ORIGINAL PAPER

Differences in diet composition and foraging patterns between sexes of the Magellanic penguin (*Spheniscus magellanicus*) during the non-breeding period as revealed by δ^{13}C and δ^{15}N values in feathers and bone

L. Silva · F. Saporit · D. Vales · M. Tavares · P. Gandini · E. A. Crespo · L. Cardona

Abstract In diving seabirds, sexual dimorphism in size often results in sex-related differences of foraging patterns. Previous research on Magellanic penguins, conducted during the breeding season, failed to reveal consistent differences between the sexes on foraging behavior, despite sexual dimorphism. In this paper, we tested the hypothesis that male and female Magellanic penguins differ in diet and foraging patterns during the non-breeding period when the constraints imposed by chick rearing activities vanish. We used stable isotope ratios of carbon and nitrogen in feather and bone to characterize the diet and foraging patterns of male and female penguins in the South Atlantic at the beginning of the 2009–2010 and 2010–2011 post-breeding seasons (feathers) and over several consecutive breeding and migratory seasons (bone). The mean δ^{13}C and δ^{15}N values of feathers showed no differences between the sexes in any of the three regions considered or in the diet composition between the sexes from identical breeding regions; however, Bayesian ellipses showed a higher isotopic niche width in males at the beginning of the post-breeding season. Stable isotope ratios in bone revealed the enrichment of males with δ^{13}C compared with females across the three regions considered. Furthermore, the Bayesian ellipses were larger for males and encompassed those of females in two of the three regions analyzed. These results suggest a differential use of winter resources between the sexes, with males typically showing a larger diversity of foraging/migratory strategies. The results also show that dietary differences between male and female Magellanic penguins may occur once the constraints imposed by chick rearing activities cease at the beginning of the post-breeding season.

L. Silva (✉) · D. Vales · E. A. Crespo
Laboratorio de Mamíferos Marinos, Centro Nacional Patagónico (CONICET), Boulevard Brown 2915, U9120ACF Puerto Madryn, Chubut, Argentina
e-mail: laura.silva.poly@gmail.com

F. Saporit · L. Cardona
Departamento de Biología Animal Institut de Recerca de la Biodiversitat, Faculty of Biology, Universidad de Barcelona, Avenida Diagonal 643, 08028 Barcelona, Spain

M. Tavares
Centro de Estudos Costeiros, Limnológicos e Marinhos, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (CECLIMAR/IB/UFRGS) e Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul (GEMARS), Av. Tramandaí, 976, Imbé 95625-000, Brazil

P. Gandini
Centro de Investigaciones de Puerto Deseado, Universidad Nacional de la Patagonia Austral-Unidad Académica Caleta Olivia, Av. Prefectura s/n, 9050 Puerto Deseado, Santa Cruz, Argentina

P. Gandini
Wildlife Conservation Society, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Puerto Deseado, Argentina

Introduction

Body size is important to explain between-species differences in diving performance (Schreer and Kovacs 1997; Watanuki and Burger 1999) and within-species differences, in which individuals differ significantly in body size because of age or sex (Bearhop et al. 2006; Drago et al. 2009a, b; Walker and Boersma 2003). For some penguin species, males are larger than females (Williams 1995), and this sexual dimorphism has been related to differences

in the foraging patterns and diets between sexes of certain species, such as *Pygoscelis adelia* (Clarke et al. 1998) and *Pygoscelis papua* (Clarke et al. 1998), but not of others, such as *Eudyptes chrysolophus* (Bearhop et al. 2006) and *Aptenodytes patagonicus* (Cherel et al. 2002).

Male Magellanic penguins (*Spheniscus magellanicus*) are 21 % heavier than females (Forero et al. 2001) and may potentially dive deeper and longer (Walker and Boersma 2003). Nevertheless, extensive studies using time–depth recorders and satellite telemetry indicate that adult male and female penguins typically have similar foraging and diving patterns during the chick rearing period (from mid-December to February), although differences have been observed in some colonies during the incubation period (from mid-September to December) and when chicks are close to fledging (from mid-February to March) (Walker and Boersma 2003; Wilson et al. 2005; Boersma and Rebstock 2009; Boersma et al. 2009; Sala et al. 2012; Raya Rey et al. 2012). Furthermore, stable isotope analyses have revealed only subtle, although statistically significant differences between male and female diets at the end of the chick rearing period (Forero et al. 2002).

A possible reason for these minor differences in foraging behavior and diet of adult Magellanic penguins during the chick rearing period is the convergence imposed on both sexes by the need to periodically return to the colony to feed the chicks (Pütz et al. 2002; Wilson et al. 2005; Boersma and Rebstock 2009; Boersma et al. 2009). Such a restriction gradually vanishes from the late chick rearing period to the post-breeding period (from mid-March to August) when the penguins remain in coastal areas but are completely aquatic (Pütz et al. 2000, 2007). Satellite telemetry has not revealed major differences in the winter foraging areas of males and females from identical colonies, but only a few individuals have been tracked (Pütz et al. 2000, 2007). Furthermore, dietary information corresponding to post-breeding grounds is limited to that supplied by the stomach contents of dead yearling Magellanic penguins that were stranded in Brazil (Fonseca et al. 2001; Pinto et al. 2007; Mäder et al. 2010).

The stable isotope analysis of feathers has become a powerful method to investigate the interbreeding foraging ecology of adult seabirds with sequential molt (Cherel et al. 2000; Bearhop et al. 2006; Quillfeldt et al. 2005; González-Solís et al. 2011). Feather keratin is metabolically inert after synthesis, and hence, the isotopic composition of feathers reflects diet during the molt process (Hobson and Clark 1992a; Bearhop et al. 2002). However, Magellanic penguins molt all their feathers over 15–20 days during the post-breeding season (Scolaro 1984; Adams and Brown 1990), which makes it impossible to establish diet over a long period from feather analysis. Bone offers an alternative approach because it integrates diet over several years

(Tieszen et al. 1983; Hobson and Clark 1992b), including both reproductive and non-reproductive periods. Accordingly, adult male and female Magellanic penguins are expected to differ in the stable isotope ratios of carbon and nitrogen in bone tissue if they differ in diet during the post-breeding period, assuming minor differences during the breeding season, as documented by other authors, a pattern that should be recorded in bone over the years. Therefore, this paper aims to use stable isotope ratios in feathers and bone to test the hypothesis that male and female Magellanic penguins differ in their foraging strategies during the non-breeding period.

Materials and methods

Study area and species biology

Magellanic penguins breed from 41° 32′S to 64° 59.72′W in the Atlantic (Schiavini et al. 2005) and can reach southeastern Brazil in winter during the migration period (Fonseca et al. 2001; Pinto et al. 2007; Mäder et al. 2010). The penguin samples used on this study were gathered from breeding colonies distributed from Cabo Virgenes (52° 22′S, 68° 24′W) to San Matías Gulf (41° 32′S, 64° 59.72′W) and were clustered into three major breeding areas: northern Patagonia, central Patagonia and southern Patagonia (Fig. 1). We also collect samples from migration grounds, Rio Grande do Sul, in southeastern Brazil (Fig. 1).

The breeding season begins in September with the arrival of adults to their breeding colonies and extends until late February or March when the chicks leave their colonies (Fig. 2; Scolaro 1984). The establishment of breeding pairs and egg laying corresponds to the early stage of the breeding season (September to October) and are characterized by fasting in both sexes (prior to egg laying for males and during egg laying for females), whereas during the incubation period (October and November) both members of the breeding pair alternate incubation with foraging trips (Scolaro 1984). The last period of the breeding season is devoted to chick rearing (December to February) and is characterized by increased foraging efforts from the adults, who feed the chicks and themselves. The post-breeding season comprises two major periods the chick fledging period (January to February) and molting in adults (March to April). There is a weight-gain period (gain in condition) during the final period of the post-breeding season followed by a fasting period. The weight-gain period is necessary to address the energy costs of synthesis and the growth of new feathers, which begins at sea (Cherel et al. 2005), whereas the fasting period is associated with plumage exchange, which

occurs on the breeding grounds (Adams and Brown 1990; Cherel et al. 2005). Importantly, new feathers emerge at sea during the weight-gain period and finish growing on the breeding grounds during the fasting period. When molting is complete, the penguins abandon their breeding grounds and the migration period begins, which occurs until the following September (Fig. 2).

Fig. 1 Study area, showing the four regions considered. Potential preys were collected from southern Brazil (*R1*), northern Patagonia (*R2*), central Patagonia (*R3*) and southern Patagonia (*R4*). Penguin samples were collected only from northern, central and southern Patagonia, as denoted by *black dots*

Sample collection

All samples of penguins and potential prey species were collected during fieldwork from 2009 to 2011 with the corresponding permissions granted by conservation agencies from the provinces of Rio Negro, Chubut, and Santa Cruz (Argentina), and of Rio Grande do Sul (Brazil).

Potential prey species were identified according to previous published dietary information (Frere et al. 1996; Fonseca et al. 2001; Pinto et al. 2007; Wilson et al. 2005) and included small pelagic fishes Argentine anchovy (*Engraulis anchoita*), Argentine hake (*Merluccius hubbsi*) shorter than 30 cm of total length, Fuegian sprat (*Sprattus fuegensis*), and Brazilian sardine (*Sardinella brasiliensis*), a demersal fish (*Eleginops maclovinus*), and demersal-pelagic squids [Argentine short-fin squid (*Illex argentinus*), South American long-fin squid (*Loligo sanpaulensis*), Patagonian squid (*L. gahi*) and slender inshore squid (*L. plei*)]. All these samples were provided by local fishermen or collected for the present study on board by staff from the CENPAT except for *E. anchoita* from Brazil, for which we used the stable isotope ratios reported by Bugoni et al. (2010).

No manipulations of living Magellanic penguins occurred when collecting feather or bone samples because all were collected from dead adults observed at the breeding colonies or dead and stranded adults at their migratory grounds. Age class was assessed consistent with plumage color (Scolaro 1978). Subsequently, each specimen was sexed using biometric characteristics (Bertellotti et al. 2002), which were insensitive to the slight latitudinal differences in body size reported for species in the Atlantic (Gandini et al. 1992). The Magellanic penguins were
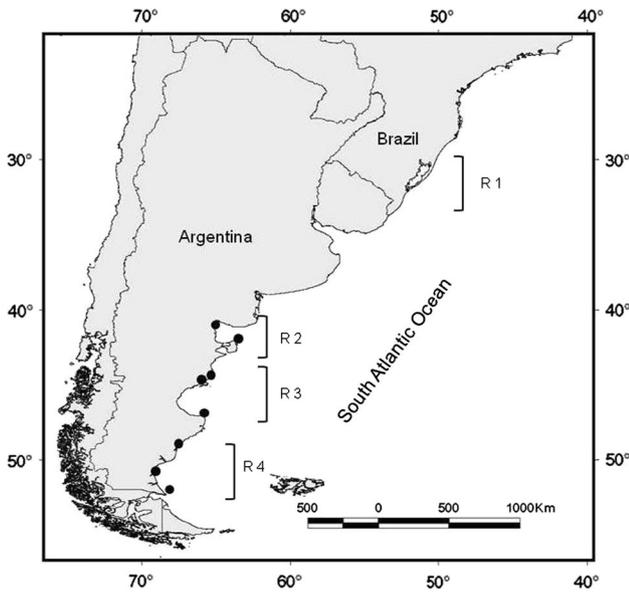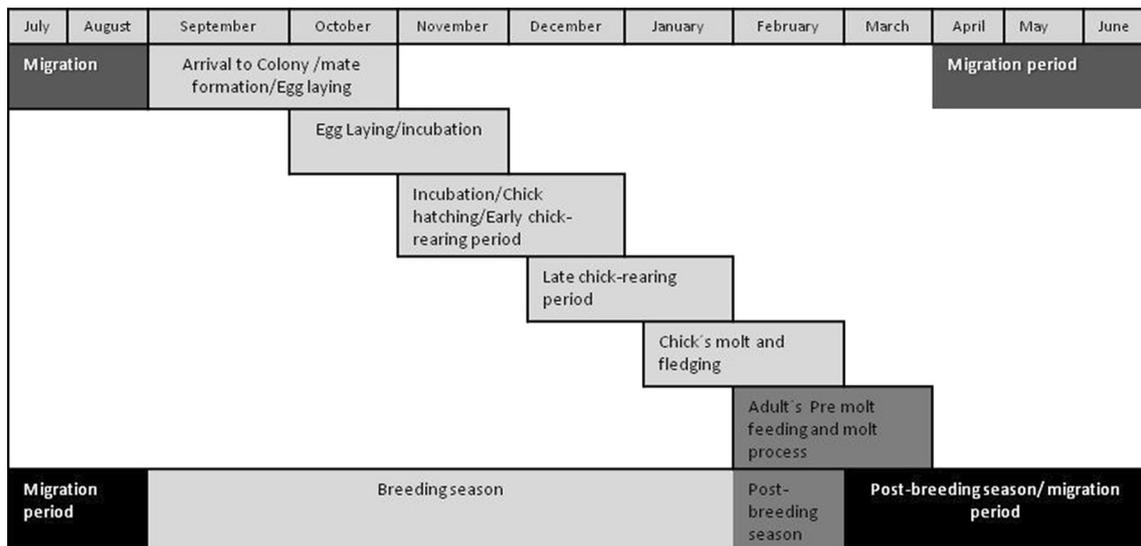

Fig. 2 Annual life cycle of Magellanic penguins from Patagonia (Argentina) showing: a detail of the different stages constituting the breeding season (in *light gray*), and its temporal relation to post-breeding season (in *dark gray*) and migration period (in *black*)

pooled into three groups according to the geographic location of the colony, northern, central and southern Patagonia (Fig. 1), the geographic scale of latitudinal changes in diet observed by Frere et al. (1996) and the three above-described major breeding areas. Ten females and 10 males were selected from each of the three regions considered, both for feather and bone stable isotope analyses.

We selected body feathers and bone tissue to perform the stable isotopes analysis because these tissues integrate dietary information over different time scales. Feathers are composed of keratin, which is an inert tissue that preserves an unchanged mean isotopic signature that corresponds to the time of synthesis (Cherel et al. 2005; Mizutani et al. 1992). Therefore, the time window integrated by the feathers depended on the duration of the molt process. Molting lasts from 15 to 20 days in Magellanic penguins, and all the feathers are simultaneously replaced at the beginning of the post-breeding period (Scolaro 1984). However, bone experiences a low turnover rate and integrates the isotopic signature of diet over several years (Bearhop et al. 2002; Cherel et al. 2005; Tieszen et al. 1983). We selected the mantle muscle from squids and white dorsal muscle from fish because these tissues offer the best proxy for stable isotope ratios in potential prey species (Pinnegar and Polunin 1999).

Stable isotope analysis

The prey samples were stored in a freezer at −20 °C until the feathers and bone could be extracted from the carcasses, cleaned and dry preserved at room temperature. Once in the laboratory, all samples (feathers, bone, fish muscle and squid mantle) were dried in a stove at 60 °C for 36 h and ground into a fine powder using a mortar and pestle. All feathers were washed in a chloroform–methanol (2:1) solution, rinsed thoroughly in distilled water to remove external contamination and dried in an oven at 60 °C to a constant mass. The tips (distal portion, see Cherel et al. 2005) of the feathers were then cut into small pieces. Four to five feathers per individual were processed for stable isotope analysis.

The lipids from muscle, mantle and bone were extracted with a chloroform/methanol (2:1) solution (Bligh and Dyer 1959) because lipids are depleted in ^{13}C compared with other molecules (DeNiro and Epstein 1977; Tieszen et al. 1983). Bone contains inorganic carbon that may contribute undesirable variability to the $\delta^{13}C$ (Lorrain et al. 2003). Consequently, the bone samples were previously soaked in 0.5N hydrochloric acid (HCl) for 24 h to decarbonize them (Ogawa and Ogura 1997; Newsome et al. 2006). Because HCl treatment adversely affects the $\delta^{15}N$ values (Bunn et al. 1995), each sample was divided into two sub-samples: one for carbon analysis after decarbonation and the other for nitrogen analysis without decarbonation. Approximately 0.7–1 mg of dried bone and 0.3–0.35 mg of feathers and prey muscle were weighed into tin cups (3.3 × 5 mm), combusted at 900 °C and analyzed in a continuous flow stable isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA; Thermo Finnigan, Bremen, Germany). Atropine was used as a system check for elemental analyses. The samples were processed at Centres Científics i Tecnològics of the University of Barcelona.

Stable isotope abundances, expressed in delta (δ) notation, in which the relative variations of stable isotope ratios are expressed in per mille (‰) deviations from predefined international standards, were calculated as:

$$\delta X = \left[\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] \times 10^3$$

where X is ^{13}C or ^{15}N, R sample is the heavy to light isotope ratio of the sample (C^{13}/C^{12} or N^{15}/N^{14}), and R standard is the heavy to light isotope ratio of the reference standards, which were VPDB (Vienna Pee Dee Belemnite) calcium carbonate for ^{13}C and atmospheric nitrogen (air) for ^{15}N. International isotope secondary standards of known C^{13}/C^{12} C ratios, as given by the IAEA (International Atomic Energy Agency), namely polyethylene (IAEA CH$_7$, $\delta^{13}C = -31.8‰$), graphite (IAEA USGS 24, $\delta^{13}C = -16.1‰$) and sucrose (IAEA CH 6, $\delta^{13}C = -10.4‰$), were used for calibration at a precision of 0.2 ‰. For nitrogen, international isotope secondary standards of known N^{15}/N^{14} ratios, namely (NH$_4$) 2SO$_4$ (IAEA N1, $\delta^{15}N = +0.4‰$ and IAEA N2, $\delta^{15}N = +20.3‰$ and KNO$_3$ (IAEA NO$_3$, $\delta^{15}N = +4.7‰$), were used to a precision of 0.3 ‰.

Data analyses

A nested ANOVA was used to test the importance of geographic distribution (region) and species identity on the average $\delta^{13}C$ and $\delta^{15}N$ values of the prey species. A two-way ANOVA followed by a post hoc Tukey test was used to assess the effect of sex and region on feather and bone stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$).

The relative contribution of potential prey to the diet of female and male Magellanic penguins was calculated using the Bayesian mixing model SIAR (Stable Isotope Analysis in R; Parnell et al. 2008). Only a selection of the most important prey species was included, according to information extracted from previously published conventional diet studies for breeding (Frere et al. 1996) and migratory grounds (Pinto et al. 2007; Fonseca et al. 2001; Mäder et al. 2010), because the use of too many prey species in the model reduces the information provided by the outcome (Parnell et al. 2008). Diet reconstruction through mixed models is only possible if trophic discrimination factors

are known, i.e., how the stable isotope ratios of carbon and nitrogen vary from diet-to-predator. The diet-to-feather and diet-to-bone (collagen) discrimination factors determined experimentally for gulls fed with perch (Hobson and Clark 1992b) were used in the present study. Finally, SIBER (Stable Isotope Bayesian Ellipses in R; Jackson et al. 2011) was used to calculate the Bayesian ellipses and isotopic overlap between sexes at the beginning of the post-breeding season (feathers) and year-round (bone). The Bayesian ellipses measure the breadth of the isotopic niche at the population level.

Results

The stable isotope ratios of potential prey species of Magellanic penguins (Fig. 1) differed among regions and prey species from the identical region (two-way nested ANOVA;

Table 1 Summary of nested ANOVA results to test for differences in the stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) of the considered potential prey species among regions and species

Prey	SS	df	MS	F	P
$\delta^{13}C$					
Model	29.05	16	1.82	8.54	<0.001
Region	3.24	3	1.08	5.07	0.003
Species (region)	25.81	13	1.99	9.34	<0.001
Error	13.4	63	0.21		
Total	42.44	79			
$\delta^{15}N$					
Model	372.14	16	23.26	40.5	<0.001
Region	180.41	3	60.14	104.72	<0.001
Species (region)	191.73	13	14.75	25.68	<0.001
Error	36.18	63	0.57		
Total	408.31	79			

Table 1). The post hoc tests revealed that specimens from southern Brazil were typically depleted in ^{15}N compared with those from the other three areas, whereas those from southern Brazil and central Patagonia were enriched in [13]C (Table 2). Furthermore, the topology of the prey community in the isoscape changed regionally because squids were more enriched in ^{13}C than *E. anchoita* in central and northern Patagonia, whereas the opposite result was true for southern Brazil. Nevertheless, *I. argentinus* was always the most depleted in [15]N (Fig. 3).

Despite the above-reported regional differences, adult Magellanic penguins from the three breeding areas did not differ in mean feather $\delta^{13}C$ and $\delta^{15}N$ values without any effect of sex (Tables 3, 4). SIAR indicated mixed fish and squid diets for penguins in the three regions at the beginning of the post-breeding season without a major difference in the average diet composition of males and females in any region (Fig. 4). The Bayesian ellipses calculated by SIBER for male penguins were much larger and encompassed those of female penguins in the three areas (Fig. 6), although the 95 % credibility intervals did not overlap in northern Patagonia. Consequently, the overlap between the sexes was highly asymmetrical and much larger for females (northern Patagonia: 95.9 %, central Patagonia: 100 % and southern Patagonia: 89.8 %) than for males (northern Patagonia: 35.6 %, central Patagonia: 21.6 % and southern Patagonia: 51.7 %), hence revealing a wider isotopic niche for males compared with females.

Sex and region had no statistically significant effects on bone $\delta^{15}N$ values (Table 4), although both sex and region had statistically significant effects on bone $\delta^{13}C$ values with no significant interaction term (Table 4). A post hoc Tukey test revealed that the bone tissue of penguins from northern and central Patagonia was enriched with ^{13}C compared with that of penguins from southern Patagonia and males were always more enriched with ^{13}C than females from the identical region (Table 5). SIAR revealed a prevalence of
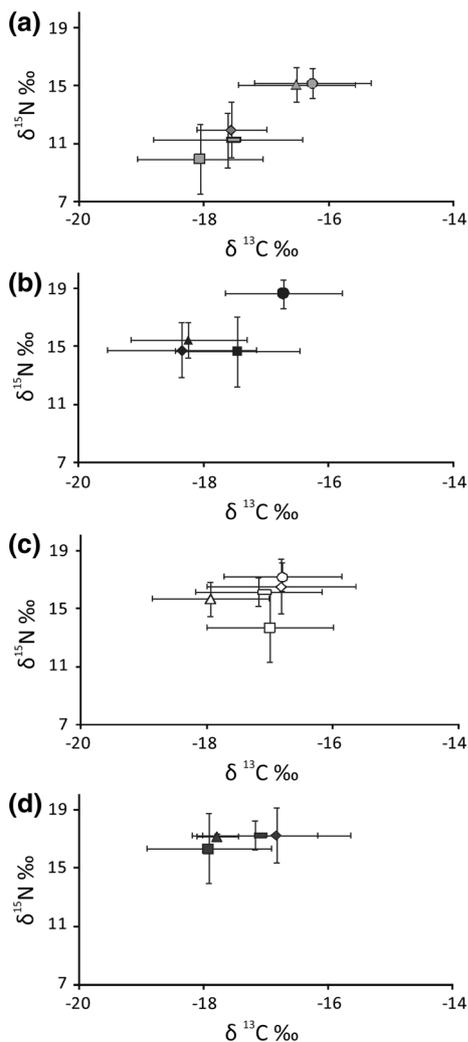
Table 2 Tukey test results for region on the stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in the prey of Magellanic penguins. Means with common letters are not significantly different ($p \leq 0.05$)

Region	$\delta^{13}C$ mean	n	SE		
Buenos Aires	−17.69	17	0.12	A	
Southern Patagonia	−17.43	18	0.11	A	B
Northern Patagonia	−17.15	25	0.09		B
Brazil	−17.12	20	0.10		B

Region	$\delta^{15}N$ mean	n	SE		
Brazil	12.87	20	0.17	A	
Northern Patagonia	15.86	25	0.15		B
Buenos Aires	15.87	17	0.20		B
Southern Patagonia	16.86	18	0.18			C

Fig. 3 Mean bivariated stable isotope ratios of the main prey of Magellanic penguins off southern Brazil (**a**), northern Patagonia (**b**), central Patagonia (**c**) and southern Patagonia (**d**). Species name: Brazil (*light gray circle, L. sampaulensis*; *light gray square, I. argentinus*; *light gray triangle, E. ancohíta*; *light gray diamond, S. brasiliensis*; *light gray rectangle, L. plei*); northern Patagonia (*filled circle, L. sampaulensis*; *filled square, I. argentinus*; *filled triangle, E. ancohíta*; *filled diamond, M. hubbsi*); central Patagonia (*open circle, L. sampaulensis*; *open square, I. argentines*; *open triangle, E. ancohíta*; *open diamond, M. hubbsi*; *open rectangle, L. gahi*); southern Patagonia (*dark gray square, I. argentines*; *dark gray triangle, S. fueguensis*; *dark gray diamond, M. hubbsi*; *dark gray rectangle, L. gahi*). Sample size 5 for all the species, except for the *Merluccius hubbsi* in northern Patagonia (*n* = 2) and southern Patagonia (*n* = 3)

small schooling fish in the annual diet of penguins of both sexes in the three regions, as integrated in the stable isotope ratios of bone tissue. Although differences in the $\delta^{13}C$ values of males and females did not result in major differences in the composition of diets calculated by SIAR (Fig. 5), the Bayesian ellipses of males were larger and encompassed those of females in northern and southern Patagonia, whereas the opposite result was true for central Patagonia

(Fig. 6). Furthermore, the overlap between the Bayesian ellipses of males and females from identical breeding areas, calculated from the stable isotope ratios in bone, was highly asymmetrical, typically larger for females (northern Patagonia: 87.0 %, central Patagonia: 42.1 % and southern Patagonia: 76.5 %) than for males (northern Patagonia: 42.8 %, central Patagonia: 74.3 % and southern Patagonia: 27.3 %) and always smaller than that observed when the stable isotope ratios in feathers were used (Fig. 6).

Discussion

Previous studies based on stomach content analyses have reported contrasting diet compositions between individuals deriving from northern, central and southern Patagonia (Frere et al. 1996; Wilson et al. 2005), but these reports only investigated the breeding season. The results reported here confirm statistically significant differences in the stable isotope ratios of potential prey from those three areas, and hence, the feathers that were formed while on those regions were expected to capture that variability. However, the stable isotope ratios in feathers did not vary regionally. The explanation for this apparent contradiction most likely resides in the timing of the molt process in this species, which occurs once the breeding season is over and the parents are free from chick rearing activities (Scolaro 1984). Accordingly, the isotopic composition of the feathers represents diet composition at that time (Cherel et al. 2000; Quillfeldt et al. 2005) and does not necessarily correspond to that revealed by the stomach contents of reproductively active adults, whose foraging behavior is constrained by the need to return to the colony to feed the chicks (Frere et al. 1996; Wilson et al. 2005). This result may also explain why the stomach content analyses conducted during the breeding season reported a prevalence of fish in the diet of Magellanic penguins breeding along Patagonia (Frere et al. 1996; Wilson et al. 2005), whereas mixed fish and squid diets have emerged from the stable isotope analysis reported here. Differences in the diet composition delivered to chicks and that consumed by the adults have also been reported for other penguin species (Cherel et al. 2002).

Independent of the reason why the stable isotope ratios of feathers did not vary along the coastline of Argentina, the absence of statistically significant differences in the stable isotope ratios of male and female feathers indicated no major differences in the average diet composition of adult penguins at the beginning of the post-breeding season, which is a result consistent with the similarity of diving and foraging patterns previously reported for most of the breeding season (Wilson et al. 1995; Walker and Boersma 2003; Wilson et al. 2005; Boersma et al. 2009; Boersma and Rebstock 2009; Sala et al. 2012; Raya Rey et al. 2012).

Table 3 Stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) in feathers and bone of adult Magellanic penguins from breeding colonies in Argentina

	Northern Patagonia		Central Patagonia		Southern Patagonia	
	Females	Males	Females	Males	Females	Males
Feathers						
δ^{13}C	−15.10 ± 0.73 (10)	−14.96 ± 0.98 (10)	−15.39 ± 0.62 (10)	−14.87 ± 1.57 (10)	−16.19 ± 0.94 (10)	−15.39 ± 1.64 (10)
δ^{15}N	18.88 ± 1.40 (10)	18.98 ± 2.37 (10)	19.45 ± 1.01 (10)	19.16 ± 2.13 (10)	19.27 ± 1.12 (10)	19.21 ± 1.11 (10)
Bone						
δ^{13}C	−12.45 ± 0.93 (10)	−12.31 ± 1.00 (10)	−12.74 ± 0.99 (10)	−12.22 ± 0.56 (10)	−13.54 ± 0.70 (10)	−12.74 ± 1.12 (10)
δ^{15}N	16.38 ± 1.19 (10)	16.48 ± 2.41 (10)	17.16 ± 0.56 (10)	16.95 ± 0.51 (10)	16.71 ± 0.39 (10)	16.77 ± 0.57 (10)

Data are shown as mean ± standard deviation. Sample size is always 10

Forero et al. (2002) reported significant differences for stable isotope ratios in the blood of adult males and females at the end of the chick rearing period, but the differences were extremely small (average δ^{13}C males = −16.2 ‰, average δ^{13}C females = −16.5 ‰) and resulted from a 2 % difference in the consumption of anchovies.

Despite the high similarities in diet composition suggested by SIAR, the Bayesian ellipses of males at the beginning of the post-breeding season were larger than those of females in the three areas. Furthermore, the size of the Bayesian ellipses of each sex did not vary among areas and the Bayesian ellipse of the females was always included in that of the males. Because the Bayesian ellipses do not measure the diversity of resources used by the population but the diversity of individual patterns of resource use, these results suggest that males exhibited a much larger diversity of foraging strategies than females at the beginning of the post-breeding period, although this trend did not result in differences in the average composition of the diet. Large penguins can regularly dive as deep as 50 m, which is a capacity that does not preclude them from also foraging in shallower waters (Stokes and Boersma 1999; Walker and Boersma 2003; Wilson et al. 2005). Conversely, small adult penguins can only forage at the top of the water column, which is typically shallower than 20 m (Stokes and Boersma 1999; Walker and Boersma 2003; Wilson 2003; Wilson et al. 2005). Thus, the larger Bayesian ellipses of males at the beginning of the post-breeding season might have emerged from a broader spectrum of diving capabilities linked to a larger body size. Deploying time–depth recorders at the beginning of the post-breeding season will be useful to test this hypothesis.

The bone tissue of adult males showed a ^{13}C enrichment pattern across areas compared with that of females. That pattern was consistent across regions, although the δ^{13}C values of both sexes were lower in southern Patagonia

Table 4 Summary statistics of two-way ANOVA to test the effect of region and sex on the stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) in the feathers and bone of Magellanic penguins from Argentina

	SS	df	MS	F	P
Feathers					
δ13C					
Model	5.70	5	1.14	0.89	0.492
Sex	0.46	1	0.46	0.36	0.549
Region	5.02	2	2.51	1.97	0.149
Sex × region	0.72	2	0.36	0.28	0.754
Error	65.00	51	1.27		
Total	70.69	56			
δ15 N					
Model	13.18	5	2.64	1.15	0.349
Sex	0.19	1	0.19	0.08	0.775
Region	11.07	2	5.54	2.41	0.100
Sex × region	1.17	2	0.59	0.25	0.776
Error	117.38	51	2.30		
Total	130.55	56			
Bones					
δ13C					
Model	11.47	5	2.294	2.811	0.025
Sex	3.55	1	3.553	4.353	0.042
Region	6.82	2	3.411	4.179	0.021
Sex × region	1.09	2	0.549	0.672	0.515
Error	44.07	54	0.816		
Total	55.54	59			
δ15N					
Model	2.09	5	0.419	0.304	0.908
Sex	0.34	1	0.338	0.245	0.622
Region	1.61	2	0.803	0.583	0.561
Sex × region	0.15	2	0.075	0.55	0.947
Error	74.33	54	1.377		
Total	76.43	59			

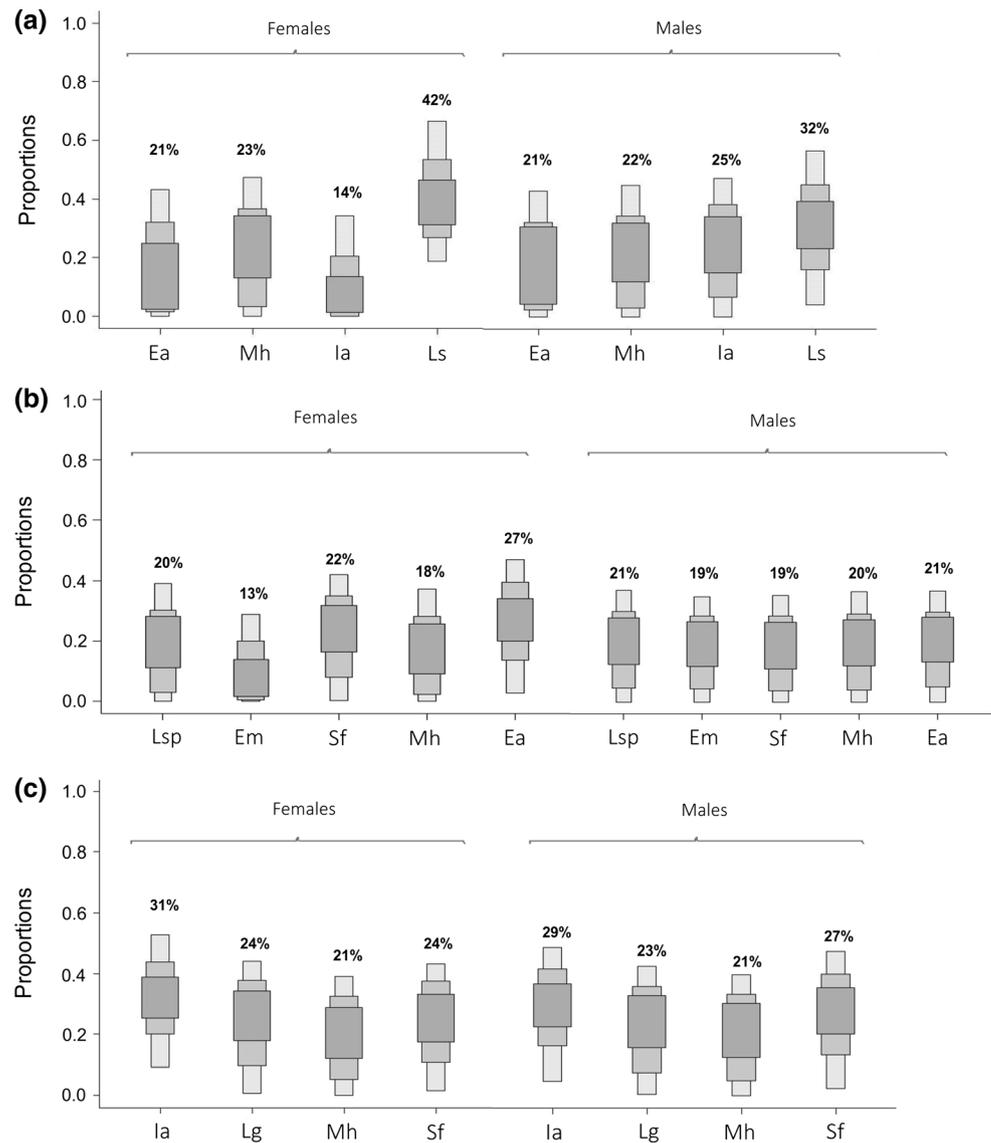**Table 5** Tukey test results for sex and region on the stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) in the bone of Magellanic penguins from Argentina

	δ^{13}C mean	n	SE		
Sex					
Female	−15.56	30	0.16	A	
Male	−15.07	30	0.16		B
Region					
Southern	−15.79	20	0.2	A	
Central	−15.13	20	0.2	A	B
North	−15.03	20	0.2		B

Means with common letters are not significantly different ($p < 0.05$)

than in northern or central Patagonia. Because the feath-
ers suggested no major differences in diet composition
at the beginning of the post-breeding season and previous

studies indicated that adult males and females typically have
similar foraging and diving patterns throughout the breed-
ing season (Wilson et al. 1995, 2005; Walker and Boersma

Fig. 5 Feasible contribution of potential preys to the diet of males and females Magellanic penguin as revealed by stable isotope ratios of carbon and nitrogen in bone from individuals breeding in northern (**a**), central (b) and southern (c) Patagonia. The 95, 75 and 50 % credibility intervals for the calculated feasible contribution of each prey are shown. (Ea: *E. ancohíta*; Mh: *M. hubbsi*; Ia: *I. argentinus*; Ls: *L. sanpaulensis*; Lsp: *Loligo spp*; Em: *E. maclovinus*; Sf: *S. fueguensis*; Lg: *L. gahi*; Lp: *L. plei*; Sb: *S. brasiliensis*.)
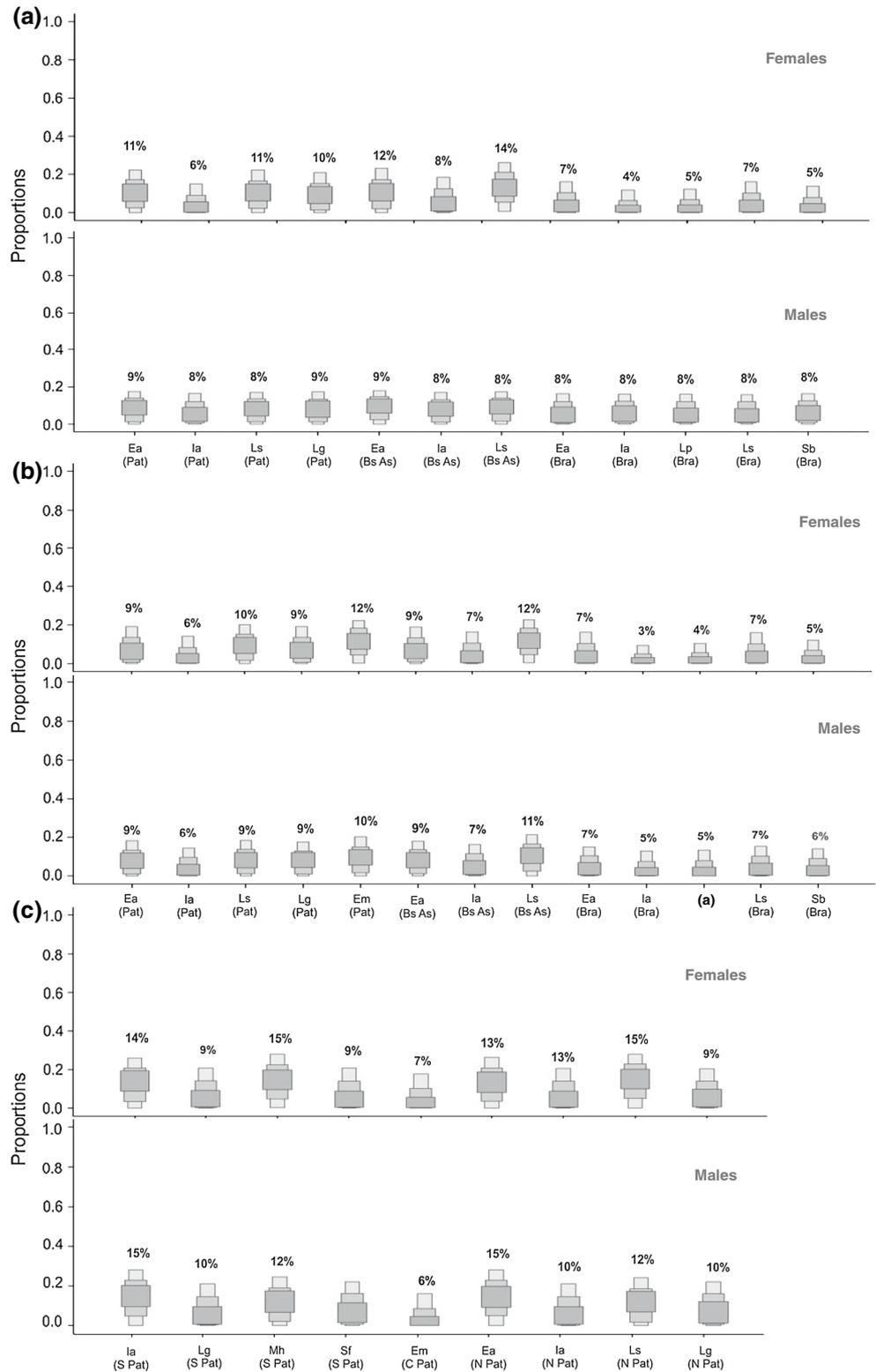
2003; Boersma et al. 2009; Boersma and Rebstock 2009; Sala et al. 2012; Raya Rey et al. 2012), the contrasting stable isotope ratios in bone tissue must result from major dietary differences in winter diets. During the post-breeding season, the penguins move across different feeding grounds (Pütz et al. 2000, 2007) and stable isotope ratios in bone are expected to incorporate dietary changes and the isotopic variation observed among regions. Potential prey species

Fig. 6 Bayesian ellipses of adult Magellanic penguins according to stable isotope ratios in feathers (*left panels*) and bone (*right panels*) from the three breeding areas: northern (*upper panels*), central (*middle panels*) and southern Patagonia (*lower panels*)
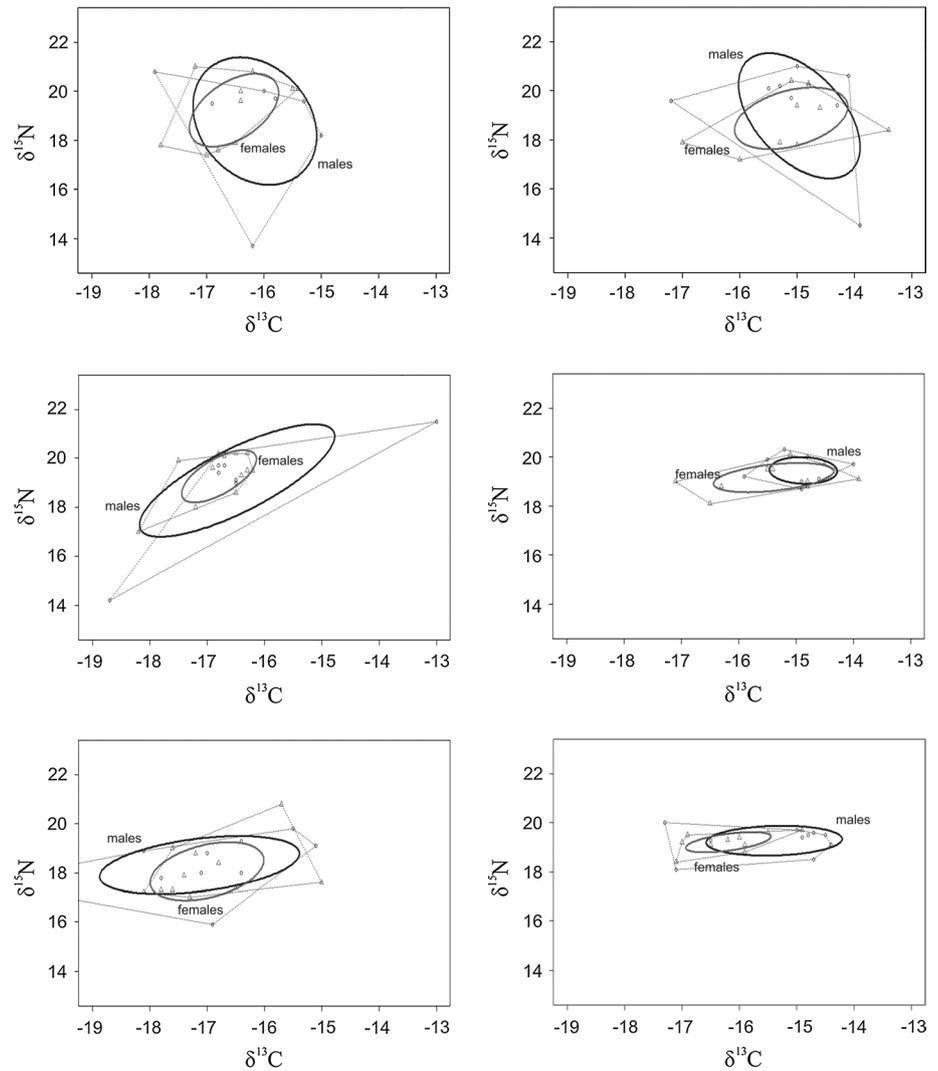
from southern Brazil are enriched with ^{13}C compared with potential prey from northern and southern Patagonia. This result suggests that the above-reported differences of average bone δ^{13}C values between breeding areas emerge because penguins that breed in northern and central Patagonia most likely made an extended use of southern Brazil during the winter (Williams 1995; Stokes et al. 1998; Pütz et al. 2000) than those from southern Patagonia, which is a result consistent with previous studies (Pütz et al. 2007). Nevertheless, the large Bayesian ellipses revealed by SIBER for males and females breeding in northern Patagonia compared with those from the other two regions might reflect a larger diversity of migratory patterns in the penguins breeding in northern Patagonia. Conversely, the smaller Bayesian ellipses calculated for the other two regions might suggest more homogenous migratory patterns among the breeding individuals. However, a larger sample size is necessary to further examine this hypothesis concerning isotopic niche widths (see Syväranta et al. 2013).

The δ^{13}C bone tissue differences between sexes are small and may be biologically meaningless. Certainly, SIAR revealed similar annual diets for both sexes in the three areas, but this result could be an artifact caused by the migratory habitats of the penguins across regions with contrasting baselines of stable isotope ratios and the incorporation of many potential prey species into the mixed models (Parnell et al. 2008). However, satellite telemetry indicates the use of similar winter foraging regions by males and females during the initial months of migration (Pütz et al. 2000, 2007), but nothing is known about the rest of the year or penguins from the other two breeding areas. Interestingly, the foraging grounds in Brazil and central Patagonia are characterized by high δ^{13}C values in potential prey species and males nesting in northern and southern Patagonia have larger Bayesian ellipses than females from the identical regions. In this scenario, statistically significant differences between the sexes may emerge because of a larger diversity of migratory

strategies in males. The analysis of stable isotope ratios in the claws of penguins arriving at the breeding colonies at the beginning of the breeding season could be beneficial to track their winter foraging grounds and test the hypothesis that males and females from identical breeding areas differ in their winter dispersal patterns.

In conclusion, the overall evidence suggests that the foraging strategies of male and female Magellanic penguins converge during the breeding season when both parents become central place foragers because of limitations imposed by chick feeding. However, small differences in the bone δ^{13}C values were observed between males and females, which potentially indicated different foraging patterns during the non-breeding season once the constraints imposed by chick rearing activities ceased. The most notable difference was a larger diversity of foraging and migratory strategies among males.

References

Adams NJ, Brown CR (1990) Energetics of molt in penguins. In: Davis LS, Darby JT (eds) Penguin biology. Academic Press, San Diego, pp 297–315

Bearhop S, Waldron S, Votier SC, Furness RW (2002) Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. Physiol Biochem Zool 75(5):451–458

Bearhop S, Phillips RA, McGill R, Cherel Y, Dawson DA, Croxall JP (2006) Stable isotopes indicate sex-specific and long-term individual foraging specialization in diving seabirds. Mar Ecol Prog Ser 311:157–164

Bertellotti M, Tella J, Godoy J, Blanco G, Forero M, Donázar J, Ceballos O (2002) Determining sex of Magellanic penguins using molecular procedures and discriminant functions. Waterbirds 25:479–484

Bligh E, Dyer W (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Boersma PD, Rebstock GA (2009) Foraging distance affects reproductive success in Magellanic penguins. Mar Ecol Prog Ser 375:263–275

Boersma PD, Rebstock G, Frere E, Moore SE (2009) Following the fish: penguins and productivity in the South Atlantic. Ecol Monogr 79(1):59–76

Bugoni L, McGill RA, Furness RW (2010) The importance of pelagic longline fishery discards for a seabird community determined through stable isotope analysis. J Exp Mar Biol Ecol 391:190–200

Bunn SE, Loneragan NR, Kempster MA (1995) Effects of acid washing on stable isotope ratios of C and N in penaeid shrimp and seagrass: implications for food-web studies using multiple stable isotopes. Limnol Oceanogr 40:622–625

Cherel Y, Hobson KA, Weimerskirch H (2000) Using stable-isotope analysis of feathers to distinguish moulting and breeding origins of seabirds. Oecologia 122:155–162

Cherel Y, Pütz K, Hobson KA (2002) Summer diet of king penguins (*Aptenodytes patagonicus*) at the Falkland Islands, southern Atlantic Ocean. Polar Biol 25(12):898–906

Cherel Y, Hobson KA, Bailleul F, Groscolas R (2005) Nutrition, physiology, and stable isotopes: new information from fasting and molting penguins. Ecology 86(11):2881–2888

Clarke J, Manly B, Kerry K, Gardner H, Franchi E, Corsolini S, Focardi S (1998) Sex differences in Adélie penguin foraging strategies. Polar Biol 20:248–258

DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197:261–263

Drago M, Cardona L, Crespo EA, Aguilar A (2009a) Ontogenic dietary changes in South American sea lions. J Zool 279:251–261

Drago M, Crespo EA, Aguilar A, Cardona L, García N, Dans SL, Goodall N (2009b) Historic diet change of the South American sea lion in Patagonia as revealed by isotopic analysis. Mar Ecol Prog Ser 384:273–286

Fonseca VS, Petry MV, Jost AH (2001) Diet of the Magellanic penguin on the coast of Rio Grande do Sul, Brasil. Waterbirds 24:290–293

Forero MG, Tella JL, Donázar JA, Blanco G, Bertellotti M, Ceballos O (2001) Phenotypic assortative mating and within-pair sexual dimorphism and its influence on breeding success and offspring quality in Magellanic penguins. Can J Zool 79:1414–1422

Forero MG, Hobson KA, Bortolotti GR, Donázar JA, Bertellotti M, Blanco G (2002) Food resource utilisation by the Magellanic penguin evaluated through stable-isotope analysis: segregation by sex and age and influence on offspring quality. Mar Ecol Prog Ser 234:289–299

Frere E, Gandini PA, Lichtschein V (1996) Variación latitudinal en la dieta del Pingüino de Magallanes (*Spheniscus magellanicus*) en la costa Patagónica, Argentina. Ornitol Neotrop 7:35–41

Gandini PA, Frere E, Holik TM (1992) Implicancias de las diferencias en el tamaño corporal entre colonias para el uso de medidas morfométricas como método de sexado en *Spheniscus magellanicus*. Hornero 13:211–213

González-Solís J, Smyrli M, Militão T, Gremillet D, Tveraa T, Phillips RA, Boulinier T (2011) Combining stable isotope analyses and geolocation to reveal kittiwake migration. Mar Ecol Prog Ser 435:251–261

Hobson KA, Clark RG (1992a) Assessing avian diets using stable isotopes I: turnover of 13 C in tissues. Condor 94:181–188

Hobson KA, Clark RG (1992b) Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. Condor 94:189–197

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. J Anim Ecol 80(3):595–602

Lorrain A, Savoye N, Chauvaud L, Paulet Y, Naulet N (2003) Decarbonation and preservation method for the analysis of organic C and N contents and stable isotope ratios of low-carbonated suspended particulate material. Anal Chim Acta 491:125–133

Mäder A, Sander M, Casa Jr G (2010) Pinguins-de-magalhães arribados na costa do Rio Grande do Sul: composição da dieta e ecologia alimentar. III Congresso Brasileiro de Oceanografia Rio Grande

Mizutani H, Fukuda M, Kabaya Y (1992) ^{13}C and ^{15}N enrichment factors of feathers of 11 species of adult birds. Ecology 73:1391–1395

Newsome SD, Etnier MA, Aurioles-Gamboa D, Koch PL (2006) Using carbon and nitrogen isotope values to investigate maternal strategies in northeast Pacific otariids. Mar Mammal Sci 22:556–572

Ogawa N, Ogura N (1997) Dynamics of particulate organic matter in the Tamagawa Estuary and inner Tokyo Bay. Estuar Coast Shelf Sci 44:263–273

Parnell A, Inger R, Bearhop S, Jackson AL (2008) SIAR: stable isotope analysis in R. http://cran.r-project.org/web/packages/siar/index.html

Pinnegar JK, Polunin NVC (1999) Differential fractionation of δ^{13}C and δ^{15}N among fish tissues: implications for the study of trophic interactions. Funct Ecol 13:225–231

Pinto MBLC, Siciliano S, Di beneditto PM (2007) Stomach contents of the Magellanic penguin *Spheniscus magellanicus* from the northern distribution limit on the Atlantic coast of Brazil. Mar Ornithol 35:77–78

Pütz K, Ingham RJ, Smith G (2000) Satellite tracking of the winter migration of Magellanic penguins *Spheniscus magellanicus* breeding in the Falkland Islands. Ibis 142:614–622

Pütz K, Ingham RJ, Smith JG (2002) Foraging movements of Magellanic penguins *Spheniscus magellanicus* during the breeding season in the Falkland Islands. Aquatic Conserv Mar Freshw Ecosyst 12:75–87

Pütz K, Schiavini A, Raya Rey A, Lüth BH (2007) Winter migration of magellanic penguins (*Spheniscus magellanicus*) from the southernmost distributional range. Mar Biol 152:1227–1235

Quillfeldt P, McGill R, Furness RW (2005) Diet and foraging areas of Southern Ocean seabirds and their prey inferred from stable isotopes: review and case study of Wilson's storm-petrel. Mar Ecol Prog Ser 295:295–304. doi:10.3354/meps295295

Raya Rey A, Pütz K, Scioscia G, Lüthi B, Schiavini A (2012) Sexual differences in the foraging behaviour of Magellanic Penguins related to stage of breeding. Emu 112:90–96

Sala JE, Wilson RP, Frere E, Quintana F (2012) Foraging effort in Magellanic penguins in coastal Patagonia, Argentina. Mar Ecol Prog Ser 464:273–287

Schiavini A, Yorio P, Gandini P, Raya Rey A, Boersma PD (2005) Los Pingüinos de las costas Argentinas: estado poblacional y conservación. Hornero 20:5–23

Schreer JF, Kovacs KM (1997) Allometry of diving capacity in air-breathing vertebrates. Can J Zool 75:339–358

Scolaro J (1978) El Pingüino de Magallanes (*Spheniscus magellanicus*) IV. Notas biológicas y de comportamiento. Serie Científica. Publicaciones ocasionales del Instituto de Biología Animal. Serie científica 10:1–6

Scolaro J (1984) Revisión sobre la biología de la reproducción del pingüino de Magallanes (*Spheniscus magellanicus*) El ciclo biológico anual. Contribución Centro Nacional Patagónico 91:1–26

Stokes DL, Boersma PD (1999) Where breeding Magellanic penguins *Spheniscus magellanicus* forage: satellite telemetry results and their implications for penguin conservation. Mar Ornithol 27:59–65

Stokes DL, Boersma PD, Davis LD (1998) Satellite tracking of Magellanic penguins migration. Condor 100:376–381

Syväranta J, Lensu A, Marjomäki TJ, Oksanen S, Jones RI (2013) An empirical evaluation of the utility of convex hull and standard ellipse areas for assessing population niche widths from stable isotope data. PLoS One 8(2):e56094. doi:10.1371/journal.pone.0056094

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotope in animal tissue: implications for $\delta13$C analysis of diet. Oecologia 57:32–37

Walker B, Boersma P (2003) Diving behavior of Magellanic penguins (*Spheniscus magellanicus*) at Punta Tombo, Argentina. Can J Zool 81:1471–1483. doi:10.1139/z03-142

Watanuki Y, Burger AE (1999) Body mass and dive duration in alcids and penguins. Can J Zool 77:1838–1842

Williams TD (1995) The penguins. Oxford University Press, Oxford

Wilson RP (2003) Penguins predict their performance. Mar Ecol Prog Ser 249:305–310

Wilson RP, Scolaro JA, Peters G, Laurenti S, Kierspel M, Gallelli M, Upton J (1995) Foraging areas of Magellanic penguins *Spheniscus magellanicus* breeding at San Lorenzo, Argentina, during the incubation period. Mar Ecol Prog Ser 1299:1–6

Wilson RP, Scolaro JA, Grémillet D, Kierspel MAM, Laurenti S, Upton J, Gallelli H, Quintana F, Frere E, Müller G, Straten MT, Zimmer I (2005) How do Magellanic penguins cope with variability in their access to prey? Ecol Monogr 75:379–401