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ORIGINAL ARTICLE



## Relevance of forage fish in the diet of Magellanic penguins breeding in northern Patagonia, Argentina

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### ABSTRACT

We quantified the trophic niche of Magellanic penguins (*Spheniscus magellanicus*) breeding and moulting in Golfo San Jorge, Argentina, through conventional stomach content and stable isotope analysis. A total of 112 adults were flushed during the early and late chick stages of 2011 and 2012 at Isla Vernacci Norte, and at least 15 prey taxa were found, including fishes, cephalopods, crustaceans and polychaetes. Overall, Argentine anchovy (*Engraulis anchoita*) showed the highest contribution in terms of importance by mass (68.1–85.3%, depending on chick stage and year), except for the old chick stage in 2011 when the shortfin squid (*Illex argentinus*) was the main prey consumed (56.0%). Based on carbon and nitrogen isotopic values from a total of 256 blood samples, corresponding to young and old chicks and to adults of both sexes sampled throughout the incubation, chick and moult stages at the above mentioned colony and years, Bayesian mixing model outputs showed that Argentine anchovy was always the main prey (48–86%). Bayesian mixing model outputs obtained from adults of both sexes and their chicks during the late chick stage of 2013 at Isla Vernacci Norte, Isla Tova and Isla Leones also showed that Argentine anchovy was the main prey consumed. This is the first comprehensive assessment of Magellanic penguin diet composition in northern Patagonia, quantifying the relative contribution of prey in the diet of adults and chicks at different stages of the annual cycle and years, and confirms the relevance of a forage fish such as the Argentine anchovy in its trophic ecology.

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## Introduction

Penguins are significant components of coastal and marine ecosystems (Croxall & Lishman 1987; Brooke 2004) and their feeding ecology has been studied extensively in several species worldwide (Ratcliffe & Trathan 2011; García Borboroglu & Boersma 2013). Knowledge of their dietary requirements is needed to adequately interpret their role in marine food webs and to understand trophic interactions within seabird assemblages, as well as potential interactions with fisheries. As in most seabird species, penguin dietary composition may be affected by several sources of variation (Shealer 2002; Ratcliffe & Trathan 2011). The use of food resources by breeding seabirds has been shown to vary among locations (Bertelotti & Yorio 1999; Tremblay & Chérel 2003), throughout the breeding season (Suryan et al. 2002; Karnovsky et al. 2008), between sexes (Bearhop et al. 2006; Phillips et al. 2011) and

among age classes (Davoren & Burger 1999; Wilson et al. 2004; Gatto & Yorio 2016). The composition and sources of variation in seabird diets have been assessed through conventional and biochemical methods (Barrett et al. 2007; Karnovsky et al. 2012), and given their respective limitations, their combination is recommended to minimize biases and adequately assess diet requirements (Sydeman et al. 1997; Polito et al. 2011; Karnovsky et al. 2012).

The Magellanic penguin *Spheniscus magellanicus* (Forster, 1781) is a widely distributed species in southern South America (Boersma et al. 2013a) and the most abundant breeding seabird in Argentine Patagonia (Yorio et al. 1999). The Magellanic penguin is also one of the main ecotourism attractions along the Atlantic coasts of Patagonia, generating important revenues at local and regional scales (Yorio et al. 2001a). This species has been internationally recognized as 'Near Threatened' (BirdLife International 2016), with main

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threats driven primarily by commercial fishing, pollution of the marine environment, unregulated tourism and recreational activities and, more recently, the potential consequences of climate change (Boersma et al. 2013a). Diet assessments have been conducted along its geographic range in the south Atlantic, including Patagonia (Gosztonyi 1984; Frere et al. 1996; Scolaro et al. 1999; Wilson et al. 2005; Scioscia et al. 2014; Ciancio et al. 2015) and the Malvinas (Falkland) Islands (Thompson 1993; Clausen & Pütz 2002), showing a variable contribution of fish, squid and crustaceans depending on the coastal region considered. In the north of its distributional range in Patagonia (42–46°S), diet studies indicate that breeding Magellanic penguins feed primarily on Argentine anchovy *Engraulis anchoita* Hubbs & Marini, 1935 (Gosztonyi 1984; Frere et al. 1996; Scolaro et al. 1999; Wilson et al. 2005), which is considered the most abundant and most ecologically important pelagic fish resource off Argentina (Hansen et al. 2001; Koen-Alonso & Yodzis 2005). The previously mentioned diet studies were conducted through the analysis of stomach contents, and were mostly based on a relatively small sample size, restricted to a single year or only to the chick stage, and/or only quantified the frequency of occurrence and numerical importance of prey species. Only the study by Forero et al. (2002) provided an assessment using stable isotope analysis during the late chick stage in northern colonies, but did not present an independent prey assessment based on conventional diet methods. Stomach content analysis is a time-consuming method that provides detailed information on prey species composition but from only the last feeding event, while stable isotope analysis of blood and feathers is a less-invasive method that, depending on the tissue selected, provides information on prey consumed over variable temporal scales, but is inadequate for estimating the fine-scale taxonomic composition of the seabird diet (Inger & Bearhop 2008). Further studies on the trophic niche of this key top predator considering the different sources of variation and complementing methodological approaches are needed to adequately understand its diet requirements and role in coastal ecosystems of the south-western Atlantic Ocean.

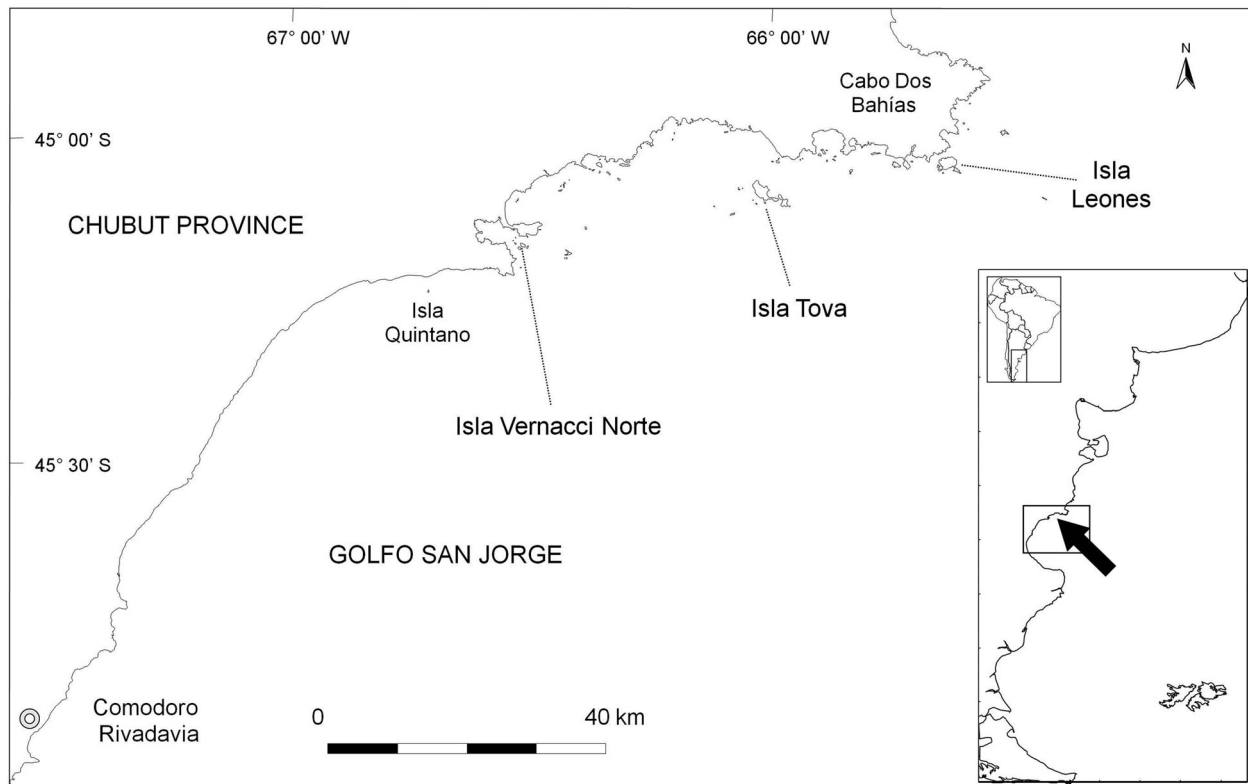
The northern sector of Golfo San Jorge is considered one of the most significant coastal sectors for marine biodiversity in Argentina (Campagna et al. 2006), and includes a marine protected area where a significant proportion of the Patagonian population of the Magellanic penguin breeds (Yorio et al. 1998). The area is subject to an important commercial fishery and is used by over 100 trawl vessels targeting Argentine

hake *Merluccius hubbsi* Marini, 1933 and Argentine red shrimp *Pleoticus muelleri* (Spence Bate, 1888) (Góngora et al. 2012), and although the Patagonian stock of Argentine anchovy is not currently an important commercial target, an experimental fishery aimed at developing this stock was implemented in Golfo San Jorge in recent years (Skewgar et al. 2007). Despite the regional significance as a breeding ground for Magellanic penguins, their diet requirements in Golfo San Jorge are still unknown. Several studies have shown the significance of forage fish for seabird populations (Cury et al. 2011), and thus it is crucial to understand the role that Argentine anchovy plays in the feeding ecology of Magellanic penguins in this relevant coastal sector. In this study we quantified through conventional and stable isotope methods the diet composition and trophic niche of Magellanic penguins breeding in northern Golfo San Jorge, and assessed the importance of Argentine anchovy in their diet.

## Materials and methods

### Study area

The Golfo San Jorge extends from Cabo Dos Bahías (44°55'S, 65°32'W) to Cabo Tres Puntas (47°06'S, 65°52'W) (Figure 1) and includes an area of more than 32,000 km<sup>2</sup>. Magellanic penguins nest on islands located in the northern coastal sector of the gulf, from Cabo Dos Bahías to Isla Quintano (45°13'S, 66°03'W) (Figure 1). In this sector, penguins breed at 19 colonies of between 30 and 57,000 nests totalling over 190,000 breeding pairs (Schiavini et al. 2005; Pozzi et al. 2015). These estimates of breeding pairs represent over 20% of the Magellanic penguin population breeding in Patagonia, Argentina (950,000 breeding pairs) and about 15% of the global breeding population (1,300,000 pairs; Boersma et al. 2013a). All nesting sites are included within the Patagonia Austral marine park, a 750 km<sup>2</sup> protected area under the joint administration of the federal and provincial governments. The study was conducted at a Magellanic penguin colony consisting of 6200 pairs on Isla Vernacci Norte (45°11'S, 66°31'W), located near the mouth of Caleta Malaspina (Figure 1). Additional information was gathered at the colonies of Isla Tova (45°06'S, 66°00'W) and Isla Leones (45°03'S, 65°36'W) (Figure 1), consisting of over 45,800 and 57,200 breeding pairs, respectively (Yorio et al. 1998; Pozzi et al. 2015). Isla Vernacci Norte and Isla Leones are separated by 70 km, and Isla Tova is located in between, about 40 and 30 km from the former two islands, respectively (Figure 1).



**Figure 1.** Map of the study area showing the location of Magellanic penguin colonies at Isla Vernacci Norte, Isla Tova and Isla Leones, Golfo San Jorge, Argentina.

### **Sampling design**

The composition and variation in Magellanic penguin diet were studied using stomach content samples, obtained through the water offloading technique (Wilson 1984), and blood samples for stable isotope analysis. To assess the seasonal and between year variation in diet composition, samples were obtained at Isla Vernacci Norte during 2011 and 2012 in three stages of the reproductive cycle (Yorio et al. 2001b): incubation (first week of November), young chicks (< 30 days of age; third week of December), and old chicks (> 30 days of age; third week of January). In addition, samples were obtained during the moult stage (third week of March). Stomach and blood samples from adults of both sexes, in addition to blood samples from chicks, were obtained during the two defined chick stages in 2011 and 2012 (see below). During the incubation and moult stages of the same two years, only blood samples were obtained from adults of both sexes. During the 2013 breeding season, and only during the old chick stage, blood samples from adults and chicks were obtained at nests from Isla Vernacci Norte, Isla Tova and Isla Leones to assess differences among breeding locations and between adults and chicks

(see below). Year refers to the calendar year of the beginning of the breeding season, e.g. 2011 is the 2011–2012 season.

### **Conventional diet sampling**

Adult penguins were captured on the beach when they were returning from a foraging trip and heading inland to their nest sites. Birds were weighed with a 6 kg spring scale to the nearest 10 g. Bill length (culmen) and bill depth were measured with digital calipers to the nearest 0.1 mm. Individuals were sexed based on morphometric measurements following Bertellotti et al. (2002). Penguins were flushed with seawater between one and three times until the water was clear, indicating the stomach was empty, using a 5.3 mm surgical catheter attached to a 250 ml syringe. After flushing, penguins were supplemented with a solution to prevent dehydration. A total of 175 adult penguins were flushed. Samples were drained through a 0.5 mm mesh sieve, weighed with a spring scale to the nearest 10 g and preserved in 70% ethanol for later analysis.

In the laboratory, each complete stomach sample was dissected in a tray under a zoom binocular microscope ( $\times 15$  magnification) and food remains were

identified to the lowest taxonomic level possible, based on whole individuals or using fish otoliths and cranial bones, squid beaks, crustacean carapaces, and polychaete mandibles and chetae. Prey items were identified with the aid of a reference collection and published information (Clarke 1986; Boschi et al. 1992; Gosztanyi & Kuba 1996).

Frequency of occurrence (%F), numerical importance (%N) and importance by mass (%M) were calculated for each prey type (Duffy & Jackson 1986). The relative contribution by mass of the main prey was estimated using a subsample of fish cranial bones and otoliths, and of squid beaks with slight erosion, measured to the nearest 0.01 mm. With a few exceptions, cephalopod beaks present in the stomach content samples were still attached to the body or inside the buccal mass. Mass of Argentine anchovy and Argentine hake was calculated applying the equations presented in Koen-Alonso et al. (1998) when using otoliths and in González-Zevallos et al. (2010) when using Argentine hake cranial bones. The mass of hawkfish *Nemadactylus bergi* (Norman, 1937) was calculated using otoliths following Koen-Alonso et al. (2000). Mass of Patagonian redfish *Sebastes oculatus* Valenciennes, 1833 was estimated using cranial bones from equations by Ciancio & Fernández (2015, unpublished data). Squid mass was estimated from beak lower rostral lengths using equations based on Pineda et al. (1996) and Ivanovic & Brunetti (1997). The masses of white shrimp *Peisos petrunkevitchi* Burkenroad, 1945, Argentine red shrimp and the polychaete *Laeonereis* sp. were obtained from a sample of whole individuals, and averages used to estimate %M. The calculation of %M only included prey species for which well-preserved diagnostic parts were available to perform calculations. However, prey species that could not be included showed a %N of less than 1%, except for the fish *Odonesthes* sp., which mostly showed a %N of less than 6% or F% of less than 12%.

We tested for differences in the importance by mass of prey types between sexes, stages and years using the analysis of similarities (ANOSIM) procedure with the PRIMER 6 package (Clarke & Gorley 2006). Similarity percentages (SIMPER) were employed to determine the prey species that contributed most to the dissimilarities between groups (Clarke 1993; Clarke & Warwick 2001). Prey richness was defined as the observed number of prey species, and prey diversity was calculated using the inverse of the Simpson Diversity Index (Krebs 1999). Unidentified prey species were not included in the former analyses. To allow for the comparison between stages, prey richness was rarefied (Krebs 1999), scaling the larger sample to the size of the

smaller one using EcoSim software (Gotelli & Entsminger 2010). Differences in richness were tested using 95% confidence intervals given by this software. Means are reported  $\pm 1$  SD.

### **Stable isotope analysis**

In order to complement the information obtained through stomach pumping with information reflecting diet over a longer time period and to determine prey used for self-feeding by adult birds, blood samples were collected to assess the  $^{13}\text{C}$  and  $^{15}\text{N}$  isotopic composition, which reflected the diet during the studied stages at the studied colonies. Stable isotope analysis allowed the estimation of the trophic niche of adult self-feeding and chicks (prey consumed and isotopic niche). Whole-blood samples integrate the isotopic composition of the prey ingested by an individual during approximately a month before the sample is collected (Hobson & Clark 1992). Whole blood samples were obtained from a subsample of the Magellanic penguin adults that were stomach-flushed and from chicks randomly selected in the Isla Vernacci Norte colony. Only one chick per nest, the largest in the brood, was sampled. In addition, blood samples were obtained from adults of both sexes from Isla Vernacci Norte during the incubation and moult stages in 2011 and 2012, and from one breeding adult and its single or older chick during the late chick-rearing period at Isla Vernacci Norte, Isla Tova and Isla Leones in 2013. Samples during the moult stage were obtained from adult birds at their nests that were assumed to have recently started moulting, as indicated by slightly erected feathers and swollen flippers. A total of 256 blood samples were obtained. Whole-blood samples (0.5–1 ml) were extracted from the metatarsal vein of each individual and conserved in 70% ethanol. Blood preservation in 70% ethanol has been recommended when freezing is not possible, as it has no significant effects on its isotopic signatures (Hobson et al. 1997; Halley et al. 2008). Samples were dried at 60°C over 24 h and then ground in a micromortar (Hobson et al. 1997). A subsample of  $1 \pm 0.2$  mg was set in a tin capsule for stable isotope analysis. Sample analyses were performed by the Stable Isotope Facility of the University of California, Davis (USA). Stable isotope abundance is expressed using standard  $\delta$  notation relative to carbonate Vienna PeeDee Belemnite and atmospheric nitrogen. The internal laboratory standards used were bovine liver, USGS-41 glutamic acid, Nylon 5

and glutamic acid. Observed analytical errors were 0.06‰ and 0.14‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively.

The relative contribution of the different prey to the isotope mixture was analysed using Bayesian mixing models within the R package SIAR (Parnell & Jackson 2013) following the guidelines suggested by Phillips et al. (2014). Before running the isotopic mixing models, the sensitive analysis proposed by Smith et al. (2013) was conducted in order to evaluate the feasibility of the proposed isotopic mixing polygon. Prior information on the proportion of prey in the diet of penguins was not included in the isotopic mixing models, nor was element concentration dependence, because the concentration of C relative to N among prey were similar (see Phillips & Koch 2002). We used the diet–tissue discrimination factor for Magellanic penguin blood ( $0.41\text{‰} \pm 0.12$  (lipid-free) for  $\delta^{13}\text{C}$  and  $2.31 \pm 0.17$  (lipid-free) for  $\delta^{15}\text{N}$ , obtained using blood samples from captive Magellanic penguins fed Argentine anchovies (Ciancio et al. 2016). In order to keep the parameters to be estimated to a minimum (see Phillips et al. 2014), only four of the main prey species contributing to Magellanic penguin diet assessed through stomach contents analysis were used in the isotopic mixing models: Argentine anchovy and Argentine hake, being the two most represented fish species in samples, squids (*Illex* and *Doryteuthis* mixture), and white shrimp. Information on the isotopic composition of Argentine anchovy (dorsal muscle) corresponded to prey obtained during this study in the study area ( $\delta^{13}\text{C} = -17.7\text{‰}$ ,  $\delta^{15}\text{N} = 16.4\text{‰}$ , SD for both =  $0.4\text{‰}$ ). Lipids from Argentine anchovy samples were extracted using chloroform–methanol (2:1) (Post et al. 2007). The isotopic composition of Argentine hake ( $\delta^{13}\text{C} = -16.5 \pm 1.2\text{‰}$ ,  $\delta^{15}\text{N} = 17.5 \pm 1.2\text{‰}$ ) and squid (*Illex* and *Doryteuthis* mixture) ( $\delta^{13}\text{C} = -17.0 \pm 2.1\text{‰}$ ,  $\delta^{15}\text{N} = 16.3 \pm 2.1\text{‰}$ ) were obtained from Forero et al. (2004). The isotopic composition of white shrimp was obtained from a set of individuals captured in Golfo San Jorge (Ciancio et al. 2008;  $\delta^{13}\text{C} = -18.0 \pm 0.2\text{‰}$ ,  $\delta^{15}\text{N} = 13.0 \pm 0.4\text{‰}$ ).

Mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope values were compared using Welch's *t*-test, the Wilcoxon test, the Kruskal–Wallis test or the Paired-Sample Wilcoxon test (Crawley 2013). Isotopic niches were compared using the hypothesis-testing framework proposed by Turner et al. (2010). Differences in centroid location, which provide information on isotopic position, and eccentricity, which provides insight into differences in the underlying distribution of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data, were tested using nested linear models and residual permutation procedures (see Turner et al. 2010 for statistical details). Means are reported  $\pm 1$  SD.

## Results

### *Diet and trophic niche throughout the breeding cycle and between years*

A total of 175 adults were captured and flushed, but stomach contents could be obtained in only 59 and 53 individuals in 2011 and 2012, respectively. During 2011, the wet mass of stomach content samples averaged  $255.0 \pm 250.9$  g ( $n = 29$ ; range 10–1145) in the young chick stage and  $152.0 \pm 154.1$  g ( $n = 30$ ; range 7–710) in the old chick stage, while in 2012 the average mass of stomach content samples was  $225.6 \pm 151.9$  g ( $n = 26$ ; range 19.5–540) in the young chick stage and  $204.8 \pm 151.5$  g ( $n = 27$ ; range 30–575) in the old chick stage. No significant differences were found in the mass of samples between the two chick stages (Mann–Whitney Test, 2011:  $U = 947.5$ ;  $p = 0.15$ ; 2012:  $U = 321$ ;  $p = 0.59$ ).

During 2011, a total of 1858 prey items comprising at least 14 prey taxa were found (eight fishes, three cephalopods and three crustaceans) while in 2012 the 1964 prey items found corresponded to 13 prey taxa (seven fishes, three cephalopods, two crustaceans and one polychaete), totalling at least 15 prey taxa considering both years (Table I). From the samples collected, prey species accumulation curves indicated that sampling was extensive enough to represent the major prey items, as indicated by all accumulation curves reaching a plateau. Prey richness and diversity were similar between chick stages in both 2011 and 2012 ( $p > 0.05$ ; Table I). Overall, fish were the most frequent prey in both chick stages and breeding seasons ( $> 87\%$ ), and Argentine anchovy was the most frequent species, varying between 66.7% and 93.1% depending on the stage and year (Table I). The analysis of stomach content samples indicated that diet composition in terms of contribution by mass was similar between sexes during the chick rearing period of both years (two-way crossed ANOSIM, 2011: Global  $R = -0.011$ ;  $p = 0.53$ ; 2012: Global  $R = 0.073$ ;  $p = 0.05$ ). Data from both sexes were therefore pooled for the subsequent analyses.

During the young chick stage of 2011, Argentine anchovy and Patagonian redfish showed the largest contribution by number (35.9% and 34.3%, respectively), followed by *Bovichtus argentinus* MacDonagh, 1931 and the Patagonian squid *Doryteuthis sanpaulensis* (Brakonieccki, 1984) (12.5% and 10.0%, respectively), while in 2012 the diet composition was dominated by Argentine anchovy (69.9%) followed by the white shrimp and Argentine hake (9.0% and 5.8%, respectively) (Table I). During the old chick stage of 2011, the

**Table I.** Frequency of occurrence and numerical importance of prey types and values for richness and diversity of prey recorded in stomach content samples of Magellanic penguins breeding at Isla Vernacci Norte, Argentina, during the chick stage in 2011 and 2012.

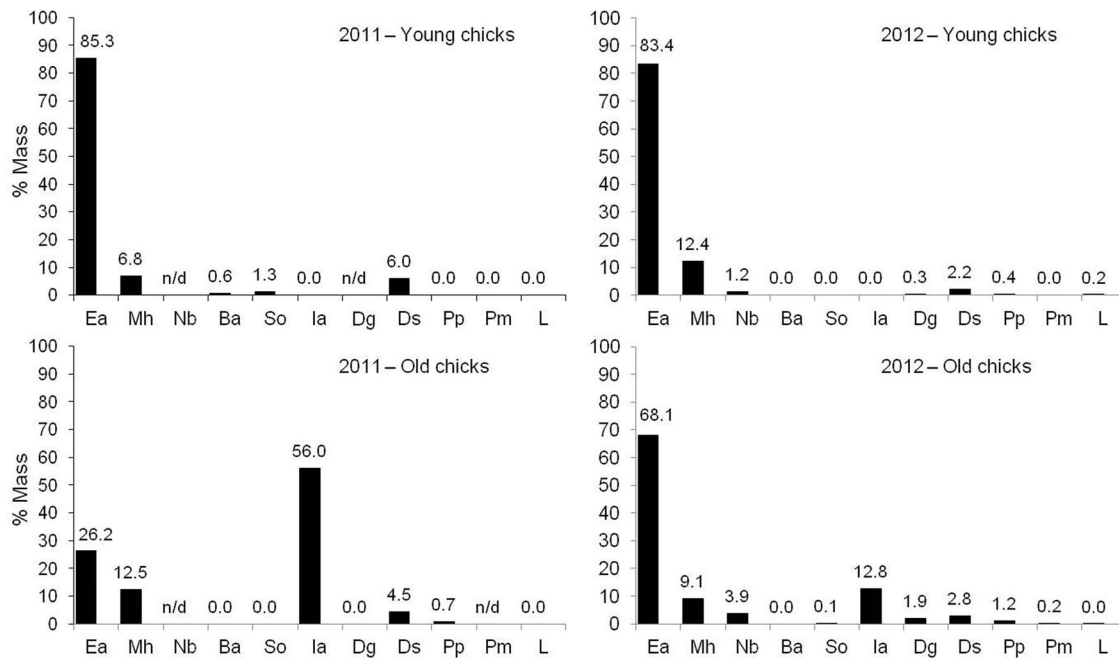
Prey	2011				2012			
	Young chicks		Old chicks		Young chicks		Old chicks	
	%F (n = 29)	%N (n = 1222)	%F (n = 30)	%N (n = 636)	%F (n = 26)	%N (n = 1018)	%F (n = 27)	%N (n = 946)
Fish								
<i>Engraulis anchoita</i> Hubbs & Marini, 1935	93.1	35.9	66.7	24.2	88.5	69.9	92.6	44.5
<i>Merluccius hubbsi</i> Marini, 1933	6.9	1.6	26.7	3.5	34.6	5.8	25.9	3.3
<i>Nemadactylus bergi</i> (Norman, 1937)	24.1	0.9	13.3	0.6	38.5	2.2	25.9	2.9
<i>Bovichtus argentinus</i> MacDonagh, 1931	24.1	12.4	10.0	0.5	7.7	0.7	18.5	3.8
<i>Odontesthes</i> sp.	10.3	1.9	6.7	28.1	11.5	1.3	29.6	5.6
<i>Ramnogaster arcuata</i> (Jenyns, 1842)	10.3	0.3	0.0	0.0	15.4	0.6	3.7	0.4
<i>Sebastes oculatus</i> Valenciennes, 1833	10.3	34.3	20.0	1.9	0.0	0.0	18.5	2.0
<i>Patagonotothen cornucola</i> (Richardson, 1844)	0.0	0.0	3.3	0.2	0.0	0.0	0.0	0.0
Unidentified fish	13.8	1.6	13.3	0.1	19.2	0.6	3.7	0.1
Cephalopods								
<i>Illex argentinus</i> (Castellanos, 1960)	0.0	0.0	33.3	4.9	0.0	0.0	7.4	0.6
<i>Doryteuthis gahi</i> (d'Orbigny, 1835)	3.4	0.2	0.0	0.0	11.5	0.4	22.2	0.6
<i>Doryteuthis sanpaulensis</i> (Brakonieccki, 1984)	44.8	10.0	30.0	5.0	26.9	4.2	25.9	2.5
Unidentified cephalopods	3.4	0.1	0.0	0.0	3.85	0.1	7.4	0.3
Crustaceans								
<i>Peisos petrunkevitchi</i> Burkenroad, 1945	3.4	0.7	16.7	29.9	30.8	9.0	25.9	33.0
<i>Pleoticus muelleri</i> (Spence Bate, 1888)	0.0	0.0	3.3	0.2	0.0	0.0	3.7	0.1
<i>Munida gregaria</i> (Fabricius, 1793)	3.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Unidentified crustaceans	0.0	0.0	0.0	0.0	7.7	0.2	0.0	0.0
Polychaetes								
<i>Laeonereis</i> sp.	0.0	0.0	0.0	0.0	11.5	4.9	3.7	0.1
Unidentified polychaetes	0.0	0.0	0.0	0.0	3.85	0.1	3.7	0.1
Total		1202		629		1008		941
Observed richness		11		11		10		13
Estimated richness		10.5 [9–11]		–		10 [10–10]		–
Diversity (Simpson)		3.6		4.2		1.9		3.2

white shrimp, silverside (*Odontesthes* sp.) and Argentine anchovy showed the largest numerical contributions (29.9%, 28.1% and 24.2%, respectively), while in 2012 the Argentine anchovy showed the largest contribution (44.5%) followed by the white shrimp and silverside (33.0% and 5.6%, respectively) (Table I). All other species showed a contribution by number of less than 5% during both chick stages in the two years.

In terms of contribution by mass, Argentine anchovy showed the highest contribution (68.1–85.3%), except for the old chick stage in 2011 (26.2%) when the shortfin squid *Illex argentinus* (Castellanos, 1960) was the main prey consumed (56.0%) (Figure 2). The importance by mass of all other prey species was less than 10%, except for Argentine hake in the old chick stage of 2011 and young chick stage of 2012 (12.5% and 12.4%, respectively) and the shortfin squid in the old chick stage of 2012 (12.8%). During the first season, the importance by mass of the different prey items was significantly different between the young and old chick stages (ANOSIM, Global  $R = 0.196$ ;  $p = 0.001$ ). The prey species that contributed most to the observed differences in diet composition between breeding stages, as designated by SIMPER, were Argentine anchovy (54.7%) and shortfin squid (22.9%),

accounting for approximately 75% of the differences in diet composition. During 2012, the importance by mass of the different prey items was similar between the two chick stages (ANOSIM, Global  $R = 0.006$ ;  $p = 0.28$ ). The analysis of stomach content samples showed that diet composition in terms of importance by mass during the chick stage differed between the two study years (two-way crossed ANOSIM, Global  $R = 0.04$ ,  $p = 0.02$ ) (Figure 2). The prey types designated by SIMPER as contributing most to the observed difference in diet composition were Argentine anchovy (55.3%) followed by shortfin squid (17.9%).

Stable isotope analysis indicated that sexes did not differ in their isotopic niche, tested using both centroid position and eccentricity ( $p > 0.05$ , using 1000 permutations), and therefore data from both sexes were pooled for subsequent analyses. In general,  $\delta^{13}\text{C}$  values of adults and chicks varied between  $-18.5\text{‰}$  and  $-16.4\text{‰}$ , whereas  $\delta^{15}\text{N}$  values varied between  $17.5\text{‰}$  and  $20.3\text{‰}$  (Figure 3a; Supplementary Figures S1 and S2). General mixing polygon sensitivity analysis (using 1500 iterations) showed that isotopic values of all individual samples, given the diet-tissue discrimination factors (DTDFs) and prey isotopic values used, were included in more than 95% of the simulated



**Figure 2.** Importance by mass (%) of prey recorded in stomach content samples of Magellanic penguins feeding their chicks at Isla Vernacci Norte, Argentina, during the young and old chick stages in 2011 ( $n = 29$  and  $30$ , respectively) and 2012 ( $n = 26$  and  $27$ , respectively). Ea: *Engraulis anchoita*, Mh: *Merluccius hubbsi*, Nb: *Nemadactylus bergi*, Ba: *Bovichtus argentinus*, So: *Sebastes oculatus*, Ia: *Illex argentinus*, Dg: *Doryteuthis gahi*, Ds: *Doryteuthis sanpaulensis*, Pp: *Peisos petrunkevitchi*, Pm: *Pleoticus muelleri*, L: *Laeonereis* sp.; n/d: no data.

mixing polygons, validating all proposed mixing models (Figure 3b).

Based on the isotopic values corresponding to adults and chicks during all stages in both years (Table II), the Bayesian mixing model outputs showed that Argentine anchovy was the main prey, particularly for adult penguins during the incubation stage (Figures 4 and 5). The results of the Bayesian mixing models obtained from chick whole-blood samples in both years were in agreement with those obtained from stomach content samples of adults feeding chicks (Figures 2 and 5), validating the isotope mixing model outputs.

During both years, adults showed significant differences among the four stages in their values of  $\delta^{13}\text{C}$  (2011: Kruskal–Wallis,  $H = 37.22$ ;  $df = 3$ ;  $p < 0.0001$ ; 2012:  $H = 48.70$ ;  $df = 3$ ;  $p < 0.0001$ ) and  $\delta^{15}\text{N}$  (2011: Kruskal–Wallis,  $H = 18.41$ ;  $df = 3$ ;  $p < 0.001$ ; 2012:  $H = 10.86$ ;  $df = 3$ ;  $p < 0.05$ ). In 2011, both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were more depleted during moult than during the three breeding stages (Paired-Sample Wilcoxon Test,  $p < 0.0001$ ). In 2012,  $\delta^{13}\text{C}$  values differed among stages (Paired-Sample Wilcoxon Test,  $p < 0.05$ ) except between the young and old chick stages (Paired-Sample Wilcoxon Test,  $p > 0.05$ ), with the observed differences being largest in relation to moult ( $p < 0.00001$ ); significant differences in  $\delta^{15}\text{N}$  values were only observed between the incubation and moult

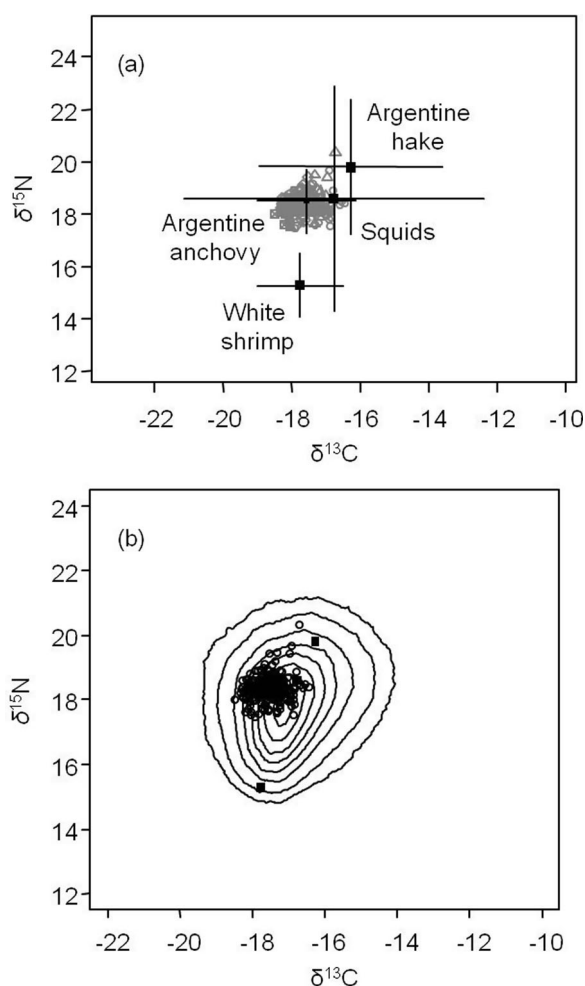
stages (Paired-Sample Wilcoxon Test,  $p < 0.05$ ). Centroid locations indicated that in 2011 the isotopic position of moulting adults differed from adults during the three breeding stages ( $p > 0.01$ , using 1000 permutations) and in 2012 it differed among all stages ( $p > 0.01$ , using 1000 permutations), except between the young and old chick stages ( $p > 0.05$ , using 1000 permutations). In addition, in both seasons no changes in the shape of the isotopic niches among breeding stages were detected (all  $p < 0.05$ , using 1000 permutations).

In both years, young and old chicks differed in the isotopic values of  $\delta^{13}\text{C}$  (2011: Welch's  $t$ -test,  $t = -4.93$ ;  $df = 11.28$ ;  $p < 0.0001$ ; 2012: Wilcoxon test,  $W = 19$ ;  $p < 0.05$ ) and  $\delta^{15}\text{N}$  (2011: Welch's  $t$ -test,  $t = -2.55$ ;  $p < 0.05$ ; 2012: Wilcoxon test,  $W = 14$ ;  $p < 0.01$ ), with old chicks showing more enriched  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Young and old chicks showed a different isotopic niche, as shown by the centroid locations considering both isotopes ( $p < 0.001$ , using 1000 permutations). No differences in the eccentricity of the isotopic niche were observed between young and old chicks ( $p > 0.05$ , using 1000 permutations).

### Diet and trophic niche across colonies

The Bayesian mixing model outputs showed that Argentine anchovy was the main prey consumed by adults and chicks in the three colonies studied





**Figure 3.** Dual stable isotope plot of  $\delta^{15}\text{N}$  (‰) and  $\delta^{13}\text{C}$  (‰) showing the isotopic values of whole blood of adult Magellanic penguins breeding at Golfo San Jorge, Argentina, and their potential prey. (a) Isotopic mixing diagram. Open circles: incubating adults; open triangles: adults during the young chick stage; diamonds: adults during the old chick stage; crossed squares: moulting adults; crosses: young chicks; inverted triangles: old chicks. Potential prey values corrected for fractionation are represented by solid squares (values are means and error bars  $\pm$  SD). (b) Simulated mixing region for the biplot in Figure 3a. The positions of individual penguins (open circles) and the average source values (solid squares) are shown. Probability contours are at the 5% level (outermost contour) and at every 10% level.

**Table II.** Stable isotope values (mean  $\pm$  SD) of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) of adult and chick Magellanic penguins at Isla Vernacci Norte, Golfo San Jorge, Argentina, in 2011 and 2012.

Year	Incubation		Young chicks		Old chicks		Moult	
	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Adults								
2011	18.3 $\pm$ 0.6	-17.4 $\pm$ 0.5	18.7 $\pm$ 0.6	-17.4 $\pm$ 0.2	18.5 $\pm$ 0.2	-17.5 $\pm$ 0.1	18.1 $\pm$ 0.3	-18.1 $\pm$ 0.2
2012	18.1 $\pm$ 0.3	-17.0 $\pm$ 0.3	18.3 $\pm$ 0.2	-17.2 $\pm$ 0.2	18.3 $\pm$ 0.2	-17.3 $\pm$ 0.2	18.4 $\pm$ 0.2	-17.9 $\pm$ 0.2
Chicks								
2011			18.6 $\pm$ 0.2	-18.0 $\pm$ 0.3	18.9 $\pm$ 0.3	-17.6 $\pm$ 0.1		
2012			18.3 $\pm$ 0.2	-17.9 $\pm$ 0.3	18.5 $\pm$ 0.4	-17.6 $\pm$ 0.2		

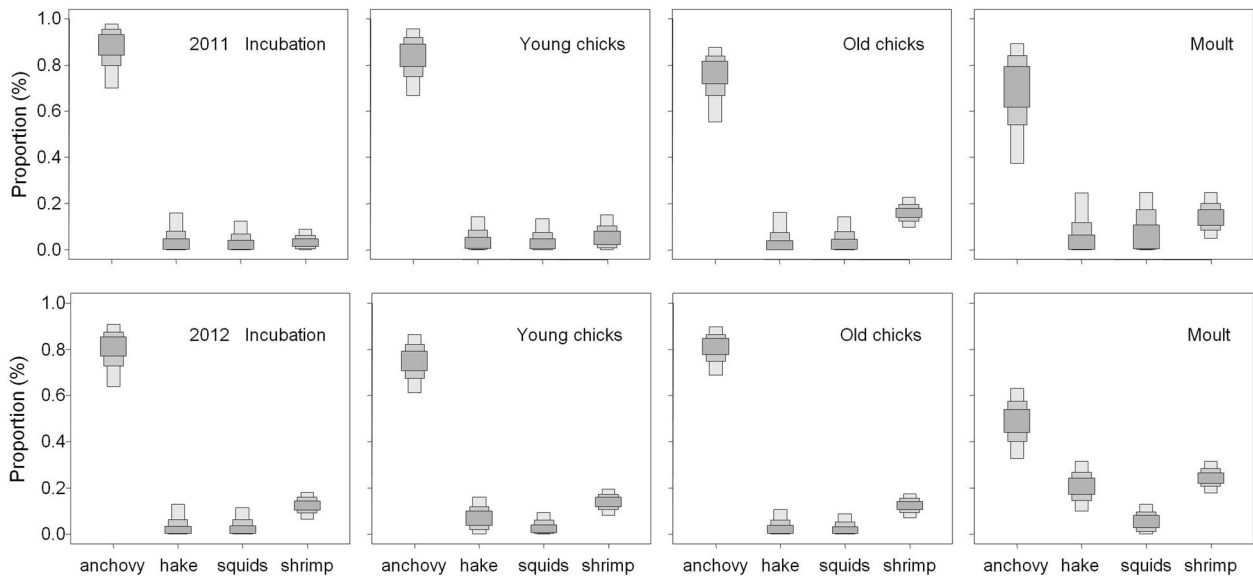
Note:  $n = 18$  for adults in all stages and years, except in the old chick stage of 2012 when  $n = 20$ , and  $n = 10$  for chicks in both stages and years.

(Table III). The estimated proportions of prey were similar among colonies (Table III), although the adult and chick diet at Isla Tova showed a relative increased diversity in the use of prey, with a reduction in the relative importance of Argentine anchovy and an increase in the estimated proportions of Argentine hake and white shrimps (Table III)

There were no differences in the values of both  $\delta^{13}\text{C}$  (Kruskal–Wallis,  $H = 3.31$ ;  $df = 2$ ;  $p = 0.19$ ) and  $\delta^{15}\text{N}$  (Kruskal–Wallis,  $H = 2.31$ ;  $df = 2$ ;  $p = 0.31$ ) among adults of the three breeding colonies. However, chicks showed significant differences in the values of  $\delta^{13}\text{C}$  (Kruskal–Wallis,  $H = 9.95$ ;  $df = 2$ ;  $p < 0.01$ ) but similar values of  $\delta^{15}\text{N}$  (Kruskal–Wallis,  $H = 0.88$ ;  $df = 2$ ;  $p = 0.67$ ). In particular, chicks from Isla Leones showed enriched values of  $\delta^{13}\text{C}$  compared to chicks from Isla Vernacci Norte (Paired Wilcoxon test,  $p < 0.05$ ). Adult penguins showed similar isotopic niches at the different colonies, presenting similar centroid location (all  $p > 0.05$ , using 1000 permutations) and similar eccentricity (all  $p > 0.05$ , using 1000 permutations). Chicks from Isla Leones showed a different isotopic niche from those from Isla Vernacci Norte, based on their different centroid positions ( $p < 0.01$ , using 1000 permutations). No differences were found in the eccentricity of the isotopic niche among the chicks of the different colonies (all  $p > 0.05$ , using 1000 permutations).

## Discussion

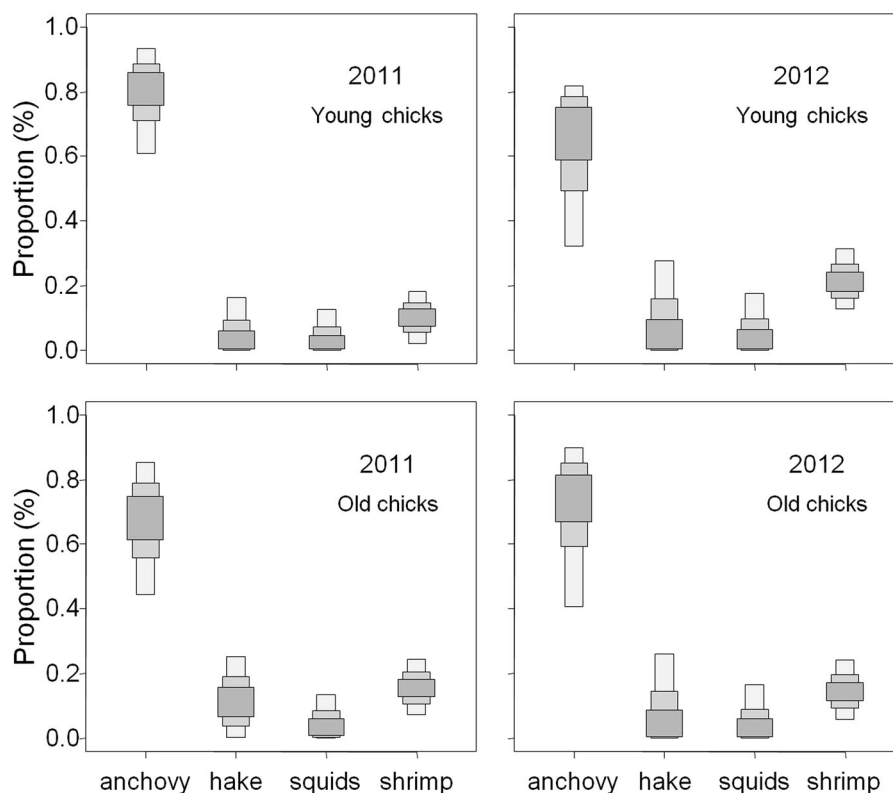
Our results based on conventional stomach analysis showed that Magellanic penguins breeding in Golfo San Jorge provisioned their chicks mostly with Argentine anchovy, in agreement with previous diet assessments at colonies located north of the study area (Gosztanyi 1984; Scolaro & Badano 1986; Frere et al. 1996; Scolaro et al. 1999; Wilson et al. 2005). Stable isotope analyses confirmed that Argentine anchovy was the main prey delivered to chicks and, moreover, that adults also fed mainly on this forage fish throughout the breeding cycle and moult in both study years.



**Figure 4.** Results of SIAR Bayesian isotope mixing models (50%, 75% and 95% credibility intervals) showing the estimated prey contributions to the self-feeding diets of Magellanic penguin adults from Isla Vernacci Norte during the incubation, young chick, old chick and moult stages of the 2011 and 2012 breeding seasons. Anchovy: *Engraulis anchoita*; hake: *Merluccius hubbsi*; squids: *Illex* and *Doryteuthis* mixture; shrimp: *Peisos petrunkevitchi*.

The similar pattern of prey consumption observed during the late chick stage at the three studied colonies located along the 70 km coastal sector also points to the relevance of Argentine anchovy for the Magellanic penguin population breeding in northern Golfo San Jorge. The Patagonian stock of Argentine anchovy extends from 41 to 48°S, but little information is available on its spatio-temporal distribution in Golfo San Jorge throughout the Magellanic penguin breeding cycle. However, Argentine anchovy distribution and abundance is related to sharp thermal gradients (Hansen et al. 2001), and a thermal front develops in spring and summer in the northern sector of Golfo San Jorge as a result of tidal mixing generated by coastal topography (Glembocki et al. 2015). Magellanic penguins breeding in the Isla Vernacci area have been shown to forage in waters within the northern sector of the gulf (Yorio et al. 2010; Sala et al. 2012), likely taking advantage of this near-shore oceanographic process. Among the other fish consumed by Magellanic penguins in Golfo San Jorge, Argentine hake and silver-side have previously been recorded in their diet at other breeding locations (Gosztonyi 1984; Scolaro & Badano 1986). Argentine hake, in particular, was identified as an important diet component during the late chick stage at Cabo Dos Bahías, representing nearly 50% of importance by mass (Frere et al. 1996), but showed only a secondary contribution to the diet of penguins breeding at our study locations.

In addition to fish, although in lower proportions, Magellanic penguins consumed cephalopods and crustaceans. Cephalopods are consumed to a greater or lesser extent by most penguin species (Croxall & Lishman 1987), and previous diet studies on Magellanic penguins indicate that this prey group may be more important than fish at some colonies located in southern Patagonia (e.g. shortfin squid and *Doryteuthis* sp.; Frere et al. 1996; Scolaro et al. 1999) and in the Malvinas (Falkland) Islands (e.g. *Gonatus* sp. and *Doryteuthis gahi* (d'Orbigny, 1835); Thompson 1993; Putz et al. 2001). Shortfin squid and *Doryteuthis* spp. are secondary prey in coastal Patagonia. Because they are energetically less valuable compared to fish (Heath & Randall 1985; Cherel & Ridoux 1992), cephalopods may constitute an alternative prey in situations where fish availability is temporarily reduced, as suggested by the combined results from conventional and stable isotope analyses. Results also showed that crustaceans contributed relatively little to the diet of Magellanic penguin chicks. Crustaceans can also be a significant component in the diet of several penguin species (Croxall & Lishman 1987), but in this study only the white shrimp showed a contribution, albeit small, to the diet of Magellanic penguin chicks. Despite the significant abundance of Argentine red shrimp and squat lobster *Munida gregaria* (Fabricius, 1793) in the study area (Boschi et al. 1981; Ravalli et al. 2013; Diez et al. 2016), these species were identified in only one stomach content and of minor



**Figure 5.** Results of SIAR Bayesian isotope mixing models (50%, 75% and 95% credibility intervals) showing the estimated prey contributions to the diets of Magellanic penguin chicks from Isla Vernacci Norte during the 2011 and 2012 breeding seasons. Anchovy: *Engraulis anchoita*; hake: *Merluccius hubbsi*; squids: *Illex* and *Doryteuthis* mixture; shrimp: *Peisos petrunkevitchi*.

numerical importance, suggesting their low relevance as food for Magellanic penguins in northern Golfo San Jorge. In contrast, studies based on stomach content analysis show that squat lobsters can represent up to 20% of the overall diet composition of Magellanic penguins breeding in the Malvinas (Falkland) Islands (Clausen & Pütz 2002), and that it is an important component in their diet in Tierra del Fuego, although mainly during the incubation stage (Scioscia et al. 2014).

Previous studies on Magellanic penguin diet have shown it can vary among the stages of the breeding cycle and between years (Thompson 1993; Clausen &

Pütz 2002; Scioscia et al. 2014), as has also been recorded for other penguin species (Ratcliffe & Trathan 2011). Stomach content analysis indicated that differences between young and old chicks in the first season were given by an increase in shortfin squid associated with a reduction in the consumption of Argentine anchovy. Although several studies have shown the durability of squid beaks in the digestive system (Furness et al. 1984; van Heezik & Seddon 1989), this is unlikely the cause of their high contribution during that particular chick stage, as all beaks found in samples were still attached to the body or

**Table III.** Prey contributions to the diets and stable isotope values (mean  $\pm$  SD) of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) of Magellanic penguin adults and chicks from Isla Vernacci Norte, Isla Tova and Isla Leones, Golfo San Jorge, Argentina, during the 2013 breeding season, estimated using SIAR Bayesian isotope mixing models.

	Isla Leones		Isla Tova		Isla Vernacci Norte	
	Adults	Chicks	Adults	Chicks	Adults	Chicks
n	10	10	10	8	16	16
Prey contributions						
Argentine anchovy	71 $\pm$ 12	71 $\pm$ 12	68 $\pm$ 10	66 $\pm$ 13	79 $\pm$ 6	73 $\pm$ 6
Argentine hake	9 $\pm$ 8	7 $\pm$ 7	9 $\pm$ 6	11 $\pm$ 8	4 $\pm$ 4	5 $\pm$ 4
Squids	6 $\pm$ 5	6 $\pm$ 5	5 $\pm$ 4	6 $\pm$ 5	4 $\pm$ 3	4 $\pm$ 3
White shrimp	13 $\pm$ 4	16 $\pm$ 4	17 $\pm$ 4	17 $\pm$ 5	13 $\pm$ 3	17 $\pm$ 3
Stable isotope values						
$\delta^{15}\text{N}$ (‰)	18.2 $\pm$ 0.3	18.4 $\pm$ 0.2	18.4 $\pm$ 0.3	18.4 $\pm$ 0.2	18.3 $\pm$ 0.3	18.4 $\pm$ 0.2
$\delta^{13}\text{C}$ (‰)	-17.4 $\pm$ 0.2	-17.6 $\pm$ 0.2	-17.3 $\pm$ 0.2	-17.7 $\pm$ 0.2	-17.5 $\pm$ 0.2	-17.9 $\pm$ 0.1

inside the buccal mass. However, the contribution by this prey throughout the chick-rearing period may have been overemphasized by the stomach content analysis, as this methodology provides information from only the last feeding event (Karnovsky et al. 2012) and does not allow an adequate diet description in the event of a high temporal variability in prey availability. Stable isotope analysis also indicated differences between both chick stages, and the more enriched  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in old chicks could have resulted from the consumption of larger Argentine anchovies and/or a slight increase in the consumption of Argentine hake, an opportunistic carnivore (Sanchez 2009). Isotopic analysis also showed that moulting adults differed from individuals sampled during the three breeding stages, which could be due to changes in the prey consumed as well as possible changes in DTDFs resulting from metabolic processes related to moult initiation. Despite these observed differences, Argentine anchovy dominated prey composition in adult and chick diets in all stages analysed in both study years.

The analysis of stomach samples indicated that Magellanic penguins fed on at least 15 different prey taxa, 2–3 times more species than those recorded in previous studies at other colonies in northern Patagonia (Gosztonyi 1984; Frere et al. 1996; Scolaro et al. 1999; Wilson et al. 2005). The higher trophic spectrum recorded in this study could be due to geographical differences in prey composition, to differences in the timing of sampling within the chick stage and/or to differences in sample gathering and processing (e.g. some studies flushed penguins only once, examined stomach contents on site and only by visual inspection and/or analysed a subsample of each stomach content). Moreover, most previous studies were conducted in the 1970s (Scolaro & Badano 1986) and 1980s (Gosztonyi 1984; Frere et al. 1996; Scolaro et al. 1999), so changes in the food supply of Magellanic penguins cannot be ruled out. In addition, previous studies of Magellanic penguin diet composition when provisioning chicks at colonies in northern Patagonia were based on prey frequency of occurrence and/or numerical importance (Gosztonyi 1984; Scolaro & Badano 1986; Scolaro et al. 1999; Wilson et al. 2005), except for the study by Frere et al. (1996), which also included their importance by mass. In the present study, the relative contribution of different prey to chick diet differed depending on the measurement used. Prey species such as Patagonian redfish, white shrimp or silverside presented relatively high values of numerical importance while others, such as the squid *D. sanpaulensis*, showed relatively high frequencies of

occurrence. However, they showed a low importance by mass due to their small size or occasional presence and, therefore, a low contribution to the diet of penguin chicks. This highlights the value of quantifying the importance by mass of prey species in addition to assessing their frequency of occurrence and numerical importance, so as to properly determine the relative contribution of the different prey consumed. Previous conventional diet studies were also based on the sampling of penguins returning to the colony to feed their offspring (Gosztonyi 1984; Scolaro & Badano 1986; Frere et al. 1996; Scolaro et al. 1999; but see Wilson et al. 2005), likely representing food delivered to chicks and not necessarily adult diet, thus providing only a partial understanding of prey requirement at the population level. The combination of diet methods proved valuable to assess the potential effects of the different sources of variation in diet composition and help overcome the biases from both methodologies, as has been highlighted in other studies (Bond & Jones 2009; Polito et al. 2011; Flemming & van Heezik 2014).

This study provides for the first time a comprehensive assessment of Magellanic penguin diet composition in northern Patagonia, quantifying the relative contribution of prey in the diet of adults and chicks throughout the nesting season, and confirms the relevance of a forage fish such as the Argentine anchovy in its trophic ecology. Our results agree with previous studies that argued the important role that the Argentine anchovy plays in the diet of this penguin species at other breeding locations in northern Patagonia (Frere et al. 1996; Forero et al. 2002; Wilson et al. 2005). The Magellanic penguin also relies mainly on forage fish elsewhere along its distributional breeding range, such as the Fuegian sprat *Sprattus fuegensis* (Jenyns, 1842) in southern continental Patagonia and Tierra del Fuego in Argentina (Frere et al. 1996; Wilson et al. 2005; Scioscia et al. 2014; Ciancio et al. 2015), and on the Peruvian anchovy *Engraulis ringens* Jenyns, 1842 and South American pilchard *Sardinops sagax* (Jenyns, 1842) in central Chile (Wilson et al. 1995). Forage fish are also key prey in other *Spheniscus* penguins breeding in Southern Africa, Chile, Peru and the Galapagos Islands (Randall & Randall 1986; Wilson et al. 1989; Herling et al. 2005; Boersma et al. 2013b). Many forage fish are targeted by commercial fisheries worldwide (Pikitch et al. 2012), including the northern stock of the Argentine anchovy (Hansen 2004; Pastous Madureira et al. 2009). Currently, the southern stock of the Argentine anchovy is not an important commercial target, although it is considered an alternative species to other partially overexploited fish in central Patagonia (Skewgar et al. 2007; Nelson

Bovcon 2016, personal communication). Several authors have pointed out the negative effects of fisheries targeting forage fish on seabird populations (e.g. Crawford 2004; Cury et al. 2011), so Magellanic penguin food requirements should be considered if an anchovy fishery is in fact developed in the study area.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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