SPATIAL AND SEASONAL VARIATION IN ADÉLIE PENGUIN DIET AS INFERRED FROM STABLE ISOTOPE ANALYSIS OF EGGSHELL

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Abstract: Spatial and seasonal variation was analyzed in Adélie penguin diets using δ¹⁵N and δ¹³C isotopic values of eggshell collected from different sites in Antarctica. A total of 148 eggshells were collected over 10 sites comprising a significant portion of the Adélie penguin breeding range in the Antarctic Peninsula, northern Ross Sea, central Ross Sea and southern Ross Sea regions. Eggshell samples were then subjected to stable isotope analysis to obtain δ¹⁵N and δ¹³C values to infer Adélie penguin diets and foraging habitats, respectively. A strong correlation between lower δ¹³C values and higher latitudes was found, supporting results of previous studies. The δ¹⁵N values of the eggshells derived from the northern Ross Sea region differed significantly from those of the other three regions, indicating that Adélie penguins in that region relied more on krill than at other locations. Furthermore, δ¹⁵N estimates of diet composition during the pre-breeding season often differed from previous studies that estimated diet composition during the breeding season, implying that there are likely dietary changes after the onset of breeding at many locations.

Key Words: Adélie penguin; Pygoscelis adeliae; stable isotope analysis; diet.

INTRODUCTION

Stable Isotope Analysis

Stable isotope analysis (SIA) is an investigative method that uses intrinsic markers to provide dietary information on an organism (Hobson 1999). SIA relies on the assumption that the isotopic content of the prey is transferred to the tissues of the consumer upon consumption (Hobson 1995). It has several advantages over conventional methods, such as analysis of stomach contents, because it is noninvasive, it is not biased towards less digestible food, and it allows the study of diets outside of the breeding season (Quillfeldt et al. 2005). SIA is very useful in studying avian species, which often have wide geographical ranges that make them more difficult to study (Emslie and Patterson 2007).

Carbon and nitrogen isotopes are commonly used in dietary studies (Hobson and Clark 1992a,b) and δ¹³C has been shown to indicate foraging location. Values of δ¹³C in marine food webs can indicate horizontal trends, such as inshore versus offshore feeding, and vertical trends, such as benthic versus pelagic feeding. Values of δ¹³C have also been shown to be more enriched in lower latitude areas (Cherel and Hobson 2007). We focus on δ¹³C mainly as an indicator of latitude. Alternatively, δ¹⁵N values are used primarily as indicators of prey trophic level because of the δ¹⁵N enrichment of approximately 3% that occurs for each step up in trophic level (Hobson and Clark 1992a). Krill occupy a lower trophic level than fish, so their tissues are depleted in δ¹⁵N compared to fish (Ainley et al. 2003).

Stable isotope analysis was used in this case to analyze the diet of Adélie penguins (Pygoscelis adeliae) for spatial and seasonal differences. The δ¹⁵N and δ¹³C isotopic values of eggshell collected from different sites in Antarctica were compared. Isotopic content of the eggshells provided information on pre-breeding season diet of females and, when compared with findings of previous studies on breeding season diet, was able to determine if diets change after the onset of breeding.

Penguin Ecology

The Adélie penguin is a well studied bird, but very little is known about its habitat and feeding behavior during the winter months as compared to summer months. These penguins migrate during the summer to their natal colonies for the breeding season (Ainley 2002). They are central place foragers while at their breeding colonies, feeding in areas immediately surrounding their breeding grounds. This pattern of behavior makes it easier for researchers to conduct studies on them in the summer than in the winter. These penguins are no longer confined during the winter months by their breeding sites, have a widespread distribution, and their diving habits make them difficult to observe while foraging (Cherel et al. 2007). Adélie penguins are an important animal to study because they play a crucial role in the delicate ecosystem of Antarctica and can serve as indicators for changes in the flow of energy through the food web (Lynnes et al. 2004).

Breeding

Adélie penguins spend more than 90% of their lives at sea, but they must come ashore for breeding in the
austral spring, usually around October (Ainley 2002). Upon completion of her clutch, which usually consists of two eggs, the female leaves to forage and the male takes over incubation of the eggs and guarding of the nest (Vleck and Vleck 2002). Males and females trade off responsibility of the nest for foraging trips and the chicks usually hatch after 30 to 39 days of incubation. The chick rearing period then begins and the parents must then provide food for their chicks (Ainley 2002).

Diets

Adélie penguin diet consists almost exclusively of krill and fish (Ainley 2002). Dominant species of krill consumed differs regionally, with Antarctic krill (Euphausia superba) dominating in pelagic waters such as the foraging grounds in the Antarctic Peninsula region, and Crystal krill (Euphausia crystallorophias) dominating in neritic waters such as the foraging grounds in the Ross Sea. Antarctic silverfish (Pleuragramma antarcticum) is the main species of fish in Adélie penguin diet. Fish and krill differ in their isotopic signatures because of the different trophic levels that they occupy, making them distinguishable from each other in isotopic studies (Quillfeldt 2005).

High krill availability has been demonstrated after winters of extensive pack ice, but in years with restricted pack ice and low krill availability Adélie penguins have been shown to rely more heavily on fish in their diets (Ainley 2002). This allows Adélie penguin diet to be used as an indicator of krill availability, and when studies such as these are analyzed alongside concurrent changes in the level of sea ice, effects of global warming can also be predicted (Fraser and Hofmann 2003).

METHODS

Eggshells were chosen as the tissue to be used for SIA because they were widely available and their collection did not require the disturbance of penguins. Isotopes in avian eggshell originate from the diet of the female immediately prior to egg laying, so eggshell is an appropriate tissue for SIA in studies of pre-breeding season diet (Schaffner and Swart 1991). Isotopes absorbed directly from recent diet are rapidly incorporated into formation of the eggshell. The organic matrix of the eggshell, which was the portion of the eggshell used for SIA in this dietary study, is synthesized from proteins in the diet or body reserves of the laying female (Emslie and Patterson 2007).

Sample Collection

Hatched, depredated, addled, or infertile Adélie penguin eggs were opportunistically collected between 2000 and 2007 at 10 sites in Antarctica: King George Island (n = 30), Cape Bird (n = 10), and Cape Crozier Island (n = 10) in the southern Ross Sea, Adélie Cove (n = 10), Edmonson Point (n = 10), and Inexpressible Island (n = 10) in the central Ross Sea, and Cape Hallett (n = 15) in the northern Ross Sea (Fig. 1). Care was taken to ensure that each sample collected was from a different egg, that the eggs were from the site being studied, and that all eggs were those of Adélie penguins and not of another avian species (Fig. 1).

Sample Preparation

Eggshell membranes were removed using a Dremel tool with a sanding attachment. Eggshells were then soaked in distilled water and cleaned of surface debris and ground into small fragments (~2 mg) using an analytical mill. Inorganic calcium carbonate from the eggshell was removed through a process of acidification. Approximately 10 mg of cleaned eggshell fragments were dissolved in a silver capsule (9 mm × 5 mm) using the drop-by-drop method employed by Jacob et al. (2005). A minimum of four 20 µL aliquots of 6 N HCl were added, one at a time after each aliquot stopped bubbling. Acid was then evaporated at room temperature under a fume hood for 24 hr, and then for 48 hr in an oven at 50°C.

Sample Analysis

The prepared samples were analyzed for their carbon and nitrogen isotope content (δ¹³C and δ¹⁵N) using isotope ratio mass spectrometry by combusting them to a gaseous state using an elemental analyzer and then processing via a continuous flow stable isotope ratio mass spectrometer. A raw ratio of the heavy to the light isotope was measured and these ratios were normalized to VPDB for δ¹³C and AIR for δ¹⁵N using USGS-40, USGS-41, and glutamic acid reference materials (Jardine and Cunjak 2005). Stable isotope values follow the δ notation and were derived from the following equation: δ_X = [(R_sample/R_standard) - 1] × 1,000, where X is ¹³C or ¹⁵N and R is the ratio of ¹²C/¹³C or ¹⁴N/¹⁵N.

Statistical Analyses

Variation in δ¹³C and δ¹⁵N between and within regions that contained more than one study site were analyzed using one-way ANOVA. The sources of variation were then determined using a Tukey-Kramer Multiple-Comparison Test. Linear regression was used to analyze the relationships of δ¹³C and δ¹⁵N with latitude. Data were examined for normality and equal variance and comparable non-parametric methods were employed when necessary. Statistical significance was assumed at p < 0.05 for all tests. All statistical analyses were performed using NCSS statistical analysis and graphics software.
RESULTS

The average $\delta^{13}C$ and $\delta^{15}N$ values obtained for each site exhibited separation, indicating that there are differences in foraging tendencies of Adélie penguins between sites and regions (Table 1; Fig. 2).

Variation between regions

There were significant differences in $\delta^{15}N$ values among the five regions studied ($F_{1,148} = 50.84$, $p < 0.0001$). The northern Ross Sea region caused most of this variability, indicating dramatically different $\delta^{15}N$ values than all of the other regions. The Antarctic Peninsula and southern Ross Sea regions also differed significantly from each other in their $\delta^{15}N$ values.

Significant differences also were found for $\delta^{13}C$ values between the four regions (Kruskal-Wallis One-Way ANOVA, $F_{3,148} = 60.00$, $p < 0.0001$). The Antarctic Peninsula differed from all other regions and the central Ross Sea region also differed from most of the other regions. The northern and southern Ross Sea regions also differed from the central Ross Sea region. The differences in $\delta^{13}C$ values between the regions seem to be driven by latitude (Kruskal-Wallis Multiple-Comparison $Z$-Value Test).
Variation within regions

The three sites in the Antarctic Peninsula region demonstrated significant differences in their $\delta^{15}$N values ($F_{2,73} = 8.17, p < 0.001$). Anvers Island and Devil Island were similar, but King George Island differed from both sites. There were also significant differences in the $\delta^{15}$N values at the three sites in the southern Ross Sea region ($F_{2,30} = 15.77, p < 0.0001$). Cape Bird and Cape Crozier were similar, but Beaufort showed differences to both sites. No significant differences in the $\delta^{15}$N values existed in the three sites in the central Ross Sea region ($F_{2,30} = 0.40, p = 0.7$). The northern Ross Sea region only contained one study site, so variability within this region could not be analyzed.

Correlations with Latitude

No significant correlation was found between $\delta^{15}$N and latitude ($F_{1,10} = 0.65, R^2 = 0.08, p = 0.4$; Fig. 3A). However, a significant negative correlation was found between average $\delta^{13}$C values and latitude of sites ($F_{1,10} = 8.27, R^2 = 0.51, p = 0.02$; Fig. 3B).

**DISCUSSION**

Eggshell isotopic values provide information on the diet of female Adélie penguins prior to their arrival at the breeding colony. These results can be compared to previous dietary studies (Table 2) conducted during the breeding season to determine if there are differences between breeding season and pre-breeding season diet. Overall, our findings do not correlate well with previous dietary studies at these same sites during the breeding season. These results indicate that Adélie penguins at many locations are likely to exhibit a dietary shift after the onset of breeding.

**Diet composition**

Comparing relative $\delta^{15}$N levels in eggshells from different regions, the diet of Adélie penguins was determined to rely more on fish or on krill in those areas. Higher $\delta^{15}$N levels implied feeding at a higher trophic level (i.e., fish) as compared to feeding at a lower trophic level (i.e., krill).

Adélie penguins in the northern Ross Sea fed significantly more on krill than Adélie penguins in all of the other regions studied because much lower $\delta^{15}$N values were obtained for the eggshells derived from the northern Ross Sea region. This result somewhat agrees with a previous study in the northern Ross Sea at Cape Hallett where penguin diet consisted of 97% krill (Logan in Puddicombe and Johnstone, 1988). There were also significantly lower $\delta^{15}$N values in the southern Ross Sea.

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**Table 1.** Eggshell sample sizes with means and standard deviations (SD) given in % for $\delta^{13}$C and $\delta^{15}$N values for each site by region.

<table>
<thead>
<tr>
<th>Region</th>
<th>Site</th>
<th>Sample Size</th>
<th>Mean of $\delta^{13}$C</th>
<th>SD of $\delta^{13}$C</th>
<th>Mean of $\delta^{15}$N</th>
<th>SD of $\delta^{15}$N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antarctic Peninsula</td>
<td>Anvers Island</td>
<td>30</td>
<td>-24.02</td>
<td>0.46</td>
<td>8.67</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>Devil Island</td>
<td>30</td>
<td>-24.68</td>
<td>0.42</td>
<td>8.80</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>King George Island</td>
<td>13</td>
<td>-24.49</td>
<td>0.39</td>
<td>7.93</td>
<td>0.56</td>
</tr>
<tr>
<td>Central Ross Sea</td>
<td>Adélie Cove</td>
<td>10</td>
<td>-24.80</td>
<td>0.35</td>
<td>8.16</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>Edmonson Point</td>
<td>10</td>
<td>-24.68</td>
<td>0.41</td>
<td>8.25</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Inexpressible Island</td>
<td>10</td>
<td>-24.82</td>
<td>0.42</td>
<td>8.34</td>
<td>0.39</td>
</tr>
<tr>
<td>Northern Ross Sea</td>
<td>Cape Hallett</td>
<td>15</td>
<td>-25.57</td>
<td>0.32</td>
<td>5.99</td>
<td>0.78</td>
</tr>
<tr>
<td>Southern Ross Sea</td>
<td>Beaufort</td>
<td>10</td>
<td>-25.69</td>
<td>0.27</td>
<td>6.68</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Cape Bird</td>
<td>10</td>
<td>-25.25</td>
<td>0.29</td>
<td>8.12</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Cape Crozier</td>
<td>10</td>
<td>-25.49</td>
<td>0.31</td>
<td>8.44</td>
<td>0.75</td>
</tr>
</tbody>
</table>

**Fig. 2.** Variation between $\delta^{13}$C and $\delta^{15}$N values of Adélie penguin eggshells for each region sampled. Mean values are shown with error bars representing standard deviation for Antarctic Peninsula ($\delta^{13}$C: $-24.37 \pm 0.52$, $\delta^{15}$N: 8.59 ± 0.72), central Ross Sea ($\delta^{13}$C: $-24.77 \pm 0.39$, $\delta^{15}$N: 8.25 ± 0.42), northern Ross Sea ($\delta^{13}$C: $-25.57 \pm 0.32$, $\delta^{15}$N: 5.99 ± 0.78), and southern Ross Sea ($\delta^{13}$C: $-25.48 \pm 0.33$, $\delta^{15}$N: 7.74 ± 1.06) regions.
than in the Antarctic Peninsula, implying that the Adélie penguins in the southern Ross Sea fed slightly more on krill than those in the Antarctic Peninsula. Previous studies completed during the breeding season do not support these findings, instead revealing that Adélie penguins in the Antarctic Peninsula were feeding more on krill than those in the southern Ross Sea (Table 2).

Beaufort and Cape Hallet eggshells had the lowest $\delta^{15}$N values of all study sites, indicating that diets of penguins breeding at these sites had the highest percentage of krill in their diets. Previous studies during the breeding season did not agree with this finding and discovered the highest percentage of krill in the diets of Adélie penguins at Cape Bird and King George Island (Table 2).

The three sites within the central Ross Sea were not significantly different in $\delta^{15}$N isotopic values, suggesting that Adélie penguin diet in this area was homogenous. It is possible that similarities in these isotope values existed because these sites are in the same water mass and likely share similar diets (Ainley et al. 2003).

A significant difference was established between $\delta^{15}$N values at Beaufort and the other two sites in the southern Ross Sea region, Cape Crozier and Cape Bird. These results contrast with Ainley et al.’s (2003) findings that there were no significant differences in the isotope values between the three colonies during the breeding season. A possible explanation for this difference is that in the pre-breeding season these populations of penguins have different diets than they do when they return to their breeding colonies. During the breeding season, which is when Ainley’s study was performed, the diets may become more homogenous because the birds are all feeding in a more restricted area immediately around their breeding grounds. During the breeding season these penguins are also more likely to be feeding in the same water mass and utilizing the same kinds of food sources.

Eggshells from King George Island had lower $\delta^{15}$N values than those from Anvers Island and Devil Island in the Antarctic Peninsula region, suggesting that Adélie penguins that breed at King George Island ate more krill. Literature values support our findings that penguin diets at King George Island are composed of more krill than diets at Anvers Island and Devil Island (Table 2).

Table 2. Summary of the composition of Adélie penguin diets at various sites as determined from previous dietary studies.

<table>
<thead>
<tr>
<th>Site</th>
<th>Diet Composition</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>King George Island</td>
<td>E. superba 99% euphausiads 96% mostly notothienid fish with some E. superba</td>
<td>Volkman et al. 1980 Eklund 1945 in Puddicombe and Johnstone 1988</td>
</tr>
<tr>
<td>Devil Island</td>
<td>E. superba 99% fish 4% mostly krill</td>
<td>Coria et al. 1995 Logan in Puddicombe and Johnstone 1988</td>
</tr>
<tr>
<td>Anvers Island</td>
<td>E. superba 99% fish 4% mostly krill</td>
<td>Eklund 1945 in Puddicombe and Johnstone 1988 Clarke et al. 1998</td>
</tr>
<tr>
<td>Terra Nova Bay (Adelie Cove, Edmonson Point, and Inexpressible Island)</td>
<td>mostly krill</td>
<td>Emison 1968 in Ainley 2002</td>
</tr>
<tr>
<td>Cape Hallett</td>
<td>krill 97% fish 2%</td>
<td>Emison 1968 in Ainley 2002</td>
</tr>
<tr>
<td>Beaufort Is</td>
<td>E. crystallorophias 64% P. antarcticum 32%</td>
<td>Emison 1968 in Ainley 2002</td>
</tr>
<tr>
<td>Cape Bird</td>
<td>E. crystallorophias 99%</td>
<td>van Heezik 1988</td>
</tr>
<tr>
<td>Cape Crozier</td>
<td>E. crystallorophias 60% P. antarcticum 39%</td>
<td>Emison 1968 in Ainley 2002</td>
</tr>
</tbody>
</table>
Since we only analyzed diets of female Adélie penguins, which other investigators have shown to feed more heavily on krill than males (Clarke et al. 1998), bias towards female diets should be taken into consideration when interpreting the diet of these populations as a whole. Male diets would have higher $\delta^{15}N$ values if they consumed a higher proportion of fish.

Foraging area

Eggshell samples from sites at higher latitudes had lower $\delta^{13}C$ values and ones that were derived from sites at lower latitudes had higher $\delta^{13}C$ values (Fig. 3). This verified the findings of Quillfeldt et al. (2005) who established a linear correlation between $\delta^{13}C$ and latitude. Cherel and Hobson (2007) proposed that $\delta^{13}C$ values are not necessarily related to latitude in a linear fashion, but instead abrupt changes in $\delta^{13}C$ values occur at certain fronts where water masses change. If true, this could explain some of the slight departures from the linear relationship in our study.

Cherel et al. (2007) found that penguins consumed the same prey throughout the year, but that there were larger variances in $\delta^{13}C$ during winter than in summer. This implied that prey is more variable during the pre-breeding season, which also may explain some of the variation in the $\delta^{13}C$ values. Once the penguins arrive at their breeding colonies their range is limited and a narrower range of $\delta^{13}C$ values would be expected.

Fractionation is the modification of an isotopic signal from the tissues of the prey to the tissues of the consumer (Rau et al. 1992). Fractionation values have been shown to differ among species, diets, stress levels, and tissue types (Hobson and Clark 1992b). The isotopic fractionation factor for Adélie penguin eggshell has not yet been established, so we could not determine the exact percent composition of krill and fish in the diet of Adélie penguins. The composition of diets (inferred by $\delta^{15}N$ values) could only be compared at each site relative to each other because of this limitation. Future studies need to determine the fractionation factor for Adélie penguin eggshell so that more accurate estimates of diet composition can be made from isotopic values. Standardized methods need to be established for reporting diet composition so that results of different studies can be compared more easily.

While it is likely that variation in pre-breeding diets does exist between years, we did not test for this in the current study. Eggshells studied were collected over multiple years, and thus we relied upon the assumption that variation in isotope values between the different regions would outweigh the annual variation in isotope values. Future work examining seasonal variation in isotope values are needed to examine if inter-annual change in diet might have an effect on the regional trends we observed in this study.

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LITERATURE CITED


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