lucenai*), a corvina de rio (*Pachyurus bonariensis*), o mexilhão dourado (*Limnoperma fortunei*) e a ameijoia asiática (*Corbicula fluminea*) já estão estabelecidas na área (Burns *et al.* 2006a; Garcia *et al.* 2006a; Harayashiki *et al.* 2014).

O Parque Nacional da Lagoa do Peixe possui uma variedade de habitats que abrigam uma fauna bastante rica, incluindo algumas espécies consideradas criticamente ameaçadas. A raia *Rhinobatos korkelii*, mundialmente ameaçada de extinção, se junta a cerca de doze (12) espécies dos mais variados grupos animais, que são consideradas ameaçadas no Brasil. Dada a sua importância como área de proteção de espécies ameaçadas, o parque recebeu reconhecimento internacional; sendo incluído no programa Ramsar (*Wetland of International Importance*), e considerado uma Reserva da Biosfera pelo UNESCO, além de ser identificado pela *Birdlife International* como uma importante área de alimentação e descanso para as aves.

Portanto, o objetivo geral deste trabalho foi o de avaliar a estrutura trófica de peixes do PNLP e ESEC-TAIM, utilizando isótopos estáveis, com a aplicação e proposição de alternativas metodológicas em análises já amplamente utilizadas.

No primeiro capítulo, as teorias de fluxo de matéria e estruturação trófica foram testadas para guildas tróficas de peixes em uma lagoa e um banhado durante diferentes hidroperíodos (inundação/seca) na ESEC-TAIM. Neste trabalho, modelos de mistura foram aplicados para traçar a variabilidade na contribuição de produtores primários terrestres e medidas de espaço isotópico foram utilizadas para comparar a estrutura trófica das guildas em diferentes ambientes e entre hidroperíodos. Além disso, foi feita a proposição do uso de valores estimados de posição trófica em substituição aos valores brutos de $\delta^{15}\text{N}$ nas elipses isotópicas, resolvendo parcialmente a limitação da comparação de áreas com valores de *baseline* distintos.

No segundo capítulo, foi avaliada a variabilidade no consumo e assimilação de diferentes recursos (animais vs. vegetais) e na posição trófica de uma espécie de peixe omnívoro ao longo do gradiente estuarino da principal lagoa do PNLP. Neste trabalho a foi averiguado a influência do uso de diferentes FDT sobre (1) as estimativas de assimilação de recursos animais e vegetais e (2) as posições tróficas estimadas, sendo proposto um método para cálculo de posição trófica que considera a proporção estimada de contribuição de cada fonte, tendo por base o respectivo FDT associado com cada uma das fontes.

CAPÍTULO 1

Influence of flooding on trophic structure and allochthonous contribution to fishes in a subtropical wetland and shallow lake.

Manuscrito redigido e submetido na língua e formato indicado para revista *Freshwater Biology*.

*O número de linhas foi retirado para melhor adequação ao formato da tese.

Influence of flooding on trophic structure and allochthonous contribution to fishes in a subtropical wetland and shallow lake.

Rodrigo Ferreira Bastos^{* a, b, c}, Alexandre Miranda Garcia^b, Nelson Ferreira Fontoura^a, Kirk O. Winemiller^c

^a Programa de Pós Graduação em Biociências (Zoologia), Faculdade de Biociências - FABIO, Pontifícia Universidade Católica do Rio Grande do Sul – PUCRS, Porto Alegre, Brazil.

^b Laboratório de Ictiologia, Instituto de Oceanografia - IO, Universidade Federal do Rio Grande – FURG, Rio Grande, Brazil.

^c Department of Wildlife and Fisheries Sciences, Texas A&M University – TAMU, College Station, USA.

* Corresponding author: rfbastos@furg.br

SUMMARY

1. Inland aquatic habitats are connected with surrounding ecosystems through organic matter flow. In ecosystems subjected to seasonal floods, allochthonous contributions to aquatic communities can differ according to hydrological conditions.

2. Stable isotope techniques can be used to trace organic matter flow and food chain length in food webs. Ratios of carbon stable isotopes (^{12}C , ^{13}C) are used to trace the origin of organic matter, and the ratio of nitrogen stable isotopes (^{14}N , ^{15}N) provides a proxy for trophic position. Bayesian statistics have improved mixing models and metrics of plant and consumer relationships within community isotopic space.

3. However some assumptions are still required when using such approaches. Here we proposed the use of trophic position estimates instead of purely Nitrogen isotopic values on the Bayesian standard ellipses (SIBER) in order to trace trophic structure changes driven by floods in subtropical coastal ecosystems.

4. We surveyed a shallow lake and a wetland community, collecting terrestrial and aquatic producers and consumers and analyzing their isotopic composition during winter/wet and summer/dry seasons.

5. Our results showed that lake trophic structure was more complex and with greater food chain length. An increase of terrestrial contribution to consumer biomass was observed in the wetland during winter/wet period, but, unexpectedly, the opposite pattern was found in lake. Food chain length was higher during the winter/wet period at both sites. This suggests a general influence of flooding on the vertical structure of food webs.

6. Finally, the proposed methodology improved metrics for estimating positions within community isotopic space (SIBER analysis), particularly with regard to the vertical dimension of trophic structure.

Key-words: autochthonous resource, flood pulse, food web, SIAR, trophic niche

Introduction

A major question in aquatic ecology is the origin of organic matter (autochthonous/aquatic vs. allochthonous/terrestrial) that sustains communities. The relationship between terrestrial and aquatic habitats can be variable due to water body morphology, hydrology, and climate characteristics. There are several general hypotheses regarding the origin of organic matter that sustaining fluvial communities (Vannote *et al.* 1980; Junk *et al.* 1989; Thorp & Delong 1994; Junk & Wantzen 2008) but less general patterns are observed for lakes. Lakes can be variable in shape, size, depth, dynamic, nutrients and connection with adjacent aquatic and terrestrial systems, all of which can lead to different trophic states (Carlson 1977) in which different primary producers dominate and support the aquatic community. In small and shallow lakes, where macrophytes are dominant, communities tend to be supported by this source, whereas larger and deep lakes are predominantly supplied by phytoplankton in the pelagic zone and macrophytes in littoral zones (Vander Zanden & Vadeboncoeur 2002; Kruger *et al.* 2015). Rodrigues, Motta-Marques & Fontoura (2015) showed different trophic states between portions (north and south) of a large shallow lake of the TAIM hydrological system in southern Brazil. They attributed such differences to different primary producers that dominated in each portion, with more macrophytes, clearer water and lower chlorophyll *a* concentration in the southern extreme, and phytoplankton dominating the northern reach where there was higher chlorophyll *a* concentration, lower water transparency, and absence of submerged macrophytes (Rodrigues *et al.* 2015).

Usually, terrestrial organic matter is incorporated in aquatic systems through the detritus compartment (Vannote *et al.* 1980) or through direct consumption of terrestrial animals (Nakano, Miyasaka & Kuhara 1999) and fruits (Correa & Winemiller 2014). This terrestrial contribution is usually higher when flooding promotes exchanges between terrestrial and aquatic habitats (Junk *et al.* 1989; Thomaz, Bini & Bozelli 2007; Davidson *et al.* 2012). Besides that, the availability and quality of terrestrial sources together with the ability of consumers to exploit new resources in surrounding area are factors that determine the actual importance of the physical connectance for food web structure (Hoeninghaus *et al.* 2011; Abrantes *et al.* 2013)

By increasing physical connections between habitats, floods also affect vertical trophic structure, or food chain length. With different resources available, there also is a greater possibility for organisms to exploit resources at different trophic levels, which could change food chain length. Two major hypotheses that have been proposed regarding the vertical trophic structure: the productivity hypothesis and the ecosystem size hypothesis. The relationship between average food chain length and community stability also has been debated. There is evidence that productivity, ecosystem-size and stability are all positively related to food chain length but in different ways (Pimm & Lawton 1977; Post *et al.* 2000; Sabo *et al.* 2010). In a recent meta-analysis, Takimoto & Post (2013) found a strong but variable effect of ecosystem size, no consistent effect of disturbance (stability), and a weak but consistent effect of resource availability (productivity) across different ecosystems.

More than half century ago, Craig (1953) reported differences in carbon isotopic composition of plants that use different photosynthetic pathways: C₃ vs. C₄ (O'Leary 1988). Since then, isotopic difference between primary producers have been used to

trace the flow of organic matter originating from different sources through food webs. Carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) have been analyzed most often because (1) they are abundant in organic materials, and (2) display predictable shifts in isotopic ratios between food and consumer (DeNiro & Epstein 1978, 1981). Consistent carbon isotopic differences have been shown between primary producers in pelagic and littoral zones of lakes (Paterson & Whitfield 1997) and between hydrological conditions (dry/wet periods) in wetlands (Wantzen *et al.* 2002). Nitrogen isotopic ratio shift in a fairly consistent manner between adjacent trophic levels, which provides a means to estimate the vertical trophic positions of consumers and food chain length in food webs (Post 2002).

In order to test the predictions about organic matter flow and trophic length in shallow lakes and wetlands, we conducted a community survey during summer/dry and winter/wet seasons in two subtropical aquatic habitats. We collected aquatic and terrestrial primary producers, invertebrates and fishes consumers in a shallow lake and in a wetland inside a protected area, and analyzed their carbon and nitrogen isotopic composition. The reserve was created in 1978 to protect important coastal ecosystems in southern Brazil that have suffered major anthropogenic impacts, such as water diversion for rice irrigation, fishing, hunting and forestry (Garcia *et al.* 2006b). Effective conservation and management of these ecosystems will depend on improved understanding of processes shaping aquatic communities within the reserve.

We hypothesize that (1) trophic structure is more complex in the lake than wetland area, (2) terrestrial input to the aquatic food web is greater during flood period in both sites, and (3) the influence of flooding is greater in the wetland where terrestrial contributions are larger. Bayesian isotopic mixing models (SIAR) and

isotopic space metrics (SIBER) (Parnell *et al.* 2008; Jackson *et al.* 2011) were used to evaluate changes in trophic structure and organic matter flow among seasons and sites.

Methods

Study area and sampling design

Taim Ecological Station is located within a national reserve that protects a large portion of the Taim Hydrological System. The area has a heterogeneous and productive landscape encompassing a variety of habitats such as inland beaches, dunes, forests, grasslands and, especially, lakes and wetlands harboring exceptional biological diversity (Ludwig *et al.* 2004; Beatriz & Schettini 2005; Garcia *et al.* 2006a; b; Borges & Colares 2007; Weber *et al.* 2009; Kutter, Bemvenuti & Moresco 2009; Bastos *et al.* 2011; Rodrigues & Bemvenuti 2011). In this area, the combination of precipitation, evaporation and wind patterns lead to seasonal hydrologic pulses. During austral winter, high rainfall and low evaporation result in accumulation of water within the wetland, which in turn promotes hydrological connectivity among lakes, wetlands and riparian terrestrial habitats. The opposite tends to occur during summer when lower precipitation and higher evaporation reduce water levels and hydrological connectivity of the landscape (Bastos, Calliari & Garcia 2014). In general, winter is also the wet period and summer is the dry period. Wind can also be an important factor promoting the exchange of water, organic matter, nutrients and even organisms between adjacent habitats (Bastos *et al.* 2014; Rodrigues *et al.* 2015). This seasonal hydrological pulse may also be affected by *El Niño* and *La Niña* climatic events, which causes positive and negative

rainfall anomalies in southern Brazil, Uruguay and Argentine (Garcia, Vieira & Winemiller 2003; Vieira, Garcia & Grimm 2008).

In order to investigate potential effects of the hydrological pulse on food web structure of fish assemblages, we conducted field sampling during winter/wet and summer/dry periods at two locations of Taim Hydrological System: Caçapava wetland (hereafter wetland) and Nicola lake (hereafter lake) (Figure 1). The sampled wetland is a complex system of ephemeral and permanent water bodies inside an area of approximately of 2 Km². Its average deep is 0.4 m and maximum registered deep of 1 m during winter/wet/wet. The studied lake is a perennial system with an area of approximately 2 Km² that can change substantially (300%) along the hydrological pulse. Its average deep is 0.5 m, but can reach 1.3 m during the wet period. Both aquatic systems are embedded within an extensive grass land area dominated in biomass by species of the family Poaceae. In contrast, macrophytes are the dominant vegetation within both aquatic systems. Prior stable isotopic surveys at this region (unpublished data) showed that most grassland species (Poaceae) have stable isotope carbon ratios ($\delta^{13}\text{C}$) enriched in ¹³C, which are typical of plants with C₄ photosynthetic pathways. In contrast, the highly abundant macrophytes found within these aquatic systems are characterized by average $\delta^{13}\text{C}$ values depleted in ¹³C, which are commonly found in plants exhibiting C₃ photosynthetic pathways (Garcia *et al.* 2006b; Rodrigues *et al.* 2014). We gave special attention to this potential isotopic aquatic-terrestrial gradient produced by spatial segregation of macrophytes and grassland in our sampling of primary producers and basal food sources.

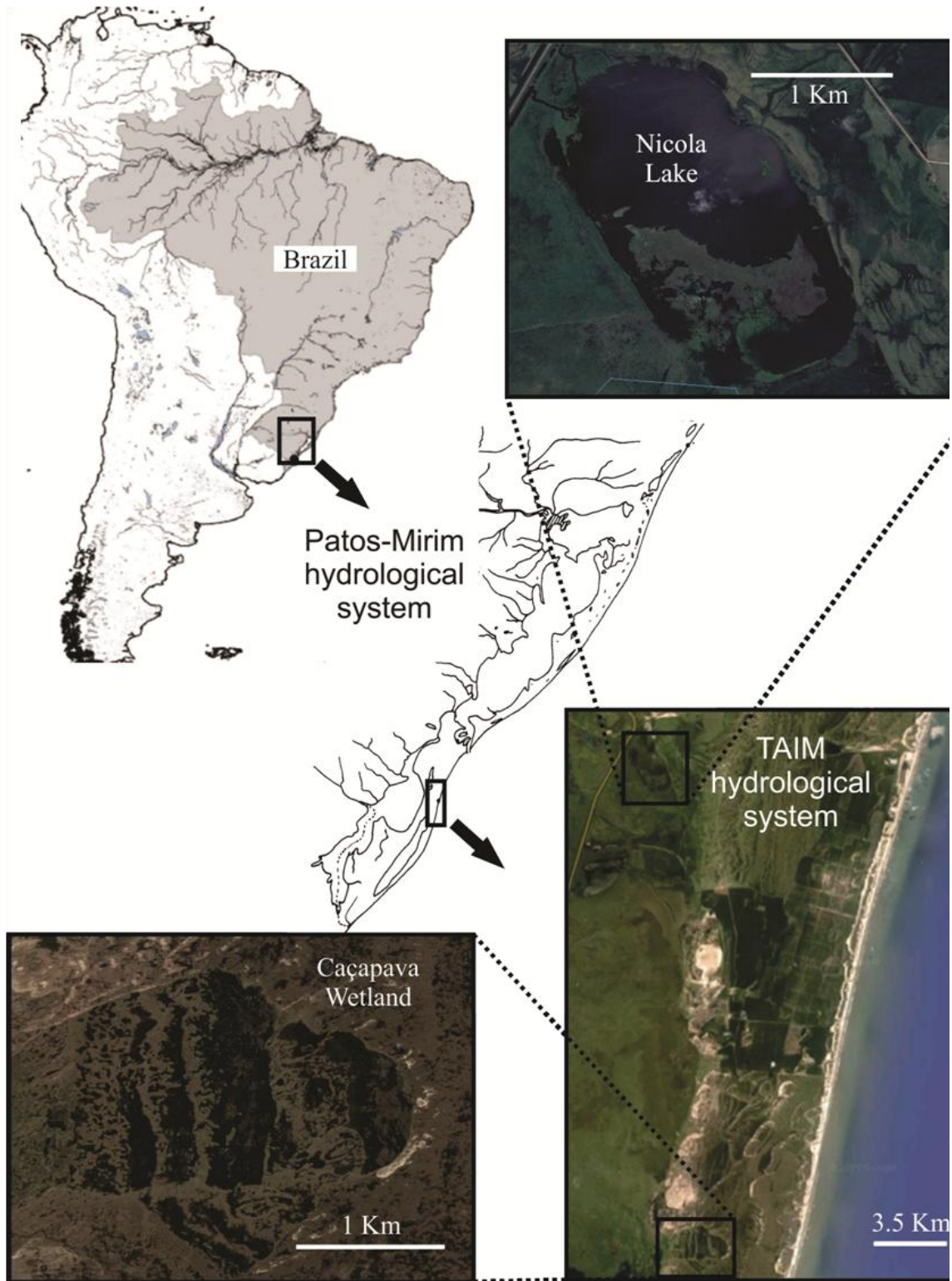


Figure 1. Map showing sites surveyed, Nicola Lake and Caçapava Wetland inside the TAIM hydrological system and Patos-Mirim hydrological system, southern Brazil, South America.

At all sites and seasons, dominant terrestrial plants from the surrounding (mostly C₄ Poaceae plants) were collected along the margins and surrounding grasslands of both wetland and lake. Aquatic basal food sources including dominant macrophytes (mostly C₃ plants), algae (as free filamentous or mixtured as biofilm), suspended particulate organic matter (SPOM) and benthic particulate organic matter (BPOM) were collected from the water, following the methods described in chapter 2 (this thesis). Other abundant and potential basal food source available was Capybaras' faeces (*Hydrochoerus hydrochaeris*, Rodentia). Several faeces pellets were usually observed along the margins of both aquatic systems and also floating in the water, especially during winter/wet/wet. Thereby, samples of such source were collected from the water and along the margins during all field trips.

Fish and macroinvertebrates were sampled using a rapid assessment method (Price & Harris 2009) involving several fishing gears as described in Bastos, Calliari & Garcia (2014) and also cast nets. Sampling was conducted within ca. 5 hrs on each sampling occasion in each aquatic system. This procedure allowed us to obtain representative sampling coverage of consumers at the wetland and in the lake. Additional manual sampling for insects, snails and clams were conducted in littoral and surrounding areas of both sites. At Caçapava wetland, however, due to logistical constraints, it was not possible to collect invertebrate terrestrial consumers, but only aquatic invertebrates. Also, zooplankton nets were used just during winter/wet surveys because during the summer/dry period, detritus was abundant throughout the water column and obstructed the sampler.

Samples preparations for isotopic analyses are described in chapter 2 (this thesis) and consisted briefly in to dry (60°C for 48hrs) and powder the selected tissue for each

consumer (muscles for fish and macroinvertebrates) and primary producers (leaves for plants, filaments for filamentous algae and bulk samples for biofilm). After being weighted (approximately 0.01 and 0.5 mg for animal and plants, respectively) within tin capsules, samples were sent to Analytical Chemistry Laboratory, Institute of Ecology, University of Georgia, for measurement of stable isotope ratios ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$). Results were reported as parts per mil (‰) deviations from the corresponding standard: $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3$. Standards were PeeDee Belemnite (C) and molecular nitrogen in air (N). Standard deviations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ based on analysis of samples replicates were $0.70 \pm 0.65\text{‰}$ and $0.32 \pm 0.51\text{‰}$, respectively.

Data analysis

The most abundant aquatic producers (C_3 macrophytes and algae), along with the most abundant terrestrial plants (C_4 grasses) were considered as primary producers sources. Other conspicuous and potential sources, such as Capybaras faeces, SPOM and BPOM were considered as organic basal sources.

Consumers were separated by major taxa (fish vs. invertebrates) and fishes were subsequently classified into six trophic functional guilds (TFG) based on the literature (Moresco & Bemvenuti 2005; Corrêa & Piedras 2008; Kutter *et al.* 2009; Bastos *et al.* 2011; Bastos, Miranda & Garcia 2013; Corrêa *et al.* 2012; Rodrigues *et al.* 2012) and on stomach contents from the individuals collected in the current study. The six fish's TFG were detritivorous, omnivorous, invertivorous, zooplanktivorous, invertivorous/piscivorous (those invertivorous that eventually consume fish) and piscivorous. Invertebrates were separated by taxa, size and habitat into six functional

groups: macrocrustaceans, microcrustaceans, aquatic filter clam, aquatic grazer snails, aquatic insects and terrestrial arthropods.

Patterns in carbon and nitrogen stable isotope ratios of basal food sources and consumers at each site and season were primarily investigated using biplots (Garcia *et al.* 2006b). Sources of organic carbon assimilated by consumers were indicated by the relative positions of taxa on the x-axis ($\delta^{13}\text{C}$ values), whereas trophic level was indicated by the relative position on the y-axis ($\delta^{15}\text{N}$). The average $\delta^{15}\text{N}$ of invertebrate primary consumers was used as baseline to estimate trophic position for each site and season following the equation: $\text{TP} = \lambda + ((\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}}) / \text{TDF})$, where λ is the baseline trophic level, which was set at 2 for invertebrate primary consumers; $\delta^{15}\text{N}_{\text{consumer}}$ is the $\delta^{15}\text{N}$ valued of a consumer specimen; $\delta^{15}\text{N}_{\text{baseline}}$ is the average $\delta^{15}\text{N}$ value from invertebrate primary consumers at the corresponding site and season; and TDF is the trophic discrimination factor for $\delta^{15}\text{N}$ (Post 2002). Invertebrate primary consumers were used as a baseline because this group can accurately integrate temporal variations in producers' stable isotopic composition (Vander Zanden & Rasmussen 2001; Post 2002; Abrantes, Barnett & Bouillon 2014). The invertebrate species or groups considered as primary consumers (amphipoda, zooplankton, aquatic clam - *Corbicula fluminea*, aquatic grazer snails - *Heliobia australis* and *Pomacea canaliculata*, insects – Coleoptera and Gryllidae) were used to construct the baseline.

Estimates of trophic positions were made for each individual and then averaged for categories described above. Average trophic positions of consumer categories and $\delta^{15}\text{N}$ values from baseline that were present at all sites and seasons and in sufficient number (> 5 individuals per site and season) were compared among sites and season using analysis of variance (ANOVA) considering normality and homocedasticity

assumptions. When a significant main effect was observed, multiple comparisons (Tukey post-hoc test) were performed (Zar 2010). When assumptions were not achieved non-parametric ANOVA (Kruskal-Wallis) was performed (Zar 2010).

In order to estimate relative contributions of terrestrial (allochthonous) vs. aquatic (autochthonous) primary producers to consumers, Bayesian stable isotope mixing models were computed using the package SIAR version 3.2.1 (Parnell *et al.* 2008). To achieve higher resolution and better ecological inferences from mixing models, primary producers with similar isotopic composition and similar ecological roles were grouped *a priori* as suggested by Phillips *et al.* (2005). Hence, algae and plants C₃ were considered as aquatic primary producer sources and plants C₄ as terrestrial primary producer source in all models. Usually, samples of suspended particulate organic matter (SPOM) are commonly used as a proxy for isotopic composition of algae and organic matter in the water column (Kaehler, Pakhomov & McQuaid 2000; Cresson *et al.* 2012), since it is difficult to have pure samples of phytoplankton. However, in subtropical shallow (<1.5 m) wetlands and lakes winds promote mixture of the entire water column (De Vicente, Amores & Cruz-Pizarro 2006; Dubois *et al.* 2012). Therefore, in these shallow and well-mixed aquatic systems, SPOM is likely to be composed of a mixture of organic matter coming from both pelagic and benthic pathways. In this way, Capybaras faeces, SPOM and BPOM, with uncertain origin, were considered mixtures of primary producers and other organic compounds, such as animal carcasses and excrement, and therefore these were excluded from this analysis. The considered TDFs were 2.54±1.59 for nitrogen ($\delta^{15}\text{N}$) (Vanderklift & Ponsard 2003) and 0.40±1.3 for carbon ($\delta^{13}\text{C}$) (Post 2002) stable isotope ratios. Such TDFs values are supposed to occur between subsequent trophic levels, representing the

per trophic level change. In order to account for such variation, we multiplied TDF values per the consumers' trophic position estimated for each site and season.

Isotopic niches of fish assemblages at each site and seasons were estimated based on bayesian standard ellipse areas (SEA_B ; Jackson *et al.* 2011), which were calculated using the SIBER package (Stable Isotope Bayesian Ellipses in R; Jackson *et al.* 2011) of SIAR (Parnell *et al.* 2008). SEAs are comparable to the univariate standard deviation and contain approximately 40% of the data compared with other isotopic space metrics, such as convex hull, and therefore provide a better and more standardized description of population/community trophic structure (Jackson *et al.* 2011). Moreover, SEAs are not affected by bias associated with the number of groups, allowing comparisons among assemblages or guilds with different members (Jackson *et al.* 2011). The small sample size-corrected SEA (SEA_C) and its overlap also were calculated for each site and season (Jackson *et al.* 2011). When SEA_C of one particular site and season (SEA_{C1}) overlapped with another particular site and season (SEA_{C2}), the overlap was given as percentage of SEA_{C1} and SEA_{C2} in which overlap occurred. It is important to note that isotopic space provides insights on a species, guild or community ecological niche, but does not solve all the issues that the niche theory aims to answer (Newsome *et al.* 2007). Hence, this isotopic space metrics should not be considered synonymous with trophic niches, but rather a proxy allowing inferences about key aspects of species or ecological community niches (Jackson *et al.* 2011). Although there is debate in the literature about the effectiveness and applicability of isotopic metrics (Layman *et al.* 2007; Layman & Post 2008; Hoeninghaus & Zeug 2008) they have been used increasingly in studies of trophic ecology with stable isotopes (Newsome *et al.* 2012; Layman *et al.* 2012; Abrantes *et al.* 2014) providing useful insights.

Results

Overall, 559 samples that included primary producers (81), other basal food sources (SPOM and BPOM) (35), invertebrates (77) and fishes (366) were collected for carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratio analysis (Tables 1 and 2). As expected, C_3 and C_4 plants were consistently positioned in the lower and upper limits, respectively, of $\delta^{13}\text{C}$ variation range at both sites (wetland and lake) and seasons (winter/wet and summer/dry) (Figure 2). $\delta^{13}\text{C}$ values ranged from -31.3‰ (*Myriophyllum* spp.) to -10.8‰ (Poaceae) in winter/wet/wet and from -28.7‰ (unidentified terrestrial plant) to -10.3‰ (Poaceae) in summer/dry at the wetland. A similar pattern was observed in the lake, with $\delta^{13}\text{C}$ values ranging from -29.6‰ (*Salvinia herzogii*) to -12.1‰ (Poaceae) during winter/wet/wet and from -29.1‰ (*Azolla filiculoides*) to -11.8‰ (Poaceae) in summer/dry. All macrophytes samples were grouped as C_3 plants with exception of *Potamogeton* spp. that had $\delta^{13}\text{C}$ values more enriched than typical C_3 and very close to C_4 range. C_3 plants had $\delta^{13}\text{C}$ values ranging from -31.3‰ (*Myriophyllum* spp.) to -23.57 (*Hydrocotyle ranunculoides*), and values of C_4 plants ranged from -18.4‰ (*Potamogeton* spp.) to -10.3‰ (Poaceae).

Other organic basal food sources had intermediate $\delta^{13}\text{C}$ values (Figure 2). With the exception of the wetland during winter/wet, all other sites/periods showed some $\delta^{13}\text{C}$ range overlap, such as SPOM and BPOM in wetland during summer/dry, and algae, BPOM, capybaras feces and even C_4 plants from the lake in both seasons (Figure 2). Algae, in particular, had the higher $\delta^{13}\text{C}$ standard deviation among all basal food sources (Table 1) ranging from -23.7‰ during winter/wet to -15.74‰ during summer/dry in the wetland (Figure 2).

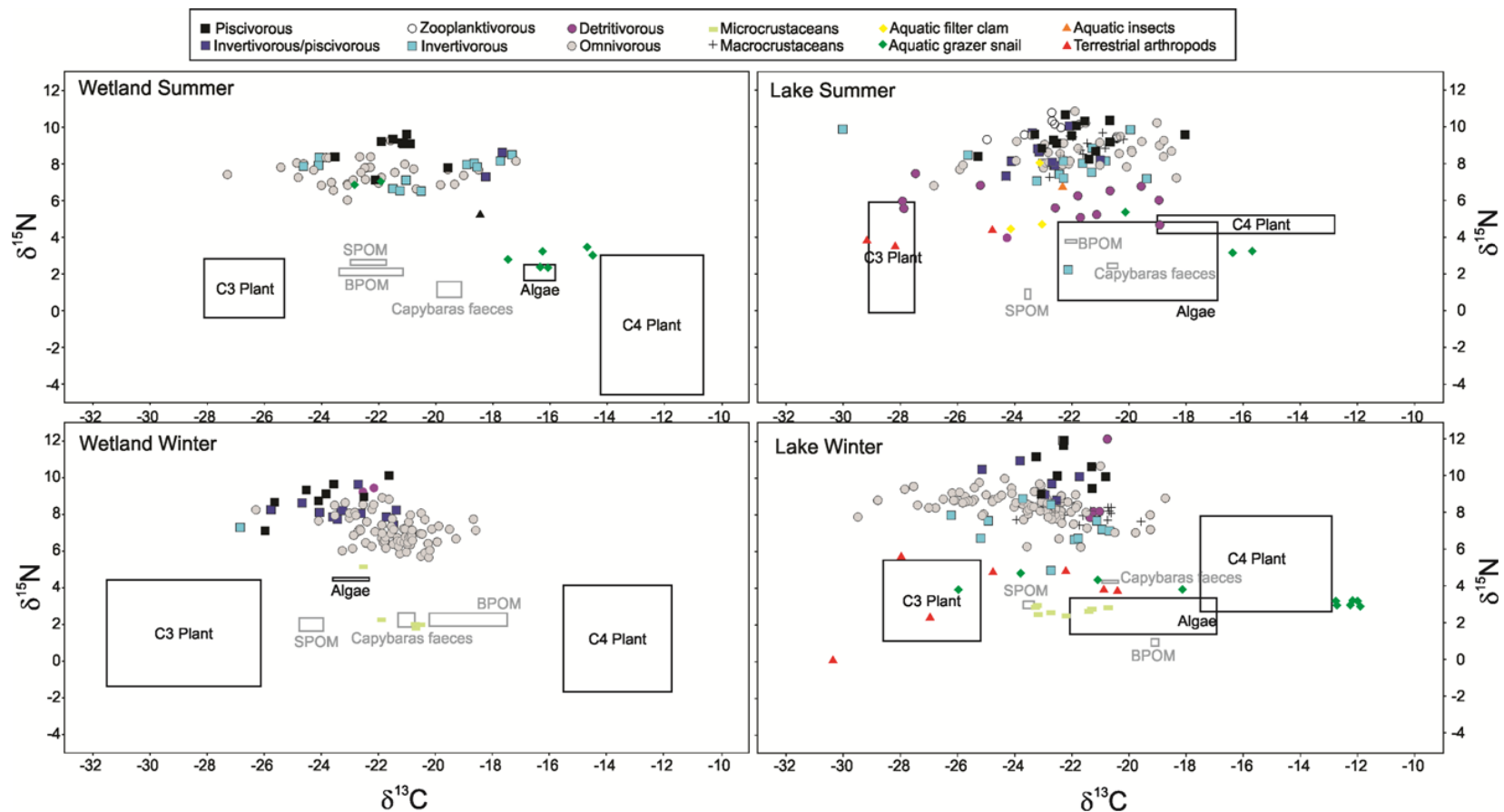


Figure 2. Average \pm standard deviation (s.d.) of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios values of primary producers (black open boxes) and basal sources (gray open boxes). Individual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of consumers groups are presented with the same symbol as described above. Samples taken in the Nicola Lake and Caçapava wetland, Taim Hydrological System, southern Brazil.

Overall, primary producer had the greatest variation on $\delta^{15}\text{N}$ values among all studied food web components. Sources from wetland had $\delta^{15}\text{N}$ values ranging from -4.1‰ (unidentified terrestrial plant) to 6.4‰ (Poaceae) in winter/wet and from -4.4‰ (*Potamogeton* sp.) to 6.6‰ (Poaceae) in summer/dry. A similar pattern was observed in the lake, with a range from -1.9‰ (*Bacopa* sp.) to 7.2‰ (*Potamogeton* sp.) in winter/wet/wet and from -1.96‰ (*Azolla filiculoides*) to 5.82‰ (*Sagittaria montevidensis*) in summer/dry. In general, C_3 and C_4 plants had higher $\delta^{15}\text{N}$ range and standard deviations than SPOM, BPOM and capybaras feces. Also, algae showed higher variation in the lake regardless of season (Table 1, Figure 2).

Almost all consumers had $\delta^{13}\text{C}$ values falling within the isotopic variation exhibited by basal food sources. Only in the lake did we observe consumer $\delta^{13}\text{C}$ values slightly ($\sim 1\text{‰}$) outside the basal source range (Figure 2, Table 2). Invertebrate consumers from the lake had broader ranges of $\delta^{13}\text{C}$ (winter/wet = insect gryllidae, -30.4‰; snail *Heleobia australis*, -11.9‰) (summer/dry = insect Coleoptera, -29.2‰; snail *Pomacea canaliculata*, -15.7‰) than invertebrate consumers at the wetland (winter/wet = zooplankton, -22.5‰; -20.5‰) (summer/dry = snail *Heleobia australis*, -22.8‰; snail *Pomacea canaliculata* -14.5‰) in both seasons (Figure 2).

With the exception of macrocrustaceans, most invertebrates had lower $\delta^{15}\text{N}$ values than fishes (Figure 2). Invertebrates ranged from 1.7‰ to 5.12‰ (zooplankton) at wetland during winter/wet and from 2.3‰ (grazer snail *Pomacea canaliculata*) to 7.0‰ (grazer snail *Heleobia australis*) during summer/dry. In the lake, invertebrates (terrestrial insect, gryllidae) ranged from 0.1‰ to 5.8‰ during winter/wet and from 3.2‰ (grazer snail *Pomacea canaliculata*) to 8.0‰ (filter clam *Corbicula fluminea*) during summer/dry. Macrocrustaceans were caught only in the lake and showed $\delta^{15}\text{N}$

ranging from 7.3‰ (*Trichodactylus* spp.) to 9.7‰ (*Palaemonetes argentinus*) in summer/dry and from 7.4‰ to 8.7‰ (*Palaemonetes argentinus*) in winter/wet (Figure 2, Table 2).

All fish species had $\delta^{13}\text{C}$ values positioned within the carbon isotopic variability of primary sources. $\delta^{13}\text{C}$ values at wetland ranged from -26.8‰ (*Hyphessobrycon igneus*) to -18.6‰ (*Cnesterodom decenmaculatus*) during winter/wet and from -27.3‰ (*Cichlasoma portalegreense*) to -17.2‰ (*Jenynsia multidentata*) during summer/dry. At the lake, $\delta^{13}\text{C}$ values ranged from -29.5‰ to -18.7‰ (*Astyanax eigenmanniorum*) during winter/wet and from -30.0‰ (*Mimagoniates inequalis*) to -18.0‰ (*Oligossarcus jenynsii*) during summer/dry (Figure 2, Table 2). Overall, fish species exhibited higher variation in $\delta^{15}\text{N}$ values during winter/wet at both sites. $\delta^{15}\text{N}$ had higher isotopic variation in the lake than wetland. $\delta^{15}\text{N}$ values at wetland ranged from 5.7‰ (*Jenynsia multidentata*) to 10.1‰ (*Hoplias* cf. *malabaricus*) during winter/wet and from 6.0‰ (*Cichlasoma portalegreense*) to 9.6‰ (*Hoplias* cf. *malabaricus*) during summer/dry. A similar pattern was observed in the lake with $\delta^{15}\text{N}$ values ranging from 5.0‰ (*Hyphessobrycon igneus*) to 12.1‰ (*Ciphocarax voga*) during winter/wet and from 2.2‰ (*Microglanis cottoides*) to 10.85‰ (*Coridoras paleatus*) during summer/dry.

Table 1. Sample size (N), isotopic values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and standard deviation (s.d.) of main producers in the Taim Hydrological System, southern Brazil. Bold and italic values represent sample size and mean and standard deviation of isotopic values by functional group.

ORGANIC SOURCES	Wetland										Lake									
	Winter/wet					Summer/dry					Winter/wet					Summer/dry				
	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.
Primary producers	22	-23.8	7.1	1.8	2.8	18	-20.2	6.9	0.7	2.6	29	-23.1	5.0	3.3	2.0	12	-22.0	6.1	3.4	2.2
<i>Algae</i>	3	-23.0	0.6	4.4	0.1	3	-16.4	0.5	2.1	0.4	9	-19.5	2.5	2.6	1.0	3	-19.7	2.8	2.7	2.1
Filamentous algae	3	-23.0	0.6	4.4	0.1	3	-16.4	0.5	2.1	0.4	9	-19.5	2.5	2.6	1.0					
Periphyton																3	-19.7	2.8	2.7	2.1
<i>C₃ Plants</i>	13	-28.8	2.7	1.5	2.9	9	-26.7	1.4	1.2	1.6	16	-27.0	1.7	3.3	2.1	5	-28.3	0.8	2.9	3.0
<i>Azolla filiculoides</i>																1	-29.1	-	-2.0	-
<i>Hydrocotyle ranuculoides</i>											3	-24.6	1.0	5.2	1.3					
<i>Sagittaria montevidensis</i>											1	-27.3	-	5.1	-	1	-28.3	-	5.8	-
<i>Salvinia herzogii</i>											3	-29.1	0.4	3.8	0.2	1	-28.4	-	2.3	-
<i>Schoenoplectus californicus</i>						3	-25.3	0.2	-0.7	0.5										
<i>Bacopa</i> spp.											4	-26.6	0.8	0.6	1.7					
<i>Juncus</i> spp.																1	-27.0	-	4.4	-
<i>Ludwigia</i> spp.	3	-30.6	0.1	0.5	0.2						3	-26.8	0.4	4.7	0.7					
<i>Myriophyllum</i> spp.	2	-31.0	0.0	2.6	0.3	3	-26.3	0.3	1.5	0.5										
Aquatic C3 plants unid.	5	-26.5	3.2	4.1	1.2						2	-28.5	0.7	2.1	0.7					
Terrestrial C3 plants unid.	3	-29.3	0.9	-2.7	1.2	3	-28.4	0.5	2.7	0.9						1	-28.9	-	3.9	-
<i>C₄ Plants</i>	6	-13.6	1.9	1.2	2.9	6	-12.4	1.8	-0.8	3.8	4	-15.2	2.3	5.3	2.6	4	-15.9	3.1	4.7	0.5
Poaceae	2	-11.4	0.9	1.8	6.5	1	-10.3	-	6.6	-	1	-12.1	-	1.5	-	1	-11.8	-	4.4	-
<i>Chara</i> spp.	1	-13.2	-	0.7	-															
<i>Potamogeton</i> spp.	3	-15.1	0.3	1.0	0.3	5	-12.8	1.6	-2.3	1.2	3	-16.3	1.1	6.6	0.6	3	-17.3	1.8	4.8	0.5

Table 1. Continuation

ORGANIC SOURCES	Wetland										Lake									
	Winter/wet					Summer/dry					Winter/wet					Summer/dry				
	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.
Basal food sources	13	-22.5	2.3	2	0.4	8	-21.3	1.6	1.9	0.7	7	-21.7	1.8	3.3	1.2	7	-22.3	1.3	2.2	1.3
SPOM	7	-24.4	0.4	1.9	0.4	2	-22.4	0.6	2.6	0.1	3	-23.5	0.2	3.1	0.2	3	-23.5	0.1	0.9	0.3
BPOM	2	-18.8	2.1	2.1	0.3	3	-22.3	1.1	2.1	0.2	1	-19.1	-	1.0	-	2	-22.0	0.2	3.8	0.1
Capybaras faeces	4	-21.1	0.5	2.2	0.4	3	-19.5	0.4	1.2	0.4	3	-20.7	0.3	4.3	0.1	2	-20.6	0.2	2.4	0.1

Table 2. Sample size (N), isotopic values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and standard deviation (s.d.) of main consumers in the Taim Hydrological System, southern Brazil. Bold values represent sample size and mean and standard deviation of isotopic values by functional group. Invertebrates indicated with “*” were used to construct the primary consumer baseline in each site and season.

CONSUMERS	Wetland										Lake									
	Winter/wet					Summer/dry					Winter/wet					Summer/dry				
	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.
FISHES																				
Piscivorous	8	-23.4	1.4	9.3	0.6	9	-21.4	1.1	8.7	0.8	8	-22.1	0.9	10.6	1.1	14	-21.9	1.6	9.4	0.7
<i>Hoplias cf. malabaricus</i>	8	-23.4	1.4	9.3	0.6	9	-21.4	1.1	8.7	0.8	3	-22.4	1.0	10.6	0.5	4	-22.5	0.5	9.3	0.5
<i>Oligosarcus jenynsii</i>											5	-21.9	0.9	10.5	1.3	10	-21.6	1.9	9.4	0.8
Invertivorous/piscivorous	12	-23.2	1.3	8.2	0.6	2	-18.0	0.4	7.9	0.9	7	-23.0	1.1	10.2	1.1	10	-23.0	1.0	8.5	0.8
<i>Charax stenopterus</i>																1	-23.2	-	8.8	-
<i>Crenicichla lepidota</i>	2	-21.6	0.2	7.6	0.3	2	-18.0	0.4	7.9	0.9	1	-22.9	-	9.1	-	1	-21.0	-	8.2	-
<i>Pimelodella australis</i>																1	-22.1	-	10.1	-

Table 2. Continuation

CONSUMERS	Wetland										Lake										
	Winter/wet					Summer/dry					Winter/wet					Summer/dry					
	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	
FISHES																					
Invertivorous/piscivorous	12	-23.2	1.3	8.2	0.6	2	-18.0	0.4	7.9	0.9	7	-23.0	1.1	10.2	1.1	10	-23.0	1.0	8.5	0.8	
<i>Pimelodus pintado</i>											3	-23.8	1.4	11.2	0.8						
<i>Rhamdia quelen</i>	1	-24.7	-	8.6	-						2	-22.6	0.1	9.2	0.6	7	-23.3	0.7	8.2	0.7	
<i>Tracheleopterus lucenai</i>	9	-23.3	1.2	8.3	0.6						1	-21.7	-	10.1	-						
Zooplanktivorous																8	-22.5	1.5	9.9	0.5	
<i>Odontesthes</i> spp.																2	-24.3	0.9	9.4	0.2	
<i>Platanichthys platana</i>																6	-21.9	1.1	10.0	0.5	
Invertivorous	1	-26.8	-	7.3	-	12	-20.7	2.6	7.6	0.7	12	-22.9	1.8	7.4	1.1	14	-22.5	2.6	7.7	1.8	
<i>Bunocephalus iheringii</i>																5	-21.5	1.0	8.2	1.0	
<i>Characidium rachovii</i>						4	-21.1	0.4	6.7	0.3											
<i>Geophagus brasiliensis</i>											3	-23.1	0.6	8.7	0.2	1	-22.3	-	8.1	-	
<i>Hyphessobrycon igneus</i>	1	-26.8	-	7.3	-	6	-20.2	3.3	8.1	0.3	9	-22.8	2.1	7.0	0.9	1	-19.4	-	7.2	-	
<i>Microglanis cottoides</i>																3	-22.5	0.6	5.5	2.8	
<i>Mimagoniates inaequalis</i>																2	-27.8	3.1	9.2	1.0	
<i>Pseudocorynopoma doriae</i>																2	-21.1	0.3	8.5	0.5	
<i>Synbranchus marmoratus</i>						2	-21.4	3.9	7.9	0.1											
Omnivorous	79	-21.5	1.2	7.0	0.8	35	-22.4	2.1	7.5	0.7	82	-23.8	2.2	8.5	0.8	35	-21.7	2.1	8.7	0.9	
<i>Astyanax eigenmanniorum</i>	19	-21.6	1.4	6.9	0.8	5	-23.1	1.6	8.1	0.3	24	-24.8	2.2	8.6	0.4	6	-21.6	1.5	9.4	0.8	
<i>Astyanax fasciatus</i>											2	-22.4	1.9	9.7	1.3						
<i>Australoheros acaroides</i>						11	-21.5	1.7	7.2	0.7											
<i>Cheirodon interruptus</i>	7	-21.9	0.3	6.5	0.2	6	-23.7	1.1	7.9	0.2	24	-24.4	2.3	8.7	0.6	8	-23.3	1.9	8.4	0.5	

Table 2. Continuation

CONSUMERS	Wetland										Lake										
	Winter/wet					Summer/dry					Winter/wet					Summer/dry					
	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	
FISHES																					
Omnivorous	79	-21.5	1.2	7.0	0.8	35	-22.4	2.1	7.5	0.7	82	-23.8	2.2	8.5	0.8	35	-21.7	2.1	8.7	0.9	
<i>Cichlasoma portalegrense</i>						5	-24.6	1.7	6.8	0.6											
<i>Cnesterodon decemmaculatus</i>	5	-20.7	1.8	7.2	0.6	2	-22.0	0.7	7.6	0.2											
<i>Corydoras paleatus</i>																7	-22.9	1.9	8.4	1.3	
<i>Hyphessobrycon luetkenii</i>											27	-23.1	0.9	8.2	0.9						
<i>Jenynsia multidentata</i>	48	-21.5	1.2	7.1	0.8	6	-20.2	2.2	7.5	0.5	5	-20.3	1.6	7.7	0.9	12	-19.8	1.4	8.5	0.8	
<i>Loricariichthys</i> spp.																2	-22.8	0.8	9.3	0.3	
Detritivorous	2	-22.3	0.3	9.3	0.1						4	-21.1	0.3	9.1	2.0	12	-22.8	3.4	6.0	0.8	
<i>Cyphocharax voga</i>	2	-22.3	0.3	9.3	0.1						2	-21.1	0.4	10.0	3.0	4	-27.1	1.3	6.5	0.9	
<i>Hypostomus commersonii</i>											2	-21.1	0.1	8.2	0.0	8	-20.7	1.4	5.8	0.7	
INVERTEBRATES																					
Macrocrustaceans											15	-21.6	1.2	8.1	0.4	12	-21.6	0.9	8.8	0.7	
<i>Trichodactylus</i> spp.																3	-22.6	0.2	7.9	0.6	
<i>Macrobrachium</i> spp.																3	-21.8	0.3	9.4	0.2	
<i>Palaeomonetes argentinus</i>											15	-21.6	1.2	8.1	0.4	6	-21.0	0.6	9.0	0.4	
Microcrustaceans	5	-21.3	0.9	2.6	1.4						8	-22.3	1.0	2.8	0.2						
Amphipoda*											8	-22.3	1.0	2.8	0.2						
Zooplankton*	5	-21.3	0.9	2.6	1.4																
Aquatic filter clam																4	-23.6	0.7	5.3	1.9	
<i>Corbicula fluminea</i> *																4	-23.6	0.7	5.3	1.9	
Aquatic grazer snails						8	-17.5	3.2	3.9	1.9	10	-16.3	5.5	3.6	0.6	3	-17.4	2.4	3.9	1.3	
<i>Heliobia australis</i> *						2	-22.4	0.7	6.9	0.1	6	-12.3	0.4	3.2	0.1	1	-20.1	-	5.4	-	

Table 2. Continuation

CONSUMERS	Wetland					Lake														
	Winter/wet			Summer/dry			Winter/wet			Summer/dry										
	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.					
Aquatic grazer snails						8	-17.5	3.2	3.9	1.9	10	-16.3	5.5	3.6	0.6	3	-17.4	2.4	3.9	1.3
<i>Pomacea canaliculata</i> *						6	-15.9	1.1	2.9	0.5	4	-22.2	3.4	4.3	0.4	2	-16.0	0.5	3.2	0.1
Aquatic insects						1	-18.4	-	5.3	-						2	-23.6	1.7	5.6	1.7
Belostomatidae																1	-24.8	-	4.4	-
Ephemeroptera						1	-18.4	-	5.3	-										
Odonata																1	-22.3	-	6.8	-
Terrestrial arthropods											7	-24.8	3.8	3.7	1.9	2	-28.7	0.7	3.7	0.2
Araneae											1	-24.8	-	4.9	-					
Coleoptera*																2	-28.7	0.7	3.7	0.2
Formicidae											1	-22.2	-	5.0	-					
Gryllidae*											5	-25.3	4.4	3.3	2.1					

Omnivorous and piscivorous were the most common fish guilds at each site during both seasons, and this allowed for spatiotemporal comparison of trophic positions, trophic linkages with basal food sources derived from stable isotope mixing models, and isotopic niches based on stable isotope ellipses. The Kruskal-Wallis test revealed that primary consumers baseline had no significant difference between sites but significantly higher values during summer/dry (lake: 4.5 \pm 1.6‰; wetland: 3.9 \pm 1.9‰) than winter/wet (lake: 3.14 \pm 0.9‰; wetland: 2.64 \pm 1.4‰) ($p = 0.023$). Average trophic position of fish guilds showed an opposite pattern (Figure 3, Table 3). ANOVA showed no interaction between effects, so trophic positions of omnivores and piscivores were tested separately by site and season. Omnivores had higher average trophic position during winter/wet (3.9) than summer/dry (3.5) ($p = 0.001$). The piscivore guild revealed the same pattern with higher average trophic position during winter/wet (4.8) than summer/dry (3.9) ($p = 0.001$) (Figure 3). There was no significant difference in trophic position between sites for piscivores ($p = 0.145$), but omnivores had slightly higher values in the lake (3.9) than wetland (3.6) ($p = 0.001$).

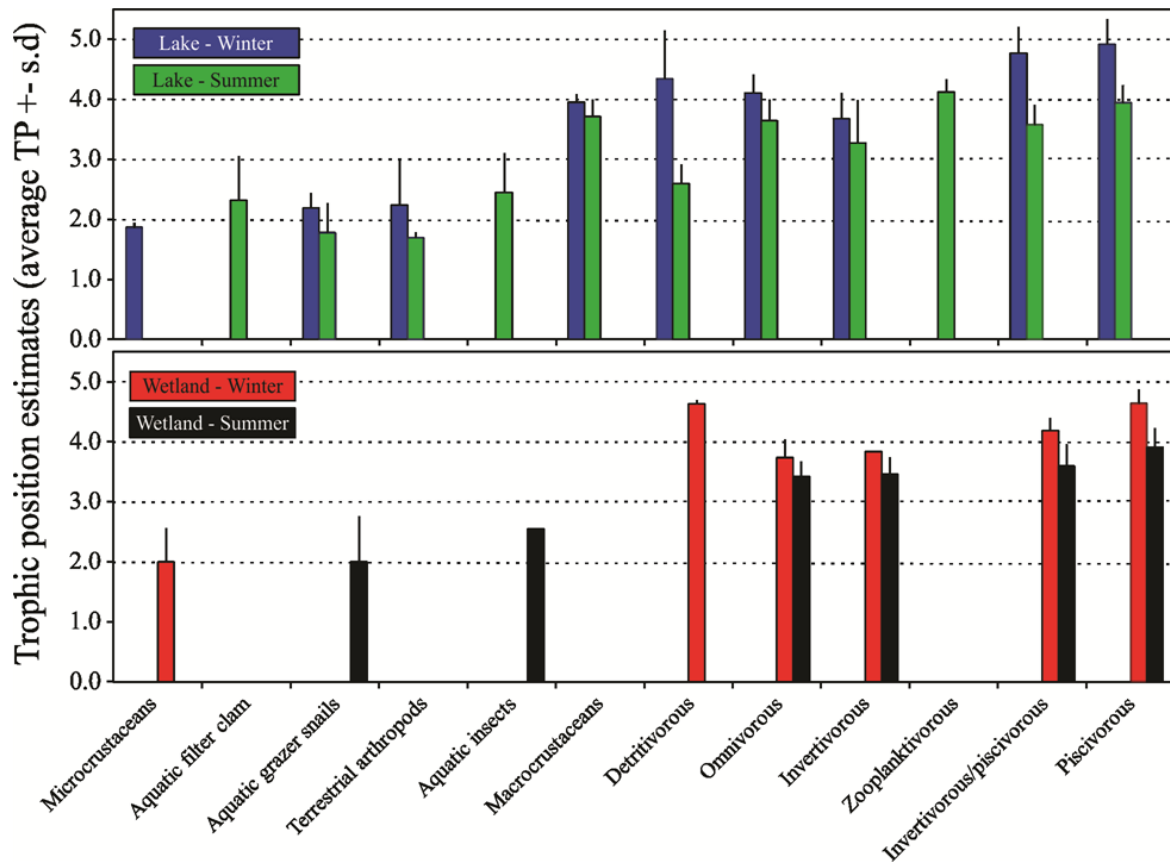


Figure 3. Average of estimated trophic positions (TP) and standard deviations (s.d.) of invertebrate consumer groups and fish trophic guilds along sites and seasons. Samples taken in the Nicola Lake and Caçapava wetland, Taim Hydrological System, southern Brazil.

Table 3. Sample size (N), mean trophic position (TP) and standard deviation (s.d.) of main consumers in the Taim Hydrological System, southern Brazil. Bold values represent mean and standard deviation values by functional group.

CONSUMERS	Wetland						Lake					
	Winter/wet			Summer/dry			Winter/wet			Summer/dry		
	N	TP	s.d.	N	TP	s.d.	N	TP	s.d.	N	TP	s.d.
FISHES												
Piscivorous	8	4.6	0.2	9	3.9	0.3	8	4.9	0.4	14	3.9	0.3
<i>Hoplias cf. malabaricus</i>	8	4.6	0.2	9	3.9	0.3	3	5.0	0.2	4	3.9	0.2
<i>Oligosarcus jenynsii</i>							5	4.9	0.5	10	4.0	0.3
Invertivorous/piscivorous	12	4.2	0.2	2	3.6	0.4	7	4.8	0.4	10	3.6	0.3
<i>Charax stenopterus</i>										1	3.7	-
<i>Crenicichla lepidota</i>	2	4.0	0.1	2	3.6	0.4	1	4.3	-	1	3.5	-

Table 3. Continuation

CONSUMERS	Wetland						Lake					
	Winter/wet			Summer/dry			Winter/wet			Summer/dry		
	N	TP	s.d.	N	TP	s.d.	N	TP	s.d.	N	TP	s.d.
FISHES												
Invertivorous/piscivorous	12	4.2	0.2	2	3.6	0.4	7	4.8	0.4	10	3.6	0.3
<i>Crenicichla lepidota</i>	2	4.0	0.1	2	3.6	0.4	1	4.3	-	1	3.5	-
<i>Pimelodella australis</i>										1	4.2	-
<i>Pimelodus pintado</i>							3	5.2	0.3			
<i>Rhamdia quelen</i>	1	4.4	-				2	4.4	0.3	7	3.5	0.3
<i>Tracheleopterus lucenai</i>	9	4.2	0.2				1	4.7	-			
Zooplanktivorous										8	4.1	0.2
<i>Odontesthes</i> spp.										2	4.0	0.1
<i>Platanichthys platana</i>										6	4.2	0.2
Invertivorous	1	3.8	-	12	3.5	0.3	12	3.7	0.4	14	3.3	0.7
<i>Bunocephalus iheringii</i>										5	3.5	0.4
<i>Characidium rachovii</i>				4	3.1	0.1						
<i>Geophagus brasiliensis</i>							3	4.2	0.1	1	3.4	-
<i>Hyphessobrycon igneus</i>	1	3.8	-	6	3.7	0.1	9	3.5	0.4	1	3.1	-
<i>Microglanis cottoides</i>										3	2.4	1.1
<i>Mimagoniates inequalis</i>										2	3.8	0.4
<i>Pseudocorynopoma doriae</i>										2	3.6	0.2
<i>Synbranchus marmoratus</i>				2	3.6	0.0						
Omnivorous	79	3.7	0.3	35	3.4	0.3	82	4.1	0.3	35	3.6	0.3
<i>Astyanax eigenmanniorum</i>	19	3.7	0.3	5	3.7	0.1	24	4.2	0.2	6	3.9	0.3
<i>Astyanax fasciatus</i>							2	4.6	0.5			
<i>Australoheros acaroides</i>				11	3.3	0.3						
<i>Cheirodon interruptus</i>	7	3.5	0.1	6	3.6	0.1	24	4.2	0.2	8	3.6	0.2
<i>Cichlasoma portalegreense</i>				5	3.1	0.2						
<i>Cnesterodon decemmaculatus</i>	5	3.8	0.2	2	3.5	0.1						
<i>Corydoras paleatus</i>										7	3.5	0.5
<i>Hyphessobrycon luetkenii</i>							27	4.0	0.4			
<i>Jenynsia multidentata</i>	48	3.8	0.3	6	3.4	0.2	5	3.8	0.4	12	3.6	0.3
<i>Loricariichthys</i> spp.										2	3.9	0.1
Detritivorous	2	4.6	0.1				4	4.3	0.8	12	2.6	0.3
<i>Cyphocharax voga</i>	2	4.6	0.1				2	4.7	1.2	4	2.8	0.3
<i>Hypostomus commersonii</i>							2	4.0	0.0	8	2.5	0.3
INVERTEBRATES												
Macrocrustaceans							15	4.0	0.1	12	3.7	0.3
<i>Cyrtograpsus angulatus</i>										3	3.3	0.2
<i>Macrobrachium</i> spp.										3	3.9	0.1
<i>Palaeomonetes argentinus</i>							15	4.0	0.1	6	3.8	0.2
Microcrustaceans	5	2.0	0.6				8	1.9	0.1			
Amphipoda*							8	1.9	0.1			
Zooplankton*	5	2.0	0.6									

Table 3. Continuation.

CONSUMERS	Wetland						Lake					
	Winter/wet			Summer/dry			Winter/wet			Summer/dry		
	N	TP	s.d.	N	TP	s.d.	N	TP	s.d.	N	TP	s.d.
Microcrustaceans	5	2.0	0.6				8	1.9	0.1			
Amphipoda*							8	1.9	0.1			
Zooplankton*	5	2.0	0.6									
Aquatic filter clam										4	2.3	0.7
<i>Corbicula fluminea</i> *										4	2.3	0.7
Aquatic grazer snails				8	2.0	0.8	10	2.2	0.2	3	1.8	0.5
<i>Heliobia australis</i> *				2	3.2	0.0	6	2.0	0.1	1	2.3	-
<i>Pomacea canaliculata</i> *				6	1.6	0.2	4	2.5	0.2	2	1.5	0.0
Aquatic insects				1	2.5	-				2	2.4	0.7
Belostomatidae										1	2.0	-
Ephemeroptera				1	2.5	-						
Odonata										1	2.9	-
Terrestrial arthropods							7	2.2	0.8	2	1.7	0.1
Araneae							1	2.7	-			
Coleoptera*										2	1.7	0.1
Formicidae							1	2.7	-			
Gryllidae*							5	2.0	0.8			

Stable isotope mixing models were performed for omnivorous and piscivorous fish separately, considering the trophic discrimination factors expected to occur between each's estimated trophic position and primary producers (Table 4). The mixing models revealed differences on assimilation of organic matter derived from various primary producers between sites and seasons (Figure 4, Table 5). Overall, aquatic primary production sources (C₃ plants + algae) rather than terrestrial primary production sources (C₄ plants) were the most assimilated in all situations (ranging from 3 to 77%, IC95%). C₃ plants were the source most assimilated by consumers, ranging from 29% in wetland during winter/wet to 77% in wetland during summer/dry. Algae also were important, with lower contributions to fish in the wetland during summer/dry (3 – 41%) and higher in the wetland during winter/wet (16 - 62%). C₄ plants contributed less to consumer

biomass in the lake, with estimated percentages ranging from zero during winter/wet to 45 during summer/dry. In general, the contribution of C₄ plants to fish biomass in the wetland increased from summer/dry to winter/wet, but the opposite pattern was observed at lake for omnivorous fish (Figura 4, Table 5). C₃ plants were the dominant source during summer/dry at wetland site for both guilds (Figure 4, Table 5).

Table 4. Trophic discrimination factors (TDFs) used on stable isotope mixing models for each fish consumer group, site and season. Samples taken in the Nicola Lake and Caçapava wetland, Taim Hydrological System, southern Brazil.

GUILD	Wetland				Lake			
	Winter/wet		Summer/dry		Winter/wet		Summer/dry	
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Piscivorous	1.18	7.50	1.28	8.15	1.13	7.15	0.94	5.95
Omnivorous	0.81	5.17	1.09	6.91	0.80	5.09	0.82	5.19

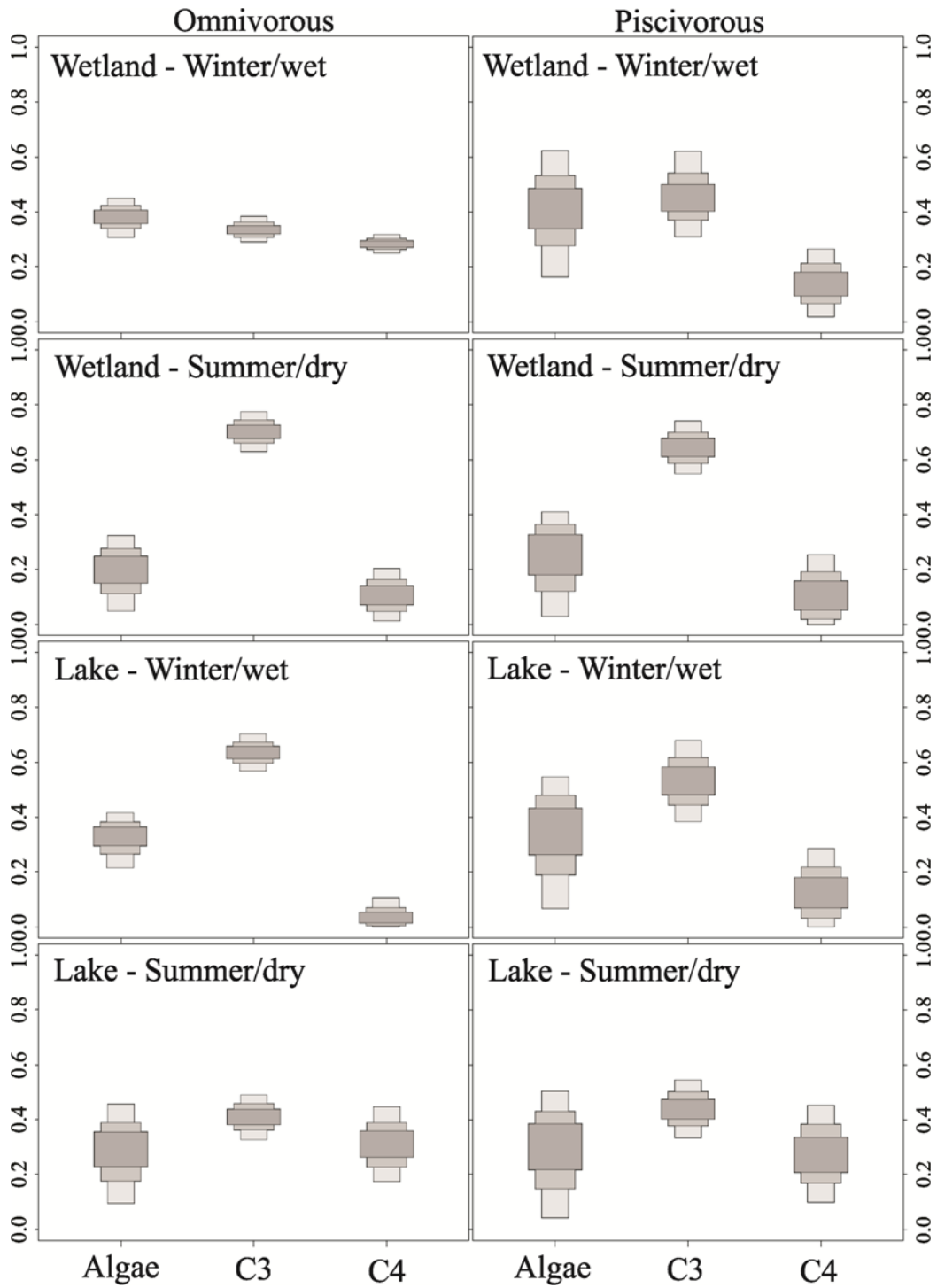


Figure 4. Relative contributions of primary producer sources to Omnivorous and Piscivorous fish guild on each situation: wetland during winter/wet; wetland during summer/dry; lake during winter/wet and lake during summer/dry. Bayesian credible intervals of the feasible contributions of each primary producer source to the fish guilds: 50 (darkest gray), 75 (medium gray) and 95% (lightest gray). Samples taken in the Nicola Lake and Caçapava wetland, Taim Hydrological System, southern Brazil.

Table 5. Stable isotope mixing models contribution estimates of predominantly primary producers of aquatic (C_3 plants and Algae) and terrestrial (C_4 plants) habitats for each fish consumer group, site and season. Samples taken in the Nicola Lake and Caçapava wetland, Taim Hydrological System, southern Brazil.

GUILD	Wetland				Lake			
	Winter/wet		Summer/dry		Winter/wet		Summer/dry	
	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Piscivorous								
Algae	0.16	0.62	0.03	0.41	0.05	0.54	0.04	0.50
C_3 Plant	0.31	0.62	0.55	0.74	0.35	0.68	0.33	0.54
C_4 Plant	0.02	0.27	0.01	0.25	0.02	0.32	0.01	0.45
Omnivorous								
Algae	0.31	0.45	0.05	0.32	0.27	0.47	0.09	0.46
C_3 Plant	0.29	0.38	0.63	0.77	0.52	0.66	0.33	0.49
C_4 Plant	0.25	0.32	0.01	0.20	0.00	0.09	0.18	0.45

The standard ellipses differed in size, shape, position and overlap in the isotopic space ($\delta^{13}C$ vs. TP units) (Figure 5) among sites and periods. For all fish assemblages and guilds (Figure 5A), the isotopic area (SEA_C) was greater in lake (L) than wetland (W), and greater during summer/dry (lake = 4.07; wetland = 2.24) than winter/wet (lake = 2.99, wetland = 1.69) at both sites (Figure 5, A). Isotopic areas occupied by fish assemblages were wider in both dimensions ($\delta^{13}C$ and TP units), and fish were more depleted in $\delta^{13}C$ in lake (Figure 5A). Between-site overlap of isotopic ellipses was higher during summer/dry, with 93% of the wetland area overlapping with 52% of the lake area, than during winter/wet, where 19% of the lake area overlapped with 25% of the wetland. Overlap between seasons was higher for the wetland, with 30% of summer/dry area overlapped with 40% of winter/wet area, than for lake, where 19% of summer/dry area overlapped with 25% of winter/wet area (Figure 5A).

Although the isotopic space occupied by omnivores was smaller than the area occupied by the entire fish assemblage, the two revealed similar patterns of spatiotemporal variation (lake summer/dry = 2.23; lake winter/wet = 1.93; wetland summer/dry = 1.71; wetland winter/wet = 1.10) (Figure 5B). TP and $\delta^{13}\text{C}$ dimensions had similar amplitudes between sites and season, with exception of winter/wet in wetland, in which a narrow $\delta^{13}\text{C}$ range was observed. However, the TP dimension was greater during winter/wet for both sites. Conversely, $\delta^{13}\text{C}$ dimension values were lower during winter/wet than summer/dry in the lake, and higher during winter/wet than summer/dry in the wetland. Overlap between seasons was higher for wetland (19% of summer/dry and 30% of winter/wet) than for lake (6% of summer/dry and 7% of winter/wet) (Figure 5B). Overlap between sites was higher during summer/dry (62% of wetland and 47% lake) than winter/wet (17% of wetland and 10% of lake).

Isotopic areas occupied by piscivores differed between seasons for the wetland (summer/dry = 1.08; wetland winter/wet = 0.65) to a greater extent than for the lake (summer/dry = 1.44; winter/wet = 1.14) (Figure 5C). The range of $\delta^{13}\text{C}$ was relatively narrow for the lake during winter/wet and for the wetland during summer/dry. The TP dimension was widest for lake and narrowest for wetland during winter/wet. There was no SEA_C overlap between summer/dry and winter/wet at both sites, and this was largely due to separation in the vertical dimension of isotopic space (TP axis) (Figure 5C). SEA_C overlap between sites was higher during summer/dry, when 84% of wetland overlapped with 66% of lake area. During winter/wet, overlap was lower with 27% of wetland and 15% of lake overlapping.

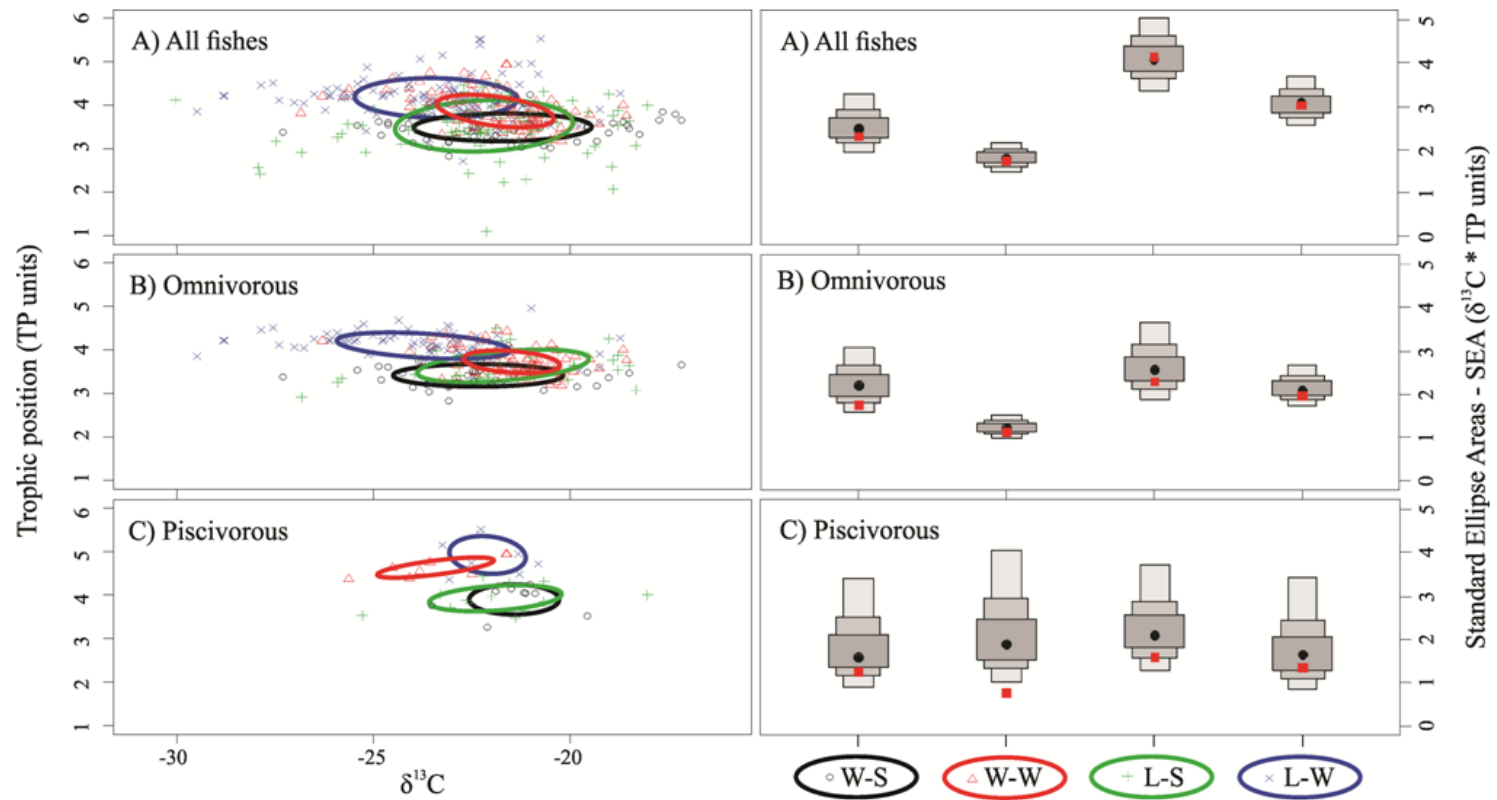


Figure 5. Estimated individual trophic position and $\delta^{13}\text{C}$ values of fishes (left graphics). Solid lines enclose the standard ellipses area corrected for small samples (SEAc), containing c. 40% of the data, showing the isotopic space for all the fish assemblages (A), omnivorous (B), and piscivorous (C) guilds by site (wetland – W; lake – L) and season (summer/dry – S; winter/wet – W). Density plots (right graphics) showing the credibility intervals (50 (darkest gray), 75 (medium gray) and 95% (lightest gray)) and modes (black dots) of the estimated bayesian standard ellipse areas (SEA_B) and the mode (red squares) of the estimated bayesian standard ellipse area corrected for small samples (SEA_C) by site and season. Samples taken in the Nicola Lake and Caçapava wetland, Taim Hydrological System, southern Brazil.

Discussion

$\delta^{13}\text{C}$ values of aquatic (mostly C_3) and terrestrial (mostly C_4) plants at both sites during both seasons were in accordance with values reported previously from this region (Garcia *et al.* 2007; Hoeninghaus *et al.* 2011; Rodrigues *et al.* 2014; Oliveira *et al.* 2014). Previous studies reported freshwater C_3 macrophytes at the lower extreme of carbon range of primary producers; however, C_4 plants were only reported for brackish habitats. We obtained $\delta^{13}\text{C}$ values in accordance with terrestrial plants that use the C_4 photosynthetic pathway (grass - Poaceae) plus certain freshwater macrophytes that use the C_3 pathway (*Potamogeton* spp.). Our grass samples had carbon isotopic values similar with reported for estuarine C_4 plants in this region (Garcia *et al.* 2007; Hoeninghaus *et al.* 2011) with $\delta^{13}\text{C}$ values ranging from -10.3‰ to -12.1‰. In contrast, *Potamogeton* spp. samples were more variable and enriched in $\delta^{13}\text{C}$ (-12.93 to -18.41) than typical C_3 plants. Cremona *et al.* (2009) reported a similar mean value for *Potamogeton perfoliatus* ($-16.5 \pm 1.1\text{‰}$) similar with our mean value ($-14.99 \pm 2.26\text{‰}$). Although classified *Potamogeton* as a C_3 plant, Beer & Wetzel (1982) noted that *Potamogeton* revealed a high initial signal for malate and PEPcase activities compared with other macrophytes they analyzed. PEPcase activity is typical for C_4 plants, and this might be the reason *Potamogeton* had a relatively heavy carbon isotope ratio. Heavy ratios also suggest that *Potamogeton* species, which are submersed macrophytes, could use both CO_2 and HCO_3^- as inorganic Carbon sources (Marcenko *et al.* 1989). The $\delta^{13}\text{C}$ values of HCO_3^- is usually different and more variable than CO_2 values (Marcenko *et al.* 1989). Consequently, a preference for HCO_3^- over CO_2 could cause ^{13}C -enriched values in *Potamogeton* species.

A similar pattern was observed for algae, which had intermediate $\delta^{13}\text{C}$ values (-23.7 to -15.74‰). Algae often reveal a large range in $\delta^{13}\text{C}$ values, because of their capability to use either CO_2 or HCO_3^- as an inorganic carbon source (Marcenko *et al.* 1989). Algae can have values as low as -40‰ (Ou & Winemiller *in press*) and as high as -10‰ in estuaries (Hoeinghaus *et al.* 2011; Claudino, Abreu & Garcia 2013).

As expected, SPOM, BPOM and capybara feces had carbon isotope ratios reflecting a mixture of C_3 , C_4 and algae. SPOM and BPOM had similar isotopic values, particularly during the summer/dry period. Low water levels during this period, combined with wind action, probably promoted water column mixing in these shallow systems as well as greater exchange between pelagic and benthic compartments. In Mangueira Lake, the largest lake inside the Taim hydrological system, when the wind blows from the south, water piles up in the northern portion of the lake and invades the adjacent Taim wetlands (Rodrigues *et al.* 2015). After a change in the wind direction, usually to the northeast, the water flows back into Mangueira Lake, carrying organic matter and nutrients (Rodrigues *et al.* 2015). This effect of wind on organic matter flow, between Mangueira Lake and adjacent wetlands also appears to occur at smaller scales as noted here and in other shallow aquatic environments (Vizzini & Mazzola 2003; De Vicente *et al.* 2006).

We obtained a large range of $\delta^{15}\text{N}$ values for primary producer samples. Such variation was not observed in Magueira Lake (Rodrigues *et al.* 2014). Previous research involving greater spatio-temporal coverage found wide variation in $\delta^{15}\text{N}$ of primary producers (Claudino *et al.* 2013; unpublished data). Algae was the primary producer with greatest $\delta^{15}\text{N}$ variability, probably because our algae samples, such as periphyton, could have been contaminated with prokaryotic and eukaryotic microbes

and small metazoans normally present in a freshwater aquatic biofilm sample (Safi *et al.* 2014). In fact, Brito *et al.* (2006) found that algae biomass in periphyton samples of a tropical stream was between 3.9 and 17% periphyton biomass. The lower range and standard deviation of other organic basal sources (SPOM, BPOM and capybaras feces) could indicate that these pools derive from mixtures of the three primary producer groups.

In general, the $\delta^{13}\text{C}$ range of consumers fell within the ranges of primary producers, indicating that our sampling covered the most important sources of organic matter entering aquatic food webs. Exceptions were terrestrial insects, such as Gryllidae (-30.4‰) and Coleoptera (-29.2‰), that had values near the lower end of the ranges for plants. These insects could have fed within terrestrial habitats distant from wetland areas (Brito *et al.* 2006; Ometto *et al.* 2006; Correa & Winemiller 2014).

$\delta^{15}\text{N}$ seemed to provide a reasonable proxy for vertical trophic structure, or trophic position (TP); however, there were some exceptions. $\delta^{15}\text{N}$ of macrocrustaceans and detritivorous fishes were higher than expected based on their presumptive TPs. Several papers have shown that trophic discrimination factors can be higher for herbivores and detritivores that rely on low quality organic material, which can produce unexpected high $\delta^{15}\text{N}$ values (Mill *et al.* 2007; Lujan, German & Winemiller 2011; Prado *et al.* 2012; Hussey *et al.* 2014). Among Taim consumers, the highest $\delta^{15}\text{N}$ obtained was for *Ciphocarax voga* (12.1‰), a detritivorous fish that should be at the second trophic level (Corrêa & Piedras 2008). This species had $\delta^{15}\text{N}$ values as low as 5.56‰. Although to a lesser degree, this species' trophic position was also overestimated in Mangueira lake, due to mean $\delta^{15}\text{N}$ (8.2‰) and was the fish

species with higher standard deviation (Rodrigues *et al.* 2014). Such a large range for $\delta^{15}\text{N}$ could be caused by large variation observed for $\delta^{15}\text{N}$ of detritus samples that may have included organisms at different trophic levels. It also could have been influenced by trophic discrimination factors that can be more variable for detritivores feeding on poor quality food sources and having higher excretion rates (Mill *et al.* 2007).

Fish $\delta^{15}\text{N}$ values were more variable and generally higher during winter/wet at both sites. Fish guilds had higher trophic positions during winter/wet in both sites. Such evidence suggests that there was an overall contraction of vertical trophic structure during the summer/dry period and expansion during winter/wet. Such seasonal variation could be due to a greater availability of macrophytes and algae during the summer/dry period, which could cause a higher consumption of that during this period by omnivores. This change could consequently shorten the vertical structure during summer/dry. Another factor that could explain such pattern is that during summer/dry more links, nodes and connectance are typically found in estuarine ecosystems (Akin & Winemiller 2006). For omnivores, higher number of available resources food can lead to an expansion in horizontal structure but at the same time a contraction in the vertical structure of food webs. Besides the temporal variation in both sites, there was difference between lake and wetland. The lower trophic position of omnivorous in wetland site suggested that this guild fed over sources at higher trophic levels in lake and lower trophic levels in wetland. As mentioned before, the lake is bigger and more stable than wetland, since the wetland can even totally dry during prolonged periods with water deficit.

Therefore, our results might reflect the predictions about ecosystem size and also disturbance, since we don't have arguments to infer different productivity (or resource availability) between sites. However, the productivity is lower during winter/wet in this region, which means that productivity could be inversely related to food chain length in these small lakes and wetlands. Meanwhile during winter/wet, with the flood pulse, the increase of aquatic area and the connectance with terrestrial environments seemed to overlap the autochthonous productivity factor, including the productivity of terrestrial environments around them.

As expected, aquatic primary producers were the most important carbon source for the entire community. Although their focus were not on that question and their sampling design were limited, the previous studies by Garcia *et al.* (2006) and Rodrigues *et al.* (2014) studying the same lake (Nicola) and other lake (Mangueira) that is also part of Taim Hydrological System, respectively, showed that fish $\delta^{13}\text{C}$ values were in accordance with C_3 macrophyte values range. In fact, C_3 macrophytes were also the most important source in Parana River's floodplain (Manetta, Benedito-Cecilio & Martinelli 2003).

In general, algae was the second source in importance for the omnivorous and piscivorous fishes here. Probably, there were functional groups, like groups that segregate their foraging area or that occupy specific microhabitat, inside omnivorous and piscivorous guilds that could use algae as the most important carbon source. For example, in Lopes *et al.* (2015) they found algae (as periphyton) as the most important carbon source for bottom-feeding fishes in a tropical floodplain.

The terrestrial plants (C_4) was the source with less estimated contribution in all situations, but as hypothesized, a higher importance was achieved during winter/wet in wetland site. Although we didn't test because of the sample size, another evidence of, at least availability, higher terrestrial input during winter/wet was the apparently more enriched carbon signature of BPOM during winter/wet in both sites. Such fact could be explained by the increase contribution of terrestrial grasses (C_4) into detritus food chain when aquatic environments floods over grasslands, especially in wetland site, where BPOM samples were more enriched and variable during winter/wet.

However, the estimated C_4 contribution in lake was slightly higher during summer/dry. Even with C_3 e algae as the most assimilated primary producers during Summer/dry, the increase of C_4 contribution was unexpected. A possible explanation is that during this period our C_4 samples were more depleted than other situations, even overlapping with algae values. Such overlap could promote a bad model resolution since the sources had similar isotopic values.

It is important to notice that we did not evaluated the dissolved organic carbon (DOC) source here. Isotopic values of SPOM and DOC can be distinct (Ziegler & Fogel 2002; Marinho *et al.* 2010). Such carbon source had as primary constituent, the terrestrial primary producers in a tropical coastal lagoon (Marinho *et al.* 2010). Such terrestrial carbon source could be, at least available, for the communities, since our mixing models would be able to trace the terrestrial primary producer importance no matter if dissolved or particulated. There are evidences that, even with high availability, C_4 plants are less assimilated than C_3 plants (Forsberg *et al.* 1993; Clapcott & Bunn 2003).

In general, the isotopic “niche” followed our hypothesis that the isotopic space occupied by the fish assemblage would be larger in lake than wetland. Besides, the standard ellipses were more carbon depleted in lake, indicating greater contribution of aquatic macrophytes in the former. Isotopic ellipses for wetland fishes were slightly displaced to right, indicating greater contributions of terrestrial plants. The TP dimension was also broader in lake, indicating an environment with more trophic levels and so more stable (Sternler, Bajpai & Adams 1997). Nonetheless, the ellipse areas were higher during summer/dry, a pattern unexpected by our first hypothesis, since with the terrestrial connection the isotopic space would enlarge. In the meantime, the foundations of the criticism on extrapolate isotopic space metrics to niche space theory (Hoeninghaus & Zeug 2008) could explained the isotopic area contraction during winter/wet even with hypothetical niche enlargement. We could not discard the niche enlargement during winter/wet, since many species or resource groups can share isotopic values while few species or resource groups can show more distinct isotopic values (Hoeninghaus & Zeug 2008). In this way, communities using more resources can show lower isotopic spaces than communities using just few but isotopically distinct resources.

This issue was partially removed since we changed the $\delta^{15}\text{N}$ values for the estimated TP values in the isotopic space, eliminating part of the assumption on the vertical dimension of space. However, the horizontal dimension of isotopic space remained with assumptions that eventually cannot be adequate, as on the examples proposed by Hoeninghaus & Zeug (2008) and consequently could be occurred here. Meanwhile, the mixing model results showed a higher terrestrial incorporation during winter/wet, at least in wetland. Thereby, the higher terrestrial incorporation during

winter/wet could be the factor reducing the isotopic area. A possible explanation is that with the flood and consequently homogenization of the environment, the individual variability, promoted by the heterogeneity during summer/dry, was lower during winter/wet, reducing the isotopic space even with higher terrestrial contribution. The homogenization promoted by water level was previously reported for floodplains (Thomaz *et al.* 2007) and it is known that complexity of trophic structure in streams is related to environmental heterogeneity (Zeni & Casatti 2014).

The ellipse area overlaps between sites were higher during summer/dry than winter/wet, indicating higher trophic distinctness during flood. When comparing seasons, there was more overlap between seasons in wetland than lake, which could indicate a higher modification between seasons in lake. Such modification in lake was driven mostly by TP dimension since there was more change in $\delta^{13}\text{C}$ dimension in wetland than lake, indicating that there was more trophic level differences in lake while the use of resources was the dimension that majority change between season in wetland. The omnivorous guild had ellipse areas lower than the entire assemblage and seemed to conduct the general pattern, since the same variation pattern in both dimensions was observed. The effect of higher TP's during winter/wet was more evident for the piscivorous guild that showed no overlap between seasons strictly driven by the increase in trophic position dimension.

Overall, this study demonstrates that hydrological pulse in shallow lakes and wetlands promote trophic structure changes. The Bayesian approach, including mixing models and standard ellipses, allowed us to trace the increase in terrestrial contribution, especially for the wetland area as we hypothesize, and to identify the expansion in trophic levels during the flood season, making possible to identify

differences/similarities in trophic structure between shallow lakes and wetland areas along hydroperiods. However, certain assumptions and caveats need to be kept in mind, such as the baseline ranges in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Layman *et al.* 2007; Hoeninghaus & Zeug 2008). Our proposition on the use of estimated trophic position instead of purely $\delta^{15}\text{N}$ values partially solve the assumption, since there is also assumptions on the use of trophic discrimination factors, that can be variable (Caut *et al.* 2009). Thereby this methodology provides an advance in community wide metrics approaches based on stable isotope data, overlaying some caveats and instigating the improvement of isotopic space metrics through SIBER analysis.

Acknowledgments

We thank Paula Pereira, Cindy Marques, Mauricio Lang, Mario Vinicius Lopes Condini for field and laboratory work assistance and also to Estação Ecológica do Taim - ICMBio staff for support during fieldwork. This research was financial supported by CNPq Universal 14/2012 and Fundação o Boticário de proteção a Natureza (1026_20142). Rodrigo Ferreira Bastos thanks CNPq (grant n° 140474/2012-9) for doctoral scholarship and CAPES - PDSE (grant n° BEX 9936/14-9) for abroad scholarship. Alexandre Miranda Garcia thanks CNPq (grant n° 305888/2012-9) for scholarships. This research was permitted by Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio under authorization number 37456-4.

References

- Abrantes, K.G., Barnett, A. & Bouillon, S. (2014) Stable isotope-based community metrics as a tool to identify patterns in food web structure in east African estuaries. *Functional Ecology*, **28**, 270–282.
- Abrantes, K.G., Barnett, A., Marwick, T. & Bouillon, S. (2013) Importance of terrestrial subsidies for estuarine food webs in contrasting East African catchments. *Ecosphere*, **4**, 1–33.
- Akin, S. & Winemiller, K.O. (2006) Seasonal variation in food web composition and structure in a temperate tidal estuary. *Estuaries and Coasts*, **29**, 552–567.
- Bastos, R.F., Calliari, L.J. & Garcia, A.M. (2014) Marine intrusion and freshwater discharge as opposite forces driving fish guilds distribution along coastal plain streams. *Hydrobiologia*, **726**, 245–258.
- Bastos, R.F., Condini, M. V., Junior, A.S.V. & Garcia, A.M. (2011) Diet and food consumption of the pearl cichlid *Geophagus brasiliensis* (Teleostei: Cichlidae): relationships with gender and sexual maturity. *Neotropical Ichthyology*, **9**, 825–830.
- Bastos, R.F., Miranda, S.F. & Garcia, A.M. (2013) Dieta e estratégia alimentar de *Characidium rachovii* (Characiformes, Crenuchidae) em riachos de planície costeira do sul do Brasil. *Iheringia, série Zoologia*, **103**, 335–341.
- Beer, S. & Wetzel, R.G. (1982) Photosynthesis in submersed macrophytes of a temperate lake. *Plant physiology*, **70**, 488–492.
- Borges, L. do V. & Colares, I.G. (2007) Feeding Habits of Capybaras (*Hydrochoerus hydrochaeris*, Linnaeus 1766), in the Ecological Reserve of Taim (ESEC - Taim) - South of Brazil. *Brazilian Archives of Biology and Technology*, **50**, 409–416.
- Brito, E.F., Moulton, T.P., De Souza, M.L. & Bunn, S.E. (2006) Stable isotope analysis indicates microalgae as the predominant food source of fauna in a coastal forest stream, south-east Brazil. *Austral Ecology*, **31**, 623–633.
- Carlson, R.E. (1977) A trophic state index for lakes. *Limnology and Oceanography*, **22**, 361–369.
- Caut, S., Angulo, E. & Courchamp, F. (2009) Variation in discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, **46**, 443–453.
- Clapcott, J.E. & Bunn, S.E. (2003) Can C4 plants contribute to aquatic food webs of subtropical streams? *Freshwater Biology*, **48**, 1105–1116.

- Claudino, M.C., Abreu, P.C. & Garcia, A.M. (2013) Stable isotopes reveal temporal and between-habitat changes in trophic pathways in a southwestern Atlantic estuary. *Marine Ecology Progress Series*, **489**, 29–42.
- Corrêa, F., Claudino, M.C., Bastos, R.F., Huckembeck, S. & Garcia, A.M. (2012) Feeding ecology and prey preferences of a piscivorous fish in the Lagoa do Peixe National Park, a Biosphere Reserve in Southern Brazil. *Environmental Biology of Fishes*, **93**, 1–12.
- Corrêa, F. & Piedras, S.R.N. (2008) Alimentação de *Cyphocarax voga* (Hensel, 1869) (Characiformes, Curimatidae) no arroio Corrientes, Pelotas, Rio Grande do Sul, Brasil. *Biotemas*, **21**, 117–122.
- Correa, S.B. & Winemiller, K.O. (2014) Niche partitioning among frugivorous fishes in response to fluctuating resources in the Amazonian floodplain forest. *Ecology*, **95**, 210–224.
- Craig, H. (1953) The geochemistry of the stable carbon isotopes. *Geochimica et Cosmochimica Acta*, **3**, 53–92.
- Cremona, F., Hamelin, S., Planas, D. & Lucotte, M. (2009) Sources of organic matter and methylmercury in littoral macroinvertebrates: a stable isotope approach. *Biogeochemistry*, **94**, 81–94.
- Cresson, P., Ruitton, S., Fontaine, M.-F. & Harmelin-Vivien, M. (2012) Spatio-temporal variation of suspended and sedimentary organic matter quality in the Bay of Marseilles (NW Mediterranean) assessed by biochemical and isotopic analyses. *Marine pollution bulletin*, **64**, 1112–21.
- Davidson, T.A., MacKay, A.W., Wolski, P., Mazebedi, R., Murray-Hudson, M. & Todd, M. (2012) Seasonal and spatial hydrological variability drives aquatic biodiversity in a flood-pulsed, sub-tropical wetland. *Freshwater Biology*, **57**, 1253–1265.
- DeNiro, M.J. & Epstein, S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, **42**, 495–506.
- DeNiro, M.J. & Epstein, S. (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, **45**, 341–351.
- Dubois, S., Savoye, N., Grémare, a., Plus, M., Charlier, K., Beltoise, a. & Blanchet, H. (2012) Origin and composition of sediment organic matter in a coastal semi-enclosed ecosystem: An elemental and isotopic study at the ecosystem space scale. *Journal of Marine Systems*, **94**, 64–73.
- Forsberg, B.R., Araujo-Lima, C.A.R.M., Martinelli, L.A., Victoria, R.L. & Bonassi, J.A. (1993) Autotrophic Carbon Sources for Fish of the Central Amazon. *Ecology*, **74**, 643–652.

- Garcia, A.M., Hoeninghaus, D.J., Vieira, J.P. & Winemiller, K.O. (2007) Isotopic variation of fishes in freshwater and estuarine zones of a large subtropical coastal lagoon. *Estuarine, Coastal and Shelf Science*, **73**, 399–408.
- Garcia, A.M., Hoeninghaus, D.J., Vieira, J.P., Winemiller, K.O., Motta-Marques, D.M.L. & Bemvenuti, M.A. (2006) Preliminary examination of food web structure of Nicola Lake (Taim Hydrological System, south Brazil) using dual C and N stable isotope analyses. *Neotropical Ichthyology*, **4**, 279–284.
- Garcia, A.M., Vieira, J.P. & Winemiller, K.O. (2003) Effects of 1997-1998 El Niño on the dynamics of the shallow-water fish assemblage of the Patos Lagoon Estuary (Brazil). *Estuarine, Coastal and Shelf Science*, **57**, 489–500.
- Hoeninghaus, D.J., Vieira, J.P., Costa, C.S., Bemvenuti, C.E., Winemiller, K.O. & Garcia, A.M. (2011) Estuary hydrogeomorphology affects carbon sources supporting aquatic consumers within and among ecological guilds. *Hydrobiologia*, **673**, 79–92.
- Hoeninghaus, D.J. & Zeug, S.C. (2008) Can stable isotope ratios provide for community-wide measures of trophic structure? Comment. *Ecology*, **89**, 2353–7; discussion 2358–9.
- Hussey, N.E., Macneil, M.A., Mcmeans, B.C., Olin, J.A., Dudley, S.F.J., Cliff, G., Wintner, S.P., Fennessy, S.T. & Fisk, A.T. (2014) Rescaling the trophic structure of marine food webs. *Ecology Letters*, **17**, 239–250.
- Jackson, A.L., Inger, R., Parnell, A.C. & Bearhop, S. (2011) Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *The Journal of animal ecology*, **80**, 595–602.
- Junk, W.J., Bayley, P.B. & Sparks, R.E. (1989) The flood pulse concept in river-floodplain systems. *Proceedings of the International Large river Symposium* (ed D.P. Dodge), pp. 110–127. Canadian Special Published in Fisheries and Aquatic Science.
- Junk, W.J. & Wantzen, K.M. (2008) The flood pulse concept: New aspects , approaches and applications - An update. *The riverine ecosystem synthesis toward conceptual cohesiveness in river science* (eds J.H. Thorp, M.C. Thoms & M.D. Delong), pp. 117–141. Academic press, London.
- Kaehler, S., Pakhomov, E.A. & Mcquaid, C.D. (2000) Trophic structure of the marine food web at the Prince Edward Islands (Southern Ocean) determined by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. *Marine Ecology Progress Series*, **208**, 13–20.
- Kruger, B.R., Werne, J.P., Branstrator, D.K., Hrabik, T.R., Chikaraishi, Y., Ohkouchi, N. & Minor, E.C. (2015) Organic matter transfer in Lake Superior's food web: Insights from bulk and molecular stable isotope and radiocarbon analyses. *Limnology and Oceanography*, **00**, 00-00.

- Kutter, M.T., Bemvenuti, M.D.A. & Moresco, A. (2009) Feeding strategy of the jundiá *Rhamdia quelen* (Siluriformes, Heptapteridae) in costal lagoons of southern Brazil. *Acta Scientiarum. Biological Sciences*, **31**, 41–47.
- Layman, C. a, Araujo, M.S., Boucek, R., Hammerschlag-Peyer, C.M., Harrison, E., Jud, Z.R., Matich, P., Rosenblatt, A.E., Vaudo, J.J., Yeager, L. a, Post, D.M. & Bearhop, S. (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biological reviews of the Cambridge Philosophical Society*, **87**, 545–62.
- Layman, C.A., Arrington, D.A., Montaña, C.G. & Post, D.M. (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology*, **88**, 42–48.
- Layman, C.A. & Post, D.M. (2008) Can Stable Isotope Ratios Provide for Community-Wide Measures of Trophic Structure? Reply. *Ecology*, **89**, 2358–2359.
- Lopes, C.A., Manetta, G.I., Figueiredo, B.R.S., Martinelli, L.A. & Benedito, E. (2015) Carbon from littoral producers is the major source of energy for bottom-feeding fish in a tropical floodplain. *Environmental Biology of Fishes*, **98**, 1081–1088.
- Ludwig, T.A. V, Flores, T.L., Filho, H.M. & Veiga, L.A.S. (2004) Inventário florístico das diatomáceas (Ochrophyta) de lagoas do Sistema Hidrológico do Taim, Rio Grande do Sul, Brasil: Coscinodiscophyceae. *Iheringia, Série Botânica*, **59**, 97–106.
- Lujan, N.K., German, D.P. & Winemiller, K.O. (2011) Do wood-grazing fishes partition their niche?: Morphological and isotopic evidence for trophic segregation in Neotropical Loricariidae. *Functional Ecology*, **25**, 1327–1338.
- Manetta, G.I., Benedito-Cecilio, E. & Martinelli, M. (2003) Carbon sources and trophic position of the main species of fishes of Baía River, Paraná River floodplain, Brazil. *Brazilian journal of biology*, **63**, 283–290.
- Marcenko, E., Srdoc, D., Golubic, S., Pezdic, J. & Head, M.J. (1989) Carbon uptake in aquatic plants deduced from their natural ¹³C and ¹⁴C content. *Radiocarbon*, **31**, 785–794.
- Marinho, C.C., Meirelles-Pereira, F., Gripp, A. da R., Guimarães, C. de C., Esteves, F. de A. & Bozelli, R.L. (2010) Aquatic macrophytes drive sediment stoichiometry and the suspended particulate organic carbon composition of a tropical coastal lagoon. *Acta Limnologica Brasiliensia*, **22**, 208–217.
- Mill, A.C., Pinnegar, J.K. & Polunin, N.V.C. (2007) Explaining isotope trophic-step fractionation: why herbivorous fish are different. *Functional Ecology*, **21**, 1137–1145.

- Moresco, A. & Bemvenuti, M.D.A. (2005) Morphologic features and feeding analysis of the black catfish *Trachelyopterus lucenai* Bertoletti, Pezzi da Silva & Pereira (Siluriformes, Auchenipteridae). *Acta Limnologica Brasiliensia*, **17**, 37–44.
- Nakano, S., Miyasaka, H. & Kuhara, N. (1999) Terrestrial-aquatic linkages: Riparian arthropod inputs alter trophic cascades in a stream food web. *Ecology*, **80**, 2435–2441.
- Newsome, S.D., Martínez del Rio, C., Bearhop, S. & Phillips, D.L. (2007) A niche for isotopic ecology. *Frontiers in Ecology and Environment*, **5**, 429–436.
- Newsome, S.D., Yeakel, J.D., Wheatley, P. V & Tinker, M.T. (2012) Tools for quantifying isotopic niche space and dietary variation at the individual and population level. *Journal of Mammalogy*, **93**, 329–341.
- O’Leary, M.H. (1988) Carbon isotopes in photosynthesis. *BioScience*, **38**, 328–336.
- Oliveira, M.C.L.M. de, Bastos, R.F., Claudino, M.C., Assumpção, C.M. & Garcia, A.M. (2014) Transport of marine-derived nutrients to subtropical freshwater food webs by juvenile mullets: a case study in southern Brazil. *Aquatic Biology*, **20**, 91–100.
- Ometto, J.P.H.B., Ehleringer, J.R., Domingues, T.F., Berry, J. a., Ishida, F.Y., Mazzi, E., Higuchi, N., Flanagan, L.B., Nardoto, G.B. & Martinelli, L.A. (2006) The stable carbon and nitrogen isotopic composition of vegetation in tropical forests of the Amazon Basin, Brazil. *Biogeochemistry*, **79**, 251–274.
- Ou, C. & Winemiller, K.O. (2016) Seasonal Hydrology Shifts Production Sources Supporting Fishes in Rivers of the Lower Mekong Basin. *Canadian Journal of Fisheries and Aquatic Sciences*, *in press*.
- Parnell, A.C., Inger, R., Bearhop, S. & Jackson, A. (2008) SIAR: Stable isotope analysis in R.
- Phillips, D.L., Newsome, S.D. & Gregg, J.W. (2005) Combining sources in stable isotope mixing models: alternative methods. *Oecologia*, **144**, 520–7.
- Pimm, S.L. & Lawton, J.H. (1977) Number of trophic levels in ecological communities. *Nature*, **268**, 329–331.
- Post, D.M. (2002) Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, **83**, 703–718.
- Post, D.M., Pace, M.L. & Hairston, N.G. (2000) Ecosystem size determines food-chain length in lakes. *Nature*, **405**, 1047–1049.
- Prado, P., Carmichael, R., Watts, S., Cebrian, J. & Heck, K. (2012) Diet-dependent $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ fractionation among sea urchin *Lytechinus variegatus* tissues:

- implications for food web models. *Marine Ecology Progress Series*, **462**, 175–190.
- Price, A.R.G. & Harris, A. (2009) Decadal changes (1996–2006) in coastal ecosystems of the Chagos archipelago determined from rapid assessment. *Aquatic Conservation: Marine and freshwater Ecosystems*, **19**, 637–644.
- Rodrigues, F.L. & Bemvenuti, M.D.A. (2011) Diet and feeding strategy of the silverside *Odontesthes bonariensis* (Valenciennes 1835) (Atheriniformes: Atherinopsidae) from the Mirim Lagoon, Rio Grande do Sul, Brazil. *Pan American Journal of Aquatic Sciences*, **6**, 161–169.
- Rodrigues, L.R., Fontoura, N.F. & Motta-Marques, D.M.L. (2012) Feeding dynamics of *Oligosarcus jenynsii* (Günther, 1864) in a subtropical coastal lake assessed by gut-content analysis and stable isotopes. *International journal of plant, animal and environmental sciences*, **2**, 126–134.
- Rodrigues, L.R., Fontoura, N.F. & Motta-Marques, D. (2014) Food-web structure in a subtropical coastal lake: How phylogenetic constraints may affect species linkages. *Marine and Freshwater Research*, **65**, 453–465.
- Rodrigues, L.R., Motta-Marques, D.M.L. & Fontoura, N.F. (2015) Fish community in a large coastal subtropical lake: how an environmental gradient may affect the structure of trophic guilds. *Limnetica*, **34**, 495–506.
- Sabo, J.L., Finlay, J.C., Kennedy, T. & Post, D.M. (2010) The Role of Discharge Variation in Scaling of Drainage Area and Food Chain Length in Rivers. *Science*, **330**, 965–967.
- Safi, L.S.L., Fontoura, N.F., Severo, H.J. & Utz, L.R.P. (2014) Temporal structure of the peritrich ciliate assemblage in a large Neotropical lake. *Zoological Studies*, **17**.
- Sterner, R.W., Bajpai, A. & Adams, T. (1997) The enigma of food chain length: absence of theoretical evidence for dynamic constraints. *Ecology*, **78**, 2258–2262.
- Takimoto, G. & Post, D.M. (2013) Environmental determinants of food-chain length: A meta-analysis. *Ecological Research*, **28**, 675–681.
- Thomaz, S.M., Bini, L.M. & Bozelli, R.L. (2007) Floods increase similarity among aquatic habitats in river-floodplain systems. *Hydrobiologia*, **579**, 1–13.
- Thorp, J.H. & DeLong, M.D. (1994) The riverine productivity model: An heuristic view of carbon sources and organic processing in large river ecosystems. *Oikos*, **70**, 305–308.
- Vanderklift, M.A. & Ponsard, S. (2003) Sources of variation in consumer-diet delta ¹⁵N enrichment: a meta-analysis. *Oecologia*, **136**, 169–82.

- Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R. & Cushing, C.E. (1980) The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 130–137.
- De Vicente, I., Amores, V. & Cruz-Pizarro, L. (2006) Instability of shallow lakes: A matter of the complexity of factors involved in sediment and water interaction? *Limnetica*, **25**, 253–270.
- Vieira, J.P., Garcia, A.M. & Grimm, A.M. (2008) Evidences of El Niño Effects on the Mullet Fishery of the Patos Lagoon Estuary. *Brazilian Archives of Biology and Technology*, **51**, 433–440.
- Vizzini, S. & Mazzola, A. (2003) Seasonal variations in the stable carbon and nitrogen isotope ratios ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) of primary producers and consumers in a western Mediterranean coastal lagoon. *Marine Biology*, **142**, 1009–1018.
- Wantzen, K.M., Machado, F.A., Voss, M., Boriss, H. & Junk, W.J. (2002) Seasonal isotopic shifts in fish of the Pantanal wetland, Brazil. *Aquatic Sciences*, **64**, 239–251.
- Weber, L.I., Hildebrand, C.G., Ferreira, A., Pedarassi, G., Levy, J.A. & Colares, E.P. (2009) Microsatellite genotyping from faeces of *Lontra longicaudis* from southern Brazil. *Iheringia, série Zoologia*, **99**, 5–11.
- Vander Zanden, M.J. & Rasmussen, J.B. (2001) Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, **46**, 2061–2066.
- Vander Zanden, M.J. & Vadeboncoeur, Y. (2002) Fishes as integrators of benthic and pelagic food webs in lakes. *Ecology*, **83**, 2152–2161.
- Zar, J.H. (2010) *Bioestatistical Analysis*, Fifth. Prentice Hall, Upper Saddle River, New Jersey, USA.
- Zeni, J.O. & Casatti, L. (2014) The influence of habitat homogenization on the trophic structure of fish fauna in tropical streams. *Hydrobiologia*, **726**, 259–270.
- Ziegler, S.E. & Fogel, M.L. (2002) Seasonal and diel relationships between the isotopic compositions of dissolved and particulate organic matter in freshwater ecosystems. *Biogeochemistry*, **64**, 25–52.

CAPÍTULO 2

Are you what you eat? Effects of trophic discrimination factors on estimates of food assimilation and trophic position of an omnivorous fish

Manuscrito redigido e submetido na língua e formato indicado para revista *Journal of Animal Ecology*.

*O número de linhas foi retirado para melhor adequação ao formato da tese.

**Are you what you eat? Effects of trophic discrimination
factors on estimates of food assimilation and trophic position
of an omnivorous fish**

Rodrigo Ferreira Bastos^{* a, b}, Fabiano Corrêa^{b, c}, Alexandre Miranda Garcia^b, Kirk O.
Winemiller^d

^a Programa de Pós Graduação em Biociências (Zoologia), Faculdade de Biociências -
FABIO, Pontifícia Universidade Católica do Rio Grande do Sul – PUCRS, Porto
Alegre, Brazil.

^b Laboratório de Ictiologia, Instituto de Oceanografia - IO, Universidade Federal do
Rio Grande – FURG, Rio Grande, Brazil.

^c Programa de Pós Graduação em Zootecnia e Recursos Pesqueiros, Universidade
Federal de Pelotas – UFPEL, Pelotas, Brazil.

^d Department of Wildlife and Fisheries Sciences, Texas A&M University – TAMU,
College Station, USA.

* Corresponding author: rfbastos@furg.br

Summary

1. A key factor for estimates of assimilation of resources and trophic position based on stable isotope data is the trophic discrimination factor (TDF). TDFs are assumed based on literature reviews, but may vary depending on a variety of factors, including the type of diet.
2. We analyzed effects of alternative TDFs on estimates of assimilated resources and trophic positions for an omnivorous fish, *Jenynsia multidentata*, that reveals dietary variation among locations across a salinity gradient of a coastal lagoon in southern Brazil. We also compared estimates of foods ingested vs. foods assimilated.
3. Food assimilation was estimated using carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios of food sources and consumer muscle tissue and an isotopic mixing model (SIAR); consumer trophic position (TP) was estimated from consumer and production source $\delta^{15}\text{N}$ values. Diet was estimated using an index of relative importance based on frequency of occurrence and volumetric and numeric proportions of food items from stomach contents.
4. The effect of variation in TDF on food assimilation and TP was tested using three alternative TDFs reported in review papers. We then created a new method that used food source-specific TDFs (reported separately for herbivores and carnivores) weighted in proportion to estimated assimilation according to mixing model estimates to estimate TP (hereafter TP_{PA}).
5. We found that plant material was not assimilated in a proportion similar to its importance in the diet of fish at a freshwater site, and the new method yielded

best assimilation estimates. Animal material made greatest contributions to fish biomass irrespective of TDFs used in the mixing model.

6. The new method produced TP estimates consistent with differences in estimated food assimilation along the salinity gradient. Our findings support the idea that food source-specific TDFs should be used in trophic studies of omnivores.

Key-words: carnivory, diet shift, herbivory, isotopic fractionation, omnivory, trophic enrichment.

Introduction

Analysis of elemental stable isotopes is widely used for estimation of flows of organic material in food webs (DeNiro & Epstein 1978, 1981), with carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) particularly useful owing to (1) their abundance in organic material and (2) relatively predictable shifts in isotopic ratios between tissues of foods and consumers (Fry 2006). This shift during the process of food digestion, incorporation and assimilation has been termed *trophic fractionation*, and an estimate of its magnitude is called the trophic discrimination factor (TDF) (Post 2002). TDF is a critical parameter used for estimation of both food assimilation and consumer trophic position. Most researchers now estimate food assimilation using isotopic mixing models that adopt Bayesian statistical approaches that can incorporate uncertainty associated with TDF and other inputs (Bond & Diamond 2011; Parnell *et al.* 2013). Computation of trophic position based on isotopic data relies on values assigned for TDFs as well as appropriate isotopic references (i.e., trophic position baselines) (Post 2002).

Almost 20 years after a call for more laboratory experiments (Gannes *et al.* 1997; Wolf *et al.* 2009), studies have revealed multiple factors affecting TDFs, including variation in food quality (e.g., protein content, amino acid profile), metabolic state (e.g., anabolic vs. catabolic), food intake rate, developmental stage, body mass, sex and even controversial ones, such as isotopic composition (Robbins *et al.* 2005; Gaye-Siessegger *et al.* 2007; Caut *et al.* 2009; Kelly & Martínez del Rio 2010; Wessels & Hahn 2010; McMahon *et al.* 2010; Florin *et al.* 2011; Newsome *et al.* 2011; Codron *et al.* 2011; Poupin *et al.* 2011). The trophic discrimination factor

for nitrogen (TDF_N) can vary considerably between trophic levels, with values often greater for primary consumers and smaller for higher trophic levels (Hussey *et al.* 2014). Several reviews of TDF_N showed inconsistent results when comparing consumers fed plant material with those fed foods derived from animal tissue (Vander Zanden & Rasmussen 2001; Post 2002; Vanderklift & Ponsard 2003; Caut *et al.* 2009). Studies involving fish have revealed different TDF_N for herbivores and carnivores, with the latter generally having lower values (Varela *et al.* 2011; Madigan *et al.* 2012) and herbivores having higher TDF_N compared to values reported in the literature (Mill *et al.* 2007; Lujan *et al.* 2011; Prado *et al.* 2012). However, laboratory experiments can test only a few parameters simultaneously, and trophic ecology is influenced by multiple factors. Moreover, captive studies often provide food *ad libitum*, which increases excretion rate, a factor that strongly influences TDF_N (Ponsard & Averbuch 1999; Olive *et al.* 2003; Mill *et al.* 2007)

To the best of our knowledge, no prior study has addressed the relationship between TDF and food quality (i.e. animal origin vs. plant origin) for animals that change diet along temporal or spatial environmental gradients. To investigate the influence of TDF on estimation of trophic position and food assimilation, we analyzed isotopic and dietary variation of an omnivorous fish in relation a salinity gradient in a coastal ecosystem. The one-sided livebearer, *Jenynsia multidentata* (Jenyns, 1842), a dominant species in fresh and brackish waters along the coast of southern South America (Garcia *et al.* 2004; Bastos *et al.* 2014), feeds on both plants (e.g., algae and seagrass) and animals (e.g., microcrustaceans, insects, polychaetes) (Aranha & Caramaschi 1999; Mai, Garcia & Vieira 2006). Plant and animal material differ in nutritional quality, with animal tissue generally containing more protein, and plant

tissues containing large fractions of cellulose and other compounds that are difficult or expensive for most animals to digest. Based on our findings, we propose a new approach for estimating trophic position that uses outputs from stable isotope mixing models and takes into account food-specific TDFs.

Materials and methods

FIELD COLLECTIONS AND SAMPLE PROCESSING

Samples were obtained monthly from April 2008 to May 2009 at Lagoa do Peixe National Park (LPNP) located on the coastal plain of Rio Grande do Sul state, Brazil (Fig. 1). Three sites were surveyed in Lagoa do Peixe: (1) lagoon mouth (LM) - the narrow channel that intermittently connects the main lagoon with the sea, (2) estuarine zone (EZ) – a mixohaline area located between the mouth and upper freshwater reaches, and (3) freshwater wetland (FW) fringing the upstream boundary of the lagoon (Fig. 1).

Jenynsia multidentata specimens were captured using a beach seine (9-m long, 2.4-m high, mesh size = 13 mm in wings and 5 mm in center) and beam trawl (0.9 x 0.9 m opening, with size mesh = 5 mm). Captured specimens were immediately euthanized in an ice bath, transported to the lab on ice, and then stored in a freezer. After thawing, each specimen was measured (total length, TL, mm), weighed (g) and dissected to remove the digestive tract for stomach contents analysis. Approximately 5 g of muscle tissue was extracted from the dorso-lateral region of each specimen for isotopic analysis. For specimens <30 mm TL, a composite sample of muscle tissue

from 2–5 individuals was obtained in order to have sufficient material for analysis of isotopic composition.

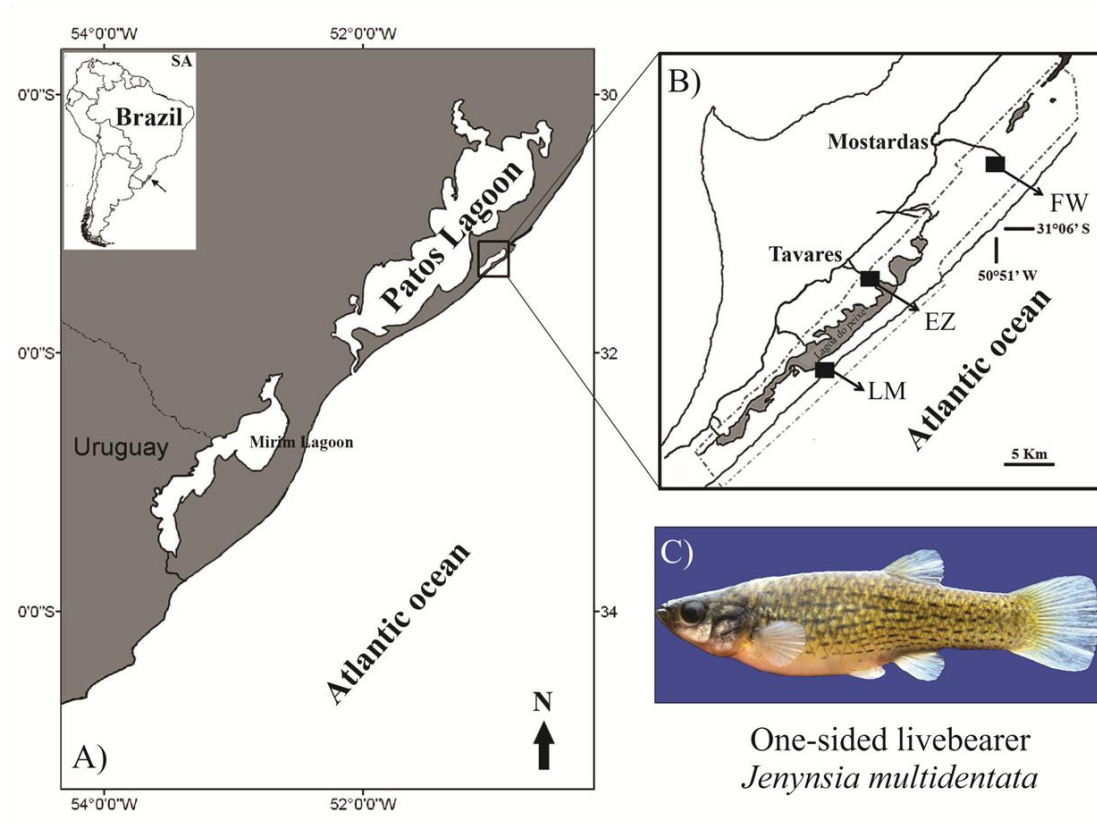


Fig. 1. Map showing the Patos-Mirim lagoon complex in Brazil, South America (SA) (A) and the coastal lagoon studied inside Lagoa do Peixe National Park limits (dashed line), located between Mostardas and Tavares cities (B). Species studied, the one-sided livebearer, *Jenynsia multidentata* (C).

In order to estimate trophic positions based on stable isotope ratios of nitrogen ($\delta^{15}\text{N}$), tissue samples were obtained for basal production sources at each survey location (leaves from floating, emergent, and submerged macrophytes; filamentous algae; periphyton; suspended particulate organic matter (POM)). Additionally, major dietary items of *J. multidentata*, such as polychaete worms, amphipods and insects,

were collected manually from sediments and macrophytes, and tissue was obtained for isotopic analysis.

ESTIMATES OF INGESTED RESOURCES

A total of 121 stomachs were analyzed to quantify the relative importance of food items ingested by *J. multidentata*. A stereoscopic binocular microscope was used to identify food items to the lowest feasible taxonomic level. Inorganic material and partially digested, unidentifiable organic matter were recorded as present or absent and excluded from subsequent analyses. The relative importance of each food category was calculated by the Index of Relative Importance (IRI) (Pinkas, Oliphant & Iverson 1970). We recorded the frequency of occurrence (F) of food categories in stomachs as percentages of total stomachs examined (Hyslop 1980). For each stomach sample, we recorded the number of items or major fragments of each food category (N) and the area (mm²) (A) of each item or category when material was spread evenly over a Petri dish at a depth of approximately 1 mm. If an item was thicker than 1 mm, the item was broken into smaller pieces to achieve a thickness of 1 mm (Hellawell & Abel 1971). When thickness was <1 mm, thickness was estimated visually (e.g., 0.25, 0.5, or 0.75 mm). The volume (V) of each food category then was calculated as thickness x A. IRI was calculated using the formula: $IRI = \%F \cdot (\%N + \%V)$, where %N was the ratio between the total number of a given food category and the total number among all categories items from all stomachs in the sample, and %A was the ratio between the total area occupied by a given food item or category and the total area occupied by all food items from all stomachs in the sample. Finally, IRI was expressed as a percentage (%IRI) obtained from the ratio between the calculated IRI

for a given food category and the total sum of the IRI calculated for all food categories.

EFFECTS OF TDFs ON ESTIMATES OF FOOD ASSIMILATION AND TROPHIC POSITION

Muscle tissue samples were obtained from the flanks of 89 specimens of *J. multidentata* captured from the three survey sites. Muscle samples and whole bodies of invertebrates (n=48), samples of filamentous algae (n=7), periphyton (n=32) and macrophytes (n=59) were rinsed with distilled water to remove foreign material. POM samples (n=42) were obtained by filtering water through a pre-combusted (450 °C, 4 h) Whatman glass fiber filter (GF/F) with the aid of a manual vacuum pump. Samples were placed in sterile Petri dishes, and dried in an oven at 60 °C for a minimum of 48 h. Dried samples were ground to a fine powder with a mortar and pestle and stored in clean Eppendorf tubes. Sub-samples were pressed into Ultra-Pure tin capsules (Costech, Valencia, CA) and sent to the Analytical Chemistry Laboratory, Institute of Ecology, University of Georgia, for measurement of stable isotope ratios ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$). Stable isotope ratios ($R = ^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$) were reported as parts per thousand (‰) relative to the corresponding standard $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3$. Standards were PeeDee Belemnite (C) and molecular nitrogen in air (N). Standard deviations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ based on analysis of internal standard replicates were 0.14‰ and 0.13‰, respectively. Organisms were grouped by studied location (LM, EZ, FW), and bi-plots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of sampled tissues and organisms were compared across sites.

To estimate relative contributions of food resources to consumers, we used the Bayesian stable isotope mixing model in R (SIAR, version 3.2.1) (Parnell *et al.* 2008). To achieve higher resolution and ecological meaning in mixing models, Phillips and collaborators (2005) recommended *a posteriori* pooling of production sources with similar isotopic composition and similar ecological roles. Based on results from stomach contents analysis, and considering the nutritional quality differences between primary producers and animals, we considered two main food resource categories for the mixing model: (1) algae (filamentous algae and periphyton) and (2) animals (amphipods, polychaete worms and insects).

Trophic position (TP) of *J. multidentata* was estimated using the following equation (Post 2002): $TP = \lambda + ((\delta^{15}N_{Jenynsia} - \delta^{15}N_{Baseline}) / TDF_N)$, where λ is the baseline trophic level which is set at 1 for primary producers; $\delta^{15}N_{Jenynsia}$ is the $\delta^{15}N$ value of a fish specimen; $\delta^{15}N_{Baseline}$ is the average $\delta^{15}N$ value from primary producers at the corresponding site; and TDF_N is the trophic discrimination factor for $\delta^{15}N$.

TDFs is a strongly influential variable in multiple source mixing models and trophic position estimates (Caut *et al.* 2009; Parnell *et al.* 2013; Hussey *et al.* 2014). To account for potential bias from error associated with choice of TDF values, four combinations of TDFs for both carbon and nitrogen isotopic ratios were used to estimate source assimilation and trophic positions. Considering that TDF could be associated with differences in food digestibility, food quality, trophic level, assimilation and excretion (Caswell *et al.* 1973; Fantle *et al.* 1999; Olive *et al.* 2003; Hussey *et al.* 2014), omnivores could span a range of TDF values depending on relative amounts of plant and animal material in their diets. Therefore, an appropriate model to estimate source assimilation for omnivores would be one that assumes a

different TDF for each plant and animal sources. We computed mixing models and TP estimates using four different combinations of TDF values. We used the average of TDF values reported in literature reviews to produce three combinations of TDF for Nitrogen (TDF_N) and for Carbon (TDF_C), respectively (combination 1 = 2.75 and 0.40, combination 2 = 2.9 and 0.47, and combination 3 = 3.4 and 0.75) (Table 1).

Table 1. List of TDF's values used in each combination used for mixing models and trophic position estimation. All mixing models with exception of method 4 used the same TDF's for both sources. For method 4, the mean of values reported for herbivorous fishes was used to estimate proportional assimilation of algae, and the mean of values reported for carnivorous fishes was used to estimate proportional assimilation of animal food. The average of carbon values reported in reviews papers was used as the TDF_C of algae (see Materials and Methods).

Mixing model/ TP estimate combinations	Literature sources	TDF _N mean	TDF _N s.d.	TDF _C mean	TDF _C s.d.
1	(Caut <i>et al.</i> 2009)	2.75	0.10	0.75	0.11
2	(Vander Zanden & Rasmussen 2001)	2.90	0.30	0.47	0.19
3	(Post 2002)	3.40	1.00	0.40	1.30
	<i>Average of reviews</i>	<i>3.02</i>	<i>0.47</i>	<i>0.54</i>	<i>0.53</i>
4/TP _{PA} method	Herbivorous X Carnivorous fishes				
	Herbivorous fish - used to estimate algae contribution (Keegan & DeNiro 1988; Jennings <i>et al.</i> 1997; Pinnegar & Polunin 2000; Mill <i>et al.</i> 2007)	4.78	1.30	-	-
	Carnivorous fish – used to estimate animals contributions (Varela <i>et al.</i> 2011; Madigan <i>et al.</i> 2012)	1.77	0.30	0.82	0.47

The SIAR mixing model allows potential food sources to have unique values for average and standard deviation of TDF_C and TDF_N . Therefore, we used the mean of published TDFs for carnivorous fishes (1.77 for N; 0.82 for C) for animal food categories, and the mean of published TDFs for herbivorous fishes (4.78 for N) for algae food categories (Table 1). We were unable to find a literature report for TDF_C of strictly herbivorous fish; therefore, we used the mean of values reported in the literature fish irrespective of trophic niche (0.54). Calculation of TP requires a single TDF_n value, and thereby ignores differences in trophic discrimination associated with different sources. In order to address such issues, a novel method was created based on the assumption that foods with different qualities implies in different TDF_N values.

Proportional contributions (95% credibility) to *J. multidentata* tissue from alternative sources (animal preys vs. algae) estimated from SIAR were used to produce specific TDF_N (TDF_N carnivorous, TDF_N herbivorous) as follow: effective $TDF_N = ((TDF_N$ carnivorous $\times C_{animal}) + (TDF_N$ herbivorous $\times C_{algae})) / C_{animal} + C_{algae}$ where TDF_N carnivorous is the specific TDF_N for the animal source; C_{animal} is the proportional contribution of animal material to fish tissue; TDF_N plant is the specific TDF_N for the algae source; C_{algae} is the proportional contribution of algae to fish tissue. In this way, the TP estimate method proposed here (TP_{PA}) is weighted by proportional assimilation of resources. Because the SIAR model output (95% credibility) is an interval of feasible contributions, we generated three TP_{PA} estimates (TP_{PA-low} , TP_{PA-med} and $TP_{PA-high}$) using separately the lowest, median, and highest values of C_{animal} combined with the highest, median and lowest values of C_{algae} , respectively.

Non-parametric analysis of variance (Kruskal-Wallis ANOVA) was used to compare $\delta^{15}N$ of *J. multidentata* and primary producers (isotopic baseline) among sites

as well as TP estimates based on the four different combinations. When a significant main effect was observed, multiple comparisons were performed (Zar 2010).

Results

INGESTED RESOURCES

Examination of 121 *J. multidentata* specimens yielded 22 food categories within 116 stomachs that contained food. The diet of *J. multidentata* was comprised of both plant (e.g., algae) and animal matter (Table 2, Fig. 2A), with composition differing among locations along the longitudinal fluvial gradient (Fig. 2A).

Filamentous algae and other plant material dominated the diet of fish from the freshwater wetland (FW, 70.65%), but invertebrates such as amphipods (15.7%) also were ingested. In the estuarine zone (EZ) fish ingested mostly invertebrates (86.3%), including polychaetes (29.4%), amphipods (23.2%) and cladocerans (7.5%). Fish from the lagoon mouth (LM) had fed exclusively on animal prey, especially polychaete worms (96.4%). Amorphous particulate organic matter was more frequently ingested in the FW (80.0%) compared to EZ (47.1%) and LM (57.1%) sites. Sand grains were more frequent in stomachs of individuals from the LM (78.6%) than other sites (17.65% at EZ, absent at FW).

Table 2. Index of relative importance (IRI) of food items found in the stomach contents of *Jenynsia multidentata* caught at the freshwater wetland (FW), estuarine zone (EZ) and lagoon mouth (LM). The index was calculated for three grouping levels (1) bold font indicates food items grouped in a first level and first analyzed just as plants or animals; (2) Italic fonts indicates food items grouped in a second level and analyzed as plants and other lower animal level of classifications (*Crustacea*, *Insecta*, *Other Arthropods*, *Polychaeta* and *Fish*); (3) Normal fonts indicates food items grouped and analyzed at the higher level of classification possible.

Food items	Index of Relative Importance		
	IRI%		
	Freshwater Wetland FW	Estuarine Zone EZ	Lagoon Mouth LM
Plants	70.65	13.68	-
<i>Plants</i>	<i>81.03</i>	<i>21.71</i>	-
Filamentous algae	80.95	32.09	-
Macrophyte fragments	-	0.76	-
Animals	29.35	86.32	100.00
<i>Crustacea</i>	<i>15.65</i>	<i>61.12</i>	-
Amphipoda	15.69	23.17	-
Ostracoda	-	0.45	-
Cladocera	-	7.49	-
Copepoda	-	3.32	-
<i>Insecta</i>	<i>1.98</i>	<i>1.09</i>	-
Aquatic Insects			
Diptera larvae	1.99	-	-
Diptera pupae		0.02	
Ephemeroptera larvae	-	1.61	-
Trichoptera larvae	-	0.08	-
Insect larvae (unidentified)	-	0.39	-
Insect larvae remains	-	0.05	-
Terrestrial Insects			
Coleoptera	-	0.10	-
Hymenoptera	-	0.02	-
Insect (unidentified)	-	0.05	-

Table 2. Continuation

Food items	Index of Relative Importance		
	IRI%		
	Freshwater Wetland FW	Estuarine Zone EZ	Lagoon Mouth LM
<i>Other Arthropods</i>	-	-	3.61
Aranae	-	0.04	-
Arthropoda (unidentified)	-	-	1.66
<i>Polychaeta</i>	1.34	15.93	96.39
<i>Heteromastus similis</i>	1.37	0.04	-
<i>Laonereis acuta</i>	-	28.86	74.61
Polychaeta (unidentified)	-	0.50	-
Polychaeta remains	-	0.02	23.73
<i>Fish</i>	-	0.45	-
Eggs	-	0.41	-
Fish remains	-	0.04	-

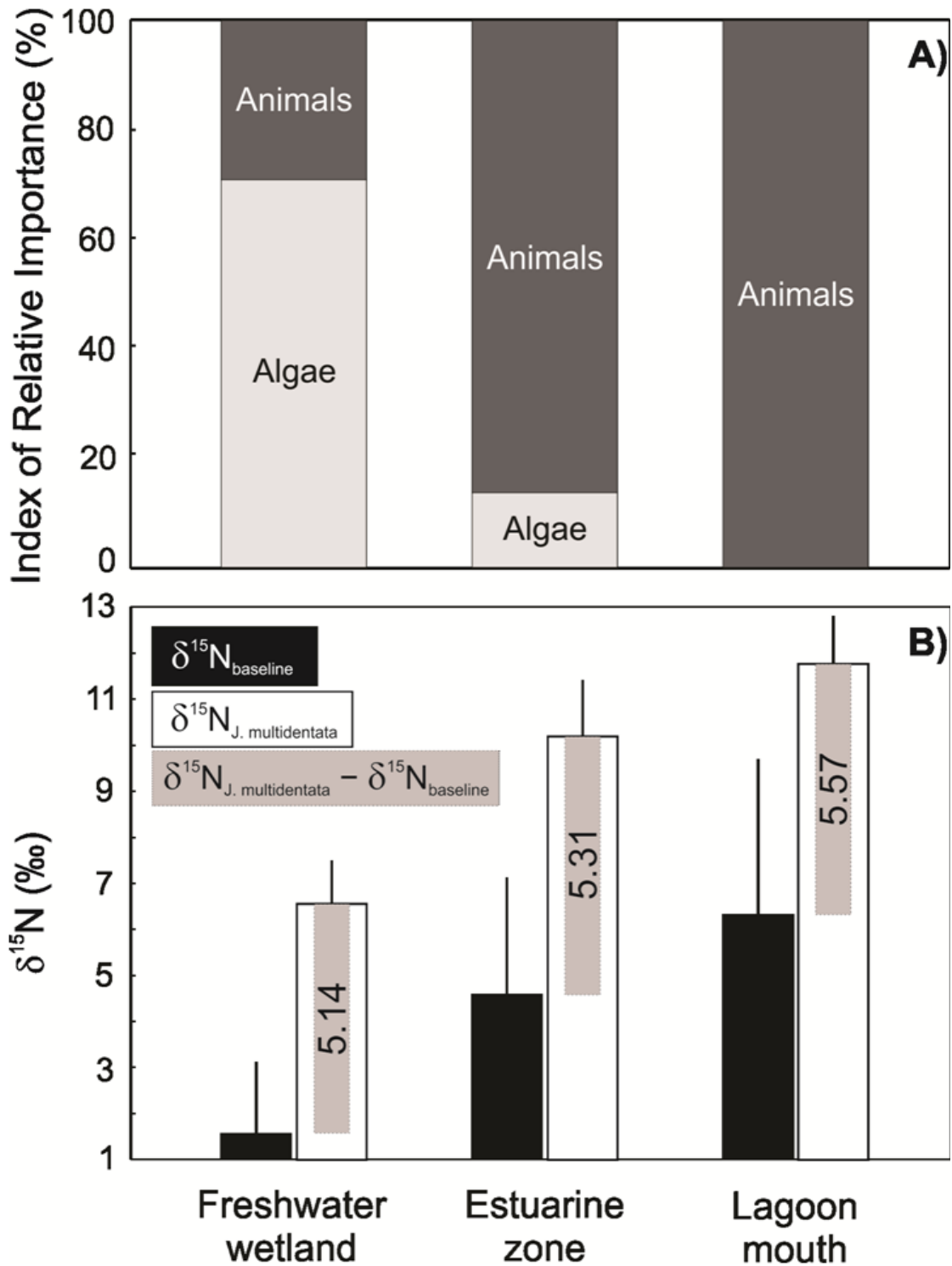


Fig. 2. (A) Index of relative importance (IRI%) of major food categories (pooled as filamentous algae (light gray bars) vs. animal prey (dark grey bars)) from stomach contents of *Jenynsia multidentata* from three sites along a freshwater-estuarine gradient. (B) Average $\delta^{15}\text{N}$ (vertical bars = 1 s.d.) of tissues from the primary producer isotopic baseline (dark bars), *Jenynsia multidentata* (white bars), and the

difference between them (grey bars) at the three sites along the freshwater-estuarine gradient.

SPATIAL VARIATION OF $\delta^{15}\text{N}$

Tissue samples from 89 *J. multidentata* specimens produced 49 samples for stable isotope analysis (27 individual samples and 22 composite samples) from the three survey sites (Table 3). Nitrogen isotopic ratios increased significantly from freshwater to lagoon mouth (Kruskal-Wallis, $H = 22.10$, $p < 0.05$) (Fig. 2B). Post-hoc tests showed statistically significant differences ($p < 0.05$) between $\delta^{15}\text{N}$ mean values for fish from FW (6.8‰) and EZ (10.07‰), and FW vs. LM (11.78‰), but not EZ vs. LM (Table 3, Fig. 2B). Mean $\delta^{15}\text{N}$ of the primary producers isotopic baseline also varied spatially, with a trend of ^{15}N enrichment observed from upstream to downstream locations (FW = 1.66‰, EZ = 4.76‰ and LM = 6.21‰) (Table 3, Fig. 2B). Post-hoc tests revealed statistically significant differences among sites ($p < 0.05$) (Table 3, Fig. 2B).

Table 3. Isotopic values from samples collected on each one sampling site. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ represent the difference between samples and standards mentioned on materials and methods section and are presented as ‰. s.d. = standard deviation; N = number of samples analyzed; ni = number of individuals.

Taxon	Freshwater wetland					Estuarine zone					Lagoon mouth				
	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N(ni)	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N(ni)	$\delta^{13}\text{C}$	s.d.	$\delta^{15}\text{N}$	s.d.	N(ni)
Consumer															
<i>Jenynsia multidentata</i>	-22.13	1.72	6.80	0.95	9(14)	-14.48	2.54	10.07	1.15	32(64)	-11.80	1.63	11.78	0.90	8(11)
Animal Preys															
Polychaeta	-	-	-	-	0	-15.88	1.22	8.49	1.56	20	-14.38	1.07	10.17	0.59	5
Amphipoda	-29.22	1.56	1.53	0.06	2	-	-	-	-	0	-	-	-	-	0
Insects (aquatic instars)	-27.16	3.58	4.00	1.56	17	-19.64	0.63	6.19	0.58	4	-	-	-	-	0
Primary producers (preyed by consumer)															
Periphyton	-33.57	3.74	0.83	1.35	8	-20.12	4.77	5.11	4.73	21	-16.89	2.99	8.20	0.56	3
Algae	-28.25	-	-0.20	-	1	-18.72	1.86	7.38	3.10	3	-17.52	1.36	5.64	2.86	3
Primary producers															
C3 macrophytes	-30.53	2.56	1.60	1.74	20	-26.62	3.17	6.00	2.90	18	-26.70	2.50	2.30	2.60	7
C4 macrophytes	-17.31	6.27	1.73	2.35	4	-14.96	4.37	4.07	1.96	7	-12.76	0.33	8.87	3.33	3
Organic matter source															
POM	-24.78	3.72	2.19	2.07	14	-20.70	3.40	4.31	1.82	19	-22.46	4.35	7.51	2.75	9
Baseline															
Average of C3, C4, POM, algae and periphyton	-28.16	5.77	1.66	1.84	47	-21.44	5.25	4.76	2.63	68	-20.36	6.12	6.21	3.48	25

EFFECT OF TDFs ON MIXING MODEL ESTIMATES

Mixing models outputs, regardless of the TDF values employed, indicated that *J. multidentata* at all three locations had assimilated material derived mostly from animal food resources (Fig. 3). Considering 95% credibility intervals, the contribution of filamentous algae to *J. multidentata* tissue ranged from 0% to 38%, whereas assimilation of animal food categories ranged from 68% to 100% (Fig. 3). Compared to the combinations that used a single (unweighted) TDF value for each element, the new mixing model method that considered food-specific TDFs for each source yielded greater contributions of algae to fishes from FW site (0-38%, Fig. 3), lowest contributions of algae to EZ (0-14%) and LM fish (0-21%), and greatest contribution of animal food categories to EZ (86-100%) and FW fish (79-100%). The proposed method tended to produce narrower 95% credibility intervals, which indicates higher resolution for estimates compared to those obtained from the other three combinations of C and N TDFs (Fig. 3).

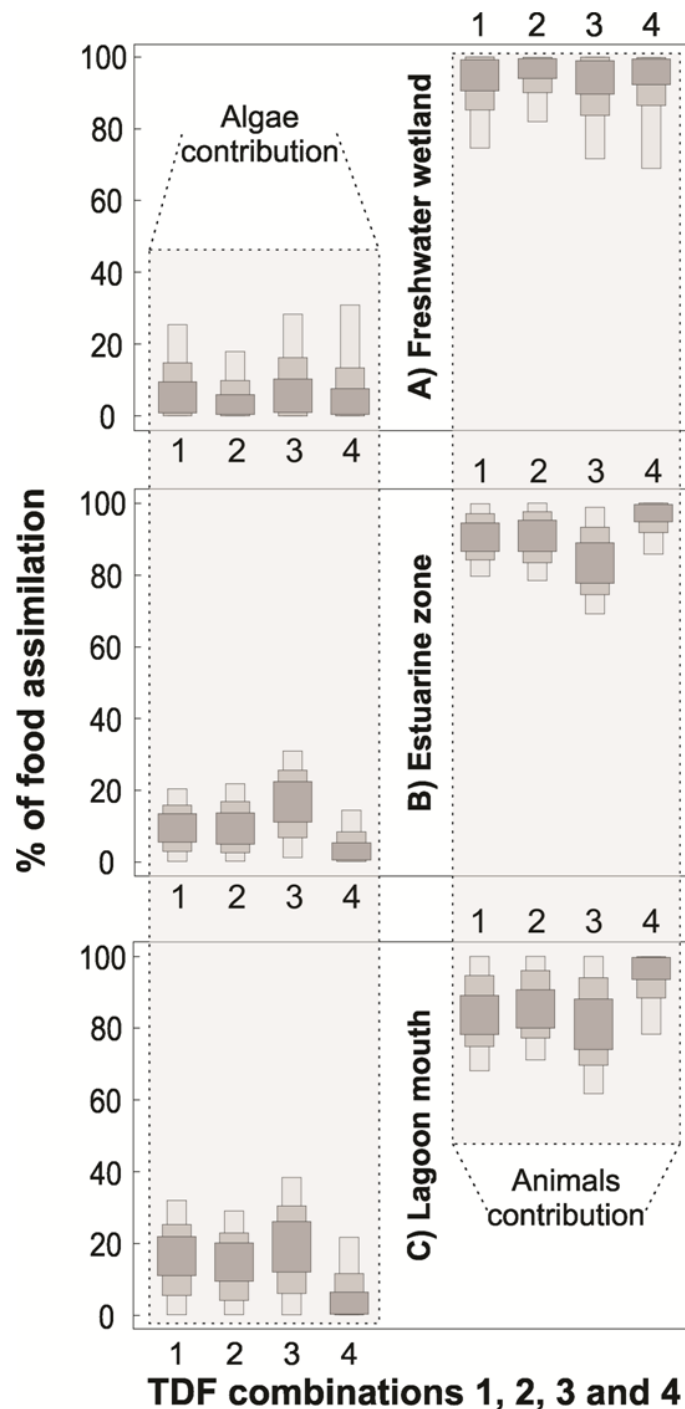


Fig. 3. Estimated relative contributions of algae (left column) and animal prey (polychaets, amphipods and insects combined; right column) assimilated by *Jenynsia multidentata* at three locations along the estuarine freshwater gradient - A) Freshwater wetland, B) Estuarine zone, C) Lagoon mouth. Numbers 1, 2, 3 and 4 refer to mixing model estimates generated using different trophic discrimination factors (see Table 1). Each plot shows 50% (dark grey), 75% (medium grey) and 95% (light grey) bayesian credibility intervals of feasible contributions of each source. See Materials and Methods section and Table 1 for details about the 4 combinations.

EFFECTS OF TDFS ON TROPHIC POSITION ESTIMATES

The effective TDF_N estimated based on proportional assimilation of resources were 2.40, 2.16 and 2.43 for the TP_{PA-low} , and 2.30, 1.98 and 2.09 for TP_{PA-med} estimates in FW, EZ and LM sites, respectively. For $TP_{PA-high}$ estimate, effective TDF_N were the same (1.77) as for strictly carnivorous fishes, since mixing model estimate number 4, which considered carnivorous vs. herbivorous TDFs, revealed 100% of animal contribution at the upper limit of estimate for all sites.

Fish TP varied significantly between locations (Kruskal-Wallis, $p < 0.05$) only when the TP_{PA} method was used, with post-hoc tests revealing a significant difference between FW and EZ sites ($p < 0.05$) and a marginally significant difference between FW and LM sites ($p = 0.06$) (Table 4). The four different combinations resulted in significantly different TP estimates ($p < 0.05$) (Fig. 4), with TP_{WAS} method producing values approximately one trophic level higher than other combinations (Fig. 4).

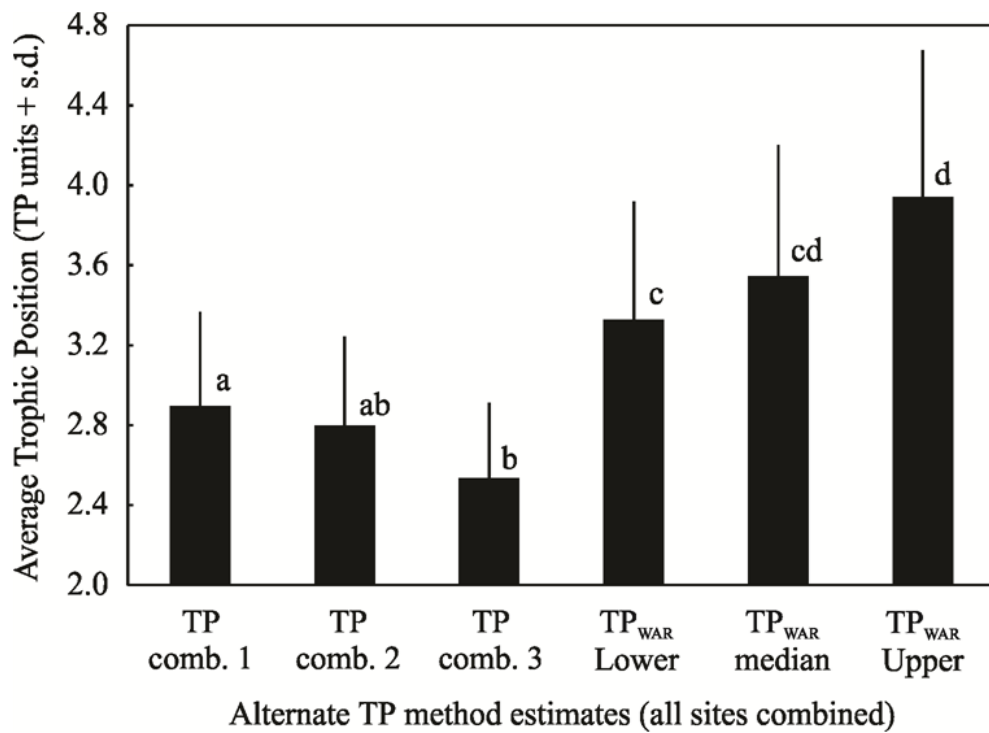


Fig. 4. Average trophic position (TP) estimated (vertical bars = 1 s.d.) obtained by each of 4 combinations with data combined for all survey sites. Bars denoted by the same letter are not significantly different according to multiple comparisons of p values.

Table 4. Trophic position (TP) (mean - μ , and standard deviation - s.d.) for *Jenyssia multidentata* from three locations calculated according to four combinations of TDFs.

Sampling site	TP combination 1		TP combination 2		TP combination 3		TP _{PA} method					
							Low		Median		High	
	μ	s.d.	μ	s.d.	μ	s.d.	μ	s.d.	μ	s.d.	μ	s.d.
Freshwater Wetland	2.87	0.35	2.77	0.33	2.51	0.28	3.14	0.40	3.24	0.41	3.91	0.54
Estuarine Zone	2.93	0.42	2.83	0.40	2.56	0.34	3.46	0.53	3.68	0.58	4.00	0.65
Lagoon Mouth	2.76	0.78	2.67	0.74	2.42	0.63	2.99	0.88	3.32	1.03	3.73	1.21

Discussion

Omnivorous *J. multidentata* in Lagoa do Peixe revealed a major shift from a diet dominated by plants to one dominated by animal prey along a longitudinal gradient from freshwater marsh to the estuary and its connection to the sea. The four combinations used for isotopic analysis of food assimilation produced estimates that were discordant with estimates based on dietary analysis. Choice of trophic discrimination factors (TDFs) affected estimates on trophic position estimates more than it affected estimates of food assimilation from isotopic mixing models. Despite the herbivorous to carnivorous diet shift along the environmental gradient, $\delta^{15}\text{N}$ differences between the consumer and primary producers isotopic baseline were similar across locations.

Even though algae were the dominant item ingested by fish at the freshwater site, all of our isotopic mixing models estimated that animal prey were the most important resource assimilated. At least four factors could explain this apparent discrepancy between diet composition from SCA and food assimilation inferred from SIA. First, differential digestibility and nutritional quality (protein content) of food items affect their assimilation (Bowen, Lutz & Ahlgren 1995). SCA and SIA data reveal trophic processes that operate on different time scales. SCA generally provides a snapshot of food ingested over preceding minutes or hours (Hellawell & Abel 1971; Hyslop 1980), whereas SIA integrates assimilation of food resources over weeks to months (Hesslein *et al.* 1991; Weidel *et al.* 2011; Madigan *et al.* 2012; Heady & Moore 2013). Second, foods such as algae and macrophytes digest more slowly and, therefore, remain within stomachs longer than animal prey, which would result in their overestimation by SCA (Hellawell & Abel 1971; Hyslop 1980). Third, some of the amorphous particulate organic matter in stomachs of fish from the freshwater habitat could

have derived from easily digested animal material rather than plant material as generally assumed. Fourth, some food items in fish stomachs could have been ingested incidentally during foraging bouts that targeted invertebrate prey (Colombini & Chelazzi 2003; Baeta *et al.* 2006; Condini *et al.* 2011). Polychaete worms were the most important item within stomachs of fish from the lagoon mouth, and sand also was frequently ingested at this location. Given that sand has no direct nutritional value, this material had been ingested incidentally, and we speculate that algae, at least part of, within stomachs of fish from the freshwater site had been ingested incidentally during capture of invertebrate prey. Finally, bias in TDF values employed in isotopic mixing models could influence accuracy assimilation estimates (Martínez del Río *et al.* 2009; Layman *et al.* 2012).

Additional evidence supporting the hypothesis that fish ingest but do not assimilate significant amount of plant material is the lack of spatial variation in fish trophic position. Average trophic position was not lower at the freshwater wetland where greater fractions of plant material had been ingested (IRI ca. 70%). Because the $\delta^{15}\text{N}$ difference between fish and local basal production sources was nearly the same at all sites, it seems unlikely that isotopic variation in basal sources or TDFs influenced this finding.

Among the four combinations used to estimate trophic position, only the TP_{PA} method revealed differences among sites. This method considers food-specific TDFs rather than a single TDF based on a reported average. In some cases, use of reported TDFs resulted in TP estimates for *J. multidentata* around 2.0, which would indicate an almost entirely herbivorous diet. A recent study of a marine food web found that use of a single TDF value to estimate consumer TP can bias results (Hussey *et al.* 2014). Use of a single TDF value regardless of the quality of ingested food types may be responsible for discrepancies between TP estimates from SCA and SIA (Rybczynski *et al.* 2008; Winemiller *et al.* 2011; this study). The use of

TDFs based on assimilation of resources estimates for plant vs. animal foods (TP_{PA} method) yielded TP estimates for *J. multidentata* indicative of a carnivorous rather than omnivorous diet. SCA indicated that algae, crustaceans and polychaete worms were the major dietary components in FW, EZ and LM respectively. A strictly herbivorous diet would yield TP = 2, and a strictly carnivorous diet would yield a TP above 3, because most of invertebrates preys are also omnivorous or even carnivorous, which will lead fish to be considered at some level between secondary (TP = 3) and tertiary (TP = 4) consumer. TP_{PA-med} produced an average TP estimate of 3.54, which is consistent with assimilation of polychaetes and other omnivorous invertebrate prey. The other three combinations produced TP values of 2.89, 2.79 and 2.53, which are inconsistent with our findings for assimilation of food sources.

Trophic position should vary according to dietary composition, food quality and assimilation dynamics, and the TP_{PA} method should improve TP estimates, especially for omnivorous species. TP estimates are known to be highly sensitive to choice of TDF as well as variation in estimates of proportional assimilation of food types (Caut *et al.* 2009; Parnell *et al.* 2013; Hussey *et al.* 2014). The TP_{PA} method not only employs food-specific TDFs, but also produces a range of feasible TPs (95% credibility intervals). The method should improve estimates not only for omnivores, but any consumer that has dietary shifts involving food types having different assimilation dynamics. Research on trophic ecology that combines SCA and SIA greatly increases precision and accuracy of biomass assimilation and TP estimates. More experimental studies that manipulate diets to track assimilation dynamics are needed to improve and refine the TP_{PA} method proposed here.

Acknowledgements

We thank João Paes Vieira, Daniel Loebmann, Sônia Huckembeck, Marlucy Coelho Claudino and Mario Vinicius Lopes Condini for field and laboratory work assistance and also to Lagoa do Peixe National Park staff for support during fieldwork. This research was financial supported by CNPq Universal 15/2007 and International Foundation for Science – IFS (grant n° A/4419-1). Fabiano Correa thanks CAPES, Rodrigo Ferreira Bastos thanks IFS (grant n° A/4419-1) and Alexandre Miranda Garcia thanks CNPq (grant n° 305888/2012-9) for scholarships. This research was permitted by Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA under authorization number 14443-1.

Data Accessibility

All data used in this manuscript are present in the manuscript and its supporting information.

References

- Aranha, J.M.R. & Caramaschi, E.P. (1999) Estrutura populacional, aspectos da reprodução e alimentação dos Cyprinodontiformes (Osteichthyes) de um riacho do sudeste do Brasil. *Revista Brasileira de Zoologia*, **16**, 637–651.
- Baeta, A., Cabral, H.N., Marques, J.C. & Pardal, M.A. (2006) Feeding ecology of the green crab, *Carcinus maenas* (L., 1758) in a temperate estuary, Portugal. *Crustaceana*, **79**, 1181–1193.
- Bastos, R.F., Calliari, L.J. & Garcia, A.M. (2014) Marine intrusion and freshwater discharge as opposite forces driving fish guilds distribution along coastal plain streams. *Hydrobiologia*, **726**, 245–258.
- Bond, A.L. & Diamond, A.W. (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecological Applications*, **21**, 1017–1023.
- Bowen, S.H., Lutz, E. V & Ahlgren, M.O. (1995) Dietary Protein and Energy as Determinants of Food Quality: Trophic Strategies Compared. *Ecology*, **76**, 899–907.
- Caswell, H., Reed, F., Stephenson, S.N. & Werner, P.A. (1973) Photosynthetic pathways and selective herbivory: a hypothesis. *American Naturalist*, **107**, 465–480.
- Caut, S., Angulo, E. & Courchamp, F. (2009) Variation in discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, **46**, 443–453.
- Codron, D., Poupin, N., Bos, C., Mariotti, F., Huneau, J.-F., Tomé, D. & Fouillet, H. (2011) The nature of the dietary protein impacts the tissue-to-diet ^{15}N discrimination factors in laboratory rats. *PloS one*, **6**, e28046.
- Colombini, I. & Chelazzi, L. (2003) Influence of marine allochthonous input on sandy beach communities. *Oceanography and Marine Biology: an Annual Review*, **41**, 115–159.
- Condini, M.V.L., Seyboth, E., Vieira, J.P. & Garcia, A.M. (2011) Diet and feeding strategy of the dusky grouper *Mycteroperca marginata* (Actinopterygii: Epinephelidae) in a man-made rocky habitat in southern Brazil. *Neotropical Ichthyology*, **9**, 161–168.
- DeNiro, M.J. & Epstein, S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, **42**, 495–506.
- DeNiro, M.J. & Epstein, S. (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, **45**, 341–351.
- Fantle, M.S., Dittel, A.I., Schwalm, S.M., Epifanio, C.E. & Fogel, L. (1999) A food web analysis of the juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole

- animals and individual amino acids. *Oecologia*, **120**, 416–426.
- Florin, S.T., Felicetti, L.A. & Robbins, C.T. (2011) The biological basis for understanding and predicting dietary-induced variation in nitrogen and sulphur isotope ratio discrimination. *Functional Ecology*, **25**, 519–526.
- Gannes, L.Z., O'Brien, D.M. & Martínez Del Rio, C. (1997) Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. *Ecology*, **78**, 1271–1276.
- Garcia, A.M., Vieira, J.P., Winemiller, K.O. & Raseira, M.B. (2004) Reproductive cycle and spatiotemporal variation in abundance of the one-sided livebearer *Jenynsia multidentata*, in Patos Lagoon, Brazil. *Hydrobiologia*, **515**, 39–48.
- Gaye-Siessegger, J., Focken, U., Abel, H. & Becker, K. (2007) Starvation and low feeding levels result in an enrichment of ^{13}C in lipids and ^{15}N in protein of Nile tilapia *Oreochromis niloticus* L. *Journal of Fish Biology*, **71**, 90–100.
- Heady, W.N. & Moore, J.W. (2013) Tissue turnover and stable isotope clocks to quantify resource shifts in anadromous rainbow trout. *Oecologia*, **172**, 21–34.
- Hellawell, J.M. & Abel, R. (1971) A rapid volumetric method for the analysis of the food of fishes. *Journal of Fish Biology*, **3**, 29–37.
- Hesslein, R.H., Capel, M.J., Fox, D.E. & Hallard, K.A. (1991) Stable isotopes of Sulfur, Carbon, and Nitrogen as indicators of trophic level and fish migration in the lower Mackenzie River basin, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 2258–2265.
- Hussey, N.E., Macneil, M.A., Mcmeans, B.C., Olin, J.A., Dudley, S.F.J., Cliff, G., Wintner, S.P., Fennessy, S.T. & Fisk, A.T. (2014) Rescaling the trophic structure of marine food webs. *Ecology Letters*, **17**, 239–250.
- Hyslop, E.J. (1980) Stomach contents analysis—a review of methods and their application. *Journal of Fish Biology*, **17**, 411–429.
- Jennings, S., Reñones, O., Morales-Nin, B., Polunin, N.V.C., Moranta, J. & Coll, J. (1997) Spatial variation in the ^{15}N and ^{13}C stable isotope composition of plants, invertebrates and fishes on Mediterranean reefs: Implications for the study of trophic pathways. *Marine Ecology Progress Series*, **146**, 109–116.
- Keegan, W.F. & DeNiro, M.J. (1988) Stable Carbon- and Nitrogen-isotope ratios used to study coral reef and terrestrial components of prehistoric Bahamian diet. *American Antiquity*, **53**, 320–336.
- Kelly, L.J. & Martínez del Rio, C. (2010) The fate of carbon in growing fish: an experimental study of isotopic routing. *Physiological and biochemical zoology: PBZ*, **83**, 473–80.

- Layman, C.A., Araujo, M.S., Boucek, R., Hammerschlag-Peyer, C.M., Harrison, E., Jud, Z.R., Matich, P., Rosenblatt, A.E., Vaudo, J.J., Yeager, L. a, Post, D.M. & Bearhop, S. (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biological reviews of the Cambridge Philosophical Society*, **87**, 545–62.
- Lujan, N.K., German, D.P. & Winemiller, K.O. (2011) Do wood-grazing fishes partition their niche?: Morphological and isotopic evidence for trophic segregation in Neotropical Loricariidae. *Functional Ecology*, **25**, 1327–1338.
- Madigan, D.J., Litvin, S.Y., Popp, B.N., Carlisle, A.B., Farwell, C.J. & Block, B. a. (2012) Tissue turnover rates and isotopic trophic discrimination factors in the endothermic teleost, pacific bluefin tuna (*Thunnus orientalis*). *PloS one*, **7**, e49220.
- Mai, A.C.G., Garcia, A.M. & Vieira, J.P. (2006) Ecologia alimentar do barrigudinho *Jenynsia multidentata* (Jenyns, 1842) (Pisces: Cyprinodontiformes) no estuário da Laguna dos Patos, Rio Grande do Sul, Brasil. *Comunicações do Museu de Ciências e Tecnologia da PUCRS*, **19**, 3–18.
- Martínez del Rio, C., Wolf, N., Carleton, S. a & Gannes, L.Z. (2009) Isotopic ecology ten years after a call for more laboratory experiments. *Biological reviews of the Cambridge Philosophical Society*, **84**, 91–111.
- McMahon, K.W., Fogel, M.L., Elsdon, T.S. & Thorrold, S.R. (2010) Carbon isotope fractionation of amino acids in fish muscle reflects biosynthesis and isotopic routing from dietary protein. *Journal of animal ecology*, **79**, 1132–1141.
- Mill, A.C., Pinnegar, J.K. & Polunin, N.V.C. (2007) Explaining isotope trophic-step fractionation: Why herbivorous fish are different. *Functional Ecology*, **21**, 1137–1145.
- Newsome, S.D., Fogel, M.L., Kelly, L. & Martínez Del Rio, C. (2011) Contributions of direct incorporation from diet and microbial amino acids to protein synthesis in Nile tilapia. *Functional Ecology*, **25**, 1051–1062.
- Olive, P.J.W., Pinnegar, J.K., Polunin, N.V.C., Richards, G. & Welch, R. (2003) Isotope trophic-step fractionation: A dynamic equilibrium model. *Journal of Animal Ecology*, **72**, 608–617.
- Parnell, A.C., Inger, R., Bearhop, S. & Jackson, A. (2008) SIAR: Stable isotope analysis in R.
- Parnell, A.C., Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W., Jackson, A.L., Grey, J., Kelly, D.J. & Inger, R. (2013) Bayesian stable isotope mixing models. *Environmetrics*, **24**, 387–399.
- Phillips, D.L., Newsome, S.D. & Gregg, J.W. (2005) Combining sources in stable isotope mixing models: alternative methods. *Oecologia*, **144**, 520–7.
- Pinkas, L., Oliphant, M.S. & Iverson, I.L.K. (1970) Food habits of Albacore, Bluefin tuna,

- and Bonito in California waters. *Fish Bulletin*, **152**, 1–105.
- Pinnegar, J.K. & Polunin, N.V.C. (2000) Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. *Oecologia*, **122**, 399–409.
- Ponsard, S. & Averbuch, P. (1999) Should growing and adult animals fed on the same diet show different $\delta^{15}\text{N}$ values? *Rapid Communications in Mass Spectrometry*, **13**, 1305–1310.
- Post, D.M. (2002) Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, **83**, 703–718.
- Poupin, N., Bos, C., Mariotti, F., Huneau, J.-F., Tomé, D. & Fouillet, H. (2011) The nature of the dietary protein impacts the tissue-to-diet ^{15}N discrimination factors in laboratory rats. *PLoS one*, **6**, e28046.
- Prado, P., Carmichael, R., Watts, S., Cebrian, J. & Heck, K. (2012) Diet-dependent $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ fractionation among sea urchin *Lytechinus variegatus* tissues: implications for food web models. *Marine Ecology Progress Series*, **462**, 175–190.
- Robbins, C.T., Felicetti, L.A. & Sponheimer, M. (2005) The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. *Oecologia*, **144**, 534–40.
- Rybczynski, S.M., Walters, D.M., Fritz, K.M. & Johnson, B.R. (2008) Comparing trophic position of stream fishes using stable isotope and gut contents analyses. *Ecology of Freshwater Fish*, **17**, 199–206.
- Vanderklift, M.A. & Ponsard, S. (2003) Sources of variation in consumer-diet $\delta^{15}\text{N}$ enrichment: A meta-analysis. *Oecologia*, **136**, 169–82.
- Varela, J.L., Larrañaga, A. & Medina, A. (2011) Prey-muscle carbon and nitrogen stable-isotope discrimination factors in Atlantic bluefin tuna (*Thunnus thynnus*). *Journal of Experimental Marine Biology and Ecology*, **406**, 21–28.
- Weidel, B.C., Carpenter, S.R., Kitchell, J.F. & Vander Zanden, M.J. (2011) Rates and components of carbon turnover in fish muscle: Insights from bioenergetics models and a whole-lake ^{13}C addition. *Canadian Journal of Fisheries and Aquatic Sciences*, **68**, 387–399.
- Wessels, F.J. & Hahn, D.A. (2010) Carbon 13 discrimination during lipid biosynthesis varies with dietary concentration of stable isotopes: Implications for stable isotope analyses. *Functional Ecology*, **24**, 1017–1022.
- Winemiller, K.O., Zeug, S.C., Robertson, C.R., Winemiller, B.K. & Honeycutt, R.L. (2011) Food-web structure of coastal streams in Costa Rica revealed by dietary and stable isotope analyses. *Journal of Tropical Ecology*, **27**, 463–476.
- Wolf, N., Carleton, S.A. & Martínez Del Rio, C. (2009) Ten years of experimental animal

isotopic ecology. *Functional Ecology*, **23**, 17–26.

Vander Zanden, M.J. & Rasmussen, J.B. (2001) Variation in delta $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, **46**, 2061–2066.

Zar, J.H. (2010) *Bioestatistical Analysis*, Fifth. Prentice Hall, Upper Saddle River, New Jersey, USA.

CONCLUSÕES

A proposta inicial da presente tese de traçar a assimilação de fontes de produção primária e recursos secundários por peixes presentes em duas importantes unidades de conservação do estado do Rio Grande do Sul, apoiadas em teorias gerais, foi alcançada. Além disso, ao longo do desenvolvimento do trabalho, novas propostas e adaptações metodológicas surgiram pela necessidade de suprir algumas falhas e pressupostos das técnicas recentemente mais utilizadas.

Além disso, foi atingida uma maior compreensão da estrutura trófica da Estação Ecológica do Taim”, à medida em que os resultados aqui presentes se juntam a alguns trabalhos prévios sobre tal assunto na região do Taim (Garcia *et al.* 2006b; Kutter *et al.* 2009; Bastos *et al.* 2011; Rodrigues *et al.* 2012, 2014). O objetivo de identificar as fontes de produção primária e as variações das contribuições terrestres para lagoas e banhados em diferentes condições hidrológicas também foram cumpridas. Os resultados corroboraram nossa hipótese inicial de que ambientes menores e mais rasos, como banhados, seriam mais dependentes do ambiente terrestre e apresentariam menores “nichos” isotópicos do que as lagoas, o que conseqüentemente foi ao encontro das hipóteses de comprimento de estrutura trófica relacionadas ao tamanho, produtividade e estabilidade. As proposições das teorias acerca do pulso de inundação (Tockner, Malard & Ward 2000; Thomaz *et al.* 2007; Junk & Wantzen 2008), foram verificadas aqui, em que uma maior importância terrestre foi observada durante o inverno/cheia, principalmente no banhado. Além disso, a proposta de substituição de valores absolutos de $\delta^{15}\text{N}$ por valores de posição trófica estimados a partir destes, resolve um dos

pressupostos para a comparação dos resultados entre diferentes situações, que é considerar os *baselines* de cada uma destas. Entretanto, o pressuposto de que os fatores de discriminação trófica são iguais e com valor fixo, mesmo para grupos com ecologias alimentares distintas, permanece nas análises deste capítulo. Para resolver esse problema, foi proposto, no capítulo seguinte, um novo método para cálculo de posição trófica de consumidores que podem se alimentar de diferentes recursos, e assim estarem sujeitos a variações nos fatores de discriminação trófica,.

O estudo da dieta, assimilação de recursos e posição trófica, bem como suas variações ao longo de um gradiente salino, junta-se a apenas um estudo prévio publicado sobre características tróficas de peixes no Parque Nacional da Lagoa do Peixe (PNLP) (Corrêa *et al.* 2012). A espécie apresentou uma dieta omnívora, variando entre quase herbívora até completamente carnívora ao longo do gradiente estuarino. Contudo, as análises de assimilação dos recursos mostrou que a espécie assimilou proporcionalmente os itens animais em maior quantidade do que ingeriu, ao passo que os itens vegetais foram mais consumidos do que assimilados. Os resultados da comparação dos valores de discriminação trófica utilizados (Vander Zanden & Rasmussen 2001; Post 2002; Caut *et al.* 2009), principalmente de $\delta^{15}\text{N}$ para carnívoros (Varela *et al.* 2011; Madigan *et al.* 2012) e herbívoros (Keegan & DeNiro 1988; Pinnegar, Campbell & Polunin 2001; Mill *et al.* 2007), mostrou que estes alteram as estimativas de assimilação de recursos e, principalmente de posição trófica. Foi proposta também uma nova metodologia para estimar as posições tróficas, visando a superação de limitações da técnica quando diferentes recursos e consequentemente fracionamentos estão envolvidos. A metodologia sugerida utiliza as estimativas de assimilação de cada recurso, calculadas por meio do pacote SIAR (Parnell *et al.* 2008),

como peso para cada um dos fatores de discriminação associados com cada tipo de recurso. Assim, as estimativas de posição trófica ficaram mais próximas do esperado a partir das estimativas de ingestão e, principalmente, de assimilação de recursos. Entretanto, esse método depende de estimativas cada vez mais precisas dos fatores de discriminação trófica que ocorrem quando os consumidores mudam de dieta.

Em suma, ao final da presente tese, pelo menos três tipos de contribuição científica são identificados emergindo nos capítulos. O primeiro tipo de contribuição está relacionado à geração e desenvolvimento de ciência tanto de base como aplicada, mas de caráter mais regional. As perguntas levantadas desde a elaboração dos projetos e respostas obtidas ao longo dos capítulos geram um maior conhecimento acerca dos habitats e fauna estudados em duas importantes áreas de preservação do estado do Rio Grande do Sul, contribuindo assim de forma direta para a gestão da Estação Ecológica do Taim e do Parque nacional da Lagoa do Peixe. Num segundo momento, com as hipóteses e contextualizações trabalhadas ao longo dos dois capítulos, são identificadas contribuições no campo da ciência de base num âmbito mais abrangente de maneira que um diálogo de interesse comum com os pares é estabelecido. Complementarmente, contribuições de caráter metodológico emergem nos dois capítulos, revelando o potencial da aplicação das propostas aqui presentes no desenvolvimento de um campo específico da ciência de base e, por consequência, nas ciências aplicadas. Por fim, os avanços gerados, apesar de suprirem algumas carências, sugerem que ainda há muito a ser explorado, tanto no conhecimento básico acerca dos habitats e fauna estudada quanto nas questões teóricas de fluxo de matéria, estrutura trófica, assimilação de recursos e, logo, no aperfeiçoamento das técnicas analíticas de dados isotópicos.

REFERÊNCIAS BIBLIOGRÁFICAS

- Abelha, M.C.F., Agostinho, A.A. & Goulart, E. (2001) Plasticidade trófica em peixes de água doce. *Acta Scientiarum. Biological Sciences*, **23**, 425–434.
- Abrantes, K.G., Barnett, A., Marwick, T. & Bouillon, S. (2013) Importance of terrestrial subsidies for estuarine food webs in contrasting East African catchments. *Ecosphere*, **4**, 1–33.
- Bastos, R.F., Condini, M. V, Junior, A.S.V. & Garcia, A.M. (2011) Diet and food consumption of the pearl cichlid *Geophagus brasiliensis* (Teleostei: Cichlidae): relationships with gender and sexual maturity. *Neotropical Ichthology*, **9**, 825–830.
- Bond, A.L. & Hobson, K.A. (2012) Reporting stable-isotope ratios in ecology: Recommended terminology, guidelines and best practices. *Waterbirds*, **35**, 324–331.
- Buchmann, F.S.C., Barbosa, V.P. & Villwock., A. (1998) Sedimentologia e paleoecologia durante o máximo transgressivo holoceno na Lagoa Mirim, RS, Brasil. *Acta Geologica Leopoldensia*, **21**, 21–26.
- Burns, M.D.M., Garcia, A.M., Bemvenuti, M.A., Vieira, J.P., Motta-Marques, David Moresco, A. & Condini, M.V.L. (2006a) Bivalvia, Mytilidae, *Limnoperna fortunei*: distribution extension. *Checkl List*, **2**, 41–43.
- Burns, M.D.M., Garcia, A.M., Vieira, J.P., Bemvenuti, M.A., Motta-Marques, D.M.L. & Condini, V. (2006b) Evidence of habitat fragmentation affecting fish movement between the Patos and Mirim coastal lagoons in southern Brazil. *Neotropical Ichthology*, **4**, 69–72.
- Caut, S., Angulo, E. & Courchamp, F. (2009) Variation in discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, **46**, 443–453.
- Codron, D., Poupin, N., Bos, C., Mariotti, F., Huneau, J.-F., Tomé, D. & Fouillet, H. (2011) The nature of the dietary protein impacts the tissue-to-diet ^{15}N discrimination factors in laboratory rats. *PloS one*, **6**, e28046.
- Corrêa, F., Claudino, M.C., Bastos, R.F., Huckembeck, S. & Garcia, A.M. (2012) Feeding ecology and prey preferences of a piscivorous fish in the Lagoa do Peixe National Park, a Biosphere Reserve in Southern Brazil. *Environmental Biology of Fishes*, **93**, 1–12.
- Craig, H. (1953) The geochemistry of the stable carbon isotopes. *Geochimica et Cosmochimica Acta*, **3**, 53–92.
- DeNiro, M.J. & Epstein, S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, **42**, 495–506.

- DeNiro, M.J. & Epstein, S. (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, **45**, 341–351.
- Florin, S.T., Felicetti, L.A. & Robbins, C.T. (2011) The biological basis for understanding and predicting dietary-induced variation in nitrogen and sulphur isotope ratio discrimination. *Functional Ecology*, **25**, 519–526.
- Fontoura, N.F., Rodrigues, L.R., Batista, C.B., Persch, T.S.P. & Janowicz, M.E. (2015) Integrating ontogenetic shift, growth and mortality to determine a species' ecological role from isotopic signatures. *PloS one*, **10**, e0125059.
- Fry, B. (2006) *Stable Isotope Ecology*. Springer, New York.
- Gannes, L.Z., O'Brien, D.M. & Martínez Del Rio, C. (1997) Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. *Ecology*, **78**, 1271–1276.
- Garcia, A.M., Bemvenuti, M.A., Vieira, J.P., Motta-Marques, D.M.L., Burns, M.D.M., Moresco, A. & Condi, M.V.L. (2006a) Checklist comparison and dominance patterns of the fish fauna at Taim wetland, south Brazil. *Neotropical Ichthyology*, **4**, 261–268.
- Garcia, A.M., Hoeninghaus, D.J., Vieira, J.P., Winemiller, K.O., Motta-Marques, D.M.L. & Bemvenuti, M.A. (2006b) Preliminary examination of food web structure of Nicola lake (Taim hydrological system, south Brazil) using dual C and N stable isotope analyses. *Neotropical Ichthyology*, **4**, 279–284.
- Gaye-Siessegger, J., Focken, U., Abel, H. & Becker, K. (2007) Starvation and low feeding levels result in an enrichment of ^{13}C in lipids and ^{15}N in protein of Nile tilapia *Oreochromis niloticus* L. *Journal of Fish Biology*, **71**, 90–100.
- Hahn, N.S., Pavanelli, C.S. & Okada, E.K. (2000) Dental development and ontogenetic diet shifts of *Roebooides paranensis* Pignalberi (Osteichthyes, Characinae) in pools of the upper Rio Paraná floodplain (state of Paraná, Brazil). *Revista brasileira de biologia*, **60**, 93–9.
- Harayashiki, C.A.Y., Varela Junior, A.S., Burns, M.D. de M. & Vieira, J.P. (2014) Establishing evidence of a non-native species *Pachyurus bonariensis* Steindachner, 1879 (Perciformes, Sciaenidae) in Mirim Lagoon, Rio Grande do Sul (Brazil). *BioInvasions Records*, **3**, 103–110.
- Hoeninghaus, D.J., Vieira, J.P., Costa, C.S., Bemvenuti, C.E., Winemiller, K.O. & Garcia, A.M. (2011) Estuary hydrogeomorphology affects carbon sources supporting aquatic consumers within and among ecological guilds. *Hydrobiologia*, **673**, 79–92.
- Hoeninghaus, D.J. & Zeug, S.C. (2008) Can stable isotope ratios provide for community-wide measures of trophic structure? Comment. *Ecology*, **89**, 2353–7; discussion 2358–9.
- Hussey, N.E., Macneil, M.A., Mcmeans, B.C., Olin, J.A., Dudley, S.F.J., Cliff, G., Wintner, S.P., Fennessy, S.T. & Fisk, A.T. (2014) Rescaling the trophic structure of marine food webs. *Ecology Letters*, **17**, 239–250.

- Hutchinson, G.E. (1957) Concluding remarks. *Cold Springs Harbor Symposium of Quantitative Biology*, **22**, 415–427.
- Jackson, A.L., Inger, R., Parnell, A.C. & Bearhop, S. (2011) Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *The Journal of animal ecology*, **80**, 595–602.
- Jennings, S., Reñones, O., Morales-Nin, B., Polunin, N.V.C., Moranta, J. & Coll, J. (1997) Spatial variation in the ¹⁵N and ¹³C stable isotope composition of plants, invertebrates and fishes on Mediterranean reefs: Implications for the study of trophic pathways. *Marine Ecology Progress Series*, **146**, 109–116.
- Junk, W.J., Bayley, P.B. & Sparks, R.E. (1989) The flood pulse concept in river-floodplain systems. *Proceedings of the International Large river Symposium* (ed D.P. Dodge), pp. 110–127. Canadian Special Published in Fisheries and Aquatic Science.
- Junk, W.J. & Wantzen, K.M. (2008) The flood pulse concept: New aspects , approaches and applications - An update. *The riverine ecosystem synthesis toward conceptual cohesiveness in river science* (eds J.H. Thorp, M.C. Thoms & M.D. DeLong), pp. 117–141. Academic press, London.
- Keegan, W.F. & DeNiro, M.J. (1988) Stable Carbon- and Nitrogen-Isotope Ratios Used to Study Coral Reef and Terrestrial Components of Prehistoric Bahamian Diet. *American Antiquity*, **53**, 320–336.
- Kelly, L.J. & Martínez del Rio, C. (2010) The fate of carbon in growing fish: an experimental study of isotopic routing. *Physiological and biochemical zoology : PBZ*, **83**, 473–80.
- Kutter, M.T., Bemvenuti, M.D.A. & Moresco, A. (2009) Feeding strategy of the jundiá *Rhamdia quelen* (Siluriformes, Heptapteridae) in costal lagoons of southern Brazil. *Acta Scientiarum. Biological Sciences*, **31**, 41–47.
- Layman, C.A., Arrington, D.A., Montaña, C.G. & Post, D.M. (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology*, **88**, 42–48.
- Madigan, D.J., Litvin, S.Y., Popp, B.N., Carlisle, A.B., Farwell, C.J. & Block, B. a. (2012) Tissue turnover rates and isotopic trophic discrimination factors in the endothermic teleost, pacific bluefin tuna (*Thunnus orientalis*). *PloS one*, **7**, e49220.
- Mao, Z., Gu, X., Zeng, Q., Zhou, L. & Sun, M. (2011) Food web structure of a shallow eutrophic lake (Lake Taihu, China) assessed by stable isotope analysis. *Hydrobiologia*, **683**, 173–183.
- McMahon, K.W., Fogel, M.L., Elsdon, T.S. & Thorrold, S.R. (2010) Carbon isotope fractionation of amino acids in fish muscle reflects biosynthesis and isotopic routing from dietary protein. *Journal of animal ecology*, **79**, 1132–1141.
- Mill, A.C., Pinnegar, J.K. & Polunin, N.V.C. (2007) Explaining isotope trophic-step fractionation: why herbivorous fish are different. *Functional Ecology*, **21**, 1137–1145.

- Newsome, S.D., Fogel, M.L., Kelly, L. & Martínez Del Rio, C. (2011) Contributions of direct incorporation from diet and microbial amino acids to protein synthesis in Nile tilapia. *Functional Ecology*, **25**, 1051–1062.
- Newsome, S.D., Martínez del Rio, C., Bearhop, S. & Phillips, D.L. (2007) A niche for isotopic ecology. *Frontiers in Ecology and Environment*, **5**, 429–436.
- O’Leary, M.H. (1988) Carbon isotopes in photosynthesis. *BioScience*, **38**, 328–336.
- Parnell, A.C., Inger, R., Bearhop, S. & Jackson, A. (2008) SIAR: Stable isotope analysis in R.
- Parnell, A.C., Inger, R., Bearhop, S. & Jackson, A.L. (2010) Source partitioning using stable isotopes: coping with too much variation. *PloS one*, **5**, e9672.
- Parnell, A.C., Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W., Jackson, A.L., Grey, J., Kelly, D.J. & Inger, R. (2013) Bayesian stable isotope mixing models. *Environmetrics*, **24**, 387–399.
- Phillips, D.L. & Gregg, J.W. (2003) Source partitioning using stable isotopes: coping with too many sources. *Oecologia*, **136**, 261–9.
- Phillips, D.L., Newsome, S.D. & Gregg, J.W. (2005) Combining sources in stable isotope mixing models: alternative methods. *Oecologia*, **144**, 520–7.
- Pimm, S.L. & Lawton, J.H. (1977) Number of trophic levels in ecological communities. *Nature*, **268**, 329–331.
- Pinnegar, J.K., Campbell, N. & Polunin, N.V.C. (2001) Unusual stable isotope fractionation patterns observed for fish host-parasite trophic relationships. *Journal of Fish Biology*, **59**, 494–503.
- Pinnegar, J.K. & Polunin, N.V.C. (2000) Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. *Oecologia*, **122**, 399–409.
- Polis, G.A. & Winemiller, K.O. (1996) *Food Webs: Integration of Patterns and Dynamics*. Chapman and Hall, New York.
- Post, D.M. (2002) Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, **83**, 703–718.
- Post, D.M., Pace, M.L. & Hairston, N.G. (2000) Ecosystem size determines food-chain length in lakes. *Nature*, **405**, 1047–1049.
- Poupin, N., Bos, C., Mariotti, F., Huneau, J.-F., Tomé, D. & Fouillet, H. (2011) The nature of the dietary protein impacts the tissue-to-diet ¹⁵N discrimination factors in laboratory rats. *PloS one*, **6**, e28046.
- Robbins, C.T., Felicetti, L.A. & Sponheimer, M. (2005) The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. *Oecologia*, **144**, 534–40.

- Rodrigues, L.R., Fontoura, N.F. & Motta-Marques, D.M.L. (2012) Feeding dynamics of *Oligosarcus jenynsii* (Günther, 1864) in a subtropical coastal lake assessed by gut-content analysis and stable isotopes. *International journal of plant, animal and environmental sciences*, **2**, 126–134.
- Rodrigues, L.R., Fontoura, N.F. & Motta-Marques, D. (2014) Food-web structure in a subtropical coastal lake: How phylogenetic constraints may affect species linkages. *Marine and Freshwater Research*, **65**, 453–465.
- Rodrigues, L.R., Motta-Marques, D.M.L. & Fontoura, N.F. (2015) Fish community in a large coastal subtropical lake: how an environmental gradient may affect the structure of trophic guilds. *Limnetica*, **34**, 495–506.
- Sabo, J.L., Finlay, J.C., Kennedy, T. & Post, D.M. (2010) The Role of Discharge Variation in Scaling of Drainage Area and Food Chain Length in Rivers. *Science*, **330**, 965–967.
- Sabo, J.L., Finlay, J.C. & Post, D.M. (2009) Food chains in freshwaters. *Annals of the New York Academy of Sciences*, **1162**, 187–220.
- Seeliger, U. & Costa, C.S.B. (2003) Alterações de habitats devido às atividades antrópicas na costa sul do Brasil. *Ecossistemas Brasileiros: manejo e conservação* (ed V. Claudino-Sales), p. 392. Expressão Gráfica e Editora, Fortaleza.
- Takimoto, G. & Post, D.M. (2013) Environmental determinants of food-chain length: A meta-analysis. *Ecological Research*, **28**, 675–681.
- Thomaz, S.M., Bini, L.M. & Bozelli, R.L. (2007) Floods increase similarity among aquatic habitats in river-floodplain systems. *Hydrobiologia*, **579**, 1–13.
- Thorp, J.H. & DeLong, M.D. (1994) The riverine productivity model: an heuristic view of carbon sources and organic processing in large river ecosystems. *Oikos*, **70**, 305–308.
- Tockner, K., Malard, F. & Ward, J. V. (2000) An extension of the flood pulse concept. *Hydrological Processes*, **14**, 2861–2883.
- Vanderklift, M.A. & Ponsard, S. (2003) Sources of variation in consumer-diet delta 15N enrichment: a meta-analysis. *Oecologia*, **136**, 169–82.
- Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R. & Cushing, C.E. (1980) The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 130–137.
- Varela, J.L., Larrañaga, A. & Medina, A. (2011) Prey-muscle carbon and nitrogen stable-isotope discrimination factors in Atlantic bluefin tuna (*Thunnus thynnus*). *Journal of Experimental Marine Biology and Ecology*, **406**, 21–28.
- Villanueva, A.O.N., Motta-Marques, D.M.D. & Tucci, C.E.M. (2000) The Taim wetland conflict: A compromise between environment conservation and irrigation. *Water International*, **25**, 610–616.

- Wantzen, K.M., Machado, F.A., Voss, M., Boriss, H. & Junk, W.J. (2002) Seasonal isotopic shifts in fish of the Pantanal wetland, Brazil. *Aquatic Sciences*, **64**, 239–251.
- Wessels, F.J. & Hahn, D.A. (2010) Carbon 13 discrimination during lipid biosynthesis varies with dietary concentration of stable isotopes: Implications for stable isotope analyses. *Functional Ecology*, **24**, 1017–1022.
- Winemiller, K.O. (1989) Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan llanos. *Environmental Biology of fishes*, **26**, 177–199.
- Winemiller, K., Akin, S. & Zeug, S. (2007) Production sources and food web structure of a temperate tidal estuary: Integration of dietary and stable isotope data. *Marine Ecology Progress Series*, **343**, 63–76.
- Winemiller, K.O., Hoeninghaus, D.J., Pease, A.A., Esselman, P.C., Honeycutt, R.L., Gbanaador, D., Carrera, E. & Payne, J. (2011) Stable isotope analysis reveals food web structure and watershed impacts along the fluvial gradient of a Mesoamerican coastal river. *River Research and Applications*, **27**, 791–803.
- Winemiller, K.O. & Polis, G.A. (1996) Food webs: What can they tell us about the world? *Food webs: integration of patterns and dynamics* (eds G.A. Polis & K.O. Winemiller), pp. 1–22. Chapman & Hall, New York.
- Wolf, N., Carleton, S.A. & Martínez Del Rio, C. (2009) Ten years of experimental animal isotopic ecology. *Functional Ecology*, **23**, 17–26.
- Vander Zanden, M.J., Cabana, G. & Rasmussen, J.B. (1997) Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios ($\delta^{15}\text{N}$) and literature dietary data. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 1142–1158.
- Vander Zanden, M.J. & Rasmussen, J.B. (2001) Variation in delta $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, **46**, 2061–2066.