Penguin eggshell membranes reflect homogeneity of mercury in the marine food web surrounding the Antarctic Peninsula

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Abstract
Remote regions such as the Antarctic have become increasingly important for investigations into far-reaching anthropogenic impacts on the environment, most recently in regard to the global mercury cycle. Spatial patterns of mercury availability in four regions of the Antarctic Peninsula were investigated using three species of sympatrically breeding Pygoscelis penguins as biomonitors. Eggshells with intact membranes from Adélie, Gentoo, and Chinstrap penguins were examined from 24 breeding colonies in the South Orkney Islands, South Shetland Islands, eastern Antarctic Peninsula, and western Antarctic Peninsula during the 2006/2007 austral summer. In addition, we compared eggshell membrane mercury concentrations with eggshell stable isotope values (δ13C and δ15N) to determine if species-specific trophic or foraging habitat preferences influenced female mercury exposure prior to breeding. With few exceptions, mercury concentrations were found to be fairly homogeneous throughout the Antarctic Peninsula, suggesting little spatial variation in the risk of exposure to dietary mercury in this food web. Mercury concentrations in Gentoo and Adélie penguins were similar while Chinstrap penguins tended to have higher eggshell membrane mercury concentrations than their congeners. However, inter and intra-specific differences in eggshell membrane mercury concentration were not related to eggshell δ13C or δ15N values, a likely result of all three species foraging at similar trophic positions. The lack of regional-scale differences in mercury availability in this marine ecosystem may be a reflection of generally uniform atmospheric deposition and upwelling of regionally homogeneous deep water rather than from geographically distinct point sources.

1. Introduction
As our understanding of the long-range transport of environmental contaminants has grown, remote regions such as Antarctica have become increasingly important in far-reaching anthropogenic impacts on the environment (Szefer et al., 1993; Sun et al., 2006; Yin et al., 2007; Bargagli, 2008). For example, contaminants such as PCBs and other organochlorines were first detected in Antarctic avifauna and marine mammals in the mid-1970s, sparking ecotoxicological investigations into the transport and fate of anthropogenically-derived contaminants in this “pristine” ecosystem (Riseborough et al., 1976; Court et al., 1997). Elevated levels of DDT and DDE have been found in penguins tissues throughout the Antarctic (Sladen et al., 1966; Court et al., 1997; Geisz et al., 2008). While several studies have documented the accumulation of mercury in Antarctic biota, our understanding of mercury availability in this marine food web is often restricted to studies conducted on a small geographic scale or with limited sample sizes (for examples see Becker et al., 2002; Aubail et al., 2011).

Penguins are suitable biomonitors for mercury availability in the Antarctic marine food web as they are the dominant avifauna in Antarctica, nesting in large, readily accessible colonies and have previously been shown to accumulate biologically relevant concentrations of mercury (Bargagli et al., 2000; Ainley, 2002; Ancora et al., 2002; Becker et al., 2002; Scheiffler et al., 2005; Metcheva et al., 2006). In the Antarctic Peninsula (AP), the three Pygoscelis penguin species consume predominantly Antarctic krill (Euphausia superba) and small amounts of fish during the breeding season (Volkman et al., 1980; Ainley, 2002; Miller et al.,

HIGHLIGHTS
► We examined regional patterns of mercury availability in the Antarctic Peninsula.
► Three species of Pygoscelis penguins were used as biomonitors.
► Chinstrap penguins tended to have higher mercury than Adélie and Gentoo penguins.
► Mercury concentrations were fairly homogeneous throughout the Antarctic Peninsula.

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Antarctic krill are the dominant primary consumers in this food web, occupy a lower trophic level, and generally have lower tissue mercury concentrations in comparison to most penguin prey fish species (Rau et al., 1992; Barrera-Oro, 2002; Brasso et al., 2012). However, a recent study using stable isotope analysis of eggshell suggests the pre-breeding diet of Gentoo (Pygoscelis papua) and Adélie (P. adeliae) penguins can contain a significant proportion of fish (Polito et al., 2011a). Thus these species may be foraging at a higher trophic level and at a more elevated risk of exposure to mercury than data on breeding season diet alone would suggest. In addition, species-specific differences in foraging preferences may also play a significant role in mercury exposure. While Adélie and Chinstrap (P. antarctica) penguins are both migratory during the winter, the latter species tends to feed on more mesopelagic fish (primarily in the family Myctophidae; Miller and Trivelpiece, 2008) whereas Adélie penguins feed on offshore pelagic fish (primarily in the family Nototheniidae; Karnovsky, 1997). In contrast, Gentoo penguins typically remain close to their breeding colonies year round and forage in nearshore benthic habitats (Williams, 1995; Ainley, 2002; Miller et al., 2005). Even so, risk of exposure to mercury could be independent of foraging behavior, trophic position or habitat use as environmental conditions vary among breeding colonies and wintering areas due to factors such as ocean circulation, coastal currents and bottom topography which could ultimately lead to spatial heterogeneity of mercury availability.

The purpose of the present study was to investigate inter-species and spatial patterns of mercury availability across the AP using eggshell membrane samples from three species of sympatrically breeding Pygoscelis penguins. Though most studies utilize homogenized albumen and yolk to determine the mercury concentration of an egg (as an indicator of female dietary mercury exposure; Evers et al., 2003), the accessibility and transport of whole eggs at penguin colonies can be logistically impractical while eggshells with intact membranes are abundant and easy to collect. Further, eggshell and eggshell membrane have been shown to reliably document differences in dietary mercury exposure in female birds (Morera et al., 1997; Akearok et al., 2010; Brasso et al., 2012). When utilizing eggshell membrane from these species it was important to consider the timing of egg laying relative to dietary uptake of mercury to accurately interpret the source of the mercury signature. The mercury concentration in egg tissues of Adélie and Chinstrap penguins reflects stored energy reserves rather than recent dietary uptake as these species spend the winter at sea and fast for ≤2 weeks during migration and prior to egg laying (Asheimer and Grau, 1983; Trivelpiece and Trivelpiece, 1990). Thus, mercury concentration in egg tissues in these two species are reflective of late-winter, pre-breeding dietary exposure to mercury while mercury signatures from non-migratory Gentoo penguins, only fasting for approximately 5 days prior to egg laying (Trivelpiece and Trivelpiece, 1990; Davis and Renner, 2003), are more reflective of mercury availability at the breeding colony. Therefore, the use of all three species as biomonitor allows the investigation into mercury exposure near breeding colonies as well as winter exposure at sea.

Our study had two main objectives: (1) to examine spatial patterns of mercury availability in the AP by comparing intraspecific mercury levels in Pygoscelis penguins breeding in the South Orkney Islands, South Shetland Islands, and the eastern and western AP, and (2) to assess interspecific differences in dietary mercury exposure. To complement these objectives, we compared eggshell membrane mercury concentrations to eggshell stable isotope values to determine if spatial and/or species-specific variation in Pygoscelis penguin trophic position (δ15N) and foraging habitat (δ13C) influences female mercury exposure prior to breeding.

2. Methods

2.1. Study site and sample collection

Eggshells were collected from 24 Adélie, Chinstrap, and Gentoo penguin colonies throughout the AP during the austral summer of 2006/2007 (November 2006 to February 2007). The AP can be divided into four geographic regions across which the ranges of these three penguin species overlap: the South Orkney Islands, South Shetland Islands, the eastern AP and western AP (Fig. 1, Table S1). Samples were collected by researchers aboard the National Geographic Endeavor tour ship and several field stations in the AP; approximately 10–20 eggshells from hatched, depredated, or abandoned eggs were opportunistically collected from each species in a given breeding colony. In mixed species colonies, only eggshells found in close proximity to nests in single species areas of the colony were collected to ensure accurate identification. Eggshells were cleaned of organic debris in the field using a toothbrush and water then air dried and stored in individually labeled plastic bags until preparation for mercury and stable isotope analyses.

2.2. Mercury analysis

In preparation for mercury analysis, approximately 0.20 g of membrane was removed from each eggshell by soaking a large fragment of the eggshell in deionized water and using a stainless steel scalpel to completely separate the membrane from the shell. The eggshell membrane was scrubbed with a toothbrush and deionized water to remove any remnants of albumen or yolk to ensure an accurate mercury signal from this tissue. All eggshell membranes were allowed to air dry under a fume hood for 24 h and stored in Ziploc bags until mercury analysis. Eggshell membranes were analyzed for total mercury via atomic absorption spectrophotometry on a Direct Mercury Analyzer DMA-80 at the College of William and Mary (Williamsburg, VA, USA). Because nearly all mercury in avian eggs is in the form of methylmercury, a measurement of total mercury concentration was used as a proxy for this highly bioavailable form (Bond and Diamond, 2009). Each set of 20 samples analyzed was preceded and followed by two method blanks, a sample blank, and two samples each of standard reference material (DORM-3, DOLT-4; fish protein, and dogfish liver certified reference materials, respectively, provided by National Research Council Canada). Total mercury concentrations are reported as parts per million (ppm) fresh weight (fw). Mean percent recoveries for standard reference materials were 95.0±2.9% (DORM-3) and 96.8±2.3% (DOLT-4). The relative percent difference between 26 pairs of duplicate samples was 0.69±0.54%. Detection limit of the assay was 0.005 ng mercury.

2.3. Stable isotope analysis

We added eggshell stable isotope values (δ13C and δ15N) from Chinstrap penguins to an existing dataset of Adélie and Gentoo penguin eggshell stable isotope values to complement the results of our mercury analysis (see Polito et al., 2011a). The organic fraction of penguin eggshell reflects the same dietary signature as eggshell membrane and is thus a suitable tissue for comparison with eggshell membrane mercury concentration (Polito et al., 2009).

Prior to stable isotope analysis eggshell membranes were removed from the shell using a Dremel tool with a sanding attachment. Eggshells were rinsed in distilled water, cleaned of remaining surface debris, and ground to a powder using an analytical mill. Eggshells were acidified prior to analysis to remove carbonates by dissolving ~10 mg of cleaned eggshell in a silver capsule through titration with 50 μl aliquots of 6 N HCl. Acidified samples were stored at room temperature under a fume hood for 24 h, then dried for at least 48 h in an oven at 60 °C. Acidified samples were not rinsed prior to drying and isotopic analysis to avoid biasing δ15N values (Jacob et al., 2005). The mean C:N value of acidified eggshells was 3.8±0.2, which closely approached the assumed C:N value for pure protein of 3.7 (Fry et al., 2003).

Approximately 5 mg of eggshell was loaded into tin cups, flash-combusted, and analyzed for δ13C and δ15N through an interfaced Thermo Delta V Plus continuous-flow stable isotope ratio mass spectrometer. Raw δ values were normalized on a two-point scale using depleted and enriched glutamic acid standard reference materials United States
Geological Survey (USGS)-40 ($\delta^{13}C$: $-26.389 \pm 0.042$; $\delta^{15}N$: $-4.5 \pm 0.1$) and USGS-41 ($\delta^{13}C$: $37.626 \pm 0.049$; $\delta^{15}N$: $47.6 \pm 0.2$). Sample precision based on duplicate standard and sample materials was 0.1% and 0.2% for $\delta^{13}C$ and $\delta^{15}N$, respectively. Stable isotope abundances are expressed using a $\delta$ notation in per-milliliter units (‰) based on the following equation: $\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$; where $X$ is $^{15}N$, and $R$ is the corresponding ratio of $^{15}N$: $^{14}N$ or $^{13}C$: $^{12}C$. The $R_{\text{standard}}$ value was based on Vienna PeeDee Belemnite (VPDB) for $^{13}C$ and atmospheric $N_2$ for $^{15}N$.

2.4. Statistical analysis

Mercury concentrations were log transformed to produce data with a more normalized distribution; log-transformed values were used in all statistical analyses described below. Comparisons of mercury concentration in eggshell membranes and $\delta^{13}C$ and $\delta^{15}N$ of eggshells were conducted by grouping samples by species (Adélie, Gentoo, and Chinstrap) and region (South Orkneys, South Shetlands, eastern AP, and western AP) in separate univariate ANOVAs with 1 factor and 10 levels (1 level for each species/region combination as not all species were present in each region) using SPSS software (version 16.0; Chicago, IL). Tukey’s HSD was used for post hoc comparisons. This design allowed for comparison among species within each region as well as with conspecifics among regions.

We used a generalized linear modeling approach (Proc GLM) to investigate the relative influence of species, region, trophic position ($\delta^{15}N$), and foraging habitat ($\delta^{13}C$) on mercury concentration using SAS (Version 9.1, SAS Institute 1999). We parameterized a global model using log transformed mercury as the dependent variable, species and region as grouped factors, $\delta^{15}N$ and $\delta^{13}C$ as covariates, and all two-way interaction terms. All possible model subsets of our global model were compared using Akaike Information Criteria (AIC; Akaike, 1973). The model with the lowest AIC score was selected as the model most strongly supported by the data. Models with $\Delta$AIC scores (difference in AIC between a given model and the model with the lowest AIC) $\leq 2.0$ were considered competitive with the most strongly supported.
model and any model with a ΔAIC ≤ 10.0 was considered well supported. Model fits were further assessed by AIC weight (ωi) which is a measure of the relative likelihood that a given model is the best among a set of models fitted (Burnham and Anderson, 2002). As not all species were present in each region the effects of species and region were potentially confounded. Therefore, we did not assess model subsets that included both species and region as factors or their interaction. All means are presented ± SD and statistical significance was defined at p < 0.05.

3. Results

3.1. Intra- and interspecific variation in eggshell membrane mercury concentration

Significant differences in mercury were detected among species/region combinations (F9,457 = 37.69, p < 0.001; Table 1). However, post-hoc comparisons indicated that only Chinstrap penguins showed significant differences in mercury across the three regions in which they were sampled. Mercury in Chinstrap penguins breeding in the South Orkney Islands was significantly lower than Chinsprats in the South Shetland Islands and the western AP (Table 1). In contrast, for both Gentoo and Adélie penguins no differences in eggshell membrane mercury concentration were detected across sampling regions. When averaged across all regions, Chinstrap penguins (0.070 ± 0.056 ppm) generally had higher eggshell membrane mercury concentrations than Gentoo (0.016 ± 0.016 ppm) and Adélie penguins (0.023 ± 0.020 ppm; Fig. 2). Furthermore, when examined within regions inter-specific comparisons found that Chinstrap penguins had higher mercury concentrations than Gentoo and Adélie penguins in two of the three regions of the AP in which they overlap (Table 1). No differences in mercury were detected between Adélie and Gentoo penguins in the South Shetland Islands or the eastern AP, while mercury concentrations in Adélie penguins were significantly higher than Gentoo penguins in the western AP (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Region, species</th>
<th># Sites</th>
<th>n</th>
<th>Hg (ppm)</th>
<th>δ15N (%)</th>
<th>δ13C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Orkney Islands</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adélie penguin</td>
<td>2</td>
<td>30</td>
<td>0.028 ± 0.016&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.3 ± 0.8&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>-24.8 ± 0.5&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chinstrap penguin</td>
<td>1</td>
<td>14</td>
<td>0.018 ± 0.010&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.2 ± 0.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>-24.1 ± 0.4&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>South Shetland Islands</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adélie penguin</td>
<td>1</td>
<td>15</td>
<td>0.015 ± 0.008&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.9 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-24.5 ± 0.4&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chinstrap penguin</td>
<td>5</td>
<td>69</td>
<td>0.083 ± 0.054&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.8 ± 0.7&lt;sup&gt;de&lt;/sup&gt;</td>
<td>-24.1 ± 0.6&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gentoo penguin</td>
<td>6</td>
<td>84</td>
<td>0.015 ± 0.009&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.7 ± 0.8&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>-23.5 ± 0.5&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Eastern Antarctic Peninsula</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Adélie penguin</td>
<td>5</td>
<td>73</td>
<td>0.019 ± 0.017&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.2 ± 0.6&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>-24.8 ± 0.6&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gentoo penguin</td>
<td>1</td>
<td>14</td>
<td>0.025 ± 0.017&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.6 ± 0.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>-24.5 ± 0.5&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Western Antarctic Peninsula</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adélie penguin</td>
<td>5</td>
<td>74</td>
<td>0.026 ± 0.022&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.9 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-23.9 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chinstrap penguin</td>
<td>1</td>
<td>10</td>
<td>0.052 ± 0.049&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.4 ± 0.5&lt;sup&gt;de&lt;/sup&gt;</td>
<td>-24.4 ± 0.2&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gentoo penguin</td>
<td>6</td>
<td>74</td>
<td>0.013 ± 0.009&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.9 ± 0.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>-23.1 ± 0.5&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
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</table>

3.2. Intra- and interspecific variation in eggshell stable isotope values

Significant differences in eggshell δ15N and δ13C values were detected among species/region combinations (δ15N: F9,457 = 9.93, p < 0.001; δ13C: F9,457 = 47.65, p < 0.001). Adélie penguin eggshell δ15N and δ13C values were higher in the western AP relative to other regions, while Gentoo penguin eggshell δ15N values were higher in the South Shetland Islands relative to other regions (Table 1). Gentoo penguin eggshell δ15N values did not differ among regions. Chinstrap penguin eggshell δ15N values were higher in the South Orkney Islands relative to the western AP, while δ15C values did not differ across sampling regions. When averaged across all regions, eggshell δ15N values were similar among species (Chinstrap: 8.8 ± 0.7%; Gentoo: 8.8 ± 0.7%; Adélie: 8.5 ± 0.7%; Fig. 2) while δ13C values were slightly higher in Gentoo penguins (−23.5 ± 0.6‰) relative to Chinstrap (−24.1 ± 0.5‰) and Adélie penguins (−24.4 ± 0.7‰; Fig. 2). Within regions, interspecific comparisons found that Adélie penguins breeding in the South Orkney and South Shetlands Islands had significantly lower δ15N values than Gentoo and/or Chinstrap penguins in these two regions (Table 1). However, interspecific variation in eggshell δ13C values was more complex with differences observed within three of the four regions examined (Table 1).
3.3. Effects of species, region, trophic position, and foraging habitat on mercury concentration

The model for mercury concentration most strongly supported by the data included species as the sole factor (Table 2). Species was also a significant predictor of eggshell membrane mercury concentration ($F_{2,457} = 5.12$ to $113.35$, $p<0.003$ in all models) in all of the other GLMs that received support (Table 2; $\Delta$AIC ≤ 10). In these five models relationships between mercury concentration and $\delta^{15}$N or $\delta^{13}$C were not significant ($F_{1,457} = 0.24$ to $1.77$, $p>0.18$ in all models), though two models included a significant interaction term (species × $\delta^{15}$N: $F_{2,457} = 5.02$ to $5.45$, $p<0.01$ in both models). To investigate this interaction further, linear regression analyses were conducted for each species with mercury as the dependent variable and $\delta^{15}$N as the independent variable. Only for Adélie penguins was a significant relationship found between $\delta^{15}$N and mercury ($R^2=0.05$, $p=0.003$); however, the low $R^2$ value suggests that the model provides little explanatory power.

4. Discussion

4.1. Regional homogeneity of mercury in the AP

The present study represents the first large-scale assessment of mercury availability in the marine food web surrounding the Antarctic Peninsula. With few exceptions, to be discussed herein, mercury concentrations were fairly homogeneous throughout the AP in 2006/2007 suggesting little spatial variation in the risk of exposure to dietary mercury in this marine food web. As a non-migratory species, Gentoo penguins provided a true regional assessment of mercury in the present study. Gentoo penguins forage within 25 km of their breeding colony year round (Davis and Renner, 2003; Tanton et al., 2004) and it is highly unlikely that the foraging ranges of Gentoo penguins in each region would overlap. Thus, the lack of significant differences in mercury concentrations in this species coupled with the similar trophic level of diet ($\delta^{15}$N) observed among regions suggests homogeneity of mercury availability at a regional scale throughout the AP. However, some small-scale variability in mercury within regions may exist as in one case (Biscoe Point, Anvers Island) differences in mercury concentrations across Gentoo penguin breeding colonies were statistically significant (Table S1). For Gentoo penguins, breeding locations are often in close proximity and individuals from multiple breeding colonies within a region can share foraging grounds (e.g. Petermann Island (15) and Pleneau Island (12) within the western AP; Fig. 1). Owing to these factors, breeding colony was used only as an organizational construct during sample collection rather than as a means of assessing spatial variation in mercury in the AP.

In contrast, Adélie and Chinstrap penguins spend the pre-breeding months away from their colonies and therefore their eggshell membrane mercury concentrations are likely relatively independent of breeding colony location. These two migratory species offer insight into over-winter mercury exposure over a broader geographic area during the late-winter period prior to their return to breeding colonies. For example, recent studies have suggested that there may be two distinct over-wintering populations of Adélie penguins in the AP; one population breeding along the western AP and wintering in the Bellingshausen Sea and the other breeding in the South Orkney Islands, South Shetland Islands, and eastern AP wintering in the Weddell Sea (see review in Polito et al., 2011a). Therefore, if a significant degree of regional heterogeneity in mercury existed, differences in over-winter mercury uptake would be expected between Adélie penguins in the western AP and the other three regions. However, despite over-wintering in geographically distinct regions of the AP, exposure to dietary mercury appeared to be homogeneous in Adélie penguins breeding across all four regions of the AP.

Only in the Chinstrap penguin did mercury concentrations differ among regions; Chinstrap penguins in the South Orkney Islands had lower mercury than those in the South Shetland Islands and western AP. It is possible that the pre-breeding ranges of colonies from the South Orkney Islands and other regions of the AP do not overlap or that the sampling of only a single breeding site within the South Orkney Islands and western AP biased our ability to identify regional level trends. Further, individual Chinstrap penguins within a breeding colony often employ divergent migratory strategies: short-distance migration remaining ≥ 70 km from the breeding colony or long-distance migration northeast into open water along the Scotia Arc (Trivelpiece et al., 2007). A higher prevalence of individual level variability in migratory behaviors in Chinstrap penguins could explain their generally higher degree of within-site variation in eggshell membrane mercury concentrations relative to their congeners.

It is unlikely that the lower mercury concentration in Chinstrap penguins sampled in the South Orkney Islands was the result of foraging at a lower trophic position as the eggshell $\delta^{15}$N value, and thus trophic level of diet, at this site was among the highest in this study (Table 1). Further, no differences were detected in foraging habitat use, as inferred by $\delta^{13}$C, for Chinstrap penguins in these three regions. At this time it is unclear why mercury concentrations in Chinstrap penguins in the South Orkney Islands appear lower than those in the South Shetland Islands and western AP, especially as this pattern was not detected in Adélie or Gentoo penguins. Sampling from additional sites and years within each region may provide insight into the validity of this finding and allow for the investigation of inter-annual variation in mercury in this marine food web.

4.2. Interspecific differences in dietary mercury uptake

Overall, mercury concentrations in Gentoo and Adélie penguins did not differ, though Chinstrap penguins generally had higher mercury concentrations than their congeners. A previous study with Gentoo penguins found albumen mercury concentration to be representative of whole egg mercury and positively correlated with the mercury concentration found in eggshell membranes (Brasso et al., 2012). Brasso et al. (2012) documented eggs with average albumen mercury concentrations of 0.16 ppm to have correspond eggshell membrane mercury concentrations of 0.01 ppm, similar to the concentrations found in the present study. Therefore, the mercury concentrations we observed in this study likely fall below reported adverse effect levels reported for whole eggs (moderate risk 0.60–1.30 ppm, high risk 1.30–2.00 ppm; Evers et al., 2003). As such, the interspecific differences in mercury discussed herein are unlikely to be biologically relevant (i.e., causing significant reproductive or developmental impairment; Evers et al., 2003, 2008). However, it is worth considering these interspecific differences to gain a better understanding of the factors governing mercury exposure in the AP marine food web.

Table 2

Summary of AIC model selection statistics used to investigate the influence of species, diet ($\delta^{15}$N), and foraging habitat ($\delta^{13}$C) on mercury concentration in Pygoscelis penguins. $\Delta$AIC is the difference in AIC score between a given model and the model with the lowest AIC score. AIC weight indicates the relative percent support for each model. Only models with a $\Delta$AIC ≤ 10 are reported.

<table>
<thead>
<tr>
<th>Model structure</th>
<th>AIC</th>
<th>$\Delta$AIC</th>
<th>AIC weight (ess)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species, $\delta^{15}$N, $\delta^{13}$C</td>
<td>249.50</td>
<td>0.00</td>
<td>0.71</td>
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<tr>
<td>Species, $\delta^{15}$N</td>
<td>252.80</td>
<td>3.30</td>
<td>0.14</td>
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<tr>
<td>Species, $\delta^{13}$C</td>
<td>254.10</td>
<td>4.60</td>
<td>0.07</td>
</tr>
<tr>
<td>Species, $\delta^{15}$N, $\delta^{13}$C</td>
<td>254.90</td>
<td>5.40</td>
<td>0.05</td>
</tr>
<tr>
<td>Species, $\delta^{15}$N, $\delta^{13}$C, Species + $\delta^{15}$N</td>
<td>256.70</td>
<td>7.20</td>
<td>0.02</td>
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<tr>
<td>Species, $\delta^{15}$N, $\delta^{13}$C</td>
<td>259.10</td>
<td>9.60</td>
<td>0.01</td>
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</tbody>
</table>
There were no clear intra- or interspecific relationships between eggshell membrane mercury concentrations and measures of penguin trophic position ($\delta^{15}N$) and foraging habitat use ($\delta^{13}C$). This study found that female Chinstrap penguins in the AP forage at a similar trophic position ($\delta^{15}N$) as their congeners. In addition, the relatively higher $\delta^{13}C$ values observed in Gentoo penguins suggest that they forage more in benthic, inshore habitats relative to the offshore, pelagic habitats where Adélie and Chinstrap penguins are distributed prior to breeding (Cherel et al., 2007; Polito et al., 2011a). Despite foraging at a similar trophic position as Gentoo and Adélie penguins and utilizing offshore, pelagic habitats similar to Adélie penguins, Chinstrap penguins had significantly higher eggshell membrane mercury concentrations (Fig. 2). This finding was confirmed via our GLM analysis, which suggested that variation in eggshell membrane mercury concentrations were best explained by species-specific differences which were independent of regional variation, trophic position ($\delta^{15}N$) and/or foraging habitat use ($\delta^{13}C$).

One possible explanation for this trend of species-specific differences in mercury exposure, despite similar tissue $\delta^{15}N$ and $\delta^{13}C$ values, is the consumption of isotopically similar prey species that differ in mercury concentration. While all Pygoscelis penguin species in the AP consume Antarctic krill, species-specific preferences for certain size classes of krill or prey fish species could lead to differences in mercury exposure. Gentoo penguins often consume benthic fish species such as Lepidonotothen squamifrons, while Adélie penguins predominantly forage on pelagic fishes such as Fleugogramma antarcticum (Karnovsky, 1997). In contrast, Chinstrap penguins preferentially prey on myctophid fish (such as Electrona antarctica; Karnovsky, 1997) which are abundant in the mesopelagic realm of the open waters surrounding the AP (Barrera-Oro, 2002). While these three prey fish species have generally similar tissue isotopic values (Polito et al., 2011b), recent studies have found enhanced bioaccumulation of mercury by fishes in the mesopelagic relative to pelagic or epipelagic realms (Montiero et al., 1996; Choy et al., 2009). Further investigations are needed to determine if dietary exposure in Chinstrap penguins is influenced by elevated mercury concentration in myctophid species relative to other prey fish and to evaluate the importance of mercury gradients in the water column in the Antarctic pelagic marine food web.

4.3. Conclusions

In marine systems such as the Southern Ocean, most biologically available mercury (methylmercury) comes from current-driven transfer from coastal waters, in situ production by microbes, or from the upwelling of deep water (Cossa et al., 2011). As such, regional scale differences in circulation, currents, upwelling and primary productivity can lead to heterogeneity in methylmercury distribution which is reflected in predator tissues. For example, Ferriss and Essington (2011) found regional variability in the muscle tissue of big-eye tuna (Thunnus obesus) and yellowfin tuna (Thunnus albacares) with the highest levels occurring in the highly productive, low dissolved oxygen, upwelling waters of the eastern equatorial Pacific. As there is evidence of regional scale differences in the physical oceanography and primary production across our four study regions within the AP (Zhou et al., 2002; Thompson et al., 2009; Montes-Hugo et al., 2009) it was surprising that Pygoscelis penguin eggshell membrane mercury concentrations were fairly homogeneous throughout the Peninsula. Even so, recent attention has been given to the possibility of significant bacterial methylation of mercury in the Upper Circumpolar Deep Water in the Southern Ocean. This nutrient-rich, oxygen poor water mass is transported to coastal surface waters during the spring and summer months and may function as a widespread source of mercury in the Antarctic marine food web (Cossa et al., 2011). Therefore regional-scale differences in oceanographic features may have little effect on mercury availability and distribution in this marine ecosystem relative to the likely uniform influence of atmospheric deposition and upwelling of regionally homogeneous Upper Circumpolar Deep Water.

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