Seasonal consistency and individual variation in foraging strategies differ among and within *Pygoscelis* penguin species in the Antarctic Peninsula region

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Abstract

Past research during the breeding season in the Antarctic Peninsula region indicates that Gentoo Penguins (*Pygoscelis papua*) are generalist foragers, while Adélie (*P. adeliae*) and Chinstrap (*P. antarcticus*) Penguins tend to specialize on Antarctic krill (*Euphausia superba*). However, little is known about the degree of temporal consistency in the diets and foraging habitats of these three species, particularly at the individual level. Such year-round and inter-annual dietary understanding is important to help interpret contrasting trends in their populations. We used carbon and nitrogen stable isotope analysis of blood and feathers to evaluate seasonal shifts in diet and individual foraging consistency within *Pygoscelis* penguin species breeding in the South Shetland Islands, as well as among three geographically distinct Gentoo Penguin populations in the Western Antarctic Peninsula and South Shetland Islands. We found that Gentoo Penguins exhibited a generalist foraging strategy with individual consistency, Adélie Penguins exhibited an intermediate generalist foraging strategy with little individual consistency, and Chinstrap Penguins exhibited a specialized diet with little variation among individuals. Our results also indicated that all three species have greater variation in foraging habitat usage during the post-breeding season compared to the breeding season. Finally, we observed differences in the degree of seasonal shifts in population level diet and consistency in foraging strategies at the individual level across the three Gentoo Penguin populations examined. This suggests that *Pygoscelis* penguins can differ in diets and foraging habitat usage not only at the population level among species, sites, and seasons, but also in the level of variation within populations, and in the degree of seasonal consistency among individuals.

Introduction

Research into the diets and foraging ecologies of Antarctic penguins can provide vital information about their vulnerability to ecological pressures and help explain declines in certain species or populations. For example, the diet of Adélie (*Pygoscelis adeliae*) and Chinstrap Penguins (*P. antarcticus*) are well documented at the population level, particularly in the South Shetland Islands and Western Antarctic Peninsula, where past studies indicate that both species forage primarily on Antarctic Krill in offshore areas (Volkman et al. 1980; Trivelpiece et al. 1987; Karnovsky 1997). Antarctic krill have declined over the past 30 years (Atkinson et al. 2004), which may be the driver of recent population declines in Adélie and Chinstrap Penguins in this region (Trivelpiece et al. 2011). In contrast, Gentoo Penguin (*Pygoscelis papua*) populations are stable and even increasing in this same region (Lynch et al. 2013). Several possible explanations for this trend have been explored including flexible timing of breeding in Gentoo Penguins as well as extended parental provisioning (Polito and Trivelpiece 2008; Hinke et al. 2012). In addition, recent studies suggest that the more generalist foraging strategy employed by Gentoo Penguins may add to their adaptability to environmental changes (Miller et al. 2009, Polito et al. 2015).

The diets and foraging ecology of *Pygoscelis* penguins have been well studied in the Antarctic Peninsula region. In general, dietary studies within the Western Antarctic Peninsula and South Shetland Islands, which are based mostly on stomach content analyses, have indicated that Gentoo Penguins have a mixed diet of Antarctic krill and fish that varies among populations and years, while Adélie and Chinstrap tend to forage predominately on krill (Volkman et al. 1980, Lishman, 1985; Karnovsky 1997; Hinke et al. 2007; Miller et al. 2008, 2009, 2010; Polito et al. 2011b; Polito et al. 2015). Studies focused on foraging habitat demonstrate that, in general,

71 Gentoo Penguins forage in deeper benthic, inshore habitats, while Chinstrap and Adélie Penguins
72 forage further offshore in shallower, pelagic areas. However, past studies have focused primarily
73 on the breeding season and only a few have focused on diet or foraging habitat during the post-
74 breeding or non-breeding period (Miller et al. 2009, 2010; Gorman et al. 2014; Juáres et al. 2016;
75 Negrete et al. 2016). Even so, existing studies do indicate that Gentoo Penguin populations
76 exhibit a generalist diet with substantial variation in their foraging ecology across breeding
77 locations (Pütz et al. 2001; Clausen and Pütz 2002, 2003; Miller et al. 2009; Polito et al. 2015).
78 Individual niche variation is frequently found within generalist populations and is
79 commonly assessed by quantifying the degree of foraging variation among individuals and the
80 presence of individual consistency over time (Bolnick et al. 2003; Sargeant 2007). While one
81 study by Carravieri et al. (2013) detected individual dietary consistency in Gentoo Penguins in
82 the sub-Antarctic Kerguelan Islands, no such studies have been undertaken in the Antarctic
83 Peninsula region. Specifically, past studies of *Pygoscelis* penguins in this region lack an analysis
84 of dietary and foraging habitat consistency of individuals through time. Quantifying the degree
85 of niche variation and consistency at the individual level is important because individual
86 variation within populations has the potential to determine a species' population level response
87 to changes in food availability or other environmental change (Bolnick et al. 2002; Bearhop et al.
88 2004). For example, within-population differences in behavioral plasticity are thought to be an
89 important factor stabilizing populations in the face of changing environmental conditions
90 (Dingemanse and Wolf 2013).
91 Although the foraging ecology of individual marine predators can be challenging to track
92 over time, stable isotope analyses (SIA), provides a robust tool for measuring animal diets
93 through time from a single capture event. Prior studies have demonstrated that SIA is an

94 effective method to quantify the diets and foraging niches of *Pygoscelis* penguins (Polito et al.
95 2011c, Polito et al. 2015; Negrete et al. 2016). Nitrogen stable isotope values ($\delta^{15}N$) in
96 consumers exhibits a strong positive correlation with trophic level (Minagawa and Wada 1984;
97 Hobson et al. 1994). Carbon stable isotope values ($\delta^{13}C$) change little with increasing trophic
98 level but instead provide a proxy of marine foraging habitat use due to differences in baseline
99 $\delta^{13}C$ values between inshore/benthic and offshore/pelagic habitats (France 1995; Cherel and
100 Hobson 2007). In addition, the $\delta^{13}C$ and $\delta^{15}N$ values of specific tissues reflect the diets of
101 consumers at the time of synthesis such that comparing tissues synthesized at different times
102 from a single individual can assess foraging niche consistency over time (Newsome et al. 2007;
103 Hückstädt et al. 2012; Kernaléguen et al. 2016). For example, Bearhop et al. (2006) detected
104 individual consistency and distinct foraging specialization in South Georgian shags
105 (*Phalacrocorax georgianus*) and Kerguelen shags (*P. verrucosus*) by comparing the $\delta^{13}C$ and
106 $\delta^{15}N$ values of blood (i.e. breeding season) and feathers (i.e. post-breeding season) sampled from
107 the same individuals. A similar analysis on sympatric *Pygoscelis* populations would help to
108 quantifying seasonal diet variation and the degree of consistency at the individual level and help
109 to inform these species' responses to recent changes in krill availability in the Antarctic
110 Peninsula region.
111 To address this gap, we use SIA of blood and feathers to evaluate seasonal shifts in diet
112 at the population level and individual level dietary consistency within *Pygoscelis* species
113 (Adélie, Chinstrap and Gentoo Penguins) breeding in sympatry in the South Shetland Islands,
114 Antarctica. In addition, we investigate these same parameters among three Gentoo Penguins'
115 populations in the Western Antarctic Peninsula and South Shetland Islands, Antarctica. Based on
116 previous dietary studies of *Pygoscelis* penguins, we predict that Gentoo Penguins will have

greater degree of among individual variation and individual consistency in diets relative to Adélie and Chinstrap Penguins (Miller et al. 2010; Polito et al. 2015). Furthermore, we predict that the degree of among individual variation and extent of individual consistency will vary among the Gentoo Penguin populations examined, which we derive from the evidence of geographic variation in diet among Gentoo Penguins breeding locations (Pütz et al. 2001; Clausen and Pütz 2002, 2003; Miller et al. 2009; Polito et al. 2015). We define "individual consistency" as among-individual differences that remain consistent between seasons even when there is an overall shift in diet of the population level between seasons.

Methods

Study Site and Sample Collection

In December 2010, we collected samples from breeding Adélie, Chinstrap, and Gentoo Penguins at Admiralty Bay, King George Island, South Shetland Islands (62.1°S, 58.25°W) (Fig. 1). In December 2014 we collected samples from breeding Gentoo Penguins at three sites in the Western Antarctic Peninsula and South Shetland Islands: Damoy Point, Wiencke Island (64.8°S, 63.5°W), Georges Point, Rongé Island (64.7°S, 62.7°W), and Stinker Point, Elephant Island (61.1°S, 55.2°W) (Fig. 1). At each breeding site, we collected three white body feathers and 1ml of whole blood from 15-22 individuals per species. We sampled adults when both members of the pair were at the nest and preferentially sampled the adult who had recently returned from the sea. All Chinstrap Penguins sampled during late-incubation, while Adélie and Gentoo Penguins were sampled within two days of chicks hatching. The half-life of stable isotope values in whole blood of a 3-5 kg penguin is predicted to be approximately 10-30 days based on allometric and empirical studies (Carleton and Martinez del Rio 2005, Barquete et al. 2013). Therefore, whole

blood samples provides a proxy for dietary information during the incubation period of the 2010 breeding season at Admiralty Bay and the 2014 breeding season at Wiencke, Rongé and Elephant Islands. Body feathers are grown following the breeding season (i.e. late February through March; Hinke et al. 2015) and are metabolically inert after synthesis (Polito et al. 2011a). As such body feathers collected for this study represent reflect diets during the post-breeding stages of the 2009 breeding season at Admiralty Bay and 2013 breeding season at Wiencke, Rongé and Elephant Islands, respectively. Combined these two tissues provide proxies of individual's diet and foraging habitat usage during the post-breeding (feathers) and breeding (blood) seasons that can be compared to evaluate individual consistency.

Molecular sexing was conducted for populations at Admiralty Bay and Elephant Island and resulted in roughly equal sampling of sexes (i.e. Polito et al. 2011). Molecular sexing was not possible for Wiencke or Rongé Island populations; however we sampled adults when both members of the pair were at the same nest and used relative body and bill size differences within pairs to approximate equal sampling of the sexes (Polito et al. 2011). Due to the differences in the sexing methods used and the higher uncertainty in sex identification at Wiencke or Rongé Island we chose not to examine sex related trends as part of this study.

Sample Preparation and Isotopic Analysis

We soaked body feathers in a mixture of 2:1 chloroform-methanol for 24 hours to remove lipids, then rinsed each segment in 2:1 chloroform-methanol and allowed them to air dry for 24 hours (Cherel et al. 2005). We then subsampled pieces of the vane of equal size from three body feathers to obtain a total of 0.5-0.8 mg per individual. We dried blood samples to constant weight at 50°C in an analytical oven for 48 hours and then ground the dried blood into a powder and

sub-sampled 0.6 mg of blood per individual for analysis. Samples were analyzed using a PDZ Europa ANCA-GSL and Costech ECS4010 elemental analyzers interfaced to a PDZ Europa 20-20 and Thermo Finnigan Delta Plus XP continuous flow stable isotope ratio mass spectrometers. Raw δ values were normalized using glutamic acid, bovine liver, and nylon 5 as reference materials (USGS-40: $\delta^{13}C$ = -26.39±0.09‰, $\delta^{15}N$ = -4.52±0.12‰; USGS-41: $\delta^{13}C$ = 37.63±0.10‰, $\delta^{15}N$ = 47.57±0.22‰; bovine liver: $\delta^{13}C$ = -21.69±0.20‰, $\delta^{15}N$ = 7.72±0.30‰; nylon 5: $\delta^{13}C$ = -27.72±0.20‰, $\delta^{15}N$ = -10.31±0.30‰). Sample precision based on internal repeats and duplicate standard reference materials across all samples was 0.1‰ for $\delta^{15}N$ and 0.2‰ for $\delta^{13}C$. Stable isotope ratios are expressed in the δ notation in per mil units (‰) according to the following equation:

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

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 Peedee belemnite (VPDB) for ^{13}C and atmospheric N_2 for ^{15}N.

Standardizing stable isotope values between blood and feathers

Dietary isotopic discrimination, which is the difference between the isotopic values of diet and consumers, can vary significantly across tissue types even when synthesized under the same diet (Hobson and Clark 1992; Bond and Jones 2009; Polito et al. 2009). Several studies have examined the isotopic values of feathers and blood synthesized under the same diet (Sanpera et al. 2007; Bugoni et al. 2008; Quillfeldt et al. 2008; Kohler et al. 2011; Cruz et al. 2012; Cherel et al. 2014). Based on a meta-analysis of these studies, we used the following regressions from Cherel et al. (2014) to standardize values to directly compare between $\delta^{15}N$ and $\delta^{13}C$ values obtain from the feather and blood tissues of the same individual penguin:

$$feather\ \delta^{15}\mathrm{N} = 1.014\ (\pm 0.056)\ \times\ blood\ \delta^{15}\mathrm{N}\ + 0.447\ (\pm 0.665)$$

$$feather\ \delta^{13}\mathrm{C} = 0.972\ (\pm 0.020)\ \times\ blood\ \delta^{13}\mathrm{C} + 0.962\ (\pm 0.414)$$

Statistical Analysis

Prior to analysis, we examined for normality in all populations using the Shapiro-Wilks test. We also examined for homogeneity of variance using Bartlett's test for normally distributed populations and Levene's test for non-normally distributed populations. In order to determine whether populations are more generalized or specialized relative to one another, we calculated coefficient of variation (CV; $\sigma/\bar{x}$) as a proxy for individual variation within populations of species for post-breeding (feather) season and breeding (blood) season δ^{15}N and δ^{13}C absolute values. Differences in CV were assessed qualitatively as there are no robust statistical analyses that can directly test for significant differences between CVs (Donnelly and Kramer 1999). As such, we calculated standard ellipse area Bayesian estimations (SEA_b) to provide a bivariate quantitative metric that allows for the comparison of niche area among and within species and populations (Jackson et al. 2011). Bivariate analyses were completed using the SIBER package (Stable Isotope Bayesian Ellipses in R) within the SIAR package in R.

To test for shift in population level diet and foraging habitat between seasons in the three penguin species sampled from King George Island in 2010, we compared population means of post-breeding (feather) δ^{15}N and δ^{13}C values to the standardized breeding season (blood) δ^{15}N and δ^{13}C values using paired t-tests for normally distributed populations with equal variance and Wilcoxon signed rank tests for populations that were non-normal.

To test whether individual diet and foraging habitat is consistent between seasons and/or

individual diet and foraging habitat are consistent relative to each other, we tested for relationships among individual's post-breeding and breeding season $\delta^{15}N$ and $\delta^{13}C$ values using Pearson Correlation for normally distribute populations with equal variance and Kendall rank correlations for populations that were non-normal or had unequal variance. We conducted these same analyses for three Gentoo Penguin populations sampled in 2014 in order to test for geographic variation in the presence of population level and individual level diet and foraging consistency. All statistical analyses were performed in R software ver. 3.3.2 (R Core Team 2016). Significance was assumed at the $p \leq 0.05$ level and all means are presented ±SD.

Results

Comparison of three species at King George Island in 2010

Gentoo Penguins had the highest CVs for post-breeding season (feather) and breeding season (blood) $\delta^{15}N$ values, followed by Adélie Penguins and Chinstrap Penguins (Table 1). Adélie and Chinstrap Penguins had nearly twice the CV for post-breeding season $\delta^{13}C$ values compared to Gentoo Penguins. All three species displayed a much lower and similar CV for breeding season $\delta^{13}C$ values. Isotopic niche areas (SEA_b) during the post-breeding were larger than niche areas during the breeding season in all three species (Adélie: $p = 0.0003$; Chinstrap*: P* = 0.001; Gentoo*: P* = 0.089; Fig 2a). There was no significant difference in SEAb during the post-breeding season between Adélie and Chinstrap Penguins (*P* = 0.119), Adélie and Gentoo Penguins (*P* = 0.177) or Chinstrap and Gentoo Penguins (*P* = 0.374). Gentoo Penguins had a larger SEAb than Chinstrap Penguins during the breeding season (*P* = 0.015), but there were no significant differences in SEAb during the breeding season between Adélie and Chinstrap Pengiuns (*P* = 0.158) or Adélie and Gentoo Penguins (*P* = 0.110).

Gentoo Penguin $\delta^{15}N$ differed between the post-breeding season and breeding season (Paired t test, $t_{20} = 3.53$, $P = 0.002$; Fig. 3). Concurrently, Gentoo $\delta^{13}C$ values differed between the post-breeding season and breeding season (Wilcoxon signed-ranks test, $T = 19.5$, $N = 21$, $P = 0.0003$). While Adélie Penguin $\delta^{15}N$ did not differ significantly between the post-breeding and breeding seasons (Paired t test, $t_{21} = -0.23$, $P = 0.9$), the $\delta^{13}C$ values were significantly lower during the post-breeding season relative to the breeding season by 1.2‰ (Paired t test, $t_{21} = -4.8$, $P = <0.0001$). Chinstrap Penguin $\delta^{15}N$ during the post-breeding season was significantly lower relative to the breeding season by 0.3‰ (Paired t test, $t_{19} = -5.34$, $P < 0.0001$), but there was no significant difference between the post-breeding and breeding seasons in $\delta^{13}C$ values (Wilcoxon signed-ranks test, $T = 100$, $N = 20$, $P = 0.86$). There was a significant correlation in individual Gentoo Penguin $\delta^{15}N$ (Pearson correlation, $r = 0.8$, $N = 21$, $P < 0.001$) and $\delta^{13}C$ values (Kendall rank correlation, $r_t = 0.47$, $N = 21$, $P = 0.006$) between the two seasons examined (Fig. 3). In contrast, there was no correlation in individual Adélie Penguin $\delta^{15}N$ (Pearson correlation, $r = 0.09$, $N = 22$, $P = 0.68$) and $\delta^{13}C$ values (Kendall rank correlation, $r_t = -0.09$, $N = 22$, $P = 0.61$) or Chinstrap Penguin $\delta^{15}N$ (Pearson correlation, $r = 0.18$, $N = 20$, $P = 0.44$) and $\delta^{13}C$ values (Kendall rank correlation, $r_t = -0.21$, $N = 20$, $P = 0.24$) between seasons (Fig. 3.

Comparison of three Gentoo Penguin populations in 2014

Gentoo Penguins at Elephant Island had the highest CV for $\delta^{15}N$ during both the post-breeding and breeding season by more than double that of the populations at Wiencke and Rongé Island (Table 1). The population at Rongé Island had the highest CV for $\delta^{13}C$ during the post-breeding season, followed by Wiencke and Elephant Island. Wiencke Island population had the highest $\delta^{13}C$ CV during the breeding season, followed by Rongé and Elephant Island. Isotopic

niche areas (SEA_b) during the post-breeding was large than niche areas during the breeding season at Wiencke Island (p = 0.039) and Rongé Island (p = 0.004), but did not differ in size at Elephant Island (p = 0.085; Fig 2b). SEA_b during the post-breeding season was larger at Elephant Island than Wiencke Island (P = 0.036). There was no significant difference in SEA_b during the post-breeding season between Wiencke and Rongé Island (P = 0.235) or Rongé and Elephant Island (P = 0.139). During the breeding season, SEAb at Elephant Island was greater than both Wiencke Island (P = 0.026) and Rongé Island (P = 0.014). There was no significant difference in SEA_b during the breeding season between Wiencke and Rongé Island (0.382).

Gentoo Penguins at Wiencke Island had a significantly higher $\delta^{15}N$ in the post-breeding season compared to the breeding season (Paired t test, t_{18} = 5.03, P < 0.0001) by 0.4‰. In addition, the $\delta^{13}C$ values of this population were significantly lower during the post-breeding season relative to the breeding season by 2.6‰ (Paired t test, t_{19} = -9.9, P < 0.0001). Gentoo Penguins at Rongé Island also had significantly higher $\delta^{15}N$ and $\delta^{13}C$ values in the post-breeding season compared to the breeding season (Paired t test, t_{18} = 4.1, P = 0.0006; Wilson signed-ranks test, T = 8, P = 0.0002, respectively). $\delta^{15}N$ values of Gentoo Penguins at Elephant Island were significantly lower during the post-breeding season relative to the breeding season by 1.2‰ (Paired t test, t_{14} = -7.23, P < 0.0001). Gentoo Penguin $\delta^{13}C$ values at Elephant Island were also significantly lower during the post-breeding season relative to the breeding season 1.0‰ (Paired t test, t_{14} = -6.03, P < 0.0001).

There was no correlation between seasons in individual $\delta^{15}N$ values (Pearson correlation, r = 0.23, N = 19, P = 0.34) or $\delta^{13}C$ values (Kendall rank correlation, r_t = 0.14, N = 15, P = 0.435.) at Wiencke Island (Fig. 4). Similarly, there was no correlation between seasons in individual $\delta^{15}N$ values (Pearson correlation, r = 0.04, N = 19, P = 0.85) or $\delta^{13}C$ values (Kendall

rank correlation, $r_t = 0.33$, $N = 19$, $P = 0.071$) at Rongé Island (Fig. 4). In contrast, Gentoo Penguins at Elephant Island exhibited a significant correlation in individual $\delta^{15}N$ values between seasons (Pearson correlation, $r = 0.60$, $N = 15$, $P = 0.02$), but no correlation in individual $\delta^{13}C$ values between seasons (Kendall rank correlation, $r_t = 0.35$, $N = 15$, $P = 0.109$; Fig. 4).

Discussion

Gentoo Penguins in the Western Antarctic Peninsula and South Shetland Islands are often regarded as generalist foragers with a mixed and varying diet of Antarctic krill and fish, whereas Adélie and Chinstrap are thought to specialize primarily on krill within this region, particularly during the breeding season (Volkman et al. 1980; Lishman 1985; Karnovsky 1997; Miller et al. 2008, 2009, 2010; Polito et al. 2011b; Polito et al. 2015). In addition, vertical and temporal differences in foraging habitat usage also aid in differentiating the foraging niches of the three species when they occur in sympatry (Wilson 2010). However, very few studies have investigated foraging variation among individuals within populations of *Pygoscelis* species (Polito et al. 2015) or temporal consistency at the population and individual level (e.g. Polito et al. 2011; Juáres et al. 2016; Negrete et al. 2016). Our results suggest that *Pygoscelis* penguins can differ in foraging ecology not only at the population level among species, sites and seasons, but also in the level of individual variation within populations, and in the degree of foraging consistency within individuals.

Comparison of foraging strategies at King George Island

Comparisons of coefficients of variation (CV) and isotopic niche area (SEA_b) provide additional support for differences in the relative width of foraging niches among the three species

301 as indicated by previous studies (Volkman et al. 1980; Miller et al. 2010; Polito et al. 2015).
302 SEA_b results suggest that three species have a similar sized population niche during the post-
303 breeding season, while Gentoo Penguins maintain a larger population niche relative to Chinstrap
304 Penguins during the breeding season. When examining $\delta^{15}N$ values among the three species at
305 King George Island, Gentoo Penguins had the highest CV, Chinstrap Penguins had the lowest
306 CV, and Adélie Penguins CV were intermediate of their congeners during both seasons
307 examined (Table 1; Fig. 3). These results suggest that Adélie Penguins are more generalized in
308 their diet relative to Chinstrap Penguins, while Gentoo Penguins are more generalized in their
309 diet relative to both Adélie and Chinstrap Penguins.). Previous studies support our findings that
310 Gentoo Penguins have greater variability in diet than Adélie and Chinstrap Penguins in the South
311 Shetland Islands during the breeding season (Volkman et al. 1980; Polito et al. 2015; Negrete et
312 al. 2016). However, while past studies indicate that Adélie Penguins diets are more diverse in
313 regions outside of the Antarctic Peninsula (e.g. Ainley 2002; Jarman et al. 2013) to our
314 knowledge there are no previous studies showing that Adélie Penguins are more generalized in
315 diet relative to Chinstrap Penguins.
316 When examining $\delta^{13}C$ values among the three species, Adélie and Chinstrap Penguins
317 had greater CV than Gentoo Penguins during the post-breeding season, while Gentoo Penguins
318 had a larger CV than Adélie and Chinstrap Penguins during the breeding. These results suggest
319 that during the post-breeding period individual Adélie and Chinstrap Penguins use a wider range
320 of foraging habitats relative to individual Gentoo Penguins at King George Island, but Gentoo
321 Penguins (Table 1). These findings are similar to those of Juáres et al. (2016) at Stranger Point,
322 King George Island, which found Adélie Penguins had a greater variation in body feather $\delta^{13}C$
323 values relative to Gentoo Penguins. In combination, these results agree with tracking studies

which indicate that Adélie and Chinstrap Penguins disperse broadly during the post-breeding period (Hinke et al. 2015) while Gentoo Penguins remain close to their breeding colonies and forage in open-water, near-shore habitats (Wilson et al. 1998a).

Seasonal and individual variation at King George Island

Our results indicated that the Adélie Penguin $\delta^{15}N$ values do not differ between seasons at the population level with no evidence of individual-level consistency in this dietary proxy. Past studies examining stomach contents during the chick-rearing period have found Adélie Penguin diets are dominated by Antarctic Krill in the South Shetland Islands (Volkman et al. 1980; Karnovsky 1997). However, the CV and SEA_b values observed in our study suggest that Adélie Penguins in this region may have a relatively more diverse trophic level of diet during the incubation and post-breeding periods; similar to what has been suggested for the pre-breeding season (Polito et al. 2011b).

Adélie penguin $\delta^{13}C$ results indicate that foraging habitats shifts between seasons at the population level with no evidence of individual level consistency. The lower $\delta^{13}C$ values and higher CV found in the post-breeding season relative to the breeding season suggest that Adélie Penguins disperse widely and forage in more pelagic/offshore habitats during this time. This agrees with a recent study of at Ardley Island, where $\delta^{13}C$ values indicated Adélie Penguins foraged further off shore during the post-breeding season compared to the incubation stage (Negrete et al. 2016). Furthermore, Tierney et al. (2008) found the incubation and guard $\delta^{13}C$ values Adélie Penguins from MacRoberston Island, East Antarctica, were higher than during arrival to breeding locations but lower than those from crèche. The above isotopic studies confirm tracking studies and indicate that foraging ranges are restricted during incubation and

chick rearing relative to outside of the breeding season (Trivelpiece et al. 1987; Hinke et al. 2015)

Chinstrap Penguin $\delta^{15}N$ values indicated a small but significant seasonal shift in population level diets with no evidence of individual level consistency (Table 1; Fig. 3). The observed difference in $\delta^{15}N$ values of 0.3‰ likely reflects a 5-10% shift between krill and fish prey between seasons based on previous isotopic mixing-model analyses of Chinstrap Penguin diets in the South Shetland Islands (Polito et al. 2011c). Combined with a relatively narrow CV for both seasons, this suggests that Chinstrap Penguins have a narrow diet that is relatively consistent between seasons. This interpretation is consistent with many studies that have found Chinstrap Penguin in the South Shetland Islands have a narrow diet composed primarily of Antarctic krill during the breeding season (Volkman et al. 1980; Trivelpiece et al. 1987; Miller et al. 2008; Miller et al. 2010; Polito et al. 2015). $\delta^{13}C$ results suggest Chinstrap Penguin population level isotopic foraging habitat use does not change between seasons, which agrees similar findings by Negrete et al. (2016), but individuals are not seasonally consistent relative to each other. Combined with a large range in post-breeding season $\delta^{13}C$ values and a relatively large CV and SEA_b values, Chinstrap Penguins appear to have considerable seasonal variability in individual foraging habitat, which is supported by a previous study of both tracking and $\delta^{13}C$ data that found Chinstrap Penguins at King George Island exhibit individual variation in movement patterns and the population generally occupies a broad geographic range of foraging habitats (Hinke et al. 2015). Although statistical tests suggest that this Chinstrap Penguin population does not shift its foraging habitat seasonally, $\delta^{13}C$ values appear to reflect the same pattern seen in Adélie Penguins. Breeding season $\delta^{13}C$ values and SEA_b values are substantially narrower and less variable than post-breeding $\delta^{13}C$ and SEA_b values, suggesting a similar

foraging restriction due to incubation/chick-rearing responsibilities during the breeding season.

Gentoo Penguins $\delta^{15}N$ results suggest that population level diets differed between the post-breeding and breeding seasons. These results differ from those of Juáres et al. (2016), which found no differences in the $\delta^{15}N$ values of Gentoo Penguins between these two time periods at Stranger Point, King George Island, South Shetland Islands. The $\delta^{13}C$ values and SEA_b of Gentoo Penguin in our study indicate that foraging habitats usage did not differ between these two seasons. This agrees with similar findings by Negrete et al. (2016) as Gentoo Penguins often remain close to their breeding colonies outside of the breeding season (Wilson et al. 1998a). While Juáres et al. (2016) and Negrete et al. (2016) did not examine individual consistency; our results also indicate that individual Gentoo Penguin were consistent, relative to each other in their diets ($\delta^{15}N$) and foraging habitats usage ($\delta^{13}C$) between seasons. In addition, similar to Adélie and Chinstrap Penguins, $\delta^{13}C$ values in Gentoo Penguins reveals a slight truncation during the breeding season, suggesting foraging range restriction due to incubation responsibilities.

Comparisons among Gentoo Penguin populations

We found evidence of seasonally consistent foraging niche differences among individual Gentoo Penguins at King George Island in 2010. However comparisons among the three other Gentoo Penguin populations in the Western Antarctic Peninsula and the South Shetland Islands in 2014 indicated that the degree of among individual variation and extent of individual consistency can vary among populations. For example, we found a greater degree of individual variation in $\delta^{15}N$ values at Elephant Island relative to Gentoo Penguins at Wiencke and Rongé Island during both the post-breeding and breeding periods (Fig. 4). Specifically $\delta^{15}N$ value CVs

393 at Elephant Island were more than twice as high as the Wiencke and Rongé populations,
394 indicating a wider dietary diversity and variation among individuals. Furthermore, Elephant
395 Island also had a larger isotopic niche area (SEA_b) than Wiencke Island during the post-breeding
396 season and both Wiencke and Rongé Island during the breeding season. A comparison of $\delta^{13}C$
397 values CV suggest that all populations demonstrate a similar level of variation in foraging habitat
398 use during the post-breeding season, while Gentoo Penguins at Wiencke Island exhibit a higher
399 degree of foraging habitat use variation during the breeding season relative to Gentoo Penguins
400 at Rongé and Elephant Island (Table 1; Fig. 4).
401 Furthermore, $\delta^{15}N$ values suggest that the population level diets differed between the
402 post-breeding and breeding periods for each of the three breeding location examined. However,
403 only at Elephant Island did among-individual differences in $\delta^{15}N$ values remain consistent
404 between seasons (Fig. 4). A similar trend was not observed in foraging habitat usage as defined
405 by $\delta^{13}C$ values. All three populations demonstrated seasonal shifts in population level foraging
406 habitat use, but among-individual differences in $\delta^{13}C$ values did not remain consistent between
407 seasons. Similar to King George Island, $\delta^{13}C$ values were higher during the breeding season at all
408 sites suggesting individuals may be constrained to more near shore locations due to incubation
409 duties.
410 The observed differences in the population level foraging niche width and the extent of
411 individual consistency may be due to regional differences in productivity, foraging habitats and
412 prey availability. For example, studies on Galapagos sea lions (*Zalophus wollebaeki*) found a
413 high degree of variation in foraging strategies and the level of individual specialization between
414 populations throughout the Galapagos Archipelago whose habitats vary in prey availability and
415 physical oceanographic characteristics (Dellinger and Trillmich 1999; Salazar 2005; Villegas-

Amtmann et al. 2008). In addition, studies of Gentoo penguins in the sub-Antarctic Kerguelen Islands found differences in oceanographic condition between open and closed-sea foraging habitats that influenced the degree of diet variation between breeding populations (Lescroël et al. 2004, Lescroël and Bost 2005). As such, it is possible that prey abundance and availability may differ between the foraging habitat with differing oceanographic condition in the Western Antarctic Peninsula and South Shetland Islands. The Western Antarctic Peninsula is known for a relatively year round abundance of Antarctic krill (Lascara et al. 1999). Gentoo Penguin populations at Wiencke and Rongé Island are most likely foraging primarily on krill, which would explain their relatively narrow variation in $\delta^{15}N$ values. The South Shetland Islands are in close proximity to an ocean convergence zone, resulting in the Southern Antarctic Convergence Front (SACCF) and Southern Boundary of the ACC (SB) (Fig. 1). These fronts are associated with high densities of Antarctic krill, but with high variability between seasons (Dietrich et al. 2014). Differences in benthic habitat availability between breeding sites and regions are also likely to further mediate prey availability (Lescroël et al. 2004, Miller et al. 2010). Variability in the abundance and availability of krill may select for greater diversity in diet among individual penguins in order to reduce competition, which could explain the high variability in $\delta^{15}N$ values for Gentoo Penguins at Elephant Island as well as King George Island. This also suggests that studies examining multiple Adélie and Chinstrap Penguins populations in the Western Antarctic Peninsula and South Shetland Islands are merited to determine if similar trends in within-species variation in foraging strategies exist.

Stable isotope analysis in Pygoscelis penguins

Past foraging ecology studies of *Pygoscelis* penguins using SIA have also included

mixing models, which incorporate $\delta^{15}N$ and $\delta^{13}C$ values of prey items to estimate the contribution of prey sources to a predator's diet (e.g. Polito et al. 2011b; Juáres et al. 2016). In addition another common approach is to use stable isotope data to calculate standardized trophic level and prey $\delta^{13}C$ values (Hobson and Bond 2012). While potentially informative, these approaches require the comprehensive and concurrent sampling of prey items to allow for robust estimates of consumer diets and/or trophic level while accounting for variation in isotopic baselines. While we were not able to collect concurrent prey samples in this study, past research indicates consistent isotopic differences between the krill and fish prey consumed by penguins (Polito et al. 2011c), and limited spatial and temporal differences in the stable isotope values of Antarctic krill in our study regions (Polito et al. 2013). While not conclusive, this indicates that the isotopic variability observed across and within penguin populations in our study are less likely to result from spatial and temporal differences in the stable isotope values of prey species.

Another potential uncertainty when using mixing models or calculating trophic level using SIA are isotopic discrimination factors. While tissue and taxa-specific isotopic discrimination factors are available for Gentoo Penguin feathers (Polito et al. 2011a), there are no published discrimination factors for *Pygoscelis* penguin blood. Past studies of *Pygoscelis* penguins have used whole blood discrimination factors derived from captive studies of other piscivorous birds (Brasso and Polito 2013; Juáres et al 2016; Negrete et al. 2016) and it is possible that there result would differ if taxa-specific discrimination factors were available. Given the above issues, we therefore chose to use the conservative approach outlined by Cherel et al. (2014) of standardizing feather and blood $\delta^{15}N$ and $\delta^{13}C$ values to allow for direct comparisons between the post-breeding and breeding periods. In addition, our correlation results would not differ by using standardized vs. un-standardized stable isotope values and as such the

462 patterns between and within species observed in our study is not an artifact of the standardization
463 methods. Nonetheless, we suggest that future research should aim to include concurrent prey
464 sampling as well as controlled dietary studies of the target species and tissues in order to obtain
465 more robust estimates of penguin diets using SIA.
466
467 **Conclusions**
468 This study is the first to use stable isotope analysis of blood and feather of all three
469 *Pygoscelis* penguins to compare temporal foraging consistency within individuals and between
470 populations. Our results indicate species-specific differences at King George Island: Gentoo
471 Penguins exhibit a generalist foraging strategy with individual level consistency, Adélie
472 Penguins exhibit an intermediate generalist foraging strategy with little individual consistency,
473 and Chinstrap Penguins exhibit a specialized diet with little variation among individuals.
474 However, unlike trophic variation, we found similarities in foraging habitat across all three
475 species, with greater variation in foraging habitat at the population level during the post-breeding
476 season compared to the breeding season. Finally, we found variation in trophic level of diet
477 ($\delta^{15}N$) between Gentoo Penguin populations, with the two populations in the Western Antarctic
478 Peninsula exhibiting a narrow range of trophic level with little individual variation, relative to
479 the population on Elephant Island, South Shetland Islands, which had a large range in trophic
480 level, and evidence of individual foraging consistency. Though generalist populations
481 comprising individual specialists have been documented in other top marine predators (Woo et
482 al. 2008; Newsome et al. 2009; Hückstädt et al. 2012; Kernaléguen et al. 2016) true evidence of
483 individual specialization has only recently been observed in penguins (Dehnhard et al. 2016).
484 Future research on individual foraging specialization in *Pygoscelis* penguins should focus on

Gentoo Penguins across their larger breeding distribution to identify the environmental mechanisms that may act to mediate the degree of individual variation within these generalist populations. Identifying and understanding these mechanisms that drive variation in foraging strategy in *Pygoscelis* penguins may aid in our understanding of how these species will respond to recent climate driven changes in the Antarctic ecosystem.

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Compliance with Ethical Standards

The authors declare that they have no conflicts of interest.

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References

Ainley D (2002) The Adélie Penguin: Bellwether of Climate Change. Columbia University Press.

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. doi:10.1038/nature02996

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. doi:10.1016/j.jembe.2013.06.021

Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H (2004) Determining trophic niche width: a novel approach using stable isotope analysis. J Anim Ecol 73:1007-1012. doi: 10.1111/j.0021-8790.2004.00861.x

Bearhop S, Phillips RA, McGill R, Cherel Y, Dawson DA, Croxall JP (2006) Stable isotopes indicate sex-specific and long-term individual foraging specialisation in diving seabirds. Mar Ecol Prog Ser 311:157-164. doi: 10.3354/meps311157

Bolnick DI, Svanback R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML (2003) The ecology of individuals: Incidence and implications of individual specialization. Amer Nat 161:1-28. doi: 10.1086/343878

Bond AL, Jones IL (2009) A practical introduction to stable-isotope analysis for seabird biologists: approaches, cautions and caveats. Mar Ornithol 37:183-188.

Brasso RL, Polito MJ (2013) Trophic calculations reveal the mechanism of population-level variation in mercury concentrations between marine ecosystems: case studies of two polar seabirds. Mar Pollut Bull 75:244-249. doi: 10.1016/j.marpolbul.2013.08.003

538 Bugoni L, McGill R, Furness RW (2008) Effects of preservation methods on stable isotope
539 signatures in bird tissues. Rapid Commun Mass SP 22: 2457-2462. doi:10.1002/rcm.3633
540 Carleton SA, del Rio CM (2005) The effect of cold-induced increased metabolic rate on the rate
541 of ^{13}C and ^{15}N incorporation in house sparrows (*Passer domesticus*). Oecologia 144(2),
542 226-232. doi: 10.1007/s00442-005-0066-8
543 Carravieri A, Bustamante P, Churlaud C, Cherel Y (2013) Penguins as bioindicators of mercury
544 contamination in the Southern Ocean: Birds from the Kergeulen Islands as a case study.
545 Sci Total Environ 454:141-148. doi: 10.1016/j.scitotenv.2013.02.060
546 Cherel Y, Hobson KA (2007) Geographical variation in carbon stable isotope signatures of
547 marine predators: a tool to investigate their foraging areas in the Southern Ocean. Mar
548 Ecol Prog Ser 329:281–287. doi:10.3354/meps329281
549 Cherel Y, Hobson KA, Hassani S (2005) Isotopic discrimination between food and blood and
550 feathers of captive penguins: implications for dietary studies in the wild. Physiol
551 Biochem Zool 78:106-115. doi: 10.1086/425202
552 Cherel Y, Jaquemet S, Maglio A, Jaeger A (2014) Differences in $\delta^{13}C$ and $\delta^{15}N$ values between
553 feathers and blood of seabird chicks: implications for non-invasive isotopic
554 investigations. Mar Biol 161:229-237. doi: 10.1007/s00227-013-2314-5
555 Clausen AP, Pütz K (2002) Recent trends in diet composition and productivity of Gentoo,
556 Magellanic and rockhopper Penguins in the Falkland Islands. Aquat Conserv: Mar Fresh
557 Ecosys 12:51-61. doi: 10.1002/aqc.476
558 Clausen A, Pütz K (2003) Winter diet and foraging range of Gentoo Penguins (*Pygoscelis*
559 *papua*) from Kidney Cove, Falkland Islands. Polar Biol 26:32-40. doi: 10.1007/s00300-
560 002-0443-2

561 Croxall JP, Davis RW, O'Connell MJ (1988) Diving patterns in relation to diet of Gentoo and
562 macaroni penguins at South Georgia. Condor 90:157-167.
563 Cruz LL, McGill RA, Goodman SJ, Hamer KC (2012) Stable isotope ratios of a tropical marine
564 predator: confounding effects of nutritional status during growth. Mar Biol 159:873-880.
565 doi: 10.1007/s00227-011-1864-7
566 Dellinger T, Trillmich F (1999) Fish prey of the sympatric Galápagos fur seals and sea lions:
567 seasonal variation and niche separation. Can J Zool 77:1204–1216. doi:10.1139/z99-095
568 Dehnhard N, Eens M, Sturaro N, Lepoint G, Demongin L, Quillfeldt P, Poisbleau M (2016) Is
569 individual consistency in body mass and reproductive decisions linked to individual
570 specialization in foraging behavior in a long-lived seabird?. Ecol Evol 6(13):4488-4501.
571 doi:10.1002/ece3.2213
572 DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in
573 animals. Geochim Cosmochim Acta 45:341-51. doi:10.1016/0016-7037(81)90244-1
574 Dietrich K, Brooks C, Bystrom I, Driscoll R, Ferm N, Hinke J, Janssen M, Lombard D, Pesce A,
575 Romain S, Thoresen L (2014) Distribution and Catch Rates of Zooplankton around the
576 South Shetland Islands, Antarctica. NOAA Techn Memo NMFS SWFSC. 524:18-27.
577 Dingemanse NJ, Wolf M (2013) Between-individual differences in behavioural plasticity within
578 populations: causes and consequences. Anim Behav (2013) 85:1031-1039.
579 doi:10.1016/j.anbehav.2012.12.032
580 Donnelly SM, Kramer A (1999) Testing for multiple species in fossil samples: an evaluation and
581 comparison of tests for equal relative variation. Am J Phys Anthropol 108:507-529. doi:
582 10.1002/(SICI)1096-8644(199904)108:4<507::AID-AJPA8>3.0.CO;2-0

583 France RL (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb
584 implications. Mar Ecol Prog Ser 124:307–312. doi:10.3354/meps124307
585 Gorman KB, Williams TD, Fraser WR (2014) Ecological sexual dimorphism and environmental
586 variability within a community of Antarctic penguins (genus Pygoscelis). PloS One
587 9:e90081. doi:10.1371/journal.pone.0090081
588 Hinke JT, Salwicka K, Trivelpiece S, Watters GM, Trivelpiece W (2007) Divergent responses of
589 *Pygoscelis* penguins reveal a common environmental driver. Oecologia 153:845-855. doi:
590 10.1007/s00442-007-0781-4
591 Hinke JT, Polito MJ, Reiss CS, Trivelpiece SG, Trivelpiece WZ (2012) Flexible reproductive
592 timing can buffer reproductive success of *Pygoscelis* spp. penguins in the Antarctic
593 Peninsula region. Mar Ecol Prog Ser 454:91-104. doi: 10.3354/meps09633
594 Hinke JT, Polito MJ, Goebel ME, Jarvis S, Reiss CS, Thorrold SR, Trivelpiece WZ, Watters GM
595 (2015) Spatial and isotopic niche partitioning during winter in chinstrap and Adélie
596 penguins from the South Shetland Islands. Ecosphere 6:1-32. doi: 10.1890/ES14-
597 00287.1
598 Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes I: turnover of 13C in
599 tissues. Condor 94:181-188. doi: 10.2307/1368807
600 Hobson KA, Piatt JF, Pitocchelli J (1994) Using stable isotopes to determine seabird trophic
601 relationships. J Anim Ecol 63:786-798. doi: 10.2307/5256
602 Hobson KA, Bond AL (2012) Extending an indicator: year-round information on seabird trophic
603 ecology from multiple-tissue stable-isotope analyses. Mar Ecol Prog Ser 461: 233-243.
604 doi:10.3354/meps09835

605 Hückstädt LA, Koch PL, McDonald BI, Goebel ME, Crocker DE, Costa DP (2012) Stable
606 isotope analyses reveal individual variability in the trophic ecology of a top marine
607 predator, the southern elephant seal. Oecologia 169:395–406. doi: 10.1007/s00442-011-
608 2202-y
609 Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among
610 and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. J Anim Ecol,
611 80(3):595-602. doi: 10.1111/j.1365-2656.2011.01806.x
612 Jarman SN, McInnes JC, Faux C, Polanowski AM, Marthick J, Deagle BE, Southwell C,
613 Emmerson L (2013) Adélie penguin population diet monitoring by analysis of food DNA
614 in scats. PLoS One 8(12):p.e82227. doi: 10.1371/journal.pone.0082227
615 Juáres MA, Santos M, Mennucci JA, Coria, NR, Mariano-Jelicich, R (2016) Diet composition
616 and foraging habitats of Adélie and gentoo penguins in three different stages of their
617 annual cycle. Mar Biol 163:105. doi:10.1007/s00227-016-2886-y
618 Karnovsky NJ (1997) The fish component of Pygoscelis penguin diets. Dissertation, Montana
619 State University-Bozeman, College of Letters and Science
620 Kernaléguen L, Dorville N, Ierodiaconou D, Hoskins AJ, Baylis AMM, Hindell MA, Semmens
621 J, Abernathy K, Marshall GJ, Cherel Y, Arnould JPY (2016) From video recordings to
622 whisker stable isotopes: a critical evaluation of timescale in assessing individual foraging
623 specialization in Australian fur seals. Oecologia 180:657-670. doi:10.1007/s00442-015-
624 3407-2
625 Kohler SA, Connan M, Hill JM, Mablouke C, Bonnevie B, Ludynia K, Kemper J, Huisamen J,
626 Underhill LG, Cherel Y, McQuaid, Jaquemet S (2011) Geographic variation in the

trophic ecology of an avian rocky shore predator, the African black oystercatcher, along the southern African coastline. Mar Ecol Prog Ser 435:235-249. doi: 10.3354/meps09215

Lascara CM, Hofmann EE, Ross RM, Quetin LB (1999) Seasonal variability in the distribution of Antarctic krill, *Euphausia superba*, west of the Antarctic Peninsula. Deep Sea Res Part 1 46:951-984. doi:10.1016/S0967-0637(98)00099-5

Lescroël A, Ridoux,V, Bost CA (2004) Spatial and temporal variation in the diet of the gentoo penguin (*Pygoscelis papua*) at Kerguelen Islands. Polar Biol 27(4):206-216. doi: 10.1007/s00300-003-0571-3

Lescroël A, Bost CA (2005) Foraging under contrasting oceanographic conditions: the gentoo penguin at Kerguelen Archipelago. Mar Ecol Progr Ser, 302:245-261. doi: 10.3354/meps302245

Lishman GS (1985) The food and feeding ecology of Adélie penguins (*Pygoscelis adeliae*) and Chinstrap Penguins (*Pygoscelis antarctica*) at Signy Island, South Orkney Islands. J Zool 205:245–263. doi: 10.1016/S0967-0637(98)00099-5

Lynch HJ, Naveen R, Casanovas P (2013) Antarctic site inventory breeding bird survey data, 1994-1013. Ecology 94:2653. doi: 10.1890/13-1108.1

Miller AK, Trivelpiece WZ (2008) Chinstrap Penguins alter foraging and diving behavior in response to the size of their principal prey, Antarctic krill. Mar Biol 154: 201-208. doi: 10.1007/s00227-008-0909-z

Miller AK, Karnovsky NJ, Trivelpiece WZ (2009) Flexible foraging strategies of Gentoo Penguins *Pygoscelis papua* over 5 years in the South Shetland Islands, Antarctica. Mar Biol 156:2527–2537. doi: 10.1007/s00227-009-1277-z

649 Miller AK, Kappes MA, Trivelpiece SG, Trivelpiece WZ (2010) Foraging-Niche Separation of
650 Breeding Gentoo and Chinstrap Penguins, South Shetland Islands, Antarctica. Condor
651 112:683–695. doi: 10.1525/cond.2010.090221
652 Minagawa M, Wada E (1984) Stepwise enrichment of 15N along food chains: further evidence
653 and the relation between δ15N and animal age. Geochim Cosmochim Acta 48:1135-
654 1140. doi: 10.1016/0016-7037(84)90204-7
655 Negrete P, Sallaberry M, Barceló G, Maldonado K, Perona F, McGill RA, Quillfeldt P, Sabat P
656 (2016) Temporal variation in isotopic composition of *Pygoscelis* penguins at Ardley
657 Island, Antarctic: Are foraging habits impacted by environmental change? Polar Biol 3:1-
658 4. doi:10.1007/s00300-016-2017-8
659 Newsome SD, Martínez del Rio C, Bearhop S, Phillips DL (2007) A niche for isotopic ecology.
660 Front Ecol Environ 5:429–436. doi: 10.1890/060150.1
661 Newsome SD, Tinker MT, Monson DH, Oftedal OT, Ralls K, Staedler MM, Fogel ML, Estes JA
662 (2009) Using stable isotopes to investigate individual diet specialization in California sea
663 otters (*Enhydra lutris nereis*). Ecology 90:961-974. doi:10.1890/07-1812.1
664 Orsi AH, Harris U (2015) Locations of the various fronts in the Southern Ocean Australian
665 Antarctic Data Centre - CAASM Metadata
666 (https://data.aad.gov.au/metadata/records/southern_ocean_fronts)
667 Polito MJ, Trivelpiece WZ (2008) Transition to independence and evidence of extended parental
668 care in the Gentoo Penguin (*Pygoscelis papua*). Mar Biol 154(2):231.
669 doi:10.1007/s00227-008-0919-x
670 Polito MJ, Fisher S, Tobias CR, Emslie SD (2009) Tissue-specific isotopic discrimination factors
671 in gentoo penguin (*Pygoscelis papua*) egg components: Implications for dietary

reconstruction using stable isotopes. J Exp Mar Biol Ecol 372:106-112. doi:10.1016/j.jembe.2009.02.014

Polito MJ, Abel S, Tobias CR, Emslie SD (2011a) Dietary isotopic discrimination in Gentoo Penguin (*Pygoscelis papua*) feathers. Polar Biol 34:1057–1063. doi:10.1007/s00300-011-0966-5

Polito MJ, Abel S, Lynch HJ, Naveen R, Emslie SD (2011b) 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. doi:10.3354/meps08863

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

Polito MJ, Reiss CS, Trivelpiece WZ, Patterson WP, Emslie SD (2013) Stable isotopes identify an ontogenetic niche expansion in Antarctic krill (Euphausia superba) from the South Shetland Islands, Antarctica. Mar Biol 160:1311-1323. doi:10.1007/s00227-013-2182-z

Polito MJ, Trivelpiece WZ, Patterson WP, Karnovsky NJ, Reiss CS, Emslie SD (2015) Contrasting specialist and generalist patterns facilitate foraging niche partitioning in sympatric populations of Pygoscelis penguins. Mar Ecol Prog Ser 519: 221-237. doi:10.3354/meps11095

Pütz K, Ingham RJ, Smith JG, Croxall JP (2001) Population trends, breeding success and diet composition of Gentoo *Pygoscelis papua*, magellanic *Spheniscus magellanicus* and rockhopper *Eudyptes chrysocome* penguins in the Falkland Islands. A review. Polar Biol 24:793-807. doi:10.1007/s003000100293

694 Quillfeldt P, Bugoni L, McGill RA, Masello JF, Furness RW (2008) Differences in stable
695 isotopes in blood and feathers of seabirds are consistent across species, age and latitude:
696 implications for food web studies. Mar Biol 155:593–598 doi: 10.1007/s00227-008-
697 1048-2
698 Salazar SK (2005) Variación temporal y espacial del espectrotrófico del lobo marino de
699 Galápagos. Masters thesis, Instituto Politécnico Nacional, La Paz.
700 Sanpera C, Ruizi X, Moreno R, Jover L, Waldron S (2007) Mercury and stable isotopes in
701 feathers of Audouin's gulls as indicators of feeding habits and migratory connectivity.
702 Condor 109:268-275. doi:10.1650/0010-5422(2007)109[268:MASIIF]2.0.CO;2
703 Tierney M, Southwell C, Emmerson LM, Hindell MA (2008). Evaluating and using stable-
704 isotope analysis to infer diet composition and foraging ecology of Adélie penguins
705 *Pygoscelis adeliae*. Mar Ecol Prog Ser 355:297-307. doi: 10.3354/meps07235
706 Trivelpiece WZ, Trivelpiece SG, Volkman NJ (1987) Ecological segregation of Adélie, Gentoo,
707 and Chinstrap Penguins at King George Island, Antarctica. Ecol 68: 351–361. doi:
708 10.2307/1939266
709 Trivelpiece WZ, Hinke JT, Miller AK, Reiss CS, Trivelpiece SG, Watters GM (2011) Variability
710 in krill biomass links harvesting and climate warming to penguin population changes in
711 Antarctica. Proc Natl Acad Sci USA 108:7625–7628. doi: 10.1073/pnas.1016560108
712 Villegas-Amtmann S, Costa DP, Tremblay Y, Salazar S, Aurioles-Gamboa D (2008) Multiple
713 foraging strategies in a marine apex predator, the Galapagos sea lion *Zalophus*
714 *wollebaeki*. Mar Ecol Prog Ser 363:299-309. doi:10.3354/meps07457
715 Volkman NJ, Presler P, Trivelpiece WZ (1980) Diets of pygoscelid penguins at King George
716 Island, Antarctica Condor: 373-378.

Wilson RP, Alvarrez B, Latorre L, Adelung D, Culik B, Bannasch R (1998) The movements of gentoo penguins *Pygoscelis papua* from Ardley Island, Antarctica. Polar Biol 19:407–413. doi: 10.1007/s003000050266

Wilson R P (2010) Resource partitioning and niche hyper-volume overlap in free-living Pygoscelid penguins. Functional Ecology 24(3):646-657. doi:10.1111/j.1365-2435.2009.01654.x

Woo KJ, Elliott KH, Davidson M, Gaston AJ, Davoren GK (2008) Individual specialization in diet by a generalist marine predator reflects specialization in foraging behaviour. J Anim Ecol 77(6):1082-91. doi:10.3354/meps0722

738 **Table 1**. Mean and standard deviation, range (in parentheses), and coefficient of variation (CV%) of non-breeding season **(**feather)
739 and breeding season (blood) $\delta^{15}N$ and $\delta^{13}C$ values for all three species at King George Island and three Gentoo populations at
740 Wiencke, Rongé and Elephant Island. Absolute values were used to calculate CV.
741

			Non-breeding (feather) mean ± SD	CV	Breeding (blood) mean ± SD	CV
$\delta^{15}N$	Adélie	22	9.1 ± 0.4 (8 to 10.1)	4.6	8.5 ± 0.3 (7.8 to 9.1)	4.0
	Chinstrap	20	9.0 ± 0.2 (8.6 to 9.3)	2.4	8.7 ± 0.2 (8.3 to 9.0)	2.0
	Gentoo	21	9.4 ± 0.6 (8.6 to 11.3)	6.2	8.5 ± 0.6 (8.0 to 10.6)	7.4
$\delta^{13}C$	Adélie	22	-24.1 ± 1.1 (-27.4 to -22.4)	4.6	-24.5 ± 0.3 (-25.1 to -24.1)	1.2
	Chinstrap	20	-23.3 ± 1.0 (-24.5 to -21.5)	4.3	-24.9 ± 0.2 (-25.4 to -24.7)	0.8
	Gentoo	21	-23.5 ± 0.7 (-24.5 to -21.8)	2.9	-24.7 ± 0.4 (-25.1 to -23.1)	1.8
$\delta^{15}N$	Wiencke	19	9.9 ± 0.3 (9.4 to 10.6)	2.9	9.0 ± 0.2 (8.7 to 9.4)	2.2
	Rongé	19	10.1 ± 0.3 (9.5 to 10.7)	3.4	9.1 ± 0.2 (8.6 to 9.5)	2.6
	Elephant	15	10.0 ± 0.7 (8.1 to 10.9)	7.5	10.6 ± 0.7 (8.4 to 11.2)	6.6
$\delta^{13}C$	Wiencke	19	-24.3 ± 0.7 (-25.3 to -22.7)	2.9	-24.3 ± 0.3 (-24.9 to -23.8)	1.3
	Rongé	19	-24.0 ± 0.9 (-25.0 to -22.4)	3.7	-24.8 ± 0.2 (-25.1 to -24.6)	0.6
	Elephant	15	-24.3 ± 0.6 (-24.9 to -23.1)	2.4	-25.0 ± 0.2 (-25.5 to -24.8)	0.8

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Figure Legends

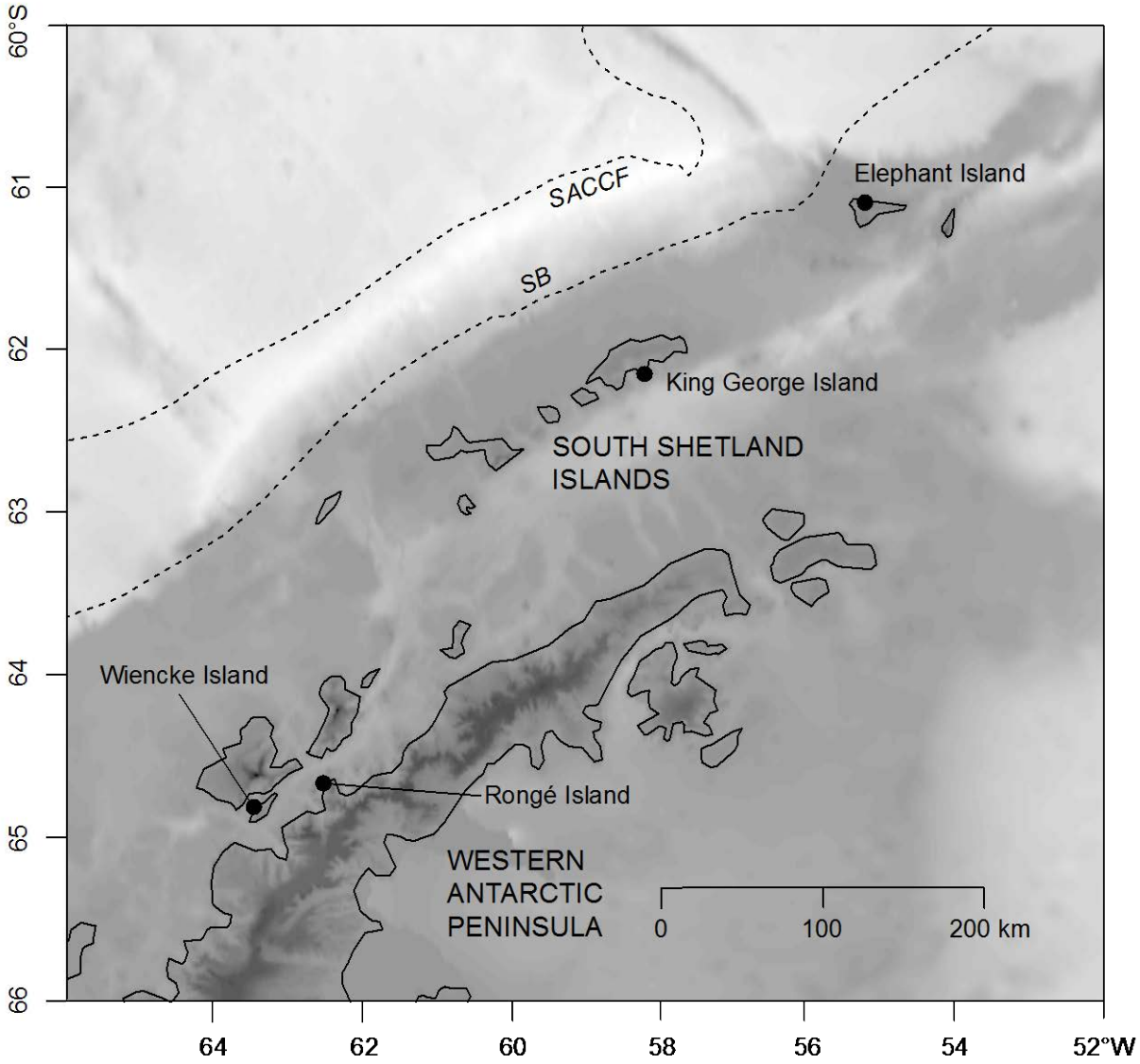
Fig. 1 Map of study sites: Wiencke Island, Rongé Island, King George Island, and Elephant Island. Dashed lines indicate major currents and fronts: Southern Antarctic Circumpolar Current Front (SACCF) and Southern Boundary (SB) from Orsi and Harris (2015).

Fig. 2 Standard ellipse area Bayesian estimations (SEA_b) for Adélie, Chinstrap, and Gentoo Penguins at King George Island, South Shetland Islands, Antarctica, 2009/2010, and for Gentoo Penguins at three breeding sites in the South Shetland Island and Western Antarctic Peninsula, 2013/2014. Blue indicates feather (post-breeding) values and red indicates blood (breeding) values. Black dots represent their mode and boxes represent 50%, 75% and 95% credible intervals.

Fig. 3 Standardized $\delta^{15}N$ and $\delta^{13}C$ values for Adélie, Chinstrap, and Gentoo Penguins at King George Island, South Shetland Islands, Antarctica, 2010. Lines connect post-breeding season (feather) values and breeding season (blood) for each sampled individual. Blood $\delta^{15}N$ and $\delta^{13}C$ values were standardized following Cherel et al. (2014).

Fig. 4 Standardized $\delta^{15}N$ and $\delta^{13}C$ values for Gentoo Penguins at three breeding sites in the South Shetland Island and Western Antarctic Peninsula, 2014. Lines connect post-breeding season (feather) values and breeding season (blood) for each sampled individual. Blood $\delta^{15}N$ and $\delta^{13}C$ values were standardized following Cherel et al. (2014).

768 **Fig 1.**



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782 **Fig. 2**
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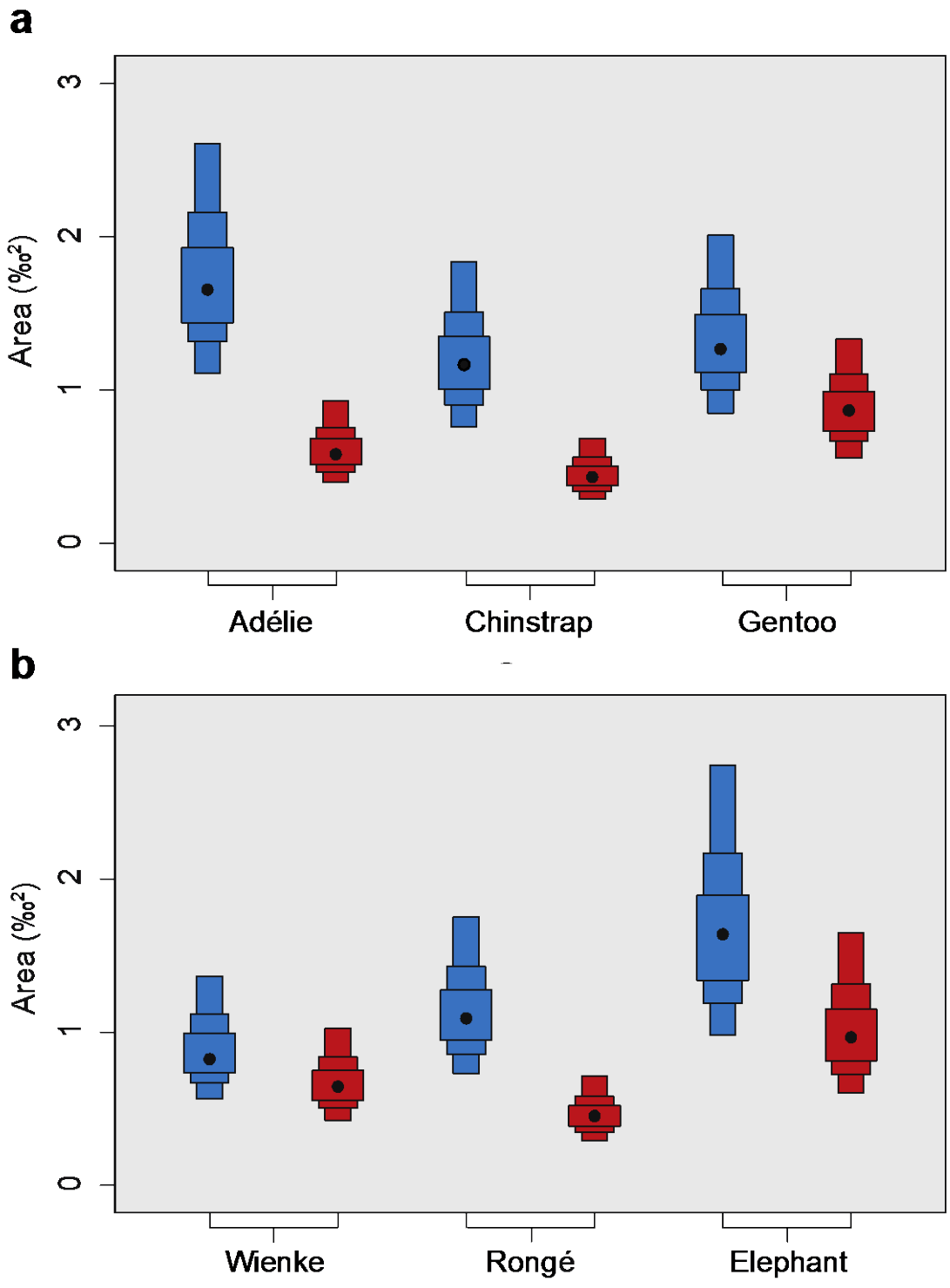

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Fig. 3

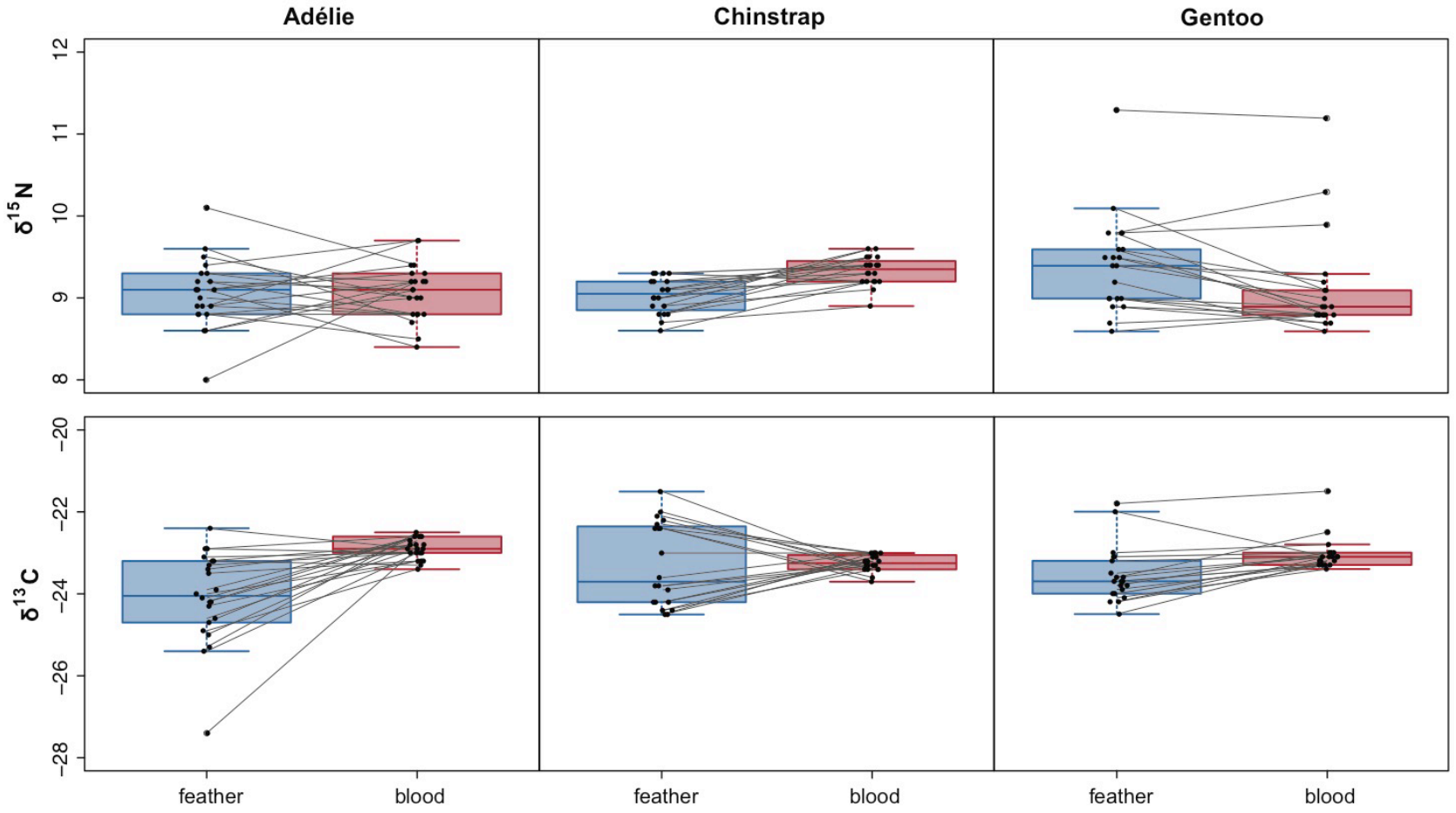
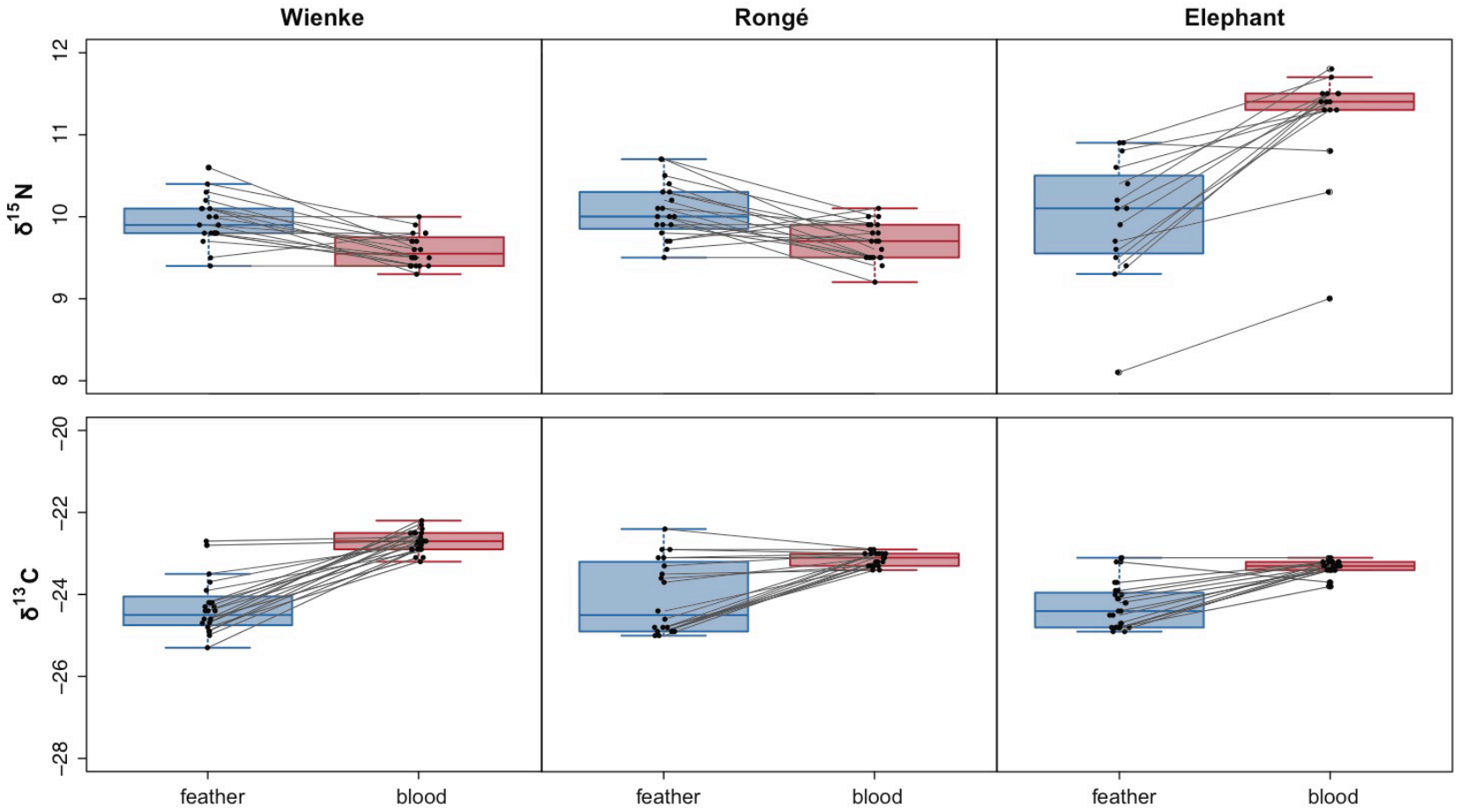

Adélie
Chinstrap
Gentoo
δ15N
δ13C
12
11
10
9
8
−20
−22
−24
−26
−28
feather
blood
feather
blood
feather
blood

Fig. 4


Wienke
Rongé
Elephant
δ15N
δ13C
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feather
blood
feather
blood
feather
blood