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**RELAÇÕES TRÓFICAS ENTRE MAMÍFEROS
MARINHOS SIMPÁTRICOS DO ESTUÁRIO
DA LAGOA DOS PATOS E ÁREA COSTEIRA
ADJACENTE, RIO GRANDE DO SUL, BRASIL**

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RESUMO

O estuário da Lagoa dos Patos (PLE) (32° S), e sua área costeira adjacente, são usados principalmente por três espécies de mamíferos marinhos. A toninha (*Pontoporia blainvillei*) habita águas marinhas rasas e se alimenta de teleósteos e cefalópodes de pequeno porte. O boto (*Tursiops truncatus*) e o leão-marinho Sul-Americano (*Otaria flavescens*) habitam a região costeira principalmente, ocupando tanto a área estuarina como a região costeira adjacente, consumindo peixes teleósteos. A análise de isótopos estáveis de carbono e nitrogênio tem sido amplamente aplicada em estudos de ecologia trófica de mamíferos marinhos. Normalmente os tecidos dos predadores são mais enriquecidos nos isótopos mais pesados do que as presas e essa diferença é geralmente chamada de fator de discriminação trófica (*Trophic Discrimination Factor* - TDF). Os valores $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$ dos predadores, juntamente com as de suas presas potenciais, podem ser usados para estimar a contribuição relativa de cada presa à dieta do predador através da aplicação de modelos de mistura isotópica. Estes modelos, no entanto, são altamente sensíveis aos valores do TDF. Assim, a escolha correta desses valores é um requisito básico nestes modelos de mistura para estimar as proporções de presas na dieta do consumidor. Neste trabalho, foi utilizada a análise de isótopos estáveis de carbono e nitrogênio na pele de toninhas, botos e leões-marinhos para avaliar a composição das suas dietas, o compartilhamento de recursos e a sobreposição de nicho isotópico. A fim de aplicar corretamente os modelos de mistura isotópica, diferentes métodos não-experimentais para estimar valores de TDF adequados foram testados. A área de sobreposição entre as elipses TDF-corrigidas e aquelas das presas foram usadas para testar TDFs obtidos da literatura e métodos matemáticos alternativos, a fim de avaliar quais os valores mais adequados para a pele e músculo de toninhas e leões-marinhos. A

utilização de valores de TDF reportados a partir de experimentos de alimentação controlada em focídeos de cativeiro resultou em nenhuma sobreposição entre as elipses leão-marinho TDF-corrigidas e as de suas presas para ambos os tecidos. Alguma sobreposição foi observada no caso da toninha usando TDFs publicados derivados de experimentos de alimentação controlada em cativeiro do boto. Os TDFs mais adequados foram aqueles calculados a partir da diferença entre a média ponderada dos valores isotópicos das presas e os valores isotópicos dos predadores: $\Delta^{13}\text{C}_{\text{pele}} = 1,3 \text{ ‰}$ e $\Delta^{15}\text{N}_{\text{pele}} = 4,5 \text{ ‰}$ e $\Delta^{13}\text{C}_{\text{músculo}} = 2,5 \text{ ‰}$ e $\Delta^{15}\text{N}_{\text{músculo}} = 4,6 \text{ ‰}$ de leões-marinhos; e $\Delta^{13}\text{C}_{\text{pele}} = 2,2 \text{ ‰}$ e $\Delta^{15}\text{N}_{\text{pele}} = 2,9 \text{ ‰}$ e $\Delta^{13}\text{C}_{\text{músculo}} = 1,6 \text{ ‰}$ e $\Delta^{15}\text{N}_{\text{músculo}} = 2,2 \text{ ‰}$ em toninhas. Em seguida, aplicaram-se estes valores de TDF para descrever a dieta de toninhas e botos e leões-marinhos, respectivamente, por meio da aplicação de modelos de mistura isotópica aos valores isotópicos em pele destes predadores. Estes mostraram uma composição de dieta diversificada para o leão-marinho e a toninha com poucas presas principais. Por outro lado, a dieta do boto revelada pelo modelo de mistura demonstrou uma dominância de *Trichiurus lepturus*, com pouca contribuição de outras espécies de peixes. Também foi calculada a área de nicho isotópico como um *proxy* para o nicho ecológico das espécies, a fim de avaliar a sua sobreposição ou segregação e compartilhamento de recursos. Os leões-marinhos apresentaram os maiores valores isotópicos de nitrogênio e o maior nicho isotópico. Por outro lado, os botos apresentaram os menores valores isotópicos de nitrogênio, o menor nicho isotópico, não tendo nenhuma sobreposição com o leão-marinho. A toninha ocupou uma posição intermediária, com sobreposição parcial de nicho com o boto. Essa segregação de nicho e compartilhamento de recursos parece permitir a coexistência dessas espécies-chave de mamíferos marinhos no PLE e zonas costeiras adjacentes. A investigação dos hábitos alimentares e as relações tróficas destes

componentes importantes desta rede trófica costeira devem auxiliar ações de manejo e conservação dirigidas à proteção deste rico ecossistema estuarino.

Palavras-chave: isótopos estáveis, *Otaria flavescens*, *Pontoporia blainvillei*, *Tursiops truncatus*, Fatores de discriminação trófica, composição da dieta, nicho isotópico

ABSTRACT

The estuary of the Patos lagoon (PLE) (32° S), and its adjacent coastal area, are mainly used by three species of marine mammals. The franciscana (*Pontoporia blainvillei*) inhabits shallow marine waters and usually fed upon of small teleosts and cephalopods. The bottlenose dolphin (*Tursiops truncatus*) and the South American sea lion (*Otaria flavescens*) mainly inhabit the coastal region occupying both estuarine and adjacent coastal areas, consuming mainly teleost fishes. The analysis of stable carbon and nitrogen isotopes has been widely applied in trophic ecology studies of marine mammals. Predator tissues are typically enriched in the heavier isotopes than their diets and this difference is usually called trophic discrimination factor (TDF). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of predators, together with those of their potential preys, can be used to estimate the relative contribution of each prey to the predator diet by applying isotope mixing models. These models, however, are highly sensitive to the values of TDF, thus the correct choice of these values is a basic requirement when applying stable isotope mixing models to estimate the proportions of prey in the consumer's diet. In this work, we used stable carbon and nitrogen isotopes analysis in skin of bottlenose dolphins, franciscanas and sea lions to assess their diet composition, resource partitioning and isotopic niche overlap. In order to correctly apply isotopic mixing models, we first tested different non-experimental methods for estimating suitable TDF values. The overlap area of TDF-corrected and prey Bayesian ellipses were used to test TDFs from literature and alternative mathematical methods in order to evaluate the most suitable TDF value for skin and muscle of franciscanas and sea lions. Published TDF values provided by control feeding trials using Phocids resulted in no overlap between TDF corrected sea lion

ellipses and those of their prey for both tissues. Some overlap was observed in the case of the franciscana using published experimental TDFs derived from bottlenose dolphins. The most suitable TDFs were calculated through the difference between predator isotopic values and weighed mean prey isotopic values: $\Delta^{13}\text{C}_{\text{skin}} = 1,3 \text{ ‰}$ and $\Delta^{15}\text{N}_{\text{skin}} = 4,5 \text{ ‰}$ and $\Delta^{13}\text{C}_{\text{muscle}} = 2,5 \text{ ‰}$ e $\Delta^{15}\text{N}_{\text{muscle}} = 4,6 \text{ ‰}$ for sea lions; $\Delta^{13}\text{C}_{\text{skin}} = 2,2 \text{ ‰}$ and $\Delta^{15}\text{N}_{\text{skin}} = 2,9 \text{ ‰}$ and $\Delta^{13}\text{C}_{\text{muscle}} = 1,6 \text{ ‰}$ and $\Delta^{15}\text{N}_{\text{muscle}} = 2,2 \text{ ‰}$ for franciscanas. We then applied these TDF to run mixing models to describe the diet of franciscanas, bottlenose dolphins and sea lions based on isotopic values of skin. The isotopic mixing models showed a diversified diet composition of South American sea lion and franciscana with few main preys. On the other side, the bottlenose dolphin diet revealed by the mixing model showed a dominance of *Trichiurus lepturus*, with few contributions of other fish species. We also calculated the isotopic niche area as a proxy for the ecological niche of the species in order to evaluate their overlap/segregation and resource partitioning. The sea lion showed the highest nitrogen isotopic values and the largest isotopic niche. On the other hand, bottlenose dolphins had the lowest nitrogen isotopic values, the smallest isotopic niche and had no niche overlap with the sea lion. The franciscana occupied an intermediate position, with partial niche overlap mainly with the bottlenose dolphin. This niche segregation and resource partition seem to allow the coexistence of these key-species of marine mammals in the PLE and coastal adjacent areas. The investigation of the feeding habits and trophic relationships of these important components of this coastal food web should aid management and conservation actions directed to the protection of this rich estuarine ecosystem.

Keywords: stable isotopes, *Otaria flavescens*, *Pontoporia blainvillei*, *Tursiops truncatus*, Trophic Discrimination Factors, diet composition, isotopic niche

APRESENTAÇÃO

A presente Dissertação foi estruturada em um capítulo e dois anexos em formato de artigo científico. O Capítulo 1, redigido em língua portuguesa, apresenta uma introdução geral, hipóteses e objetivos, material e métodos e uma síntese dos principais resultados. Os anexos I e II correspondem ao primeiro e segundo artigo da dissertação, respectivamente, e estão redigidos em língua inglesa.

CAPÍTULO 1

1. INTRODUÇÃO GERAL

O nicho ecológico de uma determinada espécie pode ser definido como um espaço hipervolumétrico e n-dimensional, que inclui a amplitude de condições (e.g. temperatura, profundidade, distância da costa) e recursos (e.g. tipo ou tamanho de presa consumida) necessárias à sua sobrevivência (Hutchinson, 1957). Entretanto, um recurso em quantidades limitadas pode derivar em uma competição entre indivíduos da mesma espécie (competição intra-específica) ou de espécies diferentes (competição inter-específica). Assim, as dimensões do nicho ecológico podem ser ampliadas ou reduzidas em função das interações com outras espécies, de forma a permitir a coexistência de espécies potencialmente competidoras (Begon et al., 2006). Segundo o princípio da exclusão competitiva, as espécies precisam diferir em alguma dimensão do nicho ecológico para que possam coexistir por longos períodos, de forma que possam minimizar a competição entre elas (Hardin, 1960). Dois mecanismos principais permitem uma diferenciação de nicho: (1) a partição de recursos, onde espécies que habitam uma mesma região exploram os recursos de forma diferente (e.g. predam sobre presas de tamanhos diferentes) e (2) a diferenciação espacial ou temporal na exploração dos recursos. Desta forma, espécies simpátricas minimizam a sobreposição de nicho permitindo a sua coexistência em determinados habitats (Harper et al., 1961).

No estuário da Lagoa dos Patos (PLE), localizado no sul do Brasil ($\sim 32^\circ$ S), e suas adjacências costeiras, diversas espécies de mamíferos marinhos costeiros coexistem, fazendo uso dos abundantes recursos que o local oferece (Pinedo, 1998) (Fig.1).



Figura 1. Leão-marinho Sul-Americano (*Otaria flavescens*) descansando no Molhe Leste (Rio Grande RS) (A), toninha (*Pontoporia blainvillei*) incidentalmente capturada em redes de pesca (B) e botos (*Tursiops truncatus*) no estuário da Lagoa dos Patos (C).

Os mamíferos são importantes componentes dos ecossistemas marinhos. Eles ocupam principalmente níveis tróficos elevados, alguns sendo predadores dos topos das cadeias alimentares. A toninha (*Pontoporia blainvillei*), espécie endêmica da costa sudoeste do Oceano Atlântico, distribui-se em águas rasas até os 35 m de profundidade (Danilewicz et al., 2009), não tendo registros da sua presença no estuário ao menos desde 1976 (Pinedo, 1998). A espécie alimenta-se habitualmente de pequenos teleósteos, cefalópodes e crustáceos, sendo estes últimos especialmente importantes para os filhotes (Basso, 2005). O boto (*Tursiops truncatus*) e o leão-marinho (*Otaria flavescens*) são

predadores generalistas de alto nível trófico, que têm suas dietas constituídas por poucas presas dominantes e relativamente alta quantidade de espécies menos abundantes (Barros & Wells, 1998; Szteren, 2006). Na região do PLE os peixes teleósteos são a principal fonte de alimento para ambas as espécies, porém o leão-marinho inclui também elasmobrânquios, cefalópodes, crustáceos e, inclusive, rejeitos da pesca na sua dieta (Lopez, 2013). Os dois predadores habitam principalmente a região costeira ocupando tanto a área estuarina quanto as áreas costeiras adjacentes (Rosas et al., 1994; Di Tullio et al., 2015). Porém, a comunidade de botos que habita a região do PLE tem um habitat restrito às águas rasas, com uma profundidade média de 4 m (Di Tullio et al., 2015) enquanto registros de forrageio do leão-marinho indicam que a espécie utilizaria também áreas da plataforma até os 100 m (Campagna et al., 2001; Rodríguez et al., 2013). A dieta desses mamíferos marinhos é também influenciada pela sazonalidade das presas, que ocupam a região em diferentes épocas do ano (Basso, 2005; Lopez, 2013).

Apesar de ter sido observado que há sobreposição parcial do nicho alimentar dos botos e leões-marinhos na região do PLE, com algumas espécies principais e até tamanhos médios compartilhados, certas diferenças foram observadas (Lopez, 2013). O leão-marinho parece ter um nicho trófico mais amplo que o boto, possivelmente devido à utilização dos rejeitos de pesca (Szteren, 2006; Lopez, 2013), que lhe confere presas que, normalmente, não fazem parte de sua dieta. A toninha, por sua vez, se alimenta de presas menores e mais variadas, incluindo cefalópodes (Basso, 2005) e, portanto, é provável que ocupe um nicho trófico diferente e em posição trófica inferior ao dos outros predadores.

Tradicionalmente, os estudos das relações tróficas entre vertebrados marinhos têm se baseado nas informações aportadas pelos conteúdos estomacais ou de fezes (p.ex.

Pauly et al., 1998). Porém, esses métodos refletem apenas a alimentação dos últimos dias. Além disso, partes rígidas de presas de difícil digestão (p. ex. bicos de cefalópodes, otólitos de teleósteos) podem se acumular no trato, superestimando a sua importância na dieta, ao contrário de presas de mais fácil digestão ou sem partes duras, que podem ser subestimadas (Pierce et al., 2004; Sekiguchi & Best, 1997). Devido a essas limitações outros métodos (p. ex. análise de ácidos graxos, sequenciamento de DNA, análise de isótopos estáveis) são atualmente utilizados em conjunto assim concedendo maior robustez aos estudos.

Dentre eles, a análise de isótopos estáveis de carbono e nitrogênio, comumente referidos pela notação delta ($\delta^{13}\text{C}$ e $\delta^{15}\text{N}$, respectivamente), vem sendo amplamente aplicada em estudos de ecologia trófica de mamíferos marinhos (Newsome et al., 2010). As composições isotópicas dos predadores estão diretamente relacionadas às aquelas presentes nas suas dietas (Peterson & Fry, 1987), estando os tecidos do predador tipicamente enriquecidos nos isótopos mais pesados (^{13}C , ^{15}N) como produto da discriminação isotópica dos isótopos mais leves (^{12}C , ^{14}N) durante o metabolismo (Ben-David & Flaherty, 2012). A discriminação isotópica é normalmente menor para ^{12}C (aprox. 0,5-1‰, DeNiro & Epstein, 1978), comparada à discriminação sofrida pelo ^{14}N (aprox. 2-5‰, DeNiro & Epstein, 1981). Além disso, fatores como fontes de nutrientes, composição de produtores primários e características oceanográficas regionais determinam paisagens isotópicas ou *isoscapes* (Graham et al., 2010). Regiões costeiras, em particular apresentam valores isotópicos de carbono geralmente mais elevados do que ambientes oceânicos em função tanto da sua composição de produtores quanto a $[\text{CO}_2]$ em ambientes bentônicos (France, 1995; Graham et al., 2010; McMahon et al., 2013). Assim, os valores de $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$ são utilizados em conjunto para indicar a

base das redes tróficas nas quais o predador está se alimentando ($\delta^{13}\text{C}$) e também para evidenciar a posição trófica ocupada pelo predador ($\delta^{15}\text{N}$). Consequentemente, estes isótopos têm o potencial de prover informações tanto da dieta quanto do uso do habitat dos predadores.

Assim, o espaço isotópico delimitado pelos valores destas duas variáveis, chamado de nicho isotópico, vem sendo utilizado como uma aproximação do nicho ecológico (Bearhop et al., 2004). A razão desta utilização é que os componentes do nicho ecológico correspondentes às áreas de forrageamento (componente espacial) e ao uso de recursos (componente trófico) (Hutchinson, 1957) podem ser aproximados através dos valores de $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$ (Bearhop et al., 2004, Layman et al., 2007; Newsome et al., 2007). O nicho isotópico pode ser evidenciado através da área criada a partir do mínimo polígono convexo contendo os valores individuais de $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$ (Layman et al., 2007) ou por meio da utilização de uma abordagem bayesiana calculando elipses isotópicas (Jackson et al., 2011). Esta última tem se tornado a principal alternativa de análise nos últimos anos devido principalmente à sua robustez frente à presença de outliers e/ou variações intraespecíficas (Syväranta et al., 2013).

Os valores de $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$ dos predadores, em conjunto com o das presas potenciais, também podem ser utilizados para estimar a contribuição relativa destas para a dieta do predador por meio de modelos de mistura (Parnell et al., 2010). Estes modelos, porém, são sensíveis aos valores dos fatores de discriminação, de forma que a escolha correta destes valores torna-se fundamental para estimar corretamente as proporções das presas por meio desta metodologia (Bond & Diamond, 2011; Olin et al., 2013). Os fatores de discriminação variam de acordo com o tipo de tecido, influenciados principalmente

pela taxa metabólica, presença de elementos estruturais (p. ex. conteúdo lipídico e proteico) e incorporação proteica na dieta (Martinez del Rio et al., 2009).

1.1. HIPÓTESES

No contexto exposto acima, duas hipóteses principais nortearam este estudo:

- i.* O nicho isotópico do leão-marinho é mais amplo do que aquele do boto ou da toninha;
- ii.* Há sobreposição de nicho isotópico entre o boto e o leão-marinho, porém ela é menor ou nula entre estes e a toninha.

1.2. OBJETIVOS

Objetivo geral

Estudar as relações tróficas entre o boto, a toninha e o leão-marinho que utilizam o estuário da Lagoa dos Patos (RS – Brasil) e adjacências.

Objetivos específicos

- (1) Determinar os fatores de discriminação trófica (TDF) entre pele e músculo de leão-marinho e toninha e suas principais presas;
- (2) Analisar a composição da dieta das três espécies de mamíferos marinhos;
- (3) Avaliar a amplitude e sobreposição de nicho isotópico entre as três espécies de mamíferos marinhos.

2. MATERIAL E MÉTODOS

Área de Estudo

O PLE (32° S) está localizado na porção sul do estado do Rio Grande do Sul, Brasil. A costa adjacente do estuário é formada por extensas praias arenosas, limitadas ao

norte pela barra da Lagoa do Peixe (33° S) e ao sul pelo Arroio Chuí (31° S). A região é considerada uma importante área reprodutiva com alta produtividade de diversas espécies de peixes marinhos e cefalópodes (Seeliger et al., 2004).

Amostragem

Amostras de pele e músculo de toninha (machos e fêmeas) e leão-marinho (machos) foram obtidas das carcaças encalhadas encontradas durante monitoramentos sistemáticos entre os anos de 2012 e 2015, abrangendo todas as épocas do ano. Toninhas capturadas acidentalmente em redes de pesca de emalhe pela frota pesqueira que atua na mesma região de estudo também foram incluídas. Amostras de pele de boto foram obtidas durante monitoramentos embarcados no estuário e na região costeira adjacente, utilizando uma balestra e flechas com a ponteira especialmente adaptada para coletar biópsias de pequenos cetáceos (Fruet et al., 2014). Apenas indivíduos adultos de ambos predadores foram utilizados para evitar vieses resultantes de variações ontogenéticas na dieta. Para tal, toninhas e leões-marinhos foram selecionados com base no seu comprimento total (Danilewicz, 2003; Danilewicz et al., 2004; Grandi et al., 2010). Os botos foram visualmente classificados em juvenil ou maduro durante a amostragem no campo, baseado no seu tamanho relativo e/ou comportamento (p. ex. pares de mãe e filhote).

As espécies de peixes e cefalópodes representando aproximadamente 95% do Índice de Importância Relativa (IRI), de acordo com estudos prévios utilizando análise de conteúdo estomacal (Bassoi, 2005; Lopez, 2013; Secchi et al., 2016), foram obtidas em indústrias de processamento de pescado que operavam na mesma região da área de estudo durante o ano de 2015. Foram coletadas presas com comprimento total dentro do intervalo de valores reportado na dieta dos predadores em períodos quentes e frios do ano

com objetivo incorporar possíveis variações sazonais nos seus valores isotópicos. Todas as amostras foram mantidas em congelador a -20°C até o processamento para análise de isótopos estáveis.

Análise de isótopos estáveis

As amostras de mamíferos marinhos e suas presas foram secas a 60°C por 48 horas. Para evitar variações nos valores de $\delta^{13}\text{C}$ em decorrência do conteúdo lipídico dos tecidos, as amostras de leão-marinho e toninhas tiveram os lipídeos extraídos através de um método modificado de Bligh e Dyer (1959), utilizando um extrator *Soxhlet*, com uma mistura 2:1 de clorofórmio e metanol. As amostras de boto não foram extraídas por causa da sua baixa razão C:N (média dos valores de C:N = 3,57), que é próxima àquela considerada como proteína pura (C:N < 3.5; Post et al. 2007). As amostras das presas também não tiveram seus lipídios extraídos (C:N médio = 3,43), com exceção das amostras de *Mugil sp.*, que devido ao seu alto conteúdo lipídico tiveram que ser extraídas.

Todas as amostras foram secas a 60°C e transformadas em pó, utilizando graal e pistilo. Aproximadamente 0,5-0,7 mg de cada amostra foi inserida em cápsulas de estanho e analisadas em um espectrômetro de massas acoplado em um analisador elementar no *Stable Isotope Core, Washington State University*. As composições isotópicas foram apresentadas na notação convencional delta (δ) em per mil (‰), em relação a Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$) e N_2 atmosférico ($\delta^{15}\text{N}$). Padrões internos de composições de carbono e nitrogênio conhecidas (acetanilide e queratina, respectivamente) foram analisadas entre amostras para estimar a precisão instrumental. O desvio padrão dos padrões internos foram $\leq 0.1\text{‰}$ para $\delta^{13}\text{C}$ e $\delta^{15}\text{N}$.

Comparação entre tecidos dos mamíferos marinhos

Os dados são apresentados como média $\pm$ desvio padrão (SD), e o nível de significância assumido foi de 0,05. Todas as análises estatísticas foram realizadas no ambiente estatístico R v.3.3.1 (R Development Core Team, 2016). Após ter os pressupostos de normalidade (teste de Shapiro-Wilks) e homocedasticidade (teste de Levene) verificados, foram realizados testes-t de Student para examinar diferenças entre os valores isotópicos entre pele e músculo dos predadores.

Fatores de discriminação trófica (Anexo I)

O manuscrito do anexo I comparou fatores de discriminação trófica (TDF) em pele e músculo de toninha e leão-marinho. Utilizando os valores isotópicos para cada tecido de cada predador e suas principais presas, foram construídas elipses isotópicas, e a área de sobreposição entre elas foi utilizada para comparar os TDFs testados, sob o pressuposto de que aquelas elipses do predador, corrigidas pelos diferentes valores de TDF, que apresentassem maior área de sobreposição com a área total das elipses das presas seriam aquelas construídas com TDFs mais adequados à espécie/tecido. Três conjuntos de valores de TDF foram testados: no método 1, TDFs provenientes da literatura foram aplicados. No método 2, sugerimos valores de TDF baseados na média dos valores isotópicos ponderada pelo Índice de Importância Relativa de cada presa. No método 3, utilizamos o pacote DESiR para estimar TDFs em músculo.

Composição da dieta e nicho isotópico (Anexo II)

No anexo II, a contribuição relativa de cada presa na dieta de boto, leão-marinho e toninha foi determinada através de modelos Bayesianos de mistura isotópica - MixSIAR. Os TDFs obtidos no anexo I (Método 2) foram utilizados nos modelos, junto

aos valores isotópicos das presas e da pele dos predadores. No caso do boto, o TDF obtido para pele de toninha, foi utilizado.

Adicionalmente, o nicho isotópico de cada espécie foi determinado, utilizando SIBER (Stable Isotope Bayesian Ellipses in R, Jackson et al., 2011) no pacote SIAR (Stable Isotope Analysis in R, Parnell et al., 2010). Foram construídas elipses isotópicas com objetivo de comparar o tamanho e a sobreposição do nicho de cada espécie de mamífero marinho.

3. SÍNTESE DOS RESULTADOS

Anexo I: Tissue-specific isotope trophic discrimination factors in two marine mammals from the Western South Atlantic

- O método 1, em que os valores isotópicos dos predadores foram corrigidos com os valores de TDF obtidos através da literatura, resultou em pouca área de sobreposição das elipses isotópicas da pele de leão-marinho com as de suas presas e nenhuma sobreposição no caso do músculo. O mesmo método produziu grande sobreposição entre as elipses isotópicas corrigidas em pele e músculo de toninha e suas presas.
- TDFs foram estimados no método 2 utilizando a diferença entre os valores isotópicos nos tecidos dos predadores e das presas ponderadas pela importância relativa da presa obtida por meio de estudos de conteúdo estomacal, reportados na literatura. Os valores estimados foram de $\Delta^{13}\text{C} = 1,0 \text{ ‰}$ e $\Delta^{15}\text{N} = 4,7\text{‰}$ em músculo e $\Delta^{13}\text{C} = 2,2\text{‰}$ e $\Delta^{15}\text{N} = 4,5\text{‰}$ em pele de leão-marinho. Para toninha, os valores estimados foram $\Delta^{13}\text{C} = 1,6 \text{ ‰}$ e $\Delta^{15}\text{N} = 2,2\text{‰}$ em músculo e $\Delta^{13}\text{C} = 2,2 \text{ ‰}$ e $\Delta^{15}\text{N} = 2,9\text{‰}$ em pele. Para ambos tecidos e espécies, o método 2 resultou

em alta sobreposição com as elipses isotópicas presas e aparentemente são os valores mais realistas.

- Por meio do pacote DeSIR estimaram-se valores de TDFs de músculo para toninhas e leão-marinho (método 3). Os valores estimados para leão-marinho foram insuficientes para produzir sobreposição com as elipses de suas presas. Para a toninha, o TDF estimado produziu sobreposição parcial com as elipses, principalmente com aquelas presas que possuem maior valor isotópico de carbono.

Anexo II. Trophic relationships of key-species of marine mammals from a subtropical estuary and adjacent coastal areas in the western South Atlantic Ocean

- A composição da dieta estimada para o leão-marinho teve várias espécies indicadas como importantes, porém com algum destaque para *Paralanchurus brasiliensis*.
- Em linhas gerais, o modelo isotópico da toninha reportou *Loligo sp.* e *Cynoscion guatucupa* como as presas que mais contribuem para a dieta, porém não apresentando uma diferença importante com respeito à contribuição das restantes espécies.
- O modelo isotópico do boto foi o que apresentou maior discrepância na composição da dieta em comparação com o encontrado utilizando a metodologia tradicional. Diferentemente do observado através da análise de conteúdo estomacal, *Trichiurus lepturus* foi a presa que mais contribuiu para a dieta do boto, seguida por *Mugil liza*, sendo que as outras presas tiveram pouca importância.

- A elipse isotópica do leão-marinho foi a que apresentou maior área (4,44‰²) (corroborando *Hipótese i*), seguida pela da toninha (3,89‰²), enquanto a do boto foi a de menor área (1,34‰²). Além disso, o leão-marinho ocupou a posição trófica mais alta, enquanto o boto foi a mais baixa, estando a toninha em posição intermediária, com base nos valores isotópicos de nitrogênio.
- Não houve sobreposição entre as elipses isotópicas do leão-marinho e do boto (rejeita *Hipótese ii*). A elipse da toninha teve uma sobreposição de 0,72‰² com a do leão-marinho, representando 18,5% e 16,2% de suas áreas, respectivamente. Em relação à elipse do boto, houve uma sobreposição de 0,79‰² com a da toninha, representando 69% e 20,3% de suas elipses, respectivamente.

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ANEXO I

Tissue-specific isotope trophic discrimination factors in two marine mammals from the
Western South Atlantic

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Abstract

The analysis of stable carbon and nitrogen isotopes has been widely applied in trophic ecology studies of marine mammals. Predator tissues are typically enriched in the heavier isotopes than their diets and this difference (Δ) is usually called trophic discrimination factor (TDF). Few studies calculated TDFs using controlled feeding for marine mammals, due to their low number or difficulty of maintenance in captivity. In this study, we collected skin and muscle samples of marine mammals and muscle of their main preys for stable isotope analysis. Overlap area of TDF-corrected and prey isotopic ellipses were used to test TDFs from literature and alternative mathematical methods in order to evaluate the most suitable TDF value for skin and muscle of franciscana dolphins (*Pontoporia blainvillei*) and South American sea lions (*Otaria flavescens*). Published TDF values provided by control feeding trials resulted in no overlap between TDF-corrected sea lion ellipses and those of their prey for both tissues. Some overlap was observed in the case of the franciscana using published experimental TDFs derived from other cetacean species. The most suitable TDFs were $\Delta^{13}\text{C} = 1.3\text{‰}$ and $\Delta^{15}\text{N} = 4.5\text{‰}$ for skin and $\Delta^{13}\text{C} = 2.5\text{‰}$ and $\Delta^{15}\text{N} = 4.6\text{‰}$ for muscle of sea lions. For franciscanas, the best TDFs for skin were $\Delta^{13}\text{C} = 2.2\text{‰}$ and $\Delta^{15}\text{N} = 2.9\text{‰}$ and for muscle were $\Delta^{13}\text{C} = 1.6\text{‰}$ and $\Delta^{15}\text{N} = 2.2\text{‰}$. The methodology that we adapted for this study provided more reliable TDFs for these two species and its application is recommended whenever experimentally derived values are not available.

Keywords: stable isotopes, *Otaria flavescens*, *Pontoporia blainvillei*, marine mammals, Trophic Discrimination Factor, diet.

1. Introduction

Traditionally, studies of trophic relationships among marine vertebrates have been based on information obtained by stomach or feces content (e.g. Pauly et al., 1998). However, due to their recognized limitations, complementary methods are currently being used in combination thus providing greater robustness to ecological studies. The analysis of carbon and nitrogen stable isotopes, commonly referred by delta notation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) has been widely applied to trophic ecology studies of marine mammals (Newsome et al., 2010). The isotopic compositions of predators are directly related to those present in their diets (Peterson & Fry, 1987). Furthermore, predator tissues are typically enriched in heavier isotopes due to the isotopic discrimination of the lighter isotopes during metabolism (Ben-David & Flaherty, 2012). This difference between predator and prey isotopic composition is called Trophic Discrimination Factor (TDF) (Martinez del Río et al., 2009) and is larger in the case of nitrogen isotopes than for carbon isotopes (DeNiro & Epstein, 1978; DeNiro & Epstein, 1981). Consequently, nitrogen stable isotopes are commonly used to estimate the trophic position of a consumer in the food web, whereas carbon isotope values have the potential to provide information of the basal sources, and thus can indicate its habitat use (Peterson & Fry, 1987; Post, 2002).

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of predators, together with those of their potential preys, can also be used to estimate the relative contribution of each prey to the predator diet by applying isotope mixing models (Parnell et al., 2010). These models, however, are highly sensitive to the values of TDF, thus the correct choice of these values is a basic requirement when applying stable isotope mixing models to estimate the proportions of prey in the consumer's diet (Bond & Diamond, 2011; Olin et al., 2013). In addition, diet-

tissue discrimination factors are required for estimating a species trophic position by means of nitrogen stable isotopic compositions (Newsome et al., 2010). The TDFs will vary depending on the tissue, influenced mainly by metabolic rate (Healy et al., 2016), structural elements (e.g. lipid and protein content) and protein incorporation into the diet (Martinez del Rio et al., 2009).

TDFs are commonly estimated using controlled feeding studies. In these experiments, captive animals are maintained under a known diet in controlled conditions (Browning et al., 2014). However, this alternative is especially difficult to conduct with marine mammals, given logistical and ethical issues that involve keeping these animals in captivity. Nevertheless, some experimentally derived TDF values are available for phocids (Hobson et al., 1996), otariids (Stricker et al., 2015; Beltran et al., 2016) and dolphin species (Browning et al., 2014; Caut et al., 2011; Giménez et al., 2016). In spite of these published TDF values being highly valuable to the application of stable isotope analysis for dietary studies, they were estimated for tissues that are easily sampled with non-lethal procedures (i.e. skin, blood, vibrissae) (Beltran et al., 2016; Browning et al., 2014). Unfortunately, there are no TDF estimates for other commonly available tissues obtained from carcasses found stranded or derived from incidental catches, such as tooth, muscle and bone (Hobson et al., 1996). In this context, alternative methods for testing suitable TDF values that best predict diet composition of high trophic level predators were proposed (e.g. sharks, Olin et al., 2013; franciscana dolphin, *Pontoporia blainvillei*, Troina et al., 2016). Furthermore, Healy et al. (2016) proposed a statistical Bayesian model based on the relationship of several published experimentally derived TDF values for birds and mammals and their relationship with ecological and physiological variables, phylogeny and experimental conditions.

The diet of the franciscana dolphin is known from several locations along its distribution in the Western South Atlantic coast (Di Benedetto et al., 2011; Paso-Viola et al., 2014; Rodriguez et al., 2002). The population that inhabits the Rio Grande do Sul, the southernmost state in Brazil, has been subject to dietary studies since the late 1970s. These studies revealed that franciscana feeds upon small teleosts and cephalopods. Shrimps are also preyed by juveniles (Basso, 2005). In this region, South American sea lions, *Otaria flavescens*, (hereafter “sea lion”) from two non-breeding colonies also forage in coastal areas (Vaz-ferreira, 1981). Individuals from the colony located in the jetties, at the entrance of the Patos Lagoon (Southern Brazil), forage in the estuary and mainly in the adjacent marine coast (Rosas et al., 1994). Stomach content analysis of individuals from this region confirms that the sea lion is a generalist high trophic level predator, and has its diet composed mainly by a few dominant fish prey and a variety of other less abundant fish species (Lopez, 2013). Therefore, these predators are good model species for our objective of testing suitable TDF values for tissues that are not easily obtained in published experimental studies, but are commonly obtained from carcasses found washed ashore during beach surveys (i.e. skin and muscle).

2. Material and methods

2.1. Sampling

Skin and muscle samples of franciscanas and sea lions were obtained from stranded carcasses found during systematic beach surveys between Lagoa do Peixe (31°26'S) and Chuí (33°44'S), southern Brazil (Fig. 1) between 2012 and 2015. Paired skin and muscle samples from the same animal were obtained whenever possible. Franciscanas incidentally caught in fish nets were also included. All samples were

obtained during warm (November to April) and cold (May to October) months from 2012 to 2015. We used only adult specimens (i.e. franciscana males >128.2 cm and females >138.9 cm [Danilewicz, 2003; Danilewicz et al., 2004] and male sea lions >183.5 cm [Grandi et al., 2010]) in order to avoid biases resulting from ontogenetic diet variations.

Muscle samples were obtained from the main prey of the two predators, selected from studies based on stomach contents data of these species (Basso, 2005; Lopez, 2013), summarizing around of 95% of the Index of Relative Importance (IRI). Individuals of fish and cephalopod species, were obtained from commercial fisheries that operate in the study area, according to the size range consumed by each marine mammal species. Furthermore, prey were obtained in cold and warm months of 2014 and 2015, as a means of including the probable range of variation in isotope values in prey over the seasons. All samples were stored in -20°C freezer until processing for stable isotope analysis.

2.2. *Stable isotope analysis*

Marine mammal and prey samples were dried at 60° C for 48 h. To avoid biases in carbon stable isotope values derived from the lipid content of the tissues, marine mammal samples were lipid extracted using a modified method from Bligh and Dyer (1959), through a *Soxhlet* extractor, with a 2:1 chloroform and methanol mixture. No lipid extraction was performed on prey muscle because mean C:N ratios from all samples were < 3.5 (mean prey muscle C:N = 3.43), indicating low fat content (Post et al. 2007) All samples were dried again at 60°C and powdered with a mortar and pestle. About 0.5-0.7 mg of each sample was inserted into tin capsules and analyzed with a mass spectrometer coupled to an elemental analyzer at the Stable Isotope Core, Washington State University. The isotopic compositions were reported in the conventional delta (δ) per mil notation

(‰), relative to Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$) and atmospheric N_2 ($\delta^{15}\text{N}$). Internal standards of known carbon and nitrogen composition (acetanilide and keratin) were analyzed between samples in order to estimate instrument precision. The standard deviation of these internal standards was $\leq 0.1\text{‰}$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

2.3. Data analysis

2.3.1. Comparison between marine mammal tissues

Data are presented as mean $\pm$ standard deviation (SD), and statistical significance was assumed at the 0.05 level. All analyses were carried out within the R statistical environment v.3.3.1 (R Development Core team, 2016). After confirming that the assumptions of normality (tested using Shapiro-Wilks' test) and homoscedasticity (tested using Levene's test) were met, we used paired Student's *t*-tests to examine the differences between skin and muscle isotopic values.

2.3.2. Trophic discrimination factors (TDF)

In order to evaluate the most suitable TDFs for franciscana and sea lion skin and muscle, we adapted a methodology applied by Olin et al. (2013), using SIBER (Stable Isotope Bayesian Ellipses in R, Jackson et al., 2011) in SIAR package (Stable Isotope Analysis in R, Parnell et al., 2010). The method is based on the construction of standard ellipses that represent the isotopic niche of the species, based on individual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. These ellipses are obtained using the raw isotopic data in the case of each prey species and using TDF-corrected isotopic values in the case of the predator. The TDF that produces the greatest area of overlap between ellipses of the predator and its preys is considered the most suitable. Therefore, we generated standard ellipse areas corrected for small sample sizes (SEAc) for each prey using raw isotope data and SEAc for sea lions

and franciscanas using TDF-corrected carbon and nitrogen isotopic values. We did this for each predator separately and using different TDFs derived from the literature and obtained by applying alternative methodologies (see section 2.3.2.1).

The total area of the combined ellipses of prey was calculated using an image processing software (*Image J* 1.48v). The area of the predator ellipse and that of the overlap with the total prey area were then measured. By estimating these areas, the percentage of overlap of the ellipses of predator and prey were then calculated (Fig. 2).

2.3.2.1. TDFs used for correcting predator ellipses

Method 1. Several prey-to-predator TDFs for some marine mammal species were obtained from controlled feeding experiments (Browning et al., 2014; Caut et al., 2011; Giménez et al., 2016; Hobson et al., 1996). However, most of them were conducted analyzing isotopic values in tissues that are easily collected from live animals and that allow for repeated sampling, mainly blood and its components (i.e. plasma, red cells). Therefore, limited data is available of TDFs for skin and muscle of both pinnipeds and cetaceans. For sea lions, we tested TDFs calculated for the skin ($\Delta^{13}\text{C} = 2.8\text{‰}$; $\Delta^{15}\text{N} = 2.3\text{‰}$) and muscle ($\Delta^{13}\text{C} = 1.3\text{‰}$; $\Delta^{15}\text{N} = 2.4\text{‰}$) of harp seals (*Pagophilus groenlandicus*) in an experiment conducted by Hobson et al. (1996); unluckily, no estimates of SD values were provided by the authors. In the case of the franciscana, we used the skin-to-diet TDF ($\Delta^{13}\text{C} = 1.01\text{‰}$; $\Delta^{15}\text{N} = 1.57\text{‰}$), calculated in a feeding trial using captive bottlenose dolphins, *Tursiops truncatus* (Giménez et al., 2016). The unique muscle TDF in cetacean available in literature was calculated from the muscle tissue isotopic values of a captive killer whale, *Orcinus orca*, that have died during an experiment (Caut et al., 2011), thus

we used skin TDF from Giménez et al. (2016) also for calculating TDF-corrected ellipses for franciscana muscle samples.

Method 2. Diet-to-predator TDFs were estimated through equation 1, by calculating the mean difference between predator isotopic values and weighed mean prey isotopic values. We used the index of relative importance of each prey in the diet of each predator, based on literature (Basso, 2005; Lopez, 2013) for weighing each $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ prey values (Tables 1 and 2). The TDF standard deviation was obtained through the mean difference between predator and weighed prey isotopic values.

Equation 1:

$$\Delta^{13}\text{C or } \Delta^{15}\text{N} = \left(\sum \frac{A}{n} \right) - \left(\sum \frac{B * C}{D} \right)$$

Where, A is the stable isotope value of predator sample, n is the number of samples of a tissue/species, B is the stable isotope value of prey sample, C is the IRI of each prey, and D is the total IRI for all preys.

Method 3. The DEsIR (Discrimination Estimation in R, Healy et al., 2016) package was used to predict suitable TDFs for muscle of franciscanas and sea lions. Briefly, a Bayesian linear modelling of the influence of species' ecology, physiology, phylogeny and experimental conditions on published TDF values was used to develop a Bayesian imputation for estimating unknown TDF values for birds and mammals (Healy et al., 2016). As this tool was developed considering several tissues including muscle, but not skin, we only applied this method for calculating TDFs for muscle samples.

3. Results

Muscle isotopic values of sea lions ranged from -16.5‰ to -13.2‰ for $\delta^{13}\text{C}$ and from 19.5‰ to 21.2‰ for $\delta^{15}\text{N}$. The carbon isotopic values of skin were significantly higher (t -test: $t = 3.29$, $p < 0.01$) ranging from -15.7‰ to -12.4‰. The $\delta^{15}\text{N}$ values of skin ranged from 18.3‰ to 21.9‰ and were not significantly different from muscle nitrogen isotopes (t -test: $t = -0.40$, $p = 0.70$) (Table 1). In franciscana, the $\delta^{13}\text{C}$ values were similar between tissues (t -test: $t = 1.75$, $p = 0.09$) and ranged from -15.8‰ to -10.6‰ for skin and from -15.9‰ to -10.8‰ for muscle. $\delta^{15}\text{N}$ values, however, were significantly higher (t -test: $t = 1.71$, $p = 0.04$) in skin (16.7‰ to 20.1‰) than in muscle (16.6‰ to 19.0‰) (Table 2).

Muscle ellipse areas of sea lion ($\text{SEAc} = 0.86\text{‰}^2$) and franciscana ($\text{SEAc} = 1.91\text{‰}^2$) were smaller than skin ellipses areas ($\text{SEAc} = 4.44\text{‰}^2$ and $\text{SEAc} = 3.89\text{‰}^2$, for sea lion and franciscana, respectively) (Figs. 3 and 4).

No overlap between prey and predator ellipses was observed using raw isotopic data (i.e. not TDF-corrected) of franciscanas and sea lions (Figs. 3 and 4). Stable isotope values of sea lions adjusted using TDF from literature (*Method 1*) resulted in no overlap between the predator ellipse derived from TDF-corrected isotopic muscle or skin values and those of its preys (Table 4, Fig. 3). On the other hand, the franciscana TDF-corrected muscle and skin isotopic values using published TDF values resulted in high overlap of prey ellipses (Table 4, Fig. 4).

The trophic discrimination factors estimated using the mean weighed difference between prey and predator isotopic values (Table 3, *Method 2*) were $\Delta^{13}\text{C} = 1.0 \pm 0.8\text{‰}$ and $\Delta^{15}\text{N} = 4.7 \pm 0.4\text{‰}$ for muscle and $\Delta^{13}\text{C} = 2.2 \pm 1.0\text{‰}$ and $\Delta^{15}\text{N} = 4.5 \pm 1.3\text{‰}$ for skin in sea lions (Fig. 3 and Table 3). For franciscana, the estimated TDFs using this method

were $\Delta^{13}\text{C} = 1.6 \pm 0.8\text{‰}$ and $\Delta^{15}\text{N} = 2.2 \pm 0.7\text{‰}$ for muscle and $\Delta^{13}\text{C} = 2.2 \pm 1.32\text{‰}$ and $\Delta^{15}\text{N} = 2.9 \pm 1.1\text{‰}$ for skin (Fig. 4 and Table 3). Furthermore, predator TDF-corrected ellipses using TDF derived from *Method 2* for both tissues and species resulted (Table 4, Figs. 3 and 4).

The TDFs estimated with the DEsIR package for muscle (*Method 3*) were $\Delta^{13}\text{C} = 0.6 \pm 1.4\text{‰}$ and $\Delta^{13}\text{C} = 0.8 \pm 1.5\text{‰}$ for sea lions and franciscanas, respectively and $\Delta^{15}\text{N} = 2.7 \pm 1.2\text{‰}$ for sea lions and $2.7 \pm 1.4\text{‰}$ for franciscanas (Table 3). The predator ellipses constructed using these TDF-corrected values resulted in no overlap with prey ellipses in the case of the sea lion (Table 4, Fig. 3). The TDF-corrected ellipse of franciscanas, partially overlapped those of their prey, despite a higher overlap was observed with ellipses of prey that have more ^{13}C -enriched carbon isotopic values (Table 4, Fig. 4).

4. Discussion

Olin et al. (2013) proposed the application of isotopic ellipses to assess appropriate TDF values for species that are logistically and/or biologically unavailable for conducting controlled feeding experiments. By measuring the overlap between the TDF-corrected predator ellipses and those derived from raw prey isotopic data it was possible to evaluate the most suitable tissue-specific TDF value. In the present work we applied this method in order to assess suitable diet-tissue discrimination values for nitrogen and carbon for skin and muscle of two coastal marine mammals were estimated using non-experimental methods. By analyzing the degree of overlap between the ellipses generated using predator TDF-corrected isotopic values and those of their prey, our results showed that experimentally obtained TDFs for cetaceans (Browning et al., 2014) produced a high overlap in the case of the Franciscana skin and muscle (Fig. 3). However, the application of TDFs values obtained from controlled feeding studies of seals (Hobson

et al., 1996) produced almost no overlap between sea lion and prey ellipses for both tissues (Fig.4). Therefore, alternative methods for estimating TDFs were considered. Overall, the TDFs resulting from the weighed mean difference between predator and prey isotopic values were higher than published and DEsIR values.

Few studies have calculated TDFs for pinnipeds, and most of them have focused on blood and its components (Beltran et al., 2016; Lesage et al., 2002) or whiskers (Beltran et al., 2016, Hobson et al., 1996; Stricker et al., 2015). The correction of isotopic values of both tissues of sea lions using only the experimentally derived TDFs for skin and muscle (Hobson et al., 1996) was insufficient to overlap corrected sea lion ellipses with those of its prey, especially due to an inappropriate $\Delta^{15}\text{N}$ value (Fig. 3). A necessary condition for applying mixing models for estimating the proportion of food sources that makes up the diet of any predator, TDF-corrected isotopic values of the consumer isotopic must fall within the range of prey isotopic values (Phillips et al., 2014). In the present study, we included the most common prey species for sea lions in the region, identified from a significant number of stomach contents. Moreover, samples were collected along both warm and cold seasons and specimens were selected based on the length interval that sea lions consume, bases on stomach content analysis (Lopez, 2013). Therefore, inadequate TDF values are probably the main cause for the lack of overlap found using published values derived from captive seals feeding experiments. These animals are generally maintained in constant environmental conditions and diet, differently of wild animals. The low sample size of captivity experiments also makes it difficult to distinguish individual differences (Hobson et al., 1996; Newsome et al., 2010). Moreover, the sea lions that inhabit the study area are recognized for seasonal movements to the Uruguayan coast and Northern Argentina (Rosas et al., 1994), where they could access

more $\Delta^{15}\text{N}$ -enriched preys (Zenteno et al., 2014; Franco-Trecu et al., 2017). Increased TDF values also could be associated with the quality of diet protein of sea lions (Robbins et al., 2005; Kurle et al., 2014), not tested here.

Applying the method proposed by Healy et al. (2016) also provided low TDFs for nitrogen and carbon isotopes, resulting in no overlap area of muscle and skin sea lion corrected ellipse and those of their preys. The package developed by these authors (DEsIR) was on a meta-analysis of published TDF data for birds and mammals, thus producing similar low prey-to-muscle discrimination factors to the single control feeding study available for pinnipeds (Hobson et al., 1996).

Sea lion diet-to-bone trophic discrimination factors were calculated by Zenteno et al. (2014) by means of the difference between the weighed mean isotopic values of prey and that found in bone samples of South American sea lions. Their estimated $\Delta^{15}\text{N}$ were similar to the values calculated here using Method 2 ($\Delta^{15}\text{N}_{\text{prey-to-bone}} = 4.4\text{‰}$ [Zenteno et al., 2014])). Furthermore, Stricker et al. (2015) experimentally calculated diet-to-vibrissae trophic discrimination factor in another otariid, the Steller sea-lion, *Eumetopias jubatus*, obtaining nitrogen TDFs also higher ($\Delta^{15}\text{N} = 3.7\text{‰}$) than those obtained for seals by Hobson et al. (1996). Despite the low n sampling of the last (9 for skin, 2 for muscle), differences in physiology and energy use of phocids and otariids could affect the isotopic discrimination (Berta et al., 2006). In the light of these results and in the absence of any other estimation of TDFs derived from control feeding experiments, our results provide a more realistic estimate of these parameters, especially in the case of $\Delta^{15}\text{N}$ values, for skin and muscle of sea lions.

In the case of franciscanas, the correction of isotopic carbon and nitrogen values with experimentally derived TDFs provided by Giménez et al. (2016) produced low overlap between franciscana corrected ellipses and those of its prey in the case of skin samples and some overlap in the case of muscle ellipses. The values estimated by the Method 2, using weighed prey stable isotope values, produced higher overlap area for both tissues. We used the skin TDF values for both tissues (Giménez et al., 2016) in method 1, hence we hypothesize that differences in metabolism and isotopic turnover (Newsome et al., 2010) may have masked results and caused higher overlap with muscle ellipses. In fact, the method 2 muscle TDF have intermediate values between that tested in method 1 and the estimated for skin in method 2. An earlier study by Caut et al. (2011) reported diet-to-skin ($\Delta^{13}\text{C} = 2.43\text{‰}$ and $\Delta^{15}\text{N} = 3.18\text{‰}$) and diet-to-muscle ($\Delta^{13}\text{C} = 1.26\text{‰}$ and $\Delta^{15}\text{N} = 1.23\text{‰}$) discrimination factors for a killer whale (*Orcinus orca*) that had died during the experiment, which could bias values found. Constant diet, commonly formed by only one prey species, provided in captivity experiments, in turn of multiple sources commonly ingested by wild live predators, could cause such discrepancy in TDF values (Robbins et al., 2010; Olin et al., 2013). Moreover, Troina et al. (2016) analyzed tooth stable carbon and nitrogen isotopes in Franciscana dolphins for estimating diet using Bayesian mixing models. The authors tested different published TDF values, concluding that the nitrogen TDF that most accurately estimated prey proportions for this dolphin was higher than those experimentally provided (Browning et al., 2014).

The application of DEsIR package (Healy et al., 2016) to estimate TDF values for Franciscana muscle samples provided similar nitrogen TDFs to those obtained by Method 2. However, the lower $\Delta^{13}\text{C}$ obtained resulted in a low overlap with prey ellipses, mainly with those showing lower $\delta^{13}\text{C}$ values. The variation in ellipse area overlapping between

the methods that we tested, corroborate the potential biases in use of inappropriate TDF values in trophic level estimation or trophic relationships studies (Olin et al., 2013). Therefore, by applying discrimination factors estimated through Method 2, it is possible to achieve a better estimate of the prey composition of this species by stable isotope analysis of skin and muscle. This finding is especially important in the case of this small cetacean as its captive rearing is extremely unsuccessful thus alternative methods for estimating these parameters are necessary in order to correctly apply isotopic analysis for diet reconstruction.

5. Conclusions

The methodology that we adapted for this study provided more reliable trophic discrimination factors for species that do not have specific studies, or are not possible to maintain captive (i.e. franciscana). Furthermore, we recommend the application of this method in order to get more realistic TDF values whenever experimentally derived ones are not available.

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Table 1

Predator (skin and muscle) and prey (muscle) data used to compute the Trophic Discrimination Factor (TDF) on the basis of tissues' stable isotope compositions. Published Index of Relative Importance (IRI) for adult male South American sea lions (*Otaria flavescens*) from southern Brazil were used for weighing prey isotope values.

	n	IRI ^a	$\delta^{13}\text{C} \pm \text{SD} (\text{‰})$	$\delta^{15}\text{N} \pm \text{SD} (\text{‰})$
<i>O. flavescens</i>				
Skin	11		-13.6±1.0	20.1±1.3
Muscle	14		-14.8±0.8	20.3±0.4
Prey species				
<i>Paralonchurus brasiliensis</i>	24	0.61	-15.8±0.7	15.6±0.8
<i>Micropogonias furnieri</i>	17	0.14	-15.5±0.5	15.5±0.6
<i>Macrodon atricauda</i>	27	0.11	-16.1±0.6	15.8±0.8
<i>Cynoscion guatucupa</i>	25	0.05	-16.7±0.3	15.7±0.5
<i>Trichiurus lepturus</i>	21	0.04	-17.0±0.7	15.3±0.7
<i>Urophycis brasiliensis</i>	12	0.03	-15.6±0.4	14.8±0.9

^aLopez (2013)

Table 2

Predator (skin and muscle) and prey (muscle) data used to compute the Trophic Discrimination Factor (TDF) on the basis of tissues' stable isotope compositions. Published Index of Relative Importance (IRI) for adult franciscana dolphins (*Pontoporia blainvillei*) from southern Brazil were used for weighing prey isotope values.

	n	IRI ^a	$\delta^{13}\text{C} \pm \text{SD} (\text{‰})$	$\delta^{15}\text{N} \pm \text{SD} (\text{‰})$
<i>P. blainvillei</i>				
Skin	14		-13.6±1.5	18.3±1.1
Muscle	13		-14.5±1.3	17.7±0.7
Prey species				
<i>Cynoscion guatucupa</i>	25	0.46	-16.7±0.3	15.6±0.3
<i>Trichiurus lepturus</i>	16	0.26	-17.1±0.8	15.5±0.6
<i>Urophycis brasiliensis</i>	6	0.11	-15.5±0.4	14.7±0.8
<i>Paralonchurus brasiliensis</i>	33	0.09	-16.0±0.6	14.9±1.0
<i>Anchoa mitchilli</i>	4	0.03	-17.1±0.1	16.1±0.2
<i>Porichthys porosissimus</i>	5	0.02	-17.4±0.2	13.9±0.8
<i>Umbrina canosai</i>	7	0.01	-16.4±0.9	15.9±0.6

^aBassoi (2005)

Table 3

Trophic Discrimination Factors (TDF) for *Otaria flavescens* and *Pontoporia blainvillei* tested in the present study using the proposed methods in tissues. When available, SD values are shown after mean TDF values.

Species	Method	Skin TDF		Muscle TDF	
		$\Delta^{13}\text{C}$ (‰)	$\Delta^{15}\text{N}$ (‰)	$\Delta^{13}\text{C}$ (‰)	$\Delta^{15}\text{N}$ (‰)
<i>O. flavescens</i>	1	2.8 ^a	2.3 ^a	1.3 ^a	2.4 ^a
	2	2.2±1.0	4.5±1.3	1.0±0.8	4.7±0.4
	3			0.6±1.4	2.7±1.2
<i>P. blainvillei</i>	1	1.01±0.37 ^b	1.57±0.52 ^b		
	2	2.2±1.32	2.9±1.1	1.6±0.8	2.2±0.7
	3			0.8±1.5	2.7±1.4

^aHobson et al. (1996). SD values not provided in the original paper.

^bGiménez et al. (2016)

Table 4

Percentage of overlap between total prey isotopic ellipses and sea lion (*Otaria flavescens*) and franciscana (*Pontoporia blainvillei*) isotopic ellipses estimated based on individual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

		Skin		Muscle		
		1	2	1	2	3
Method						
<i>O. flavescens</i>						
	Predator	0%	72%	0%	100%	0%
	Total prey	0%	72%	0%	18%	0%
<i>P. blainvillei</i>						
	Predator	21%	85%	49%	100%	92%
	Total prey	14%	58%	15%	15%	29%

Figure legends

Figure 1. Stretch of coast area located between Chui river and the Lagoa do Peixe bar, southern Rio Grande do Sul state, Brazil, that was surveyed for collecting samples from stranded carcasses.

Figure 2. The percentage of overlap between the sum of the prey ellipse areas (colored ellipses) and predator ellipse area (black dashed ellipse) (A) were calculated by measuring the total prey ellipse area (in black) and the predator ellipse area (black dashed) (B). The area of overlap (black area) was then measured (C).

Figure 3. Isotopic ellipses for sea lion (*Otaria flavescens*) (black) muscle (left) and skin (right) corrected using different Trophic Discrimination Factors (TDF) obtained from different methods and ellipses of main prey (Red *Paralichthys brasiliensis*; Green *Micropogonias furnieri*; Dark blue *Trichiurus lepturus*; Light blue *Macrodon atricauda*; Pink *Cynoscion guatucupa*; Yellow *Urophycis brasiliensis*).

Figure 4. Isotopic ellipses for franciscana, *Pontoporia blainvillei*, (black) muscle (left) and skin (right) corrected using different Trophic Discrimination Factor (TDF) obtained from different methods and ellipses of main prey (Red *Loligo sp.*; Green *Trichiurus lepturus*; Dark blue *Umbrina canosai*; Light blue *Porichthys porosissimus*; Pink *Cynoscion guatucupa*; Yellow *Urophycis brasiliensis*; Light gray *Paralichthys brasiliensis*; Dark gray *Anchoa mitchilli*).

Figure 1

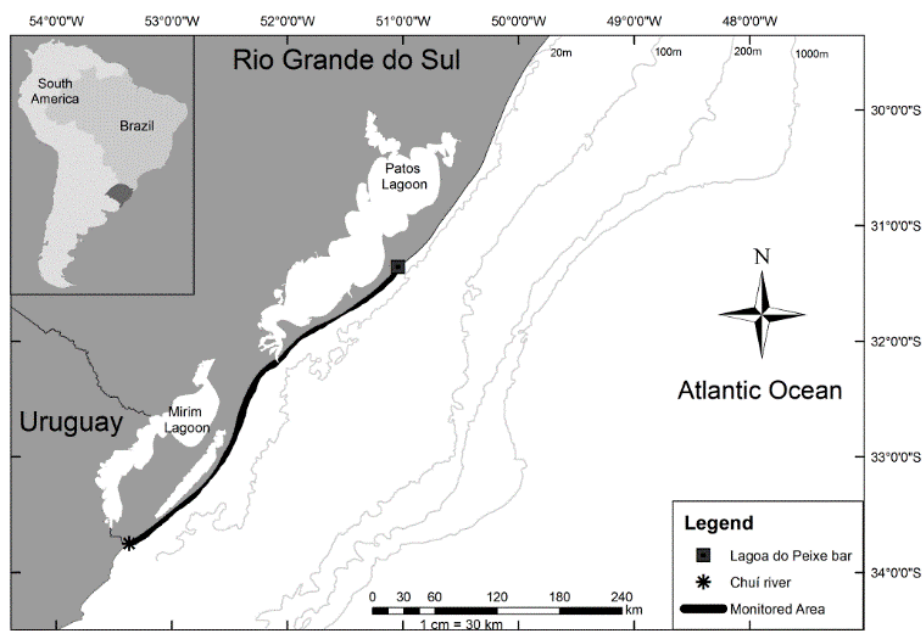


Figure 2

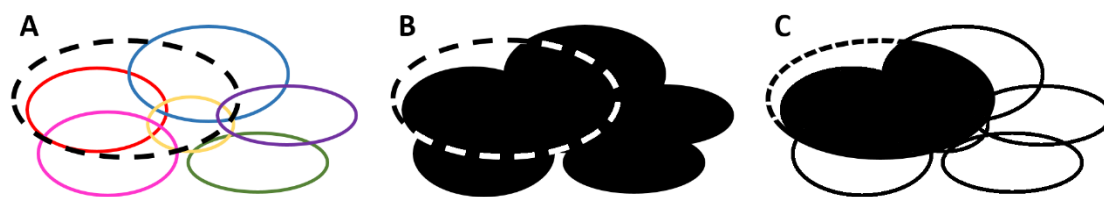


Figure 3

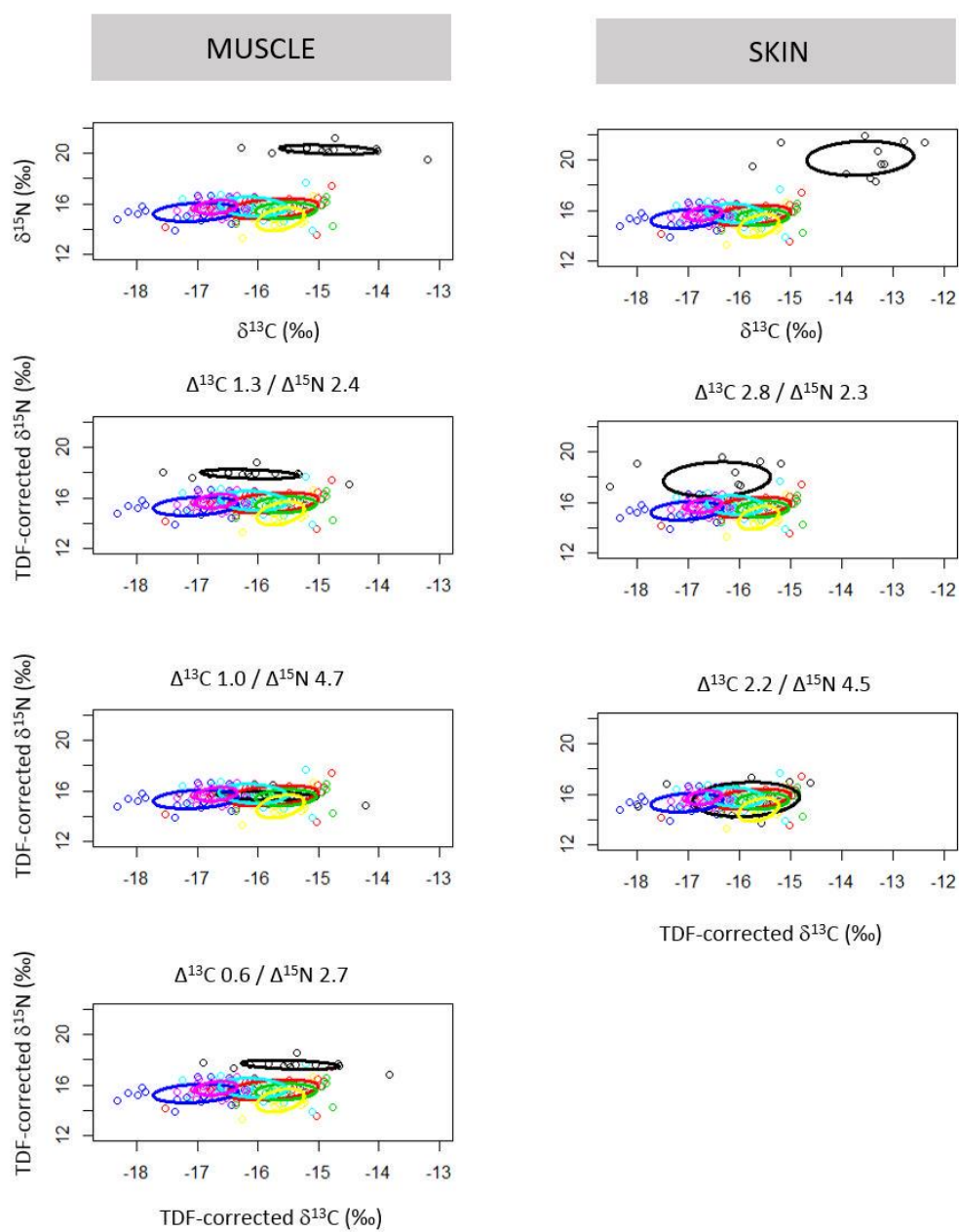
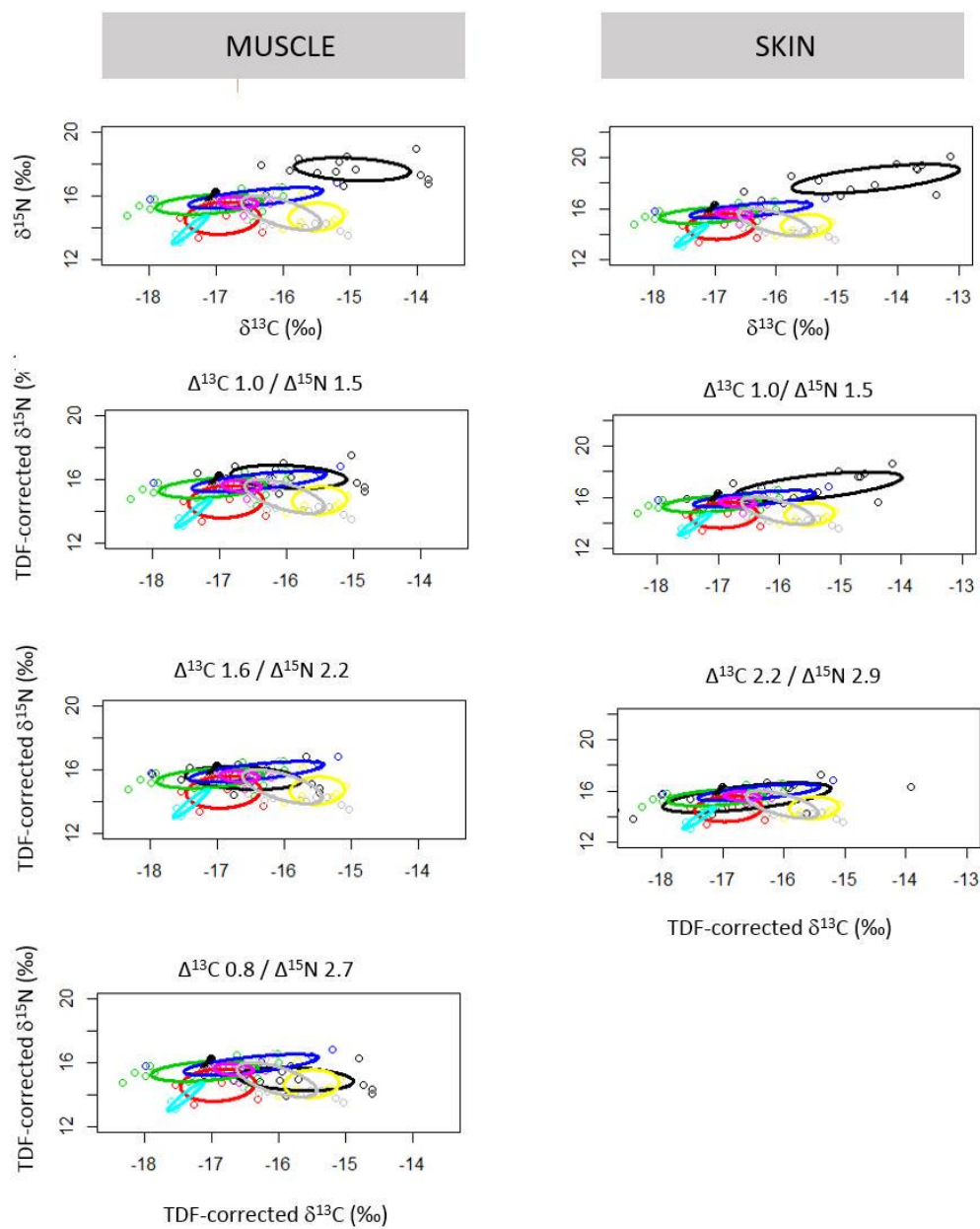


Figure 4



ANEXO II

Trophic relationships of key-species of marine mammals from a subtropical estuary and adjacent coastal areas in the western South Atlantic Ocean

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Abstract

The Patos lagoon estuary (PLE) (32° S) and its adjacent coastal area are mainly used by three species of marine mammals. The franciscana (*Pontoporia blainvillei*) inhabits shallow marine waters and usually fed upon of small teleosts and cephalopods. The bottlenose dolphin (*Tursiops truncatus*) and the South American sea lion (*Otaria flavescens*) inhabit the coastal region occupying both estuarine and adjacent coastal areas, consuming mainly teleost fishes. In this work, we used stable isotope analysis of carbon and nitrogen in skin of bottlenose dolphins, franciscanas and sea lions to assess their diet composition, resource partitioning and isotopic niche overlap. The isotopic mixing models showed a diversified diet for the South American sea lion and a diet with few main preys for the franciscana. On the other side, the bottlenose dolphin diet revealed by the mixing model showed a dominance of *Trichiurus lepturus*, with few contributions of other fish species. The sea lion showed the highest nitrogen isotopic values and the largest isotopic niche. On the other hand, bottlenose dolphins had the lowest nitrogen isotopic values, the smallest isotopic niche and had no niche overlap with the sea lion. The franciscana occupied an intermediate position, with partial niche overlap mainly with the bottlenose dolphin. This niche segregation and resource partition seem to allow the coexistence of these key-species of marine mammals in the PLE and coastal adjacent areas. The investigation of the feeding habits and trophic relationships of these important components of this coastal food web should aid management and conservation actions directed to the protection of this rich estuarine ecosystem.

Keywords: Stable isotopes, *Pontoporia blainvillei*, *Tursiops truncatus*, *Otaria flavescens*, diet composition, isotopic niche

1. Introduction

In the Patos Lagoon Estuary (PLE), located in southern Brazil (32° S), and its coastal adjacent waters, many species of coastal marine mammals coexist, making use of the abundant resources available in this system (Pinedo, 1998). The franciscana (*Pontoporia blainvillei*), an endemic species of the Southwestern Atlantic Ocean, is distributed in shallow waters up to 35 m deep (Danilewicz et al., 2009), having no records of their presence in the estuary at least since 1976 (Pinedo, 1998). The species usually fed upon small teleosts, cephalopods and crustaceans, the latter being especially important for calves (Basso, 2005). The bottlenose dolphin (*Tursiops truncatus*) and the sea lion (*Otaria flavescens*) are generalist high trophic level predators, which diets consist of a few dominant prey and several less abundant species (Barros and Wells, 1998; Szteren et al., 2006). At PLE, teleost fish are the main food source for both species, but the sea lion also includes elasmobranchs, cephalopods, crustaceans, and even discarded fisheries in their diet (Lopez, 2013). These two predators mainly inhabit the coastal region occupying both the estuarine area and adjacent coastal areas (Rosas et al., 1994; Di Tullio et al., 2015). However, bottlenose dolphins of the PLE occur in a narrow coastal zone of shallow waters with an average depth of 4 m (Di Tullio et al., 2015), whereas foraging records of sea lions indicate that the species also utilize continental shelf waters up to 100 m deep (Campagna et al., 2001; Rodríguez et al., 2013). The diet of the marine mammals of this region is also influenced by the seasonal abundance of some of their main prey, which occupy the area at different times of the year (Basso, 2005; Lopez, 2013; Secchi et al., 2016).

The sea lion appears to have a broader trophic niche than the dolphin, possibly due to the use of discarded fish (Szteren, 2006; Lopez, 2013), which gives them the

opportunity of obtaining prey that are not usually part of their diet. The franciscana, in turn, feeds on smaller and more varied preys, including cephalopods (Basso, 2005) and therefore is likely to occupy a different and lower food niche than the other predators.

The franciscana is classified as vulnerable in IUCN red list, due to anthropogenic interactions, especially incidental mortality in gillnets. In the region of the PLE, there is a high mortality rate of franciscanas dolphins associated with fisheries (Secchi & Wang, 2002). Moreover, the South American sea lion population located in coastal Uruguay, from which the individuals from the PLE area come from, has declined dramatically because the hunting. Therefore, continues decreasing at an annual rate of 1.7% being the competition with fisheries and resources reduction probably constitute the main reasons for the lack of recovery of this population (Riet-Sapirza et al., 2013).

Traditionally, studies of trophic relationships among marine vertebrates have been based on information obtained by stomach or feces content (e.g. Pauly et al., 1998). However, due to their recognized limitations, complementary methods (e.g. stable isotope analysis) are currently used in combination with traditional ones to provide greater robustness to ecological studies. The analysis of stable isotope ratios of carbon and nitrogen, commonly referred by the delta notation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) has been widely applied to trophic ecology studies of marine mammals (Newsome et al., 2010). The isotopic compositions of predators are directly related to those present in their diets (Peterson & Fry, 1987). However, predator tissues are typically enriched in heavier isotopes (^{13}C , ^{15}N) due to the isotopic discrimination of the lighter isotopes (^{12}C , ^{14}N) during metabolism (Ben-David & Flaherty, 2012). This difference between predator and prey isotopic composition is called Trophic Discrimination Factor (TDF) (Martinez del

Río et al., 2009) and is larger in the case of nitrogen than for carbon isotopes (DeNiro & Epstein, 1978; DeNiro & Epstein, 1981).

Baseline carbon isotope values result from combination of producers with different photosynthetic pathways (Fry & Sherr, 1984), thus, major aquatic habitat types have distinct $\delta^{13}\text{C}$ values (seagrass habitat > kelp forest > nearshore marine > offshore marine > freshwater, Fry & Sherr, 1984; Clementz & Koch, 2001; Michener & Kaufman, 2007). Consequently, while stable isotope ratios of nitrogen ($\delta^{15}\text{N}$) are commonly used to estimate the trophic position of a consumer in the food web, stable isotope ratios of carbon ($\delta^{13}\text{C}$) have the potential to provide information on assimilation of basal food sources, and thus can indicate its habitat use (Barros et al. 2010; Peterson & Fry, 1987; Post, 2002).

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of predators, together with those of their potential preys, can also be used to estimate the relative contribution of each prey to the predator diet by applying isotope mixing models (Parnell et al., 2010). These models, however, are highly sensitive to the values of TDF, thus the correct choice of these values is a key requirement when applying stable isotope mixing models to estimate the proportions of prey in the consumer's diet (Bond & Diamond, 2011; Olin et al., 2013).

The ecological niche is considered an n-dimensional space, which represents the main variables or resources for a species (Hutchinson, 1957). Some of these dimensions can be approached by analyzing the $\delta^{15}\text{N}$ values (trophic component) and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (spatial component) (Bearhop et al., 2004; Newsome et al., 2007). This isotopic niche can be represented in a multivariate space forming a δ -space with isotopic values as axes thus allowing for the indirect evaluation of the trophic overlap/segregation and

resource partitioning among predators (Newsome et al., 2007). Furthermore, the variance of isotopic values among individuals of the population and the resultant isotopic niche area can be used as a measure of niche width, and thus of the specialist/generalist nature of the species' diet (Bearhop et al., 2004).

In this study, we analyzed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in marine mammal skin samples, a tissue with mean turnover rates that provides information on diet assimilated from 1 to 2 months (Alves-Stanley and Worthy, 2009; Giménez et al., 2016). Using this stable isotope analysis approach, the objectives of the research were to (1) describe the food habits of bottlenose dolphins, franciscanas and South-American sea lions inhabiting a subtropical estuarine environment and its adjacent coastal regions, and (2) evaluate the extent of trophic niche overlap and resource partitioning among these high trophic level marine mammal species. The investigation of the trophic relationships of top predators is important for understanding the structure and trophic dynamics of coastal ecosystems and should aid management and conservation actions directed to the protection of these ecosystems.

2. Material and methods

2.1 Study area and sampling

Patos Lagoon estuary (PLE, $\sim 32^\circ \text{S}$) is located at the southern portion of Rio Grande do Sul state, Brazil. The adjacent coast of PLE is formed by extent sandy beaches, limited at the north by the Peixe lagoon bar (31°S) and at the south by the Chuí bar (33°S), forming thus approximately 350 km of monitored coast (Fig. 1). The region is considered an important nursery area with high productivity of various species of marine fishes and cephalopods (Seeliger et al., 2004).

Skin samples of franciscanas (males and females) and sea lions (males) were obtained from stranded carcasses found during systematic beach surveys between Peixe lagoon bar and Chuí bar (Fig. 1). Franciscanas incidentally caught in fish gillnets by the local fleet were also included. Bottlenose dolphin skin samples were obtained during boat surveys along the PLE and coastal adjacent areas, using a crossbow with tips especially adapted for biopsies of small cetaceans (Fruet et al., 2014). All samples were obtained during warm (November to April) and cold (May to October) months from 2012 to 2015. Only adult specimens were used in order to avoid biases resulting from ontogenetic diet variations. Therefore, franciscanas and sea lions were selected based on their total length (Danilewicz, 2003; Danilewicz et al., 2004, Grandi et al., 2010). Bottlenose dolphins were visually classified as juvenile/mature individuals during field sampling, based on their relative size and/or behavior (e.g. mother-calf pairs).

Muscle samples were obtained from the main prey of the two predators, selected from studies based on stomach contents data of these species (Basso, 2005; Lopez, 2013), summarizing around of 95% of the Index of Relative Importance (IRI). Individuals of fish and cephalopod species, were obtained from commercial fisheries that operate in the study area (coastal adjacent area), according to the size range consumed by each marine mammal species. Furthermore, prey were obtained in winter and summer months of 2014 and 2015, as a means of including the probable range of variation in isotope values in prey over the seasons. All samples were stored in -20°C freezer until processing for stable isotope analysis.

2.2. Stable isotope analysis

Marine mammal and prey samples were dried at 60° C for 48 h. To avoid biases in $\delta^{13}\text{C}$ values derived from the lipid content of the tissues, sea lion and franciscana samples were lipid extracted using a modified method from Bligh and Dyer (1959), through a *Soxhlet* extractor, with a 2:1 chloroform and methanol mixture. No lipid extraction in bottlenose dolphin samples (C:N = 3.6) was performed because their mean C:N ratio is near to theoretical pure protein (C:N < 3.5; Post et al. 2007). No lipid extraction was performed on prey muscle because mean C:N ratios from all samples were 3.43, with exception of *Mugil sp.* samples (mean C:N = 8.6), that had lipids extracted because of their high C:N values. All samples were dried at 60°C and powdered with a mortar and pestle. About 0.5-0.7 mg of each sample was weighed into tin capsules and analyzed with a mass spectrometer coupled to an elemental analyzer at the Stable Isotope Core, Washington State University. The isotopic compositions were reported in the conventional delta (δ) per mil notation (‰), relative to Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$) and atmospheric N_2 ($\delta^{15}\text{N}$). Internal standards of known carbon and nitrogen composition (acetanilide and keratin, respectively) were analyzed between samples in order to estimate instrument precision. The standard deviation of these internal standards were $\leq 0.1\text{‰}$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

2.3. Data analysis

2.3.1. Comparison among marine mammal tissues

Data are presented as mean $\pm$ standard deviation (SD), and significance was assumed at the 0.05 level. All statistical analyses were carried out within the R statistical environment v.3.3.1 (R Development Core team, 2016).

2.3.2. Diet composition

To determine relative contributions of different resources to the diet of each predator, we used MixSIAR, a Bayesian stable isotope mixing model that uses a mixed effects framework to estimate relative contributions of potential prey items to the consumer population (Stock and Semmens, 2013). Information obtained using conventional methods (e.g. stomach content analysis) were included as informative prior distributions in order to give more robustness to the model (Moore and Semmens, 2008). Published IRI values were calculated by prey group (i.e. cephalopods and fish) (Basso, 2005; Lopez, 2013; Secchi et al., 2016) due to the larger digestion time of cephalopod beaks (Sekiguchi and Best, 1997) therefore increasing the risk of an overestimation of the relative importance of this group. Thus, we used the frequency of occurrence of the preys, based on stomach content analysis of the predators (Basso, 2005; Lopez, 2013; Secchi et al., 2016) as informative priors. This procedure is especially useful while dealing with isotopically similar prey (Franco-Trecu et al., 2013). Species-specific prey-to-predator trophic discrimination factors (TDF) for the skin of each species were used in the model (Table 4). Each model was based on 500,000 iterations, thinned by 15 and with an initial discard of 50,000 iterations, resulting in 30,000 posterior draws of the posterior distribution.

2.3.3. *Isotopic niche and overlap*

The isotopic niche of each species were determined by the SIBER (Stable Isotope Bayesian Ellipses in R, Jackson et al., 2011) in SIAR package (Stable Isotope Analysis in R, Parnell et al., 2010). The method is based on the construction of standard ellipses that represent the isotopic niche of the species, based on individual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. We generated standard ellipse areas corrected for small sample sizes (SEAc) for sea lions, franciscanas and bottlenose dolphins. The isotopic overlap (i.e. measure of niche overlap)

among the three species was calculated (Jackson et al., 2011) and then the percentage of the total predator area represented by the overlap area was obtained for each pair of predators.

3. Results

Mean skin isotopic nitrogen values for sea lions were the highest among the three species, ranging from 18.3‰ to 21.9‰. In contrast, nitrogen isotopic values of bottlenose dolphins were the lowest, ranging from 15.6‰ to 20.3‰. The $\delta^{15}\text{N}$ values of franciscanas showed intermediate values, ranging from 16.7‰ to 20.1‰. The skin carbon isotopic values of sea lions varied between -15.7‰ and -12.4‰. Franciscana and bottlenose dolphin $\delta^{13}\text{C}$ values ranged from -16.5‰ to -11.7‰ and -15.8‰ and 13.3‰, respectively (Table 5).

MixSIAR model for sea lions showed a relative similar contribution of their main prey, although *P. brasiliensis* and *T. lepturus* showed the highest and lowest contribution, respectively. In the case of the franciscana, *Loligo sp.* and *C. guatucupa* seemed to be the most important prey in the diet of this species. For bottlenose dolphins, *T. lepturus* was the most important prey item, followed by *Mugil sp.* and *M. furnieri*. In general, proportions of preys found using the isotopic Bayesian mixing model were similar to those found using traditional methods (e.g. stomach contents) (Fig. 2).

The isotopic MixSIAR model showed differentiate main contributions of preys for each predator. *Paralichthys brasiliensis* and *Micropogonias furnieri* were more important for sea lion than for another predators. *Cynoscion guatucupa*, *Macrodon atricauda* and *Urophycis brasiliensis* also showed a considerable contribution to its diet, demonstrating the less selective diet of this predator. In the case of *Trichiurus lepturus*, they had the highest importance in bottlenose dolphin diet, and low contribution to

another predators. In contrast, *Anchoa marinii*, *Loligo sp.* and *Cynoscion guatucupa* mainly contributed for franciscana diet, despite of the latter also has important contribution to sea lion diet (Fig. 2).

Bottlenose dolphins had the smallest ellipse area ($SEAc = 1.34\text{‰}^2$), while sea lions ellipse had the largest isotopic niche area ($SEAc = 4.44\text{‰}^2$). The isotopic niche area of the franciscana was larger than that of the bottlenose dolphin but smaller than sea lion's ($SEAc = 3.89\text{‰}^2$). There was no overlap between bottlenose dolphin and sea lion ellipses. Nevertheless, bottlenose dolphin and franciscana ellipses overlapped in 0.79‰^2 , representing 59% and 20.3% of each ellipse area, respectively. The ellipse area overlap between franciscana and sea lion was 0.72‰^2 , which represents 18.5% and 16.2% of franciscana and sea lion ellipse areas, respectively (Fig. 3).

4. Discussion

The present study assessed the diet composition and resource partitioning of three species of marine mammals that inhabit the Patos Lagoon Estuary and its adjacent coastal waters. By using published stomach content data (Basso, 2005; Lopez, 2013; Secchi et al., 2016) and carbon and nitrogen stable isotope analysis of skin samples from franciscanas, sea lions and bottlenose dolphins, we showed a complete segregation in the isotopic space between the bottlenose dolphin and the sea lion, and some degree of resource partitioning between these two and the franciscana.

The assimilation of prey by the sea lion inferred from Bayesian mixing models was similar to that observed in Lopez (2013) using stomach content data (Figures 2a and 2b). Both methods showed that *Paralichthys brasiliensis* was the most important prey in sea lion diet, besides some difference in its relative proportion with respect to the other prey items consumed. This fish is usually discarded by fishermen because of its low

commercial value and small size (Haimovici et al., 2005) and sea lions take advantage of this food source. Furthermore, operational interactions between the sea lions and fisheries are common along its distribution (Sepúlveda et al., 2007; Rodríguez et al., 2013; Machado et al., 2016) and this include the consumption of fish trapped in gillnets and the entanglement and/or intentional kill of some specimens (Rosas et al., 1994; Szteren and Páez, 2002). Indeed, fishes of commercial value are also preyed by sea lions in the area, including *Micropogonias furnieri*, *Cynoscion guatucupa* and *Macrodon atricauda* (Chao et al., 2015). Thus, potential ecological interactions might also take place through a resource competition between local fisheries and these large predators (Szteren et al., 2004).

Franciscana dolphins consumed mainly *Cynoscion guatucupa* and *Loligo sp.*, according to the stable isotope data and stomach content analyses (Figures 2c and 2e), despite a lower contribution of squid indicated by the isotope mixing model. The high frequency of squid beaks recovery from stomach contents is usually related to the lower digestion rates of these structures compared to that of fish otoliths (Sekiguchi and Best, 1997; Tollit et al., 1997). Therefore, the difference found between methods in the relative contribution of cephalopods could be due to the overestimation of its importance to diet by using conventional methods (i.e. stomach or scat contents) (Troina et al., 2016).

A high contribution of demersal-pelagic fishes, such as *Cynoscion guatucupa*, for franciscanas was revealed by MixSIAR models. Previous studies based on traditional methods also reported the importance of both demersal-pelagic and demersal prey in its diet (Rodríguez et al., 2002; Bassoi, 2005; Paso-Viola et al., 2014; Franco-Trecu et al., 2017). Results of our stable isotope analysis indicate a slight higher contribution of *Anchoa mitchilli* with respect to the importance reported from stomach content analysis

by Bassoi (2005). This was probably due to the recognized seasonality in the diet composition of the franciscanas in their southern distribution (Bassoi, 2005; Paso-Viola et al., 2014, Franco-Trecu et al., 2017). During cold months a higher abundance of spawning engraulids occurs near freshwater discharges (e.g. Patos lagoon and Río de la Plata estuaries) (Lima and Castello, 1995; Jaureguizar et al., 2002) becoming more available to dolphins. Indeed, a higher number of franciscanas analyzed here were collected during cold months (64%) which could explain the difference found between both methods.

The relative importance of prey obtained from both methods for bottlenose dolphins, identified *Trichiurus lepturus* as the predominant prey item in the case of the MixSIAR and a more evenly distributed diet, with *Micropogonias furnieri* as the most important prey in the case of the stomach content analysis (Secchi et al., 2016) (Fig. 2f). Although an increasing relative importance of *T. lepturus* and a decreasing trend in the contribution of *M. furnieri* was detected in the last decade (Secchi et al., 2016) the dominance of former in our results could also be influenced by the TDF used while running the MixSIAR models. Alternative TDF values (e.g. Browning et al., 2014) should be tested in order to find more realistic results in the case of this predator before further interpretations could be done.,

When comparing species' position in the isotopic space the isotopic niche of the sea lion clearly differed from the one of the dolphins. Furthermore, the sea lion showed a high $\delta^{15}\text{N}$ position in the isotopic space (i.e. high trophic level) and had the largest ellipse area among the three species. This pattern could be explained by the larger body size and mouth diameter of this predator conferring him probably greater prey catch and handle capacity (Weise et al., 2010; Saporiti et al., 2016). On the other side, male sea lions make

important post-reproductive movements from Uruguayan rookeries, over the continental shelf, to southern Brazilian coasts in cold months (Rosas et al., 1994). The large habitat used by the sea lion maybe allowed these individuals to assess preys with different isotopic composition, therefore, producing a large isotopic space (Franco-Trecu et al., 2013; Zenteno et al., 2014).

Following the same rationale of body size and predation capacity, we expected a large overlap in the isotopic area between the bottlenose dolphin and the sea lion. However, a complete segregation in the isotopic space was found between these predators, despite the fact that they share some dominant preys even of the same body size interval (Lopez, 2013; Secchi et al., 2016). The position of the bottlenose dolphin ellipse towards more depleted (i.e. lower $\delta^{13}\text{C}$ values) carbon isotope values could be explained by the consumption of more demersal-pelagic preys compared to sea lions (e.g. *Trichiurus lepturus*, Table 3). Due to the known higher carbon isotope values found in benthic compared to pelagic food sources (France, 1995), this difference could be transferred up the food web resulting in pelagic consumers with lower $\delta^{13}\text{C}$ values. On the other hand, bottlenose dolphin nitrogen isotopic values could have been influenced by preys with low trophic position (e.g. the mullet, *Mugil sp.*, which has illiophagus habits), that is only consumed by this predator and seems to be ignored by the sea lion. However, our results did not indicate a high proportion of this item in their diet in spite of Secchi et al. (2016) having reported *Mugil sp.* as an important prey mainly for female bottlenose dolphins inhabiting PLE. Alternatively, as stated before, the chosen TDF value used in MixSIAR models could have underestimate the contribution of this fish to the diet of the dolphins, thus providing contradictory results. Another interpretation for the complete segregation and higher nitrogen isotope values found for the sea lions in

comparison to those of the bottlenose dolphins could be the larger foraging ground experimented by the pinnipeds that could include variations in the isotopic baselines within these regions (Zenteno et al., 2014; Franco-Trecu et al, 2017). Mean prey isotopic values reported by Zenteno et al. (2014) obtained from samples collected in southern Brazil and northern Argentina differed in approximately $\sim 1\text{‰}$ for both carbon and nitrogen isotope values, being the latter more depleted in ^{13}C and enriched in ^{15}N . Although this difference seems not to be highly significant it could have made a contribution to the extremely high nitrogen isotopic values in comparison with their preys (Tables 1 and 5) showed by the sea lions, resulting in surprisingly large TDF values (see Appendix I).

With respect to the size of the isotopic niche, the smaller ellipse area of the bottlenose dolphins may be linked to lower prey variation found for this species, showing a high dominance of *T. lepturus*. The high variation in stable isotope carbon ratios in the franciscana could be explained by the high variability of prey groups including the consumption of demersal-pelagic, pelagic and demersal fishes and squids (Haimovici et al., 1996; Bassoi, 2005; Troina et al., 2016). Sample size of bottlenose dolphins was significantly larger than those of the other two species. Jackson et al. (2011) highlighted that when $n \rightarrow \infty$, the niche area tends to converge to the real value of the population ellipse. On the other hand, low sample sizes (e.g. franciscanas and sea-lions) could have resulted in the inclusion of odd isotopic values for the calculation of the ellipse area, being less representative, and may be larger, than the real niche area of the population.

Due to its considerable smaller size and, consequently, smaller prey size, we expected the franciscana to be in the lowest trophic position. However, its ellipse was placed between that of the sea lion and the bottlenose dolphin (Fig. 3). The franciscana

isotopic niche showed an important overlap with that of the bottlenose dolphin and a small overlap with the sea lion. Although franciscanas and bottlenose dolphins share some prey (Fig. 3), the relative importance of them in their diets was different between these predators (Basso, 2005; Lopez, 2013; Franco-Trecu et al., 2017). This reinforces the importance of complementing the approach given by the construction of the isotopic niche ellipses with the information provided by a detailed description and comparison of the prey items consumed by the predators (Bearhop et al., 2004; Newsome et al., 2010; Franco-Trecu et al., 2017). The association of these methods is especially important whenever dealing with isotopically similar prey species that could result in a high overlap in the isotopic niche area of the predators that are consuming them, even when they show different diets.

5. Conclusions

This study provides information of niche differentiation and resource partitioning among the three main marine mammal species inhabiting the Patos lagoon estuary and its adjacent marine area. The approach used combining traditional methods (i.e. stomach content analysis) with stable isotope analysis allowed a more refined view of their diet composition and feeding relationships. We showed that, although the sea lion and the bottlenose dolphins share some prey, their main preference is towards distinct fish species, probably due to their different foraging behavior. Indeed, preying upon discarded fish by local fisheries is common for sea lions, whereas bottlenose dolphins seem not to present this trophic behavior. Furthermore, bottlenose dolphins prefer coastal shallow waters, including sheltered estuarine areas, while franciscanas use coastal areas up to 35m depth and do not enter the estuary. This niche segregation and resource partition seem to allow the coexistence of these key-species of marine mammals in the PLE and coastal

adjacent areas. The investigation of the feeding habits and trophic relationships of these important components of this coastal food web should aid management and conservation actions directed to the protection of this rich estuarine ecosystem.

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Tables and figures

Table 1

Prey data used to estimate the diet composition based on stable isotope analysis. Published frequency of occurrence (%FO) of prey for adult male South American sea lions (*Otaria flavescens*) from southern Brazil were used as informative priors in the MixSIAR model. Information on the ecological group (EG) of each prey was also included. D = demersal, DP = demersal-pelagic.

Prey species	n	%FO ^a	TL ± SD (cm)	δ ¹³ C ± SD (‰)	δ ¹⁵ N ± SD (‰)	EG
<i>P. brasiliensis</i>	24	82.9	18.3±2.5	-15.8±0.7	15.6±0.8	D
<i>M. furnieri</i>	17	45.7	26.7±2.1	-15.5±0.5	15.5±0.6	D
<i>M. atricauda</i>	27	48.6	19.5±3.2	-16.1±0.6	15.8±0.8	D
<i>C. guatucupa</i>	25	31.4	18.1±3.8	-16.7±0.3	15.7±0.5	DP
<i>T. lepturus</i>	21	28.6	65.4±11.6	-17.0±0.7	15.3±0.7	DP
<i>U. brasiliensis</i>	12	45.7	24.1±5.2	-15.6±0.4	14.8±0.9	D

^aLopez (2013)

Table 2

Prey data used to estimate the diet composition based on stable isotope analysis. Published frequency of occurrence (%FO) of prey for adult franciscanas (*Pontoporia blainvillei*) from southern Brazil were used as informative priors in the MixSIAR model. Information on the ecological group (EG) of each prey was also included. D = demersal, DP = demersal-pelagic, P = pelagic.

Prey species	n	%FO ^a	TL ± SD (cm)	δ ¹³ C ± SD (‰)	δ ¹⁵ N ± SD (‰)	EG
<i>C. guatucupa</i>	25	65.0	17.4±4.1	-16.7±0.3	15.6±0.3	DP
<i>T. lepturus</i>	16	37.0	64.1±14.9	-17.1±0.8	15.5±0.6	DP
<i>U. brasiliensis</i>	6	28.0	21.3±2.3	-15.5±0.4	14.7±0.8	D
<i>P. brasiliensis</i>	33	34.0	15.2±5.1	-16.0±0.6	14.9±1.0	D
<i>Loligo sp.</i>	19	82.0	7.2±1.9	-16.8±0.4	15.2±1.3	P
<i>A. marinii</i>	4	28.0	7.75±0.13	-17.1±0.1	16.1±0.2	P
<i>P. porosissimus</i>	5	21.0	19.5±1.5	-17.4±0.2	13.9±0.8	D
<i>U. canosai</i>	7	13.0	18.3±3.1	-16.4±0.9	15.9±0.6	D

^aBasso (2005)

Table 3

Prey data used to estimate the diet composition based on stable isotope analysis. Published frequency of occurrence (%FO) for adult bottlenose dolphins (*Tursiops truncatus*) from southern Brazil were used as informative priors in the MixSIAR model. Information on the ecological group (EG) of each prey was also included. D = demersal, DP = demersal-pelagic.

Prey species	n	%FO ^{a,b}	TL ± SD (cm)	δ ¹³ C ± SD (‰)	δ ¹⁵ N ± SD (‰)	EG
<i>Menticirrhus</i> sp.	26	35.4	23.8±2.1	-15.5±0.6	15.8±0.5	D
<i>M. furnieri</i>	17	54.8	26.7±2.1	-15.5±0.6	15.5±0.6	D
<i>Mugil</i> sp.	10	32.2	38.6±5.1	-14.3±1.2	11.4±1.6	DP
<i>P. brasiliensis</i>	24	32.2	18.3±2.5	-15.8±0.7	15.6±0.8	D
<i>T. lepturus</i>	21	29.0	65.4±11.6	-17.0±0.7	15.3±0.7	DP

^aLopez (2013)

^bSecchi et al. (2016)

Table 4

Trophic Discrimination Factors (TDF) for *Otaria flavescens*, *Pontoporia blainvillei* and *Tursiops truncatus* used for estimating diet composition through MixSIAR models. SD values are shown after mean TDF values whenever available.

Species	Tissue	Δ ¹³ C (‰)	Δ ¹⁵ N (‰)
<i>O. flavescens</i>	Skin	2.2 ± 1.0 ^a	4.5 ± 1.3 ^a
<i>P. blainvillei</i>	Skin	2.2 ± 1.3 ^a	2.9 ± 1.1 ^a
<i>T. truncatus</i>	Skin	2.2 ± 1.3 ^a	2.9 ± 1.1 ^a

^a Wiegand et al. (Appendix I)

Table 5

Stable carbon (δ¹³C) and nitrogen (δ¹⁵N) isotope mean values (±SD) in skin and muscle samples of *Otaria flavescens*, *Pontoporia blainvillei* and *Tursiops truncatus* from Rio Grande do Sul, Brazil.

Species	Tissue	n	δ ¹³ C ± SD (‰)	δ ¹⁵ N ± SD (‰)
<i>Otaria flavescens</i>	Skin	11	-13.6 ± 1.0	20.1 ± 1.3
<i>Pontoporia blainvillei</i>	Skin	14	-13.6 ± 1.5	18.3 ± 1.1
<i>Tursiops truncatus</i>	Skin	75	-14.7 ± 0.5	17.4 ± 0.8

Figure legends

Figure 1. Study area located between Chuí bar (33° 45 'S) and the Peixe lagoon bar (31° 21 'S), southern Rio Grande do Sul state, Brazil.

Figure 2. Relative contribution of prey sources to the diet South American sea lion, *Otaria flavescens* (a), Franciscana dolphin, *Pontoporia blainvillei* (c), and bottlenose dolphin, *Tursiops truncatus* (e). The proportion of each fish species is shown as box plots showing the 50%, 75% and 95% credibility intervals. On the right, a comparison of frequencies of occurrence (light gray bars) and the 95% credibility interval determined by MixSIAR model (black bars) for the three predators (b, d and f, respectively).

Figure 3. Isotopic niche areas of the South American sea lion, *Otaria flavescens* (green), franciscana dolphins, *Pontoporia blainvillei* (red), and bottlenose dolphins, *Tursiops truncatus* (black), from the Patos lagoon estuary and adjacencies, southern Brazil.

Figure 1

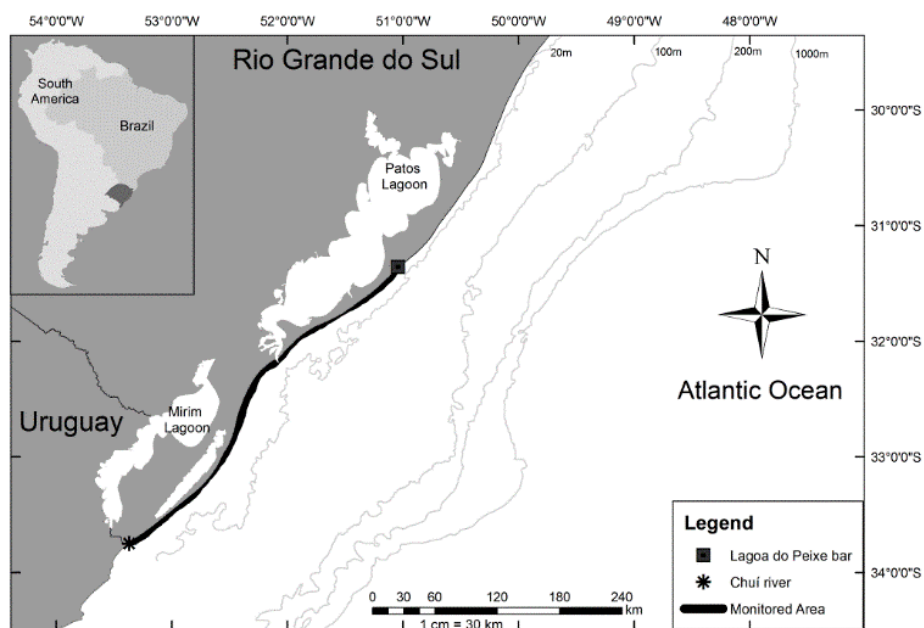


Figure 2

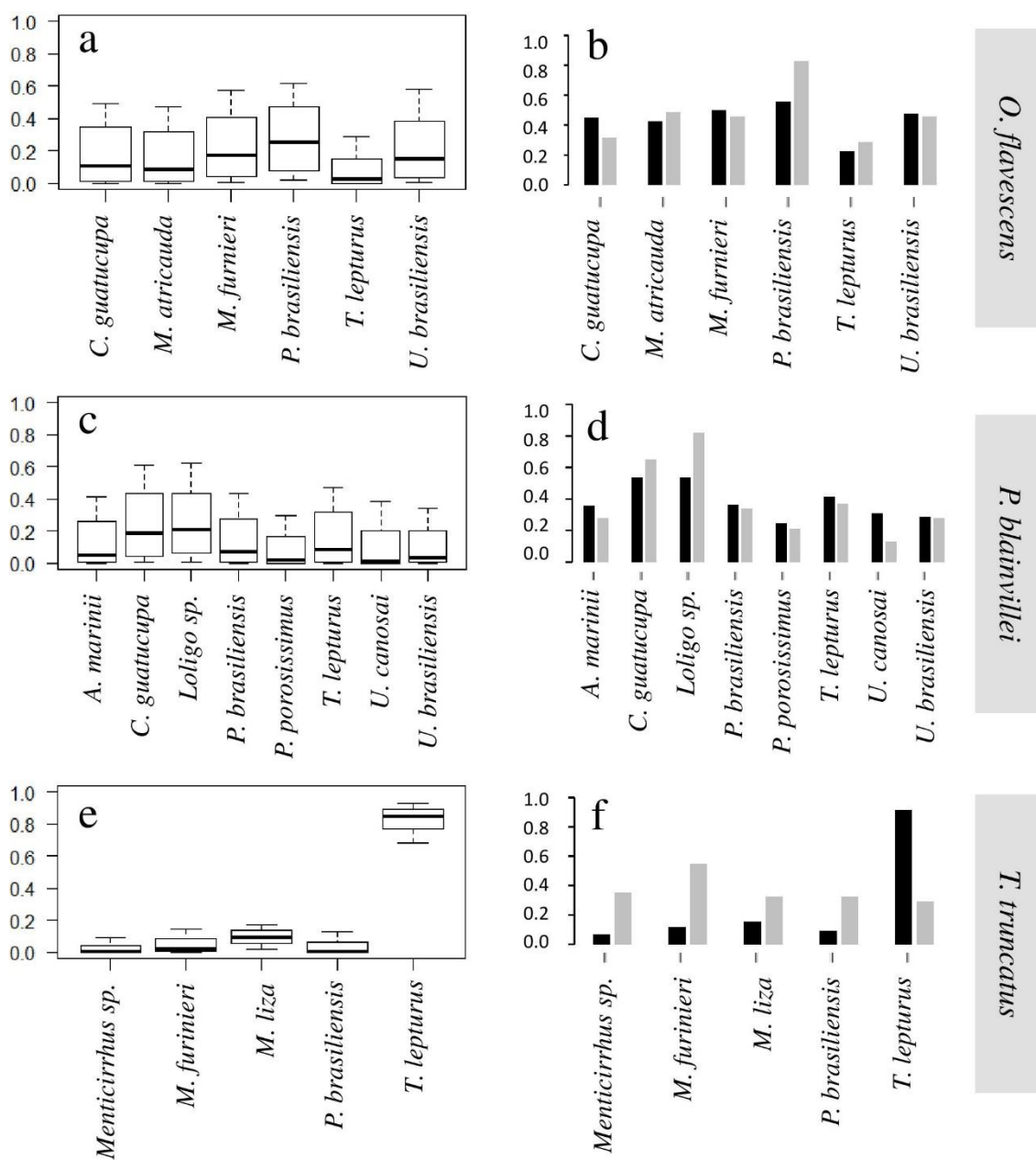


Figure 3

