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ORIGINAL PAPER

Diet composition and foraging habitats of Adélie and gentoo penguins in three different stages of their annual cycle

Mariana A. Juáres[1] · Mercedes Santos[1] · Jorge A. Mennucci[1] · Néstor R. Coria[1] · Rocío Mariano-Jelicich[2]

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Abstract We evaluated the diet of Adélie (*Pygoscelis adeliae*) and gentoo (*P. papua*) penguins at Stranger Point colony (25 de Mayo/King George Island) during different stages of their annual cycle using the stable isotope method and the conventional dietary analyses (i.e. stomach contents). Antarctic krill (*Euphausia superba*) dominated the diet of Adélie and gentoo penguins in all three studied stages (pre-breeding, breeding and post-breeding). Nevertheless, only in gentoo penguins a shift in the diet was evident from mainly krill during the breeding stage to mixed diet (i.e. krill and fish/squids) during the pre- and post-breeding stages. Results from stable isotopes suggest that Adélie penguins might be foraging in southern localities compared to their congener during the inter-breeding period. Moreover, Adélie penguins showed a large individual variability in foraging habitat during the post-breeding stage. However, our model has predicted that both species exploited similar areas during the breeding season. The results obtained during the chick-rearing stage were compared with the stomach contents of both species obtained during the same season (2011/2012). Our findings provide new knowledge on the feeding ecology and foraging habitats of pygoscelid penguins. They confirm the importance of Antarctic krill during the annual cycle and improve the understanding of life strategies and predator–prey interactions essentially out of the reproductive period, in which the information is very limited. These results can help to establish new management strategies, particularly considering the potential overlap between predators and fisheries.

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✉ Mariana A. Juáres
marianajuares@hotmail.com

[1] Departamento Biología de Predadores Tope, Instituto Antártico Argentino, Balcarce 290, C1064AAF Ciudad Autónoma de Buenos Aires, Argentina

[2] Laboratorio de Vertebrados, Instituto de Investigaciones Marinas y Costeras IIMyC (Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de Mar del Plata), Funes 3250, B7602AYJ Mar del Plata, Argentina

Introduction

In the Antarctic marine ecosystem, the energy flow tends to be dominated by a main flow from the phytoplankton through the zooplankton, mainly Antarctic krill (*Euphausia superba*), towards higher-order predators (Ducklow et al. 2013), such as pygoscelid penguins. In the western Antarctic Peninsula and islands of the Scotia Arc, the climate change has adversely affected the krill populations (Atkinson et al. 2004; Reiss et al. 2008). To understand the responses of the krill-dependent predators to face this marine environment variability, it is necessary to know the foraging behaviour of different sympatric predators, mainly if they have different life-history strategies. This will allow us to identify the species-specific responses under the same environmental conditions and will help to elucidate the specific causal mechanisms of their population trends. Moreover, due to the potential overlap between predators and fisheries, the knowledge of trophic interactions, the determination of movement patterns and the identification of foraging habitats are essential to establish effective conservation measures (Cherel and Hobson 2007).

The Adélie penguins (*Pygoscelis adeliae*) are migratory and ice-obligated species. After the breeding stage, the adults migrate to the residual pack ice, and they feed

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intensively and then fast while moulting their feathers on the pack ice (Ainley 2002). Adélie penguins that nest in the western Antarctic Peninsula migrate to the Bellingshausen Sea. Instead, the adults that breed in the South Shetland Islands (hereinafter SSI) overwinter in areas within the Bransfield Strait (Mar de la Flota) and the Weddell Sea (Fraser and Trivelpiece 1996; Polito et al. 2011a; Hinke et al. 2014, 2015). During the austral spring, the adults return to the nesting colony where they face a prolonged fasting stage at the beginning of the breeding season (e.g. Ainley 2002). Conversely, gentoo penguins (*P. papua*) are non-migratory and ice-avoiding species which remain around their natal colony during winter (e.g. Tanton et al. 2004; Hinke and Trivelpiece 2011). The adults moult in their colony just after rearing their chicks. Moreover, at the beginning of the breeding season the egg-forming females do not face a long fasting period (Trivelpiece et al. 1987). Although both species inhabit different winter habitats, during the breeding period they become central place foragers due to incubation and chick-feeding duties (e.g. Ainley 2002). In the study area, at least during breeding stages, both species are exposed to the same temporal ecological constraints since there is no clear segregation in breeding phenology of Adélie and gentoo penguins (Juáres 2013), possibly due to the great plasticity observed in the breeding chronology of gentoo penguins (Hinke et al. 2012; Lynch et al. 2012; Juáres et al. 2013). However, here and at other localities where they breed sympatrically, inter-specific differences in the foraging behaviour during the chick-provisioning stage were found. Adélie penguins usually feed in offshore and pelagic habitats, while gentoo penguins forage inshore and benthic ones (Trivelpiece et al. 1987; Juáres 2013; among others). In both species, plasticity in the feeding strategies was reported in relation to a reduction in the main prey availability (e.g. Ropert-Coudert et al. 2002; Miller et al. 2009). In the SSI, however, reports highlighted the ability of gentoo penguins to modify one or more aspects of their trophic biology allowing them to face the changes in the local food resources (Miller et al. 2009).

The predominance of Antarctic krill in the chick-rearing diet of pygoscelid penguins breeding at the SSI is undisputable (Miller et al. 2009, 2010; Trivelpiece et al. 2011; Juáres 2013; Juáres et al. 2013; among others). In general, these results were mainly obtained by stomach flushing of adult breeders, which should forage close to the reproductive colony. Few studies on the composition of the diet were conducted with this traditional method during the non-breeding period (Jablonski 1985; Ainley et al. 1992).

The analysis of stable isotope signatures has been used to assess the spatial and temporal variations in the diets of penguins during different moments of their annual cycle (e.g. Dehnhard et al. 2011; Polito et al. 2011a; Hinke et al. 2015). The stable isotope ratio in a tissue reflects the diet composition and foraging habitat of seabirds during the time of synthesis, but each tissue integrates a different timescale according to its turnover rate (Barret et al. 2007). For example, whole blood provides dietary information integrated of approximately 20 days (Barquete et al. 2013), while egg tissues give information on female diets during a period prior to breeding (e.g. Polito et al. 2009). Nitrogen ratios ($\delta^{15}N$) recorded in the tissues can be used to estimate the trophic level and diet, because consumers are enriched in $\delta^{15}N$ relative to their prey (Cherel et al. 2005). The carbon ratios ($\delta^{13}C$) show a little variation along the food webs and are also commonly used to infer the foraging habitats (i.e. inshore vs. offshore and benthic vs. pelagic) as well as the geographic location of these habitats (Cherel and Hobson 2007; Quillfeldt et al. 2010). In the marine environment, the benthic and/or inshore trophic networks are often more enriched in $\delta^{13}C$ than pelagic and/or offshore food webs. Furthermore, changes in the $\delta^{13}C$ values can serve as indicators of latitudinal variations in the foraging habitats (Cherel et al. 2000; Cherel and Hobson 2007; Quillfeldt et al. 2010; Polito et al. 2011a; Hinke et al. 2015; but see Ceia et al. 2015).

In the SSI, few studies on the foraging behaviour of pygoscelid penguins during different stages of their annual cycle were conducted using the stable isotope method (e.g. Polito et al. 2009, 2011a; Hinke et al. 2015). At Stranger Point colony (25 de Mayo/King George Island, SSI), Adélie and gentoo penguins breed sympatrically. In this colony, previous research on diet composition of both species was conducted during the breeding period (chick-provisioning stage) by using the stomach sampling methodology (Juáres 2013; Juáres et al. 2013). In addition, the pre-breeding diet of gentoo penguin was previously reported by Polito et al. (2011a). However, based on our knowledge, information on pre-breeding diet of Adélie penguins and post-breeding diet of both species at this colony is still unavailable. So, our findings provide new specific knowledge on foraging behaviour of *Pygoscelis* penguins, improving the understanding of life strategies and predator–prey relationship mainly out of the breeding period to which this information is limited.

In this study, we used the stable isotope method to characterize the trophic ecology of Adélie and gentoo penguins during the pre-breeding stage, the chick-rearing stage and post-breeding stage. Furthermore, the findings were compared with those obtained from stomach contents collected during the chick-rearing stage. We examined the isotopic variation in different tissues from adults of Adélie and gentoo penguins to test our hypothesis that, in the Stranger Point population, the Antarctic krill is the main prey of both species throughout the annual cycle.

Materials and methods

Study area

Fieldwork was carried out at Stranger Point, 25 de Mayo/King George Island (62°16′S, 58°37′W), SSI (Fig. 1), within the Antarctic Specially Protected Area N° 132 ("Potter Peninsula") during the 2011/2012 season (hereafter 2012). In this colony, approximately 3700 pairs of Adélie and 4990 pairs of gentoo penguins breed sympatrically. During this season, the beginning of the egg laying was recorded on October 23 in Adélie penguins (peak of egg laying: *c.* November 11) and on October 26 in gentoo penguins (peak of egg laying: *c.* November 7). Moreover, the beginning of the crèche stage was registered on December 22 and 30 for Adélie and gentoo penguins, respectively.

Stomach contents

Stomach contents were collected by following the Ecosystem Monitoring Program Methods (Method A8) of the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR 2004), using the stomach-flushing method described by Wilson (1984) and modified by Gales (1987). Sampling was carried out during the stages of guard and crèche of chicks, in adult birds assumed initially as breeders. Follow-up studies performed in later years to confirm the breeding status of candidate diet birds support this assumption. All samples were frozen (−20 °C) until they arrived in Buenos Aires. At the laboratory, each sample was thawed, drained and weighed (balance 200 ± 1 g). Individual prey items were separated and weighed.

For each species, the percentage of krill and fish was determined by frequency of occurrence (total number of samples containing the item/total number of samples analysed by 100) and by weight (total weight of the item/total weight of all samples by 100).

Avian tissues and prey samples

To evaluate the diet compositions throughout the annual cycle of both species, different tissues were collected from different breeding individuals: (1) post-breeding stage (i.e. after the 2011 breeding season): breast feathers from adults; (2) pre-breeding stage (i.e. prior to egg laying in the 2012 breeding season): eggshell membranes; and (3) chick-rearing stage (i.e. the 2012 breeding season): adult blood. Both feathers and blood sampled were collected during crèches stage (about 45 days after hatching). We assume that our whole blood samples integrated only dietary information from the chick-rearing stage. The eggshell membranes were collected from nests with eggs hatched, and then they were frozen (−20 °C). Breast feathers were cut from the base, and blood was collected with a syringe by venepuncture of a leg vein. Each whole blood sample was preserved in absolute ethanol (J. T. Baker®, México) until its arrival in Buenos Aires, and then it was frozen (−20 °C) until isotopic analysis was carried out. Ethanol has been regularly used as preserver since it has shown to have a negligible

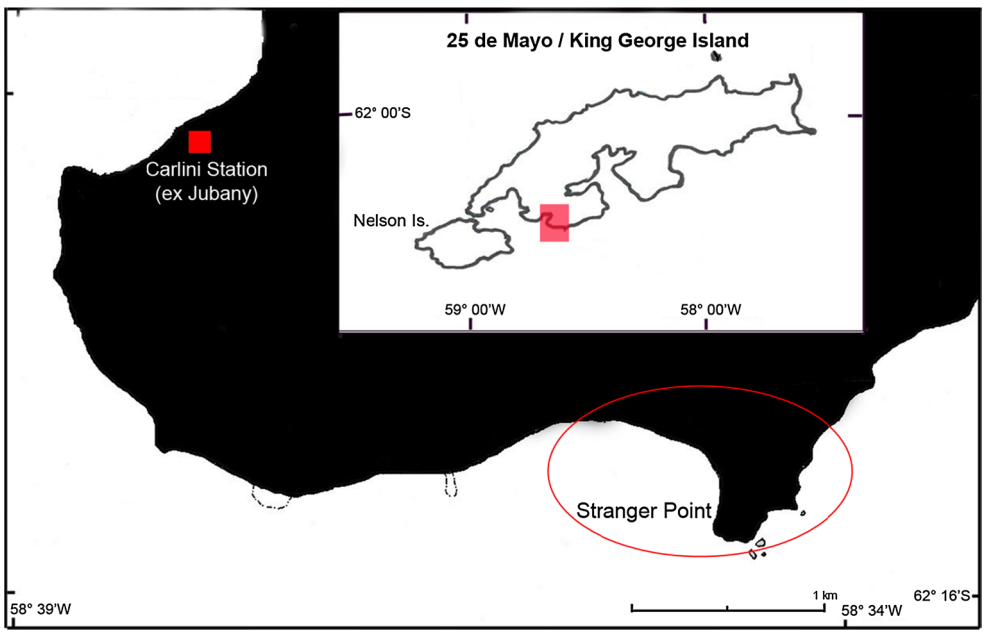

Fig. 1 Study area. Stranger Point colony, 25 de Mayo/King George Island, South Shetland Islands, Antarctica

Table 1 $\delta^{13}C$ and $\delta^{15}N$ signatures and C/N ratio (mean ± SD, ‰) of Antarctic krill and fish, principal components of Adélie and gentoo penguin diets in the South Shetland Islands used for SIAR

Species	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C/N	Habitat	Source
Euphausia superba	16	−26.6 ± 1.7	3.5 ± 0.7	3.4 ± 0.1		This study
Electrona antarctica	41	−25.5 ± 0.7	8.8 ± 0.7	3.3 ± 0.1	Pelagic	Polito et al. (2011c)
Lepidonotothen squamifrons	10	−24.2 ± 0.7	9.6 ± 0.8	3.3 ± 0.1	Demersal	Polito et al. (2011a)

effect on the stable carbon and nitrogen isotope values in a variety of tissues (Hobson et al. 1997; Kelly et al. 2006; Bugoni et al. 2010; but see Bugoni et al. 2008).

Whole specimens of Antarctic krill ($n = 16$) were collected from the beach and were stored frozen (−20 °C) during the 2012 season to determine the isotopic signature. Previous studies report *Electrona antarctica*, *Notothenia* sp., *Pleuragramma antarcticum* and *Lepidonotothen* sp. as frequent fish prey of gentoo penguins in the study area (Juáres 2013). As fish remains found in the stomach contents were highly digested, we could not use them for stable isotope analysis. Moreover, logistic difficulties prevented fish sampling during the study season. So, the inventory of potential food sources was completed with isotopic data of likely relevant species from studies conducted near the study area (Table 1). We assumed that these isotopic values were representative of fish targeted by Adélie and gentoo penguins.

Samples preparation and stable isotope analysis

All fresh tissue samples were dried in an oven at 60 °C and ground using a hand mortar (Cherel et al. 2007). In order to reduce carbon variability because of lipid variation between tissues, we conducted lipid extraction from potential prey sample (i.e. krill) using successive rinses in a 2:1 chloroform–methanol solution (Bligh and Dyer 1959). Feathers were also rinsed in a 2:1 chloroform–methanol solution in order to clean their surface and cut in small pieces. Two to three breast feathers from each individual were homogenized and used in the analyses. Egg membranes were separated from eggshells, dried and cut in small pieces following Quillfeldt et al. (2009). All samples were then weighed and packed in tin capsules for their analysis. Raw values were normalized on a two-point scale using glutamic acid reference materials with low and high values (USGS-40 with $\delta^{13}C = -28.8$ ‰ and $\delta^{15}N = -4.26$ ‰; and USGS-41 with $\delta^{13}C = 37.6$ ‰ and $\delta^{15}N = 47.6$ ‰). Replicate measurements of biologically relevant internal laboratory standards indicate a sample precision of 0.1 and 0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Stable isotope abundances were expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following equation:

$$\delta X = \left\{ \left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right\} \times 1000$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The R_{standard} values were based on the Vienna Pee Dee Belemnite (V-PDB) for $\delta^{13}C$ and Air for $\delta^{15}N$.

Model and statistical analysis

We analysed the stomach content data to test differences in the proportion of fish consumed by each species using a Fisher's exact test.

Comparisons between $\delta^{13}C$ and $\delta^{15}N$ values among tissues and species studied were made with MANOVA and Tukey as the post hoc test. Significance was assumed at $P < 0.05$. Following Hobson and Bond (2012), we performed two analyses. Firstly, we compared each tissue among species using raw values to evaluate differences between species in isotopic signatures in each stage of their annual cycle, and then we examined differences among stages in $\delta^{13}C$ or $\delta^{15}N$ for each species applying proper discrimination factors for each tissue.

To quantitatively assess the importance of different prey as food sources, we used the SIAR Bayesian stable isotope mixing model in R environment (Parnell et al. 2010). This model provides quantitative indices of food item contribution to a consumers' diet accounting for known variability in sources, fractionation and other unquantified variability within the model. Moreover, SIAR outputs represent true probability density functions, rather than a range of feasible solutions as was the case of earlier mixing models (Parnell et al. 2010). We performed a mixing model assuming two and three functional prey clusters: (1°) krill and "fish" (calculated by averaging $\delta^{13}C$ and $\delta^{15}N$ values of both pelagic, *Electrona antarctica* and benthonic, *Lepidonotothen squamifrons*, species); and (2°) krill, pelagic fish (*E. antarctica*, from Polito et al. 2011c) and benthonic fish (*L. squamifrons*, from Polito et al. 2011a). In order to perform this analysis, the isotopic signature for food sources must be rearranged by appropriate discrimination factors. The use of inadequate values will result in inaccurate interpretations (e.g. Bond and Diamond 2011). In this study, we used egg membranes ($\delta^{13}C$: 2.8 ± 0.5 ‰ and $\delta^{15}N$: 4.4 ± 0.5 ‰, from Polito et al. 2009) and feather ($\delta^{13}C$: 1.3 ± 0.5 ‰ and $\delta^{15}N$: 3.5 ± 0.5 ‰, from Polito et al. 2011b) discrimination values for *Pygoscelis papua*. Since there are no diet-blood discrimination factors available either for Adélie or

Table 2 Diet composition of chick-provisioning Adélie and gentoo penguins at Stranger Point during the 2011/2012 season

Species	Breeding stage	*n*	Frequency of occurrence (%)		Weight percentage (%)	
			Krill	Fish	Krill	Fish
Adélie	Guard stage	15	100	0	99.99	0
	Crèche stage	25	100	4.00	99.98	<0.01
	Stage combined	40	100	2.50	99.98	<0.01
Gentoo	Guard stage	14	100	14.30	99.48	0.51
	Crèche stage	25	100	24.00	92.75	7.02
	Stage combined	39	100	20.51	94.74	5.10

The frequency of occurrence (as percentage) and the weight percentage of two principal prey items (Antarctic krill and fish) are shown

n Number of stomach contents analysed

for gentoo penguins, we developed a potential scenario using a pair of discrimination factors published for other penguin species by Cherel et al. 2005 (δ^{13}C: 0.02 ± 0.7 ‰ and δ^{15}N: 2.7 ± 0.4 ‰). These values have been already used in several studies (Davies et al. 2009; Raya Rey et al. 2012). Models were run with 1,000,000 iterations, thinned by 15, with an initial discard of 40,000 resulting in 64,000 posterior draws. In all cases, the predator isotopic values were contained within the mixing space after application of discrimination corrections.

Results

Stomach contents

During the 2012 season, Antarctic krill represented the primary prey in terms of frequency of occurrence and percentage in weight for both Adélie and gentoo penguins for each breeding stage analysed (Table 2). In both species, krill comprised >92 % of the diet by weight. When we consider the complete chick-provisioning period (i.e. guard and crèche stages), fish prey was better represented in gentoo penguin than in its congener (>frequency of occurrence. Fisher's exact test, $P = 0.03$). Nevertheless, the percentage in weight of this item did not exceed 7 % (crèche stage, Table 2).

Stable isotope analysis

Stable carbon and nitrogen isotopes values for all tissues sampled in both species and their potential food sources are presented in Fig. 2.

During the pre- and post-breeding stages, Adélie penguins showed depleted values of carbon and nitrogen compared to their congener (post hoc Tukey test, $P < 0.005$ in all cases) (Table 3; Fig. 2a, b). In contrast, during the breeding stage gentoo penguins showed lower δ^{15}N values (post hoc Tukey test, $P = 0.005$), while both species showed similar δ^{13}C values (post hoc Tukey test, $P = 0.15$).

Moreover, the isotopic signatures of all tissues differed significantly within species, after applying specific discrimination factors for each tissue (Adélie: $P < 0.001$ for all cases; gentoo: $P < 0.001$ for all cases). These differences were observed between pre- and post-breeding stages, and breeding and post-breeding stages in Adélie carbon signatures (post hoc Tukey test, $P < 0.05$), while no differences between stages were observed when nitrogen signatures were considered (post hoc Tukey's test, $P > 0.05$ for all combinations). Furthermore, in Adélie penguins a great dispersion in the isotopic signatures of feathers was recorded (Fig. 2a).

According to our isotopic models, Antarctic krill dominated the diets of both species in all stages analysed (Tables 4, 5). The two-source model (i.e. krill and fish) predicted a higher contribution of krill, which exceeded 72 % of Adélie penguin diet and 60 % in its congener. In this model, the fish were better represented during inter-breeding period in gentoo penguins (Table 4). Similar results were obtained with a three-source model (i.e. krill, pelagic fish and benthonic fish; Table 5). This model also predicted a higher contribution of Antarctic krill over the fish portion of the diet of both penguins, with Adélie penguins showing larger contribution of krill compared to their congener except during the breeding stage when krill contributed similarly to the diet of both species. Although much overlap existed in credibility intervals (CI), the model predicted a similar contribution of pelagic fish during the breeding stage for both species, a higher relative contribution of benthonic fish to gentoos' diet during pre- and post-breeding stages, while a lightly higher relative contribution of pelagic fish was predicted for Adélies' diet (Table 5). Differences between models for each penguin species were observed about the inclusion of zero as a possible solution for the fish portion of penguins prey categories. CIs for benthonic fish were less precise for Adélie

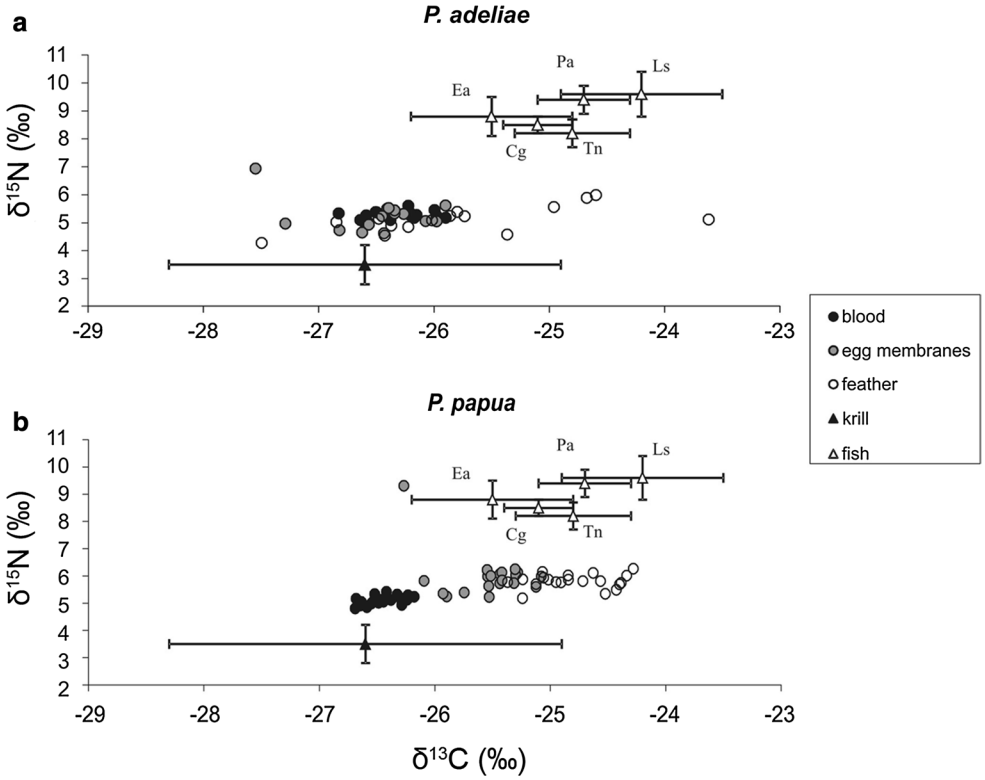

Fig. 2 Isotope signatures of feathers, egg membranes and blood of Adélie (**a**) and gentoo (**b**) penguins in relation to different prey species. Values are presented as $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD, ‰), and all penguin tissues were corrected by the application of the discrimination factors. Fish species: Ea (*Electrona antarctica*) and Ls (*Lepidonotothen squamifrons*)

Table 3 $\delta^{13}C$ and $\delta^{15}N$ signatures (mean ± SD, ‰) for Adélie and gentoo penguins during different stages of their annual cycle (prior to correction by discrimination factors)

Year	Species	Stage	Tissue type	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	*n*
2011	Adélie	Post-breeding	Feathers	−24.5 ± 0.9	8.6 ± 0.5	15
2012		Pre-breeding	Egg membranes	−23.7 ± 0.4	9.7 ± 0.6	15
2012		Breeding	Blood	−26.3 ± 0.3	7.9 ± 0.1	18
2011	Gentoo	Post-breeding	Feathers	−23.5 ± 0.3	9.3 ± 0.3	20
2012		Pre-breeding	Egg membranes	−22.7 ± 0.3	10.4 ± 0.8	20
2012		Breeding	Blood	−26.4 ± 0.2	7.7 ± 0.2	20

n Number of samples for each species and stage analysed

Table 4 Diet composition of Adélie and gentoo penguin diets during different stages of their annual cycle derived from stable isotope analysis using SIAR Bayesian mixing model (food sources considered: krill and fish)

Year	Stage	Tissue type	Species	Krill (%)	Fish (%)
2011	Post-breeding	Feathers	Adélie	78 (73–84)	21 (16–27)
			Gentoo	69 (65–74)	30 (26–35)
2012	Pre-breeding	Egg membranes	Adélie	77 (72–82)	23 (18–28)
			Gentoo	66 (61–72)	33 (28–39)
2012	Breeding	Blood	Adélie	77 (73–81)	22 (19–27)
			Gentoo	78 (75–83)	21 (17–25)

Values are presented as mean estimates with 95 % credibility intervals

penguins in all stages analysed and for both species during the breeding stage (including zero with high probability of occurrence), while CIs for pelagic fish were less precise in gentoo penguins, compared to their congener, during both pre- and post-breeding stages (Table 5).

Discussion

At Stranger Point colony, Antarctic krill dominated the diets of Adélie and gentoo penguins over their annual cycle. The stable isotope analysis suggested inter-specific differences in the foraging habitat used outside the breeding season. However, both species might be foraging in similar habitats during the chick-rearing stage since a marked isotopic separation was not recorded, which would be expected due to the foraging preferences (pelagic/offshore vs benthic/inshore habitats) of each species. Furthermore, the intra-specific comparison evidenced a shift in the gentoo penguin isotopic values suggesting differences in diet

Table 5 Diet composition of Adélie and gentoo penguin diets during different stages of their annual cycle derived from stable isotope analysis using SIAR Bayesian mixing model (food sources considered: krill, pelagic fish and demersal fish)

Year	Stage	Tissue type	Species	Krill (%)	Pelagic fish (%)	Benthonic fish (%)
2011	Post-breeding	Feathers	Adélie	69 (63–76)	16 (0.4–31)	14 (0.5–28)
			Gentoo	58 (54–63)	11 (0–26)	30 (16–43)
2012	Pre-breeding	Egg membranes	Adélie	66 (60–73)	23 (5–38)	10 (0–24)
			Gentoo	51 (43–60)	24 (0.9–46)	24 (4–43)
2012	Breeding	Blood	Adélie	66 (61 –71)	24 (8.6–37)	9.8 (0–22)
			Gentoo	69 (63–74)	22 (7–35)	9 (0–21)

Values are presented as mean estimates with 95 % credibility intervals

composition. Further studies will contribute to the understanding of the origin of these shifts as a result of different prey, foraging habitat or temporal or spatial change in baseline isotopic values.

Comparisons between species

During the pre-breeding and post-breeding stages, the $\delta^{13}C$ and $\delta^{15}N$ values were lower in Adélie penguin than in gentoo penguin, similar to those previously reported by Polito et al. (2011a). Our results potentially evidence the use of different foraging habitats during the inter-breeding period, i.e. Adélie penguin used pelagic/offshore habitats, while gentoo penguin used benthic/inshore habitat. However, values of $\delta^{13}C$ decline at higher latitudes, this being particularly evident in Antarctic and sub-Antarctic water masses (e.g. Cherel and Hobson 2007; Quillfeldt et al. 2010; but see Ceia et al. 2015). Recent studies have confirmed initial southward migration of Adélie penguin after breeding (Hinke et al. 2015). Feather isotopic values reported here were in line with this recent study, suggesting that Adélie penguin could be feeding in areas further south than its congener (which probably feeds in inshore and benthic habitats not associated with sea ice). Furthermore, a higher dispersion was observed in the feather isotopic signatures of Adélie penguins (Fig. 1). This result may reflect differences between sexes and/or individuals in the foraging area exploited prior to the moult (Quillfeldt et al. 2005; Cherel et al. 2007; Hobson and Bond 2012). Future studies using satellite telemetry will help to identify the foraging locations of male and female Adélie penguins during the pre-moult stage (i.e. post-breeding stage) and thus contribute to the corroboration of these hypotheses.

When comparing the results between species, although statistical differences in the $\delta^{15}N$ values were recorded, these differences were lower than 3 ‰, hence not enough to show a change in the trophic level. However, the stable isotope mixing model evidenced (even though there was much overlap in credibility intervals) that Adélie penguin had a higher contribution of pelagic fish while gentoo penguin showed a higher contribution of benthic fish. This difference between species in the $\delta^{15}N$ values could result from the use of different foraging habitats during the non-breeding period (Cherel et al. 2000). Nevertheless, differences in isotopic values could also arise from shifts in the isotopic value of penguins' prey and/or spatio-temporal shifts in baselines during inter-breeding stages (Cherel and Hobson 2007; Ceia et al. 2015; Hinke et al. 2015; but see Quillfeldt et al. 2015). In line with this, we highlight the need of further efforts in improving the sampling of potential prey outside the breeding season in order to enhance these results.

In contrast, during the breeding stage the $\delta^{13}C$ value was low (and similar) in both species and the $\delta^{15}N$ value was slightly higher in Adélie penguins than their congener. These findings suggest that, during the 2012 chick-rearing stage, both Adélie and gentoo penguins feed in similar habitats and had similar diets (see Tables 4, 5). During the breeding stage, both species consumed the same prey, mainly krill, and the isotope data would suggest that the foraging occurred primarily in pelagic and offshore waters (i.e. the areas typically used by Adélie penguins). The slight difference in $\delta^{15}N$ value could also indicate that gentoo penguins consumed, at least in part, more prey items of lower trophic level. However, bearing in mind the precision of 0.2 ‰ in ^{15}N values some considerations arise regarding the real difference between these values, and this result should be taken with caution. In a previous study, at Stranger Point colony the following results were found for gentoo penguins: (1) a high consumption of Antarctic krill (see also below); (2) a larger number of benthic dives than their congener (although with no significant differences in the foraging trip duration); and (3) a greater use/exploitation of the water column (Juáres 2013). Based on this evidence, it is essential to analyse simultaneously the isotopic data together with those obtained with geolocation devices to characterize the habitat use and examine ecological segregation.

Comparisons between stages

Results from the mixing models showed that in Adélie penguins, significant changes in the diet between the different stages were not evident. However, in gentoo penguins the

data supported a shift in the diet from a slightly mixed diet during pre-breeding stage to an increased proportion of krill during the breeding stage. This result may be related to the lower availability of krill near the colony at the beginning of the breeding season (i.e. September), when the ice edges provide the only habitat with predictable and abundant food resource (Trivelpiece and Fraser 1996). Polito et al. (2011a) registered that high trophic-level prey items were significantly represented in Adélie and gentoo penguins during the pre-breeding stage. For gentoo penguins, our findings were similar to those reported by these authors. Nevertheless, unlike them, our results indicate that krill dominated the diet of adult Adélie penguins during this stage.

Both species showed a similar trend of C^{13}-depletion from the post-breeding to the breeding stage (post-breeding > pre-breeding ≥ breeding). During the inter-breeding period, Adélie penguins migrate to areas southwards with respect to their colony location (Hinke et al. 2015), while gentoo penguins are non-migratory but with a high dispersion (see Hinke and Trivelpiece 2011). In both cases, a C^{13}-depleted towards the non-breeding period would be expected. During the post-breeding stages, the higher $\delta^{13}C$ values could indicate either the incorporation of high trophic level prey or a shift to a benthic carbon source (similar to that reported by Dunton 2001). According to Schmidt et al. (2011), an important benthic feeding is frequent in Antarctic krill, even during winter, with a dynamic exchange between seabed and upper waters. On the other hand, this difference could also reflect a high primary productivity in the foraging locations used outside the breeding season. During the inter-breeding period, Adélie penguins feed in shallow waters in areas associated with sea ice (e.g. Erdmann et al. 2011), while gentoo penguins exploit coastal areas (e.g. Hinke and Trivelpiece 2011). Both the near-surface ocean waters and coastal waters can register a high primary productivity in winter (e.g. Quillfeldt et al. 2010; Thiebot et al. 2012; Goutte et al. 2014). However, these hypotheses should be confirmed with telemetry and information of chlorophyll-*a* concentration or particulate organic matter.

Stomach contents analysis

The results obtained from stomach contents analysis also evidenced the importance of Antarctic krill in the diet of adults and chicks of both species during the chick-provisioning stage. In gentoo penguins, the fish item was better represented. However, a low weight percentage of this prey category was recorded in both Adélie (<0.01 %) penguins and gentoo (5.1 %) penguins. Even comparing the results with those from Juáres et al. (2013), during the 2012 season we recorded the highest percentage in fish weight (7 %) of the period 2008–2012, although with a lower frequency of occurrence.

During the chick-rearing stage, like Polito et al. (2011c), we recorded a greater contribution of fishes from the stable isotope analysis than in the stomach content analysis. This may reflect a feeding strategy in which the adults eat fish to maintain themselves and not as part of the chick diet or in which adults feed their chicks with digested prey which has a higher energy supply (Miller et al. 2010; Polito et al. 2011c). On the other hand, Quillfeldt et al. (2005) characterized the chicks' diet of Adélie and gentoo penguins in Potter Peninsula using the stable isotope ratios of feathers from chicks killed by skuas (*Stercorarius* sp.). All these previous studies suggest that chicks would be mainly fed with krill while the adults might be eating fish to maintain themselves. Our results are consistent with this hypothesis.

Conclusions

In summary, this study: (1) provides new knowledge on the diet composition of pygoscelid penguins out of the reproductive season, (2) corroborates the importance of krill in the diet of Adélie and gentoo penguins during different stages of their annual cycle, although (3) shows a slightly intra-annual shift in gentoo penguin stable isotope data, suggesting a mixed diet during pre-breeding stage and 4) suggests that there may be an intra-specific variation in the pre-moulting areas exploited by Adélie penguins.

The breeding population trends of Adélie and gentoo penguins are consistent between all localities monitored at the SSI (e.g. Trivelpiece et al. 2011; Santos et al. 2014; Juáres et al. 2015). Some researchers consider that these population changes are primarily related to the spatio-temporal reduction in sea ice (e.g. Fraser et al. 1992), while others attribute it to the decline in the biomass of Antarctic krill (Trivelpiece et al. 2011). Nevertheless, as the factors leading to the long-term fluctuations are not totally elucidated, it is important to report information on these species, fundamentally outside the breeding season. Moreover, improving the knowledge of the spatial and temporal variability of the diets of krill-dependent predators (such as pygoscelid penguins) and predator–prey interactions can contribute to new management strategies, particularly considering the spatial and temporal variation of krill fishery practices and the potential overlap with predators.

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