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

Nicho Isotópico da Lontra-neotropical, *Lontra longicaudis* (Carnivora, Mustelidae), em Diferentes Sistemas Aquáticos no Sul do Brasil

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Coorientador: Dr. Elton Pinto Colares

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APRESENTAÇÃO

A presente dissertação tem como foco o uso da ferramenta de isótopos estáveis em estudos ecológicos sobre a lontra-neotropical, *Lontra longicaudis*, e foi estruturada em dois capítulos. Ambos os capítulos foram redigidos em língua inglesa e em formato de artigo.

O capítulo 1, intitulado "Effects of acidification on the isotopic ratios of Neotropical otter tooth dentin", foi formatado de acordo com as normas do periódico "Rapid Communications in Mass Spectrometry" e apresenta uma avaliação metodológica sobre a preparação de amostras de dentina da lontra-neotropical para análise de isótopos estáveis. Para tanto, foram testados os efeitos de dois diferentes métodos de acidificação sobre as razões isotópicas de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$).

O capítulo 2, intitulado "Isotopic niche of the Neotropical otter, *Lontra longicaudis* (Carnivora, Mustelidae), in diferente coastal aquatic systems in southern Brazil", corresponde ao artigo principal da dissertação, o qual será submetido para a revista Hydrobiologia e teve como objetivo a produção de conhecimento sobre a ecologia trófica e uso de habitat da lontra-neotropical em três sistemas aquáticos, localizados na região costeira do sul do Brasil: a Ilha de Santa Catarina, a Bacia Hidrográfica do Rio Tramandaí e o Banhado do Taim. Em vista disso, foram analisadas as razões isotópicas de $\delta^{13}C$ e $\delta^{15}N$ da dentina de lontras e de músculo de suas potenciais presas a fim de investigar diferenças isotópicas entre as regiões, comparar as composições isotópicas da lontra-neotropical e de suas potenciais presas e estimar a contribuição proporcional de cada presa à dieta da lontra-neotropical em cada sistema.

RESUMO

A lontra-neotropical, *Lontra longicaudis*, é um carnívoro mustelídeo de hábito semiaquático. Embora tipicamente de água doce, a espécie tem sido registrada em ambientes com influência marinha, como praias costeiras, pequenas ilhas e estuários. Dado o exposto, a hipótese do presente estudo é que o nicho isotópico da lontra-neotropical é mais amplo em uma ilha costeira (Ilha de Santa Catarina - RS) e em uma bacia hidrográfica composta por uma série de ambientes límnicos e um estuário (Bacia do Rio Tramandaí - RS), devido à maior contribuição marinha, em comparação com uma região de banhado (Banhado do Taim – RS). Para testar esta hipótese, foram analisadas as razões isotópicas de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$) em amostras de dentina de dentes da lontra-neotropical provenientes destas três regiões. Além disso, para avaliar possíveis vieses nas análises isotópicas, avaliaram-se os efeitos da acidificação em amostras de dentina. A acidificação não afetou os valores isotópicos de carbono da dentina da lontra-neotropical, considerando-se assim, um passo desnecessário para a análise de isótopos estáveis em dentes de mamíferos carnívoros. O nicho isotópico de lontras de diferentes regiões indicou uma similaridade no uso de recursos e habitat. No entanto, o nicho isotópico foi mais amplo em lontras da Ilha de Santa Catarina, seguido do Banhado do Taim e da Bacia do Rio Tramandaí. Os resultados revelaram uma baixa contribuição marinha na dieta de alguns indivíduos da Ilha de Santa Catarina e do Banhado do Taim. Portanto, conclui-se que as lontras da ilha de Santa Catarina e Banhado do Taim utilizam principalmente ambientes de água doce como áreas de forrageamento. As lontras da Bacia do Rio Tramandaí, por outro lado, usam apenas ambientes de água doce para forragear. As diferenças encontradas entre animais de diferentes regiões provavelmente estão relacionadas a um conjunto de fatores, como características do ambiente e oportunidade ecológica.

Palavras-chave: carbono, dieta, habitat, isótopos estáveis, nitrogênio.

ABSTRACT

The Neotropical otter, *Lontra longicaudis*, is a semi-aquatic carnivore mustelid. Although typically a freshwater species, it has been recorded in environments with marine influence, such as in coastal beaches, small islands and estuaries. Given the above, the hypothesis of the present study is that the isotopic niche of the Neotropical otter is wider in a coastal island (Santa Catarina Island – SC) and in a river basin composed by a series of ponds and an estuary (Tramandaí River Basin – RS) than in a wetland (Taim Wetland – RS) due a greater occurrence of the species in the marine environment of the former areas. In order to test this hypothesis, the stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in dentin samples of otters from different regions was analyzed. Furthermore, to evaluate possible biases in isotopic analysis, the effect of acidification in Neotropical otter tooth dentin was assessed. The acidification had no effect on carbon isotopic values of Neotropical otter tooth dentin, hence an unnecessary procedure for stable isotope analysis. Long-term isotopic niche of otters from different regions indicated a similarity in the use of resources and habitat. However, the isotopic niche was wider in otters of Santa Catarina Island, followed by Taim Wetland, and Tramandaí River Basin. Results revealed a marine contribution in the diet of some individuals from Santa Catarina Island and Taim Wetland. Therefore, otters from Santa Catarina Island and Taim Wetland mainly used freshwater environments as foraging areas. The otters of the Tramandaí River Basin, on the other hand, use only freshwater environments to forage. The differences found among animals from different regions are probably related to a variety of factors, such as landscape characteristics and ecological opportunity.

Key-words: carbon, diet, habitat, nitrogen, stable isotopes.

INTRODUÇÃO GERAL

O conhecimento dos hábitos alimentares de um predador é imprescindível para avaliar o papel ecológico da espécie, como por exemplo, sua influência sobre as populações de presas (e.g., Norrdahl & Korpimäki, 2000; Ripple et al., 2014) e a competição potencial com outros carnívoros (e.g., Caro & Stoner, 2003; Kasper et al., 2016), bem como entender os requerimentos tróficos e espaciais da espécie (e.g., Ben-David et al., 1997; Valenzuela et al., 2013). Segundo a teoria de forrageamento ótimo, desenvolvida por MacArthur & Pianka (1966), os animais tendem a escolher presas que deem maior rendimento energético ao final do processo de obtenção do alimento.

A dieta dos consumidores costuma ser investigada através de análise de conteúdo estomacal ou gastrointestinal (e.g., Barros & Wells, 1998; Kasper et al., 2016) e fecal (e.g., Bagchi et al., 2003; Iversen et al., 2013). A análise de conteúdo do sistema digestório e de fezes permite a diferenciação de presas a nível individual (Klare et al., 2011) e o acesso ao tamanho das presas ingeridas (e.g., Kasper et al., 2004). No entanto, essas metodologias possuem algumas limitações que podem gerar vieses nos resultados, devido, por exemplo, à digestão diferencial das presas ou à identificação errônea de táxons consumidos (Klare et al., 2011; Bowen & Iverson, 2013). Carss & Parkinson (1996), em um experimento de alimentação controlada com a lontra-europeia (*Lutra lutra*), observaram uma série de falhas associadas à investigação da dieta por meio de análise fecal. A análise de fezes apresenta uma pobre estimativa das reais proporções (em número e biomassa) dos itens predados pelo consumidor, já que restos de um único peixe, por exemplo, podem ser detectados em várias amostras de fezes, podendo levar a uma superestimava de itens alimentares (Carss & Parkinson, 1996). Um método alternativo e complementar para a investigação de hábitos alimentares é a análise de isótopos estáveis.

Os isótopos estáveis (IE) têm demonstrado ser uma valiosa fonte de informações para entender os hábitos alimentares dos animais (e.g., Newsome et al., 2009; Carlisle et al., 2012; Chiaradia et al., 2016). Os IE são diferentes formas energeticamente estáveis de um particular elemento químico. Essas formas possuem o mesmo número de prótons e elétrons, no entanto diferem umas das outras devido ao seu número de nêutrons. Consequentemente, cada isótopo de um elemento químico possui massa atômica (A) distinta. Por exemplo, o carbono ocorre em duas formas estáveis na natureza: o leve, ^{12}C, possui seis prótons e seis nêutrons (A = 12); já o isótopo pesado, ^{13}C, apresenta seis prótons e sete nêutrons (A = 13). Os isótopos mais comumente utilizados em estudos ecológicos são os de carbono e nitrogênio. Usualmente, a razão isotópica (R) de isótopos pesados e leves ($^{13}C/^{12}C$ e $^{15}N/^{14}N$) em cada amostra é calculada por meio da seguinte fórmula:

$$\delta X(‰) = [(R_{AMOSTRA}/R_{PADRÃO}) - 1]$$

onde R é a razão dos isótopos pesados por isótopos leves e X é o elemento na forma pesada. Os dados são expressos pela notação delta (δ) ($\delta^{13}C$ e $\delta^{15}N$) em partes por mil (‰) em relação aos padrões internacionais: *Vienna Pee Dee Belemnite* (V-PDB) para C e nitrogênio atmosférico para N. As razões isotópicas em materiais biológicos são tipicamente negativas para carbono ($\delta^{13}C$) e positivas para nitrogênio ($\delta^{15}N$) (Ben-David & Flaherty, 2012).

A análise de isótopos estáveis de carbono e nitrogênio ($\delta^{13}C$ e $\delta^{15}N$) baseia-se no fato de que a composição isotópica varia de forma previsível, conforme o elemento se move através dos diversos compartimentos de um ecossistema (Peterson & Fry, 1987). Parte dessa variação na distribuição isotópica se deve ao fato de que os isótopos comportam-se de diferentes formas na natureza (Fry, 2006). Assim, durante a incorporação de carbono e nitrogênio pelos consumidores, considerando o organismo como um todo, ocorre uma depleção dos isótopos leves (^{12}C e ^{14}N) através da respiração e excreção, respectivamente (DeNiro & Epstein, 1978, 1981). A composição isotópica de cada tecido também depende da rota metabólica durante o processo de síntese, uma vez que os elementos são distribuídos desproporcionalmente entre diferentes tecidos (e.g., Schwarcz, 1991). Portanto, os IE presentes nos tecidos dos consumidores refletem aqueles encontrados na sua dieta, porém com uma pequena alteração resultante da retenção preferencial do isótopo mais pesado (^{13}C e ^{15}N). Esta diferença entre os valores isotópicos dos consumidores e a sua dieta é denominado fator de discriminação trófica (FDT) (Newsome et al., 2010). O enriquecimento no isótopo pesado é acentuado no caso do nitrogênio (~3-5‰) de forma que os valores de $\delta^{15}N$ podem ser utilizados para inferir a posição trófica dos diferentes predadores nas teias alimentares (Post, 2002). A discriminação do isótopo leve também ocorre na incorporação do carbono entre os níveis tróficos, porém de uma forma menos acentuada. Assim, os valores de $\delta^{13}C$ refletem os processos biogeoquímicos que ocorrem na base da rede trófica, sendo geralmente diferentes entre os ambientes dominados por produtores com diferenças fisiológicas (e.g., plantas com vias fotossintéticas diferentes, C3 vs. C4) (DeNiro & Epstein, 1978). Devido à discriminação por nível trófico de ^{13}C ser de aproximadamente 0-1‰, os valores isotópicos de carbono são geralmente utilizados como um indicador da base alimentar (Post, 2002) e, portanto, úteis para inferir os habitats preferenciais dos consumidores.

Os valores isotópicos dos indivíduos de diferentes populações também podem ser influenciados por características físicas e geográficas (Newsome et al., 2007). A temperatura da superfície dos corpos aquáticos varia segundo a latitude, afetando a solubilidade do CO_2 (Goericke & Fry, 1994). Em regiões frias, o CO_2 é altamente solúvel devido a fatores como baixa estratificação da coluna de água e pouca atividade fotossintética. Quando o CO_2 dissolvido é elevado, a incorporação do carbono leve associado à absorção fotossintética de CO_2 é fortemente expressa, levando a baixos valores de $\delta^{13}C$. O inverso aplica-se em altas temperaturas, com a presença de alta luminosidade e águas estratificadas, características de latitudes temperadas e tropicais (Graham et al., 2010). Além disso, animais que se alimentam exclusivamente em ambiente marinho são geralmente mais enriquecidos isotopicamente do que aqueles que forrageiam em ambiente dulcícola ou terrestre. Essas diferenças ocorrem devido a uma série de fatores, como fonte de nutrientes e composição de produtores primários (Schoeninger & DeNiro, 1984).

A assinatura isotópica de carbono dos produtores primários depende do fracionamento do carbono durante o processo fotossintético (Kelly, 2000). Por exemplo, as plantas C3, que fixam o CO_2 pela ação da enzima ribulose 1,5-bifosfato carboxilase (RUBISCO), são tipicamente empobrecidas no carbono pesado, devido à alta afinidade da enzima pelo isotopômero leve ($^{12}CO_2$). Enquanto que na via fotossintética C4, o CO_2 é inicialmente carboxilado em ácidos orgânicos pela enzima fosfoenolpiruvato (PEP) carboxilase, onde o fracionamento é fracamente expresso. Consequentemente, as plantas C3 possuem valores de $\delta^{13}C$ aproximadamente de -28‰ e as plantas C4 possuem $\delta^{13}C$ em torno de -14‰ (O'Leary, 1988). A composição de isótopos de nitrogênio das plantas também é determinada por mecanismos fisiológicos, bem como pela fonte externa de nitrogênio e pela presença de micorrizas (Evans, 2001). Assim, as diferenças isotópicas encontradas nas redes tróficas são um reflexo da comunidade de produtores primários.

Portanto, os isótopos de carbono e nitrogênio fornecem informações quantitativas sobre a utilização de recursos e sobre o habitat. Esses valores, quando plotados em um gráfico ($\delta^{13}C$ vs. $\delta^{15}N$), resultam em um *δ-espaço* comparável ao espaço *n*-dimensional denominado de nicho ecológico, definido por Hutchinson (1957), no qual os eixos representam os valores isotópicos (Newsome et al., 2007). O conceito de nicho ecológico refere-se às tolerâncias e necessidades de um organismo. O nicho real de uma espécie é multidimensional devido a sua necessidade de recursos variados (e.g., nutrientes e água) e tolerância a diferentes variáveis ambientais (e.g., pH e temperatura). Assim, o nicho ecológico é considerado como um hipervolume *n*-dimensional, onde *n* é o número de dimensões que constituem o nicho (Townsend et al., 2010). Consequentemente, o *δ-espaço isotópico* é denominado nicho isotópico, pois se refere a fatores que são comumente utilizados para definir o nicho ecológico (utilização de recursos e habitats preferenciais) (Newsome et al., 2007).

As informações alimentares e de utilização do habitat podem ser obtidas através da utilização de tecidos provenientes de dentes, por exemplo, dentina (e.g., Mendes et al., 2007; Troina et al., 2016) e esmalte (e.g., Clementz & Koch, 2001; Feranec & DeSantis, 2014), como substrato isotópico. Os dentes são estruturas compostas por uma coroa, localizada acima da gengiva, e por uma ou mais raízes abaixo da gengiva. A coroa é recoberta pelo esmalte e a raiz pelo cemento. Abaixo do esmalte e do cemento há um tecido mineralizado denominado dentina, o qual se estende da coroa até a raiz do dente (Ungar, 2010). A fração orgânica da dentina é formada principalmente por colágeno, que contém aproximadamente 40% de carbono e cerca de 15% de nitrogênio, enquanto que a fração mineral é composta por apatita (fosfato de cálcio) (Bocherens & Drucker, 2013). Devido à composição de aminoácidos da fração orgânica, constituída por grandes concentrações de serina e glicina, a dentina dos consumidores é mais enriquecida isotopicamente no ^{13}C em comparação com outros tecidos (e.g., músculo) (Wolf et al., 2015). As frações orgânicas e inorgânicas da dentina refletem diferentes contribuições das macromoléculas dietéticas. O colágeno é sintetizado a partir das proteínas da dieta do consumidor, enquanto que a apatita é assimilada a partir de proteínas, carboidratos e lipídios. Isso implica que o sinal isotópico do colágeno reflete uma composição isotópica da dieta proteica, e os isótopos C e N da apatita refletem o consumo alimentar total (Ambrose & Norr 1993, Tieszen & Fagre, 1993). Devido à composição do colágeno, alguns autores recomendam acidificar os tecidos metabolicamente inertes, para eliminar o carbono inorgânico da amostra e, assim, reduzir as fontes de variação isotópica e facilitar a interpretação dos dados (e.g., Ambrose 1990, Liden et al., 1995).

Os IE têm sido empregados em uma série de estudos acerca de espécies da Família Mustelidae, elucidando questões importantes sobre dieta e uso de habitat. Um exemplo é o trabalho realizado por Smith et al. (2015), no qual a especialização individual na utilização de recursos entre machos e fêmeas de lontra marinha, *Enhydra lutris*, foi investigada. A Família Mustelidae é representada por carnívoros de tamanho pequeno ou médio, que se caracterizam por apresentar glândulas odoríferas perianais bem desenvolvidas, focinho moderadamente longo, orelhas curtas, corpo delgado e muito musculoso, membros curtos, cinco dígitos em cada pata e garras não retráteis (Orr, 1986). A diversificação filogenética dos mustelídeos provavelmente foi consequente de uma combinação de eventos de *turnover* faunísticos e diversificação em níveis tróficos mais baixos, a qual resultou em duas subfamílias, Mustelinae e Lutrinae, com um total de cerca de 22 gêneros (Koepfli et al., 2008). O grupo originou-se na

Eurásia e atualmente estão presentes em praticamente todo o mundo, com exceção da Antártica e Oceania (Nowak, 1991). No Brasil, há ocorrência de 6 espécies de mustelídeos, sendo 4 da subfamília Mustelinae e 2 da subfamília Lutrinae (Oliveira, 2009).

A lontra-neotropical, *Lontra longicaudis*, e a ariranha, *Pteronura brasiliensis*, são as duas espécies de lontras que ocorrem no Brasil (Reis et al., 2006; Bastida et al., 2007) (Fig. 1). A lontra-neotropical distribui-se ao longo da América Central e da América do Sul, desde o México até a Argentina (Rheingantz et al., 2014). Encontra-se associada, principalmente, a sistemas de água doce, como rios, riachos, lagos e lagoas (Cimardi, 1996). Embora seja uma espécie tipicamente de água doce, sua presença tem sido registrada em ambientes com influência marinha, como em praias costeiras (e.g., Alarcon e Simões-Lopes, 2004), pequenas ilhas (e.g., Nakano-Oliveira et al., 2004; Carvalho-Junior et al., 2012) e estuários (Nakano-Oliveira et al., 2004; Silva et al., 2005; Barbieri et al., 2012), indicando que a espécie também utiliza esses ambientes. Por ser encontrada em uma grande variedade de habitats, é considerada uma espécie com ampla tolerância ecológica (Kruuk, 2006). A lontra-neotropical apresenta diversas adaptações para o hábito semiaquático, como membranas interdigitais nas patas e cauda achatada e musculosa (Cimardi, 1996).

Figura 1. *Lontra longicaudis* alimentando-se de um peixe da espécie *Cyphocharax voga* no Banhado do Taim, Rio Grande do Sul, Brasil (foto: Lauro Madureira).

As lontras são animais oportunistas que selecionam presas abundantes e de baixa motilidade no ambiente, minimizando o gasto de energia na localização e captura do alimento (Rheingantz et al., 2012; 2017). A dieta da espécie tem sido investigada no Brasil por meio de análise fecal, e demonstra que a lontra preda, principalmente, peixes e crustáceos, e ocasionalmente, outros grupos de vertebrados e invertebrados. Os principais peixes predados pela lontra, em ordem de importância, pertencem às Famílias Cichlidae, Loricariidae e Callichthyidae (Pardini, 1998; Kasper et al., 2004b; Quintela et al., 2008; Carvalho-Junior et al., 2010; Sousa et al., 2013; Rheingantz et al., 2017). Os crustáceos mais consumidos, por outro lado, são espécies das Famílias Trichodactylidae e Palaemonidae (Pardini, 1998; Quadros & Monteiro-Filho, 2001; Rheingantz et al., 2017).

A espécie exibe preferência por áreas de forrageio com vegetação ripária ou costões rochosos em seu entorno (Alarcon & Simões-Lopes, 2003). Assim, embora tipicamente de água doce, a lontra neotropical pode utilizar, como área de forrageio, regiões costeiras e estuarinas que apresentem tais características. No entanto, estudos que investiguem a dieta da espécie nesses ambientes são escassos. Alarcon & Simões-Lopes (2004) verificaram que a lontra-neotropical consome principalmente peixes da Família Sciaenidae em um ecossistema costeiro em Santa Catarina.

A importância relativa de cada espécie de presa na sua dieta pode variar de acordo com a região. Segundo Rheingantz et al. (2017), as populações próximas à região costeira apresentam maior diversidade trófica, possivelmente devido a uma maior heterogeneidade dessas áreas. Além disso, os hábitos alimentares da espécie podem ser bem definidos com pouca variação sazonal (Carvalho-Junior et al., 2013; Sousa et al., 2013) ou podem variar de acordo com as estações do ano (Rheingantz et al., 2011), provavelmente devido à disponibilidade de presas.

A lontra-neotropical mede em torno de 1,20 m de comprimento total e pesa cerca de 12 kg, sendo o macho em média 25% maior que a fêmea (Kruuk, 2006). A espécie também exibe dimorfismo sexual em relação ao tamanho do crânio, o qual pode estar associado a diferenças comportamentais, bem como a adaptações ao consumo de diferentes tipos de presas (Hernández-Romero et al., 2015). Além disso, machos tendem a se deslocar muito mais do que as fêmeas e estas, normalmente, forrageiam próximas de seus filhotes (Trinca et al., 2013).

A maturidade sexual da espécie ocorre por volta dos dois anos de vida (Cimardi, 1996). A época de reprodução geralmente é determinada pelo fotoperíodo (Amstislavsky & Ternovskaya, 2000). No entanto, a lontra-neotropical não possui estação de acasalamento pronunciada. Além disso, pode realizar implantação tardia, permitindo que os filhotes nasçam em uma época favorável do ano (Kruuk, 2006). Segundo Cimardi (1996), o período gestacional é de 60 a 65 dias e o número de filhotes é de 1 a 5. No entanto, Arcila & Ramírez (2004) registraram um período gestacional de 86 dias em uma fêmea cativa do Parque Zoológico Santa Fé em Medellín, Colômbia. A prole permanece por cerca de um ano sob os cuidados da mãe. Os filhotes, ao alcançarem dois meses de idade, começam a ingerir pequenas presas. O macho não participa do cuidado parental, tendo pouco ou nenhum contato com os filhotes. Apesar de ser solitária, grupos compostos por mãe e filhotes são frequentemente encontrados (Kruuk, 2006).

A lontra aproveita cavidades naturais ao longo das margens dos corpos d'água para descanso. Na ausência desses locais, ela pode escavar os barrancos para a formação de abrigos. Os abrigos e latrinas utilizados pela espécie são caracterizados pelas marcações odoríferas produzidas pelas glândulas perianais (Pardini & Trajano, 1999; Kasper et al., 2008). O padrão de atividade da espécie varia segundo o habitat na qual ela se encontra. Segundo Rheingantz et al. (2016), no Pantanal a lontra-neotropical apresenta hábitos diurnos, enquanto que, na Mata Atlântica apresenta hábitos noturnos. De acordo com os autores, essas diferenças podem estar associadas à influência antrópica ou ao período de atividade de suas presas. A lontra-neotropical é uma espécie muito flexível, podendo sobreviver em ambientes impactados. Alarcon & Simões-Lopes (2003), ao investigarem o uso de habitat da espécie em ambientes costeiros preservados e degradados, verificaram que a utilização de áreas com influência antrópica é comum. No entanto, há uma preferência por florestas não degradadas e áreas fluviais preservadas. Ademais, o uso de ambientes alterados só é possível desde que os impactos não afetem diretamente a cadeia trófica e disponibilidade de abrigos (Alarcon & Simões-Lopes, 2003).

No passado, a lontra-neotropical foi intensamente caçada devido ao valor de sua pele, levando à redução de sua população (Silva, 1984). Atualmente, a *L. longicaudis* é classificada como quase ameaçada na lista vermelha de espécies ameaçadas (IUCN, 2015). Apesar de ser considerada uma espécie com alta plasticidade e tolerante a distúrbios antrópicos, a degradação dos ambientes aquáticos pode se tornar uma ameaça à sua sobrevivência (Alarcon & Simões-Lopes, 2003). A destruição e poluição do seu habitat, juntamente com a expansão da malha hidroenergética, são as principais ameaças para a espécie. Além disso, por consumirem peixes capturados em redes de pesca, as lontras são frequentemente alvo de retaliação por parte dos pescadores (Rodrigues et al., 2013). Segundo Barbieri et al. (2012), os pescadores, no sul do Brasil, possuem uma percepção negativa diante da interação das lontras com a pesca de emalhe, considerando-a um dos animais mais prejudiciais à atividade. Outra ameaça à espécie é o tráfego de veículos. Quintela et al. (2012) observaram que atropelamentos são a principal causa de mortalidade de animais no sul e sudeste do Brasil.

Neste contexto, informações sobre o nicho espacial e trófico são essenciais para identificar os requerimentos ecológicos, como habitats e recursos alimentares críticos para a lontra-neotropical. Estas informações podem auxiliar no processo de estabelecimento de medidas para atenuar os conflitos com atividades humanas, sendo importantes para a conservação da espécie e seus habitats.

Até o presente momento, a ferramenta de IE não foi utilizada no estudo da ecologia trófica da *L. longicaudis*. Com o emprego deste método, dado o exposto acima, serão avaliadas as seguintes hipóteses:

- O conteúdo de carbono inorgânico da dentina da lontra-neotropical é uma fonte de variação isotópica que altera os valores de $\delta^{13}C$, sendo a acidificação da dentina um passo metodológico necessário para a análise de isótopos estáveis;
- O nicho isotópico da lontra-neotropical varia segundo o habitat no qual ela se encontra, sendo mais amplo nos exemplares de uma ilha costeira (Ilha de Santa Catarina – SC) e em uma bacia hidrográfica composta por uma série de lagoas e um estuário (Bacia do Rio Tramandaí – RS), devido a uma maior ocorrência da espécie no ambiente marinho, em comparação com uma região de banhado (Banhado do Taim – RS);
- A dieta da lontra-neotropical na Ilha de Santa Catarina e na Bacia Hidrográfica do Rio Tramandaí inclui fontes marinhas/estuarinas e de água doce, enquanto que no Banhado do Taim a lontra preda somente presas de água doce.

Objetivo geral

Caracterizar o nicho isotópico da lontra-neotropical (*Lontra longicaudis*) em diferentes sistemas aquáticos costeiros do sul do Brasil.

Objetivos específicos

- Analisar o efeito da acidificação sobre as razões isotópicas da dentina da lontra-neotropical;
- Investigar se existe diferença de nicho isotópico da lontra-neotropical entre diferentes sistemas aquáticos;

- Estimar a contribuição proporcional de cada presa aos indivíduos de localidades distintas.

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CAPÍTULO 1

Effects of acidification on the isotopic ratios of Neotropical otter tooth dentin

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Effects of acidification on the isotopic ratios of Neotropical otter tooth dentin

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ABSTRACT

RATIONALE: Stable carbon and nitrogen isotopes are widely used in ecological studies, providing important information on the trophic ecology and habitat use of species. However, some factors may lead to isotopic variability, which makes difficult the interpretation of data, such as the presence of inorganic carbon in mineralized tissues. In order to remove the inorganic carbon, acidification is a commonly used treatment.

METHODS: The effects of two methods of acidification were tested: (i) dentin acidification with 10% HCl using the “drop-by-drop” technique, and (ii) dentin acidification in an “HCl atmosphere”, by exposing the dentin to vaporous 30% hydrochloric acid. Results were compared to untreated subsamples.

RESULTS: The nitrogen isotopic ratios were statistically different between the two acidification treatments. Nonetheless, no significant changes in carbon isotopic ratios were found in acidified and untreated samples.

CONCLUSIONS: The results indicated that acidification had no effect on carbon isotopic ratios of Neotropical otter tooth dentin, while introduce a source of error in nitrogen isotopic ratios. Therefore, we conclude that acidification is an unnecessary step for C and N stable isotope analysis.

Key words: carbon, demineralization, inorganic carbon, nitrogen, stable isotopes

RATIONALE

The Neotropical otter, *Lontra longicaudis*, is a semi-aquatic carnivore mustelid, found mainly in freshwater environments.[1] Despite its wide distribution, occurring throughout Central and South America,[2] it is a little studied species. Most of the studies about the Neotropical otter are focused on diet (e.g.,[3-5]) and habitat use (e.g.,[6-8]). However, the methodology applied in these works has limitations that can generate biases in results. The analysis of diet from fecal analysis, for example, presents a poor estimation of the real proportions (in number and biomass) of the consumed items, due to the differential digestion of the ingested prey.[9] An alternative and complementary method for the investigation of feeding biology and habitat use is the analysis of stable isotopes.

Carbon and nitrogen stable isotopes are widely used in ecological studies (e.g.,[10-12]). These elements are typically incorporated by consumers through their diet. Nevertheless, during the incorporation of carbon and nitrogen by consumers, there is a loss of the light isotopes (^{12}C and ^{14}N) through the processes of respiration and excretion, respectively.[13,14]

As a result, the isotopic composition of an organism's tissue reflects that of its diet, with a small change resulted from the preferential retention of the heavier isotope (^{13}C and ^{15}N).[15]

The enrichment in the heavy isotope is accentuated in the case of nitrogen (3.4 ‰ more enriched, on average), so $\delta^{15}N$ values can be used to infer the trophic position of the different consumers in the food webs.[16] The values of $\delta^{13}C$, on the other hand, reflect the biogeochemical processes that occur at the base of the trophic web, being generally different between the environments dominated by producers with physiological differences (e.g. different photosynthetic pathways, C3 vs. C4).[13] Because the ^{13}C trophic level enrichment is, in average, approximately 0.4 ‰, carbon isotopic compositions are generally used to identify carbon pathways, sources of primary productivity [16] and thus the preferred habitats of the consumers.

Mammal teeth are composed of organic and mineral materials that are deposited throughout the whole life of animal.[17] Thus, its isotopic analysis has the potential to provide long-term diet information of consumers that are difficult to observe in the nature. Hence, several studies have used tissues from teeth, such as dentin, as an isotopic substrate (e.g.,[18,19]). Dentin is a mineralized tissue located below the enamel and the cementum, which extends from the crown to the root of the tooth,[20] constituted of the organic fraction, mainly formed by collagen, and of the mineral fraction that contains apatite (inorganic carbon).[21] The organic and inorganic fractions reflect different contributions of the dietary macromolecules. Collagen is synthesized from the proteins of the consumer's diet, whereas, apatite is assimilated from proteins, carbohydrates and lipids. This implies that the collagen signal reflects an isotopic composition of the protein diet, and the C and N apatite isotopes reflect total dietary consumption.[22,23]

Due to the composition of the collagen, some authors recommend the isolation of this compound from the inert tissues for isotopic analysis (e.g.,[24,25]), in order to reduce sources of isotope variation and to facilitate the interpretation of the data. The elimination of inorganic carbon is performed by acidifying the dentin. However, it has recently been suggested that this procedure is an unnecessary step for the analysis of stable isotopes in the case of bone and tooth collagen (e.g.,[26-28]).

Although stable isotope analysis has been employed in a number of studies about the sea otter, *Enhydra lutris* (e.g.,[10,29,30]), studies with freshwater otters are scarce. Furthermore, specific protocols for this method are not established yet. Considering that it is essential to evaluate and standardize the methodology to ensure comparability of results and to promote the use of stable isotopes as a tool for researches, the objective of the present study was to analyze the effect of acidification on the isotopic ratios of Neotropical otter tooth dentin.

METHODS

Sample collection

Teeth samples were collected opportunistically from 12 dead Neotropical otters in southern Brazil (29°48’S, 50°11’W) between 2004 and 2016. All animals collected were considered adults, based on sexual maturation, which occurs around two years of age.[31] Among these, eight were males and three females. The sex of one individual could not be determined. Bones were removed from each carcass during necropsies and macerated immersing in fresh water for about 4 months to detach all soft tissues from the calcified tissues. Remaining soft tissues were cleaned manually using abrasive tool and fresh water. After the skull was cleaned, the teeth of each individual were removed from the mandibular or maxillary bone. One tooth from each specimen, either an incisor or a premolar, was used the experiment.

Stable isotope analysis

Teeth samples were previously washed with distilled water, and dried at room temperature for 24h. Initially, the enamel and cement layers of the tooth were removed using a small bit, exposing the dentin. Then, a fraction of the dentin was sampled with a drill, resulting in a powder. The powder dentin was divided into two sub-samples: one subsample did not receive any further treatment, while the other was acidified by the "HCl atmosphere" method. This method consists in exposing the tissue to vaporous 30% hydrochloric acid (gaseous form) in a desiccator for 24 h.[32] The remaining portion of the dentin, which was not powdered, was acidified with 10% HCl using the "drop-by-drop" technique until no CO_2 gas bubbles were released,[33] and sampled using a small drill bit as well, to obtain a powder. After that, all the subsamples were dried in an oven at 60 °C for 1h. Approximately 0.5-1 mg of the resulting powder from each subsample was packed into tin capsules. The isotopic composition of the subsamples was obtained using a Costech 4010 (Costech, Valencia, CA) elemental analyzer coupled to a Thermo Scientific Delta V (Thermo Scientific, Bremen, Germany) isotope ratio mass spectrometer at the University of New Mexico Center for Stable Isotopes (UNM–CSI, Albuquerque, NM). Isotope data are expressed in delta (δ) notation which for $\delta^{13}C$ and $\delta^{15}N$ (‰) in relation to international standards = $[(R_{sample} / R_{standard}) - 1]$, where R_{sample} or $R_{standard}$ are the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in the sample or standard for carbon and nitrogen. Internationally accepted standards are Vienna Pee Dee Belemnite (V-PDB) limestone and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively. The weight percent carbon to nitrogen concentrations ratios of dentine samples was also determined and had values < 3.5 (Table 1). According to Post et al.,[34] samples of aquatic animals that have these values do not require lipid extraction or mathematical correction. Furthermore, Newsome et al.[35] suggest that mammalian tooth collagen do not require lipid extraction due to the low concentration of lipids. Thus, the samples were nonlipid extracted. Delta values were calculated using multiple-point normalization. The internal laboratory standards used were soy protein, whey protein, casein, tuna, IAEA-N1, IAEA-N2, USGS-4 and USGS-43. Analytical precision (SD) of these internal standards was measured to be < 0.2 ‰ for $\delta^{15}N$ and < 0.04 ‰ in the case of $\delta^{13}C$.

Data analysis

A Shapiro-Wilk's Normality Test was applied to verify the normality of the $\delta^{13}C$ and $\delta^{15}N$ data. According to this test the isotopic ratios of C and N of the three datasets (control, and both acidification methodologies) showed normal distributions. The effects of acidification on stable isotope ratios of dentin were determined via a repeated measures one-way ANOVA. In order to estimate the inorganic carbon content of the teeth samples, a carbonate proxy, proposed by Jacob et al.[33] was applied:

$$Carbonate\ proxy = \frac{[C\!:\!N]\ control}{[C\!:\!N]\ acidified} - 1$$

Where $[C{:}N]_{control}$ is the C:N ratio of non-treated subsamples and $[C{:}N]_{acidified}$ is the C:N ratio of acidified subsamples. Samples with a proxy ≤ 0.03 were considered as containing no significant carbonates. To verify if there is a relation between the carbon inorganic content (as evidenced by the proxy) and the C and N composition in the sample, a linear regression was applied, using the $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ (calculated as the difference between the control and acidified subsamples, for each method) and the carbonate proxy. Data were statistically analyzed using the statistical environment R, version 3.4.1,[36] except for the ANOVA and *post hoc* Tukey's test, that were run in PAST.[37] The significance level for two-sided tests was $\alpha = 0.05$.

RESULTS

The mean and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ values of Neotropical otter dentin after each method, C:N ratios, and $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values are shown in Table 1. In the present study, the "drop-by-drop" treatment was defined as method 1 and the "HCl atmosphere" as method 2. The acidification had no significant effect on carbon isotopic ratios (ANOVA, $p = 0.180$). However, the magnitude of variation between the $\delta^{13}C$ values of the acidified and untreated samples ranged from -0.55 ‰ to +3.26 ‰ after the "drop-by-drop" method, and from -0.41‰ to +0.2 8‰ after the "HCl atmosphere" method. Meanwhile, the nitrogen δ-values were statistically different between the treatments (ANOVA, $p = 0.008$). The *post hoc* Tukey's test revealed that $\delta^{15}N$ values were significantly different between the two acidification treatments (Tukey's HSD test, $p = 0.007$). Acid effects on $\delta^{15}N$ also encompassed a broad range from -1.07‰ to +4.51‰ and from -1.43‰ to -0.44 ‰ after applying "drop-by-drop" and "HCl atmosphere" acidification methods, respectively. The mean (±SD) carbonate proxy value was 0.13 (± 0.09) for "drop-by-drop" treatment and 0.18 (±0.05) for "HCl atmosphere" method. Only one sample had a carbonate proxy value ≤ 0.03.

Table 1. Mean (± SD) $\delta^{13}C$, $\delta^{15}N$, $\Delta\delta^{13}C$, $\Delta\delta^{15}N$ values (difference between the isotopic values of the control and acidified subsamples), and C:N ratios for Neotropical otter (*Lontra longicaudis*) tooth dentin samples (n = 12) under two pre-stable isotope treatments. Method 1 represents the "drop-by-drop", whereas method 2 consists in "HCl atmosphere". Both treatments use HCl to acidify the dentin. Values for control samples (no treatment) are also shown.

Treatment	**$\delta^{13}C$ (‰)**	**$\Delta\delta^{13}C$**	**$\delta^{15}N$ (‰)**	**$\Delta\delta^{15}N$**	**C:N**
Control	-20.9 ± 1.7		13.8 ± 1.8		3.4 ± 0.1
Method 1	-21.2 ± 2.1	0.3 ± 1.0	13.5 ± 1.6	0.4 ± 1.5	3.1 ± 0.3
Method 2	-20.8 ± 1.6	-0.1 ± 0.2	14.6 ± 1.6	-0.8 ± 0.3	2.9 ± 0.1

The $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values obtained by the to "drop-by-drop" treatment were negatively related to the carbonate proxy ($r^2 = 0.67$, $p = 0.001$ and $r^2 = 0.58$, $p = 0.004$, respectively). Nevertheless, these results are clearly influenced by the high values of $\Delta\delta^{13}C$ (+3.26) and $\Delta\delta^{15}N$ (+4.51) obtained by the "drop-by-drop" method for one individual (Fig. 1A and 1B). After removing this sample, no significant relationship was found between $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values and the carbonate proxy ($r^2 = 0.006$, $p = 0.830$ and $r^2 = 0.008$, $p = 0.760$, for $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$, respectively). In the case of the "HCl atmosphere" method, no significant relation was found between the inorganic carbon in Neotropical otter dentin and both $\Delta\delta^{13}C$

(r^2 = 0.010, p = 0.760) and $\Delta\delta^{15}N$ (r^2 = 0.047, p = 0.500) values (Fig. 1C and 1D).

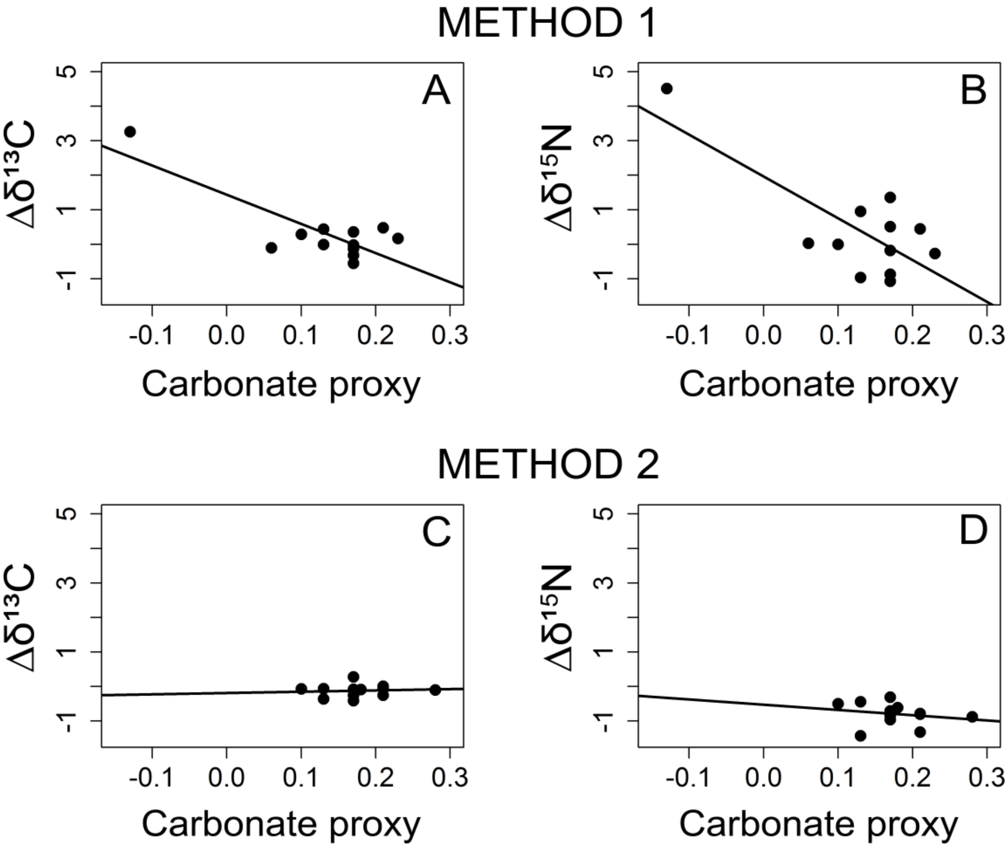

Figure 1. Effects of inorganic carbon content in Neotropical otter dentin (represented by carbonate proxy) on the difference between acidified and non-acidified samples ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$), according to "drop-by-drop" method (A and B) and to "HCl atmosphere" treatment (C and D).

DISCUSSION

The acidification purpose is to remove the inorganic carbon, changing $\delta^{13}C$ values, however our results demonstrate that this pre-analytical procedure can alter the $\delta^{15}N$ values as well. Goering et al.[38] suggested that the differences after acidification in $\delta^{15}N$ were probably caused by the leaching of organic nitrogen compounds with different isotopic values (e.g., amino acids). It is known, for example, that non-essential amino acids tend to accumulate more ^{15}N than essential amino acids.[39] Additionally, different techniques to extract collagen result in different amino acid patterns.[25] Therefore, in order to reduce this bias introduced to nitrogen ratios, a less damaging method to remove the inorganic carbon, which consists in acidifying the tissue in an "HCl atmosphere" is sometimes used (e.g.,[32,40]). In the present study both "drop-by-drop" and "HCl atmosphere" modified the $\delta^{15}N$ mean values. Nevertheless, acid effects on $\delta^{15}N$ produced smaller changes after the vaporization technique than the "drop-by-drop" method.

Although the "HCl atmosphere" method reduces the risk of removing amino acids, this may not efficiently remove inorganic carbon from samples.[41] We found no statistical differences in the $\delta^{13}C$ values between the treatments. However, there is a decrease in the mean $\delta^{13}C$ values after the first acidification technique and a little increase after the second one. The inorganic carbon is more enriched in ^{13}C than the organic carbon,[26] so decreases in $\delta^{13}C$ values indicate that the inorganic carbon was removed from the tissue. A decrease in C:N ratios can be an evidence of elimination of inorganic carbon as well,[42] as observed in the present study. However, these results should be interpreted with caution. Due to changes in nitrogen isotopic ratios, the C:N ratio may not be a reliable indication that inorganic carbon has been totally removed. In addition, the average $\delta^{13}C$ and $\delta^{15}N$ using the "drop-by-drop" method may have been influenced by the values of a single sample that showed depleted C^{13} and N^{15} values after treatment.

Therefore, the magnitude of the changes in $\delta^{13}C$ values of Neotropical otter tooth dentin after the acidification is not biologically significant. Despite the carbonate proxy indicates that dentin contains inorganic carbon, this finding showed that the dentin of this species has a low content of inorganic carbon. The carbonate proxy used in the present study was initially proposed by Jacob et al.[33] and was calculated on the basis of invertebrates and marine fishes samples. As evidenced by Mateo et al.[43] for marine invertebrates, different taxonomic groups showed distinct shifts associated with pre-stable isotope sample preparation, due to chemical heterogeneity. Thus, the proxy used may not be suitable to apply for Neotropical otter teeth samples. In this case, possibly the carbonate proxy does not reflect the actual content of inorganic carbon from teeth samples. Another possibility is that the organic and inorganic fractions have similar $\delta^{13}C$ values.

Pre-analytical procedures for stable isotope analysis can influence the measurement and interpretation of dietary information. The analysis of trophic position is an estimation that is calculated on the basis of stable isotopes of nitrogen.[16] This implies that $\delta^{15}N$ shifts can impact the assessment of trophic level.[44] In the present study, we observed an overall mean increase of 0.8 ‰ in $\delta^{15}N$ values for the "drop-by-drop" method, and one sample was 4.51 ‰ more enriched in ^{15}N after this treatment. As a result, the trophic position estimated on samples from Neotropical otter tooth dentin that was acidified by the "drop-by-drop" method has a potential bias.

CONCLUSIONS

Acidification of otter tooth dentin would not be a necessary step for stable isotope analysis. Nevertheless, if the acidification is still considered as a necessary step for the stable isotope analysis, we recommend that the samples should be acidified by the "HCl atmosphere" method, in order to avoid the effects of acidification on $\delta^{15}N$ values.

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CAPÍTULO 2

Isotopic niche of the Neotropical otter, *Lontra longicaudis* (Carnivora, Mustelidae), in different coastal aquatic systems in southern Brazil

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Isotopic niche of the Neotropical otter, *Lontra longicaudis* (Carnivora, Mustelidae), in different coastal aquatic systems in southern Brazil

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ABSTRACT

The Neotropical otter, *Lontra longicaudis*, is a typically freshwater species and has been recorded in environments with marine influence, such as in coastal beaches, small islands and estuaries. Thus, we hypothesized that the isotopic niche of the Neotropical otter is wider in a coastal island (Santa Catarina Island – SC) and in a river basin composed by a series of ponds and an estuary (Tramandaí River Basin – RS) than in a wetland (Taim Wetland – RS) due a greater occurrence of the species in the marine environment of the former areas. In order to test this hypothesis, the stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in otter dentin samples from different regions was analyzed. Long-term isotopic niche of otters was wider in Santa Catarina Island, followed by Taim Wetland, and

Tramandaí River Basin. The results revealed a marine contribution in the diet of some individuals from Santa Catarina Island and Taim Wetland. Therefore, we conclude that otters from Santa Catarina Island and Taim Wetland mainly use freshwater environments as foraging areas. The otters of the Tramandaí River Basin, on the other hand, only use freshwater environments to forage. The differences found among the otters from different regions are probably related to a variety of factors, such as landscape characteristics and intraspecific competition.

Key words: carbon, diet, habitat use, nitrogen, stable isotopes

INTRODUCTION

Knowledge of a predator's diet is essential to assess the ecological role of the species, such as its influence on prey populations (e.g., Norrdahl & Korpimäki, 2000; Ripple et al., 2014) and potential competition with other carnivores (e.g., Caro & Stoner, 2003), as well to understand the trophic and spatial requirements of the species (e.g., Ben-David et al., 1997; Valenzuela et al., 2013). Information on the trophic ecology of the consumers has been inferred through analysis of stomach or gastrointestinal contents (e.g., Barros & Wells, 1998; Kasper et al., 2016) and feces (e.g., Bagchi et al., 2003; Rheingantz et al., 2011; Iversen et al., 2013). The analysis of the contents of the digestive system and feces allows the differentiation of prey at the individual level (Klare et al., 2011) and access to the size of prey ingested (e. g., Kasper et al., 2004). However, these methodologies have limitations associated with the differential digestion of prey consumed, which can lead to an over or underestimation of the importance of certain items to the species diet (Perini et al., 2009; Klare et al., 2011).

Stable isotope analysis is an alternative and complementary tool in the study of trophic ecology (e.g., Newsome et al., 2009; Carlisle et al., 2012; Chiaradia et al., 2016). The isotopic composition of consumer's tissues reflects the food ingested and assimilated, with slight changes resulting from the preferential retention of the heavier isotope (^{13}C and ^{15}N). The trophic enrichment of the heavy isotope is accentuated in the case of nitrogen (~ 3-5 ‰), thus δ^{15}N ratios are useful to infer the trophic position of the predators in the food webs (Post, 2002). The discrimination of the light isotope also occurs during the incorporation of carbon between trophic levels, though less markedly. Thus, δ^{13}C ratios reflect biogeochemical processes occurring at the base of the trophic web, being generally distinctive between environments dominated by producers with physiological differences (e.g., plants with photosynthetic pathways, C3 vs. C4) (DeNiro & Epstein, 1978). Because the trophic level discrimination in ^{13}C is approximately 0-1 ‰, isotopic ratios reflect the characteristics of the base of the food web (Post, 2002), hence a proxy for inferring preferred habitats of consumers.

Isotopic information of mammals can be obtained through a series of tissues, such as skin (e.g., Ruiz-Cooley et al., 2004; Castro et al., 2015), vibrissae (e.g., Newsome et al., 2010; Lübcker et al., 2015), and tooth dentin (e.g., Troina et al., 2016; Evacitas et al., 2017). The choice of tissue to be analyzed depends mainly on the temporal response that is to be obtained, since different tissues reflect different temporal windows. Mammalian teeth, for example, are inert structures continuously synthesized, and thus, incorporate isotopic information about the whole life of the animal (Bocherens & Drucker, 2013). Clementz & Koch (2001) verified that it is possible to distinguish different aquatic habitats with stable isotopes in tooth enamel. Mammals that forage in freshwater habitats typically are ^{13}C-depleted compared to mammals that feed on estuaries or marine habitats (Clementz & Koch, 2001).

Since carbon and nitrogen isotopes provide quantitative information regarding resources and habitat use, their values plotted in a bidimentional graph (δ^{13}C vs. δ^{15}N), result in a δ-space equivalent to the ecological niche defined by Hutchinson (1957) (see Newsome et al., 2007). The ecological niche concept refers to the tolerances and requirements of an organism. In terms of feeding habits, generalist populations tend to be exhibit higher niche variation, due the ecological variation, than more specialized populations (Bolnick et al., 2007). Higher niche variation may also reflect an increase in individual specialization, as reported for sea otters, *Enhydra lutris* (Newsome et al., 2015).

The diet of the Neotropical otter, *Lontra longicaudis*, has been studied through fecal analysis (e.g., Rheingantz et al., 2011; Quintela et al., 2012; Sousa et al., 2013) and includes fish and crustaceans, and occasionally other groups of vertebrates and invertebrates, such as amphibians and insects (Rheingantz et al., 2017). The Neotropical otter is described as an opportunistic predator that selects abundant and low mobile prey (Rheingantz et al., 2012). Although the species is commonly found in freshwater habitats, it has been recorded in environments with marine influence, such as in coastal beaches (e.g., Alarcon & Simões-Lopes, 2004), small islands (e.g., Nakano-Oliveira et al., 2004; Carvalho-Junior et al., 2012) and estuaries (Nakano-Oliveira et al., 2004; Silva et al., 2005; Barbieri et al., 2012). The trophic diversity of the Neotropical otter is typically higher in coastal environments than more continental areas due the entry of marine food items (Rheingantz et al., 2017). The presence of the species in marine habitats is associated with the availability of vegetation and rocky shores (Alarcon & Simões-Lopes, 2003), probably due shelter availability, which may drive differences in the patterns of use of areas with marine influence.

Given the above, we hypothesized that the isotopic niche of the Neotropical otter is larger in a coastal island (Santa Catarina Island – SC) and in a river basin composed by a series of ponds and an estuary (Tramandaí River Basin – RS) than in a wetland (Taim

Wetland – RS) due a greater occurrence of the species in the marine environment of the former areas. In the present study, stable isotope compositions of carbon and nitrogen in teeth dentin of Neotropical otters sampled from those three environments were used to: (i) investigate isotopic differences among regions, (ii) compare isotopic compositions between Neotropical otters and among their potential prey, and (iii) estimate the contribution of potential prey to Neotropical otter's diet in each region.

MATERIALS AND METHODS

Study areas

This study was carried out in three different subtropical aquatic systems, located in the coastal region of southern Brazil: Santa Catarina Island, Tramandaí River Basin and Taim Wetland (Fig. 1). The climate of those three study areas is characterized by rainfalls well distributed throughout the year and temperatures around 23 ºC and 14 ºC in summer and winter, respectively (Wrege et al., 2012).

The Santa Catarina Island (27°35'S, 48°28'W) is situated parallel to the continent and it holds many aquatic environments, among which Peri and Conceição Lagoons are the most important water bodies. The Peri Lagoon is characterized by dense marginal vegetation composed of sedges (*Scirpus californicus*) and high density of cyanobacteria (Ferreira et al., 2016). The coastal region of the island is characterized by sandy beaches and rocky shores that may favor the presence of the Neotropical otter in this environment (Carvalho-Junior et al., 2012). Several species of macroalgae occur along the marine coast of the island, with Ceramiales order the most diverse group (Bouzon et al., 2016).

The Tramandaí River Basin (29°48'S, 50°11'W) comprises a series of ponds connected by channels that are communicated with the estuary of Tramandaí Lagoon through the Tramandaí River. The Camarão Channel connects the Tramandaí Lagoon to the Atlantic Ocean (Villwock, 2009). In contrast to the Santa Catarina Island, this region is characterized by the presence of dunes (Schäfer, 1988). Due to the marine and continental influence, the vegetation surrounding the water bodies of the Tramandaí River Basin is diverse. The lagoon margins are predominantly delimited by bulrush (*Schoenoplectus californicus*) and cattail (*Typha domingensis*). A variety of macrophytes (e.g., *Eichhornia* sp., *Myriophyllum* sp., *Potamogetum* sp.), and algae (*Chara* sp. and *Nitella* sp.) species occur within the freshwater limits of the system (Prado, 2009). The estuary is characterized by abundant aquatic plants and macroalgae, such as *Scirpus olneyi* (Hoeinghaus et al., 2011), *Ruppia maritima* and *Enteromorpha* sp. (Rosa-Filho et al., 2004).

The Taim Wetland (32°44'S, 52°34'W) is characterized by large portions of wetlands with interconnected ponds, vast grassland, sand dunes and coastal beaches (Marques et al., 2013). The large flooded portions are mainly covered by grass (*Zizaniopsis bonariensis*) and sedges (*Scirpus californicus*). In the peripheral regions of the wetland the vegetation is predominantly shrub with palustrine forests (e.g., *Bromelia antiacantha*, *Eryngium pandanifolium*, *Erythrina crista-galli*, *Ficus subtriplinervia*) (Gomes & Krause, 1982). The marine coast of the Taim Wetland is exposed, formed by sandy beaches lacking rocky shores. The sandy shore is inappropriate for the establishment of aquatic macrophytes. Benthic algae such as *Bryopsis plumosa* and *Gymnogongrus griffithsiae* are abundant instead (Cordazzo & Seeliger, 1995).

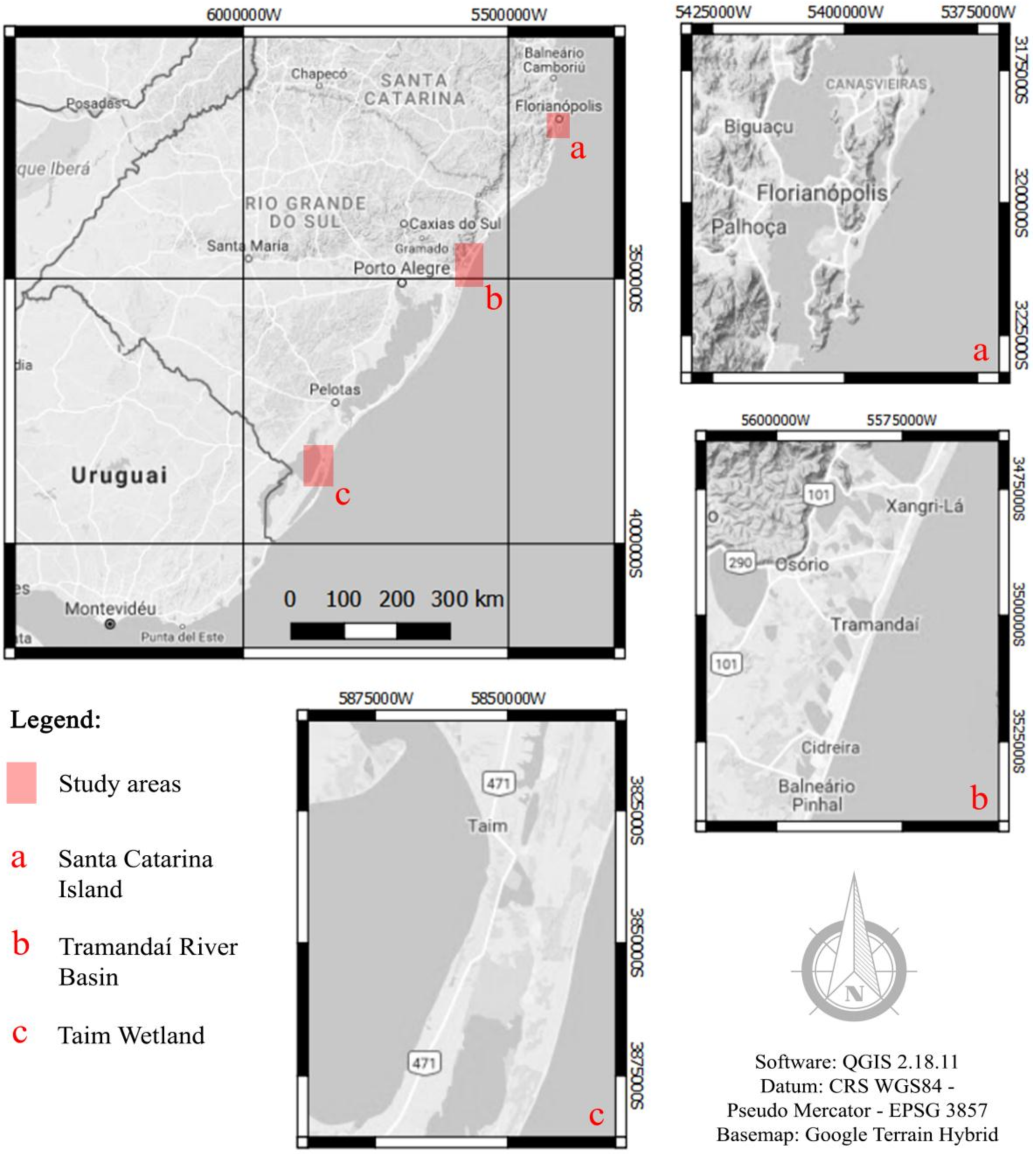

Fig. 1 Sample locations of potential prey and Neotropical otter tissues in southern Brazil

Prey and Neotropical otter tissue sampling

Otter's teeth were collected opportunistically from dead animals by different institutions from 1981 to 2016 and used for isotopic analysis and age determination (Table S1). Prey samples were obtained from fishermen of each study area during the summer and winter seasons, in 2016 and 2017, to account for effects of seasonal variations in isotopic analysis (Table 1). The prey species analyzed were selected based on previous studies on the diet of Neotropical otters in southern Brazil (Barbieri, unpublished data; Colares and Waldemarin, 2000; Quadros and Monteiro-Filho, 2001; Alarcon and Simões-Lopes, 2004; Kasper et al., 2004b; Carvalho-Junior et al., 2010; Quintela et al., 2012; Sousa et al., 2013, Peres, 2014). When the taxonomic level of prey reported in the literature was high (e.g. family, order or class) prey species were chosen based on abundance, as a proxy of availability, and body size (Garcia et al., 2006; Malabarba et al., 2013). This criterion was adopted because otters are opportunist predators that prey on abundant species in the environment (Rheingantz et al., 2012; 2017) and usually larger than 10 cm (Kruuk and Moorhouse, 1990; Kasper et al., 2004).

In Santa Catarina Island, fish were sampled from both marine and freshwater environments. In the Tramandaí River Basin, fish were also collected from two distinct aquatic environments: the Tramandaí Lagoon and adjacent freshwater lagoons. In the Taim Wetland, only freshwater prey was sampled, since there is no evidence of the Neotropical otter feeding in the adjacent marine environment.

Table 1 Mean (± SD) $\delta^{13}C$ and $\delta^{15}N$ values (‰) of potential prey of Neotropical otter collected in Santa Catarina Island, Tramandaí River Basin and Taim Wetland, grouped according to ecological group

SANTA CATARINA ISLAND						
			$\delta^{13}C$		**$\delta^{15}N$**	
Ecological Group	**Species**	***n***	**Mean**	**SD**	**Mean**	**SD**
Freshwater Herbivorous Fish	*Tilapia rendalli*	11	-23.7	1.5	7.0	0.8
Freshwater Omnivorous Fish	*Geophagus brasiliensis*	8	-22.3	0.5	9.1	0.5
Marine Carnivorous Fish	*Centropomus* sp./ *Menticirrhus americanus/ Micropogonias furnieri/ Stellifer brasiliensis*	11	-16.7	0.7	15.2	0.7
Marine Invertebrates	*Callinectes sapidus*	1	-14.7	-	10.9	-
TRAMANDAÍ RIVER BASIN						
			$\delta^{13}C$		**$\delta^{15}N$**	
Ecological Group	**Species**	***n***	**Mean**	**SD**	**Mean**	**SD**

Freshwater Omnivorous Fish	*Cyphocharax voga/ Geophagus brasiliensis/ Rhamdia quelen*	13	-23.2	2.9	8.7	1.9
Freshwater Carnivorous Fish	*Crenicichla* sp./ *Hoplias malabaricus/ Hoplosternum littorale*	6	-24.0	1.8	9.8	0.8
Estuarine Herbivorous Fish	*Mugil* sp.	5	-14.9	2.8	10.0	1.9
Estuarine Omnivorous Fish	*Genidens genidens*	6	-16.2	0.5	14.7	2.6
Estuarine Carnivorous Fish	*Micropogonias furnieri*	1	-18.7	-	12.1	-
Estuarine Invertebrates	*Callinectes sapidus*	12	-18.3	3.3	9.2	2.4
TAIM WETLAND						
			$\delta^{13}C$		$\delta^{15}N$	
Ecological Group	**Species**	***n***	**Mean**	**SD**	**Mean**	**SD**
Freshwater Omnivorous Fish	*Cyphocharax voga/ Gymnogeophagus gymnogenys/ Rhamdia quelen*	26	-24.6	1.4	9.9	1.0
Freshwater Carnivorous Fish	*Crenicichla punctata/ Geophagus brasiliensis/ Hoplias* aff. *malabaricus/ Hoplosternum littorale/ Oligosarcus jenynsii*	50	-23.3	1.6	12.3	1.5
Freshwater Invertebrates	*Palaemonetes argentinus*	1	-30.4	-	9.2	-
Marine Carnivorous Fish	*Paralonchurus brasiliensis*	5	-14.2	0.5	16.0	0.6

Values of *Palaemonetes argentinus* and *Paralonchurus brasiliensis* were obtained from Brito & Bugoni (2015) and Silva-Costa & Bugoni (2013), respectively

Age determination of Neotropical otter

The age of *Lontra longicaudis* specimens was determinate using a method adapted from Stephenson (1977) for the North American river otter, *L. canadensis*. Initially, morphological characteristics of the skull were analyzed. One-year old or younger otters show a low sagittal ridge, widely separated temporal ridges, irregular skull surface and incomplete ossification. Adult animals had their age estimated by reading growth layer groups (GLG) of cement in the canines. The first cement GLG is deposited after the dentition change, which occurs during the first year of life (Heggberget, 1984). It is estimated that GLGs of otters, consisting of one light- and one dark-colored cement layers each, are deposited annually (Stephenson, 1977, Bodkin et al., 1997, Oliveira et al., 2007). Therefore, their counting can be used to determine the age, each GLG representing one year of life of adult otters (Fig. 2).

The canines were removed and the sides were cut using a metallographic low-speed saw to obtain a central part of the tooth. Then, each part was immersed in 10 % formaldehyde for 24 h and decalcified in 5 % nitric acid. After decalcification, the central part of the tooth was cut into 25 µm thick slides using a freezing microtome. The slides were blued in ammonia, stained with Mayer's hematoxylin, washed with running tap water, subsequently submerged in 50 % glycerin for 30 min and fixed in 100 % glycerin. The sections were analyzed using an optical microscope.

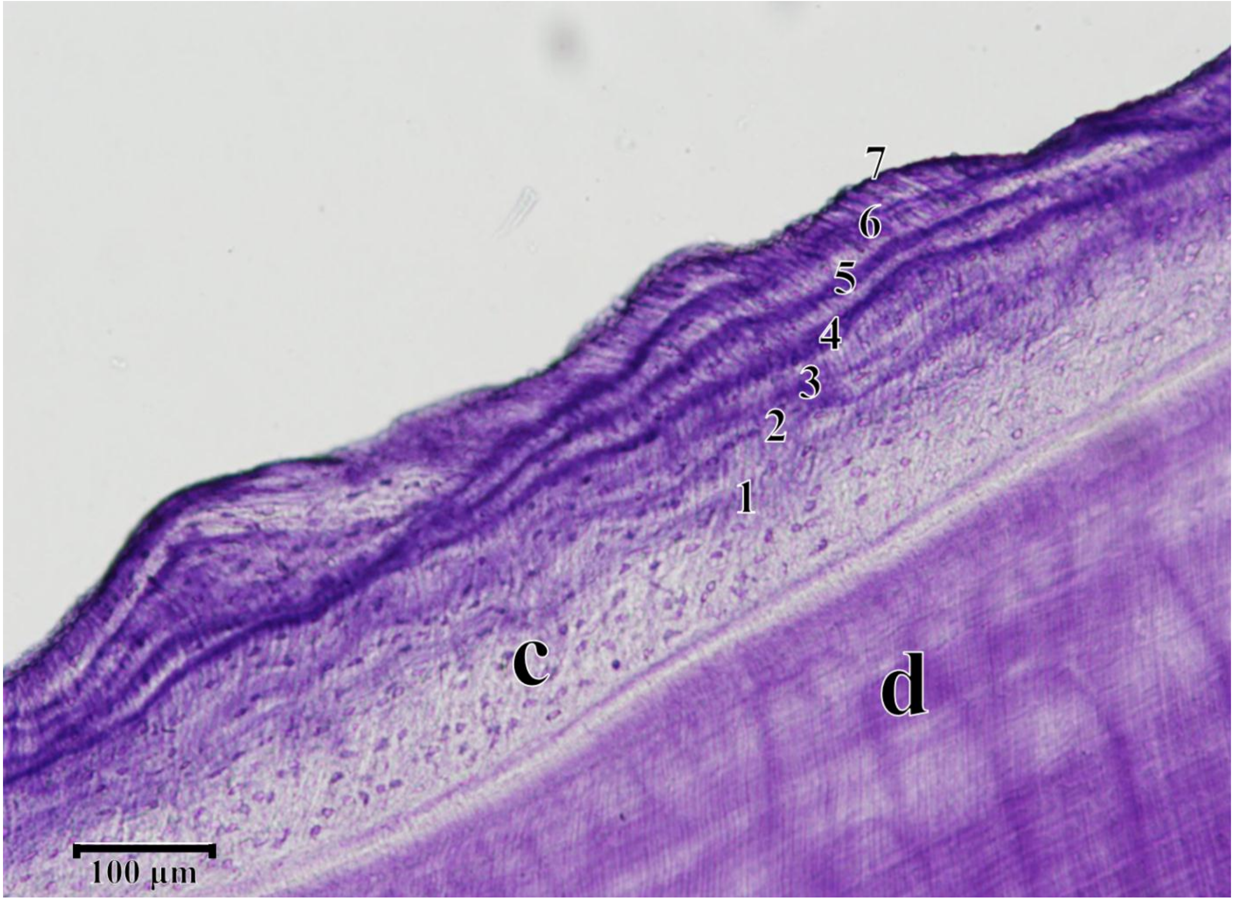

Fig. 2 Longitudinal section of a canine from a Neotropical otter (*Lontra longicaudis*) showing the dentin (d), cementum (c) and cement layers (1-7)

Stable isotope analysis

Teeth were previously cleaned with distilled water and dried at room temperature for 24 h. Subsequently, the dentin was sampled using a small drill, so that the resulting powder represented the entire life of the individual. In order to eliminate the inorganic carbon, which could alter the values of organic $\delta^{13}C$, the dentin powder was acidified by the "HCl atmosphere" method, as described by Carrasco et al. (in press). For isotopic analysis of prey, about 5 g of muscle tissue was extracted from each specimen. The samples were cleaned with distilled water and dried in oven at 60 °C for 48 h. The dehydrated muscle was grounded into a fine powder using mortar and pestle.

The weight percent carbon to nitrogen concentrations ratios of dentine and muscle samples was also determined and had values <3.5. Samples of aquatic animals that have these values do not require lipid extraction or mathematical correction (Post et al., 2007). In addition, mammalian tooth collagen typically has a low concentration of lipids (Newsome et al., 2010b). Thus, both dentine and muscle tissues were nonlipid extracted.

Approximately 1 mg of the resulting powder from each sample of tooth dentine and fish muscle was packed into tin capsules for further isotopic analysis. Isotopic compositions were obtained using a Costech 4010 (Costech, Valencia, CA) elemental analyzer coupled to a Thermo Scientific Delta V (Thermo Scientific, Bremen, Germany) isotope ratio mass spectrometer at the University of New Mexico Center for Stable Isotopes (UNM–CSI, Albuquerque, NM). The natural isotopic ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) observed in otter tissues and prey muscle were expressed by delta notation (δ) ($\delta^{13}C$ and $\delta^{15}N$, respectively), in parts per thousand (‰) of the international standards: V-PDB (Vienna Pee Dee Belemnite limestone) for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. The isotopic ratio (R) of each element

(^{13}C / ^{12}C and ^{15}N / ^{14}N) in the sample was compared to the international standards to determine the isotopic composition of the animals' tissues following the equation:

$$\delta X\ (‰) = \left[\left(R_{Sample}/R_{Standard}\right) - 1\right]$$

Where R_{Sample} and $R_{Standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the sample and standard, respectively. Delta values were calculated using multiple-point normalization. The internal laboratory standards used were soy protein, whey protein, casein, tuna, IAEA-N1, IAEA-N2, USGS-4 and USGS-43. Analytical precision (SD) of these internal standards was measured to be <0.2 ‰ for $\delta^{15}N$ and <0.04 ‰ for $\delta^{13}C$.

Data analysis

The normality and homogeneity of the data was assessed through the Shapiro-Wilk and Levene tests, respectively. The nitrogen isotopic ratios were transformed into natural logarithm, whereas the isotopic ratios of carbon were not altered for the statistical analyzes. The data of carbon isotopic ratios, even after transformation to square and logarithm, did not present a normal distribution or equal variances. Thus, we opted for a non-parametric test to analyze $\delta^{13}C$ values. Differences in $\delta^{15}N$ and $\delta^{13}C$ values among otters' dentin of different regions were determined via an analysis of variance (ANOVA) and Kruskal-Wallis test, respectively.

The isotopic niches of the Neotropical otter of different regions were calculated from the values of $\delta^{13}C$ and $\delta^{15}N$ in dentin using Stable Isotope Bayesian Ellipses in R (SIBER; Jackson et al., 2011). The standard ellipse area corrected for small sample sizes (SEAc, expressed as per mil square $‰^2$) was used as the measure of isotopic niche, which contain approximately 40% of data (Syväranta et al., 2013).

To estimate the proportional contribution of each food source to the diet of the Neotropical otter, Stable Isotope Mixing Models in R (SIMMR; Parnell and Inger, 2016) were applied using $\delta^{13}C$ and $\delta^{15}N$ values of otter dentin and prey muscle. The values used for trophic discrimination, defined as the difference between the isotopic values of the consumer and their diet, were +3.7 ± 1.6 for $\delta^{13}C$ and +3.6 ± 1.3 for $\delta^{15}N$, as reported for mammalian bone collagen (Szpak et al., 2012). A simulated mixing polygon was used to evaluate the model and the trophic discrimination factors (Smith et al., 2013). Based on ecological similarities and isotopic composition, prey species were grouped into ecological groups. All species of prey collected, and consequently all ecological groups, were incorporated to mixing model. In addition, for a better fit of the model and for the reconstruction of the diet, isotopic ratios of sources for the Taim Wetland were obtained from the literature (Silva-Costa & Bugoni, 2013; Brito & Bugoni, 2015) and incorporated into the model (Table 2).

Statistical analyzes were performed using the statistical environment R version 3.4.1 (R Core Team, 2017). The significance level for two-sided tests was $\alpha = 0.05$.

RESULTS

In the present study, 26 Neotropical otter canine teeth were analyzed for age determination. Three individuals had their age estimated due to the morphological characteristics of the skull, which indicated that they were less than one year old. All canines analyzed for age determination showed visible GLG's in the cement. Most of our samples were composed by otters between three and six years-old. The oldest individual in our sample was nine years old (Fig. 3).

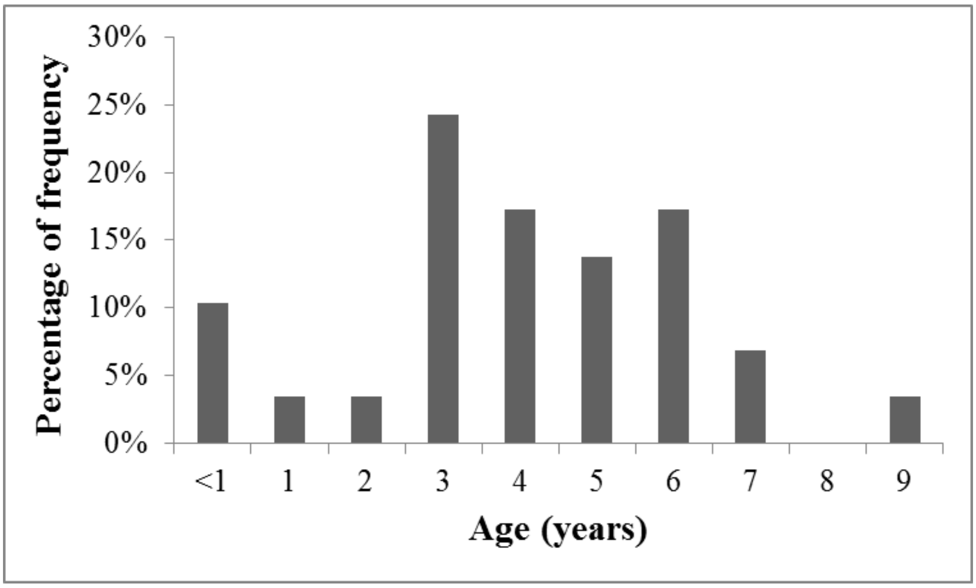

Fig. 3 Age distribution by percentage of frequency.

Altogether 29 teeth samples were analyzed isotopically: 10 from Taim Wetland, 12 from Tramandaí River Basin, and seven from Santa Catarina Island. Mean isotopic values of both δ^{13}C and δ^{15}N were similar among the study area, though variation was high, especially in Santa Catarina Island and Taim Wetland (Table S1). The δ^{13}C values ranged from -24.4 ‰ to -11.8 ‰, with a mean and standard deviation of -19.9 ± 2.9 ‰. The δ^{15}N ratios also varied broadly, from 9.8 ‰ to 19.2 ‰, with a mean and standard deviation (± SD) of 14.2 ± 1.8‰. Isotopic ratios in otter specimens analyzed were variable across the study areas. Otters from Santa Catarina showed higher mean values of δ^{13}C (δ^{13}C = -18.1 ± 4.3 ‰) and δ^{15}N (δ^{15}N = 15.0 ± 2.0 ‰), compared to otters sampled in Tramandaí River Basin (δ^{13}C = -20.6 ± 1.5 ‰, δ^{15}N = 14.1 ± 1.2 ‰) and from Taim Wetland (δ^{13}C = -20.3 ± 2.8 ‰, δ^{15}N = 13.7 ± 2.1 ‰). Differences among areas were non-significant in δ^{15}N (ANOVA, $p = 0.34$) and δ^{13}C (Kruskal-Wallis, $p = 0.74$).

Long-term isotopic niche was wider in otters of Santa Catarina Island (SEAc = 20.86 ‰^{2}), followed by Taim Wetland (SEAc = 14.51 ‰^{2}), and Lagunar System of Tramandaí (SEAc = 6.35 ‰^{2}) (Fig. 4).

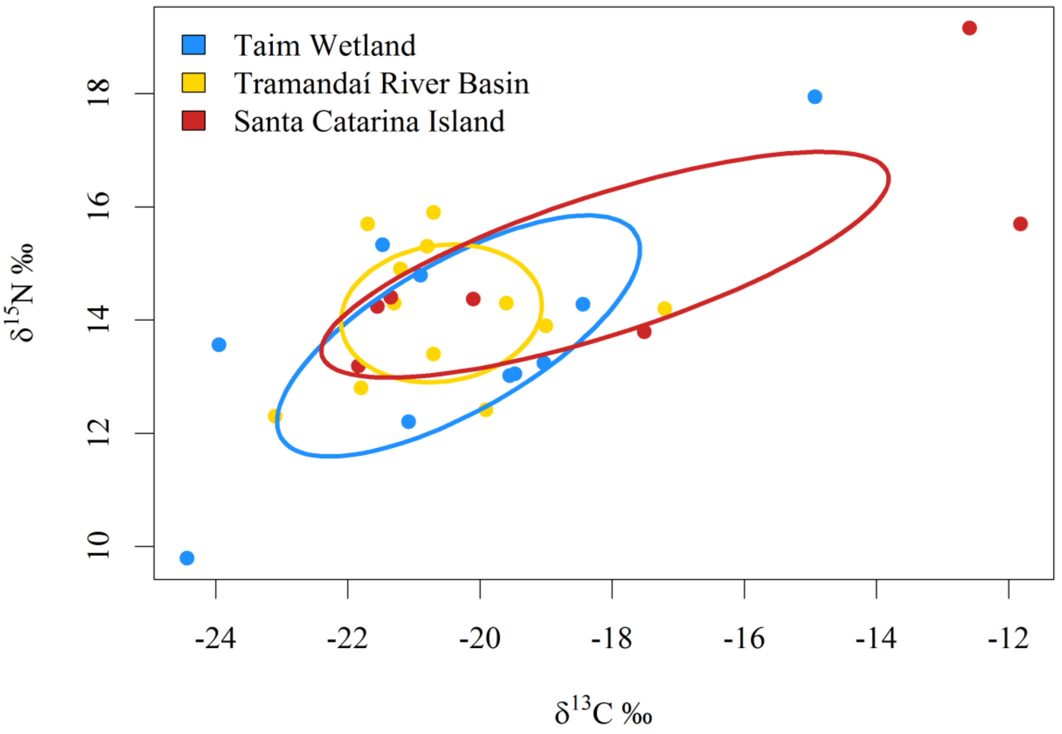

Fig. 4 Isotopic niche area, measured as standard ellipse area corrected for small sample sizes, based on $\delta^{13}C$ and $\delta^{15}N$ of Neotropical otter (*Lontra longicaudis*) dentin from different regions in southern Brazil

Although the $\delta^{13}C$ and $\delta^{15}N$ values of some animals' borderline to the simulated mixing polygons, Neotropical otter isotopic ratios fell within simulated mixing polygons calculated with potential prey isotopic ratios corrected with the trophic discrimination factors (Fig. 5). Individual isotopic ratios of the otters and their potential prey, the latter corrected by the trophic discrimination factors proposed by Szpak et al. (2012), are plotted in Fig. 6.

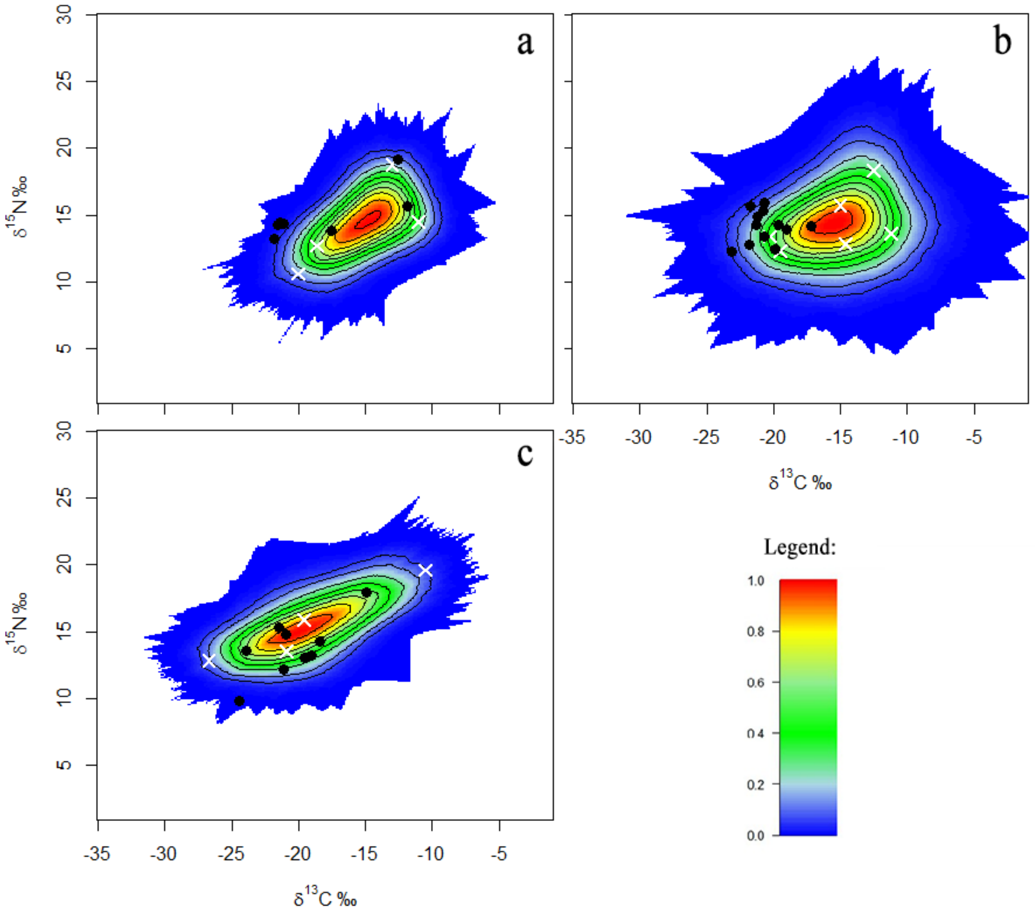

Fig. 5 Simulated mixing polygon for the Neotropical otter (*Lontra longicaudis*) dentin $\delta^{13}C$ and $\delta^{15}N$ values (black circles) and mean isotopic ratios of prey groups (white crosses) from (a) Santa Catarina Island, (b) Tramandaí River Basin and (c) Taim Wetland. Source values were corrected for a trophic discrimination factor of +3.7 ± 1.6 ‰ for $\delta^{13}C$ and +3.6 ± 1.3 ‰ for $\delta^{15}N$

The isospace plot indicates that marine and freshwater prey were isotopically discrete in Santa Catarina Island (Fig. 6a). Further, there is evidence of three foraging strategies of Neotropical otters occurring in this area: a first group related to freshwater resources and the other two relying on marine prey (fish and crustaceans), respectively. The otters dependent on marine resources were less than one-year old, found dead on the beach. Meanwhile, the group related to freshwater sources was composed by animals with age varying from less than one to five years, which were collected in different environments. In terms of population, the proportional contribution of the freshwater omnivorous fish to the diet of the Neotropical otter in the Santa Catarina Island was high, followed by marine carnivorous fish, freshwater herbivorous fishes and marine crustaceans (Fig. 7a).

The isospace of Tramandaí River Basin, on the other hand, indicates a consistency in the use of freshwater resources by the otters (Fig. 6b). In this area, the freshwater carnivorous fish represented the major contribution to the diet (Fig. 7b).

Except for two specimens, a near seven years-old female collected on the marine coast that was C^{13}-enriched and a near three years-old male with depleted $\delta^{13}C$ and $\delta^{15}N$ values, otters from the Taim Wetland were isotopically similar to freshwater fishes (Fig. 6c). The largest contribution to otter diet in Taim Wetland area was from freshwater omnivorous fish, followed by freshwater invertebrates, freshwater carnivorous fishes and marine carnivorous fish (Fig. 7c).

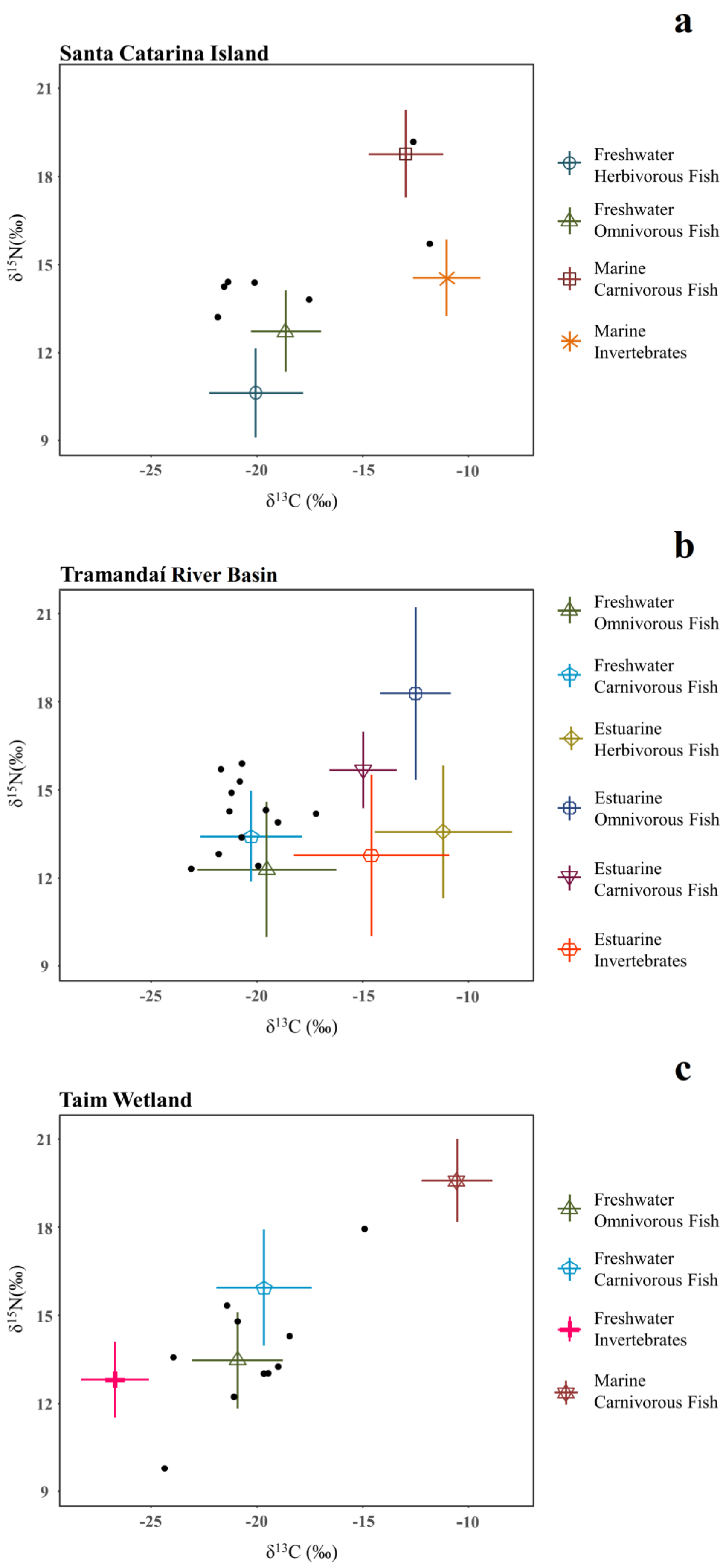
a
Santa Catarina Island
21
18
15
12
9
δ15N(‰)
-25
-20
-15
-10
δ13C (‰)
Freshwater Herbivorous Fish
Freshwater Omnivorous Fish
Marine Carnivorous Fish
Marine Invertebrates
b
Tramandaí River Basin
21
18
15
12
9
δ15N(‰)
-25
-20
-15
-10
δ13C (‰)
Freshwater Omnivorous Fish
Freshwater Carnivorous Fish
Estuarine Herbivorous Fish
Estuarine Omnivorous Fish
Estuarine Carnivorous Fish
Estuarine Invertebrates
c
Taim Wetland
21
18
15
12
9
δ15N(‰)
-25
-20
-15
-10
δ13C (‰)
Freshwater Omnivorous Fish
Freshwater Carnivorous Fish
Freshwater Invertebrates
Marine Carnivorous Fish

Fig. 6 Individual Neotropical otter (*Lontra longicaudis*) dentin $\delta^{13}C$ and $\delta^{15}N$ values (solid circles) plotted against mean isotopic values of prey groups. Source isotopic ratios were corrected for a trophic discrimination factor of +3.7 ± 1.6 ‰ for $\delta^{13}C$ and +3.6 ± 1.3 ‰ for $\delta^{15}N$

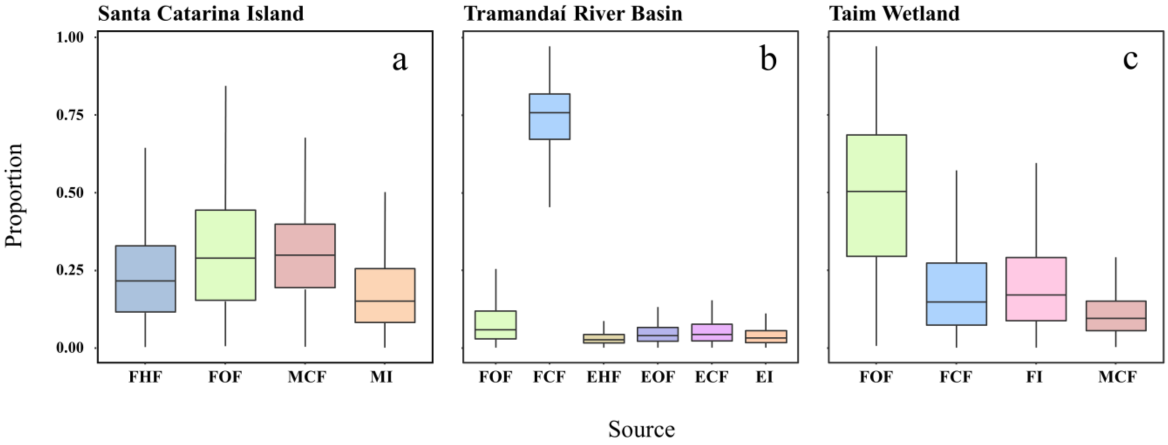

Fig. 7 Dietary proportions according to stable isotope mixing model. Sources: freshwater herbivorous fish (FHF), freshwater omnivorous fish (FOF), freshwater carnivorous fish (FCF), freshwater invertebrates (FI), marine carnivorous fish (MCF), marine invertebrates (MI), estuarine herbivorous fish (EHF), estuarine omnivorous fish (EOF), estuarine carnivorous fish (ECF) and estuarine invertebrates (EI)

DISCUSSION

The diet of the Neotropical otter has been investigated in Brazil through fecal analysis (e.g., Rheingantz et al., 2011; Sousa et al., 2013; Quintela et al., 2012), and has shown that the species exploits a wide variety of food resources in different habitats (see Rheingantz et al., 2017). However, the methodology employed in these studies does not allow the differentiation between the individuals within the population. In the present study, the approach used, coupled with the sex and age determination of specimens, provided information on variation in feeding habits of Neotropical otters at the individual level. Moreover, the analysis of metabolically inert tissues provided information on most relevant food resources for the otters over a time scale of years.

Long-term isotopic niche of otters from different regions, obtained by sampling the entire dentin, revealed a great individual variation in all analyzed populations. However, the isotopic niche was wider in otters of Santa Catarina Island, followed by Taim Wetland and Tramandaí River Basin, probably due to the individuals that present divergent isotopic values. Freshwater sources represented the major contribution to the diet in these three regions, nevertheless, in Santa Catarina Island and Taim Wetland, we found some individuals with isotopic ratios characteristic of marine environments, suggesting that these otters incorporated marine-derived nutrients.

The broader isotopic niche of individuals sampled on the Island of Santa Catarina and in the region of Taim Wetland may be result of population variation. In other words, the individuals that compose a population are not ecologically equivalent, being able to explore different resources (Bolnick et al., 2003). This variation can derive from factors such as intraspecific competition and ecological opportunity (diversity of available resources) (Araújo et al., 2011). In contrast, the population from the Tramandaí River Basin presents a narrower isotopic niche, which indicates that otters from this area exploit a low variety of resources.

Although the Tramandaí River Basin comprises a coastal region with the presence of an estuary, otters from this area were ^{13}C-depleted, indicating that the species forages only in freshwater environments. In contrast to our results, Barbieri (unpublished data) demonstrated that the otters use the Tramandaí Lagoon as feeding area, preying on catfish (*Genidens* spp.), mullet (*Mugil* spp.), whitemouth croaker (*Micropogonias furnieri*) and crab (*Callinectes* spp.). The Tramandaí Lagoon is inserted in an urban region, and consequently it is an environment impacted by the human presence. Therefore, we hypothesized that otters of this region preferentially feed on freshwater lakes, which are less impacted, and occasionally feed on the estuary during their displacements between the freshwater lakes. It is know that Neotropical otter's movements, especially of males, comprise long distances (Trinca et al., 2013). Nakano et al. (2004), through telemetry data, found that a male otter usually moved between islands that were separated by an estuary, which corroborates with our hypothesis. Due the sign of the dentin represent the whole life of the animal, occasional feeding events in the estuary could not be detected. Another possibility is that, due our small sample size and collection points, the animals residing in the Tramandaí Lagoon were not sampled. Most of our otter's samples was collected on adjacent highways to freshwater bodies.

The ecological group of freshwater carnivorous fish represented the major contribution to the diet of the Neotropical otter in the Tramandaí River Basin. This group is represented by a cichlid popularly known as pike (*Crenicichla* sp.), by the trahira (*Hoplias malabaricus*), a fish of family Erythrinidae, and by the atipa (*Hoplosternum littorale*), which belongs to family Callichthyidae. Our results were similar to those found by Sousa et al. (2013) in the Lower Arroio Grande River, southern Brazil, which verified a high frequency of food remains from trahira and fishes of Callichthyidae and Cichlidae families in otter's feces. Among the fish species mentioned, the trahira is the most frequent and abundant in the Tramandaí River Basin (Artiori, 2012), indicating that the contribution of *Hoplias malabaricus* in the diet of the Neotropical otter is greater in relation to the other taxa.

The morphological characteristics of the coast of Santa Catarina Island, associated with the high productivity of coastal waters (Gregg et al., 2005; Noernberg et al., 2007) may have favored the occupation of *L. longicaudis* in these habitats. Otters in the marine environment use a small strip of water, as observed for the Eurasian otter, *Lutra lutra*, which forages at less than 100 m from shore (Kruuk & Moorhouse, 1991). Based on the ecology of the Neotropical otter, it is likely that the species feeds in shallow waters near the rocky shores, using them as a physical barrier to ambush the prey. Additionally, the Neotropical otter use natural cavities along the banks of water bodies to rest (Pardini & Trajano, 1999; Kasper et al., 2008). Thus, the rocky shores possibly facilitate the process of catch their prey and provide natural shelters.

Suckling may have influenced the isotopic values in young individuals. Studies with mammals, especially with pinnipeds (seals and sea lions), have shown that nursing pups are a trophic level higher than their mothers, evidenced by $\delta^{15}N$ values. In contrast, there is little or no difference in $\delta^{13}C$ values between the cubs and their mothers, probably due the milk lipid composition (Cherel et al., 2015; Drago et al., 2015). In otters, milk constitutes the entire diet of the cub for about two months (Kruuk, 2006). In addition, sampling the entire dentin may have reduced the effect of suckling on the isotopic values of young otters. Assuming that the influence of suckling on $\delta^{13}C$ and $\delta^{15}N$ values for Neotropical otter teeth collagen is negligible, this effect was not considered in the interpretation of the results.

Otter cubs start to eat small prey at the age of three months. From this period onwards they begin to follow their mothers for about a year. Males, on the other hand, do not participate in parental care (Kruuk, 2006). Therefore, the presence of <1 yr old animals from the Santa Catarina Island with ^{13}C-enriched isotopic ratios, suggests that adult females also use the marine waters of the island to forage. Previous studies had already reported evidences of the species feeding in the marine waters of Santa Catarina Island (Alarcon & Simões-Lopes, 2004; Carvalho-Junior et al., 2013), and nearby (Carvalho-Junior et al., 2012). Thus, although typically freshwater, the island's coast feature may have favored the use of this habitat by females and their offspring.

In terms of population, freshwater omnivorous fish represented the major contribution to the diet of the Neotropical otter in the Santa Catarina Island. Carvalho-Junior et al. (2010)

verified by fecal analysis that cichlid fish correspond to the main otter prey in the Peri Lagoon. The most abundant cichlids in this environment are the pearl (*Geophagus brasiliensis*) and the readbreast tilapia (*Tilapia rendalli*) (Ferreira et al., 2016), which were grouped into different ecological groups in the present study. Due to the greater proportional contribution of freshwater omnivorous fish to the diet of the Neotropical otter, represented by the pearl cichlid, we suggest that *Geophagus brasiliensis* is the main species of prey in Santa Catarina Island.

Despite the lack of records of otters foraging on the marine coast adjacent to Taim Wetland, an isotopically enriched specimen was observed among the samples from this region. It is known that in southern Brazil *Mugil liza* juveniles moves from the surf zone into the costal stream, carrying marine-derived nutrients (Oliveira et al., 2014). Therefore, we suggest that the ^{13}C-enriched adult female fed on marine prey that was present in the streams that flow into the sea. This assumption is in agreement with Quintela et al. (2012) that found otter's scats in a coastal stream near to Taim region. The authors identified fish remains of Mugilidae family that are typically from marine and estuarine environments (Fischer et al., 2011). Fish remains of taxonomic groups that are typically from limnic environments (e.g., Characidae and Cichlidae) were also found, but at lower frequencies (Quintela et al., 2012).

Another individual, presenting discrepant values of the population from Taim Wetland, was found. This otter was a near three years-old male with depleted δ^{13}C and δ^{15}N values. Analyzing the collected potential prey and the information present in the literature, it was not possible to identify a source isotopically similar to this animal. Due its isotopic signature, this animal probably fed preferentially low-trophic freshwater prey.

The major contribution to the diet of the Neotropical otter in the Taim Wetland region was of freshwater omnivorous fish. This ecological group is represent by a characin (*Cyphocharax voga*), by the cichlid smooth-cheek eartheater (*Gymnogeophagus gymnogenys*) and by the South American catfish (*Rhamdia* sp.), a fish of family Heptapteridae. Quintela et al. (2008), through feces collected in Bolaxa Stream, southern Brazil, also found that cichlids, *Cyphocharax voga* and *Rhamdia* sp. are the most fishes consumed by the otter. Based on the high abundance and frequency of *Cyphocharax voga* in Taim lakes (Garcia et al., 2006), we suggest that this species corresponds to the main food source of the otter in this environment.

Intraspecific competition in the freshwater environments may be greater, since the Neotropical otter is found predominantly in lakes and streams (e.g., Gori et al., 2003; Kasper et al., 2008; Carvalho-Junior et al., 2010; Quintela et al., 2011). Newsome et al. (2015) observed that the increase in intraspecific competition is dependent of factors such as habitat characteristics, which can lead to individual diet specialization in sea otters (*Enhydra lutris*). Therefore, the characteristics of the aquatic environments of Taim Wetland and Santa Catarina Island associated with intraspecific competition may be favoring an individual specialization in Neotropical otters, reflected in a broad isotopic niche. In the Tramandaí River Basin, on the other hand, due a wide availability of freshwater environments, the otters in this region may do not require alternative foraging areas to avoid intraspecific competition.

Otters need freshwater sources to avoid physiological problems associated with the sea salt (see Kruk, 2006), thus we suggest that the C^{13}-enriched individuals interspersed between the marine and freshwater environments. As a result, the Neotropical otter may be contributing to the flow of matter and energy between these environments. The connectivity of different food webs intermediated by fish (e.g., Oliveira et al., 2014) and birds (e.g., Brito & Bugoni, 2015; Faria et al., 2016) has been reported in previous studies in southern Brazil. The results of the current study indicate that this process may be occurring through a mammal as well.

Neotropical otters consume a wide range of prey (see Rheingantz et al., 2017), thus important sources in the species diet may not have been sampled and incorporated into the mixing model. In addition, Neotropical otter and prey samples were collected in different periods, which may have influenced the isotopic values, since the isotopic fractionation is influenced by physical characteristics of the environment (Newsome et al., 2007). Another factor that should be taken into account is that both analyzed tissues (Neotropical otter dentine and potential prey muscle) encompass distinct time windows. Dentin is synthesized and deposited continuously, incorporating isotopic information throughout the animal's life (Bocherens & Drucker, 2013), while muscle is a metabolically active tissue, and consequently, has higher rates of isotopic incorporation (e.g., Buccheister & Latour, 2010; Kim et al., 2012; Madigan et al., 2012). Trophic discrimination factors are also a limitation in the interpretation of the model. The fractionation can vary depending on characteristics of the consumer, such as growth rates and diet (Robbins et al., 2005; Caut et al., 2007; 2009), so assuming constant trophic discrimination factors, in this case, calculated on the basis of mammalian collagen (Szpak et al., 2012), may generate some biases in the results. Therefore, we emphasize the importance of studies that evaluate methodological problems associated to the use of mixing models.

The current study presents the first inferences about the dietary contribution of the different sources to the Neotropical otter's diet, with the aim of identifying patters in the use of resources by the species. Using stable isotope analysis, freshwater fish proved an important prey for Neotropical otter, as evidenced in previous studies about the species diet in southern Brazil (Quadros & Monteiro-Filho, 2001; Kasper et al., 2004b; Uchôa et al., 2004; Kasper et al., 2008; Quintela et al., 2008; Carvalho-Junior et al., 2010; Sousa et al., 2013). Similar patterns were also recorded in other regions of Brazil (Helder-José & Andrade, 1997; Pardini, 1998; Louzada-Silva et al., 2003; Silva et al., 2012) and in other countries along its distribution, such as Argentina (Gori et al., 2003; Chemes et al., 2010), Colombia (Mayor-Victoria & Botero-Botero, 2010) and Mexico (Macías-Sánchez & Aranda, 1999). The high contribution of freshwater fish to the diet of Neotropical otter reflects a similarity in feeding habits among the systems studied. However, in Santa Catarina Island and in Taim Wetland, a greater isotopic variation among individuals was found, indicating a higher trophic diversity, with strong individual specialization, in these regions.

CONCLUSION

The isotopic niche of the Neotropical otter varies according to the region in which it is found, being wider in the Santa Catarina Island and Taim Wetland, due to a greater individual variation. Otters from Santa Catarina Island and Taim Wetland use mostly freshwater environments as foraging areas. However, some individuals from these regions incorporate marine-derived nutrients, indicating that the species in these areas also uses the marine coast to feed. The otters of the Tramandaí River Basin, on the other hand, have a narrower isotopic niche and seem to be restricted to freshwater environments for foraging. The differences found among the otters from different regions are probably related to a variety of factors, such as landscape characteristics and intraspecific competition.

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SUPPLEMENTARY MATERIAL

Table S1 Age (years), sex, year of collection, study area and sampling institution of Neotropical otters analyzed in the present study. $\delta^{13}C$ and $\delta^{15}N$ values (‰) of teeth dentin are also shown. UFSC (Universidade Federal de Santa Catarina), IEB (Instituto Ekko Brasil), GEMARS (Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul), UFRGS (Universidade Federal do Rio Grande do Sul), NEMA (Núcleo de Educação e Monitoramento Ambiental) and FURG (Universidade Federal do Rio Grande).

Individual	Sex	Age (years)	$\delta^{13}C$	$\delta^{15}N$	Institution	Year	Locality
910	M	5	-20.1	14.4	UFSC	1998	Santa Catarina Island
2792	M	3	-21.8	13.2	UFSC	1999	Santa Catarina Island
3090	M	<1	-12.6	19.2	UFSC	2002	Santa Catarina Island
3390	F	4	-21.4	14.4	UFSC	2004	Santa Catarina Island
157	-	<1	-11.8	15.7	UFSC	-	Santa Catarina Island
158	M	<1	-17.5	13.8	UFSC	-	Santa Catarina Island
1	-	4	-21.6	14.2	IEB	-	Santa Catarina Island
Mean (SD)			**-18.1 ± (4.3)**	**15.0 ± (2.0)**			
G1157	M	5	-21.2	14.9	GEMARS	2004	Tramandaí River Basin
G1483	M	5	-20.7	15.9	GEMARS	2004	Tramandaí River Basin
CDS166	M	3	-19.9	12.4	UFRGS	2010	Tramandaí River Basin
G1405	F	4	-20.8	15.3	GEMARS	2010	Tramandaí River Basin
G1481	M	6	-17.2	14.2	GEMARS	2011	Tramandaí River Basin
G1629	M	4	-21.3	14.3	GEMARS	2012	Tramandaí River Basin
G1666	F	3	-20.7	13.4	GEMARS	2013	Tramandaí River Basin
G1632	M	4	-21.8	12.8	GEMARS	2013	Tramandaí River Basin
-	M	1	-21.7	15.7	GEMARS	2014	Tramandaí River Basin
G1677	M	6	-19.0	13.9	GEMARS	2014	Tramandaí River Basin
G1692	F	3	-23.1	12.3	GEMARS	2016	Tramandaí River Basin
-	M	3	-19.6	14.3	GEMARS	2016	Tramandaí River Basin
Mean (SD)			**-20.6 ± (1.5)**	**14.1 ± (1.2)**			
2579	-	2	-21.1	12.2	UFSC	1981	Taim Wetland
L002	-	9	-19.5	13.1	FURG	1996	Taim Wetland

L003	M	6	-18.4	14.3	FURG	1996	Taim Wetland
L004	M	3	-21.5	15.3	FURG	1997	Taim Wetland
66550	F	7	-14.9	17.9	FURG	2013	Taim Wetland
353	M	3	-24.4	9.8	FURG	2016	Taim Wetland
960	M	6	-24.0	13.6	NEMA	2016	Taim Wetland
947	M	6	-20.9	14.8	FURG	2016	Taim Wetland
521	M	5	-19.0	13.2	FURG	2017	Taim Wetland
L005	-	7	-19.6	13.0	FURG	-	Taim Wetland
Mean (SD)			**-20.3 ± (2.8)**	**13.7 ± (2.1)**			

CONSIDERAÇÕES FINAIS E PERSPECTIVAS

A presente dissertação teve como objetivo o estudo de aspectos ecológicos da lontra-neotropical, *Lontra longicaudis*. Para isso, a ferramenta de isótopos estáveis e a contagem das linhas de cemento foram utilizadas pela primeira vez em pesquisas sobre a espécie. A contagem das linhas de cemento permitiu a estimativa de idade dos exemplares utilizados no presente estudo. Por sua vez, a análise de isótopos estáveis foi utilizada para identificar padrões na utilização de recursos e habitats pela espécie. Empregando ambas as metodologias em conjunto foi possível fazer inferências sobre a dieta e uso de habitat em nível individual.

As evidências isotópicas de longo prazo demonstraram que as lontras da Ilha de Santa Catarina (SC) e do Banhado do Taim (RS) exploram uma maior variedade de recursos alimentares, em comparação com os animais do Sistema Lagunar de Tramandaí (RS). Os resultados indicam que a espécie forrageia preferencialmente em ambientes de água doce nas três regiões analisadas, corroborando estudos prévios sobre uso de habitat e dieta da espécie no sul do Brasil. Além disso, no presente estudo, três animais provenientes da Ilha de Santa Catarina e da região do Banhado do Taim apresentaram valores enriquecidos em ^{13}C, característicos de ambientes marinhos. Esses animais eram dois filhotes e uma fêmea, o que sugere que fêmeas e filhotes utilizam a costa como área de forrageamento.

Os resultados encontrados no presente estudo sugerem que os indivíduos são especialistas e compõem uma população generalista, no entanto, a metodologia empregada não permitiu verificar a especialização individual na espécie. Assim, recomenda-se que sejam feitos estudos, utilizando a análise de isótopos estáveis, que verifiquem a especialização individual através da amostragem longitudinal de tecidos metabolicamente inertes, como vibrissas e dentina. Além disso, recomenda-se que sejam realizados mais estudos sobre o uso de ambientes costeiros pela espécie, a fim de identificar padrões no uso de habitat e recursos alimentares, e assim, auxiliar no estabelecimento de medidas de conservação da espécie e seus habitats.