Estuarine, Coastal and Shelf Science 210 (2018) 109–122

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

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

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ARTICLE INFO

Keywords:
Bycatch
Diet
Engraulis anchoita
Plastic pollution
Stable isotopes
Wintering period

ABSTRACT

Some top predators couple their migration with the migration of their main prey. Magellanic penguins, *Spheniscus magellanicus*, migrate north from their Patagonian colonies during their non-breeding period, supposedly following the Argentine anchovy, *Engraulis anchoita*, a keystone species and their main prey item in the southwestern Atlantic Ocean. However, previous studies based on stranded carcasses suggest that cephalopods are the main food item for the penguins at their Brazilian wintering grounds. Moreover, anchovy stocks are in the early stages of commercial exploitation in southern Brazil. This study aims to investigate the importance of the Argentine anchovy in the diet of Magellanic penguins, including penguins of different age classes, both healthy and debilitated individuals, and in different parts of their annual cycle, using stomach content analysis and stable isotope analysis of multiple tissues, representing different time windows. Juvenile and adult penguins were collected ($n = 54$), either stranded on the beach or incidentally killed during gillnet fishing in adjacent waters. Penguins collected at sea had higher body mass indexes compared to stranded, demonstrating that they were healthy individuals. Among adult penguins from both areas ($n = 21$), fish was the main food item in their stomachs (prey-specific index of relative importance, PSIRI = 86%), with high contribution of Argentine anchovy. Cephalopods were the main food item (PSIRI = 71%) for juveniles ($n = 20$), with a predominance of the squid *Doryteuthis sanpaulensis*. In liver, muscle and feathers of penguins, mixed models based on $\delta^{13}C$ and $\delta^{15}N$ values demonstrated large contribution of the Argentine anchovy in the diet of the juveniles (95% credibility interval = 46–98.6%) and adults (39.8–98.9%), despite the high importance of cephalopods in the juvenile diet as identified in the stomach content analysis. Adults and juveniles presented isotopic niche overlap for all the tissues analysed, despite the differences in isotopic niche areas (= niche width) among the age classes. In 29% of the stomach contents, plastic debris was also found. Our results highlight the importance of using complementary techniques to study trophic ecology, as stomach content analysis of individuals found dead on beaches may not provide reliable information on diet. The strong reliance of penguins on the Argentine anchovy as a key resource throughout its annual cycle suggests that the stocks of this small pelagic fish should be exploited sustainably, ensuring the minimal forage biomass needed not only for seabirds but also for other consumers.

1. Introduction

Resources and areas where animals forage are key factors or dimensions used for ecological niche descriptions, and they are defined as *n*-dimensional hypervolume (Hutchinson, 1957). While sedentary species or those inhabiting stable environments usually have similar niches and diets during most of their annual cycles, migratory species or inhabitants of variable and seasonal environments typically change their niches throughout the year. Alternatively, migratory species could synchronize their migrations with the movements of their main food sources (Nathan et al., 2008). Migration coupled with prey migration is postulated to occur in Magellanic penguins, *Spheniscus magellanicus*,

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https://doi.org/10.1016/j.ecss.2018.06.001
Received 10 August 2017; Received in revised form 14 May 2018; Accepted 5 June 2018
Available online 15 June 2018

which migrate northward along the southwestern Atlantic Ocean, over the continental shelf, in the winter. From March to September, penguin migration is associated with highly productive zones and the availability of their main food item, the Argentine anchovy, *Engraulis anchoita* (Boersma et al., 1990; Stokes et al., 1998; Pütz et al., 2000, 2007). Northern and southern stocks of Argentine anchovies migrate to lower latitudes following these productive zones (*sensu* Hansen, 2004). However, previous studies on the diet of Magellanic penguins in Brazilian wintering grounds, based mostly on stomach content analysis of mostly emaciated juvenile penguins, failed to demonstrate this close association between penguins and anchovies, with cephalopods reported as their main food item (Fonseca et al., 2001; Pinto et al., 2007; Di Beneditto et al., 2015). In the northern Argentinean Patagonia colonies, several studies based on flushed stomach contents demonstrated a predominance of the Argentine anchovy (Gosztonyi, 1984; Frere et al., 1996). In the southern colonies, diets vary depending on the latitude, and Magellanic penguins are considered opportunistic and non-selective, relying mainly on fish (Frere et al., 1996; Pütz et al., 2001).

The lack of congruence between dietary studies in the wintering grounds and the alleged coupled migration could be due to biased sampling. In the Brazilian wintering areas, juvenile penguins die from severe emaciation, and thus, stomach contents could indicate abnormal feeding behaviours, mostly based only on longer-lasting harder remains such as squid beaks. Notwithstanding the common use of stomach content analysis of dead organisms to study food resources consumed by seabirds (Barrett et al., 2007; Karnovsky et al., 2012) and to infer trophic niches, there are limitations associated with this technique. Stomach content analysis allows the identification of ingested remains based on undigested fragments (e.g., cephalopods beaks, fish otoliths and plastic debris), but this type of analysis is unable to detect small or fragile prey that are completely digested (Barrett et al., 2007). In addition, this method could be unreliable if based on starved, unhealthy or abnormally behaving individuals. Additionally, stomach contents only provide information on recently ingested food over a short period, underestimating the amount of food ingested by an organism (van Heezic and Seddon, 1989; Barrett et al., 2007; Karnovsky et al., 2012).

Stable isotope analysis (SIA) is a complementary method for traditional diet studies. SIA allows the investigation of niche overlap between age and sex, and it enables the gathering of information on the intraspecific differences in foraging grounds, diet specialization, and the use of isotopic niche as a proxy for trophic or spatial dimensions (Newsome et al., 2007). SIA is used to investigate trophic positions and identify sources of primary production (Hobson et al., 1994; Fry, 2006). Values of $\delta^{13}C$ ($^{13}C/^{12}C$) vary approximately 1‰ at each trophic level and reflect basal food sources; thus, environments based on different producers (e.g., marine and freshwater environments) have distinct baseline values (Peterson and Fry, 1987; Hobson, 1995; Fry, 2006, see example in Farinós-Celdrán et al., in press). The values of $\delta^{15}N$ ($^{15}N/^{14}N$) are used to represent trophic levels, varying from 2 to 5‰ between trophic levels (DeNiro and Epstein, 1981; Vander-Zanden et al., 1997). Different tissues reflect dietary integrations over different periods, and sampling multiple tissues from the same individual can thus provide a time-series analysis of diet (Dalerum and Angerbjörn, 2005). Tissues with rapid turnover, such as the liver, reflect recent diets (days to a week); muscles have slower turnover rates (weeks to months) and thus reflect medium to long-term diets; and inert tissues, such as fur, feathers and claws, reflect diets during growth periods (Mizutani et al., 1991, 1992; Hobson and Clark, 1992).

Magellanic penguins are distributed throughout southern South America and adjacent islands, where they breed from late September to February (Scolaro et al., 1980; Boersma et al., 1990). After breeding, they leave colonies to forage and store reserves, before returning to moult on land during two to three weeks of fasting (Boersma et al., 1990; Wilson et al., 2005). Then, penguins from the Atlantic South American colonies 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), where Magellanic penguins are the only regular and abundant penguin species (Barquete et al., 2006). Throughout their migration at sea, the main threats to Magellanic penguins are oil (Gandini et al., 1994, 1996) and plastic (Brandão et al., 2011) pollution, as well as bycatch in fisheries (Gandini and Frere, 1999; Cardoso et al., 2011; Crawford et al., 2017) and overfishing of their main prey (Gandini et al., 1996). Along the southern Brazilian coast, it is estimated that 19,000 Magellanic penguins become stranded each year, and the majority are juveniles (97.5%) (Mäder et al., 2010) and females (about 60–70% in Reis et al., 2011, Vanstreels et al., 2013, Nunes et al., 2015).

In Brazil, Magellanic penguins occupy areas that are also intensively used by Brazilian fishing fleets (Vasconcellos et al., 2014; Haimovici and Cardoso, 2017), where keystone species, such as the Argentine anchovy, also occur (Acha et al., 2004; Costa et al., 2016). This spatial overlap between penguins and fishing fleets increases the chances of bycatch (Cardoso et al., 2011). Argentine anchovies inhabit the inner continental shelf of South America between 23°S and 47°S and are considered important for the energy flow to higher trophic levels in coastal food webs in southern Brazil (Lima and Castello, 1995). Commercially important fish species such as hake, *Merluccius hubbsi*, bluefish, *Pomatomus saltatrix* and striped weakfish, *Cynoscion guatucupa*, feed mostly on the Argentine anchovy (Haimovici et al., 1993; Lucena et al., 2000). Reliable information on the diets of top predators, such as penguins, is needed to understand trophic interactions and to adequately interpret their role in marine food webs (Yorio et al., 2017). This information is even more important as the Argentine anchovy is in the early stages of commercial exploration in southern Brazil; thus, there is still time for ecosystem-based management practices (Carvalho and Castello, 2013; Costa et al., 2016).

In this study, dietary analyses of Magellanic penguins were conducted using complementary methods, with individuals from different age classes found dead in distinct areas and in wintering grounds in southern Brazil. The 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, to compare isotopic niches between adults and juveniles during their wintering period and to assess if stomach content analysis of emaciated and unhealthy penguins found dead on beaches can provide reliable information on the diet of this species.

2. Material and methods

2.1. Study area

Dead Magellanic penguins were collected on the coast and at sea, in Rio Grande do Sul State (RS), in 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 bottom and drift gillnet fisheries were also used in the analysis. These fisheries operated between 32°–33°S and 51°–53°W, at depth of 9–21 m. The mean ± 1 SD of total net lengths were 1.23 ± 0.6 km for drift gillnets and 10.6 ± 4 km for bottom gillnets (Fogliarini et al., unpublished data).

During the winter, oceanographic patterns over the continental shelf in southern Brazil are characterized by the mixing of sub-Antarctic shelf waters (SASW; derived by the Falkland/Malvinas Current and also transported by the western branch, the Patagonian Current) and subtropical shelf waters (STSW; influenced by the tropical Brazilian Current and coastal waters), resulting in the subtropical shelf front (STSF) (Fig. 1). The STSF is a transition zone with a latitudinal thermohaline gradient and high productivity between 33° and 36°S (Möller et al., 2008; Piola et al., 2008). This high productivity is influenced by cold waters from SASW and by the low salinity waters from La Plata River and Patos Lagoon plumes at the surface (Piola et al., 2008; Costa

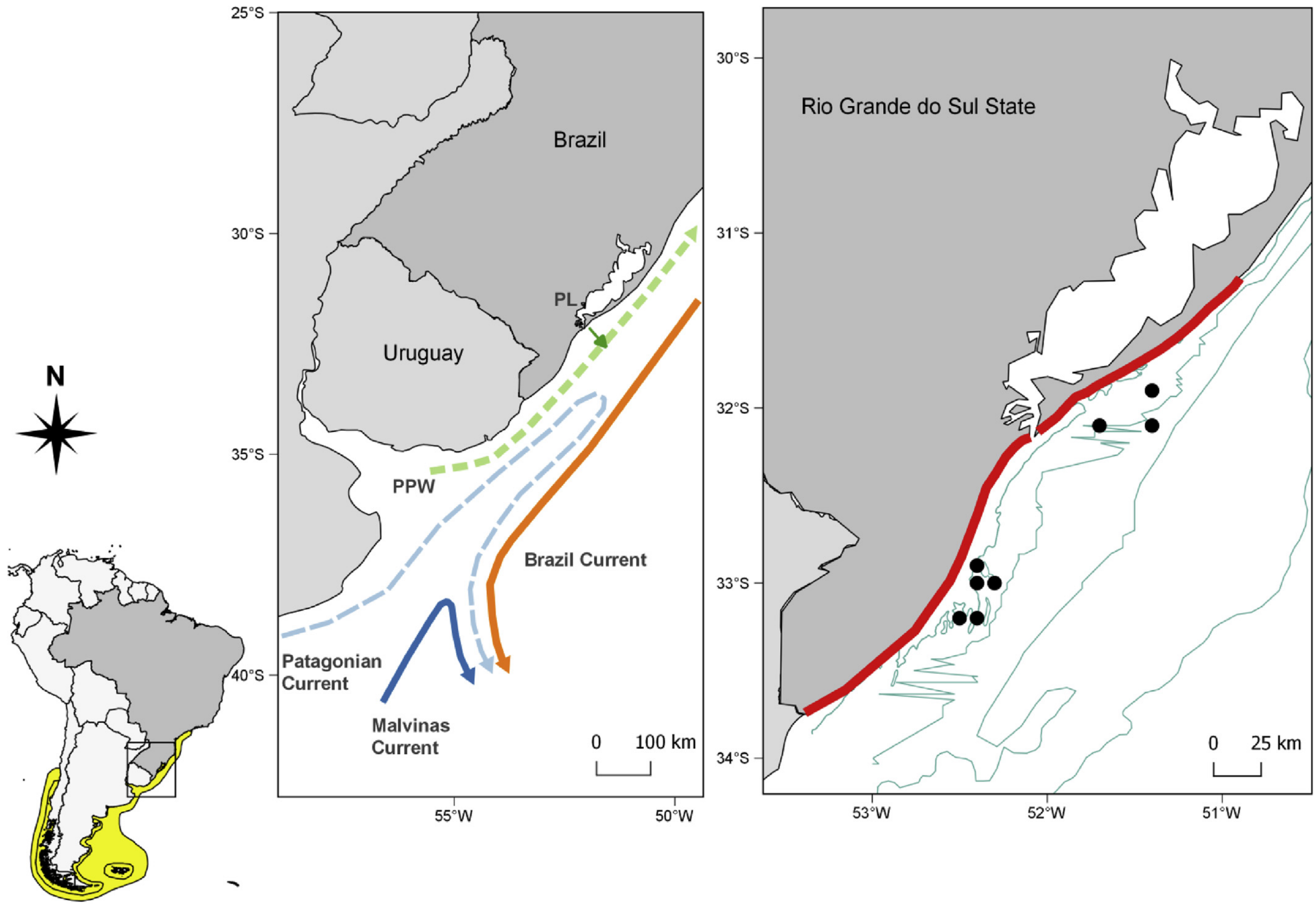

Fig. 1. Distribution map of the Magellanic penguin, *Spheniscus magellanicus*. The map on the left shows the distribution of Magellanic penguins including breeding and non-breeding periods (yellow) (BirdLife International and HBW, 2017). The central map shows a scheme of currents over the continental shelf along the wintering grounds of the penguins (for details, see Möller et al., 2008). PPW—La Plata Plume waters from the La Plata River plume flowing northward (light green); PL – Patos Lagoon outflow (dark green); Brazil Current (orange); Patagonian Current (light blue); Falkland/Malvinas Current (dark blue). The right map shows the area of beach monitoring (red) where the stranded penguins were collected. The blue lines show isobaths of 15, 20, 50 and 100 m. The black dots are the areas where penguins were collected at sea as bycatch in fishing nets. These points were calculated as the intermediate positions where nets were released and hauled (Fogliarini et al., unpublished data). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

et al., 2016). An important portion of the adult anchovy stock that migrates from Uruguay and Argentine continental shelves to Brazil is associated with this cold and productive water intrusion (Costa et al., 2016).

2.2. Sampling methods

During the austral winter and spring of 2015, a total of 34 (7 adults and 27 juveniles) Magellanic penguins were found stranded dead on the beach and collected. The penguins were selected based on the following characteristics: limited decomposition, with visually preserved skin and plumage and the absence of larvae and perforations. Additionally, 20 individuals were collected by onboard observers when they were incidentally captured by gillnet fishing vessels (18 adults and 2 juveniles) in the austral winters of 2013, 2014 and 2015. All 54 individuals were frozen and then necropsied. Contour feathers from the back, ventral area and head, pectoral muscles, liver and stomach contents were collected. Feathers were stored at room temperature, and all other tissues and stomach contents were frozen. The sex of the collected individuals was determined by gonad inspection (Proctor and Lynch, 1998).

2.3. Diet composition

In the laboratory, prey remains found in stomach were identified under a dissecting microscope to the lowest possible taxonomic level. Fishes with a low degree of digestion were identified according to Fischer et al. (2011) and with support from experts at the Ichthyology Lab at Universidade Federal do Rio Grande - FURG.

Fish *sagittal* otoliths were identified according to Naves (1999) and by comparison with a reference collection at the Waterbirds and Sea Turtles Lab and Demersal Fish Resources and Cephalopods Lab, both at FURG. The number of fish prey in each stomach was determined as the maximum number of otoliths and eye lens pairs (Bugoni and Vooren, 2004), in addition to the number of whole fish when present. The length, width and the index of digestion (ID 0–3) were measured following Bugoni and Vooren (2004), and only otoliths showing slight or no erosion (ID 0–1) were used. Otolith width was used when otolith length could not be measured.

Cephalopods were identified by chitinous beak remains according to Santos (1999), Vaske and Costa (2011) and by comparison with a reference collection from the Demersal Fish Resources and Cephalopods Lab. The number of cephalopod prey was determined as the maximum number of either upper or lower beaks for each species in the same stomach. Prey remains were measured with an ocular microscopic scale. 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*.

The estimated fish and cephalopod body masses were calculated by the equations listed in Table A.1 (Appendix A). For fish that could not be identified to the species level, the mean mass of fish from the same taxon above the species level was used. Other items and remains such as mollusc shells, plant fragments and solid waste such as plastic were recorded, counted, and identified when possible but were not included in the dietary analysis, as they were incidental or considered as secondary ingestion (Pinto et al., 2007). Anthropogenic debris fragments were classified into different categories of use, according to the European Commission (2013) as in Colferai et al. (2017).

2.4. Stable isotope analysis

Undigested prey such as fish found in the stomach contents and the squid *Doryteuthis sanpaulensis* that is fished in the region were frozen for later SIA as potential food sources.

Due to ^{13}C depletion in lipids as compared to whole tissue and aiming to reduce $\delta^{13}C$ variability in different tissues (Sotiropoulos et al., 2004; Bugoni et al., 2010), lipids were extracted from the muscle and liver samples of penguins and undigested muscle of the prey items (Table A.2) using a 2:1 chloroform:methanol solvent in a Soxhlet apparatus over 8 h. Thus, this procedure enables these tissues types and the lipid-free tissues such as feathers to be compared. Samples were then dried at 60 °C for 48 h. To remove external residuals, feathers were washed in a 0.25 M NaOH solution, and then, they were rinsed five times with distilled water and finally dried at 70 °C for 12 h. Muscle and liver samples were ground, and feather samples were cut into small pieces with scissors. Each sample was homogenized, and a subsample of approximately 0.7 mg was weighed 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 equation (1) as in Bond and Hobson (2012):

$$\delta^{13}C \text{ or } \delta^{15}N \text{ (‰)} = \left(\frac{R_{sample}}{R_{standard}}\right) - 1 \quad (1)$$

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

2.5. Data analysis

2.5.1. Body mass index

To calculate the body mass index (BMI), the body mass (g) was divided by the total length, measured from bill tip to tail feather, in mm (as in Numata et al., 2000). After calculating the BMI, 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. To correlate and represent graphically the data set of BMI values and the mass and length of adult and juvenile Magellanic penguins from the beach and sea, these variables were converted into principal components in a principal component analysis (PCA) (Package "ggplot2"; R Core Team, 2017). A total of 45 sampled penguins were used for these analyses. To test the bias of using stomach contents of emaciated penguins for diet study, adult Magellanic penguins from the sea and beach were compared in terms of the cephalopod beaks found on their stomachs.

2.5.2. Diet

Empty penguin stomachs were excluded from analysis. The relative importance of each food item (food item is defined as a category of prey, as in Bugoni and Vooren, 2004), identified at the species level or the lowest taxon possible, was calculated using the following parameters: FO = absolute frequency of occurrence (FO), as the number of stomach contents with the food item and relative as a percentage of all analysed stomach contents (FO%); contribution by number absolute (N), as the number of individual prey in relation to the total number ingested and relative contribution by number (PN%), as the percent of total number of individual prey in all the stomach contents, excluding samples in which the food item (i.e., the category) did not occur; contribution of 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%), e.g., 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. Lastly, these parameters were integrated in to the prey-specific index of relative importance (PSIRI%) (Brown et al., 2012), calculated as:

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

2.5.3. Stable isotopes

To determine the relative contribution of different food items to the diet of adult and juvenile Magellanic penguins, the Bayesian stable isotope mixing models were used in the Stable Isotope Analysis in R (SIAR) (Parnell et al., 2010). These food items were selected according to diet results, ecological characteristics and the best adjustment of the models. The SIAR models were built based on preliminary tests with isotope values of a range of potential food sources found in the stomach contents. Analyses by graphic results, plots of the proportion by sources and matrix plots of correlations between sources were carried out. The best fitted models were those based on correlation matrices with low competing sources, 'essential' sources for the model (see the SIAR manual for details on source selection, Parnell and Jackson, 2015) (Table A.2), resulting in a model composed of three food items: Argentine anchovy, squid *D. sanpaulensis* and the silverside *Odontesthes argentinensis*. The anchovy and the squid were also identified as important food items for Magellanic penguins in the same region in a previous study (Fonseca et al., 2001), while silversides, which are representative of more coastal fish species and were also present in our samples (see Results), were prey for penguins in the southern Argentina colonies (Frere et al., 1996). The contribution of each food source to the synthesis of Magellanic penguin tissues was modelled for liver, muscle and feather values. Diet-tissue discrimination factors used in the models were obtained by calculating the mean ± SD of values from controlled-diet experiments on other seabirds found in the literature (Table A.3). For the liver samples, the trophic discrimination factors used were 0.5 ± 1.2‰ for $\delta^{13}C$ and 2.5 ± 0.3‰ for $\delta^{15}N$; for the muscle samples, 1.2 ± 1.3‰ for $\delta^{13}C$ and 1.9 ± 0.7‰ for $\delta^{15}N$ were used, and for the feather samples, 1.6 ± 1.5‰ for $\delta^{13}C$ and 3.8 ± 0.7‰ for $\delta^{15}N$ were used. Different diet-to-tissue trophic discrimination factors were used because different tissues have distinct metabolic routing (Dalerum and Angerbjörn, 2005).

In addition to running models as described above for comparative purposes using the three tissues, an additional model was used for feathers with the goal of testing the importance of the Argentine anchovy during the annual cycle of Magellanic penguins. Because feathers retain their isotopic composition during synthesis, which occurs just after breeding in Argentina, feathers were also modelled with sources from Argentinean Patagonia that were recently used 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 A.2), with the same discrimination values used for feathers in this study.

To verify the variations in the $\delta^{13}C$ and $\delta^{15}N$ values, generalized linear models (GLMs) with Gaussian distribution were separately run for each isotope (McCulloch and Searle, 2001). 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 a high correlation with age (as most juveniles were sampled on the beach and adults at sea) or were not significant in models (sex). The selection of the best model was based on the Akaike information criterion – AIC. The models were then simplified by excluding variables when they were statistically non-significant at $P < 0.05$ or when 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):

$$\%\ explained = \frac{Residual\ deviance\ of\ the\ variable}{Deviance\ of\ the\ null\ model}\ x\ 100 \quad (3)$$

The isotopic niches (Newsome et al., 2007) of adult and juvenile penguins were calculated for liver, muscle and feather isotopic values using Stable Isotope Bayesian Ellipses in R – SIBER (Jackson et al., 2011). The area of isotopic niches, as a proxy of niche widths, and the percentages of area overlap between the groups were calculated from standard ellipse areas adjusted for a small sample size (SEAc), as in Mancini and Bugoni (2014). All statistical analyses of stable isotopes were carried out in R 3.4.0 software (R Core Team, 2017).

3. Results

3.1. Body mass index (BMI)

Individuals incidentally killed during gillnet fishing had higher BMIs in comparison to individuals collected on the beach (Fig. 2). In terms of the mean by age class *vs.* area, beached individuals, mostly juveniles ($n = 23$), had BMIs below the mean values of the birds sampled at sea. Only two of the five beached adults had high BMIs comparable to the adults sampled at sea. The PCA was represented graphically (Fig. A.1), where the data set was transformed in vectors, in a data matrix. This analysis demonstrated the variation in the data in relation to the mean, and showed that Magellanic penguins from both age classes from the sea were above the mean, i.e. with higher mass and BMIs.

Testing the relation of cephalopod beaks in the stomach contents of adult Magellanic penguins from the beach and sea, it was determined that a total of 84 cephalopods beaks were in the stomach of a single beached penguins (from a total of 4 penguins sampled), while only 11 cephalopod beaks were found in 7 adult penguin stomach contents from a total of 18 penguins from the sea.

Females predominated in samples (82%, $n = 28$ penguins with gonads inspected).

3.2. Diet inferred by stomach content analysis

A total of 3146 prey was found in 41 stomach content samples from the analysed Magellanic penguins. The main food items consumed were cephalopods (2396 individuals of 3 species) and teleost fish (751 individuals of 8 species) (Table 1). In the 21 stomach content samples from the adults, fish predominated (415 individuals), primarily the Argentine anchovy (124 individuals), and the second most frequent item was cephalopods (110 individuals), primarily the squid *D. sanpaulensis* (56 individuals). In the 20 stomach content samples from juveniles, the main food item was cephalopods (2286 individuals), primarily the pelagic octopus paper nautilus (2074 individuals), and the second most frequent item was fish (336 individuals), primarily the marine silverside (*O. argentinensis*) (17 individuals). In terms of the overall contribution represented by the integrated prey-specific index of relative importance PSIRI%, fish was the main prey item (PSIRI = 86.4%) for adults, followed by cephalopods (PSIRI = 13.6%), with *E. anchoita* and *D. sanpaulensis* as the main items at the species level. The most frequent and energetically important group, as indicated by the mass contribution, was *E. anchoita* (FO% = 66.7 and PM % = 43, respectively) (Table 1).

Juvenile Magellanic penguins showed differences in their diets in comparison to the adults. The main food item of the juveniles 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%). The frequency of occurrence was high for *D. sanpaulensis* (FO% = 80.0), followed by *A. nodosa* (FO% = 55.0). In relation to the mass contribution, the most important food item was *D. sanpaulensis* (PM% = 45.7), followed by *A. nodosa* (PM% = 28.6) (Table 1).

The estimated mean total length of fishes was 96 mm (ranging from

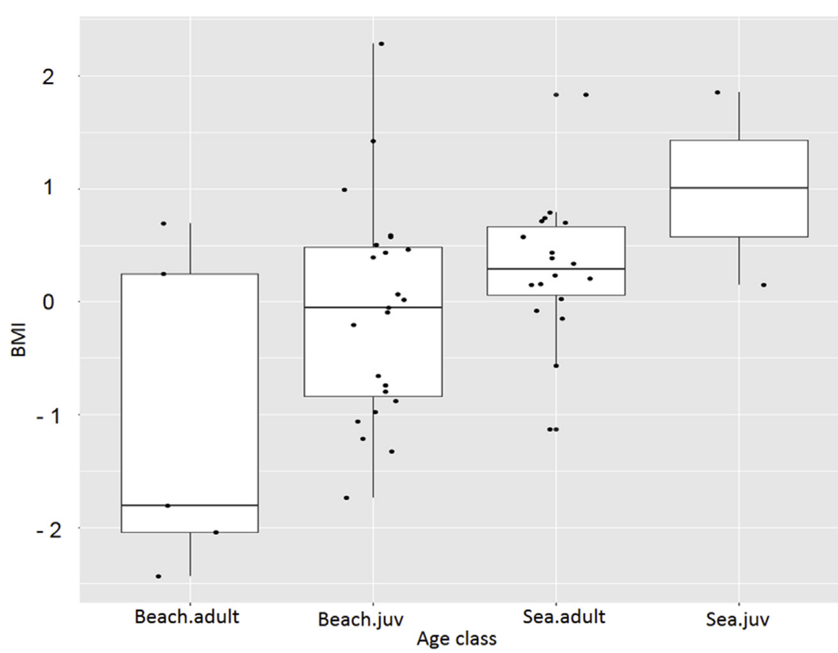

Fig. 2. Body mass index (BMI) of Magellanic penguins, *Spheniscus magellanicus*, collected between 2013 and 2015 at sea and in 2015 on the beach in southern Brazil, separated by area and age classes.

Table 1
Food items found in the stomach contents of 21 adult and 20 juvenile Magellanic penguins, *Spheniscus 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 (FO) and relative (FO%), contribution by number absolute (N) and relative (FN%), contribution by mass absolute (M in g) and relative (PM%) and prey-specific index of relative importance (PSIRI%). *Not included in the PSIRI.

Food items	Adults							Juveniles						
	FO	FO%	N	FN%	M (g)	PM%	PSIRI%	FO	FO%	N	PN%	M (g)	PM%	PSIRI%
Fishes	20	95.2	415	179	2955	170.5	86.4	16	80	336	109.2	2399.6	84.4	28.9
Engraulidae 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	947.4	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 argntinensis	–	–	–	–	–	–	–	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
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	11.1	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	513	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
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 2
Total length and body mass of fish and cephalopods in 41 stomach content samples of adult and juvenile Magellanic penguins, *Spheniscus magellanicus*. The original values of a species with one individual are given. n = number of prey.

	Total length (mm)			Mass (g)			n
	Mean	Min	Max	Mean	Min	Max	
All fish							
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	5.3	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
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	1.4	283.2	171
Argonauta nodosa	15.9	3.1	63.3	1.7	0	37.4	2098
Cephalopods NI	75.5	25.9	250.2	24.4	1.1	283.2	180

37 to 147.6 mm), and the mean total mantle length (ML) of the cephalopods was 69.8 mm (ranging from 3.1 to 250.2 mm) (Table 2). The mean size (total length for fish and ML for squids) and mass of *E. anchoita* were 105.8 mm and 7.6 g, respectively. The octopus *A. nodosa* was 15.9 mm ML and 1.7 g, and *D. sanpaulensis* was 73.8 mm ML and 23.7 g (Table 2).

3.3. Non-food items

Plant remains, other molluscs, crustaceans and fish eggs were present in low numbers and were regarded as secondary items. Plastic was found in 29% of the stomach content samples. The anthropogenic items that were found included plastic sheet fragments, nylon and fishing rope (Table 1).

3.4. Stable isotope analysis

3.4.1. Stable isotope mixing models

Stable isotope values in the penguin tissues ranged from −17.6 to −15.4‰ (n = 53) for δ^{13}C in the liver, −18.5 to −15.9‰ (n = 54) in the muscle and −18.6 to −14.0‰ in the feathers (n = 54). For δ^{15}N, the values ranged from 18.2 to 20.6‰ in the liver, 15.8–19.4‰ in the muscle and from 17.9 to 21.9‰ in the feathers (Table 3).

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

Isotopic niche areas (expressed by ‰^{2} as a proxy for niche width) showed overlap between the adult and juvenile penguins for the liver, muscle and feather samples (Fig. 5). For the liver samples, the isotopic

Table 3
Summary of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes values (mean ± SD) from liver, muscle and feather samples of Magellanic penguins, *Spheniscus magellanicus*, with characteristics such as area of sampling, age and sex.

Area	Age	*n*	Sex			Tissue					
						Liver		Muscle		Feather	
			F	M	NI	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
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

niche area for the adults (0.6‰^{2}) and juveniles (0.7‰^{2}) were similar, overlapping by 26.5% (adults over juvenile niche areas) and 23.3% (juvenile over adult areas), respectively. For the muscle samples, adults (1.4‰^{2}) had larger niche areas than juveniles (1.0‰^{2}), overlapping by 61.4% and 83.3%, respectively, in niche areas. However, the SEAc values for the feather samples differed from the other tissues, with a large difference between the adults (3.5‰^{2}) and juveniles (8.2‰^{2}), with a large overlap of adults over juvenile areas (48.6%), but half the overlap of juveniles over the adult areas (24.4%).

3.4.2. Generalized linear models (GLM)

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

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

The $\delta^{13}C$ values in adults were significantly higher than the values in the tissues of the juveniles ($P < 0.001$), while the $\delta^{15}N$ values were lower in the adults in comparison to the juveniles ($P = 0.002$). The comparison between tissues showed that the $\delta^{15}N$ values in the liver ($P = 0.030$) and muscle ($P = 0.001$) were lower than those in the feathers. The interaction between 'muscle:age' was significant for $\delta^{13}C$ ($P = 0.013$) and $\delta^{15}N$ ($P = 0.028$), while 'liver:age' was only significant for $\delta^{15}N$ ($P = 0.006$) (Table A.4).

The ANOVA of the GLM indicated that the model explained 40.8% of the variance for $\delta^{13}C$ and 23.8% of the variance for $\delta^{15}N$. For both the $\delta^{13}C$ and $\delta^{15}N$ values, the higher explanatory value of the model was by the explanatory variable 'tissue' (28% and 18.7%, respectively) (Table 5).

4. Discussion

Similar to breeding grounds in northern Patagonia where the largest colonies of Magellanic penguins are located, we demonstrated that in the wintering grounds in southern Brazil penguins also depend heavily on the Argentine anchovy. This key resource is important for both juveniles and adults and throughout the whole annual cycle of penguins. However, the conventional dietary analysis indicated that fish was the most important food item for the adults and for healthy juveniles killed in fisheries, but this technique showed that cephalopods were the main food item for the juveniles. Notwithstanding this result, the mixing models based on the stable isotope analysis for all the tissues showed that the Argentine anchovy was the most important food item for both the adult and juvenile penguins during the wintering periods at lower latitudes. This result is in contrast with previous studies, which indicated that cephalopods were the main food item, but these studies were based only on the stomach contents of individuals stranded on beaches (e.g., Fonseca et al., 2001; Di Beneditto et al., 2015). In the current study, the combined use of both techniques and the analysis of healthy individuals that were bycaught in gillnets highlighted the importance of the Argentine anchovy in the diet of Magellanic penguins, providing a more reliable picture of the diet of penguins and their role as a consumer of the keystone Argentine anchovy.

4.1. Diet composition and stable isotope analysis in the wintering areas

During the wintering period, the stomach contents of adult Magellanic penguins mainly contained the Argentine anchovy, while for juveniles, the most important food item was the squid *D. sanpaulensis*, as in previous studies on the Brazilian wintering grounds (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). The paper nautilus *A. nodosa*, had a high contribution in terms of number in this study, and the paper nautilus had also been common in previous studies based on stranded penguins in the area (e.g., Santos and Haimovici, 2002). However, the paper nautilus was not the most important food item in the diet of Magellanic penguins as showed by the PSIRI%, and this result could be due to small size of this prey. Elsewhere, for instance in the southern colonies of Santa Cruz province, 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 of cephalopods and fish (Frere et al., 1996). At the Falkland/Malvinas Islands, the importance of squid in the diet decreases from areas in the west to areas in the south, where they are replaced by fish probably due to different distributions and availability of prey (Pütz et al., 2001). All this demonstrate opportunistic behaviour, and diet varying according to changes in the availability of different food items.

The Argentine anchovy has also been documented as the main food item in the wintering grounds in the northern RS state, based on SIA in penguin claws (Silva et al., 2015), and Engraulidae was an important food item on the Rio de Janeiro (RJ) coast, Brazil, based on penguin muscle (Di Beneditto et al., 2015). Silverside fishes are also common in the distribution area of penguins, with shoals in pelagic, and estuarine-coastal waters in Argentina, as well as in the wintering grounds in Brazil (Bemvenuti, 1987; Fischer et al., 2011). The mean mantle length and mass of *D. sanpaulensis* was similar to those identified in previous studies in Brazil (Pinto et al., 2007; Di Beneditto et al., 2015) and indicate that penguins fed on adults and subadult squid (Andriguetto and Haimovici, 1996). Other previously recorded mean sizes of this squid that was preyed upon by penguins in southern Brazil were 7.5 mm and 0.4 g in Fonseca et al. (2001), and they appear unrealistically low. Regarding fish size in general, and specifically the Argentine anchovy, length and mass were similar to previous studies in the area (Fonseca et al., 2001) as well as in Patagonia (Scolaro et al., 1999), which also indicates that penguins rely on subadult and adult anchovies but juveniles of other large-sized species.

Although the stomach content analysis generated relevant information about consumed prey, 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 Heezic and Seddon, 1989; Barrett et al., 2007), which probably occurred in this and previous penguin studies based on stranded specimens, with squid beaks frequently being the only

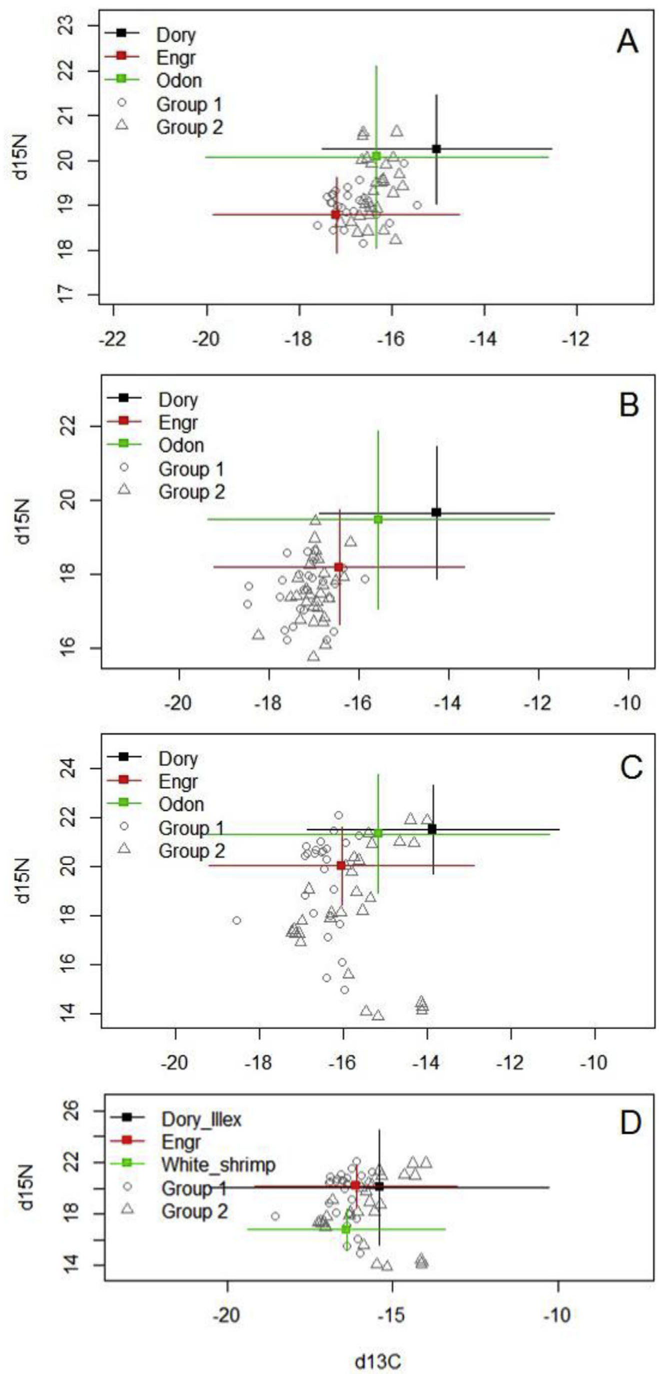

Fig. 3. Stable isotope values in the liver (A), muscle (B) and feather (C) samples of Magellanic penguins, *Spheniscus magellanicus*, in southern Brazil, and the modelling test with the Argentinean sources of feathers (D) based on a stable isotope analysis in R (SIAR). "Group 1" represents adults and "Group 2" juveniles. The values of potential food items (mean ± SD) are *D. sanpaulensis* (black), *E. anchoita* (red) and *O. argentinensis* (green). For the (D) graph, values are *Doryteuthis* sp. + *Illex* sp. (black), *E. anchoita* (red) and white shrimp (green), from Argentinean Patagonia (as in Yorio et al., 2017). The values of the sources were corrected for a trophic discriminant factor for each tissue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

material in stomachs. In addition, studies with emaciated juveniles can be more biased as birds are not feeding normally, and thus, the birds 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 the adults and juveniles. The stable isotopic values in the liver tissues represent the most recent time window, while those in the muscle represent the migratory period and those in the feather represent the periods before moulting and just after breeding. Thus, the SIAR model showed a high contribution of the Argentine anchovy to the diet of Magellanic penguins during the wintering period in the study area and during the migratory period. Despite different stable isotope values in tissues used for modelling, and the models using the same values in prey, distinct metabolic routing (as explained by Dalerum and Angerbjörn, 2005) and distinct values of trophic discriminating factors for each tissue, used as input in each model, explain the similarity in the output results of mixing models. The importance of the Argentine anchovy to the diet of juvenile Magellanic penguins feeding in Brazilian waters, during the wintering period, was recently confirmed by Silva et al. (2015), indicating 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 maintained for both the adults and juveniles. Through direct methods, i.e., stomach contents accessed by stomach flushing, the Argentine anchovy was determined to be the dominant prey species for penguins in the northern Argentinean colonies (Wilson et al., 2005; Boersma et al., 2009). These results seem to confirm that the migration of Magellanic penguins is related to the northward movement of the northern stock of anchovies in the winter, as previously hypothesized (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 their nesting grounds.

The adults and juveniles presented large overlaps in their isotopic niches, which was an outcome that had been hypothesized as both occupy waters on the inner shelf, where most Argentine anchovy shoals are located (Costa et al., 2016), and which was expected based on the high importance of the Argentine anchovy as indicated by the isotopic models. Overall, despite differences in stable isotope values among age classes and tissues, the magnitude of differences is relatively small, in comparison with postulated food items.

4.2. Temporal variation

The stable isotope analysis in the feathers showed a variation of the $\delta^{15}N$ values, with a higher standard deviation in comparison to other tissues sampled. These values can be partially influenced by variations in isotopic values at the base of the trophic chain, in different places (Hobson et al., 1994; McMahon et al., 2013), which are magnified along the upper trophic levels. 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 moulting, feather 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 food sources from southern Brazil and from Patagonia, did not alter the main conclusion, i.e., strong reliance on the Argentine anchovy. However, the higher variation in the $\delta^{15}N$ values in feathers in comparison to both liver and muscle, supposedly derived from foraging in the previous few weeks before moulting, suggests the occurrence of penguins from distinct colonies, as diets differs in distinct breeding grounds, forming a mixed stock in the wintering grounds in southern Brazil.

4.3. Age and sex variation in the diet in the wintering areas

In 2015, there were more juvenile Magellanic penguins stranded on the beach in southern Brazil than to adults, as formerly shown by Mäder et al. (2010) who estimated that 19,000 individuals were found dead on the coast and the majority were juveniles. The opposite result was

Table 4
Stable isotopic contribution of the main values at the 95% credibility interval (CI) from the stable isotope analysis in R (SIAR) for adult and juvenile Magellanic penguins, *Spheniscus magellanicus*, from liver, muscle and feather samples. The food items included from literature were used in an additional model for feather samples, in order to analyse and compare the source contribution from Argentinean Patagonia during the feather synthesis.

Food items	Liver		Muscle		Feather	
	Adults (%)	Juveniles (%)	Adults (%)	Juveniles (%)	Adults (%)	Juveniles (%)
Fishes						
E. anchoita	39.8–67	55.5–80.6	82.5–98.9	79–98.6	55.3–95.5	46–86.7
E. anchoita[a]	–	–	–	–	1.5–42	4.4–39
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
Cephalopods						
D. sanpaulensis	6.6–38	4.6–32.9	0–8	0.1–10.1	0.2–18.6	0.7–26.6
Doryteuthis sp. + *Illex* sp.[b]	–	–	–	–	13–64	18.2–59.2
Crustaceans						
Peisos petrunkevitchi[c]	–	–	–	–	21–59.4	22–56

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

found at sea, where adults were predominant in the bycatch in gillnets, similar to the results in Cardoso et al. (2011).

The values of δ^{15}N in the juvenile tissues were significantly lower in comparison to the values in the adults, although the SIAR also shows a high importance of the Argentine anchovy for both classes, despite other sources increased in importance in the models. Silva et al. (2015) found the same result based on the stable isotopes in the claws of emaciated juveniles stranded in wintering grounds, i.e., confirming the importance of fish in the penguin diet. Keratin in claws is formed well before sampling, probably in colonies, and thus is comparable to feathers sampled for the current study. As discussed previously, both results conflict with those based on stomach content analysis only, where squids are usually predominant (e.g. Fonseca et al., 2001; Pinto et al., 2007; Di Beneditto et al., 2015).

Females were predominant in our sampling, as in previous studies in the region (Vanstreels et al., 2013; Nunes et al., 2015), but the GLM analysis did not show significant differences in the SI values between the sexes. In Argentinean Patagonia, higher values of δ^{13}C and δ^{15}N were determined for adult males compared to females, indicating that males fed significantly more on fish (anchovy) than females (Forero et al., 2002). In non-breeding areas, segregation appears to occur due to segregation in foraging areas but not in food items preyed, with birds sharing the same food when in sympatry, as shown by our GLM models. Thus, the predominance of females in southern Brazil could be explained by males using more offshore areas, or remaining in the areas south of the females, closer to the breeding grounds.

4.4. Interactions with human activities

Between 2013 and 2015, the Magellanic penguins that were collected dead from gillnet fishing appeared to be healthy, with fat reserves, high BMIs and no evidence of injury during necropsy. During the winter in southern Brazil, several Magellanic penguins are caught by bottom gillnet fisheries that target stripped weakfish and by drift gillnet fisheries that target bluefish (Cardoso et al., 2011; Fogliarini et al., unpublished data). During the summer, the penguins are affected by fisheries in areas adjacent to their breeding colonies, e.g., at Golfo San Jorge in Patagonia, where the estimate of penguin mortality due to

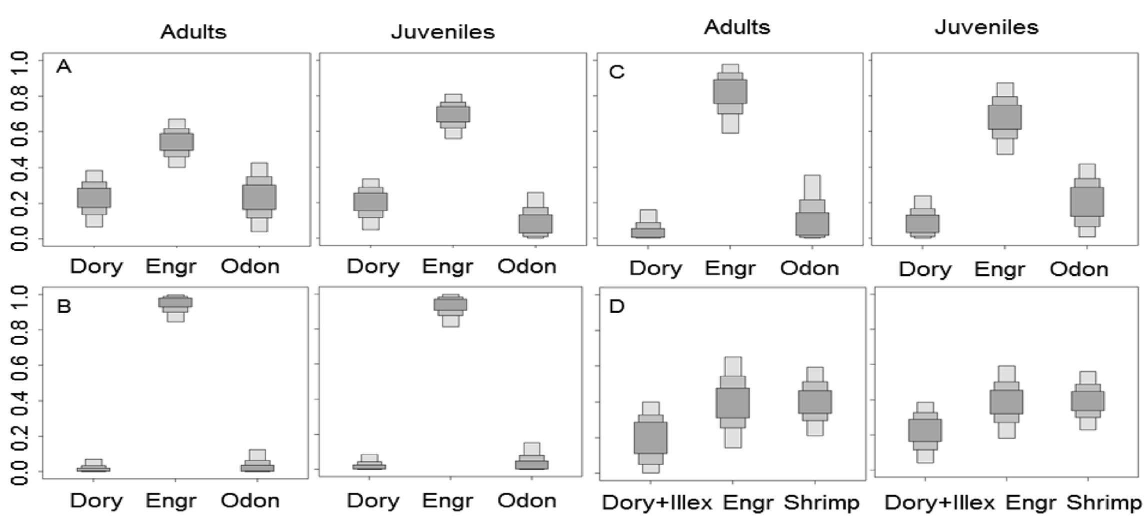

Fig. 4. Stable isotope analysis in R (SIAR) proportion, with 95, 75 and 25% credibility intervals for liver (A), muscle (B) and feather (C and D) samples, respectively, of adult and juvenile Magellanic penguins, *Spheniscus magellanicus*. Food sources are the squid *D. sanpaulensis* (Dory), fish *E. anchoita* (Engr) and *O. argentinensis* (Odon) for "A", "B" and "C", respectively. For "D", the squids *Doryteuthis* sp. + *Illex* sp. (Dory + Illex), the fish *E. anchoita* (Engr) and the shrimp *P. petrunkevitchi* (Shrimp) are from Argentinean Patagonia, as used by Yorio et al. (2017).

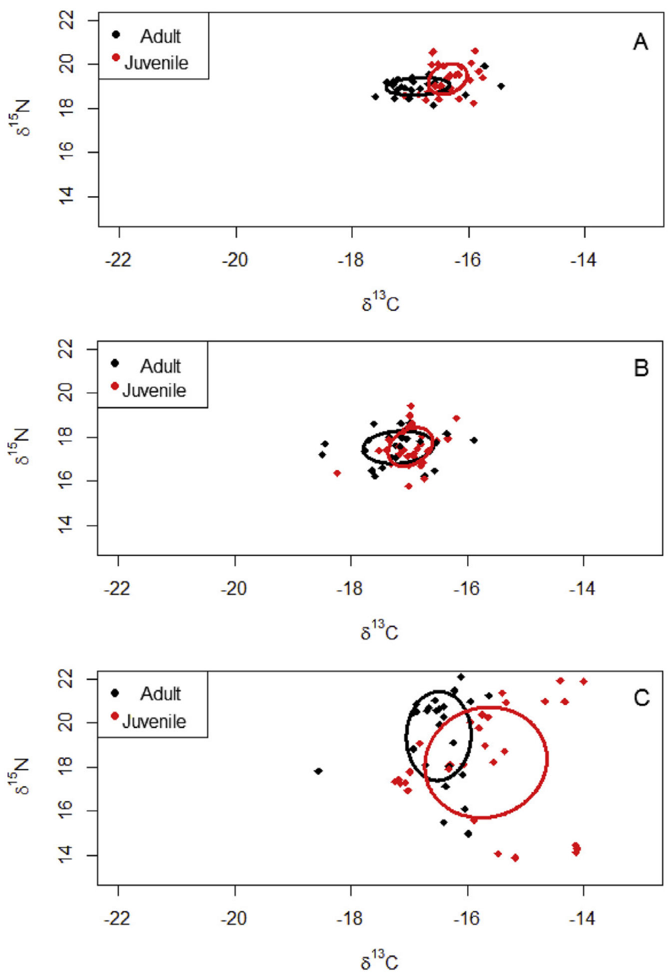

Fig. 5. Isotopic niche of Magellanic penguins, *Spheniscus magellanicus*, adults and juveniles from liver (A), muscle (B) and feather (C) samples, based on standard ellipses areas corrected for small samples sizes (SEAc) using Stable Isotope Bayesian Ellipses in R – SIBER.

shrimp fisheries was 0.3% of the breeding population every summer (Gandini and Frere, 1999). The effect of fisheries near Argentinean colonies is one of the main causes of the decrease in Magellanic penguin populations, not only due to 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, the Argentine anchovy is currently only fished to be used as live-bait for the skipjack tuna, *Katsuwonus pelamis* pole-and-line fishery (Carvalho and Castello, 2013). However, an evaluation of anchovy stocks is aimed at commercial exploitation (Madureira et al., 2009), which could represent a risk to the supply of the main food item for penguins. If these plans move forward, then the use of the Argentine anchovy by penguins should be considered, for example, in the calculations of maximum allowable catches.

Plastic and fishing-related items were found in 29% of the 41 stomach contents analysed in this study. In the state of Rio de Janeiro, 14.9% out of 175 stomach contents of Magellanic penguins had human debris (Brandão et al., 2011). A recent study in Rio de Janeiro (Di Beneditto and Siciliano, 2017) demonstrated an increase from 42.5% in 2000 to 89.1% in 2008 in the frequency of penguins with plastic in their stomachs. Plastic pollution is a chronic problem throughout the distribution of Magellanic penguins, and it also affects 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).

5. Conclusion

This study confirms the importance of using complementary techniques for reliable dietary studies, particularly when involving debilitated or stranded consumers. In this context, the stomach contents and SIA from distinct tissues are good choices. With isotopic mixed models, it was possible to verify for the first time in the wintering grounds the importance of the Argentine anchovy to both adult and juvenile Magellanic penguins, from individuals stranded on the beach and bycatch in gillnet fisheries. The models also demonstrated that dietary studies based on unhealthy (e.g., stranded) birds result in biased information. The Argentine anchovy contributed not only to the wintering grounds in southern Brazil but also during their whole annual cycle, thus confirming previous anecdotal suggestions of a coupled migration between penguins and their main prey northward in the winter. Finally, 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 keystone forage fish.

Table 5
Summary of ANOVA from a generalized linear model (GLM) for $\delta^{13}C$ and $\delta^{15}N$ values. The % explained is calculated as the ratio between the deviance/residual deviance 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 class	2	2.430	2.43	155	59.153	3.184	0.0441
Total explained	5	40.885	40.7				
$\delta^{15}N$							
Null model				160	415.00		
Main effects							
Tissue	2	77.793	18.75	158	337.21	19.070	< 0.001
Age class	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				

Acknowledgements

The authors are grateful to their colleagues that supported the study, especially G.T. Nunes, F.A. Faria, G. Leal, P. Mancini, L. Furlanetto, C. Trindade, C. Lima, S. Paz, C. Barreto, G. Oliveira and P. Echevenguá. Thanks to NEMA and ECOMEGA Lab (FURG) for the support provided during beach monitoring and collecting carcasses. We are also grateful to F. Lameiro, V. Lemos and M. Freire from the Oceanographic Institute (FURG), for the identification of food items. We thank the CNPq and the Brazilian Ministry of Environment for financial support for the fieldwork at sea (406163/2012-0). Authors are grateful to Silvina Botta, Andre Chiaradia and two anonymous reviewers for their careful review and suggestions. FPM received a CAPES scholarship. LB (310550/2015) and MH (303561/2015-7) are research fellows from Brazilian CNPq.

Appendix A

Table A.1
Allometric equations used to estimate the total length (TL) and mass (M) based on fish otolith measurements (Naves, 1999), the mantle length (ML) and mass for cephalopods from the upper (URL) and lower (LRL) rostral length of squids and the upper (UHL) and lower (LHL) hood lengths of *Argonauta nodosa* (Santos, 1999). Otl = otolith length, Otw = otolith width.

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 plei	$ML = 67.431URL^{1.2908}$	$M = 8.8096URL^{2.8564}$	
	$ML = 64.303LRL^{1.3143}$	$M = 7.9418LRL^{2.908}$	
Argonauta nodosa	$ML = 4.9237UHL^{1.2933}$	$M = 0.0377UHL^{3.4949}$	
	$ML = 9.5338LHL^{1.2314}$	$M = 0.2593LHL^{3.1856}$	

Table A.2
Stable isotope values of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) ± SD of the potential prey items found in the literature and used to calculate the best model for prey contribution in the diet of Magellanic penguins, *Spheniscus magellanicus*, from both age classes. *N* = number of individuals; Mean length = mean of total length for fish and crustaceans; mean of total mantle length for cephalopods.

Species	*n*	Mean length ± SD (mm)	$\delta^{13}C$ ± SD (‰)	$\delta^{15}N$ (‰)
Fish				
Anchoa marinii	1	79.0	−16.7	16.7
Chloroscombrus chrysurus	1	60.0	−16.8	17.9
Cynoscion guatucupa[a]	2	123.9 ± 29.5	−16.7 ± 0.1	17.4 ± 0.6
Engraulis anchoita	6	109.9 ± 17.4	−17.6 ± 0.6	16.3 ± 0.3
Engraulis anchoita[b]	–	–	−17.7 ± 0.4	16.4 ± 0.4
Mugil brevirostris	3	122.0 ± 7.8	−13.1 ± 0.1	9.5 ± 1.8
Mugil sp.	1	133.0	−13.5	11.1
Odontesthes argentinensis	12	104.1 ± 35.5	−16.8 ± 1.4	17.6 ± 1.0
Oligoplites saliens	7	57.0 ± 18.5	−16.7 ± 0.2	16.8 ± 0.3
Pomatomus saltatrix	1	88.0	−16.7	17.9
Trichiurus lepturus[a]	1	59.7	−16.7	20.3
Cephalopods				
Doryteuthis sanpaulensis[c]	7	11.1 ± 4.6	−15.5 ± 0.3	17.8 ± 0.5
Doryteuthis sp. ± *Illex* sp.[d]	–	–	−17.0 ± 2.1	16.3 ± 2.1
Crustaceans				
Artemesia longinaris[a]	12	1.0 ± 0.7	−15.4 ± 0.5	15.6 ± 0.6
Peisos petrunkevitchi[e]	–	–	−18.0 ± 0.2	13.0 ± 0.4

[a] Prey found in stomach contents of *Pomatomus saltatrix* and *Cynoscion guatucupa*, from gillnet fishing.
[b] Values used by Yorio et al. (2017).
[c] Prey provided by fishing in the region.
[d] Values used by Forero et al. (2004).
[e] Values used by Ciancio et al. (2008).

Table A.3
Discrimination factors found in the literature, used in SIAR (stable isotope analysis in R) models for the 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 1992
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 1992
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	0.1	4.4	Cherel et al. 2005
Eudyptes chrysocome	feather	fish muscle	0.6	3.5	Cherel et al. 2005
Spheniscus humboldti	feather	fish (anchovy)	2.9	4.8	Mizutani et al. 1992
Aptenodytes patagonicus	feather	fish	0.7	3.5	Cherel et al. 2005
Aptenodytes patagonicus	feather	fish muscle	0.3	2.7	Cherel et al. 2005
Larus delawarensis	feather	fish (perch)	0.2	3.0	Hobson and Clark 1992
Pygoscelys papua	feather	fish (herring)	1.3	3.5	Polito et al. 2011

Table A.4
Results from the generalized linear model (GLM) with coefficients of the model. Only significant terms ($P \leq 0.05$) are shown. The values of the intercept represent $\delta^{13}C$ and $\delta^{15}N$ for the feather tissue and for the adult age class, in comparison to the other levels.

	Estimate	Standard error	*t*-value	*P*
$\delta^{13}C$ (AIC = 309.7; 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 = 579.6; 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.

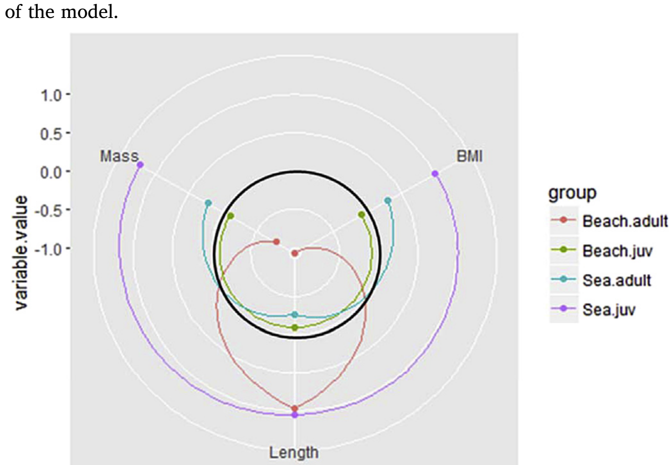

Fig. A.1. The principal component analysis (PCA) of adult and juvenile Magellanic penguins, *Spheniscus magellanicus*, that were in gillnet fishing bycatch in 2013, 2014 and 2015 and collected on the beach in 2015. The black circle represents the mean (mean = 0). Note that the values of BMI and mass are well below the mean values for beached birds.

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