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ECOLOGIA TRÓFICA DO PINGUIM-DE-MAGALHÃES (*Spheniscus magellanicus*) NO SUL DO BRASIL

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SUMÁRIO

RESUMO

O pinguim-de-Magalhães (*Spheniscus magellanicus*) migra para latitudes menores durante o período não reprodutivo, ocorrendo em áreas de grande produtividade no sul do Brasil. No inverno, a plataforma continental interna do Rio Grande do Sul, sul do Brasil, é influenciada pela Frente Subtropical de Plataforma e pela descarga do Rio da Prata e do estuário da Lagoa dos Patos, tornando-se uma importante área para a pesca e para o desenvolvimento de espécies-chave como a anchoita (*Engraulis anchoita*). A anchoita é o principal alimento do pinguim-de-Magalhães nas colônias ao norte da Argentina e supostamente também nas áreas de invernagem, embora isso não tenha sido demonstrado. Neste contexto, o objetivo deste estudo foi verificar a importância da anchoita na alimentação de pinguins juvenis e adultos durante o ciclo anual. Para determinar a ecologia trófica do pinguim-de-Magalhães, foram coletados indivíduos na praia (7 adultos e 27 juvenis) e no mar (18 adultos e 2 juvenis), provenientes da captura incidental na pesca de emalhe. Pinguins coletados no mar apresentaram índice de massa corporal maior (portanto eram indivíduos saudáveis), do que indivíduos encontrados na praia. A partir da análise de conteúdos estomacais, os indivíduos adultos (*n* = 21) tiveram peixes como principal alimento (PSIRI = 86%), e a anchoita como o principal item alimentar. Os juvenis tiveram os cefalópodes como principal presa (PSIRI = 71%), com *Doryteuthis sanpaulensis* como a mais importante. Apesar da grande importância de cefalópodes na dieta de juvenis, análises de isótopos estáveis de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$) de fígado, músculo e penas, mostraram relevante contribuição da anchoita na dieta dos indivíduos estudados (95% intervalo de credibilidade – CI = 46,0 – 98,6%), assim como dos adultos (39,8 – 98,9%). Adultos e juvenis apresentaram sobreposição de nichos isotópicos nos tecidos analisados, apesar das diferentes amplitudes entre as classes etárias. Os resultados desse estudo ressaltam a importância da utilização de técnicas complementares e a necessidade de utilizar-se de indivíduos saudáveis para inferências adequadas sobre a ecologia trófica. A grande dependência da anchoita, como recurso chave pelos pinguins, demonstra que os predadores no topo da cadeia trófica precisam ser considerados em eventuais explorações comerciais deste recurso pesqueiro.

Palavras-chave: captura incidental, conteúdo estomacal, dieta, *Engraulis anchoita*, isótopos estáveis, período não reprodutivo.

ABSTRACT

Magellanic penguins (*Spheniscus magellanicus*) migrate to lower latitudes during the wintering period, reaching high productive areas in southern Brazil. In winter, the inner shelf along the coast of Rio Grande do Sul state, southern Brazil, is influenced by the Subtropical Shelf Front and by La Plata River and Patos Lagoon discharges, becoming an important fishing area with occurrence of keystone species, such as the Argentine anchovy (*Engraulis anchoita*). The Argentine anchovy is the main food item of Magellanic penguins at northern colonies in Argentina and supposed to be also important, despite not yet demonstrated, in wintering grounds. In this context, this study aims to investigate the importance of a keystone species, the Argentine anchovy, in the diet of Magellanic penguins of different age classes, and throughout the annual cycle of the species. A total of 7 adults and 27 juveniles were collected stranded dead on the beach, and 18 adults and 2 juveniles at sea obtained from gillnet bycatch, to determine the trophic ecology of Magellanic penguins. Penguins collected at sea had higher body index in relation to individuals found on the beach, demonstrating to be healthy individuals. Among stranded and bycaught individuals, fish and the Argentine anchovy, in particular, were the main food items in stomach contents of adults (n = 21; PSIRI = 86%). On the other hand, juveniles had cephalopods as the main prey item (PSIRI = 71%), with predominance of *Doryteuthis sanpaulensis*. Despite the high importance of cephalopods in the diet of juveniles, δ^{13}C and δ^{15}N values in liver, muscle and feather, showed relevant contribution of Argentine anchovy to the diet of juveniles (95% credibility interval = 46.0 – 98.6%) and adults (39.8 – 98.9%).

Adults and juveniles showed isotopic niche overlap in all tissues analyzed, regardless of differences in the niche width areas between age classes. Our results highlight the importance of using complementary techniques for studying trophic ecology of marine vertebrates, since stomach content analysis of individuals found dead on beaches may not provide reliable information on the diet. The strong reliance of penguins on the Argentine anchovy as a key resource demonstrates that top predators should be considered in eventual commercial exploitation of this fishing resource.

Key-words: bycatch, diet, *Engraulis anchoita*, stable isotopes, stomach content, wintering period.

INTRODUÇÃO GERAL

O pinguim-de-Magalhães *Spheniscus magellanicus* (Forster, 1781), possui uma população global estimada entre 1,1 e 1,6 milhões de pares reprodutivos (BirdLife International, 2016). Nidificam em densas colônias no sul da América do Sul, tendo como limite norte na costa Atlântica a Península Valdés e ao sul, a Ilha Martillo, no Canal de Beagle, Argentina. Reproduzem-se também em ilhas *offshore*, como na Ilha dos Estados e nas Ilhas Malvinas/Falkland (Scolaro *et al.*, 1980; Gandini *et al.*, 1996) (Anexo – Fig. 1). Sua população apresenta flutuação em diferentes partes de sua distribuição, com tendências de declínio, sendo assim classificada internacionalmente na categoria de espécie “Quase ameaçada” (*Near Threatened* – NT) (BirdLife International, 2016). Suas principais ameaças são especialmente a poluição por petróleo (Gandini *et al.*, 1994; 1996), predação de ovos e filhotes (Frere *et al.*, 1992), captura incidental em artefatos de pesca (Gandini e Frere, 1999; Cardoso *et al.*, 2011), sobrepesca de seus principais recursos alimentares (Gandini *et al.*, 1996), além dos impactos do turismo nas colônias (Yorio e Boersma, 1992).

Os filhotes de pinguins-de-Magalhães adquirem a plumagem durante a fase final da reprodução, ainda no ninho. Já os adultos (Fig. 1), após o período reprodutivo deslocam-se de suas colônias e passam de 3 a 6 semanas forrageando e adquirindo gordura antes de realizar a muda total das penas, em terra (Boersma *et al*., 1990; Wilson *et al.*, 2005). Após a muda migram sobre a plataforma continental para menores latitudes, atingindo o norte da Argentina, Uruguai e sul do Brasil (Stokes *et al.*, 1998; García-Borboroglu *et al.*, 2010) (Anexo – Fig. 1). Durante o período não reprodutivo, que vai de março a setembro, tem migração relacionada à produtividade e disponibilidade de alimento, como a migração de cardumes de peixes (Stokes *et al.*, 1998; Pütz *et al.*, 2000, 2007). A anchoita (*Engraulis anchoita*) é considerada o principal item alimentar dos pinguins-de-Magalhães durante o período reprodutivo, nas colônias do norte da Argentina (Gosztonyi, 1984; Frere *et al.*, 1996).

Fig. 1. Pinguim-de-Magalhães (*Spheniscus magellanicus*) adulto (A) e juvenil (B) que ocorrem no litoral do Rio Grande do Sul, Brasil.

A região de ocorrência do pinguim-de-Magalhães no Brasil é também uma das mais importantes áreas de pesca da costa brasileira (Vasconcellos *et al.,* 2014), especialmente pelo desenvolvimento de espécies-chave para o ecossistema, como a anchoita (Acha *et al.*, 2004; Costa *et al.*, 2016). Este pequeno peixe habita a plataforma continental da América do Sul entre 23°S e

47°S (Lima e Castello, 1995). No Brasil, estudos recentes estimaram a biomassa de anchoita entre 72.000 e 814.000 toneladas (Costa *et al*., 2016). A anchoita é considerada um importante componente no fluxo de energia do ecossistema local, como base de alimentação para outros estoques pesqueiros (Lima e Castello, 1995; Costa *et al*., 2016), como o da anchova (*Pomatomus saltatrix*) e da pescada (*Cynoscion guatucupa*) (Lucena *et al.*, 2000).

A cada ano, nos meses de inverno e primavera, estima-se que 19.000 pinguins-de-Magalhães sejam encontrados mortos nas praias do Rio Grande do Sul (RS), 97,5% dos quais são juvenis (Mäder *et al.*, 2010) (Fig. 2). Apesar da sua dieta variar latitudinalmente, a espécie é considerada oportunista e pouco seletiva quanto aos requerimentos alimentares, com predomínio de peixes em relação a lulas (Frere *et al.*, 1996). No entanto, diversos estudos com conteúdos estomacais, provenientes de pinguins juvenis debilitados e encontrados mortos ao longo do litoral brasileiro, apresentaram cefalópodes como principal item alimentar (Fonseca *et al.*, 2001; Pinto *et al.*, 2007; Di Beneditto *et al.*, 2015). A variação na exploração dos recursos pelas aves marinhas pode ser influenciada pela idade e pela experiência dos indivíduos (Hodum e Hobson, 2000; Mäder *et al.*, 2010) (Fig. 2). Em colônias da espécie na Patagônia argentina, diferenças entre classes etárias na utilização de recursos alimentares já foram observadas, com adultos consumindo presas de maior valor energético, em comparação aos juvenis (Forero *et al.*, 2002). Porém, o uso de indivíduos encontrados mortos nas praias, supostamente após período de prolongado jejum, podem não refletir a real alimentação da espécie em condições normais.

Fig. 2. Pinguim-de-Magalhães (*Spheniscus magellancius*) juvenil, encontrado debilitado no litoral do Rio Grande do Sul.

Os recursos alimentares utilizados por aves marinhas podem ser estudados de formas variadas, e um dos métodos mais comuns é a análise de conteúdos estomacais de animais encontrados mortos (Barret *et al.*, 2007). Apesar desta técnica permitir identificar as presas ingeridas, a partir de estruturas não digeridas, é limitante quanto à identificação de presas de rápida digestão e apenas fornece informações sobre o alimento ingerido recentemente (van Heezik e Seddon, 1989; Barret *et al.*, 2007).

A análise de isótopos estáveis (AIE) complementa a análise de conteúdo estomacal, indicando recursos efetivamente assimilados nos tecidos do consumidor (Barret *et al.*, 2007). Os isótopos estáveis mais utilizados para inferir a composição da dieta assimilada nos tecidos dos consumidores são os de carbono ($\delta^{13}C$) e de nitrogênio ($\delta^{15}N$) (Cherel *et al*., 2005). Os valores de

$\delta^{13}C$ refletem os locais de alimentação (costeiro vs. oceânico), variando até 1‰ a cada nível trófico, enquanto os valores de $\delta^{15}N$ aumentam de 2 a 5‰ a cada nível trófico (DeNiro e Epstein, 1978; Hobson e Clark, 1992). Apesar da confiabilidade na utilização do método, deve-se levar em conta os processos fisiológicos dos indivíduos estudados, já que valores de $\delta^{15}N$ podem ser enriquecidos devido ao estresse nutricional ou jejum prolongado (Hobson *et al*., 1993). As taxas de renovação (*turnover*) dos isótopos estáveis nos tecidos variam de acordo com a atividade metabólica dos mesmos (Tieszen *et al.,* 1983). Tecidos com alta taxa de renovação, como o fígado e o músculo, variam de dias a semanas, respectivamente (Hobson e Sealy, 1991; Hobson e Clark, 1992), enquanto tecidos metabolicamente inertes como as penas, mantém os valores isotópicos do momento de sua síntese (Hobson, 1999; Cherel *et al*., 2005).

A partir da AIE, também é possível identificar diferenças intraespecíficas em áreas de alimentação e especialização da dieta, com a determinação do nicho isotópico de um subgrupo, por exemplo adultos *vs.* juvenis, machos v*s.* fêmeas (Newsome *et al.* 2007). Informações sobre os recursos e as áreas utilizadas, podem ser consideradas como dimensões para caracterização do nicho ecológico, já que este é definido como um hipervolume *n*-dimensional de condições e recursos (Hutchinson, 1957). Portanto, a análise de $\delta^{13}C$ e de $\delta^{15}N$ pode ser utilizada como um *proxy* do nicho trófico e/ou espacial (Newsome *et al.* 2007), além de fornecer um melhor entendimento do nicho quando usado em combinação com técnicas complementares, como a análise da dieta (Bearhop *et al*., 2004).

OBJETIVOS

Neste contexto, o objetivo geral do presente estudo é analisar a ecologia trófica do pinguim-de-Magalhães, com propósito de: (1) verificar a importância da anchoita na alimentação de pinguins juvenis e adultos, (2) comparar o nicho isotópico de indivíduos adultos e juvenis ao longo de seu ciclo anual, e (3) avaliar a condição corporal de indivíduos mortos na praia e capturados incidentalmente, como uma indicação das limitações do uso destes indivíduos para inferências sobre a dieta.

HIPÓTESES

Hipotetizou-se que (*i*) pinguins adultos apresentarão dieta e valores de $\delta^{13}C$ e $\delta^{15}N$ característicos de alimentação especializada em anchoita, que (*ii*) indivíduos juvenis mortos nas praias apresentarão nichos isotópicos mais amplos em comparação aos adultos no mar, por ingerirem maior proporção de cefalópodes e catabolizarem seus tecidos devido ao jejum prolongado anterior ao óbito, e que (*iii*) a análise do conteúdo estomacal de animais encontrados mortos nas praias, após período de debilidade, não fornecem um cenário adequado sobre a dieta dos indivíduos e da espécie.

MATERIAIS E MÉTODOS

Área de estudo

Para o presente estudo, pinguins-de-Magalhães foram coletados tanto na praia quanto em alto mar, no estado do Rio Grande do Sul, extremo sul do Brasil. Os indivíduos coletados no litoral foram encontrados encalhados mortos entre os municípios de Mostardas/Tavares (31°20'S; 51°05'W) e Chuí (33°45'S; 53°22'W) (Anexo – Fig. 1). Os pinguins coletados em alto mar foram resultantes de captura incidental na pesca de emalhe de fundo e de superfície. A média ± 1 desvio padrão do comprimento das redes foram de 1,23 ± 0,6 km para o emalhe de superfície e de 110,6 ± 4 km para o emalhe de fundo. Esses indivíduos foram capturados a uma profundidade de 14 a 21,9 m, entre 32°–33°S e 51°–53°W (Fogliarini *et al.*, em revisão).

Durante o inverno os padrões oceanográficos da plataforma continental no sul do Brasil são caracterizados pelas Águas Subantártica de Plataforma (SAWS, originada a partir da Corrente das Falkland/Malvinas) e Subtropical de Plataforma (STWS, influenciada pela Corrente do Brasil e composta pelas Águas Tropicais e Costeiras), formando a Frente Subtropical de Plataforma (STSF) (Anexo - Fig. 1). A STSF é uma zona de transição com gradiente termohalino latitudinal e alta produtividade entre as latitudes de 33°S e 36°S (Möller *et al.*, 2008; Piola *et al.*, 2008). A alta produtividade é influenciada diretamente pelas águas frias vindas da SAWS, com a baixa salinidade gerada pela descarga do Rio da Prata (RPP) e do estuário da Lagoa dos Patos (PL) (Piola *et al.*, 2008; Costa *et al.*, 2016).

Coleta de amostras

Durante o ano de 2015 foram coletados 34 pinguins-de-Magalhães no litoral sul do RS, dos quais 7 eram adultos e 27 juvenis, classificados com base na plumagem (Fig. 1, Harrison, 1984). Os indivíduos coletados foram encontrados mortos durante monitoramentos mensais de praia, entre a Lagoa do Peixe e o Arroio Chuí. Os espécimes estudados apresentavam visualmente baixo grau de decomposição. Entre os anos de 2013 e 2015, 20 indivíduos foram coletados durante a pesca de emalhe, dos quais 18 eram adultos e 2 juvenis. Esses indivíduos foram provenientes da captura incidental da pesca de emalhe de anchova (*Pomatomus saltatrix*) e pescada (*Cynoscion guatucupa*).

Todos os indivíduos coletados foram congelados e necropsiados em laboratório. Durante esse procedimento os indivíduos foram medidos, pesados, o sexo foi determinado por exame das gônadas (Proctor e Lynch, 1998), quando possível. Os conteúdos estomacais (n = 54) e amostras de fígado (n = 53), músculo (n = 54) foram coletados e congelados, e penas de contorno (n = 54) foram coletadas e guardadas a seco. Além destas, foram congeladas também amostras de peixes pouco digeridos encontrados nos conteúdos estomacais, e lulas *Doryteuthis sanpaulensis* provenientes da pesca na região, cedidas pelo Laboratório de Recursos Pesqueiros Demersais e Cefalópodes - FURG.

Composição da dieta

Em laboratório, os conteúdos estomacais foram analisados em lupa estereoscópica, e os itens foram quantificados e identificados até o menor nível taxonômico possível. Os peixes encontrados com baixo grau de digestão foram identificados de acordo com Fischer *et al*. (2011) e com o auxílio de especialistas do Laboratório de Ictiologia, na Universidade Federal do Rio Grande - FURG. Peixes também foram identificados através de otólitos *sagitta* remanescentes nos conteúdos, conforme Naves (1999) e as coleções de referências e especialistas do Laboratório de Aves Aquáticas e Tartarugas Marinhas e do Laboratório de Recursos Pesqueiros Demersais e Cefalópodes, na mesma instituição. Os peixes ingeridos foram quantificados por meio da contagem dos pares de otólitos e globos oculares. Os cefalópodes foram identificados através dos bicos encontrados, seguindo as descrições de Santos e Haimovici (1998), Santos (1999) e Vaske-Jr e Costa. (2011), assim como através de consulta à coleção de referência e de especialista do Laboratório de Recursos Pesqueiros Demersais e Cefalópodes. O número de indivíduos de cefalópodes em cada amostra foi obtido pela contagem de bicos superiores e inferiores de cada espécie encontrada, de cada conteúdo gastroesofágico analisado. Para cada otólito *sagitta* encontrado foi determinado seu comprimento, largura e o grau de digestão por meio da classificação em Índices de Digestão (ID), conforme Bugoni e Vooren (2004).

Para estimar o comprimento total e a massa corporal dos peixes encontrados, foram utilizadas equações alométricas conforme Naves (1999), apenas com as medidas baseadas em pares de otólitos com pouca digestão (ID = 0 e 1). Nas equações, a largura do otólito foi utilizada quando não foi possível a medição do comprimento. Para peixes não identificados, foi utilizada a massa média das presas calculadas para outros peixes de mesmo táxon. Para cefalópodes, o comprimento do manto e a massa corporal foram calculados por equações alométricas conforme descrito por Santos (1999) e Santos e Haimovici (1998). Para esses cálculos foram utilizadas as medidas de comprimento do rostro superior (URL) e inferior (LRL) para bicos de lula e, comprimento do escudo superior (UHL) e inferior (LHL) para bicos de *Argonauta nodosa*. Outros itens encontrados, tais como conchas de moluscos, invertebrados, plantas e plásticos foram registrados, contabilizados e identificados quando possível.

Análises de isótopos estáveis

As amostras de fígado, músculo e das presas tiveram seus lipídios removidos com clorofórmio:metanol (2:1) como solvente, em aparelho Sohxlet por 8 h. Essa remoção é necessária para minimizar a variação dos valores de $\delta^{13}C$ dos tecidos (Sotiropoulos *et al*., 2004) causado por valores deplecionados em ^{13}C em lipídios (Tieszen *et al.*, 1983). Posteriormente as amostras foram secas em estufa a uma temperatura de 60°C por 48 h. Com a finalidade de remover qualquer contaminação, as amostras de penas foram lavadas em solução de NaOH 0,25 M, e então lavadas cinco vezes com água destilada. Após as lavagens foram secas em estufa a 70°C durante 12 h. Amostras de fígado e músculo foram trituradas e as amostras de penas foram cortadas com tesoura em pequenos fragmentos. Posteriormente, cada amostra foi então triturada, homogeneizada, pesada em aproximadamente 0,7 mg, e acondicionada em cápsulas de estanho esterilizadas. A determinação dos valores de δ^{13}C e de δ^{15}N foi realizada em Espectrômetro de Massa de Razão Isotópica no *Stable Isotope Core Laboratory*, na Universidade de Washington (EUA). Os valores isotópicos são representados em notação δ (delta) e expressos em partes por mil (‰), utilizando os padrões internacionais de Vienna *Pee Dee Belemnite limestone* para C e N_2 atmosférico para N, conforme a equação de Bond e Hobson (2012):

$$\delta^{13}C \ ou \ \delta^{15}N(‰) = \left(\frac{R_{amostra}}{R_{padrão}}\right) - 1 \qquad \text{(eq. 1)}$$

onde $R_{amostra}$ é igual à razão dos isótopos pesados e leves da amostra, e $R_{padrão}$ é a razão dos isótopos pesados e leves dos padrões internacionais. A precisão dos valores de δ^{13}C e δ^{15}N foi de 0,2‰.

Análise dos dados

Índice de massa corporal

Para gerar um índice de massa corporal (IMC), foi utilizado o comprimento total e a massa corporal dos indivíduos coletados. O cálculo do IMC foi feito pela divisão da massa pelo comprimento total (Numata *et al.*, 2000). Os valores de IMC, massa e comprimento foram padronizadas, subtraindo a média e dividindo pelo desvio padrão, a fim de remover o efeito da classe etária nas comparações entre os locais de coleta (praia e mar). Os valores foram, enfim, convertidos em componentes principais através da Análise de Componentes Principais (PCA) (Pacote “ggplot2”; R Core Team, 2017), similar À análise realizada por Nunes *et al.* (2017).

Dieta

A importância de cada item alimentar, identificado em nível de espécie ou outra categoria taxonômica, foi calculada a partir dos seguintes parâmetros: FO = frequência de ocorrência, como a quantidade de vezes que determinado item alimentar ocorreu no total de amostras analisadas; FO% = frequência de ocorrência relativa, ou seja, o percentual da presença de determinado item alimentar em relação ao total de conteúdos analisados; N% = proporção numérica do item alimentar na dieta, em relação ao total de presas encontradas; PN% = contribuição relativa média em número, ou seja, o percentual médio do número total de determinada presa em relação às amostras, considerando-se apenas aquelas em que o item alimentar ocorreu, conforme Brown *et al.* (2012). M% = a proporção da massa de cada item alimentar em relação à massa total das amostras; PM% = contribuição relativa percentual média da massa de cada item em relação à massa total das amostras em que aquele item ocorreu; e PSIRI% = índice de importância presa-específica, calculado conforme a equação de Brown *et al*. (2012):

$$PSIRI\% = \frac{(PN\% + PM\%)\ x\ FO\%}{2} \qquad \text{(eq. 2)}$$

Isótopos estáveis

Para determinar a contribuição relativa dos itens alimentares nos tecidos do consumidor, foram utilizados modelos Bayesianos de misturas de isótopos (SIAR) no software R (Parnell *et al.*, 2010). As contribuições das fontes alimentares para a síntese do fígado, músculo e penas dos pinguins foram modeladas. As fontes utilizadas para estas análises foram selecionadas de acordo com os resultados obtidos nas análises da dieta, os itens ecologicamente distintos, os melhores ajustes após inspeção visual dos gráficos, e a melhor resolução dos modelos. O modelo mais ajustado foi composto por três espécies de presas, sendo elas a anchoita, a lula *D. sanpaulensis* e o peixe-rei *Odontesthes argentinensis*. Além dos dados da dieta, anchoita e a lula *D. sanpaulensis* são consideradas importantes itens alimentares para os pinguins-de-Magalhães na região estudada (Fonseca *et al*., 2001; Silva *et al.,* 2015), enquanto peixes-rei são representantes mais costeiros que os demais e também são consumidos por pinguins nas colônias do extremo sul da América do Sul (Frere *et al.*, 1996; Scolaro *et al.*, 1999; Scioscia *et al.*, 2014). Adicionalmente, uma vez que as penas são sintetizadas nas áreas de reprodução e visando testar a importância da anchoita na dieta do pinguim-de-Magalhães durante todo seu ciclo anual, os valores de isótopos estáveis das penas foram também modelados com os valores isotópicos de fontes da Patagônia Argentina, conforme estudo de Yorio *et al*. (2017) para o pinguim-de-Magalhães. Foram utilizados três itens alimentares: a anchoita, o camarão *Peisos petrunkevitchi* e a mistura das lulas *Illex* sp. e *Doryteuthis* sp. (Anexo 1 - Tabela 2).

Os fatores de discriminação trófica (FDT) utilizados nos modelos foram calculados a partir de médias ± 1 desvio padrão de valores obtidos em estudos de alimentação controlada de aves marinhas ecologicamente similares(Anexo 1 - Tabela 2): para os modelos baseados nas amostras de fígado, os valores de FDT foram de 0,45 ± 1,2‰ para $\delta^{13}C$ e 2,5 ± 0,28‰ para $\delta^{15}N$; para músculo foram de 1,2 ± 1,27‰ para $\delta^{13}C$ e 1,9 ± 0,71‰ para $\delta^{15}N$ e, para penas foram de 1,6 ± 1,47‰ para $\delta^{13}C$ e 3,76 ± 0,73‰ para $\delta^{15}N$.

A fim de verificar as variações nos valores de $\delta^{13}C$ e $\delta^{15}N$, foram utilizados modelos lineares generalizados - GLM (McCulloch e Searle, 2001) com distribuição Gaussiana. Foi construído um modelo para cada isótopo, considerando como variáveis explanatórias a classe etária (com 2 níveis – juvenil e adulto), os tecidos (3 níveis - fígado, músculo e penas), e suas interações. Modelos incluindo como variáveis o local de coleta e o sexo dos pinguins foram testados e posteriormente descartados, por serem altamente correlacionados (área e idade, já que a maioria dos juvenis ocorreu na praia e a maioria dos adultos no mar) ou não apresentarem significância (sexo). Os modelos foram então simplificados removendo as variáveis não significativas. A seleção do melhor modelo foi baseada no AIC (*Akaike Information Criterion*). Quando a diferença entre o AIC dos modelos foi menor que duas unidades, o modelo com menor número de variáveis foi selecionado (Zuur *et al.*, 2007). Foi realizada em seguida ANOVA dos resíduos dos modelos do GLM, fornecendo a significância dos fatores e a porcentagem explicada pelas variáveis. A porcentagem explicada por cada uma das variáveis foi calculada como a deviância dos resíduos da variável / deviância nula do modelo × 100, conforme Ye *et al*. (2001).

O nicho isotópico (Newsome *et al*., 2007) dos pinguins adultos e juvenis, foi determinado para as amostras de fígado, músculo e penas usando SIBER (*Stable Isotopes Bayesian Ellipses in* R). As dimensões e as porcentagens de sobreposição foram calculadas a partir das análises ajustadas para pequenos tamanhos de amostras (SEAc) (Jackson *et al*., 2011).

SÍNTESE DOS RESULTADOS

1. Houve um marcado padrão de ocorrência quanto à classe etária dos pinguins-de-Magalhães amostrados, com indivíduos adultos coletados em maior número no mar e predomínio de juvenis na praia.

2. Indivíduos capturados incidentalmente na pesca de emalhe apresentaram índice de massa corporal mais elevado em relação aos indivíduos encontrados na praia, demonstrando estarem em boas condições de saúde.

3. Pinguins adultos apresentaram dieta composta predominantemente por peixes (PSIRI = 86%), dentre os quais a anchoita foi o item alimentar mais importante. Já a dieta dos indivíduos juvenis teve maior contribuição de cefalópodes (PSIRI = 71%), e o principal item foi a lula *D. sanpaulensis*.

4. Modelos de mistura isotópicos indicaram que a anchoita foi a presa que mais contribuiu na dieta de pinguins-de-Magalhães tanto adultos quanto juvenis, em diferentes escalas temporais (dias – meses) indicados por tecidos com diferentes taxas de renovação.

5. Indivíduos adultos e juvenis apresentaram diferentes amplitudes de nicho isotópico. A maior sobreposição das áreas de nicho isotópico dos pinguins ocorreu com os valores das amostras de músculo. A área dos adultos foi 61,4 sobreposta pela área dos juvenis, enquanto a área de nicho dos juvenis foi sobreposta por 83,3% dos adultos, ou seja, a área do nicho dos juvenis foi uma porção menor do nicho dos adultos.

6. Indivíduos adultos apresentaram valores de δ^{13}C mais elevados em comparação aos juvenis. O oposto foi verificado para δ^{15}N, com juvenis

apresentando valores mais elevados em relação aos adultos, possivelmente devido ao catabolismo de tecidos proteicos de seus corpos anterior ao óbito por inanição.

7. Foram encontrados resíduos plásticos em 29% dos conteúdos estomacais analisados, ocorrendo com maior frequência em juvenis (FO = 40%) em relação aos adultos (FO = 19%).

8. Análises de dieta baseadas em conteúdos estomacais de animais encontrados mortos nas praias, após período de inanição ou doença, não são indicadores confiáveis da alimentação de indivíduos saudáveis e, portanto, da espécie.

9. Técnicas complementares para estudos de ecologia trófica de vertebrados são recomendados.

CONCLUSÃO E PERSPECTIVAS FUTURAS

Por meio deste estudo comparou-se pela primeira vez a dieta de indivíduos de pinguins-de-Magalhães adultos e juvenis no sul do Brasil, coletados tanto na praia como no mar, durante o período não reprodutivo. Apesar de sua dieta ser bastante estudada nas colônias na Argentina (ex., Gosztonyi, 1984; Frere *et al.*, 1996; Forero *et al.*, 2002; Yorio *et al.*, 2017), a alimentação e utilização do habitat em territórios de invernagem ainda são escassas (Silva *et al.*, 2015). Análises de conteúdos estomacais mostraram a importância dos peixes na dieta dos adultos, e de cefalópodes para os juvenis. Esses resultados encontrados para os juvenis corroboram com estudos prévios no Brasil (ex., Fonseca *et al.*, 2001; Pinto *et al.,* 2007; Di Beneditto *et al.*, 2015). Com a utilização de metodologias complementares, como a análise de isótopos estáveis e análise de conteúdos estomacais, foi possível inferir a real importância da contribuição da anchoita na dieta de pinguins adultos e juvenis. Essa contribuição foi observada em diferentes escalas temporais do ciclo anual da espécie. Diferença no índice corporal dos indivíduos saudáveis provenientes da pesca em comparação aos indivíduos debilitados coletados na praia demonstram alimentação precária e severa debilitação dos juvenis. Também foram demonstradas as limitações do uso destas carcaças encontradas nas praias para estudos de dieta de vertebrados marinhos, particularmente aqueles mortos por enfermidades ou inanição.

O conhecimento sobre as relações tróficas dos pinguins-de-Magalhães durante o período não reprodutivo tem grande importância para sua conservação. A importante contribuição da anchoita na dieta da espécie confirma a dependência da espécie ao longo de todo o ciclo anual, similar ao que já foi demonstrado em estudos de outros pinguins *Spheniscus* que também se alimentam intensamente de peixes do gênero *Engraulis* (Wilson, 1985; Paredes *et al.*, 2002). Isto demonstra a necessidade de conservação dos estoques de pequenos peixes pelágicos na região sul do Brasil, além da necessidade de mitigação dos efeitos de captura incidental na pesca de emalhe de anchova e pescada. Neste contexto, é fundamental o conhecimento dos recursos utilizados e das áreas de forrageamento dos pinguins-de-Magalhães nas áreas de invernagem para conservação da espécie durante todo seu ciclo anual e ao longo de toda sua distribuição.

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ANEXO 1

Trophic ecology of Magellanic penguins (*Spheniscus magellanicus*)

during the non-breeding period

Manuscrito redigido de acordo com as normas para submissão ao periódico

Estuarine, Coastal and Shelf Science.

Trophic ecology of Magellanic penguins (*Spheniscus magellanicus*) during the non-breeding period

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* A versão para publicação incluirá outros coautores, após terem acesso e revisarem o manuscrito.

ABSTRACT

Magellanic penguins (*Spheniscus magellanicus*), migrates during the wintering period, reaching high productive areas in southern Brazil, under the influence of the Subtropical Shelf Front and by La Plata River and Patos Lagoon discharges. This is an important fishing area with occurrence of keystone species, such as the Argentine anchovy (*Engraulis anchoita*), regarded as the main food item of Magellanic penguins at northern colonies in Argentina and supposed to be also important, despite not demonstrated, in wintering grounds. In this context, this study aims to investigate the importance of a keystone species, the Argentine anchovy, in the diet of Magellanic penguins of different age classes, and throughout the annual cycle of the species. A total of 7 adults and 27 juveniles were collected stranded dead on the beach, and 18 adults and 2 juveniles at sea, as bycatch on gillnet fishing. Penguins collected at sea had higher body index compared to individuals found on the beach, demonstrating to be healthy individuals. Among stranded and bycaught individuals, fish and the Argentine anchovy was the main food item in stomach contents of adults (n = 21; PSIRI = 86%). On the other hand, juveniles (n = 20) *presented* cephalopods as the main prey item (PSIRI = 71%), with predominance of *Doryteuthis sanpaulensis*. Despite the high importance of cephalopods in the diet of juveniles, δ^{13}C and δ^{15}N stable isotope analysis in liver, muscle and feathers, showed relevant contribution of Argentine anchovy to the diet of juveniles (95% credibility interval = 46 – 98.6%) and adults (39.8 – 98.9%). Adults and juveniles presented isotopic niche overlap in all tissues analyzed, despite differences in niche areas between age classes. Our results highlight the importance of using complementary techniques in studies on trophic ecology, as stomach content analysis of individuals found dead on beaches may not provide reliable information on the diet. The strong reliance of penguins on Argentine anchovy as a key resource throughout the year demonstrates that top predators should be considered in commercial exploitation of this fishing resource.

Key words: bycatch, diet, *Engraulis anchoita*, stable isotopes, stomach content, wintering period.

1. Introduction

Magellanic penguins *Spheniscus magellanicus* are distributed in southern South America and adjacent islands, with Atlantic coast colonies occurring from Tierra del Fuego (Scolaro *et al.*, 1980; Boersma *et al*., 1990) to the most northern colony at the Complejo Islote Lobos (41°25'S; 65°01'W), Argentina (Boersma *et al.*, 2009). It also occurs offshore, at Isla de Los Estados and at Malvinas/Falkland Islands (Scolaro *et al.*, 1980; Gandini *et al.*, 1996). Colony sizes of the Magellanic penguin presents both increase and decreases along its distribution area, with an overall population decreasing trend (Boersma, 2008). The main threats to Magellanic penguins are oil pollution (Gandini *et al.*, 1994; 1996), egg and chick predation (Frere *et al.*, 1992), bycatch in fisheries (Gandini and Frere., 1999; Cardoso *et al.*, 2011), overfishing of their main prey (Gandini *et al.*, 1996) as well as disturbance caused by tourism in colonies (Yorio and Boersma, 1992).

Magellanic penguins have a seasonal breeding schedule, breeding in late September until February (Boersma *et al.*, 1990). After breeding, they leave the colonies for a foraging trip to store up reserves, before returning to molt on land during two to three weeks (Boersma *et al*., 1990; Wilson *et al.*, 2005). Thereafter, they migrate over the continental shelf to lower latitudes, regularly reaching northern Argentina, Uruguay and southern Brazil (Stokes *et al.*, 1998; García-Borboroglu *et al.*, 2010). During the wintering period, from March to September, migration and wintering areas are associated to productivity and availability of their main prey, thus is coupled with the migrations of the northern and southern stock (*sensu* Hansen, 2004) of the Argentine anchovy *Engraulis anchoita* (Boersma *et al.*, 1990; Stokes *et al.*, 1998; Pütz *et al.*, 2000, 2007).

In Brazil, Magellanic penguins occupy areas also intensively fished by Brazilian fleets (Vasconcellos *et al.*, 2014; Haimovici and Cardoso, 2017), where are also located the development areas of key-species, such as the Argentine anchovy (Acha *et al.* 2004; Costa *et al.*, 2016). At northern Argentinean colonies, the Argentine anchovy is reported to be the main food item of Magellanic penguins during the breeding season (Gosztonyi, 1984; Frere *et al.*, 1996). This fish inhabit the inner continental shelf of South America between 23°S and 47°S (Lima and Castello 1995) and is considered an important keystone species for energy flow to higher trophic levels in along southern Brazil. Commercially important fish species such as the hake *Merluccius hubbsi* (Haimovici *et al*., 1993), the bluefish (*Pomatomus saltatrix*) and the striped weakfish (*Cynoscion guatucupa*) (Lucena *et al.*, 2000) feed mostly on Argentine anvhovy.

The diet of Magellanic penguins had been extensively studied in breeding colonies based on flushed stomach contents (e.g. Frere *et al.*, 1996; Scolaro *et al.*, 1999), and also in wintering grounds based on stomach content analysis of emaciated penguins found stranded on the beaches (e.g. Fonseca *et al.*, 2001; Pinto *et al.*, 2007; Di Beneditto *et al.*, 2015). Their diet varies depending on the latitude of the colony and is considered opportunistic and non-selective, relying mainly on fish rather than squids (Frere *et al.*, 1996). However, juveniles found along the coast of Rio Grande do Sul state, the southernmost in Brazil, during winter and spring months, showed cephalopods as main food items (Fonseca *et al.*, 2001). Additionally, individuals found in northern Brazilian states such as Rio de Janeiro, presented mostly cephalopods in stomach contents (Di Beneditto *et al.*, 2015). Because juveniles are found dead after severe emaciation and inferred abnormal feeding behavior, diet studies were mostly based only on long-lasting harder remains such as squids beaks. It is estimated that 19,000 Magellanic penguins, the vast majority of which were juveniles (97.5%), strands along southern Brazilian coast every year (Mäder *et al.*, 2010).

Factors that influence feeding behavior and prey choice by seabirds can range from behavioral to morphological characteristics (Pierotti and Annett, 1995; Forero *et al.*, 2002). Differences in exploitation of food resources, within populations, can be explained by age and experience of individuals, as observed in Magellanic penguins in breeding grounds in Patagonia, where adults consume higher proportion of fish in comparison to juveniles (Forero *et al.*, 2002).

Reliable information on diet of penguins is needed to adequately interpret their role in marine food webs and to understand trophic interactions (Yorio *et al*., 2017), as well as to support ecosystem-based management of fish stock used by both fisheries and marine top predators. One of the most common methods to study food resources consumed by seabirds is stomach content analysis of dead organisms (Barret *et al.*, 2007). Despite allowing the identification of ingested prey based on undigested remains in stomach contents, this technique is biased, unable to detect rapid digestion prey and only providing information on recently ingested food (van Heezik and Seddon, 1989; Barret *et al.*, 2007). Stable Isotope Analysis (SIA) is an auxiliary method for studies of diet and foraging grounds. SIA of tissues provides quantitative information of the relative contributions of each source to the diet (Hobson and Clark, 1992a). Values of $\delta^{13}C$ ($^{13}C/^{12}C$) reflect basal food sources, varying about 1‰ at each trophic level (e.g. coastal *vs.* limnetic environments) (Peterson and Fry, 1987; Hobson, 1995; Fry, 2006), while $\delta^{15}N$ ($^{15}N/^{14}N$) values are used to represent trophic levels, varying from 2 to 5‰ between trophic levels (DeNiro and Epstein, 1981; Vander-Zanden *et al*., 1997). Tissues with rapid turnover, such as liver, will reflect recent diet (days to a week), muscle has slower turnover rates (weeks to months) and thus reflect medium to long-term diet, and inert tissues, such as feathers, reflects the diet during their growth period (Mizutani *et al.*, 1991, 1992; Hobson and Clark, 1992b). Based on SIA it is possible investigate niche overlap through the construction of isotopic niches of subunits under investigation (e.g. adults *vs.* juveniles; males *vs.* females). Information about sources and areas where animals forage can be considered as axis and dimensions for the ecological niche description, defined as *n*-dimesional hypervolum of conditions and resources (Hutchinson, 1957). Thus, SIA allows inferences on intraspecific differences at foraging grounds, diet specialization, and the use of isotopic niche as a proxy of trophic or spatial niches (Newsome *et al.*, 2007).

The current study aims to investigate 1) the importance of a keystone species, the Argentine anchovy, in the diet of Magellanic penguins of different age classes, and throughout the annual cycle of the species; 2) compare isotopic niches between adults and juveniles during the wintering period; and 3) address if stomach content analysis of emaciated and unhealthy penguins found dead on beaches provides reliable information on the diet of the species.

2. Material and Methods

2.1 Study area

Magellanic penguins were collected on the coast and at sea, in Rio Grande do Sul state, southernmost Brazil (Fig. 1). Individuals collected on the coast were found stranded along approximately 355 km of beach, between the municipalities of Mostardas/Tavares (31°20'S; 51°05'W) and Chuí (33°45'S; 53°22'W). Penguins that were bycaught in fishing gillnets at sea were also used. This fishery operates at a distance of 1 to 3 km from the coast, between 17 and 30 m of depth, and at 32°–33°S and 51°–53°W, targeting bluefish and striped weakfish (Fogliarini *et al.*, in revision).

During winter, oceanographic patterns over the continental shelf in southern Brazil are characterized by the mixture of Sub-Antarctic Shelf Waters

(SASW; originated by the Falkland/Malvinas Current) and Subtropical Shelf Waters (STSW; influenced by Brazilian Current and Tropical and Coastal Waters), resulting in the Subtropical Shelf Front (STSF). The STSF is a transition zone with latitudinal thermohaline gradient and high productivity between 33° and 36°S (Piola *et al.*, 2008; Möller *et al.*, 2008) (Fig. 1). This high productivity is influenced by cold freshwaters from SASW and at the surface by the low salinity waters from La Plata River and Patos Lagoon plumes (Piola *et al.*, 2008; Costa *et al.*, 2016).

2.2 Sampling methods

During 2015, a total of 34 stranded dead Magellanic penguins were collected on the beach (7 adults and 27 juveniles), with age class classified by plumage (Harrison, 1984). The penguins analyzed were selected based on limited decomposition stages, with visually preserved skin and plumage, as well as absence of larvae and perforations. Additionally, 20 individuals were collected from incidental capture by gillnet fishing vessels (18 adults and 2 juveniles) between 2013 and 2015. All 54 individuals were frozen and then necropsied at the laboratory. Contour feathers, pectoral muscle, liver and stomach content were collected and frozen, and sex was determined by gonad inspection (Proctor and Lynch, 1998).

2.3 Diet composition

At the laboratory, prey remains found in stomachs were quantified and identified under a dissecting microscope to the lowest possible taxonomic level.

Fishes with low digestion degree were identified according to Fisher *et al.* (2011) and with the support from experts at the Ichthyology Lab at Universidade Federal do Rio Grande - FURG. Fish *sagitta* otoliths were identified according to Naves (1999), by comparison with reference collection at Waterbirds and Sea Turtles Lab and Demersal Fish Resources and Cephalopods Lab, both at FURG. The number of fish prey at each stomach content was determined from the maximum number of otoliths and eye lenses pairs (Bugoni and Vooren 2004), adding the number of whole fish when present. Cephalopods were identified by quitinous beak remains according to Santos (1999), Vaske-Jr and Costa. (2011) and by comparison with reference collection at Demersal Fish Resources and Cephalopods Lab. The number of cephalopod prey was determined according to the maximum number of either upper or lower beaks of each species in the same stomach content. Prey remains were measured with a microscopic scale at the ocular. Measurements of cephalopod beaks were conducted using upper (URL) and lower (LRL) rostral length for squids and upper (UHL) and lower (LHL) hood length for the paper nautilus *Argonauta nodosa*. For fish otoliths, the length, width and the index of digestion (ID 0 – 3), were measured following Bugoni and Vooren (2004). Only measurements of otoliths with slightly or none digestion (ID = 0 – 1) were used. Otolith width was used when otolith length could not be measured. The estimated fish and cephalopod body mass was calculated by equations listed in Table 1. For fish unidentified at species level, the mean mass of fish from the same taxon above species level was used. Other items and remains such as mollusk shells, plant fragments and solid waste such as plastics were recorded, counted, and identified when possible, but were not included in dietary analysis, as they are incidental or considered secondary ingestion (Pinto *et al*., 2007). Anthropogenic debris were classified as in European Commission (2013).

2.4 Stable isotopes analysis

Feathers were stored dried in zip-lock plastic bags at room temperature, while liver and muscle samples were frozen. Undigested food items such as fish found in stomach contents, and the squid *D. sanpaulensis* fished in the region and provided by Demersal Fish Resources and Cephalopods Lab, were also frozen for later SIA as potential food sources.

Lipids were extracted from muscle and liver samples of penguins and undigested muscle of prey items (Table 2) using a 2:1 chloroform:methanol solvent in a Soxhlet apparatus over 8 h. Samples were then dried at 60°C for 48 h. In order to remove external residuals, feathers were washed in a 0.25 M NaOH solution, then rinsed five times with distilled water and finally dried at 70°C for 12 h. Muscle and liver samples were ground, feather samples were cut in small pieces with scissors. Each sample was homogenized and a subsample of approximately 0.7 mg was weighted into tin cups (4 × 6 mm) and sent for analysis in an isotope ratio mass spectrometer at the Stable Isotope Core Laboratory, at Washington State University (USA). Stable isotope ratios were expressed in δ notation as parts per thousand (‰) using the international standards Vienna Pee Dee Belemnite and air, for carbon and nitrogen, respectively. Values were determined by the equation 1 as in Bond and Hobson (2012):

$$\delta^{13}C \; or \; \delta^{15}N(‰) = (\frac{R_{sample}}{R_{standard}}) - 1 \qquad \text{(eq. 1)}$$

where R_{sample} is the ratio of heavy and light isotope from the sample and, $R_{standard}$ is the ratio of heavy and light isotope from international standards. The laboratory precision for δ^{13}C e δ^{15}N values was 0.2‰.

2.5 Data analysis

2.5.1 Body index

To obtain a Body Mass Index (BMI), the total length and body mass of 45 sampled penguins were used. To calculate BMI, the mass (in g) was divided by total length – bill tip to tail feather, in mm (Numata *et al.*, 2000). To remove age class effects between sampling areas (beach and sea), values of BMI, mass and length were standardized, subtracting the mean and dividing by the standard deviation. Values were converted into principal components by Principal Components Analysis (PCA), in R software (Package “ggplot2”, R Core Team, 2017), as in Nunes *et al.* (2017).

2.5.2 Diet

The relative importance of each food item, identified as species or the lowest taxon possible, was calculated using the following parameters: FO = absolute (FO) frequency of occurrence, as the number of stomach contents with the food item, and relative (FO%) as a percent of all stomach analysed; contribution by number absolute (N), as the number of individual prey items in relation to the total number ingested, and relative contribution by number (PN%), as the percent of total number of individual prey in all stomach contents, excluding samples in which the food item did not occur; contribution by mass (M) as the proportion of the mass of a given food item in the diet, over the total mass measured or reconstructed in all stomach contents, and relative mass contribution (PM%), i.e., prey-specific mass as the proportion of each food item found in all samples, but excluding those in which the food item did not occur. And finally, all these parameters were integrated in to the prey-specific index of relative importance - PSIRI% (Brown *et al.*, 2012), calculated as:

$$PSIRI\% = \frac{(PN\% + PM\%)\ x\ FO\%}{2} \quad \text{(eq. 2)}$$

2.5.3 Stable isotopes

To determine the relative contribution of food items in the diet of adult and juvenile Magellanic penguins, Bayesian stable isotope mixing models – SIAR (Parnell *et al.*, 2010) were used. Sources used for analysis were selected according to diet analysis results (See Results section), food items with distinct ecological characteristics, and the best adjustment of models. Models were chosen based on preliminary tests wih a range of potential soruces, analysis by graphic results of SIAR data, plots of proportion by sources and matrix plots of correlations between sources, following the manual guide of the package. The best fitted model was composed by three food items: Argentine anchovy, squid *D. sanpaulensis* and the silverside *Odontesthes argentinensis*. Anchovy and the squid *D. sanpaulensis* were also identified as important food items for

Magellanic penguins in the same region in a previous study (Fonseca *et al*., 2001), while silversides are preyed by penguins in southern Argentina colonies (Frere *et al*., 1996), it is a representative of more coastal fish species, and was also present in our samples (see Results). The contribution of each source to the synthesis of Magellanic penguin tissues was modelled for liver, muscle and feather values. Discrimination factors used in models were obtained by calculating the mean ± SD of values from controlled-diet experiments on other seabirds found in the literature (Table 3). For liver samples, the discrimination factors were 0.45 ± 1.2‰ for δ^{13}C and 2.5 ± 0.28‰ for δ^{15}N, for penguin muscle 1.2 ± 1.27‰ for δ^{13}C and 1.9 ± 0.71‰ for δ^{15}N were used and, for feather samples, 1.6 ± 1.47‰ for δ^{13}C and 3.76 ± 0.73‰ for δ^{15}N were used.

In addition to running models as described above for comparative purposes using the three tissues, only varying TDF values according to tissue, an additional model was used for feathers, aiming to test the importance of Argentine anchovy during the annual cycle of Magellanic penguin. Because feathers retain isotopic composition during synthesis just after breeding in Argentina, it was also modeled with sources from Argentinean Patagonia, used recently by Yorio *et al*. (2017). Three food items were included in the model: the Argentine anchovy, the white shrimp (*Peisos petrunkevitchi*) and the mixture of *Illex* sp. and *Doryteuthis* sp. (Table 2).

In order to verify the variation in δ^{13}C and δ^{15}N values, Generalized Linear Models – GLMs (McCulloch and Searle, 2001) with Gaussian distribution, were run for each isotope separately. Explanatory variables were age classes (2 levels – adults and juveniles), tissues (3 levels - liver, muscle and feather) and their interactions. Models including area (beach and sea) and penguin sex (males and females), were excluded from the final model, as they showed high correlation with age (as most juveniles were sampled on the beach and adults at sea) or did not show significant values (sex). The selection of the best model was based on the Akaike Information Criterion – AIC. Models were then simplified by excluding variables statistically non-significant at $P < 0.05$, or whenever the difference in AIC between statistical models was smaller than two units (Zuur *et al.*, 2007). An ANOVA of the residuals from the GLM output was also run, providing the significance of values and the percentage explained by each variable. The percentage explained by each variable and their interactions were calculated as in Ye *et al.* (2001):

$$\%\, explained = \frac{Residual\ deviance\ of\ the\ variable}{Deviance\ of\ the\ null\ model}\ x\ 100 \qquad \text{(eq. 3)}$$

The isotopic niche (Newsome *et al.*, 2007) of adult and juvenile penguins was determined for liver, muscle and feather isotopic values using Stable Isotope Bayesian Ellipses in R – SIBER (Jackson *et al.*, 2011). The area of the isotopic niche and the percentages of overlap between groups were calculated from standard ellipse areas adjusted for small sample size (SEAc). All statistical analysis of stable isotopes were carried out in R 3.4.0 software (R Core Team, 2017).

3. Results

3.1 Body Mass Index

Individuals incidentally killed in gillnet fishing had higher BMI in comparison to individuals collected on the beach (Fig. 2). It was possible to note that bycaught individuals, including adults (*n* = 18) and juveniles (*n* = 2), were above the mean of BMI, in PCA analysis. Regarding the mean by age class *vs.* area, beached individuals, mostly juveniles (*n* = 23) had BMI below mean values of birds sampled at sea. Only two of the five beached adults had high BMI, comparable to adults sampled at sea, while two juveniles collected at sea had BMI comparable with healthy adults.

3.2 Diet

A total of 3146 prey were found in 41 stomach content samples from the Magellanic penguins analyzed. Main prey items consumed were cephalopods (2396 individuals of 3 species) and teleost fish (751 individuals of 8 species) (Table 4). At the 21 stomach content samples from adults, the main prey was fish (415 individuals), mostly the Argentine anchovy (124 individuals), the second most frequent was cephalopods (110 individuals), mostly the squid *D. sanpaulensis* (56 individuals). At the 20 stomach content samples from juveniles, the main prey was cephalopods (2286 individuals), mainly the pelagic octopus, the paper nautilus (*A. nodosa*) (2074 individuals), the second most frequent was fish (336 individuals), mostly the marine silverside (*O. argentinensis*) (17 individuals).

In terms of the overall contribution, represented by the integrated PSIRI%, for adults, fish was the main food item (PSIRI = 86.4%), followed by cephalopods (PSIRI = 13.6%). The main items at the species level were *E. anchoita* (PSIRI = 29.0%) and *D. sanpaulensis* (PSIRI = 7.1%). The most frequent species was *E. anchoita* (FO% = 66.7), followed by *D. sanpaulensis* (FO% = 38.1) and *Lycengraulis grossidens* (FO% = 19.0). In number (PN%), the main contributor was *A. nodosa*, accounting for 51.3%, followed by *E. anchoita* (44.4%). The most energetically important group, as indicated by the mass contribution, was *E. anchoita* (PM = 43%), followed by *A. nodosa* (PM = 27.7%) (Table 4).

Juvenile Magellanic penguins showed differences in diet in comparison to adults. The main food item was cephalopods (PSIRI = 71.1%), followed by fish (PSIRI = 28.9%). At the species level, the most important food item was *D. sanpaulensis* (PSIRI = 30.8%) and *A. nodosa* (PSIRI = 23.2%). Frequency of occurrence was high for *D. sanpaulensis* (FO% = 80.0), followed by *A. nodosa* (FO% = 55.0) and *D. plei* (FO% = 20.0). The main contributor in number was *A. nodosa* (PN% = 55.9) and silversides *Odontesthes* sp. (PN% = 33.0). In relation to mass contribution, the most important food item was *D. sanpaulesis* (PM% = 45.7), followed by *A. nodosa* (PM% = 28.6) (Table 4).

The estimated mean total length of fish was 96 mm (range 37 – 147.6 mm), and total mantle length of cephalopods was 69.8 mm (range 3.1 – 250.2 mm) (Table 5). The mean size (total length for fish and mantle length for squids) and mass, respectively, of *D. sanpaulensis,* was 73.8 mm ML and 23.7 g, the octopus *A. nodosa* was 15.9 mm ML and 1.7 g, and *E. anchoita* was 105.8 mm and 7.6 g (Table 5).

3.3 Non-food items

Plant remains, other mollusks, crustaceans and fish eggs were present in low numbers, regarded as secondary items. Plastic was found in 29% of stomach contents. Anthropogenic items found were plastic sheet fragments, nylon, and fishing rope (Table 4).

3.4 Stable isotpes analysis

3.4.1 GLM

The best fitted GLM model, for both δ^{13}C and δ^{15}N, was:

$$\delta^{15}\text{N or } \delta^{13}\text{C} \sim \text{Tissue + Age + Tissue:Age}$$

δ^{13}C values in adults were significantly higher than values in tissues of juveniles, while δ^{15}N values were lower in adults in comparison to juveniles (Table 6). Comparison between tissues showed δ^{15}N values in liver and muscle lower than feather values. The interaction between ‘muscle:age’ was significant for δ^{13}C and δ^{15}N, while ‘liver:age’ was only significant for δ^{15}N.

The ANOVA of the GLM indicated that the model explained 40.8% of the variance for δ^{13}C and 23.8% for δ^{15}N. For both SI values the higher explicability was for ‘tissue’ (28% for δ^{13}C and 18.7% for δ^{15}N) (Table 7).

3.4.2 Stable isotopes mixing models

Stable isotope values in penguin tissues varied from -17.6 to -15.4‰ (*n* = 53) for $\delta^{13}C$ in liver, -18.5 to -15.9‰ (*n* = 54) in muscle and -18.6 to -14.0‰ in feathers (*n* = 54). For $\delta^{15}N$, values varied from 18.2 to 20.6‰ in liver, 15.8 to 19.4‰ in muscle and from 17.9 to 21.9‰ in feathers (Table 6).

Stable isotope mixing models with best fit were those using three food sources to the diet of penguins: *D. sanpaulensis*, *E. anchoita* and *O. argentinensis* (Fig. 3). For all tissues modeled, the main contribution (credibility interval CI = 95%) was *E. anchoita* for both adults and juveniles, followed by *O. argentinensis*, except for liver samples in juveniles. The main contribution of anchovy was found in muscle of adults, in feathers of juveniles, and in liver of juveniles (Table 8, Fig. 4). The results of SIAR model, with isotopic values of penguins feathers and using values of food items from Argentinean Patagonia, also showed that the Argentine anchovy was the main contributor on the diet for both adults and juveniles nearby colonies (Fig. 4).

Isotopic niches showed overlapped areas between adult and juvenile penguins for liver, muscle and feather samples (Fig. 5). For liver samples, adults (0.6‰^{2}) and juveniles (0.7‰^{2}) had a similar niche area, overlapping 26.5% (adults over juvenile area) and 23.3% (juveniles over adult areas), respectively. For muscle samples, adults (1.4‰^{2}) had a greater niche width than juveniles (1.0‰^{2}), overlapping 61.4% and 83.3% in niche areas. However, SEA_C values for feather samples differed from other tissues, with a high difference between adults (3.5‰^{2}) and juveniles (8.‰^{2}), with large overlap of adults over juvenile area (48.6%) but limited overlap of juveniles over adult areas (24.4%).

4. Discussion

Fish and cephalopods composed the bulk of the diet of both adult and juvenile Magellanic penguins stranded on the beach and collected at sea in their wintering grounds in southern Brazil. Conventional dietary analysis indicated that fish was the most important food item for adults, in contrast to juveniles, that showed cephalopods as the main food item. However, mixing models based on stable isotope analysis in all tissues showed that Argentine anchovy is the most important food item for both adult and juveniles penguins during the wintering period at lower latitudes. This is in contrast with previous studies based only on stomach contents of individuals stranded on the beach, that indicated cephalopods as the main item. In this study, the use of both techniques and the analysis of healthy individuals bycaught on gillnets highlighted the importance of Argentine anchovy in the diet of Magellanic penguins.

4.1 Diet composition and stable isotope analysis in wintering areas

During the wintering period, the stomach contents of adult Magellanic penguins was mainly composed by Argentine anchovy, while for juveniles the most important food item was the squid *D. sanpaulensis*. Previous studies in Brazilian wintering grounds, using individuals stranded on the beach, indicated that the diet of Magellanic penguins was composed mainly by cephalopods, also showing *D. sanpaulensis* as an important food item (Fonseca *et al.*, 2001; Pinto *et al.*, 2007; Baldassin *et al.*, 2010; Di Beneditto *et al.*, 2015). The squid *D. sanpaulensis* occurs on a yearly basis on the shelf, and is regarded as the most abundant cephalopod in southern Brazil (Haimovici and Andriguetto, 1986; Santos and Haimovici, 2002). *Argonauta nodosa*, a pelagic octopus, had high contribution in numbers in stomach contents, and had been also commonly found in the diet of Magellanic penguins, stranded dead on the beach, in previous studies (e.g. Santos and Haimovici, 2002). Because *A. nodosa* were small in size they were not important in diet. In southern colonies of Santa Cruz province, in Argentina, penguins fed primarily on cephalopods, including *Doryteuthis* sp. (Scolaro *et al.*, 1999), while in central Santa Cruz province, penguins showed a more diverse diet, composed by cephalopods and fish (Frere *et al.*, 1996). At Falkland/Malvinas Islands, the importance of squid in the diet decreases from western to southern locations, being replaced by fish (Pütz *et al.*, 2001).

The Argentine anchovy had been also reported as the main food item consumed by Magellanic penguins in wintering grounds in northern RS state, based on SIA of the claws (Silva *et al.*, 2015). This fish was also an important food item at wintering grounds in Rio de Janeiro coast, Brazil (Di Beneditto *et al.*, 2015). Silverside fishes are also common along the distribution area of penguins, with shoals in oceanic surface and estuarine-coastal waters in Argentine, as well as in wintering grounds in Brazil (Bemvenuti, 1987; Fisher *et al.*, 2011).

In this study the mean mantle length and mass of *D. sanpaulensis* was 73.8 mm and 23.7 g, higher than in a previous studies on diet of Magellanic penguins at this region (7.5 mm and 0.4 g; Fonseca *et al.*, 2001), which seems to be unrealistic and an error in the afore mentioned study. Additionally, in Rio de Janeiro coast, squids were found with ML measuring 54.5 and 61.5 mm, and 18.1 and 14.7 g (Pinto *et al.*, 2007; Di Beneditto *et al.*, 2015). These values are similar to the current study and from a previosu study in the same area (74.4 mm and 18 g; Santos and Haimovici, 1998) in southern Brazil. The mean mantle length and mass for the paper nautilus (15.9 mm and 1.7 g) were similar to values found at Rio de Janeiro coast (24 – 18.8 mm and 3.1 – 1.8 g, respectively) (Pinto *et al.*, 2007; Di Beneditto *et al.*, 2015). Values found in this study show that penguins fed on adults and subadults of *D. sanpaulensis* (Andriguetto and Haimovici, 1996).

The total length of consumed fish varied from 37 to 143 mm, within values found by Fonseca *et al.* (2001) in Rio Grande do Sul state, with prey varying from 106.7 to 137 mm, and were also similar to values found in distinct colonies from north to south Argentine (56 – 370 mm) (Scolaro *et al.*, 1999). In relation to fish mass, the mean in our study was 10 g, within mean values found previously at the same region (6.46 – 20.2 g) (Fonseca *et al.*, 2001), and in Argentinean colonies (2.5 – 153.8 g) (Scolaro *et al.*, 1999).

Although stomach content analysis generates relevant information, an overestimation can occur towards prey with hard, less digestible structures, such as cephalopod beaks. These structures can remain in gastrointestinal tracts from days to months (van Heezik and Seddon, 1989; Barret *et al.*, 2007).

In addition, studies with emaciated juveniles can be even more biased as birds are not feeding normally, and thus could concentrate cephalopod beaks in their stomach due to limited gastric transit. By using complementary methods such as the SIA and collecting both healthy adults and debilitated juveniles, it was possible to confirm the high importance of fish, especially the Argentine anchovy, during the wintering period, for both groups. The SIAR modeling clearly showed the high contribution of the Argentine anchovy to the diet of Magellanic penguins during the wintering period at the study area, during the migratory period and just after breeding when penguins forage to sustainthe feather synthesis. The values of liver samples represent the most recent time window while those from the muscle samples represent the migratory period. The importance of this food item to the diet of juvenile Magellanic penguins feeding in Brazilian waters, during the wintering period, was recently confirmed by Silva *et al.* (2015), indicating a significant consumption of Argentine anchovy, as well as the squid *D. sanpaulensis*, in south Brazil. With isotopic mixing models, using values of food items from Argentinean Patagonia, the importance of the Argentine anchovy was mantained, for both adults and juveniles. Argentine anchovy are the dominant prey species for penguins in northern Argentinean colonies (Wilson *et al*., 2005; Boersma *et al*., 2009), and the distribution of their stocks are associated with high productivity and sea fronts (Hansen *et al.*, 2001). This result seems to confirm that the migration of Magellanic penguins is related to the northward movement of northern stock of anchovy in winter, as previously speculated (Cardoso *et al.*, 2011; Costa *et al.*, 2016), while during breeding and pre-moulting periods they feed on the southern stocks of the Argentine Anchovy, adjacent to nesting grounds.

Adults and juveniles presented some overlap in isotopic niches which is expected as both occupy waters on the inner shelf, where most Argentine anchovy shoals are located (Costa *et al.*, 2016).

4.2 Temporal variation

Stable isotope analysis in feathers showed variation of δ^{15}N values, with a higher standard deviation in comparison to other tissues sampled. These values can be partially influenced by variations on isotopic values at the base of the trophic chain, in different places (Hobson *et al*., 2004; McMahon *et al*., 2013) and by different metabolic rates of each tissue (Hobson and Clark, 1992a). As feathers are an inert tissue formed during the nesting period in juveniles and during the post-breeding/moulting period in adults, and Magellanic penguins fast on land during moult, its isotopic composition reflects the food ingested just before the synthesis period (Hobson, 1999; Cherel *et al.*, 2005). Both models for feathers in the current study, using sources from southern Brazil and also from Patagonia did not altered the main conclusion, i,e. strong reliance on Argentine anchovy. However, the higher variation in δ^{15}N values in feathers in comparison to both liver and muscle suggests the occurrence of penguins from distinct colonies, forming a mixed stock in wintering grounds in southern Brazil.

4.3 Age and sex variation in diet in wintering areas

In 2015, juvenile Magellanic penguins stranded on the beach in southern Brazil predominated in relation to adults, as formally shown by Mäder *et al.* (2010) that estimated that 19,500 individuals each year were juveniles. The opposite was found at sea, where adults predominated as bycatch in gillnets, similar to results by Cardoso *et al.* (2011).

The values of δ^{15}N in juvenile tissues were significantly lower in comparison to adults, although SIAR shows high importance of Argentine anchovy for both classes. Silva *et al.* (2015) based on stable isotopes in claws of emaciated juveniles sampled in wintering grounds found the same result, confirming the importance of fish in their diet, in opposition to results based on stomach contents only, where squids usually predominate (Fonseca *et al.*, 2001; Pinto *et al.*, 2007; Di Beneditto *et al.*, 2015).

In our study, females represented 82% of the 28 penguins with sex determined by gonad inspection. This result is similar to previous ones along the Brazilian coast, where a greater female proportion (about 70%) among stranded individuals had been reported (Reis *et al.*, 2011; Vanstreels *et al.*, 2013; Nunes *et al.*, 2015). Despite males present larger size and body mass than females (Bertellotti *et al.*, 2002), it was verified slightly differences between sexes during foraging trips in breeding grounds (Raya-Rei *et al.*, 2012) depending on food availability (Frere *et al.*, 1996; Pütz *et al.*, 2007). However, as suggested by Raya-Rei *et al.* (2012), larger individuals have larger mass-specific energy expenditure, being able to travel further and potentially catch more food. In this study, the GLM models did not show significant differences between SI values of sexes, but in Argentinean Patagonia higher values of δ^{13}C and δ^{15}N were reported for adult males compared to females, indicating that males fed significantly more on fish (anchovy) that females (Forero *et al.*, 2002). Overall, the predominance of females in southern Brazil could be explained by males using more offshore areas, or remaining in areas south of females, closer to breeding grounds.

4.4 Interactions with human activities

Between 2013 and 2015 Magellanic penguins collected dead from gillnet fishing appeared to be healthy, with fat reserves, high BMI and no evidence of injury during necropsy. During winter in southern Brazil, several Magellanic penguins are caught by bottom gillnet fisheries targeting stripped weakfish and by drift gillnets targeting bluefish (Cardoso *et al.*, 2011; Fogliarini *et al.*, in revision). During summer, penguins are affected by fisheries in areas adjacent to their breeding colonies, e.g. at Golfo San Jorge in Patagonia, where the estimative of penguin mortality by shrimp fisheries was 0.33% of the breeding population every summer (Gandini and Frere, 1999). The effect of fisheries near Argentinean colonies is one of the main causes of decrease in Magellanic penguin populations, not only as bycatch, but also due to overexploitation of their main food sources, such as the Argentine anchovy (Gandini *et al.*, 1996; Gandini and Frere 1999; Scolaro *et al.*, 1999; Yorio *et al.*, 2010; Boersma *et al*., 2015). In southern Brazil, Argentine anchovy are currently only fished to be used as live-bait for the skipjack tuna (*Katsuwonus pelamis*) pole-and-line fishery (Carvalho and Castello, 2013). However, evaluation of stocks are being performed aiming a potential commercial exploitation (Madureira *et al.*, 2009) that could represent a risk to the supply of main food item for the Magellanic penguin. If these plans go forward, the use of the Anchovy by penguins should be considered, for example, in the calculations of maximum allowable catches.

In addition to fisheries, other threat for Magellanic penguins, and maybe more important at the moment, is the environmental pollution (Pinto *et al.*, 2007). Plastic and fishing-related items were found in 29% out of 41 stomach contents analyzed in the current study. In the state of Rio de Janeiro, 14.9% out of 175 stomach contents presented human debris (Brandão *et al.*, 2011). Plastic pollution is a chronic problem along the distribution areas of Magellanic penguins, affecting also other seabirds such as albatrosses and petrels (Tourinho *et al.*, 2010), sea turtles (Bugoni *et al.*, 2001; Colferai *et al.*, 2017) and marine mammals (Denuncio *et al.*, 2011). The ingestion of plastic debris by seabirds is a global problem, not only leading individuals to death, but also reducing body condition and affecting reproduction success (Wilcox *et al.*, 2015).

This study stresses the importance of the anchovy in the winter trophic relations in southernmost Brazil including both adult and juvenile Magellanic penguins. Therefore, the stocks of this small pelagic fish should be exploited sustainably, ensuring not only the minimal forage biomass for seabirds (Cury *et al.,* 2011), but also for other top predators relying on this key-stone forage fish.

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Table 1

Allometric equations to estimate total length (TL) and Mass (M) for fish, from otolith measurements, and mantle length (ML) and mass for cephalopods from upper (URL) and lower (LRL) rostral lengths of squids and, upper (UHL) and lower (LHL) hood lengths from *Argonauta nodosa*. Otl = otolith length and Otw = otolith width. The equations for fish are from Naves (1999) and for cephalopods are from Santos (1999).

Species	Total length × Otolith length	Mass × Total length	Total length × Otolith width
Fish			
Anchoa marinii	$TL = -2.15 + 28.271Otl$	$M = 0.0000027TL^{3.146719}$	$TL = -20.53 + 54.546Otw$
Engraulis anchoita	$TL = 35.355345Otl^{1.0309666}$	$M = 0.0000076TL^{2.9566755}$	$TL = 64.55592Otw^{1.236113}$
Lycengraulis grossidens	$TL = 38.106486Otl^{1.080817}$	$M = 4.2407473(10^{-7})TL^{3.54467624}$	$TL = 55.756704Otw^{1.5481124}$
Odontesthes argentinensis	$TL = 39.71408Otl^{1.1932243}$	$M = 0.0000079TL^{2.9644835}$	$TL = 56.132136Otw^{1.5004836}$
Cephalopods			
Doryteuthis sanpaulensis	$ML = 14.408e^{1.1418URL}$	$M = 0.3804e^{2.6451URL}$	
	$ML = 13.497e^{1.0836LRL}$	$M = 0.2947e^{2.5972LRL}$	
Doryteuthis pley	$ML = 67.431URL^{1.2908}$	$M = 8.8096URL^{2.8564}$	
	$ML = 64.303LRL^{1.3143}$	$M = 7.9418LRL^{2.908}$	
Argonauta nodosa	$ML = 4.9237UHL^{1.2933}$	$M = 0.0377UHL^{3.4949}$	
	$ML = 9.5338LHL^{1.2314}$	$M = 0.2593LHL^{3.1856}$	

Table 2

Stable isotopes values of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) ± SD of potentially prey items used to calculate the best model for prey contribution in the diet of *Spheniscus magellanicus from both age classes. n:* Number of individuals. Mean length: mean of total length for *Artemesia longinaris* and the fish, and mean of total mantle length for *D. sanpaulensis*.

Species	*n*	Mean length ± *SD* (mm)	$\delta^{13}C$ (‰)	± SD	$\delta^{15}N$ (‰)	± SD
Artemesia longinaris[a]	12	1 ± 0.7	-15.4	0.5	15.6	0.6
Anchoa marinii	1	79	-16.7	0.0	16.7	0.0
Chloroscombrus chrysurus	1	60	-16.8	0.0	17.9	0.0
Cinoscion guatucupa[a]	2	123.9 ±29.5	-16.7	0.1	17.4	0.6
Doryteuthis sanpaulensis[b]	7	11.1 ± 4.6	-15.5	0.3	17.8	0.5
Doryteuthis sp. + *Illex* sp.[e]	-	-	-17	2.1	16.3	2.1
Engraulis anchoita	6	109.9 ± 17.4	-17.6	0.6	16.3	0.3
Engraulis anchoita[c]	-	-	-17.7	0.4	16.4	0.4
Mugil brevirostris	3	122 ± 7.8	-13.1	0.1	9.5	1.8
Mugil sp.	1	133	-13.5	0.0	11.1	0.0
Odontesthes argentinensis	12	104.1 ± 35.5	-16.8	1.4	17.6	1.0
Oligoplites saliens	7	57 ± 18.5	-16.7	0.2	16.8	0.3
Peisos petrunkevitchi[d]	-	-	-18	0.2	13	0.4
Pomatomus saltatrix	1	88	-16.7	0.0	17.9	0.0
Trichiurus lepturus[a]	1	59.7	-16.7	0.0	20.3	0.0

[a] Prey found in stomach contents of *Pomatomus saltatrix* and *Cynoscion guatucupa*, from gillnet fishing.

[b] Prey provided by fishing in the region.

[c] Values used by Yorio *et al.* (2017).

[d] Values used by Ciancio *et al.* (2008).

[e] Values used by Forero *et al.* (2004).

Table 3

Discrimination factors with species and controlled diet used in SIAR models for liver. muscle and feather samples of Magellanic penguins (*Spheniscus magellanicus*) found in southern Brazil.

Species	Tissue	Diet	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	References
Phalacrocorax carbo	Liver	Fish (mackerel)	1.3	2.3	Mizutani *et al.* 1991
Larus delawarensis	Liver	Fish (perch)	-0.4	2.7	Hobson and Clark 1992b
Phalacrocorax carbo	Muscle	Fish (mackerel)	2.1	2.4	Mizutani *et al.* 1991
Larus delawarensis	Muscle	Fish (perch)	0.3	1.4	Hobson and Clark 1992b
Phalacrocorax carbo	Feather	Fish (sprat)	2.6	4.9	Bearhop *et al.* 1999
Phalacrocorax carbo	Feather	Fish (mackerel)	3.6	3.6	Mizutani *et al.* 1991
Phalacrocorax carbo	Feather	Fish (mackerel)	3.8	3.7	Mizutani *et al*. 1992
Eudyptes chrysocome	Feather	Fish	0.11	4.4	Cherel *et al.* 2005
Eudyptes chrysocome	Feather	Fish muscle	0.55	3.53	Cherel *et al.* 2005
Spheniscus humboldti	Feather	Fish (anchovy)	2.9	4.8	Mizutani *et al.* 1992
Aptenodytes patagonicus	Feather	Fish	0.7	3.49	Cherel *et al*. 2005
Aptenodytes patagonicus	Feather	Fish muscle	0.26	2.65	Cherel *et al*. 2005
Larus delawarensis	Feather	Fish (perch)	0.2	3	Hobson and Clark 1992b
Pygocelys papua	Feather	Fish (herring)	1.3	3.5	Polito *et al.* 2011

Table 4

Prey items found in stomach contents of 21 adult and 20 juvenile Magellanic penguins (*Sphensicus magellanicus*) collected at sea as bycatch in gillnet fisheries and stranded dead on the beach in southern Brazil between 2013 and 2015. Frequency of occurrence absolute (N) and relative (FO%). Contribution by number absolute (N) and relative (PN%), contribution by mass absolute (M in g) and relative (PM%). and Prey-specific index of relative importance (PISIRI%). *Not included in the PSIRI.

	Adults							Juveniles						
Food items	FO	FO%	N	PN%	M (g)	PM%	PSIRI%	FO	FO%	N	PN%	M (g)	PM%	PSIRI%
Cephalopods	8	38	110	81.1	2154	82.3	13.6	19	95	2286	119.6	8650.1	133.7	71.1
Loliginidae NI	-	-	-	-	-	-	-	4	20	28	6.4	684	17.9	2.4
Doryteuthis plei	1	4.8	4	4.7	151.1	8.1	0.3	4	20	5	0.6	188.8	4.4	0.5
Doryteuthis sanpaulensis	8	38.1	56	11.9	1327.2	25.3	7.1	16	80	119	31.4	2820.3	45.7	30.8
Argonauta nodosa	2	9.5	24	51.3	40.4	27.7	3.8	11	55	2074	55.9	3491.2	28.6	23.2
Cephalopod NI	3	14.3	26	13.3	635.2	21.3	2.5	9	45	60	25.4	1465.8	37	14
Fishes	20	95.2	415	179	2955	170.5	86.4	16	80	336	109.2	2399.6	84.4	28.9
Engraulididae NI	14	66.7	86	27.6	559.4	24.3	17.3	1	5	1	0.5	6.5	0.8	0
Anchoa marinii	2	9.5	16	9	32	2.6	0.6	1	5	4	1.9	2.3	0.3	0.1
Engraulis anchoita	14	66.7	124	44.4	947.36	43	29.1	-	-	-	-	-	-	-

Lycengraulis grossidens	4	19	7	7.1	87.8	10.3	1.7	-	-	-	-	-	-	-
Atherinopsidae NI	1	4.8	6	17.1	47.9	17.4	0.8	-	-	-	-	-	-	-
Odontesthes argentinensis	-	-	-	-	-	-	-	3	15	17	18.7	163.6	15	2.5
Odontesthes sp.	3	14.3	28	30.3	223.3	31	4.4	2	10	24	33	191.4	23.4	2.8
Mugil brevirostris	-	-	-	-	-	-	-	2	10	3	6.1	44.8	6.7	0.6
Mugil sp.	-	-	-	-	-	-	-	2	10	3	8.8	38.6	8.1	0.8
Chloroscombrus chrysurus	-	-	-	-	-	-	-	1	5	1	0.6	1.3	0.2	0
Oligoplites saliens	-	-	-	-	-	-	-	1	5	12	7.7	19.2	3.5	0.3
Pomatomus saltatrix	-	-	-	-	-	-	-	1	5	1	3.1	4	1.2	0.1
Fish NI	16	76.2	148	43.5	1057	42	32.6	16	80	270	28.9	1927.9	25.2	21.6
Non-food items*														
Plant remains	2	9.5	-	-	-	-	-	8	40	-	-	-	-	-
Isopod	-	-	-	-	-	-	-	1	5	-	-	-	-	-
Mollusk	-	-	-	-	-	-	-	4	20	-	-	-	-	-
Fish eggs	1	4.8	-	-	-	-	-	-	-	-	-	-	-	-
Plastic sheets	4	19	5	-				8	40	42	-	-	-	-
Nylon	1	4.8	1	-	-	-	-	1	5	1	-	-	-	-
Fishing rope	-	-	-	-	-	-	-	1	5	1	-	-	-	-

Table 5

Total length and body mass of cephalopods and fish found in 41 stomach contents samples of adult and juvenile Magellanic penguins (*Spheniscus magellanicus*). The original values of species with one individual are given. *n* is the number of prey.

	Total length (mm)			Mass (g)			n
	Mean	Min	Max	Mean	Min	Max	
Cephalopods	69.8	3.1	250.2	22.4	0.01	283.2	2638
Loliginidae	75.5	25.9	250.2	24.4	1.1	283.2	180
Doryteuthis plei	108.4	25.9	181.3	37.8	1.1	78.7	9
Doryteuthis sanpaulensis	73.8	25.9	250.2	23.7	1.40	283.2	171
Argonauta nodosa	15.9	3.1	63.3	1.7	0.01	37.4	2098
Cephalopods NI	75.5	25.9	250.2	24.4	1.1	283.2	180
All fish	96	37	143	10	0.3	19.1	406
Engraulidae	106.3	79	137	6.5	2.3	13.8	20

Anchoa marinii	45.5	40.2	79	0.6	0.3	3.4	17
Engraulis anchoita	105.8	57.4	136.2	7.6	1.2	15.5	112
Lycengraulis grossidens	122.4	102.6	138.5	11.4	5.8	16.7	7
Atherinopsidae	107.1	40	143	8	0.7	15.3	21
Odontesthes argentinensis	120.9	94	143	9.6	3.8	15.3	17
Odontesthes sp.	107.1	40	143	8	0.7	15.3	21
Mugil brevirostris	122	117	131	14.9	13.2	17.8	3
Mugil sp.	106.6	61.3	133	12.9	3.2	19.1	4
Chloroscombrus chrysurus	60	-	-	1.3	-	-	1
Oligoplites saliens	55.3	37	89	1.6	0.6	3.8	6
Pomatomus saltatrix	88	-	-	40.2	-	-	1
Fish NI	99.5	37	143	7.1	0.3	19.1	176

Table 6

Contribution of main items values of the 95% credibility interval (CI) from SIAR for adult and juvenile Magellanic penguins (*Spheniscus magellanicus*) from liver, muscle and feather samples.

	Liver		Muscle		Feather	
Prey items	Adult (%)	Juveniles (%)	Adult (%)	Juveniles (%)	Adult (%)	Juveniles (%)
D. sanpaulensis	6.6 – 38.0	4.6 – 32.9	0.0 – 8.0	0.1 – 10.1	0.2 – 18.6	0.7 – 26.6
E. anchoita	39.8 – 67.0	55.5 – 80.6	82.5 – 98.9	79.0 – 98.6	55.3 – 95.5	46.0 – 86.7
O. argentinensis	4.7 –43.3	0.7 – 28.4	0.1 – 14.6	0.2 – 17.5	<0.1 – 39.4	2.1 – 44.7
Doryteuthis sp. + *Illex* sp.[a]	-	-	-	-	1.5 – 42	4.4 – 39.0
Engraulis anchoita[b]	-	-	-	-	13.0 – 64	18.2 – 59.2
Peisos petrunkevitchi[c]	-	-	-	-	21.0 – 59.4	22.0 – 56.0

[a] Values used by Forero *et al.* (2004)
[b] Values used by Yorio *et al.* (2017)
[c] Values used by Ciancio *et al.* (2008).

Table 7

Generalized linear model (GLM) with coefficients of the model. Only significant terms ($P \leq 0.05$) are shown. The values of intercept represents $\delta^{13}C$ and $\delta^{15}N$ for feather tissue and for adult age, in relation to comparison to the other levels

	Estimate	Standard error	*t*-value	*P*
$\delta^{13}C$ (AIC = 309.69; df = 155)				
Intercept	-17.306	0.272	-63.523	<0.001
Main effects				
Age class	0.810	0.169	4.807	<0.001
Interactions				
Tissue muscle:Age class	-0.298	0.240	-2.523	0.013
$\delta^{15}N$ (AIC = 579.55; df = 155)				
Intercept	20.621	0.630	32.740	<0.001
Main effects				
Age class	-1.206	0.390	-3.095	0.002
Tissue liver	-1.956	0.898	-2.177	0.030
Tissue muscle	-3.091	0.890	-3.470	0.001
Interactions				
Tissue liver:Age class	1.546	0.554	2.789	0.006
Tissue muscle:Age class	1.220	0.551	2.213	0.028

. df = degrees of freedom for the residuals of the model.

Table 8

Summary of stable carbon (δ^{133}C) and nitrogen (δ^{15}N) isotopes values (mean ± SD) from liver, muscle and feather samples of Magellanic penguins (*Spheniscus magellanicus*), in addition to characteristics as area of sampling, age and sex.

						Tissue					
			Sex			Liver		Muscle		Feather	
Area	Age	*N*	F	M	NI	δ^{13}C(‰)	δ^{15}N(‰)	δ^{13}C(‰)	δ^{15}N(‰)	δ^{13}C(‰)	δ^{15}N(‰)
Beach	Adult	7	3	1	3	-16.4 ± 0.5	19.1 ± 0.5	-16.8 ± 0.4	17.4 ± 0.7	-16.0 ± 0.8	19.3 ± 2.2
	Juvenile	27	6	2	19	-16.4 ± 0.4	19.3 ± 0.7	-16.9 ± 0.4	17.5 ± 0.8	-15.9 ± 0.9	18.6 ± 2.4
Sea	Adult	18	14	2	2	-16.6 ± 0.5	19.2 ± 0.6	-17.1 ± 0.5	17.6 ± 0.8	-16.1 ± 0.9	18.8 ± 2.3
	Juvenile	2	-	-	2	-16.5 ± 0.2	18.9 ± 0.3	-17.3 ± 0.3	17.4 ± 0.2	-16.5 ± 0.7	19.2 ± 2.0

Table 9

Summary of ANOVA from generalized linear model (GLM) for $\delta^{13}C$ and $\delta^{15}N$ values. The % explained is calculated as the ratio between the deviance/deviance residuals of the null model*100 (Ye *et al*. 2001).

Source of variation	df	Deviance	% explained	df of residuals	Residuals deviance	*F*	*P*
$\delta^{13}C$							
Null model				160	100.038		
Main effects							
Tissue	2	28.035	28.02	158	72.003	36.731	<0.001
Age class	1	10.420	10.42	157	61.583	27.303	<0.001
Interactions							
Tissue:Age	2	2.430	2.43	155	59.153	3.184	0.0441
Total explained	5	40.885	40.87				
$\delta^{15}N$							
Null model				160	415.00		
Main effects							
Tissue	2	77.793	18.75	158	337.21	19.070	<0.001
Age	1	3.335	0.80	157	333.87	1.635	0.203
Interactions							
Tissue:Age class	2	17.717	4.27	155	316.15	4.343	0.015
Total explained	5	98.845	23.82				

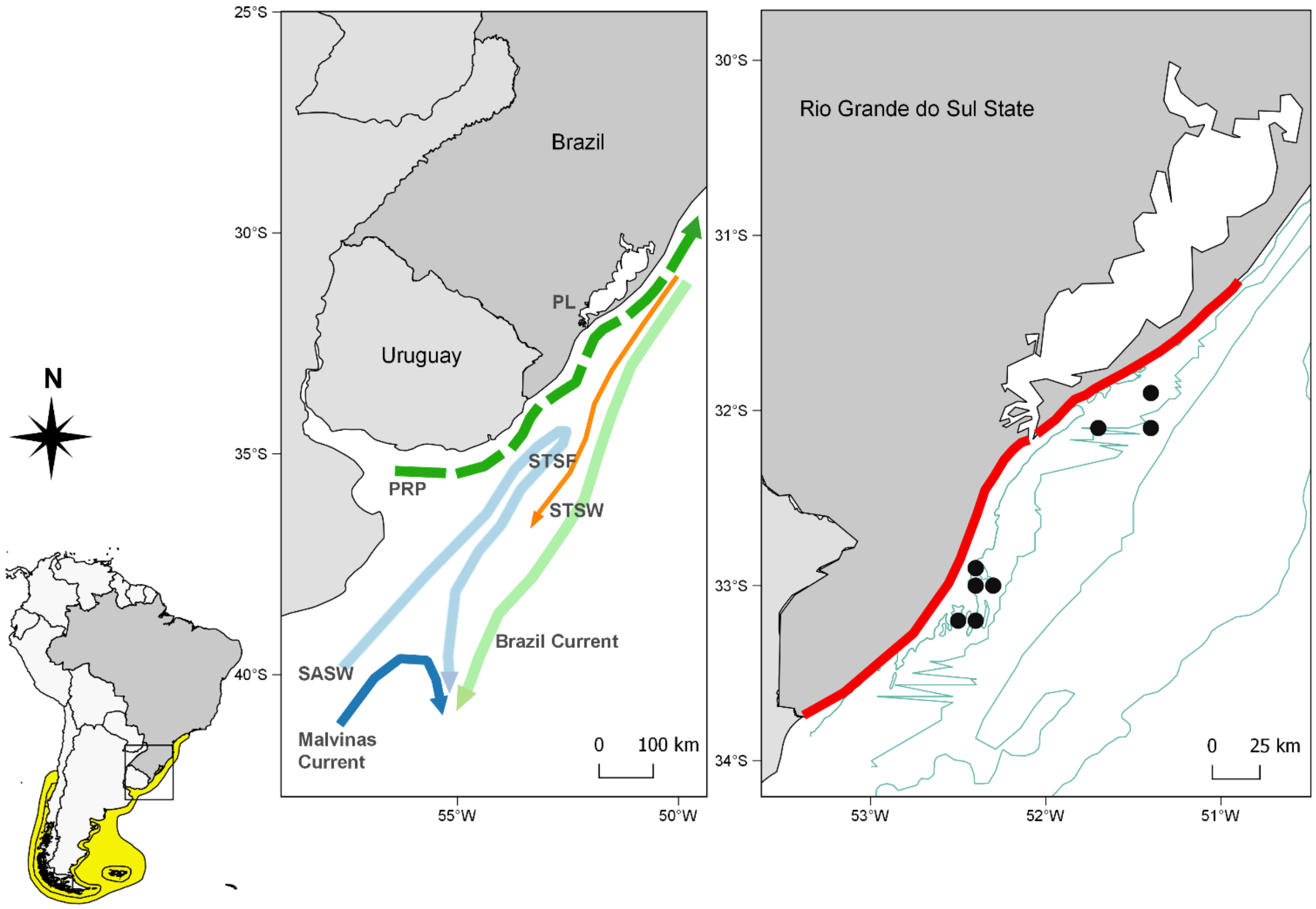

Fig. 1. Distribution map of Magellanic penguin (*Spheniscus magellanicus*). The map on the left shows the distribution of Magellanic penguins during breeding and non-breeding periods (yellow). The central map shows a scheme of oceanographic patterns over the continental shelf along the wintering grounds of penguins (for details, see Piola *et al*., 2008). PRP - La Plata River plume; PL - Patos Lagoon; STSW - Subtropical Shelf Waters; SASW - SubAntarctic Shelf Waters. The right map shows the area of beach monitoring (red) where stranded penguins were collected. The blue lines shows isobaths of 15, 20, 50 and 100 m, respectively. The black dots are the local where penguins were bycaught at sea in fishing nets. These points are calculated as the intermediate position where nets were release and hauled (Fogliarini *et al.*, in revision).

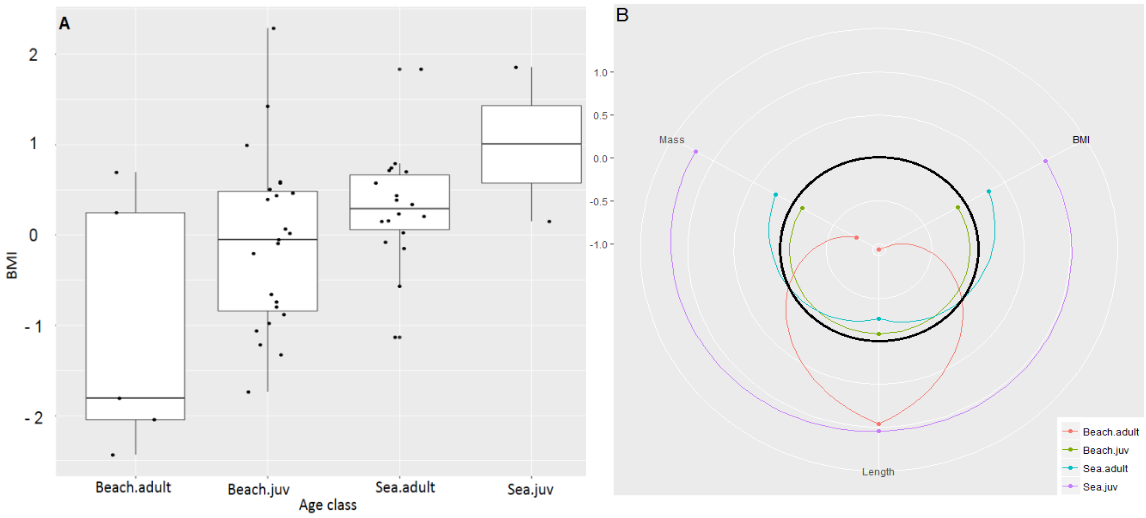

Fig. 2. Body Mass Index (BMI) of Magellanic penguins (*Spheniscus magellanicus*) collected between 2013 and 2015 at sea, and in 2015 on the beach in southern Brazil, separated by area and age classes (A). The PCA analysis (B) shows graphically in black the mean (mean = 0). Note the values of BMI and Mass well below mean values for beached birds.

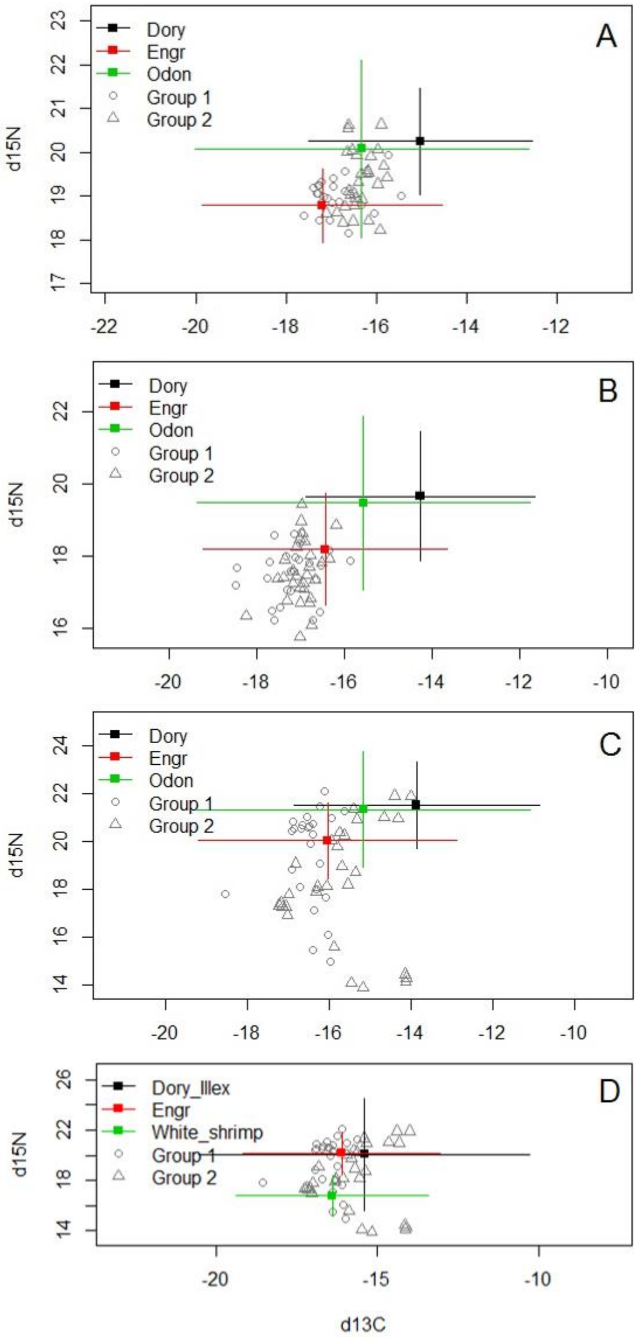

Fig. 3. Stable isotopes values in liver (A), muscle (B) and feather (C) samples of Magellanic penguins (*Spheniscus magellanicus*) in southern Brazil, and the modeling test with Argentinean sources in feathers (D), based on SIAR with adults (Group 1) and juveniles (Group 2). Values of potential prey items are *D. sanpaulensis* (black), *E. anchoita* (red) and *O. argentinensis* (green). For (D) are *Doryteuthis* sp. + *Illex* sp. (black), *E. anchoita* (red) and white shrimp (green). Values of sources were corrected for trophic discriminant factor for each tissue.

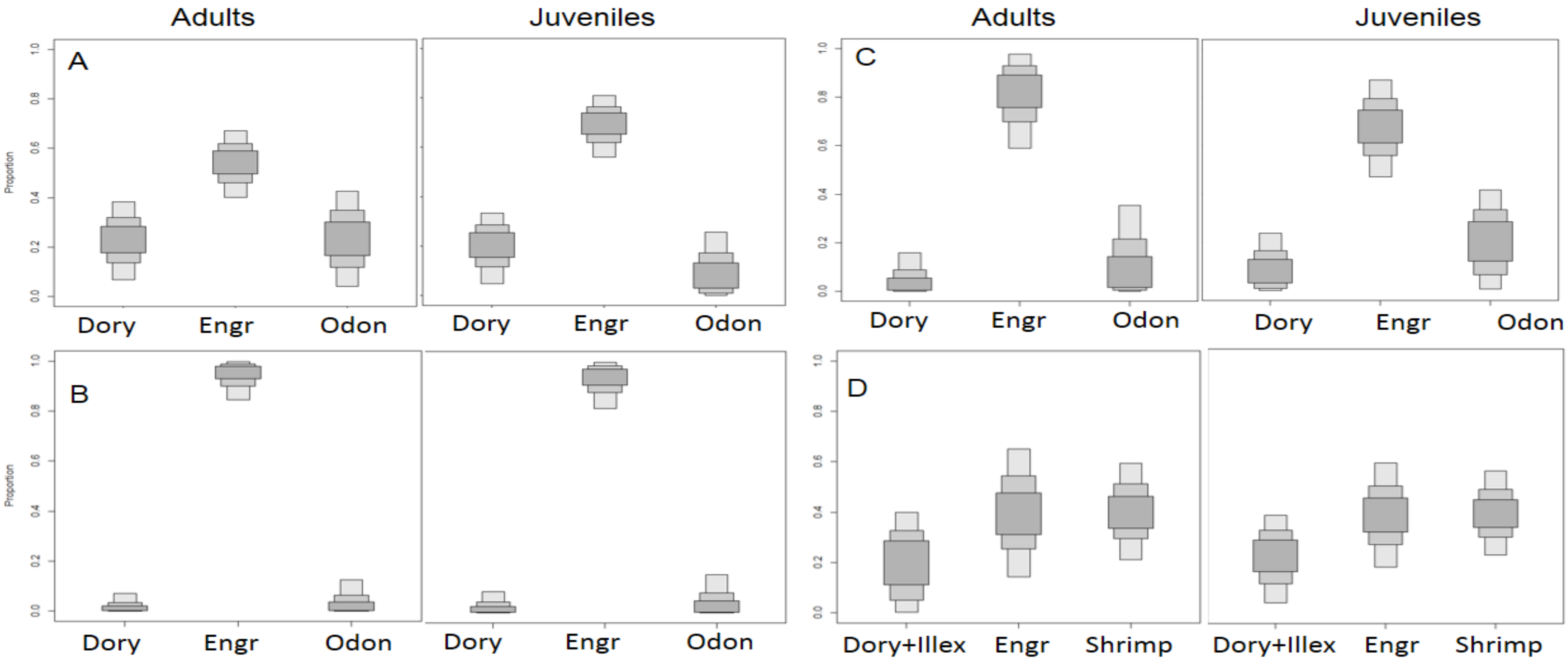

Fig. 4. SIAR mixing model plots. With 95, 75 and 25% credibility intervals from liver (A), muscle (B) and feather (C and D) samples of Magellanic penguin (*Spheniscus magellanicus*). Sources are the squid *D. sanpaulensis* (Dory), fish *E. anchoita* (Engr) and *O. argentinensis* (Odon) for "A", "B" and "C", and *Doruteuthis* sp. plus *Illex* sp. (Dory_Illex), *E. anchoita* (Engr) and the *P. petrunkevitch* (Shrimp) for "D".

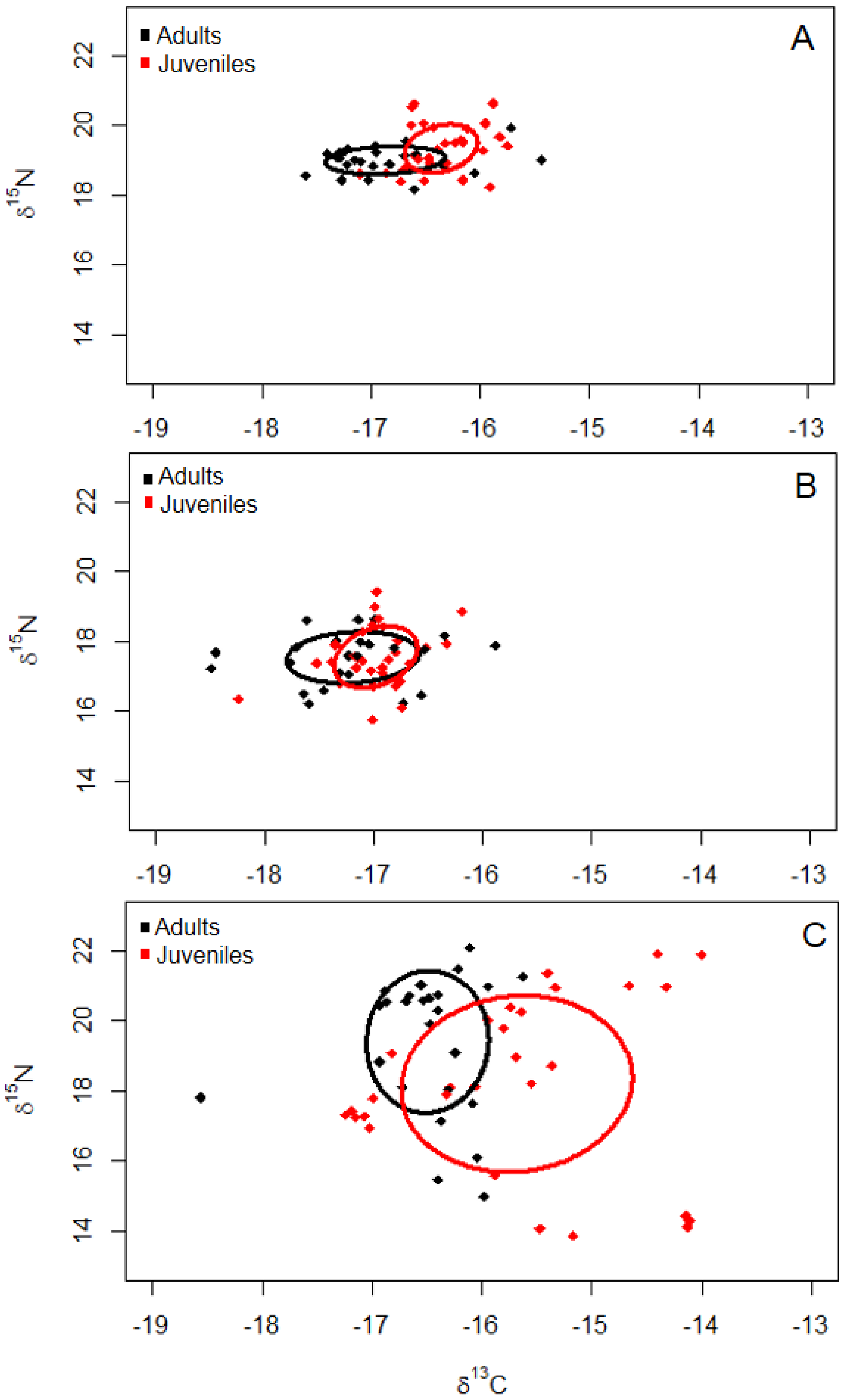

Fig. 5. Isotopic niche of Magellanic penguins (*Spheniscus magellanicus*) adults and juveniles from liver (A), muscle (B) and feather (C) samples, based on standard ellipses areas corrected for small samples sizes (SEAc) using SIBER.