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

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

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.

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
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...
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 spp., Pleuragramma antarcticum and Lepidonothen squamifrons sp. as frequent fish prey of gentoo penguins in the study area (Juárez 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 and packed in tin capsules for their analysis. Raw values were normalized on a two-point scale using glutamic acid and packed in tin capsules for their analysis. 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 and packed in tin capsules for their analysis.

### Table 1

| Species                          | n | δ13C (‰)  | δ15N (‰)  | 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)        |
| Lepidonothen squamifrons        | 10| −24.2 ± 0.7| 9.6 ± 0.8  | 3.3 ± 0.1| Demersal| Polito et al. (2011a)        |

where X is $^{13}$C or $^{15}$N and R is the corresponding ratio $^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N. The $R_{\text{standard}}$ 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, Lepidonothen 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 factors for Pygoscelis papua. Since there are no diet-blood discrimination factors available either for Adélie or
for gentoo penguins, we developed a potential scenario using a pair of discrimination factors published for other penguin species by Cherel et al. 2005 ($\delta^{13}C: 0.02 \pm 0.7$ ‰ and $\delta^{15}N: 2.7 \pm 0.4$ ‰). 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 $\delta^{15}N$ values (post hoc Tukey test, $P = 0.005$), while both species showed similar $\delta^{13}C$ 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

### Table 2

| Species  | Breeding stage | $n$  | Frequency of occurrence (%) | Weight percentage (%) |
|----------|----------------|-----|-----------------------------|-----------------------|
|          |                |     | Krill                       | Fish                  |
| Adélie   | Guard stage    | 15  | 100                         | 0                     |
|          | Crèche stage   | 25  | 100                         | 4.00                  |
|          | Stage combined | 40  | 100                         | 2.50                  |
| Gentoo   | Guard stage    | 14  | 100                         | 14.30                 |
|          | Crèche stage   | 25  | 100                         | 24.00                 |
|          | Stage combined | 39  | 100                         | 20.51                 |

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.
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

![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)](image)

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    | $-24.5 \pm 0.9$   | $8.6 \pm 0.5$      | 15 |
| 2012 | Adélie  | Pre-breeding | Egg membranes | $-23.7 \pm 0.4$   | $9.7 \pm 0.6$      | 15 |
| 2012 | Adélie  | Breeding    | Blood       | $-26.3 \pm 0.3$   | $7.9 \pm 0.1$      | 18 |
| 2011 | Gentoo  | Post-breeding| Feathers    | $-23.5 \pm 0.3$   | $9.3 \pm 0.3$      | 20 |
| 2012 | Gentoo  | Pre-breeding| Egg membranes | $-22.7 \pm 0.3$   | $10.4 \pm 0.8$     | 20 |
| 2012 | Gentoo  | Breeding    | Blood       | $-26.4 \pm 0.2$   | $7.7 \pm 0.2$      | 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
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; 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

### 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.
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 $\geq$ 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 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.

**Acknowledgments** We want to thank Lucrecia Longarzo, Martín Gray y Paula Moran, for their help in collecting samples associated with this study. The permit for this work was granted by the Dirección Nacional del Antártico (Environmental Office). The FONCYT—Agencia Nacional de Promoción Científica y Tecnológica (Grant: PICTO 2010-0111) and the Instituto Antártico Argentino—Dirección Nacional del Antártico provided financial and logistical support. We also thank the two anonymous reviewers and the editors for their helpful comments and suggestions on improving the manuscript. Finally, we are very grateful to Ana Gentile who improved the English.
References

Ainley DG (2002) The Adélie penguin: bellwether of climate change. Columbia University Press, Columbia, NY
Ainley DG, Ribic CA, Fraser WR (1992) Does prey preference affect habitat choice in Antarctic seabirds? Mar Ecol Prog Ser 90:207–221
Atkinson A, Siegel V, Pakhomov E, Rothery P (2004) Long-term decline in krill stock and increase in salps within the Southern Ocean. Nature 432:100–103
Barquete V, Strauss V, Ryan PG (2013) Stable isotope turnover in blood and claws: a case study in captive African penguins. J Exp Mar Biol Ecol 448:121–127
Barrett RT, Camphuysen KJC, Anker-Nilssen T, Chardine JW, Furness RW, Garthe S, Hiippop O, Leopold MF, Montvecchica WA, Veit RR (2007) Diet studies of seabirds: a review and recommendations. ICES J Mar Sci 64:1675–1691
Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917
Bond AL, Diamond AW (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination facets. Ecol Appl 21:1017–1023
Bugoni L, McGill RAR, Furness RW (2008) Effects of preservation methods on stable isotope signatures in bird tissues. Rapid Commun Mass Spectrom 22:2457–2462
Bugoni L, McGill RAR, 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
CCAMLR (2004) Standard methods for monitoring parameters of predator species. CCAMLR Ecosystem Monitoring Program, Hobart, Australia
Ceia FR, Ramos JA, Phillips RA, Cherel Y, Jones DC, Vieira RP, Xavier JC (2015) Analysis of stable isotope ratios in blood of tracked wandering albatrosses fails to distinguish a δ13C gradient within their winter foraging areas in the southwest Atlantic Ocean. Rapid Commun Mass Spectrom 29:2328–2336
Cherel Y, Hobson KA (2007) Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. Mar Ecol Prog Ser 329:281–287
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, Hobson KA, Hassani S (2005) Isotopic discrimination between food and blood feathers of captive penguins: implications for dietary studies in the wild. Physiol Biochem Zool 78:106–115
Cherel Y, Hobson KA, Guinet C, Vanpe C (2007) Stable isotopes document seasonal changes in trophic niches and winter foraging individual specialization in diving predator from the Southern Ocean. J Anim Ecol 76:826–836
Davies WE, Hifpner JM, Hobson KA, Ydenberg RC (2009) Seabird seasonal trophodynamics: isotopic patternns in a community of Pacific alcid. Mar Ecol Prog Ser 382:211–219
Dehnard N, Voigt CC, Poirsebleau M, Demongin L, Quillfeldt P (2011) Stable isotopes in southern rockhopper penguins: foraging areas and sexual differences in the non-breeding period. Polar Biol 34:1763–1773
Ducklow HW, Fraser WR, Meredith MP, Stammerjohn SE, Doney SC, Martinson DG, SAILLEY SF, Schofield OM, Steinberg DK, Venables HJ, Amsler CD (2013) West Antarctic Peninsula: an ice-dependent coastal marine ecosystem in transition. Oceanography 26:190–203
Dunton K (2001) δ15N and δ13C measurements of Antarctic Peninsula fauna: trophic relationships and assimilation of benthic seaweeds. Am Zool 41:99–112
Erdmann ES, Ribic CA, Patterson-Fraser DL, Fraser WR (2011) Characterization of winter foraging locations of Adélie penguins along the Western Antarctic Peninsula, 2001–2002. Deep-Sea Res II 58:1710–1718
Fraser WR, Trivelpiece WZ (1996) Factors controlling the distribution of seabirds: winter–summer heterogeneity in the distribution of Adélie penguin populations. In: Ross RM, Hofmann EE, Quetin LB (eds) Foundations for ecological research west of the Antarctic Peninsula. American Geophysical Union, Washington, pp 257–272
Fraser WR, Trivelpiece WZ, Ainley D, Trivelpiece SG (1992) Increases in Antarctic penguin populations: reduced competition with whales or a loss of ice due to environmental warming? Polar Biol 11:525–531
Gales RP (1987) Validation of the stomach flushing technique for obtaining stomach contents of penguins. Ibis 129:225–343
Goutte A, Charrassin JB, Cherel Y, Carra vieiri A, De Grissac S, Massé G (2014) Improvement of ice algal production for top predators: new insights using sea-ice biomarkers. Mar Ecol Prog Ser 513:269–275
Hinke JT, Trivelpiece WZ (2011) Daily activity and minimum food requirements during winter for gentoo penguins (Pygoscelis papua) in the South Shetland Islands, Antarctica. Polar Biol 34:1579–1590
Hinke JT, Polito MJ, Reiss CS, Trivelpiece SG, Trivelpiece WZ (2012) Flexible reproductive timing can buffer reproductive success of Pygoscelis spp. penguins in the Antarctic Peninsula region. Mar Ecol Prog Ser 454:91–104
Hinke JT, Trivelpiece SG, Trivelpiece WZ (2014) Adélie penguin (Pygoscelis adeliae) survival rates and their relationship to environmental indices in the South Shetland Islands, Antarctica. Polar Biol 37:1797–1809
Hinke JT, Polito MJ, Goebel ME, Jarvis S, Reiss CS, Thorrold SD, Trivelpiece WZ, Watters GM (2015) Spatial and isotopic niche partitioning during winter in chinstrap and Adélie penguins from the South Shetland Islands. Ecosphere. doi:10.1890/ES14-00287.1
Hobson KA, Bond AL (2012) Extending an indicator: year-round information on seabird trophic ecology from multiple-tissue stable-isotope analyses. Mar Ecol Prog Ser 461:233–243
Hobson KA, Gloutney ML, Gibbs HL (1997) Preservation of blood and tissue samples for stable-carbon and stable-nitrogen isotope analysis. Can J Zool 75(10):1720–1723
Jablonski B (1985) The diet of penguins on King George Island, South Shetland Islands. Acta Zool Crac 29:17–86
Juárez MA (2013) Biología reproductiva y ecología trófica de dos especies sympatricas del género Pygoscelis en las Islas Shetland del Sur, Antártida (Breeding biology and trophic ecology of two sympatric species of the genus Pygoscelis at the South Shetland Islands, Antarctica). PhD Thesis, Universidad Nacional de La Plata. http://hdl.handle.net/10915/27166
Juárez MA, Santos MM, Negrete J, Santos MR, Mennucci JA, Rombolá E, Longarzo L, Coria NR, Carlini AR (2013) Better late than never? Interannual and seasonal variability in breeding chronology of gentoo penguins at Stranger Point, Antarctica. Polar Biol 32:18448. doi:10.3402/polar.v32i0.18448
Juárez MA, Santos M, Negrete J, Mennucci JA, Perchivale PJ, Casaux R, Coria NR (2015) Adélie penguin population changes at Stranger Point, Antarctica. Antarct Sci 27(5):455–461. doi:10.1017/S0954102015000152
Kelly B, Dempson JB, Power M (2006) The effects of preservation on fish tissue stable isotope signatures. J Fish Biol 69:1595–1611
Lynch HJ, Fagan WF, Naveen R, Trivelpiece SG, Trivelpiece WZ (2012) Differential advancement of breeding phenology in response to climate may alter staggered breeding among sympatric pygoscelid penguins. Mar Ecol Prog Ser 454:135–145

 Springer
Miller AK, Karnovsky NJ, Trivelpiece WZ (2009) Flexible foraging strategies of Gentoo penguins Pygoscelis papua over 5 years in the South Shetland Island, Antarctica. Mar Biol 156:2527–2537
Miller AK, Kappes MA, Trivelpiece SG, Trivelpiece WZ (2010) Foraging-niche separation of breeding Gentoo and Chinstrap penguins, South Shetland Island, Antarctica. Condor 112(4):683–695
Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS ONE 5:e9672. doi:10.1371/journal.pone.0009672
Polito MJ, Fisher S, Tobias CT, Emslie SD (2009) Tissue-specific discrimination factors in gentoo penguin (Pygoscelis papua) egg components: implications for dietary reconstruction using stable isotopes. J Exp Mar Biol Ecol 372:106–112
Polito MJ, Lynch HJ, Naveen R, Emslie SD (2011a) Stable isotopes reveal regional heterogeneity in the pre-breeding distribution and diets of sympatrically breeding Pygoscelis spp. penguins. Mar Ecol Prog Ser 421:265–277
Polito MJ, Abel S, Tobias CR, Emslie SD (2011b) Dietary isotopic discrimination in gentoo penguin (Pygoscelis papua) feathers. Polar Biol 34(7):1057–1063
Polito MJ, Trivelpiece WZ, Karnovsky NJ, Ng E, Patterson WP, Emslie SD (2011c) Integrating stomach content and stable isotope analyses to quantify the diets of Pygoscelid penguins. PLoS ONE 6:e26642. doi:10.1371/journal.pone.0026642
Quillfeldt P, McGill RAR, 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
Quillfeldt P, McGill RAR, Masello JF, Poisbleau M, van Noordwijk H, Demongin L, Furness RW (2009) Differences in the stable isotope signatures of seabird egg membrane and albumen—implications for non-invasive studies. Rapid Commun Mass Spectrom 23:3632–3636
Quillfeldt P, Masello JF, McGill RAR, Adams M, Furness RW (2010) Moving polewards in winter: a recent change in the migratory strategy of a pelagic seabird? Front Zool 7:1–11. http://www.frontiersinzooiology.com/content/7/1/15
Quillfeldt P, Ekschmitt K, Brickle P, McGill RAR, Wolters V, Dehnhard N, Masello JF (2015) Variability of higher trophic level stable isotope data in space and time—a case study in a marine ecosystem. Rapid Commun Mass Spectrom 29:667–674
Raya Rey A, Polito M, Archuby D, Coria N (2012) Stable isotopes identify age- and sex-specific dietary partitioning and foraging habitat segregation in southern giant petrels breeding in Antarctica and southern Patagonia. Mar Biol 159:1317–1326
Reiss CS, Cossio AM, Loeb V, Demer DA (2008) Variations in the biomass of Antarctic krill (Euphausia superba) around the South Shetland Islands, 1996–2006. ICES J Mar Sci 65:497–508
Ropert-Coudert Y, Kato A, Bost CA, Rodary D, Sato K, Maho YL, Naito Y (2002) Do Adélie penguins modify their foraging behaviour in pursuit of different prey? Mar Biol 140:647–652
Santos MM, Korczak-Abshire M, Jáuregui MA, Trivelpiece WZ, Hinke JT (2014) How similar are CEMP indices from adjacent locations? A case of study using Pygoscelis adeliae and P. papua monitoring data from three breeding colonies on King George Island. CCAMLR WG-EMM-14/43. https://www.ccamlr.org/en/wg-emm-14/43
Schmidt K, Atkinson A, Steigenberger S, Fielding S, Lindsay MCM, Pond DW, Tarling GA, Klevjer TA, Allen CS, Nicol S, Achterneg EP (2011) Seabed foraging by Antarctic krill: implications for stock assessment, bentho-pelagic coupling, and the vertical transfer of iron. Limnol Oceanogr 56:1411–1428
Tanton JL, Reid K, Croxall JP, Trathan PN (2004) Winter distribution and behaviour of Gentoo penguins Pygoscelis papua at South Georgia. Polar Biol 27:299–303
Thiebot JB, Cherel Y, Trathan PN, Bost CA (2012) Coexistence of oceanic predators on wintering areas explained by population-scale foraging segregation in space or time. Ecology 93:122–130
Trivelpiece WZ, Fraser WR (1996) The breeding biology and distribution of Adélie penguins: adaptations to environmental variability. In: Ross RM, Hofmann EE, Quein LB (eds) Foundation for ecological research west of the Antarctic Peninsula. American Geophysical Union, Washington, pp 273–285
Trivelpiece WZ, Trivelpiece SG, Volman NJ (1987) Ecological segregation of Adélie, Gentoo, and Chinstrap penguins at King George Island, Antarctica. Ecology 68:351–361
Trivelpiece WZ, Hinke JT, Miller AK, Reiss CS, Trivelpiece SG, Waters GM (2011) Variability in krill biomass links harvesting and climate warming to penguin population changes in Antarctica. PNAS 108:7625–7628
Wilson RP (1984) An improved stomach pump for penguins and other seabirds. J Field Ornithol 55:109–112