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Investigating Species and Population Level Foraging Variation and Individual Specialization in Pygoscelis Penguins Using Stable Isotope Analysis

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INVESTIGATING SPECIES AND POPULATION LEVEL FORAGING VARIATION AND INDIVIDUAL SPECIALIZATION IN *PYGOSCELIS* PENGUINS USING STABLE ISOTOPE ANALYSIS

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Masters of Science in

The College of Oceanography and Coastal Sciences

by

Rachael W. Herman
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ABSTRACT

Gentoo Penguins (*Pygoscelis papua*) are known to be generalist foragers, while Adélie (*P. adeliae*) and Chinstrap (*P. Antarctica*) tend to specialize on krill within the Western Antarctic Peninsula and South Shetland Islands, particularly during the breeding season. However, little is known on temporal consistency in diet and foraging habitat of these species, particularly at the individual level. We used stable isotope analysis (SIA) of blood and feathers to evaluate seasonal and individual foraging consistency within Adélie, Chinstrap and Gentoo Penguins breeding in the South Shetland Islands, as well as among three Gentoo Penguins’ populations in the Western Antarctic Peninsula and South Shetland Islands. 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 seasonal consistency within individuals.

Previous dietary analyses suggest Gentoo penguins have a generalist foraging niche, which may help buffer them from recent climate-driven declines in key prey species, such as Antarctic krill (*Euphausia superba*). Ecological theory indicates that generalist populations fall under two different categories: Type A generalist populations exhibit large variation within individuals, and little variation between individuals, where Type B generalist populations are comprised of individual specialists, with large variation between individuals. We conducted SIA using tail feathers from Gentoo penguins at four geographically isolated breeding sites across the Scotia arc to assess individual variation in winter diets and determine the type of generalist strategies that Gentoo penguins utilize. Our results indicate the presence of individual specialization (type B generalism) within all four geographically distinct breeding colonies, with lower degrees of individual specialization in southern populations and higher degrees of
individual specialization in northern populations. In addition, our results also suggest that individual specialization may be driven by prey abundance and diversity, as foraging habitat in the southern populations are marked by high abundance of Antarctic krill and low prey diversity, while the northern populations forage on a wider diversity of prey.
CHAPTER 1: GENERAL INTRODUCTION

In the last four decades, the Antarctic Peninsula and South Shetland Islands have experienced substantial climate-driven ecosystem changes, in particular a decline in the amount and seasonal duration of sea-ice coverage (Stammerjohn et al. 2008, Pritchard et al. 2012). Many studies suggest these changes are the major cause of decline in the abundance of Antarctic Krill (*Euphausia superba*), a keystone species for the Antarctic marine food web (Atkinson et al. 2004; Ducklow et al. 2007, Trivelpiece et al. 2011). Concurrently, the three species of *Pygoscelis* penguins, which consume krill, have experienced substantial changes in population numbers in the Antarctic Peninsula. Both Chinstrap (*Pygoscelis antarctica*) and Adélie Penguins (*P. adeliae*) in this region are declining, and the prevailing hypothesis suggests that decreases in Antarctic krill abundance due to climate change and krill fisheries are contributing to their declines (Trivelpiece et al. 2011). However, Gentoo Penguins (*P. papua*), which also forage on krill, have not declined, and some populations are even increasing in numbers and colonizing previously uninhabited areas south of their historical range (Lynch, et al. 2012).

Gentoo Penguins appear to show resilience to changes in krill abundance and other climate-driven changes in their ecosystem. One hypothesis is that, relative to other penguin species, they have a more flexible diet and generalist foraging niche, which could help to buffer them from recent declines in krill (Miller et al. 2009, Polito et al. 2015). In contrast, Chinstrap and Adélie Penguins appear to be more dependent on krill and may be unable to adapt to declines in this principle prey item (Trivelpiece et al. 2011, Polito et al. 2015).

However, much of what we know about the foraging ecology of penguins in the Antarctic Peninsula as well as throughout the Scotia Arc is based almost exclusively on stomach content analyses restricted to the breeding season and at very few sites. Unfortunately, stomach content
analyses provide only a snapshot of recent diets and can have significant preservation biases (Polito et al. 2011). Therefore, hypotheses regarding the dietary niche of Gentoo Penguins require more rigorous testing to assess temporal dietary consistency outside of the breeding season.

Initially, population changes in Gentoo, Adélie, and Chinstrap Penguin along the Antarctic Peninsula and South Shetland Islands were attributed to climate driven decline in sea ice in the region (Montes-Hugo et al. 2009). Gentoo Penguins prefer habitat free of ice in which to forage, while Adélie Penguins rely on the presence of sea ice for foraging. However, Chinstrap Penguins also prefer ice-free habitat, so the drastic declines in their population numbers are unexplained. This suggests an alternative driving force in the substantial changes of these three species’ populations.

Reduction in the amount and seasonal duration of sea-ice has caused a marked decline in the overall biomass of Antarctic krill in the Antarctic Peninsula as well as in the southern Scotia Sea (Trivelpiece et al. 2011). Antarctic krill are largely dependent on sea-ice during a crucial stage of their life cycle, and loss of this habitat may be causing a decrease in reproductive success and recruitment (Smetacek et al. 1990). As krill are a major prey item for penguins and other predatory vertebrates, the current hypothesis is that the changes in penguin populations are the product of changes in krill availability and abundance (Trivelpiece et al. 2011). This provides an alternative explanation for the declining populations of Chinstrap and Adélie Penguins, whose diets are dominated by Antarctic krill. However, it is thought that the more variable diets of Gentoo Penguins may buffer them against declines resulting from decreases in krill (Polito et al. 2015). Observed increases in populations and range expansion in Gentoo Penguins may further
be due to an interspecific ecological release as the other Pygoscelis penguins decline in the Antarctic Peninsula and South Shetland Island region (Miller et al. 2009, 2010).

Gentoo Penguins are one of the most widespread penguin species, with a circumpolar breeding distribution and a wide latitudinal range stretching from 46°00’ S in the Crozet Islands south to 65°16’ S on the Antarctic Peninsula (Ainley et al. 1995). Gentoo Penguins are generalist which exhibit substantial variation in their diet across breeding locations in the Scotia Arc. Stomach content analyses of Gentoo Penguins of the South Shetland Islands show diets composed of mostly Antarctic krill (Euphausia superba) (Miller et al. 2009), while studies conducted on populations in South Georgia have found that diets there consist of a mix of Antarctic Krill and fish. In the Falkland Islands, where there are no Antarctic krill, Gentoo Penguin diets include mostly fish, as well as some crustaceans such as lobster krill (Munida gregaria) and cephalopods (Putz et al. 2001; Clausen and Putz 2002, 2003).

Sub-Antarctic populations of Gentoo Penguins in South Georgia and the Falkland Islands have been relatively stable (Baylis et al. 2013), while populations in the South Sandwich Islands have undergone increases in population size (Lynch et al. 2012; Forcada and Trathan 2009; Convey et al. 1999). Additionally, there is recent evidence of population increases and southward range expansion in the Antarctic Peninsula, while the populations of Adélie and Chinstrap Penguins are drastically declining in these areas (Lynch et al. 2013).

Generalist populations fall under two different categories: Type A generalist populations exhibit extensive variation within individuals and little variation between individuals, whereas Type B generalist populations are composed of individuals that specialize, with large variation between individuals (Bolnick et al. 2003). Quantifying the degree of individual variation within Gentoo Penguin populations will allow for the detection of the type of generalist strategy that a
population employs (Bearhop et al. 2004). It is important to assess which type of generalist population Gentoo Penguins fall under, as these strategies may impart differing ecological and evolutionary responses under times of environmental change. Individuals in a Type A generalist species would be able to respond to change quickly, whereas many individuals in Type B generalists would not successfully adapt to shifts in prey availability even if proportions of the populations survive. For example, individual specialization in bluegill sunfish has been shown to produce a delayed response to fluctuations in prey availability (Werner et al. 1981). Villegas-Amntmann et al. (2008) suggested that Galapagos sea lion populations decline during times of El Nino when prey availability is significantly lower due to a lack of dietary plasticity in individual specialists. Environmental change could result in significant population declines and lower genetic diversity in populations composed of Type B generalists.

Foraging strategies employed by a species may differ among geographically distinct populations. A comparison of multiple studies of Galapagos sea lions (*Zalophus wollebaeki*) throughout the Galapagos Archipelago suggests a high degree of variation in foraging strategies between populations driven by variation in prey availability and physical oceanographic characteristics (Villegas-Amtmann et al. 2008; Salazar, 2005; Dellinger and Trillmich, 1999; Kooyman and Trillmich, 1986). Quantifying variation in diet among individuals and through time would allow for a more robust measure of foraging niche width that can be compared between populations whose foraging strategies may differ in habitat and trophic composition.

Stable isotope analysis (SIA) provides an effective method for identifying diet composition and foraging niches penguins (Polito et al 2011, Polito et al. 2015). Nitrogen stable isotope values ($\delta^{15}N$) of marine consumers exhibits a strong linear relationship with trophic level (Hobson et al. 1994). This is due to “trophic enrichment” in which an organism’s body
selectively sequesters more $^{15}$N than $^{14}$N from the food it consumes, while excreting more $^{14}$N, resulting in an enrichment of $^{15}$N in the organism’s tissue (Hobson et al. 1994). The accumulated effects of enrichment result in higher relative levels of $^{15}$N in organisms foraging at higher trophic levels. Analysis of individual $\delta^{15}$N values enables the detection of variation in trophic food choice between and within species that may go undetected through other methods (Bearhop et al., 2006).

Carbon stable isotope values ($\delta^{13}$C) differs very little between trophic levels and instead can provide a means of detecting whether carbon acquired during foraging comes from offshore or inshore locations. Primary producers in inshore (benthic) systems are more efficient at $^{13}$C uptake during photosynthesis than those in offshore (pelagic) systems (France, 1995). Higher carbon isotopic values ($\delta^{13}$C) therefore indicate that individuals have been focusing foraging at inshore locations (Cherel and Hobson, 2007). In addition, lower latitude phytoplankton and particulate organic matter (POM) are more enriched in $^{13}$C compared to higher latitudes, which results in an $^{13}$C “isoscape” that increases towards lower latitudes (Rau et al. 1991). This is due to high levels of dissolved CO$_2$ in cold southern oceans, which reduce the presence of organic $\delta^{13}$C (Rau et al. 1997, Popp et al. 1999). The latitudinal gradient of $\delta^{13}$C can be used to infer latitudinal foraging positions of seabirds (Quillfeldt et al. 2005; Cheryl and Hobson 2007; Jaeger et al. 2010).

Together nitrogen and carbon isotopic values have been used to determine the 2-dimensional parameters of an isotopic niche size, an index of ecological or foraging niche that can be used to identify specialist vs. generalist strategies in many top marine predators (Bearhop et al. 2004, Newsome et al. 2007, Jaeger et al. 2009, Huckstadt et al. 2012). Moreover, this method has been effectively used to determine the specialist foraging niches of Chinstrap and
Adélie Penguins and to broadly characterize the generalist foraging strategies of Gentoo Penguins, which exhibit a much wider isotopic niche with a higher degree of variation in both prey choice and location compared to sister species (Miller et al. 2009, 2010; Polito et al. 2015). Stable isotopes have also been used to identify type A vs. type B generalists in many marine predators by determining and comparing the degree of individual variation within a population (Newsome et al. 2009; Huckstadt et al. 2012; Kernaleguen et al. 2015). My proposed research will build on these existing studies to detect individual variation and determine whether Gentoo Penguins exhibit Type A or Type B generalist strategies using stable isotope analysis.

In this thesis I conduct stable isotope analysis of three Pygoscelis penguin species to detect temporal consistency in foraging ecology, identify the level of variation in foraging strategies among individuals, and assess temporal and eco-geographical variability in generalist strategies. In Chapter 2, I compare seasonal consistency in foraging strategies between three Pygoscelis penguin species as well as between three separate Gentoo Penguin populations within the Western Antarctic Peninsula and South Shetland Islands. In Chapter 3, I determine the prevalence of Type A vs. Type B foraging niche generalization occurring in Gentoo Penguin populations across a large portion of their breeding distribution.

LITERATURE CITED


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 Penguin (P. antarctica) is 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). This relatively narrow foraging niche and dietary focus on Antarctic krill, which has declined over the past 30 years (Atkinson et al. 2004), is suggested to drive recent populations decline of 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). One possible explanation for this trend is the more generalist foraging strategy employed by Gentoo Penguins, which may allow for flexibility in diet and foraging habitat (Miller et al. 2009, Polito et al. 2015).

While the diets and foraging ecology of Pygoscelis penguins have been well studied in the Antarctic Peninsula region, many studies overlook diet and niche variation between individuals within populations, as well as variation within individuals. In addition, past studies focuses primarily on the breeding season and little is known about the diets of these species during the non-breeding period (Miller et al. 2009, 2010, Juáres et al. 2016). Even so, existing studies do indicate that Gentoo Penguin populations can exhibit substantial variation in their
foraging ecology across breeding locations (Miller et al. 2009; Putz et al. 2001; Clausen and Putz 2002, 2003; Polito et al. 2015). However, it has not been possible to assess if and how individuals within these populations vary in diets and assess the degree of individual specialization and foraging constancy within these generalist populations (Croxall et al, 1988; Wilson et al, 1998; Clausen and Putz, 2003; and Polito et al. 2015). Specifically, these past studies lack analyses of dietary and foraging habitat consistency of individuals through time, which is necessary for a robust assessment of individual specialization (Bolnick et al, 2003; Bearhop et al. 2004). Quantifying the degree of niche variation and consistency at the individual level is critical because individual variation within populations has the potential to determine a species population level response to changes in food availability or other environmental change (Bolnick et al. 2003).

Although the foraging ecology of individual marine predators can be challenging to track over time, stable isotope analyses (SIA) provides a robust tools for measuring animal diets through time from a single capture event. Prior studies have demonstrated that SIA is an effective method for quantifying the diets and foraging niches of *Pygoscelis* penguins (Polito et al 2011, Polito et al. 2015). Nitrogen stable isotope values ($\delta^{15}N$) in consumers exhibits a strong correlation with trophic level (Minagawa & Wada 1984, Hobson et al. 1994). Carbon stable isotope values ($\delta^{13}C$) differs little between trophic levels but instead provides a proxy of marine foraging habitat use due to differences in baseline $\delta^{13}C$ values between inshore/benthic and offshore/pelagic habitats (France 1995, Hobson & Cherel 2011). In addition, the $\delta^{13}C$ and $\delta^{15}N$ values of specific tissues reflect the diets of consumers at the time of synthesis such that comparing tissues synthesized at different times from a single individual can assess foraging niche consistency over time (Newsome et al. 2009; Hückstädt et al. 2012; Kernaleguen et al.
2015). For example, Bearhop et al. (2006) compared the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of blood (i.e. breeding season) and feathers (i.e. non-breeding season) in four diving seabird species at Bird Island, South Georgia to investigate the degree of foraging specialization and individual consistency within species. However, to our knowledge a similar analysis has not been conducted on sympatric Pygoscelis populations. Such an analysis would help to quantifying seasonal diet variation and the degree of consistency at the individual level and help to inform these species responses to recent changes in krill availability in the Antarctic Peninsula region.

To address this gap, we use SIA of blood and feathers to evaluate seasonal and individual foraging consistency within Pygoscelis species (Adélie, Chinstrap and Gentoo Penguin) breeding in sympathy in the South Shetland Islands, Antarctica. In addition, we also investigate these same parameters among three Gentoo Penguins’ populations in the Western Antarctic Peninsula and South Shetland Islands, Antarctica, as this species is predicted to be a generalist forager at the population level. Our goals are to: 1) determine if and how population level diet and foraging habitat use shifts seasonally and, 2) assess the degree which individual variation and foraging consistency within populations influence the population level foraging niches of Pygoscelis penguins.

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) (Figure 1.1). 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 (6-4.8°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) (Figure 2.1). At each breeding site, we collected 3 body feathers and 1ml of whole blood from 15-20 individuals per species.

Figure 2.1: 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).

In penguins, blood contains isotopic signatures of prey items consumed within approximately 20 days prior to sampling, thus in this study provides a proxy for dietary information during the late incubation period when individuals were sampled (Barquete et al.
Body feathers are metabolically inert after synthesis and reflect isotopic signatures of prey items consumed post-breeding and prior to molting in the previous year (Polito et al. 2011a). Combined these two tissues provide proxies of individual’s diet and foraging habitat usage during the non-breeding (feathers) and breeding (blood) seasons that can be compared to evaluate seasonal consistency.

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 0.5-0.6mg 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 0.6mg 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: \( \delta^{13}C = -16.65\%_o, \delta^{15}N = -6.8\%_o \); USGS-41: \( \delta^{13}C = -37.63\%o, \delta^{15}N = 47.6\%o \); bovine liver: \( \delta^{13}C = -21.69\%o, \delta^{15}N = 7.72\%o \); nylon 5: \( \delta^{13}C = -27.72\%o, \delta^{15}N = -10.31\%o \)).

Sample precision based on internal repeats and duplicate standard reference materials was 0.1‰, for both \( \delta^{15}N \) and \( \delta^{13}C \). Stable isotope ratios are expressed in the δ notation in per mil units (‰) according to the following equation:

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

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 Peedee belemnite (VPDB) for \( ^{13}C \) and atmospheric \( N_2 \) for \( ^{15}N \).
Trophic Level and Prey $\delta^{13}C$ Values

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, 1998; Bond and Jones, 2009; Polito et al. 2009). Therefore, in order to directly compare blood and feather $\delta^{15}N$ values, we converted these values to trophic level using the formula below (Hobson et al. 1994, 2002, Hobson & Bond 2012; Brasso and Polito, 2013):

$$\text{TL}_{\text{bird}} = 3 + (\delta^{15}N_{\text{bird}} - \Delta N_{\text{avian tissue}} - \delta^{15}N_{\text{primary consumer}})/\Delta N_{\text{food web}}$$

This model uses an individual bird’s $\delta^{15}N$ value to estimate its trophic level (TL) in relation to the mean $\delta^{15}N$ values of a food web-specific primary consumer ($\delta^{15}N_{\text{primary consumer}}$) and the mean $\delta^{15}N$ food web trophic discrimination per trophic transfer ($\Delta N_{\text{food web}}$), while accounting for tissue-specific $\delta^{15}N$ discrimination factors ($\Delta N_{\text{avian tissue}}$). For our analyses, we used mean $\delta^{15}N_{\text{primary consumer}}$ values of salps ($Salpa thompsoni$) collected from the Antarctic Peninsula region (2.7‰; Stowasser et al. 2012) and a mean $\Delta N_{\text{food web}}$ value of 3.4‰, which is a robust value across multiple food webs (Deniro and Epstein, 1981; Minagawa and Wada, 1984; Post, 2002; Søreide et al., 2006; Brasso and Polito, 2013). We incorporated mean blood $\Delta N_{\text{avian tissue}}$ values derived from captive studies of four piscivorous birds (+2.7; Cherel et al. 2005) and mean feather $\Delta N_{\text{avian tissue}}$ values discrimination factor from a captive feeding study of Pygoscelis penguins (+3.5; Polito et al. 2011a).

In order to directly compare blood and feather $\delta^{13}C$ values, we applied tissue-specific discrimination values to penguin tissue $\delta^{13}C$ values in order to derive expected prey $\delta^{13}C$ values following the methods of Hobson & Bond 2012, which correspond to the prey habitat in which the penguins are foraging. Once again based on the studies by Polito et al. (2011a) and Cherel et
al. (2005), we subtracted the corresponding discrimination factors from feather (+1.3‰) and blood (+0.0‰) δ\(^{13}\)C values prior to analysis.

**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 variance (CV; \(\sigma/\bar{x}\)) as a proxy for individual variation within populations of species for non-breeding (feather) season and breeding (blood) season TL and prey δ\(^{13}\)C 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).

To test for population level diet and foraging habitat consistency of the three penguin species sampled from King George Island in 2010, we compared population means of TL and prey δ\(^{13}\)C values between non-breeding (feather) and breeding (blood) seasons 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 between individual’s breeding and non-breeding season TL and δ\(^{13}\)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. 3.2.1 (R Core Team 2015). Significance was assumed at the $\alpha = 0.05$ level and all means are presented ±SD.

To aid in the interpretation and discussion of our analyses, we developed a framework in which each sample population was categorized into one of four “types” based on four possible combinations of the paired t-test/Wilcoxon signed rank test and Pearson correlation/Kendall rank correlation results (Table 2.1).

Table 2.1: Foraging “types” and interpretations based on four possible combinations of the paired t-test/Wilcoxon signed rank test and Pearson correlation/Kendall rank correlation results for TL and prey $\delta^{13}$C.

<table>
<thead>
<tr>
<th>Paired t-test/ Wilcoxon signed rank test</th>
<th>Pearson correlation/ Kendall rank correlation</th>
<th>Foraging Type</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant</td>
<td>Not</td>
<td>Type 1</td>
<td>Population diets/habitats are not seasonally consistent; individuals are not seasonally consistent relative to each other.</td>
</tr>
<tr>
<td>Not</td>
<td>Not</td>
<td>Type 2</td>
<td>Population diets/habitats are seasonally consistent; individuals are not seasonally consistent relative to each other.</td>
</tr>
<tr>
<td>Not</td>
<td>Significant</td>
<td>Type 3</td>
<td>Population diets/habitats are seasonally consistent; individuals are seasonally consistent relative to each other.</td>
</tr>
<tr>
<td>Significant</td>
<td>Significant</td>
<td>Type 4</td>
<td>Population diets/habitats are not seasonally consistent; individuals are seasonally consistent relative to each other.</td>
</tr>
</tbody>
</table>
RESULTS

Comparison of three species at King George Island in 2010

Gentoo Penguins had the highest CVs for breeding season (blood) and non-breeding season (feather) TL, followed by Adélie penguins and Chinstrap Penguins (Table 2.2). Adélie and Chinstrap Penguins had nearly twice the CV for non-breeding season prey $\delta^{13}$C values compared to Gentoo Penguins. All three species displayed a much lower and similar CV for breeding season prey $\delta^{13}$C values.

Gentoo Penguins TL and prey $\delta^{13}$C values did not differ significantly between the non-breeding season (feather) and breeding season (blood; Table 2.3). While Adélie penguin TL did not differ significantly between the non-breeding and breeding seasons, Adélie penguin prey $\delta^{13}$C values were significantly lower during the non-breeding season relative to the breeding season by 1.0‰. Chinstrap Penguin TL during the non-breeding season was significantly lower relative to the breeding season by 0.15 trophic levels, but there was no significant difference between the non-breeding and breeding seasons in prey $\delta^{13}$C values (Table 2.3). There was a significant correlation in individual Gentoo Penguin TL and prey $\delta^{13}$C values between the two seasons examined (Table 2.3; Figure 2.2). In contrast, there was no correlation in individual TL or prey $\delta^{13}$C values between seasons for either Chinstrap or Adélie Penguins (Figure 2.2).

Comparison of three Gentoo Penguin populations in 2014

Gentoo Penguins at Elephant Island had the highest CV for TL during both the non-breeding and breeding season by more than double that of the populations at Wiencke and Rongé Island (Table 2.2). The population at Rongé Island had the highest CV for prey $\delta^{13}$C during the non-breeding season, followed by Wiencke and Elephant Island. Wienke Island population had the highest prey $\delta^{13}$C CV during the breeding season, followed by Elephant and Rongé Island.
Table 2.2: Mean and standard deviation, range (in parentheses), and coefficient of variation (CV%) of TL, $\delta^{15}$N, and prey $\delta^{13}$C of non-breeding and breeding seasons for all three species at King George Island and three Gentoo populations at Wiencke, Rongé and Elephant Island.

<table>
<thead>
<tr>
<th></th>
<th>Non-breeding (feather) mean ± SD</th>
<th>CV</th>
<th>Breeding (blood) mean ± SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adélie</td>
<td>3.84 ± 0.12 (3.53 to 4.15)</td>
<td>3.2</td>
<td>3.90 ± 0.10 (3.71 to 4.08)</td>
<td>2.5</td>
</tr>
<tr>
<td>Chinstrap</td>
<td>3.83 ± 0.06 (3.71 to 3.91)</td>
<td>1.7</td>
<td>3.98 ± 0.05 (3.85 to 4.09)</td>
<td>1.3</td>
</tr>
<tr>
<td>Gentoo</td>
<td>3.94 ± 0.17 (3.71 to 4.50)</td>
<td>4.4</td>
<td>3.92 ± 0.18 (3.76 to 4.53)</td>
<td>4.7</td>
</tr>
<tr>
<td>$\delta^{15}$N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adélie</td>
<td>9.1 ± 0.4 (8 to 10.1)</td>
<td>4.6</td>
<td>8.5 ± 0.3 (7.8 to 9.1)</td>
<td>4.1</td>
</tr>
<tr>
<td>Chinstrap</td>
<td>9.0 ± 0.2 (8.6 to 9.3)</td>
<td>2.4</td>
<td>8.7 ± 0.2 (8.3 to 9.0)</td>
<td>2.0</td>
</tr>
<tr>
<td>Gentoo</td>
<td>9.4 ± 0.6 (8.6 to 11.3)</td>
<td>5.9</td>
<td>8.5 ± 0.6 (8.0 to 10.6)</td>
<td>7.3</td>
</tr>
<tr>
<td>Prey $\delta^{13}$C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adélie</td>
<td>-25.4 ± 1.1 (-28.7 to -23.7)</td>
<td>-4.4</td>
<td>-24.5 ± 0.3 (-25.1 to -24.1)</td>
<td>-1.2</td>
</tr>
<tr>
<td>Chinstrap</td>
<td>-24.6 ± 1.0 (-25.8 to -22.8)</td>
<td>-4.1</td>
<td>-24.9 ± 0.2 (-25.4 to -24.7)</td>
<td>-0.8</td>
</tr>
<tr>
<td>Gentoo</td>
<td>-24.8 ± 0.7 (-25.1 to -23.1)</td>
<td>-2.7</td>
<td>-24.8 ± 0.2 (-25.8 to -23.1)</td>
<td>-1.8</td>
</tr>
<tr>
<td>TL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wiencke</td>
<td>4.10 ± 0.10 (3.94 to 4.29)</td>
<td>2.1</td>
<td>4.05 ± 0.06 (3.97 to 4.18)</td>
<td>1.4</td>
</tr>
<tr>
<td>Rongé</td>
<td>4.14 ± 0.10 (3.97 to 4.32)</td>
<td>2.4</td>
<td>4.08 ± 0.07 (3.94 to 4.20)</td>
<td>1.7</td>
</tr>
<tr>
<td>Elephant</td>
<td>4.10 ± 0.22 (3.56 to 4.39)</td>
<td>5.4</td>
<td>4.52 ± 0.21 (3.87 to 4.70)</td>
<td>4.6</td>
</tr>
<tr>
<td>$\delta^{15}$N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wiencke</td>
<td>9.60 ± 0.3 (9.4 to 10.6)</td>
<td>2.9</td>
<td>9.0 ± 0.2 (8.7 to 9.4)</td>
<td>2.1</td>
</tr>
<tr>
<td>Rongé</td>
<td>10.1 ± 0.3 (9.5 to 10.7)</td>
<td>3.4</td>
<td>9.1 ± 0.2 (8.6 to 9.5)</td>
<td>2.6</td>
</tr>
<tr>
<td>Elephant</td>
<td>10.0 ± 0.7 (8.1 to 10.9)</td>
<td>7.5</td>
<td>10.6 ± 0.7 (8.4 to 11.2)</td>
<td>6.6</td>
</tr>
<tr>
<td>Prey $\delta^{13}$C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wiencke</td>
<td>-25.6 ± 0.7 (-26.6 to -24.0)</td>
<td>-2.7</td>
<td>-24.2 ± 0.5 (-24.9 to -22.5)</td>
<td>-2.1</td>
</tr>
<tr>
<td>Rongé</td>
<td>-25.4 ± 0.9 (-26.3 to -23.7)</td>
<td>-3.5</td>
<td>-24.8 ± 0.2 (-25.1 to -24.6)</td>
<td>-0.6</td>
</tr>
<tr>
<td>Elephant</td>
<td>-25.6 ± 0.6 (-26.2 to -24.4)</td>
<td>-2.3</td>
<td>-25.0 ± 0.2 (-25.5 to -24.8)</td>
<td>-0.8</td>
</tr>
</tbody>
</table>

Gentoo Penguins at Wiencke Island had a very small (0.05 of a trophic level), yet significant difference in their TL between the non-breeding and breeding seasons. In addition, the prey $\delta^{13}$C values of this population were significantly lower during the non-breeding season relative to the breeding season by 0.7‰. While the TL of Gentoo Penguins at Rongé Island did not differ significantly between the non-breeding and breeding seasons, prey $\delta^{13}$C during the non-breeding season were significantly higher relative to the breeding season by 0.6‰.
Table 2.3: Results for paired t-test, Wilcoxon signed rank test, Pearson correlation, Kendall rank correlations, and diet/foraging category based on Table 2.1 of TL and prey δ¹³C for all three species at King George Island, South Shetland Islands and three Gentoo populations at Wiencke, Rongé and Elephant Island. Underlined paired t-test and Pearson correlation results are presented in the results and discussion due to sample distributions that were both normal and had equal variance for the given species/population. Underlined Wilcoxon signed rank test and Kendall rank correlation results are presented in the results and discussion due to sample distributions that were both non-normal and had non-equal variance for the given species/population.

<table>
<thead>
<tr>
<th></th>
<th>Paired t-test</th>
<th>Wilcoxon signed rank test</th>
<th>Pearson correlation</th>
<th>Kendall rank correlation</th>
<th>Diet/foraging category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t</td>
<td>p-value</td>
<td>W</td>
<td>p-value</td>
<td>r</td>
</tr>
<tr>
<td><strong>TL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adélie</td>
<td>-2.0</td>
<td>0.06</td>
<td>158.5</td>
<td>0.98</td>
<td>0.09</td>
</tr>
<tr>
<td>Chinstrap</td>
<td>-8.9</td>
<td>&lt;0.001</td>
<td>10.5</td>
<td>1</td>
<td>0.18</td>
</tr>
<tr>
<td>Gentoo</td>
<td>1.1</td>
<td>0.27</td>
<td>257.5</td>
<td>0.18</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>Prey δ¹³C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adélie</td>
<td>-3.3</td>
<td>0.003</td>
<td>32.5</td>
<td>0.003</td>
<td>-0.08</td>
</tr>
<tr>
<td>Chinstrap</td>
<td>1.4</td>
<td>0.17</td>
<td>135</td>
<td>0.27</td>
<td>-1.6</td>
</tr>
<tr>
<td>Gentoo</td>
<td>-1.1</td>
<td>0.28</td>
<td>35.5</td>
<td>0.1</td>
<td>0.66</td>
</tr>
<tr>
<td><strong>TL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wiencke</td>
<td>2.2</td>
<td>0.04</td>
<td>269.5</td>
<td>0.03</td>
<td>0.2</td>
</tr>
<tr>
<td>Rongé</td>
<td>2.0</td>
<td>0.07</td>
<td>259.5</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Elephant</td>
<td>-0.5</td>
<td>&lt;0.001</td>
<td>18</td>
<td>1</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>Prey δ¹³C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wiencke</td>
<td>-8.0</td>
<td>&lt;0.001</td>
<td>33</td>
<td>1</td>
<td>0.22</td>
</tr>
<tr>
<td>Rongé</td>
<td>-2.9</td>
<td>0.009</td>
<td>43</td>
<td>0.02</td>
<td>0.46</td>
</tr>
<tr>
<td>Elephant</td>
<td>-3.4</td>
<td>0.004</td>
<td>15.5</td>
<td>0.009</td>
<td>0.17</td>
</tr>
</tbody>
</table>

21
Figure 2.2: Trophic level (TL) and prey $\delta^{13}$C values for Adélie, Chinstrap, and Gentoo Penguins at King George Island, South Shetland Islands, Antarctica, 2010. Lines connect non-breeding season (feather) values and breeding season (blood) for each sampled individual.
Figure 2.3: Trophic level (TL) and prey δ13C values Gentoo Penguins at three breeding sites in the South Shetland Island and Western Antarctic Peninsula. Lines connect non-breeding season (feather) values and breeding season (blood) for each sampled individual.
The TL and prey $\delta^{13}C$ values of Gentoo Penguins at Elephant Island were both significantly lower during the non-breeding season relative to the breeding season by 0.42 of a trophic level, and 0.6‰ respectively. Individual TL or prey $\delta^{13}C$ values were not correlated between seasons at Wiencke Island or Rongé Island (Table 2.3). In contrast, Gentoo Penguins at Elephant Island exhibited a significant correlation in individual TL values between seasons, but no correlation in individual prey $\delta^{13}C$ values.

DISCUSSION

Gentoo Penguins are often regarded as generalist foragers, whereas Adélie and Chinstrap are suggested to specialize primarily on krill within the Western Antarctic Peninsula and South Shetland Islands, 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). However, very few studies have investigated foraging variation within populations of Pygoscelis species (Polito et al. 2015) or between different times of year (e.g. Polito et al. 2011, Juáres 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 degree of foraging consistency within individuals.

Comparison of individual variation and foraging strategies of species at King George Island

Qualitative comparisons of CVs among the three species provide additional support for differences in foraging strategies as indicated by previous studies (Volkman et al. 1980, Miller et al. 2010, Polito et al. 2015). CV values comparing the level of individual variability in trophic level (TL) among the three species at King George Island showed that Gentoo Penguins had the highest CV, Chinstrap Penguins had the lowest CV, and Adélie penguin CV were intermediate of
their congeners during both seasons examined (Table 2.2; Figure 2.2). While qualitative, our results suggest that Adélie Penguins are more generalized in their trophic level of diet relative to Chinstrap Penguins, while Gentoo Penguins are more generalized in their trophic level of diet relative to both Adélie and Chinstrap Penguins. Previous studies support our findings that Gentoo Penguins have greater variability in diet than Adélie and Chinstrap Penguins in the South Shetland Islands during the breeding season (Volkman et al. 1980, Polito et al. 2015). However, while past studies indicate that Adélie Penguins diets are more diverse in regions outside of the Antarctic Peninsula (e.g. Ainley 2002) to our knowledge there are no previous studies showing that Adélie Penguins are more generalist in diet relative to Chinstrap Penguins.

Examining the CV of prey $\delta^{13}$C values as a proxy of foraging habit use among the three species at King George Island suggest that during the non-breeding period individual Adélie and Chinstrap Penguins use a wider range of foraging habitats relative to individual Gentoo Penguins (Table 2.2). This finding is similar to a previous study by Juáres et al. (2016) at Stranger Point, King George Island, in which they found Adélie penguins had a greater dispersion of body feather $\delta^{13}$C values in comparison to Gentoo Penguins. In addition, these results agree with tracking studies which indicate that while Adélie and Chinstrap Penguins disperse broadly during the non-breeding period (Hinke et al 2015), Gentoo Penguins remain close to their breeding colonies and forage in open-water, near-shore habitats (Wilson et al. 1998).

**Seasonal consistency in three species at King George Island**

We observed differences across species in population and individual-level trophic level and foraging habitat use consistency at King George Island. Adélie penguins exhibited a type 2 pattern for TL, suggesting that population diets are seasonally consistent but individual are not seasonally consistent relative to each other. Past dietary studies during the chick rearing period
using stomach content analyses of Adélie penguins have found that their diets comprise primarily Antarctic Krill, with very low percentages of fish (Volkman et al. 1980; Karnovsky, 1997). Our results are focused at two other times of the year when adults are not provisioning chicks, late incubation and post-breeding, and when combined with their CV suggests that Adélie penguins in the South Shetland Islands may have a more diverse diet that is consistent between these two seasons. This compliments a prior study by Polito et al. (2011b) in which they found that Adélie penguins in the South Shetland Islands have a heterogeneous diet of fish and krill during the pre-breeding seasons. Adélie penguins demonstrated a type 1 pattern for prey $\delta^{13}C$ in which population foraging habitat use is not seasonally consistent and individual are also not seasonally consistent relative to each other. The low values of non-breeding season prey $\delta^{13}C$ and relatively high CV suggest that Adélie penguins disperse widely and forage in more pelagic/offshore habitats, as $\delta^{13}C$ values are generally lower in offshore pelagic systems than inshore benthic systems (France 1995, Hobson & Cherel 2011). However, relatively higher breeding season $\delta^{13}C$ values and lower CV suggest Adélie penguins forage in more constrained and relatively more inshore locations at that time. This might be evidence that adults are restricted to inshore and/or shallow locations during incubation and chick rearing, but have more foraging range flexibility outside the breeding season once chicks have fledged, a pattern also observed in diet analyses and tracking studies (Trivelpiece et al. 1987; Polito et al. 2015).

Chinstrap Penguins demonstrated a type 1 pattern for TL, suggesting that population diet is not seasonally consistent, and individuals are not seasonally consistent relative to each other. However, Chinstraps TL CVs were the lowest observed and the range of TL values was less than one quarter of a trophic level for both breeding and non-breeding seasons (Table 2.2, Figure 2.2). This suggests that the Chinstrap Penguin population has very low variation between individuals.
TL values indicate that all individuals forage within one quarter of a trophic level from one another. As prey items such as Antarctic krill and fish differ by at least one trophic level, this would suggest all individuals have a relatively similar diet (Polito et al. 2011). Therefore, Chinstrap Penguins likely consistently forage within the same general trophic level between seasons, even though population may shift within the trophic level overall. Our findings are consistent with many studies that have found Chinstrap Penguin diets in the South Shetland Islands are composed primarily of Antarctic krill during the chick rearing season (Volkman et al. 1980; Trivelpiece et al. 1987; Miller et al. 2008; Miller et al. 2010; Polito et al. 2015). Chinstrap Penguin exhibited a type 1 pattern for prey \( \delta^{13}C \), suggesting population foraging habitat use is seasonally consistent and individual are not seasonally consistent relative to each other.

Combined with a large range in non-breeding season prey \( \delta^{13}C \) values and a relatively large CV value, 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 results from the Wilcoxon signed rank test suggest the Chinstrap population does not shift its foraging habitat seasonally, prey \( \delta^{13}C \) values appear to reflect the same pattern seen in Adélie penguins. Breeding season prey \( \delta^{13}C \) values are substantially narrower and less variable than prey \( \delta^{13}C \) non-breeding values, suggesting a similar foraging restriction due to incubation/chick-rearing responsibilities during the breeding season.

Gentoo Penguins exhibited a type 3 pattern for TL, suggesting that population diets are seasonally consistent, and individual are seasonally consistent relative to each other. This is supported by one study investigating seasonal consistency of diet in a population of Gentoo
Penguins at King George Island, South Shetland Islands, in which $\delta^{15}$N values of Gentoo Penguins during the non-breeding season not differ from the following breeding season (Juàres et al. 2016). However, this previous study did not examine consistency at the individual level. Gentoo Penguins also demonstrated a type 3 pattern for prey $\delta^{13}$C, which indicates that population foraging habitat is seasonally consistent, and individual are seasonally consistent relative to each other. Similar to Adélie and Chinstrap Penguins, examination of prey $\delta^{13}$C values reveals a slight truncation during the breeding season, which may also suggest some foraging restrictions due to incubation/chick-rearing responsibilities.

Foraging strategies of *Pygoscelis* penguins at King George Island

Overall, our results indicate that Chinstrap Penguins are the most specialized in their diet compared to both Adélie and Gentoo Penguins, with little variation between individuals. In contrast, Adélie penguins appear more generalist in diet relative to Chinstrap Penguins but less generalist than Gentoo Penguins, and individuals do not specialize due to a lack of individual consistency between seasons. Furthermore, our results suggest that Gentoo Penguins may be dietary generalists at the population level due to a higher degree of individual variation. Our results also indicate that individual Gentoo Penguins may be more specialized within the population, as they exhibit individual consistency between seasons. Though generalist populations comprising individual specialists have been documented in other top marine predators, no previous studies have examined this in a penguin species (Woo et al. 2008; Newsome et al. 2009; Hückstädt et al. 2012; Kernaleguen et al. 2015).
Comparison of seasonal consistency and individual variation among Gentoo populations:

Even though we found evidence of seasonal and individual consistency in diet and foraging habitat use of Gentoo Penguins at King George Island, these patterns varied considerably between Gentoo Penguins populations in the Western Antarctic Peninsula and the South Shetland Islands. Comparisons among the three Gentoo populations provide support for differences in the degree of individual variation in diet and foraging habitat. Assessments of CV values between breeding locations indicated that Gentoo Penguins at Elephant Island demonstrate higher individual variation in TL relative to Gentoo Penguins at Wiencke and Rongé Island during both the breeding and non-breeding periods (Table 2.2; Figure 2.3). Elephant Island’s TL CVs were more than twice as high as the Wiencke and Rongé populations, indicating a wider dietary diversity and variation between individuals when compared to the Western Antarctic Peninsula populations. A comparison of prey δ13C values CV among the populations suggest that all populations demonstrate a similar level of variation in foraging habitat use during the non-breeding season, while Gentoo Penguins at Wiencke Island exhibit a higher degree of foraging habitat use variation during the breeding season relative to Gentoo Penguins at Rongé and Elephant Island (Table 2.2; Figure 2.3).

The Gentoo Penguin population at Wiencke Island exhibited a type 1 pattern for TL, suggesting that the population’s diet is not seasonally consistent, and that individuals were not seasonally consistent relative to each other. However, the t-test result for this population was only weakly significant (p=0.04). Furthermore, TL range values and CVs, as well as individual patterns look noticeably similar to the population at Rongé Island, which exhibited a type 2 pattern for TL, suggesting that population diet is seasonally consistent, and individuals are not seasonally consistent relative to each other (Table 2.3; Figure 2.3). These two populations
appear to have similar dietary trends, with no individual consistency between seasons. Elephant Island demonstrated a type 4 pattern for TL, suggesting the population diet is not seasonally consistent, and individuals are consistent relative to each other. All three populations exhibited a type 2 for prey δ^{13}C, suggesting population foraging habitats shifts seasonally, and individuals are not consistent relative to each other. Overall prey δ^{13}C values were higher during the breeding season at all sites, similar to those at King George Island, suggesting individuals are also restricted to more inshore and/or shallow locations during incubation.

The within-species variation in foraging strategy we observed may be due to regional differences in productivity and prey availability. Studies on Galapagos sea lions (Zalophus wollebaeki) found a high degree of variation in foraging strategies and the level of individual specialization between populations throughout the Galapagos Archipelago whose habitats vary in prey availability and physical oceanographic characteristics (Villegas-Amtmann et al. 2008; Salazar, 2005; Dellinger and Trillmich, 1999; Kooyman and Trillmich, 1986). It is possible that prey abundance and availability may differ between the foraging habitats 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 TL. 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) (Figure 2.1). These fronts are associated with high densities of Antarctic krill, but with high variability between seasons (Dietrich et al. 2014). Variability in the abundance and availability of krill may select for greater diversity in diet between individual penguins in order to reduce competition, which could explain the high
diversity in TL 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**

Because there are no published discrimination factors for *Pygoscelis* penguin blood, we used $\delta^{15}N$ and $\delta^{13}C$ discrimination factors for whole blood derived from captive studies of four piscivorous birds for our TL and prey $\delta^{13}C$ value calculations, as suggested by Cherel et al. (2005) and used in other studies on *Pygoscelis* penguins (Brasso et al. 2013, Juáres et al 2016). It is possible that the absolute values of calculated TL and prey $\delta^{13}C$ values would differ if species-specific discrimination factors were available. However, correlation results did not differ for $\delta^{15}N$ and TL or $\delta^{13}C$ and prey $\delta^{13}C$ comparisons, suggesting that the observed relationships are not an artifact of the chosen discrimination factors. Nonetheless, we suggest that future research should aim to include controlled dietary studies of the target species in order to obtain more refined $\delta^{15}N$ and $\delta^{13}C$ discrimination factors for whole blood.

**Conclusions**

This study is the first to use stable isotope analysis of blood and feather of all three *Pygoscelis* penguins to compare temporal foraging consistency within individuals and between populations. Our results indicate species-specific differences at King George Island: Gentoo Penguins exhibit a generalist foraging strategy with individual specialization, Adélie Penguins exhibit an intermediate generalist foraging strategy with little individual consistency, and Chinstrap Penguins exhibit a specialized diet with little variation between individuals. However,
unlike trophic variation, we found similarities in foraging habitat across all three species, with greater variation in foraging habitat at the population level during the non-breeding season compared to the breeding season. Finally, we found variation in trophic level between Gentoo Penguin populations, with the two populations in the Western Antarctic Peninsula exhibiting a narrow range of trophic level with little individual variation, relative to the population on Elephant Island, South Shetland Islands, which had a large range in trophic level, and evidence of individual foraging consistency. 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.

LITERATURE CITED


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CHAPTER 3:
GEOGRAPHIC VARIATION IN GENTOO PENGUIN (*PYGOSCELIS PAPUA*) GENERALIST FORAGING STRATEGIES THROUGHOUT THE SCOTIA ARC

INTRODUCTION

While traditionally ecological studies have treated conspecific individuals as functionally equivalent, there is growing evidence to suggest that individual specialization and individual niche variation is widespread with important implications for ecology and evolutionary dynamics (Bolnick et al. 2003, Sargeant 2007). For example, specialist individuals can have an advantage over generalist individuals within populations if they are better able to exploit a particular high-profit resource. Pigeon guillemots (*Cepphus columba*) that specialized on lipid rich fish species have higher fledging rates than their generalist conspecifics (Golet et al. 2000). Likewise, spectacled salamanders with specialized foraging niches (*Salamandra perspicillata*) demonstrate better physiological condition than individuals with a broader trophic foraging niche (Costa et al. 2015).

Individual specialization and individual niche variation is frequently found within generalist populations (Bolnick et al. 2003; Sargeant 2007). Identifying individual specialization within generalist populations requires determining the degree of foraging variation within individuals relative to variation among individuals, and if these variations persist through time. Using this approach, generalist populations can be broadly grouped into two categories: Type A generalist populations exhibit extensive niche variation within individuals and little niche variation between individuals, whereas type B generalist populations are composed of individuals with specialized niches, that are smaller than the population niche as a whole (Bolnick et al. 2003; Bearhop et al. 2004). Due to the ecological importance of individual niche
specialization there is a growing interest to better understand what factors drives individuals within generalist populations to specialize. There is evidence to suggest that individual specialization may be a density-dependent strategy, in which individuals restrict their foraging niche to reduce intraspecific competition as population size increases and prey resources decrease (Parent et al. 2014). For example, Svanbäck and Bolnick (2004) found that increases in population density of Eurasian perch (*Perca fluviatilis*) were accompanied by an expansion of the population’s total niche width, while within-individual niche width remained small and specialized on a single habitat. Therefore, individual specialization and niche variation within a population may actually act as a buffer against environmental pressures affecting prey resources and habitats by enabling a population to adapt to such changes through individual foraging diversity (Durell 2000; Bolnick et al. 2003).

Stable isotope analysis (SIA) provides an effective way to assess the diet and foraging niches of individuals through time (Bearhop et al. 2004; Newsome et al. 2007, Jaeger et al. 2009, Vander Zaden et al. 2010; Hückstädt et al. 2012, Kernaleguen et al. 2015). Nitrogen isotopic values ($\delta^{15}$N) increase with trophic level and can be used as a proxy of a consumer’s diet, while carbon isotopic values ($\delta^{13}$C) in marine consumers vary with latitude and can be used to indicate differences in foraging habitat with respect to inshore versus offshore locations (France 1995; Cherel and Hobson, 2007). Together, $\delta^{15}$N and $\delta^{13}$C values can be used to determine the 2-dimensional parameters of an isotopic niche space, a comparable measurement of an ecological or foraging niche (Newsome et al. 2007). Comparing isotopic niches between and within species has allowed researchers to identify specialist vs. generalist foraging strategies at both the population (Polito et al. 2015) and individual level (Newsome et al. 2009). However, while informative, studies of individual foraging specialization often focus on a single population or
are limited in spatial or temporal scope (Vander Zaden et al. 2010; Hückstädt et al. 2012; Kernaleguen et al. 2015).

To our knowledge, no past studies have examined individual foraging specialization across a species’ distributional range to determine if populations can exhibit either type A or type B generalist strategies in response to differing environmental and density dependent conditions. We seek to address this knowledge gap, by investigating foraging niche specialization in four geographically distinct Gentoo Penguin (Pygoscelis papua) populations spanning the latitudinal extent of their breeding range. Gentoo Penguins are an ideal model species for a large-scale comparative study of individual specialization across generalist populations due to their large breeding distribution and evidence as generalist foragers at the population level (Miller et al. 2009; Lynch 2013; Polito et al. 2015). While there is evidence of considerable variation in this species’ population level foraging niches across breeding populations (Croxall et al, 1988; Wilson et al, 1998; Miller et al. 2009; Putz et al. 2001; Clausen and Putz; and Polito et al. 2015), past studies have not assessed individual diet and foraging consistency through time, which is necessary for a robust assessment of individual specialization (Bolnick et al, 2003; Bearhop et al. 2004, Jaeger et al. 2009). Furthermore, there are large latitudinal differences in factors such as population sizes, physical oceanographic features, and prey assemblage and abundance across this species breeding range, which are likely to influence the use of type A vs. type B generalist strategies.

We hypothesize that northern populations of Gentoo Penguins that are relatively large in size and whose foraging habitat comprises a high diversity of prey resources will favor individual foraging specialization (type B strategies) and lower niche overlap between individuals. In contrast, smaller southern populations whose prey resources are dominated by
*Euphaisa superba* (Antarctic krill) are more likely to exhibit type A generalist strategies with greater niche overlap between individuals. To test these hypotheses, we examine isotopic niche metrics within four geographically isolated Gentoo Penguin populations at both the population and individual levels to quantify the degree to which individual niches are specialized relative to their population’s total niche (i.e. type A vs. type B generalists; Bearhop et al. 2004; Sargeant 2007). In addition, we compare metrics of individual isotopic niche overlap within the four populations to reveal the degree to which individuals differentiate their foraging niches relative to one another within each population (Sargeant 2007).

**METHODS**

**Study area and sample collection**

Our research focuses on Gentoo Penguin breeding colonies from four geographically isolated regions in the Scotia Arc: Cuverville Island in the Western Antarctic Peninsula (64.6875°S, 62.6219°W); Cape Shirreff on Livingston Island in the South Shetland Islands (62.4589°S, 60.7886°W); Start Point on South Georgia (54.0472°S, 37.3564°W); and Cow Bay on the Falkland Islands (51.4335°S, 57.8564°W) (Figure 3.1). These oceanographic regions differ considerably both physically and ecologically (Table 3.1). The Western Antarctic Peninsula and South Shetland Islands are found just south of the Southern Antarctic Circumpolar Convergence Front (SACCF) and Southern Boundary of the ACC (SB), where there is an abundance of Antarctic krill (*Euphaisa superba*), a primary food source for *Pygoscelis* penguins (Volkman et al. 1980; Miller et al. 2008, 2010). South Georgia is located north of the SACCF and within the flow of the ACC, which transports Antarctic krill from areas around the Antarctic continent, resulting in high krill abundance in the surrounding waters as well. However, this influx recruitment can be ephemeral and vary considerably from year to year (Murphy et al.
2013). The Falkland Islands are found in shallower warmer waters north of the Polar Front and the Sub-Antarctic Front (SAF) where there is no Antarctic krill, but a wide diversity of prey items (Clausen and Pütz, 2003). In addition, Gentoo Penguin population sizes are much larger in the Falkland Islands and South Georgia at the northern extent of their range relative to the South Shetland Islands and Western Antarctic Peninsula at the southern extent of their range (Table 3.1).

Figure 3.1: Map of the four Gentoo Penguin breeding locations sampled in the study. Major currents and fronts are indicated by dotted lines: Sub-Antarctic Front (SAF), Polar Front (PF), and Antarctic Circumpolar Convergence (ACC).
Table 3.1: Summary of the differences in ecological and physical characteristics between the four major study regions. Southern Antarctic Circumpolar Convergence Front (SACCF), Polar Front (PF), Sub-Antarctic Front (SAF). Prey resources based on previous dietary studies of Gentoo Penguins in each region.

<table>
<thead>
<tr>
<th>Ecological &amp; Physical Characteristic</th>
<th>Western Antarctic Peninsula</th>
<th>South Shetland Islands</th>
<th>South Georgia</th>
<th>Falkland Islands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population Size</td>
<td>63,897</td>
<td>36,450</td>
<td>98,867</td>
<td>132,321</td>
</tr>
<tr>
<td>Currents and Fronts</td>
<td>South of SACCF</td>
<td>South of SACCF</td>
<td>Between SACCF and PF</td>
<td>North of PF and SAF</td>
</tr>
<tr>
<td>Seasonal Sea Ice Presence</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

In the austral summer of 2011/12, we captured 16-20 individual breeding adults from active nests at each colony. Sex was not determined. For each individual we collected a single central tail feather (i.e. the longest), which was stored at room temperature prior to analysis. Gentoo penguins tail feathers are grown over a period of approximately 100 days during the post-molt season. They are metabolically inert and can be sampled for SIA at discrete intervals to reflect the early winter diets of penguins throughout the tail feather growth period and allow for serial sampling to detect temporal variability or consistency in foraging (see Appendix 1; also Hinke et al. 2015).
Sample preparation and isotopic analysis

We cut tail feathers into 1cm segments and rinsed segment in 2:1 chloroform:methanol and allowed them to air dry for 24 hours (Cherel et al. 2005). We subsampled 0.5mg of feather vane material from four 1cm sections between 5.5-6.5cm, 7.5-8.5cm, 9.5-10.5cm, and 11.5-12.5cm for a total of four sections per individual feather. This sampling design allowed for the analysis of individual penguin isotopic niche during the late fall /early winter period at during four discrete 5-7 day long foraging periods distributed across 33 days of continuous feather growth during the post-molt, non-breeding season (Appendix A). Samples were analyzed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. Results are in the format of stable isotope ratios using del notation in per mil units derived from the equation: δ\text{X} = [(R_{\text{sample}}/ R_{\text{standard}}) - 1] × 1000. \ R_{\text{standard}} is the ratio \text{^{13}C}/\text{^{12}C} or \text{^{15}N}/\text{^{14}N}. \ R_{\text{standard}} values are based off of Vienna PeeDee Belemnite for δ\text{^{13}C} and atmospheric N\textsubscript{2} for δ\text{^{15}N}. Raw δ values were normalized using glutamic acid, bovine liver, and nylon 5 as reference material (USGS-40: δ\text{^{13}C} = -16.65‰, δ\text{^{15}N} = -6.8‰; USGS-41: δ\text{^{13}C} = -37.63‰, δ\text{^{15}N} = 47.6‰; bovine liver: δ\text{^{13}C} = -21.69‰, δ\text{^{15}N} = 7.72‰; nylon 5: δ\text{^{13}C} = -27.72‰, δ\text{^{15}N} = -10.31‰). Sample precision based on internal repeats and duplicate standard reference materials was 0.1‰ for both δ\text{^{15}N} and δ\text{^{13}C}.

Niche analysis at the population level

For population level analysis the isotopic values (δ\text{^{15}N} and δ\text{^{13}C}) of tail feather sections were first averaged the four feather values within individuals to produce a single mean isotopic value per individual. We used individual mean values for all of the population level comparisons described below. We compared the mean individual isotopic values between populations for both δ\text{^{15}N} and δ\text{^{13}C} values using one-way ANOVAs and Tukey's HSD (honest significant difference).
test to examine differences in all pairwise comparisons of populations. To test for variation in population-level isotopic niche size, we used the R package SIAR to calculate standard ellipse areas corrected for sample size (SEA_C) for each population, which provide a measure of the 2-dimensional core isotopic niche area of a population (Jackson et al. 2011). We then compared all posterior draws from the Bayesian approximation of this metric (SEA_b) between populations to test for significant differences in core isotopic niche area. We also constructed convex hulls for each population, which act as proxies for total niche area (TA; Layman & Allgeier 2012). Finally, we calculated each population’s mean distance to centroid (MDC) and mean nearest neighbor distance (NND), which can be interpreted as a metric for a population’s trophic diversity and the density of individual packing within a population (Laymen et al. 2007, Turner et al. 2010)

**Niche analysis at the individual level**

For individual level analysis of isotopic niches we used the δ^{15}N and δ^{13}C values of all individual tail feather sections from each individual. We first calculated the degree of individual specialization found in each population using the R-package RInSp (Zaccarelli et al. 2013). This package applies the method of Bolnick et al. (2002) to calculate an individual specialization index for a population using total niche width index for continuous data proposed by Roughgarden (1972). Specifically, the total niche width of a population (TNW) can be partitioned into two components: the variation within individuals, or the within-individual component (WIC), and the variation between-individual in a population, or the between-individual component (BIC) so that TNW = WIC + BIC (Roughgarden, 1972). The degree of individual specialization of a population is represented by the ratio of WIC/TNW so that ratios approaching zero suggest high degrees of individual specialization (Type B population) and
ratios approaching one suggest lower degrees of individual specialization or more generalism at the individual level (Type A population). For each population we calculated separate TNW, WIC, BIC and WIC/TNW metrics based on tail feather section $\delta^{15}$N and $\delta^{13}$C values. We assessed significance of WIC/TNW using a nonparametric Monte Carlo technique to generate 10,000 replicate datasets under the null hypothesis that all individuals are generalists (Type A population) and used these distributions to calculate P-values (Zaccarelli et al. 2013).

To compliment the above univariate analyses, we also calculated the total isotopic niche areas (TA) for each individual using their respective $\delta^{15}$N and $\delta^{13}$C values of individual tail feather sections. We divided individual’s TA values by their respective population’s TA to obtain an index of relative niche area to quantify the degree to which an individual’s niche is specialized relative to the population. Values approaching zero indicate individuals have smaller isotopic niches than their population’s total niche and thus a higher degrees of individual specialization (Type B population). In contrast, values approaching one indicate individual’s isotopic niche is similar in size to their population’s total niche and thus a more generalist at the individual level (Type A population). In addition we used TA to obtain an index of relative niche overlap to quantify the degree to which individuals within populations are specialized relative to each other (Sergeant, 2007). We calculated relative niche overlap as the number of individual TAs that overlapped with any single individual’s TA, divided by the number of total individuals within each population. Values approaching zero suggest a low degree of overlap between individual’s niches with individuals differentiating in resource use, while values approaching one indicate greater overlap between individual’s niches and the use of common resources among individuals. We then used ANOVA (for normally distributed data) or the Kruskal-Wallis H test (for non-normally distributed data) to test for differences in individual’s
relative niche area and relative niche overlap across the Gentoo Penguin populations. All statistical analyses were performed within the program R (Ver. 3.2.1; R Core Team 2015).

RESULTS

Population Level Niche Metrics

Tail feather $\delta^{15}$N and $\delta^{13}$C values differed significantly among populations (Table 3.2; $\delta^{15}$N: $F_{3,296} = 2035$, $p < 0.0001$, $\delta^{13}$C: $F_{3,296} = 5635$, $p < 0.001$). All four populations differed significantly from one another for both $\delta^{15}$N and $\delta^{13}$C and did not overlap. Isotopic values were correlated with the latitudinal position of each population, with the Western Antarctic Peninsula having the lowest $\delta^{15}$N and $\delta^{13}$C values, followed by the South Shetland Islands, South Georgia, and the Falkland Islands (Figure 3.2). Trophic diversity measured by MDC was significantly higher in South Georgia compared to all other sites ($p < 0.05$). MDC was also significantly higher in the South Shetland Islands compared to the Western Antarctic ($p = 0.014$). Mean nearest neighbor distance NND was significantly lower in the Western Antarctic Peninsula than South Georgia ($p = 0.009$). Total and core isotopic niche areas also differed across sites (Table 3.3). South Georgia had the largest total niche area (TA) followed by the South Shetland Islands, the Falkland Islands and the Western Antarctic Peninsula. Similarly, core niche area (SEA$_b$) showed the same spatial pattern as TA with significantly larger SEA$_b$ in South Georgia relative to the Falkland Islands and the Western Antarctic Peninsula ($p < 0.01$, $p < 0.001$, respectively) and significantly larger SEA$_b$ in the South Shetland Islands relative to the Western Antarctic Peninsula ($p < 0.01$).
Table 3.2: Individual Specialization niche parameters for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ per region. WIC = within-individual component, BIC = between-individual component, TNW = total niche width, WIC/TNW = ratio approximating degree of individual specialization within populations.

<table>
<thead>
<tr>
<th>Isotope values</th>
<th>Population</th>
<th>$n$</th>
<th>Mean ± SD</th>
<th>CV (%)</th>
<th>WIC</th>
<th>BIC</th>
<th>TNW</th>
<th>WIC/TNW</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta^{15}\text{N}$</td>
<td>Western Antarctic Peninsula</td>
<td>20</td>
<td>9.0 ± 0.3 (8.3 to 9.8)</td>
<td>3.3</td>
<td>0.045</td>
<td>0.037</td>
<td>0.083</td>
<td>0.55</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>South Shetland Islands</td>
<td>19</td>
<td>9.6 ± 0.6 (8.5 to 11.8)</td>
<td>6.3</td>
<td>0.19</td>
<td>0.21</td>
<td>0.39</td>
<td>0.47</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>South Georgia</td>
<td>16</td>
<td>12.0 ± 1.2 (10.2 to 15.2)</td>
<td>10.0</td>
<td>0.36</td>
<td>1.02</td>
<td>1.38</td>
<td>0.26</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Falkland Islands</td>
<td>20</td>
<td>16.8 ± 0.5 (15.7 to 18.5)</td>
<td>3.0</td>
<td>0.09</td>
<td>0.18</td>
<td>0.27</td>
<td>0.35</td>
<td>0.001</td>
</tr>
<tr>
<td>$\delta^{13}\text{C}$</td>
<td>Western Antarctic Peninsula</td>
<td>20</td>
<td>24.7 ± 0.4 (-25.8 to -23.9)</td>
<td>2.0</td>
<td>0.09</td>
<td>0.04</td>
<td>0.12</td>
<td>0.67</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>South Shetland Islands</td>
<td>19</td>
<td>23.3 ± 0.7 (-24.8 to -22.0)</td>
<td>3.0</td>
<td>0.28</td>
<td>0.22</td>
<td>0.5</td>
<td>0.56</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>South Georgia</td>
<td>16</td>
<td>18.7 ± 0.7 (-20.4 to -17.2)</td>
<td>3.7</td>
<td>0.15</td>
<td>0.31</td>
<td>0.45</td>
<td>0.33</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Falkland Islands</td>
<td>20</td>
<td>14.3 ± 0.4 (-15.3 to -13.4)</td>
<td>2.8</td>
<td>0.09</td>
<td>0.11</td>
<td>0.2</td>
<td>0.47</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Figure 3.2: Standard ellipse areas and convex hull areas (TA) representing niches of each population. Points represent individual averaged $\delta^{15}$N and $\delta^{13}$C values. Black = Western Antarctic Peninsula, red = South Shetland Islands, green = South Georgia, blue = Falkland Islands.

Individual niche area

WIC/TNW for $\delta^{15}$N was highest in the Western Antarctic Peninsula followed by the South Shetland Islands and the Falkland Islands and lowest at South Georgia (Table 3.2). All four regions had WIC/TNW based on $\delta^{15}$N values consistent with significant individual specialization within populations relative to a null distribution (p<0.005).
Table 3.3: Population level metrics evaluating differences in bivariate niche parameters. TA = total niche area based on individual averages, MDC = mean distance to centroid, NND = mean nearest neighbor distance, Mean SEA$_b$ = mean of posterior probability distributions for Bayesian standard ellipse area estimates including 95% credibility intervals. Groups that do not share at least 1 superscripted letter within a column are significantly different for the given variable at the 0.05 level. Ranges of values are in brackets.

<table>
<thead>
<tr>
<th>Population</th>
<th>TA</th>
<th>MDC</th>
<th>NND</th>
<th>SEA$_b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Antarctic Peninsula</td>
<td>0.39</td>
<td>0.26$^a$</td>
<td>0.10$^a$</td>
<td>0.49 [0.34-0.70] $^a$</td>
</tr>
<tr>
<td>South Shetland Islands</td>
<td>1.82</td>
<td>0.61$^b$</td>
<td>0.23$^{ab}$</td>
<td>1.05 [0.71-1.52] $^{bc}$</td>
</tr>
<tr>
<td>South Georgia</td>
<td>2.06</td>
<td>0.97</td>
<td>0.32$^b$</td>
<td>1.69 [1.12-2.47] $^b$</td>
</tr>
<tr>
<td>Falkland Islands</td>
<td>1.11</td>
<td>0.44$^{ab}$</td>
<td>0.18$^{ab}$</td>
<td>0.75 [0.52-1.07] $^{ac}$</td>
</tr>
</tbody>
</table>

WIC/TNW for $\delta^{13}$C were also the highest in the Western Antarctic Peninsula, followed by the South Shetland Islands, Falkland Islands, and South Georgia (Table 3.4). The South Shetland Islands, Falkland Islands, and Georgia showed significant individual specialization for $\delta^{13}$C WIC/TNW relative to null distributions (p<0.005), while the Western Antarctic Peninsula did not. We found no significant differences in individual’s relative niche area among Gentoo Penguin populations. All four populations had mean relative niche area values below 7% (0.3-21.0%; Table 3.4).

**Individual niche overlap**

We found significant differences in relative niche overlap between populations (Figure 3.3; Table 3.4; $F_{3/71} = 11.84$, p < 0.0001). Western Antarctic Peninsula population had a significantly higher relative niche overlap compared to all other sites (Table 3.4; South Shetland Islands, p < 0.0001; South Georgia, p = 0.004; Falkland Islands, p = 0.006).
Table 3.4: Individual relative niche and overlap metrics based on total niche area (TA). Population TA = total niche area based on all individual values. Asterisk (*) indicates a significant difference from all other populations for the given variable at the 0.05 level. Ranges of values are in brackets.

<table>
<thead>
<tr>
<th>Population</th>
<th>Population TA (%(^2))</th>
<th>Individual TA (%(^2)) Mean ± SD</th>
<th>Relative niche area (%) Mean ± SD</th>
<th>Relative niche overlap Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Antarctic Peninsula</td>
<td>2.18</td>
<td>0.15 ± 0.05 [0.01-0.35]</td>
<td>6.7 ± 4.5 [0.3-16.1]</td>
<td>0.66 ± 0.16* [0.25-0.85]</td>
</tr>
<tr>
<td>South Shetland Islands</td>
<td>5.83</td>
<td>0.35 ± 0.05 [0.05-0.95]</td>
<td>5.9 ± 4.0 [0.9-16.3]</td>
<td>0.31 ± 0.08 [0.16-0.42]</td>
</tr>
<tr>
<td>South Georgia</td>
<td>5.66</td>
<td>0.31 ± 0.04 [0.04-0.97]</td>
<td>6.1 ± 5.7 [0.7-17.1]</td>
<td>0.44 ± 0.23 [0.06-0.69]</td>
</tr>
<tr>
<td>Falkland Islands</td>
<td>3.04</td>
<td>0.19 ± 0.06 [0.03-0.64]</td>
<td>5.5 ± 4.0 [1.0-21.0]</td>
<td>0.46 ± 0.22 [0.00-0.75]</td>
</tr>
</tbody>
</table>
Figure 3.3: Convex hull areas (TA) for each individual within the four sample populations. Dotted line represents TA for entire population.

DISCUSSION

We originally hypothesized that Gentoo Penguins populations in the northern Scotia Arc, which are relatively larger in size and have a higher diversity of prey resources, will favor individual foraging specialization (type B strategies) and lower niche overlap between individuals. Conversely, we hypothesized Gentoo Penguins populations in the southern Scotia
Arc, which are relatively smaller in size and have prey resources dominated by Antarctic krill (*Euphaisa superb*), would exhibit type A generalist strategies with higher niche overlap between individuals. Contrary to these hypotheses, our results indicate that all four Gentoo Penguins populations we examined exhibited clear evidence of individual foraging specialization (e.g. type B generalist foraging strategies) based on their isotopic niches. However, consistent with our predictions, the relative degree of individual niche specialization varied between populations. Gentoo Penguin populations in the north had a higher degree of individual niche specialization relative to southern populations and the southernmost population examined (Western Antarctic Peninsula) exhibited the highest degree of foraging niche overlap among individuals.

**Population-level isotopic niches**

The linear trend observed in both δ¹⁵N and δ¹³C values corresponding to the latitudinal position of each population (the lowest being the Western Antarctic Peninsula, followed by the South Shetland Islands, South Georgia, and the Falkland Islands) is consistent with the latitudinal gradient in δ¹⁵N and δ¹³C due to baseline differences in productivity within frontal zones in which both isotopic values increase from south to north (Francois et al. 1993; Altabet and Francois, 1994). These gradients create a geographical isoscape that has been documented in other seabird species using stable isotope analysis (Quillfeldt et al. 2005, Cherel and Hobson 2007; Jaeger et al. 2010)

MDC results suggest a larger population niche in South Georgia than all other populations, and a smaller population niche in the Western Antarctic Peninsula than in the South Shetland Islands (Table 3.2). These results are further supported by SEA₅ values, where the core isotopic niche area in the South Georgia population is significantly larger than the Falkland Islands and the Western Antarctic Peninsula, and the Western Antarctic Peninsula is significantly
smaller than the South Shetland Islands. NND results suggest that individual niches are farther apart from one another in South Georgia than in the Western Antarctic Peninsula. Population niche size appears to be positively correlated with a greater degree of individual niche diversity, which may act to reduce intraspecific competition. Our results agree with several empirical studies on fish species where high population density was associated with greater variation in individual niche diversity (Svanbäck & Persson 2004; Svanbäck et al. 2008; Araújo et al. 2008; Svanbäck & Persson 2009; Frederich et al. 2010).

Individual niche specialization relative to the population

Contrary to our hypotheses, we found consistent evidence of individual specialization and type B generalism within all four of our study populations. Individual specialization indices of δ¹⁵N suggest that individuals in all populations tend to specialize trophically, or on particular prey items, consistent with a type B generalist strategy. Furthermore, all four populations had mean individual relative niche areas that were below 0.07, meaning that individuals in all populations on average utilize under 7% of the total population niche, indicative of type B generalism (Table 3.4). The degree of specialization, however, varies by population. Individual specialization indices of δ¹³C suggest Gentoo Penguin populations found in the South Shetland Islands, South Georgia, and Falkland Islands specialize in foraging location, consistent with type B generalism, whereas individuals in the Western Antarctic Peninsula population do not (Table 3.2). In addition, both individual specialization indices of δ¹⁵N and δ¹³C exhibited the same trend of increasing specialization with the lowest in the Western Antarctic Peninsula, followed by the South Shetland Islands, the Falkland Islands, and South Georgia. This trend somewhat reflects our hypotheses with regards to the northern populations with larger populations and wider diversity of prey resources (South Georgia and the Falkland Islands) being more specialized.
compared to the smaller southern populations with dominant prey sources (Western Antarctic Peninsula and South Shetland Islands). Therefore, although Gentoo Penguins appear to be type B generalists overall, differences in the degree of individual specialization observed in populations of this species may be driven by geographic differences in prey availability and population size.

Studies of Galapagos sea lions (Zalophus wollebaeki) throughout the Galapagos Archipelago suggest a high degree of variation in foraging strategies and individual specialization between populations driven by variation in prey availability and physical oceanographic characteristics (Villegas-Amtmann et al. 2008; Salazar, 2005; Dellinger and Trillmich, 1999; Kooyman and Trillmich, 1986). The waters surrounding the Western Antarctic Peninsula and South Shetland Islands are known for high abundance of Antarctic krill due to the presence of sea ice, which is crucial for the juvenile life stage of krill (Ducklow et al. 2007). It is possible that the presence of a highly available dominant prey source may exert less pressure on individuals to specialize, which would explain the lowest degrees of individual specialization we found in these regions, particularly in the Western Antarctic Peninsula population. Previous dietary studies support these findings that Gentoo Penguin diets are dominated by Antarctic krill in these regions (Miller et al. 2008, 2010). On the contrary, dietary studies of Gentoo Penguins in South Georgia have found variation in both prey choice and foraging habitat in this region. (Tanton et al. 2004; Williams et al 2008; Croxall and Prince 1980). Waters around South Georgia are also known to be abundant with krill, but this is highly variable both seasonally and year to year, which may act as a strong selective pressure for individuals to specialize given the ephemeral nature of prey abundance (Tarling et al. 2007). Therefore, individuals may have to rely on other prey items during times of low krill abundance, resulting in a wider population niche and higher levels in individual specialization, as indicated in our results.
Population size also appears correlated with degree of individual specialization, as individuals in larger populations have been shown to become more specialized, while their population niche increases (Svanbäck and Bolnick 2004; Svanbäck and Persson 2004; Parent et al. 2014). Both the Western Antarctic Peninsula and South Shetland Islands have significantly smaller populations of Gentoo Penguins with corresponding lower levels of individual specialization found in our study. South Georgia and the Falkland Islands have the largest population sizes, with corresponding high indices of individual specialization. This supports previous studies that found increases in population size results in larger population niches and higher degrees of individual specialization to reduce intraspecific competition (Svanbäck & Persson 2004; Svanbäck et al. 2008; Svanbäck & Persson 2009; Frederich et al. 2010). Our results provide evidence to suggest that individual specialization relative to the population may be a density-dependent strategy, in which individuals restrict their foraging niche to reduce intraspecific competition as population size increases and prey resources decrease.

**Individual niche specialization relative to other individuals**

We hypothesized that the two southern populations of Gentoo Penguins would have greater niche overlap between individuals relative to two northern populations. However, only the Western Antarctic Peninsula had a significantly greater degree of individual niche overlap compared to all other populations based on relative niche overlap (Table 3.4; Figure 3.3). This suggests that individuals in the Western Antarctic Peninsula exhibit more overlap in their foraging niches, resulting in less specialization among individuals compared to other three populations. This could be explained by prey resource clustering, or less pressure for resource competition between individuals specific to this region compared to other populations (Sargeant 2007). Although both the Western Antarctic Peninsula and South Shetland Islands are known for
high abundances of krill relative to northern population habitats, prey abundance and availability
does differs between the foraging habitats in the Western Antarctic Peninsula and South Shetland Islands. The Western Antarctic Peninsula is known for a high year round abundance of Antarctic krill (Lascara et al. 1999) which may result in individuals clustering around resources and minimize the need to reduce competition through niche partitioning, as are results demonstrate. 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) (Figure 3.1). These fronts are associated with high densities of Antarctic krill, but with high variability between seasons (Dietrich et al. 2014). Variability in the abundance and availability of krill may select for greater diversity in diet between individual penguins in order to reduce competition, which would explain the lower niche overlap among individuals in the South Shetland Islands. Furthermore, $\delta^{13}$C values vary substantially north and south of convergence zones and fronts (Francois et al. 1993; Altabet and Francois, 1994). This may explain why individuals in the South Shetland Islands exhibit less individual niche overlap in foraging habitat, as some individuals foraging north of these frontal zones would have higher $\delta^{13}$C values than individuals foraging primarily south of the frontal zones, which has been documented in other seabirds (Quillfeldt et al. 2005, Cherel and Hobson 2007; Jaeger et al. 2010)

Significance

Our study is the first to compare and detect population level differences in the degree of individual specialization within a species. Determining the generalist strategy of Gentoo Penguins and detecting any variation in individual specialization between geographically distinct populations may have implications for their responses to environmental change and prey availability in the Scotia Arc. The Antarctic Peninsula and South Shetland Islands are currently
undergoing a drastic climate driven decline in the amount and seasonal duration of sea ice (Stammerjohn et al. 2008, Pritchard et al. 2012). Concurrently, Antarctic krill is also declining in this region due to the loss of sea ice, which is crucial habitat for the juvenile life stage (Ducklow et al. 2007; Trivelpiece et al. 2011). As this principle prey item continues to decline, Gentoo Penguin populations may become more specialized in these regions, similar to those populations in South Georgia and the Falkland Islands. However, Gentoo Penguins in the South Georgia and the Falkland Islands are known to forage on a wider diversity of prey items and its unclear whether the Western Antarctic peninsula and South Shetland Islands harbor a comparable range of prey resources. Therefore, populations and individuals that specialize on krill in South Shetland Islands, Western Antarctic Peninsula may be at higher risk, a risk intensified because their foraging ranges overlap with both commercial krill fisheries and sea ice loss (Miller et al. 2009, 2010; Nicol and Foster 2002; Trivelpiece et al. 2011).

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CHAPTER 4:
GENERAL SUMMARY AND CONCLUSIONS

Populations of Chinstrap and Adélie Penguins have declined significantly in the Western Antarctic region over the past few decades, while Gentoo Penguin population numbers have remained stable and even increased in this region (Lynch, et al. 2012). Many studies have indicated that, during the breeding season, Gentoo Penguins are generalist foragers, while Adélie and Chinstrap tend to specialize on krill within the Western Antarctic Peninsula and South Shetland Islands (Volkman et al. 1980, Lishman, 1985; Karnovsky 1997, Miller et al. 2008, 2009, 2010; Polito et al. 2011b; Polito et al. 2015). These differences in foraging strategies may help to explain differences in population trends among these three species. Decreases in Antarctic krill abundance may be causing the declines we see in Adélie and Chinstrap Penguins, while a generalist foraging strategy exhibited by Gentoo Penguins may act as a buffer to the decline in this principle prey item. However, little is known on temporal consistency in diet and foraging habitat of these species, particularly at the individual level, which can further reveal a more detailed understanding of foraging ecology, as well as provide further information as to how these species may react to ecological pressures. The overall goal of this thesis was to investigate dietary and foraging habitat consistency in *Pygoscelis* species using stable isotope analysis and to identify the drivers of variation in foraging strategies across populations.

In chapter two, we found significant variation in foraging strategies among *Pygoscelis* penguin species in the South Shetland Islands. Gentoo Penguins demonstrated a generalist foraging strategy marked by significant individual specialization and seasonal consistency, Adélie Penguins exhibited an intermediate generalist foraging strategy with little seasonal consistency between individuals, and Chinstrap Penguins appear to be highly specialized in
trophic level and consistent between seasons. These results suggest that Chinstrap Penguins may be at the greatest risk to the decline in Antarctic krill, as they appear to consistently specialize on this prey species between seasons. Although Adélie Penguins exhibited a more variable diet than Chinstrap Penguins, the decrease in Antarctic krill abundance may still be a driving factor in their decline in population numbers, although more research is needed. The seasonal consistency in generalist diet with significant individual variability that we found in Gentoo Penguins may provide further evidence as to why this species appears to be resilient to declines in Antarctic krill.

In contrast, we found similarities in foraging habitat across all three species, with greater variation in foraging habitat at the population level during the non-breeding season compared to the breeding season. This could be due to adult penguins having a more restricted foraging range during the breeding season while they are incubating eggs. We also found evidence of significant variation in foraging strategy between populations of Gentoo Penguins in the Western Antarctic Peninsula and South Shetland Islands, suggesting that foraging ecology may be a product of local geographic and environmental conditions.

In chapter three, we further explored this by comparing foraging strategies of four geographically isolated Gentoo Penguin populations throughout the Scotia Arc, which vary in population size and prey variability and abundance. We found that all four populations exhibited type B generalism, but the degree of individual specialization varied geographically. The smaller southern populations in the Western Antarctic Peninsula and South Shetland Islands had lower degrees of individual specialization than the larger northern populations in South Georgia and the Falkland Islands. This suggests that individual specialization may be mediated by population size as a way to minimize competition between conspecifics. In addition, our results also suggest
that individual specialization may be driven by prey abundance and diversity, as foraging habitat in the southern populations are marked by high abundance of Antarctic krill and low prey diversity, while the northern populations forage on a wider diversity of prey.

Investigating population level foraging strategies in penguins using SIA of multiple tissues representing different time scales has only recently been used (Juàres et al. 2016). However, we are the first to use this method to look at individual consistency in foraging strategies, which provides a more robust understanding of a species’ and population’s ecology. Furthermore, our studies are the first to compare and detect population level differences in the degree of individual specialization within a species. Investigating individual specialization using SIA with serial sampling of metabolically inert tissue has only recently been conducted in very few studies with the vibrissae of marine mammals (Newsome et al 2009, Huckstadt et al 2012, Kerguelan et al 2015; Jaegar et al. 2009). Our results suggest that this method might be useful in other organisms in which serial sampling is possible to quantify temporal changes and confirm generalist strategies through time.

Given the current pressures on Pygoscelis penguins in the Scotia arc such as climate change and the decline in principle prey items such as Antarctic krill (Atkins et al. 2004; Ducklow et al. 2007, Trivelpiece et al. 2011), it would be important to investigate how species may be affected by these large scale changes, and whether they can adapt over time. Future directions of research should include temporal analysis of Pygoscelis penguin foraging strategies by combining the SIA sampling methods from chapter two and three, as well as repeated sampling over multiple years. This would help to understand whether populations and individuals vary or maintain foraging consistency over a larger time scale, as well as help to identify populations that are more at risk to significant environmental changes.
LITERATURE CITED


Lishman, G. S. 1985. The food and feeding ecology of Adélie penguins (Pygoscelis adeliae) and Chinstrap penguins (P. antarctica) at Signy island, South Orkney Islands. Journal of Zoology 205: 245-263.


APPENDIX:
ESTIMATING THE TIMING OF TAIL FEATHER GROWTH

We investigated tail feather growth in a captive population of Gentoo Penguins at SeaWorld in Orlando, Florida in 2012. We monitored ten individual adults (5 males and 5 females) from the start of molt. Following the beginning of molt, we measured the exposed length (cm) of the largest newly growing central tail feather of each individual at approximately 20 day intervals until 100 days after molt when tail feather growth was near completion. We assumed that the duration of feather growth is similar between captive and wild *Pygoscelis*.

We then used PROC NLIN with the Marquardt method in SAS (SAS Institute Inc. 1999) to fit von Bertalanffy growth curves to this dataset. The von Bertalanffy growth curve has been commonly used to model growth in large seabirds (Ricklefs 1967) and is formulated as:

\[ L_t = L_\infty (1 - e^{-k(t-t_0)}) \]

where \( L_t \) is the predicted length (in cm) at time \( t \) (in days), \( L_\infty \) is the mean length that would be reached if feathers grew indefinitely, \( k \) is a growth parameter of dimension time\(^{-1}\), and \( t_0 \) is the hypothetical time (in days) when feather length is zero. We modeled growth curves and compared 95% confidence intervals of parameter estimates, residual mean square error (MSE) and pseudo-\( R^2 \) as measures of goodness of fit. MSE indicated that the von Bertalanffy growth curve fit our data for males slightly better than for females and both sexes combined, while pseudo \( R^2 \) indicated that the curve fit slightly better for females than males and both sexes combined. However, 95% confidence intervals (CI) of parameter estimates overlapped considerably between all three models (Table A.1). Because of this overlap and the small differences between sexes, we used the parameter estimates derived from both sexes combined to estimate the timing of tail feather growth based on length:

\[ L_t = L_\infty (1 - e^{-k(t-t_0)}) \]
\[ t = t_0 - \frac{1}{k} \times \ln \left( \frac{L_t}{L_\infty} \right) \]

where \( t \) is the predicted time (in days) since the start of molt that a section of tail feather at length \( L_t \) was synthesized based on the predicted von Bertalanffy growth parameters \( (L_\infty = 27.2, \ k = 0.01, \ t_0 = 1.7) \); Figure A.1).

Table A.1: Parameter estimates, 95% confidence intervals (in parentheses), residual mean square error (MSE) and pseudo R\(^2\) values estimated from fitted von Bertalanffy growth equations of total feather length (cm) relative to time (days) elapsed since the onset of feather molt.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter Estimates</th>
<th>Fit Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( L_\infty )</td>
<td>( k )</td>
</tr>
<tr>
<td>Males</td>
<td>24.8 (18.6-30.9)</td>
<td>0.01 (0.01-0.02)</td>
</tr>
<tr>
<td>Female</td>
<td>30.5 (19.2-41.7)</td>
<td>0.01 (0.003-0.01)</td>
</tr>
<tr>
<td>Combined</td>
<td>27.2 (21.5-32.9)</td>
<td>0.01 (0.002-0.01)</td>
</tr>
</tbody>
</table>

We applied the above formula to estimate timing of growth for discrete sections of tail feathers that we obtained from individual wild Gentoo Penguins (Table A.2). We sampled discrete sections of the tail feathers for stable isotope analysis by using stainless steel scissors to cut four 1.0cm sections of feather shaft starting at the distal portion of each feather between 5.5-6.5cm, 7.5-8.5cm, 9.5-10.5cm, and 11.5-12.5cm. These sections represent growth occurring between 21-25, 29-33, 38-43, and 48-54 days following the onset of molt. We chose to sample sections starting at 5.5cm in order to avoid sampling feather material grown within the molt period, during which Gentoo Penguins fast for 19.5 days on average (Adams and Brown, 1990).
Table A.2. Estimated timing of growth for tail feather sections using the formula: 
\[ t = t_0 - \frac{1}{k} \times \ln \left(1 + \frac{L}{L_{co}}\right). \] Asterisk (*) indicates tail feather sections used for analysis.

<table>
<thead>
<tr>
<th>Tail feather length (cm)</th>
<th>Day that feather reaches length</th>
<th>Period of growth per 1cm feather section</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>3 days</td>
</tr>
<tr>
<td>0.5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>6</td>
<td>5 days</td>
</tr>
<tr>
<td>2.5</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>13</td>
<td>5 days</td>
</tr>
<tr>
<td>4.5</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>5.5</td>
<td>21</td>
<td>5 days*</td>
</tr>
<tr>
<td>6.5</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>29</td>
<td>6 days*</td>
</tr>
<tr>
<td>8.5</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>9.5</td>
<td>38</td>
<td>6 days*</td>
</tr>
<tr>
<td>10.5</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>11.5</td>
<td>48</td>
<td>7 days*</td>
</tr>
<tr>
<td>12.5</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>13.5</td>
<td>60</td>
<td>7 days</td>
</tr>
<tr>
<td>14.5</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>15.5</td>
<td>73</td>
<td>9 days</td>
</tr>
<tr>
<td>16.5</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>17.5</td>
<td>89</td>
<td>10 days</td>
</tr>
<tr>
<td>18.5</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>19.5</td>
<td>109</td>
<td>13 days</td>
</tr>
<tr>
<td>20.5</td>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>
Figure A.1 Fitted von Bertalanffy growth curve (solid line) and 95% confidence intervals (dotted lines) of total tail feather length relative to time since the onset of feather molt in a captive population of twelve adult (5 male and 5 females) Gentoo Penguins maintained at SeaWorld in Orlando, Florida during 2012.
VITA

Rachael was born in Florida in 1987. She grew up in Sutton, Massachusetts, and received her B.S. in Biology with Departmental Honors from the University of Massachusetts at Amherst in 2009. In 2011, she lived and worked on the remote island atoll of Laysan, located in the Northwestern Hawaiian Islands, for seven months, during which she monitored the reproductive ecology of albatross and developed her passion for seabirds and oceanography. Following this formative experience, she continued to follow her passion for seabirds and worked many positions including Island Manager for the Roseate Tern Recovery program for Massachusetts Division of Fish and Wildlife, avian technician for Alaska Maritime National Wildlife Refuge, and Research Coordinator for the California Least Tern and Snowy Plover Recovery Program with the San Diego Zoo Institute for Conservation Research. She returned to school in 2014 to join Dr. Michael Polito’s lab at Louisiana State University, where she had the incredible opportunity to study penguin foraging ecology in the Antarctic for her Master’s Thesis. She is expected to graduate in 2016.