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Differing foraging strategies influence mercury (Hg) exposure in an Antarctic penguin community☆

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ABSTRACT

Seabirds are ideal model organisms to track mercury (Hg) through marine food webs as they are long-lived, broadly distributed, and are susceptible to biomagnification due to foraging at relatively high trophic levels. However, using these species as biomonitors requires a solid understanding of the degree of species, sexual and age-specific variation in foraging behaviors which act to mediate their dietary exposure to Hg. We combined stomach content analysis along with Hg and stable isotope analyses of blood, feathers and common prey items to help explain inter and intra-specific patterns of dietary Hg exposure across three sympatric *Pygoscelis* penguin species commonly used as biomonitors of Hg availability in the Antarctic marine ecosystem. We found that penguin tissue Hg concentrations differed across species, between adults and juveniles, but not between sexes. While all three penguins species diets were dominated by Antarctic krill (*Euphausia superba*) and to a lesser extent fish, stable isotope based proxies of relative trophic level and krill consumption could not by itself sufficiently explain the observed patterns of inter and intra-specific variation in Hg. However, integrating isotopic approaches with stomach content analysis allowed us to identify the relatively higher risk of Hg exposure for penguins foraging on mesopelagic prey relative to congeners targeting epipelagic or benthic prey species. When possible, future seabird biomonitoring studies should seek to combine isotopic approaches with other, independent measures of foraging behavior to better account for the confounding effects of inter and intra-specific variation on dietary Hg exposure.

1. Introduction

Mercury (Hg) is a potent neurotoxin leading to physiological and reproductive impairments in wild birds (Brasso and Cristol, 2008; Evers et al., 2008; Wada et al., 2009). In marine systems, seabirds are often used as biomonitors to assess the distribution, bioavailability and toxicity of Hg across the world's oceans (Kojadinovic et al., 2007; Day et al., 2012; Brasso et al., 2015). Seabirds are ideal model organisms to track Hg through marine food webs as they are long-lived, have wide-spread foraging ranges, and are susceptible to biomagnification due to feeding at relatively high trophic levels (Burger and Gochfeld, 2000; Bond and Diamond, 2009).

However, using seabirds as biomonitors for Hg bioavailability requires a solid understanding of species-specific foraging ecologies and other aspects of their life-histories. This is because species living in the same breeding location could be exposed to different concentrations of dietary Hg due to differences in prey preferences, small-scale foraging habitat use, and larger-scale migration and dispersal strategies (Anderson et al., 2009; Carravieri et al., 2014). Therefore, when comparing Hg exposure across and within seabird communities inter-specific variation in foraging ecology must be explicitly taken into account. Even in single species studies, factors such as age, sex and individual-based differences in foraging

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ecology and thus Hg exposure necessitate an understanding of the degree of intra-specific variation in these factors within populations (Becker et al., 2002; Pedro et al., 2015).

The use of stable isotope analysis in applied studies of the diets and foraging ecology of seabirds has developed rapidly in recent years (Bond and Jones, 2009). Nitrogen isotopic values ($\delta^{15}N$) in seabird tissues are commonly used to infer trophic level and diets (Polito et al., 2011a; Brasso and Polito, 2013), while carbon isotopic values ($\delta^{13}C$) help trace trends in marine habitat use (inshore/benthic vs. offshore/epipelagic; Cherel and Hobson, 2007). These relationships have led stable isotope analysis to be often incorporated into modern ecotoxicological investigations (Jardine et al., 2006). Integration of stable isotope and Hg analyses have allowed researchers to track biomagnification of Hg within marine food webs (Atwell et al., 1998) and identify trophic differences among sympatric species that explain patterns of Hg exposure (Blevin et al., 2013; Pouilly et al., 2013). In addition, stable isotope-based dietary studies, including those using dietary mixing models, avoid many of the digestive and temporal biases inherent to more traditional methods such as stomach content analysis (Karnovsky et al., 2012). Even so, stable isotope-based dietary mixing models are limited by their inability to estimate the dietary contribution of specific prey species with overlapping isotopic values (Phillips et al., 2005). Often these limitations can be overcome in seabirds by combining isotopic approaches with the analysis of otoliths and other identifiable prey remains recovered from stomach contents (Polito et al., 2011a).

The objective of this study was to identify how differences in the foraging strategies of the three sympatric *Pygoscelis* penguin species influenced their exposure to Hg in the Antarctic Peninsula. *Pygoscelis* penguins are commonly used as biomonitors for Hg availability in Antarctic marine ecosystem as they forage primarily on Antarctic krill (*Euphausia superba*), nest in large, readily accessible breeding colonies, and tissues such as blood, feathers, guano and eggshells can be used to track Hg exposure across much of their annual cycle (Brasso et al., 2012, 2014; Celis et al., 2015). However, studies integrating stable isotope and Hg analyses in *Pygoscelis* penguins have often reported species-specific differences in tissue Hg concentrations which do not correlate with stable isotope based estimates of species relative trophic position (e.g. Brasso et al., 2012, 2014, 2015). This unpredicted uncoupling of trophic position and Hg concentration has hampered the interpretation and use of these species as biomonitors of Hg availability across and within studies. As such a greater understanding of how the inter and intra-specific variation in the foraging habitats and diets of these species influences their exposure to Hg in the Antarctic marine food web is required.

To address this issue we examined variation in the foraging ecology and Hg exposure of Adélie (*Pygoscelis adeliae*), chinstrap (*P. antarctica*), and gentoo (*Pygoscelis papua*) penguins breeding on King George Island in the Antarctic Peninsula region. Specifically, we use a combination of stable isotope and stomach content analysis to identify species, age and sex-specific dietary and foraging habitat trends and relate them tissue Hg concentrations reflecting both short-term dietary exposure (blood) as well as over longer annual, or inter-molt, time periods (feathers). Blood is used here as measure of short-term Hg exposure as concentrations in blood represent an integrated signal of recent dietary uptake and internal tissue redistribution of Hg (Evers et al., 2005; Ackerman et al., 2008). For example, lab dosing studies of non-molting seabirds indicate blood Hg concentrations reflect dietary Hg exposure within days of dosing with half-life of incorporation averaging between 30 and 60 days (Bearhop et al., 2000a). Due to the reasonable overlap in the timeframe of integration blood Hg and stable isotope values are often compared in ecotoxicological studies of seabird (e.g. Bond and Diamond, 2009; Hipfner et al., 2011). For example, the half-life of stable isotope values in whole blood of a 3–5 kg penguin is predicted to be approximately 10–30 days based on allometric and empirical studies (Carleton and del Rio, 2005; Barquete et al., 2013). In contrast, Hg concentrations in feathers provide a temporally integrative signal of annual or inter-molt body Hg burden in birds (Evers et al., 2008; Bond and Diamond, 2009). During molt feather Hg concentrations are highly correlated with blood Hg concentrations as both are reflective of remobilized Hg from body tissues (Bearhop et al., 2000a). Stable isotope analyses of feathers provides a similarly integrated signal of diets during the inter-molt period owing to the unique pattern of catastrophic molt and fasting in adult penguins (Stonehouse, 1967) when the bulk of feathers are synthesized via endogenous reserves (Williams et al., 1977; Cherel et al., 1994). Finally, we examine Hg concentrations and stable isotope values in the tissues of four representative penguin prey species to provide additional predictive power and explain individual and group patterns of dietary Hg exposure in these penguins.

2. Methods

2.1. Study site and sample collection

We conducted fieldwork within the Antarctic Specially Protected Area (ASPA) no. 128 along the western shores of Admiralty Bay, King George Island, South Shetland Islands, Antarctica (62°10′S, 58°27′W). All three species of *Pygoscelis* penguins breed sympatrically at this location (Trivelpiece et al., 1987). We sampled penguins during December 2010, coinciding with the late incubation period for chinstrap penguins and the early brood period for Adélie and gentoo penguins. We captured 20–22 actively breeding adults per species and 20 non-breeding juvenile gentoo penguins (approximately one year old) identified by white head patches that did not reach the eye, incomplete white eye-rings and the lack of a brood patch (Trivelpiece et al., 1985). Individuals were sexed using a combination of molecular and morphometric methods (see Polito et al., 2012 for details). From each individual, we collected plucked breast feathers and whole blood samples (<0.5 ml) by venipuncture of the interdigitary vein. Blood samples were stored frozen (−20 °C) prior to analysis.

In January 2009 we collected penguin prey samples during trawls conducted by the U.S. Antarctic Marine Living Resources (US AMLR program) around the South Shetland Islands and Northern Antarctic Peninsula. Prey species were representative of the major components of penguin's diet at Admiralty Bay: Antarctic krill (*Euphausia superba*), neritic/epipelagic fish (e.g. *Pleuragramma antarcticum*), coastal/benthic fish (e.g. *Lepidonotothen squamifroms*) and oceanic/mesopelagic myctophid fish (e.g. *Electrona antarctica*; Volkman et al., 1980; Karnovsky, 1997). While the three prey fish groups listed above differ in both horizontal and vertical habitat use, for simplicity we refer to these three groups as epipelagic, benthic mesopelagic and fish. We collected a total of ten, 10 g samples of krill (approximately 20 individuals per sample) and ten whole fish per species and stored them frozen (−80 °C) prior to analysis. While we could not assess to what effect differences in penguin and prey collection years may have had on our study, previous work suggest little inter-annual variability in the Hg and stable isotope values of *Pygoscelis* penguins and their prey (Polito et al., 2013; Brasso et al., 2014).

2.2. Mercury analysis

Prior to analysis, we cleaned adult feathers in a series of acetone and deionized water baths and allowed them to air dry under a

fume hood for ~24 h. Whole prey samples were homogenized and freeze-dried to constant weight. We analyzed individual adult feathers and aliquots of whole blood and whole prey (~0.02 g) for total Hg via atomic absorption spectrometry on a Direct Mercury Analyzer DMA-80. Only one feather was analyzed from each individual as Hg concentrations do not vary significantly among body feathers in penguins (Brasso et al., 2013). Because nearly all Hg in blood, feathers and prey tissue is present in the form of methylmercury (except possibly in krill where percent methylmercury is unknown), a measurement of total Hg concentration was used as a proxy for this highly bioavailable form (Bloom, 1992; Bond and Diamond, 2009; Payne and Taylor, 2010). 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). All Hg concentrations are reported as parts per million (ppm); blood and prey values are presented as wet weight (ww), and feathers are reported as fresh weight (fw). As blood samples were analyzed as wet weight we did not assess the effect of hematocrit values on reported Hg concentrations. Mean percent recoveries for standard reference materials were 100.5± 2.1% (DORM-3) and 103.5 ± 2.2% (DOLT-4). The relative percent difference among duplicate samples was 2.1%. Detection limit of the assay was 0.005 ng Hg.

2.3. Stable isotope analysis

Prior to isotopic analysis, the tip (oldest part) of each feather was cut and discarded, as this section is grown at sea and may not reflect endogenous reserves (Cherel et al., 2005a). We cleaned feathers using a 2:1 chloroform: methanol rinse, air-dried under a fume hood for ~24 h and cut them into small fragments with stainless steel scissors. Prior to isotopic analysis whole blood samples and prey homogenates were freeze-dried to constant weight. We extracted lipids from prey homogenates using a Soxhlet apparatus with a 1:1 Petroleum-Ether: Ethyl-Ether solvent mixture for 8 h (Seminoff et al., 2007). Lipid-extracted krill were not acidified prior to isotopic analysis. Whole blood was not lipid-extracted prior to isotopic analysis as the lipid component of avian blood is less than 1% of the total wet mass and unlikely to be of sufficient magnitude to influence $\delta^{13}C$ values (see Bearhop et al., 2000b). Furthermore, the C:N mass ratios of penguin blood in our study ranged from 3.2 to 3.5 and was not correlated with $\delta^{13}C$ value ($R^2 = 0.01$, $P = 0.521$). We flash-combusted (Thermo-Finnigan & Costech ECS4010 elemental analyzers) approximately 0.5 mg of each whole blood, feather and prey sample loaded into tin cups and analyzed for carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$) through interfaced Thermo Finnigan Delta Plus XL and Delta V Plus continuous-flow stable isotope ratio mass spectrometers (CFIRMS). Raw δ values were normalized on a two-point scale using glutamic acid reference materials with low and high values (i.e. USGS-40 ($\delta^{13}C = -26.4‰$, $\delta^{15}N = -4.5‰$) and USGS-41 ($\delta^{13}C = 37.6‰$, $\delta^{15}N = 47.6‰$)). Sample precision based on repeated sample and reference material was 0.1‰ and 0.2‰, for $\delta^{13}C$, and $\delta^{15}N$, respectively.

Stable isotope ratios are expressed in δ notation in per mil units (‰), according to the following equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \cdot 1000 \quad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The $R_{standard}$ values were based on the Vienna PeeDee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$.

2.4. Penguin dietary analysis

We used the SIAR Bayesian multi-source isotopic mixing model in the R environment (R Development Core Team, 2007) to quantify the diet composition of penguins in our study. The SIAR model estimates probability distributions of multiple source contributions to a mixture while accounting for the observed variability in source and mixture isotopic signatures, dietary isotopic fractionation, and elemental concentration (Parnell et al., 2010). We used the SIAR model with $\delta^{13}C$ and $\delta^{15}N$ values from penguin and prey tissues to predict the relative contribution of krill, epipelagic fish, benthic fish and mesopelagic myctophid fish to the diets of penguins in our study. We incorporated diet to whole blood discrimination factors derived from captive studies of four piscivorous birds (see review by Cherel et al., 2005b; $\delta^{15}N$: +2.7 ± 0.4; $\delta^{13}C$: +0.0 ± 0.7) and diet to feather discrimination factor from a captive feeding study of *Pygoscelis* penguins ($\delta^{15}N$: +3.5 ± 0.4; $\delta^{13}C$: +1.3 ± 0.5; Polito et al., 2011b) and ran 1 million iterations, thinned by 15, with an initial discard of the first 40,000 resulting in 64,000 posterior draws. Finally, we conducted a sensitivity analyses to examine the effect of variation in discrimination factors on mixing model results (e.g. Bond and Diamond, 2011) by incorporating discrimination factors ranging 1‰ higher and lower than those estimated from the above captive feeding studies.

While isotopic mixing models provide a robust estimate of the relative amounts of fish and krill in penguin diets, they can have difficulty estimating the relative contribution of different fish species to penguin diets due to overlap in stable isotopes values (Polito et al., 2011a). Therefore we used fish otoliths recovered from penguin stomach contents to assess the fish composition of penguin diets at Admiralty Bay and complement our isotopic analyses. Direct dietary information was not available for the year or individuals sampled in our study, therefore we assess long-term differences in the fish composition of penguin diets at our study site using a 21-year dataset of penguin diets during the chick-rearing period at Admiralty Bay from 1988 to 89 to 2008–09. Detailed methods are described in Hinke et al. (2007), but briefly we collected stomach samples using gastric lavage from four to five breeding adults per species, replicated on 5–8 day intervals, resulting in approximately 20–40 samples per species per year and 1699 samples over the 21 year period (Adélie penguin = 619, chinstrap penguin = 549, gentoo penguin = 479). We recovered fish otoliths by swirling diet samples in a dark-bottomed pan and identified otoliths to the lowest possible taxonomic level and three habitat groupings (benthic, epipelagic and mesopelagic myctophids) using an internal reference collection and a published guide (Williams and McEldowney, 1990; Karnovsky, 1997). We calculated the frequency occurrence and the minimum number of individuals (MNI) of each fish taxa in each penguins species diet over this 21 year period following standard methods (Polito et al., 2002). This method allowed us to assess the relative importance of epipelagic, benthic and mesopelagic fish to the diets of the adult Adélie, chinstrap and gentoo penguin populations at Admiralty Bay. Unfortunately, no data were available to help assess the fish component of juvenile gentoo penguin diets at Admiralty Bay.

2.5. Statistical analysis

Statistical analysis was conducted using SAS (Version 9.1, SAS Institute, 1999). Penguin and prey tissue Hg concentrations were log-transformed prior to analyses to generate data sets that did not differ from a normal distribution (Shapiro-Wilk, $P > 0.05$ in all cases). We used separate ANOVAs to test for differences among groups (adult Adélie penguins, adult chinstrap penguins, adult gentoo penguins, and juvenile gentoo penguins) and between sexes

for penguin blood and feather Hg, $\delta^{13}C$, and $\delta^{15}N$ values. We used similar ANOVAs approach to examine differences in Hg, $\delta^{13}C$, and $\delta^{15}N$ values among penguin prey species. Tukey's HSD was used for all post-hoc comparisons. Relationships between blood and feather Hg, $\delta^{13}C$, and $\delta^{15}N$ values, and Hg concentrations and stable isotope values within tissue were tested using Pearson correlation.

We then used a generalized linear modeling approach with a normal distribution and an identity-link function to test multiple alternative hypotheses regarding the influence of species, age, sex, trophic position ($\delta^{15}N$), and foraging habitat ($\delta^{13}C$) on Hg concentrations in penguin blood and feathers. We conducted separate analyses by tissue for both adults of all three species as well as for gentoo penguins only to test for differences between age classes. We parameterized a global model for all adults using log transformed Hg as the dependent variable, species and sex as grouped factors, $\delta^{15}N$ and $\delta^{13}C$ as covariates, and all two-way interaction terms. We parameterized a global model for gentoo penguins only using log transformed Hg as the dependent variable, age and sex as grouped factors, $\delta^{15}N$ and $\delta^{13}C$ as continuous variables, and all two-way interaction terms. Continuous variables ($\delta^{13}C$ and $\delta^{15}N$) that were significantly correlated were not included in the same models. All possible model subsets of our global model were compared using Akaike Information Criteria adjusted for small sample sizes (AIC_c; Akaike, 1973). The model with the lowest AIC_c score was selected as the most parsimonious model. Models with ΔAIC_c scores (difference in AIC_c between a given model and the model with the lowest AIC_c) $\leq$2.0 were interpreted to be equally competitive with the most parsimonious model and any model with a $\Delta AIC_c \leq 10.0$ was considered well supported. Model fits were further assessed by AIC weight (wi) 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). All means are presented ± SD and statistical significance was defined at $P < 0.05$.

3. Results

3.1. Hg concentrations in penguins and their prey

Penguin blood Hg concentration differed among penguin groups ($F_{3,83} = 3.79$, $P < 0.001$) but not between sexes ($F_{1,83} = 3.08$, $P = 0.083$) and did not exhibit a significant interaction between group and sex ($F_{3,83} = 2.73$, $P = 0.051$; Fig. 1). Similarly, penguin feather Hg concentrations differed among penguin groups ($F_{3,83} = 18.25$, $P < 0.00$) but not between sexes ($F_{1,83} = 0.64$, $P = 0.425$; Fig. 1), though there was a significant interaction between group and sex ($F_{3,83} = 3.62$, $P = 0.0169$). Inter-specific patterns were similar in both blood and feathers, with adult chinstrap penguins having the highest mean Hg concentration, followed by adult Adélie and gentoo penguins which were both significantly higher than juvenile gentoo penguins (Table 1). In addition, gentoo penguins tended to have the most variable blood and feather Hg concentrations. This was especially true of adult gentoo penguins which had ~3-5 times higher coefficient of variation (CV) relative to other penguins species (Table 1).

Penguin Hg concentrations were significantly and positively correlated between blood and feathers from the same individual ($r = 0.66$, $P < 0.001$). However, this trend appears to be driven primarily by adult gentoo penguins ($r = 0.65$, $P = 0.002$), as blood and feathers Hg concentrations were not significantly correlated within other penguin groups (All $P > 0.8$).

Hg concentrations also differed among representative penguin prey species ($F_{3,83} = 9.36$, $P < 0.001$). Antarctic krill (*E. superba*) Hg concentrations were significantly lower than all three fish species (Table 2). In addition, epipelagic fish (*P. antarcticum*) had lower Hg concentrations relative to benthic fish (*L. squamifroms*) and myctophid (*E. antarctica*) fish species (Table 2).

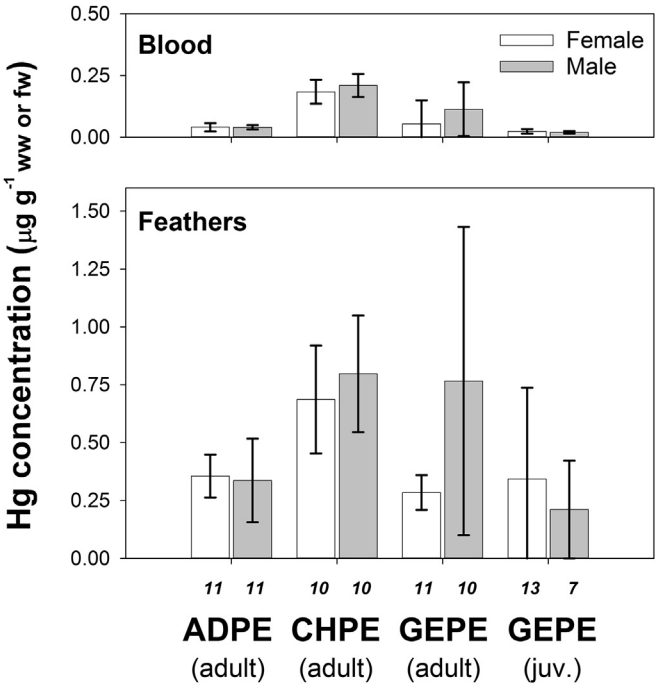

Fig. 1. Total Hg concentration in blood (μg g^{-1} ww) and feathers (μg g^{-1} fw) from three penguin species (Adélie: ADPE; Chinstrap: CHPE; Gentoo: GEPE) at King George Island, Antarctica. Sample sizes provided below each bar; significant differences between sexes are indicated with asterisks (Tukey's HSD, $p < 0.05$).

3.2. Stable isotope values in penguins and their prey

Penguin blood $\delta^{13}C$ values differed among penguin groups ($F_{3,83} = 3.79$, $P = 0.014$), but not between sexes ($F_{1,83} = 3.45$, $P = 0.067$) and did not exhibit a significant interaction between group and sex ($F_{3,83} = 0.66$, $P = 0.582$; Fig. 2). Significant differences between penguin groups were driven by higher blood $\delta^{13}C$ values found adult Adélie penguins relative to chinstrap penguins (Table 1). Similarly, penguin feather $\delta^{13}C$ values differed among penguin groups ($F_{3,83} = 4.36$, $P = 0.007$), but not between sexes ($F_{1,83} = 0.63$, $P = 0.429$) and did not exhibit a significant interaction between group and sex ($F_{3,83} = 1.02$, $P = 0.387$; Fig. 2). Significant differences between penguin groups were driven by higher feather $\delta^{13}C$ values found in adult chinstrap penguins and juvenile gentoo penguin, relative to adult Adélie penguins (Table 1). Gentoo penguins tended to have the most variable blood and feather $\delta^{13}C$ values in comparison to other species (Table 1).

Penguin blood $\delta^{15}N$ values differed among penguin groups ($F_{3,83} = 14.07$, $P < 0.001$), between sexes ($F_{1,83} = 16.99$, $P < 0.001$) and exhibited a significant interaction between group and sex ($F_{3,83} = 4.40$, $P = 0.007$; Fig. 2). Significant differences between penguin groups were driven by higher blood $\delta^{15}N$ values found in adult Adélie and chinstrap penguins relative to juvenile gentoo penguins (Table 1). While statically significant, the difference between males and females in all species combined (+0.2‰) was equal to our level of analytical precision for $\delta^{15}N$. In addition, significant differences between sexes were found only in adult Adélie and gentoo penguins, with males having higher blood $\delta^{15}N$ values in both cases (Fig. 2). Penguin feather $\delta^{15}N$ values differed among penguin groups ($F_{3,83} = 5.14$, $P = 0.003$), but not between sexes ($F_{1,83} = 1.92$, $P = 0.170$) and did not exhibit a significant interaction between group and sex ($F_{3,83} = 0.92$, $P = 0.434$; Fig. 2). Significant

Table 1
Mean ± SD, range, and coefficient of variation (CV; %) of total Hg concentration (μg g^{-1} ww or fw) and stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values (‰) in blood and feathers from three penguin species at King George Island, Antarctica. Within each tissue, means not sharing a letter were found to differ significantly (Tukey's HSD, $p < 0.05$).

Tissue	Penguin species (age)	*n*	Hg		$\delta^{13}C$		$\delta^{15}N$	
			Concentration (μg g^{-1} ww or fw)	CV	Ratio (‰)	CV (%)	Ratio (‰)	CV (%)
Blood	Adélie (adults)	22	0.040 ± 0.013^{a} (0.022–0.084)	32.76	−24.6 ± 0.3^{a} (−25.1 to −24.1)	−1.23	8.5 ± 0.3^{a} (7.8–9.1)	3.98
	Chinstrap (adults)	20	0.197 ± 0.048^{b} (0.070–0.294)	24.43	−24.9 ± 0.2^{b} (−25.4 to −24.7)	−0.79	8.7 ± 0.2^{a} (8.3–9.0)	2.15
	Gentoo (adults)	21	0.082 ± 0.104^{a} (0.019–0.342)	126.89	−24.7 ± 0.4^{ab} (−25.0 to −23.0)	−1.79	8.5 ± 0.6^{ab} (8.0–10.6)	7.39
	Gentoo (juvenile)	20	0.022 ± 0.008^{c} (0.007–0.039)	36.85	−24.8 ± 0.6^{ab} (−25.9 to −23.4)	−2.22	8.0 ± 0.2^{b} (7.7–8.5)	2.97
Feather	Adélie (adults)	22	0.346 ± 0.140^{a} (0.182–0.693)	40.58	−24.1 ± 1.1^{a} (−27.4 to −22.4)	−4.60	9.1 ± 0.4^{ab} (8.0–10.1)	4.57
	Chinstrap (adults)	20	0.742 ± 0.243^{b} (0.398–1.290)	32.79	−23.3 ± 1.0^{b} (−24.5 to −21.5)	−4.39	9.0 ± 0.2^{ab} (8.6–9.3)	2.31
	Gentoo (adults)	21	0.514 ± 0.513^{a} (0.180–1.976)	99.86	−23.6 ± 0.7^{ab} (−24.5 to −21.8)	−2.87	9.4 ± 0.6^{a} (8.6–11.3)	6.30
	Gentoo (juvenile)	20	0.297 ± 0.341^{c} (0.079–1.523)	114.85	−23.0 ± 1.0^{b} (−24.3 to −20.6)	−4.37	8.7 ± 1.0^{b} (7.7–11.3)	10.97

Table 2
Mean ± SD, range, and coefficient of variation (CV; %) of total Hg concentration (μg g^{-1} ww) and stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values (‰) in four representative penguin prey taxa. Means not sharing a letter were found to differ significantly (Tukey's HSD, $p < 0.05$).

Prey group (species)	n	Hg		$\delta^{13}C$		$\delta^{15}N$	
		Concentration (μg g^{-1} ww)	CV	Ratio (‰)	CV (%)	Ratio (‰)	CV (%)
Krill (*E. superba*)	6	0.002 ± 0.001^{a} (0.001–0.002)	34.74	−26.4 ± 0.8^{a} (−27.3 to −25.4)	−2.91	3.1 ± 0.3^{a} (2.5–3.5)	11.05
Epipelagic fish (*P. antarcticum*)	10	0.008 ± 0.002^{b} (0.005–0.011)	22.85	−25.1 ± 0.2^{b} (−25.3 to −24.8)	−0.65	9.0 ± 0.3^{b} (8.5–9.4)	3.53
Mesopelagic fish (*E. antarctica*)	10	0.031 ± 0.017^{c} (0.017–0.069)	53.31	−25.8 ± 0.6^{a} (−26.9 to −24.7)	−2.48	9.0 ± 0.4^{b} (8.2–9.3)	3.94
Benthic fish (*L. squamifons*)	10	0.041 ± 0.021^{c} (0.012–0.083)	50.37	−24.2 ± 0.7^{c} (−25.3 to −23.4)	−2.79	9.6 ± 0.8^{c} (7.9–10.5)	8.39

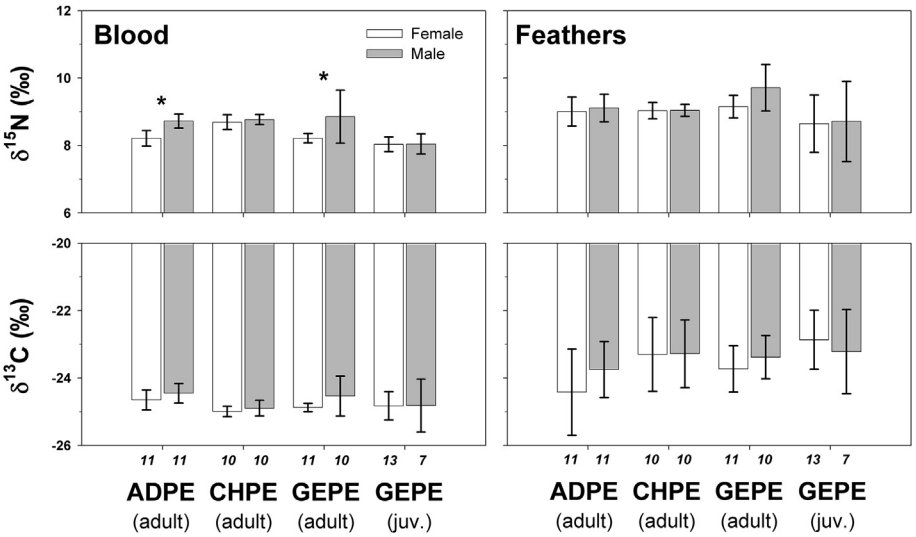

Fig. 2. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values (‰) in blood and feathers from three penguin species (Adélie: ADPE; Chinstrap: CHPE; Gentoo: GEPE) at King George Island, Antarctica. Sample sizes provided below each bar; significant differences between sexes are indicated with asterisks (Tukey's HSD, $p < 0.05$).

differences between penguin groups were driven by higher blood $\delta^{15}N$ values found in adult gentoo penguins relative to juvenile gentoo penguins (Table 1). Similar to Hg concentration and $\delta^{13}C$ values, gentoo penguins tended to have the most variable blood and feather $\delta^{15}N$ values in comparison to other species (Table 1).

Penguin stable isotope values were significantly and positively correlated between blood and feathers from the same individual for $\delta^{15}N$ ($r = 0.51$, $P < 0.001$), but not $\delta^{13}C$ ($P = 0.720$). Within penguin groups the only significant correlations found between tissue stable isotope values were $\delta^{13}C$ in adult gentoo penguins ($r = 0.63$, $P = 0.002$) and $\delta^{15}N$ for adult ($r = 0.82$, $P < 0.001$) and juvenile ($r = 0.49$, $P = 0.025$) gentoo penguins (all other comparisons $P > 0.121$). Penguin tissue $\delta^{13}C$ and $\delta^{15}N$ values were also significantly and positively correlated with each other for both blood ($r = 0.49$, $P < 0.001$) and feathers ($r = 0.32$, $P = 0.003$). Within penguin groups the only significant correlations between tissue $\delta^{13}C$ and $\delta^{15}N$ values were adult (Blood: $r = 0.94$, $P < 0.001$; Feather: $r = 0.55$, $P = 0.009$) and juvenile (Blood: $r = 0.70$, $P < 0.001$; Feather: $r = 0.82$, $P < 0.001$) gentoo penguins (all other comparisons $P > 0.152$).

Prey stable isotope values differed across taxa for both $\delta^{13}C$ ($F_{3,83} = 22.09$, $P < 0.001$) and $\delta^{15}N$ ($F_{3,83} = 239.31$, $P < 0.001$). Krill had lower $\delta^{13}C$ values than both epipelagic fish and benthic fish and $\delta^{15}N$ values that were lower than all three fish species (Table 2). Within fish species $\delta^{13}C$ values differed between all species, with epipelagic fish having the lowest values and benthic fish having the highest values (Table 2). Epipelagic and mesopelagic myctophid fish did not differ in $\delta^{15}N$ values, though both species had lower $\delta^{15}N$ values relative to benthic fish (Table 2).

3.3. Penguin diets at Admiralty Bay

Isotopic mixing models using discrimination factors estimated from captive feeding studies predicted that krill was more important than fish prey in the diet of penguins at Admiralty Bay with mean estimates ranging from 54.2 to 65.8% of the diet depending on species, age and the type of tissue examined (Fig. 3). However, isotopic mixing models had difficulty separating the fish component of penguin diets among epipelagic, mesopelagic and benthic fishes with a higher degree of overlap in 95% credibility intervals (CI) in isotopic models using both blood and feather values (Fig. 3). One exception to this generalization is the model using blood isotopic values that predicted a higher mean contribution of benthic fishes (30.0%; CI: 12.5–60.7%) relative to mesopelagic fishes (4.7%; CI: 0.0–11.6%) in the diets of adult gentoo penguins at Admiralty Bay. Predicted contributions of the proportion of krill vs. other prey (i.e. fish) were sensitive to variation in discrimination factors with changes of ~15% per 1‰ shift in discrimination factors (Table S1 of the Supplementary Material). However, differences in the relative importance of krill were consistent among penguin groups across all discrimination factors employed and all models had similar difficulty separating the fish component of penguin diets. Therefore, we restrict our further discussion to mixing models that employed the discrimination factors estimated from captive feeding studies.

From 1988 to 89 to 2008–09 a total of 1996 otoliths were recovered from 1699 stomach samples with 93.6% of otoliths identifiable to taxa in three habitat groups (epipelagic, mesopelagic, benthic fishes). When examine MNI across all stomach samples, *P. antarcticum* (91.0%) and *Notolepis coatsi* (9.0%) comprised the dominant epipelagic species. *E. antarctica* (78.0%), *Gymnoscopelus braueri* (11.9%), *Gymnoscopelus nicholsi* (5.8%) and *Protomyctophum bolini* (2.9%) comprised the dominate mesopelagic species. *Lepidonotothen* sp. (38.3%), *Notothenia* sp. (22.4%), *Trematomus newnesi* (20.8%), *Pagetopsis macropterus* (6.0%), *Chaenodraco wilsoni* (4.0%) comprised the dominate benthic species. Other taxa individually contributed less than 2% to the above groups. Using this dataset we found strong preferences in the types of fish consumed by each penguin species during the chick rearing period. Epipelagic fish were the most common fish found in adult Adélie penguin diets by frequency occurrence and percentage of individuals (Fig. 4). In contrast, mesopelagic fish were most common in adult chinstrap penguin diets and benthic fish were the most

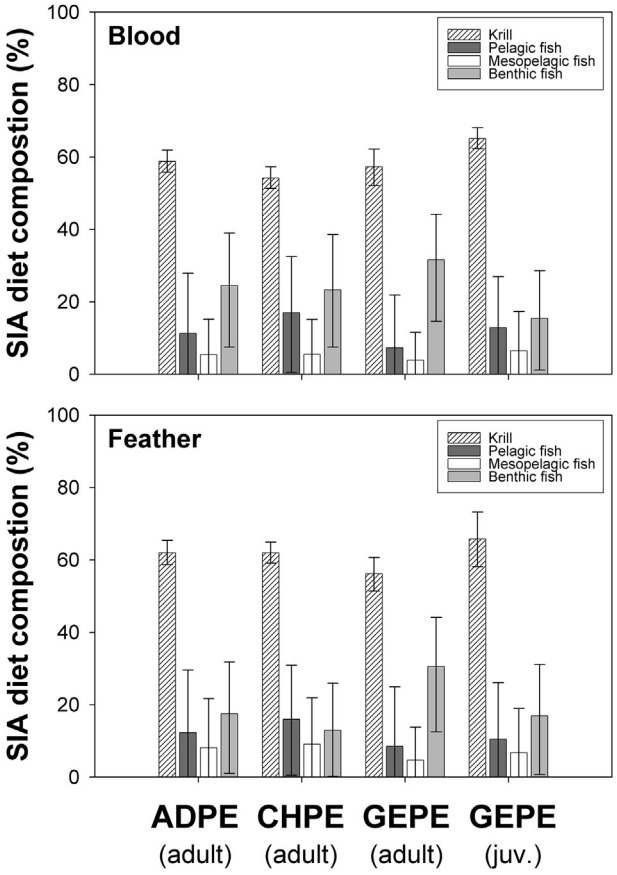

Fig. 3. Predicted dietary composition of three penguin species (Adélie: ADPE; Chinstrap: CHPE; Gentoo: GEPE) at King George Island, Antarctica based on blood and feather stable isotope values and a multi-source Bayesian isotopic mixing model. Proportions are presented as mean ± Bayesian 95% credibility intervals.

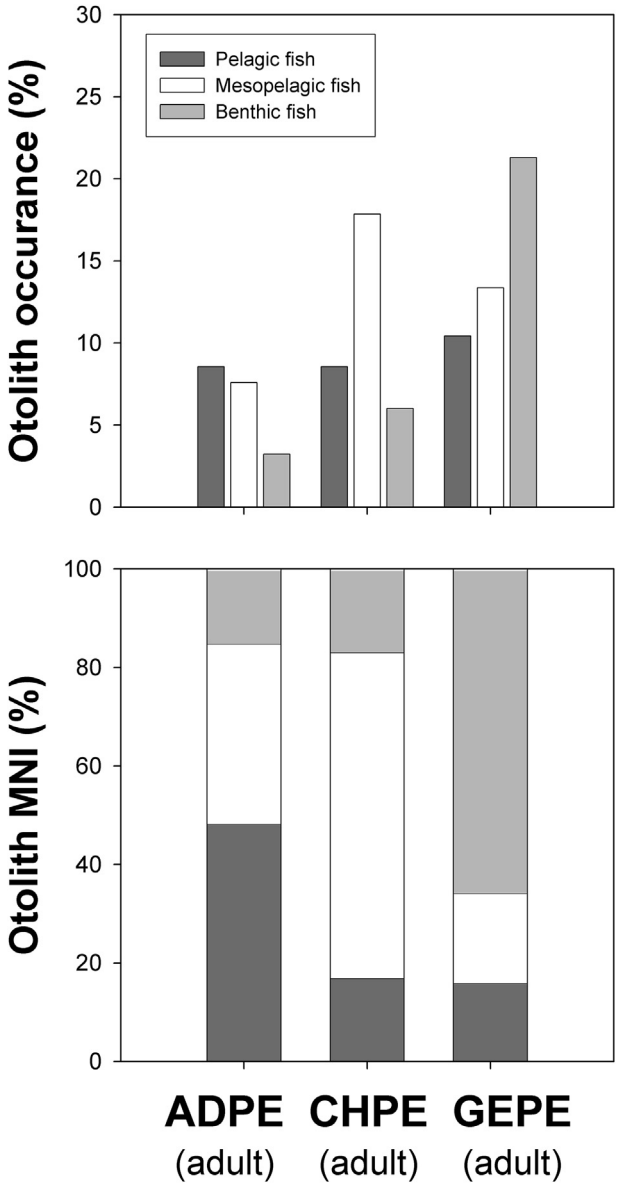

Fig. 4. The proportion of minimum number of individuals and frequency occurrence of epipelagic, mesopelagic and benthic fish species based on a 21 year dataset of fish otoliths recovered from adult stomach contents of three penguin species (Adélie: ADPE; Chinstrap: CHPE; Gentoo: GEPE) at King George Island, Antarctica.

common in adult gentoo penguin diets. Otolith data also validated our use of representative epipelagic, mesopelagic, benthic fish taxa for Hg and stable isotope analysis as *P. antarcticum* (47.4%), *E. antarctica* (60.0%), and *Lepidonotothen* sp. (28.26%) were the single most common fish prey found in adult Adélie, chinstrap and gentoo penguin stomach samples respectively.

3.4. Influence of species, age, sex, and foraging ecology on penguin Hg concentrations

When examined across all species, sexes and ages, feathers and blood Hg concentrations were significantly and positively correlated with $\delta^{15}N$ values, but not $\delta^{13}C$ values (Fig. 5). When examining only adults, based on AIC_c analyses the most parsimonious model found to explain variation in both feathers and blood Hg concentrations included species, $\delta^{15}N$ and their interaction (species*$\delta^{15}N$) as covariates (Table S2 of the Supplementary Material). For both feathers and blood, Hg concentrations differed across species and while they were not significantly correlated with $\delta^{15}N$ values in adults only, a significant species*$\delta^{15}N$ interaction was found (Table 3). This interaction was a result of significant positive correlations between Hg concentrations and $\delta^{15}N$ values in adult gentoo penguin feathers ($r = 0.75$, $P < 0.001$) and blood ($r = 0.63$, $P = 0.002$) samples and the lack of relationship between these parameters in adult Adélie and chinstrap penguins (all $P > 0.450$). While no other competitive models (i.e., $\Delta AIC_c \leq 2.0$) were found to predict adult feather Hg concentration, a model that included an effect of species, $\delta^{13}C$ and their interaction (species* $\delta^{13}C$) was

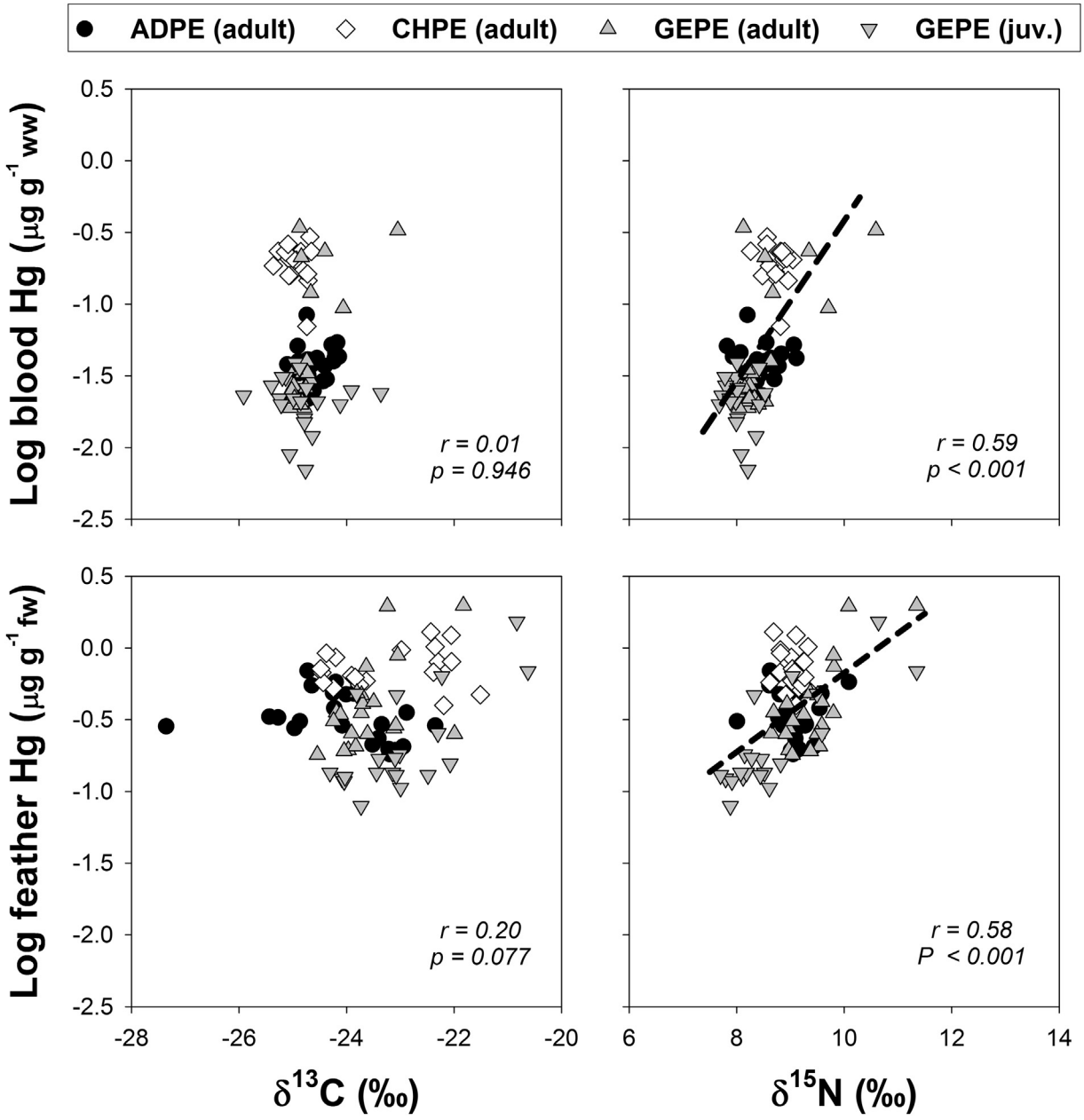

Fig. 5. Relationships between stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values (‰) and total Hg concentration in blood (μg g^{-1} ww) and feathers (μg g^{-1} fw) from three penguin species (Adélie: ADPE; Chinstrap: CHPE; Gentoo: GEPE) at King George Island, Antarctica. Test statistics reflect Pearson correlation analyses across all species and age groups.

Table 3
Model components, including: selected parameters, degrees of freedom (df), F value (F), P-value (P), parameter estimates (β) and standard errors (SE from the AIC_c selected models that best explain variation in total Hg for penguin feather and blood in adults as well as for only gentoo penguins. Na is shown for estimates of multi-level parameters.

Comparison	Tissue	Parameter	df	F	P	β	SE
All adults	Feather	Species	2	7.67	0.0011	Na	Na
		$\delta^{15}N$	1	1.44	0.2357	0.3756	0.0648
		Species*$\delta^{15}N$	1	7.31	0.0015	Na	Na
	Blood	Species	2	4.59	0.0124	Na	Na
		$\delta^{15}N$	1	1.16	0.2854	0.4401	0.0813
		Species*$\delta^{15}N$	1	4.33	0.0178	Na	Na
Gentoo only	Feather	$\delta^{15}N$	1	78.21	<0.0001	0.3228	0.0365
	Blood	Age	1	3.83	0.058	Na	Na
		$\delta^{15}N$	1	1.03	0.3177	0.1471	0.2718
		Age*$\delta^{15}N$	1	4.12	0.0496	Na	Na

competitive in explaining variation in adult blood Hg concentration ($\Delta AIC_c = 1.7$; Table S2). This alternate model for adult blood provided similar results, with a significant effect of species ($F_{2,63} = 4.61$, $P = 0.014$), a non-significant effect of $\delta^{13}C$ values ($F_{2,63} = 2.61$, $P = 0.1120$), and a significant species* $\delta^{13}C$ ($F_{2,63} = 4.75$, $P = 0.012$) due to positive correlations between blood Hg concentrations and $\delta^{13}C$ values in adult gentoo penguin feathers ($r = 0.60$, $P = 0.004$) but not other species (all $P > 0.319$). Even so AIC_c weights illustrated that the model using blood $\delta^{15}N$ values had twice as much support as the model using blood $\delta^{13}C$ values as a covariate (Table S2).

When examining feathers Hg concentrations in adult and juvenile gentoo penguins the model containing only $\delta^{15}N$ as a covariate was selected as the most parsimonious with no other competitive models found (Table S3 of the Supplementary Material). Gentoo penguin feathers Hg concentrations were positively correlated with $\delta^{15}N$ values both across (Table 3) and within age classes (Adults: $r = 0.75$, $P < 0.001$; Juveniles: $r = 0.81$, $P < 0.001$). The most parsimonious model explaining variation in gentoo penguin blood Hg concentrations included age, $\delta^{15}N$ and their interaction (age*$\delta^{15}N$) as covariates (Table S3). The only significant term in this model was the age*$\delta^{15}N$ interaction, because adult blood Hg concentrations were positively correlated with $\delta^{15}N$ values ($r = 0.63$, $P = 0.002$), but not juvenile gentoo penguin ($r = -0.18$, $P = 0.431$). A model that included the above three covariates as well two others (sex, sex*$\delta^{15}N$) was also competitive ($\Delta AIC_c = 1.6$; Table S3), but resulted in all model covariates being non-significant ($P > 0.279$).

4. Discussion

We found that tissue Hg concentrations differed across penguin species, between adults and juveniles, but not between sexes. Adult chinstrap penguins had the highest mean tissue Hg concentrations followed in descending order by adult Adélie penguins, adult gentoo penguins and juvenile gentoo penguins. Interestingly, while tissue Hg concentrations across all species and age classes were significantly and positively correlated with $\delta^{15}N$ values, these relationships were driven exclusively by adult gentoo penguins which also had the most variable tissue Hg concentrations and stable isotope values. Furthermore, similar to previous studies (Brasso et al., 2012, 2014) the higher tissue Hg concentrations found in adult chinstrap penguins could not be explained using $\delta^{15}N$ values, a proxy for trophic position, or by the stable isotope-based dietary mixing models used in this study. However, integrating data from penguin stomach content analysis with prey species Hg concentrations and stable isotope values revealed that the differences in epipelagic, benthic and mesopelagic prey selection by penguins are stronger drivers of Hg exposure, relative to trophic position alone.

4.1. Interspecific trends in penguin foraging ecology and Hg exposure

While all three species of *Pygoscelis* penguin diets are dominated by Antarctic krill in the Antarctic Peninsula region (Volkman et al., 1980), previous studies have found a general pattern of foraging niche partitioning of these species during the breeding season (Trivelpiece et al., 1987; Lynnes et al., 2002). This occurs via slight differences in diet, foraging area, and depth utilization (Kokubun et al., 2010; Miller et al., 2010; Wilson, 2010). For example, past stable isotope and conventional studies at King George Island and sites in the Antarctic Peninsula indicate higher krill consumption and greater use of offshore foraging habitats by Adélie and chinstrap penguin relative to gentoo penguins (Trivelpiece et al., 1987; Miller and Trivelpiece, 2008; Miller et al., 2010; Polito et al., 2015). The results of our otolith analysis agree with these previous studies and help to identify the differential use of epipelagic, mesopelagic, and benthic foraging habitats across the three species of *Pygoscelis* penguins.

Interestingly, the foraging habitat differences identified via otolith analysis were not as clearly delineated in the stable isotope values of penguin tissues. Isotopic mixing models predictions of the fish component of penguins diets overlapped broadly and only were able to predict a higher mean contribution of benthic fishes relative to mesopelagic fishes in the diets of adult gentoo penguins. Stable isotope-based dietary mixing models in this and other studies provided a robust estimate of the relative amounts of fish and krill in penguin diets due to their large difference in $\delta^{15}N$ values (e.g. Polito et al., 2011a, Polito et al. 2015). However, these models have difficulty estimating the relative dietary contribution of different fish species to penguin diets due to a high degree of overlap in the stable isotope values of epipelagic, mesopelagic, and benthic fishes around Antarctica (Cherel et al., 2010, 2011).

Differences in the diets and foraging ecology of *Pygoscelis* penguin species appear to have significant implication for their relative exposure to Hg in the Antarctic marine food web. For example, adult gentoo penguins often have a more mixed diet of krill and fish relative to their congeners (Trivelpiece et al., 1987) and this has the potential to lead to greater Hg exposure due their higher trophic level of diet (Carravieri et al., 2013; Brasso et al., 2015). However, while differences in relative krill vs. fish consumption has been observed in previous studies (Miller et al., 2010; Polito et al., 2015), we found no evidence of differences in trophic level (i.e. $\delta^{15}N$ values in blood or feathers) or predicted krill consumption (from isotopic mixing models) among adult Adélie, chinstrap and gentoo penguins at Admiralty Bay. Therefore the differences in Hg exposure among adult *Pygoscelis* penguins in our study are not likely related to difference in level of krill consumption or trophic levels *per se*. Instead, the observed differences in penguin species relative selection of epipelagic, mesopelagic, and benthic prey are likely stronger drivers of the observed differences in tissue Hg concentrations across *Pygoscelis* penguin species.

For example, we found that while adult Adélie, gentoo and chinstrap penguins shared a similar trophic level of diet (based on $\delta^{15}N$ values as a proxy), chinstrap penguins have relatively higher feather and blood tissue Hg concentrations. In addition, past studies suggest that this pattern of chinstrap penguins having higher Hg concentrations than Adélie and gentoo penguins is inter-annually and seasonally consistent across much the Antarctic Peninsula region (Brasso et al., 2012, 2014). While not tested until now, these past studies hypothesized that elevated Hg

concentrations in chinstrap penguin tissues may result from exploiting mesopelagic prey with higher Hg concentrations rather than foraging at a higher trophic level than their congeners (Brasso et al., 2012, 2014, 2015). Marine predators that forage in the mesopelagic realm or on mesopelagic prey have been documented to have elevated exposure to Hg compared to their trophically similar, epipelagic-feeding counterparts due to enhanced bioaccumulation of Hg at depth (Montiero et al., 1996; Montiero and Furness, 1997; Choy et al., 2009). We found strong evidence to support this hypothesis when comparing tissue Hg concentrations and stable isotope values across the three most common epipelagic, mesopelagic, and benthic fish species found in the diets of *Pygoscelis* penguins. Specifically, while epipelagic and mesopelagic myctophid fish shared similar $\delta^{15}N$ values, mesopelagic fish had higher Hg concentrations relative to epipelagic fishes. In addition, while mesopelagic fish occupied a slightly lower $\delta^{15}N$ values than benthic fish, these two prey species have similar Hg concentrations. These results substantiate the relatively higher risk of Hg exposure for chinstrap penguins foraging on mesopelagic fish relative to congeners foraging at a similar trophic level but targeting epipelagic or benthic prey species.

4.2. Intraspecific trends in penguin foraging ecology and Hg exposure

Relationships between feather and blood tissue Hg concentration and stable isotope values provide additional insights into seasonal and individual variation in penguin foraging ecology and Hg exposure. Whole blood reflects the short-term Hg exposure incorporated from food during blood formation as well as some component of residual Hg residing in other tissues (Bearhop et al., 2000a,c). Similarly, the stable isotope values of whole blood in a penguin 3–5 kg penguin integrate short-term dietary information over a period 20–60 days prior to collection (Carleton and del Rio, 2005; Barquete et al., 2013). Therefore the adult and juvenile whole blood samples examined in this study provide insight into species foraging ecology and Hg exposure during a short time period during the late incubation and the early chick-rearing periods. In contrast, the stable isotope values and Hg concentrations in adult penguin feathers reflect an integrated signal of endogenous tissue Hg and diets over the inter-molt period prior to the annual catastrophic molt in which all body feathers are lost and re-grown over a period of 2–3 weeks while fasting (Stonehouse, 1967). Furthermore, as non-breeding juvenile gentoo penguins (one year olds) have not yet completed their first adult molt, their feather stable isotope values reflect an integrated dietary history and Hg exposure from the food parents provide them during feather synthesis prior to fledgling (Hobson and Clark, 1992; Tierney et al., 2008; Polito et al., 2011a).

When examined across all species and age classes, tissue Hg concentrations and stable isotope values were significantly and positively correlated between blood (short-term signal) and feathers (long-term signal) from the same individual. This result suggests some degree of seasonal consistency in the diets and foraging ecology and Hg exposure at the individual level in *Pygoscelis* penguins. However, this global trend was driven primarily by adult and, to a lesser extent, juvenile gentoo penguins ($\delta^{15}N$ only). Interestingly, this weak but significant relationship between blood and feather $\delta^{15}N$ values in juvenile gentoo penguins in may indicate an unexpected relationship between the diets of the parents and that of their offspring post-fledging. Gentoo penguins also tended to have the most variable blood and feather Hg concentration and stable isotope values in comparison to other species. This high degree of individual variation in Hg exposure reflects similar individual variation in trophic position ($\delta^{15}N$) as individuals foraging at higher trophic positions also had higher Hg concentrations. Two previous studies, on Antarctic (Brasso et al., 2014) and sub-Antarctic (Carravieri et al., 2013) islands also have found that individual variation in trophic position led to increased variation in Hg concentrations in gentoo penguins at the population level.

Previous studies indicate the potential for sexual differences in diets in gentoo penguins, with larger males diving deeper and consuming a higher proportion of fish than females (Volkman et al., 1980; Bearhop et al., 2006; Miller et al., 2010). Such sexual differences in diets, when they occur, might be expected to lead to differential exposure to Hg between males and females (Becker et al., 2002). For example, Pedro et al. (2015) found higher Hg exposure in males relative to females as well as higher Hg exposure in individuals feeding on a higher proportion of fish. In contrast, we found little evidence for differences in stable isotope values between sexes within species and no evidence of differences in tissue Hg concentration. However, differing results across studies might be expected due to temporal and/or spatial variation in prey and foraging habitat availability that mediate the degree and consistency of sexual variation in *Pygoscelis* penguin diets as observed in other seabird species (Miller et al., 2010; Phillips et al., 2011).

While no sexual differences were apparent, we did observe differences in tissue Hg concentrations between age classes of gentoo penguins in our study. Both blood and feather Hg concentrations were significantly higher in adults relative to juveniles. However, these differences do not seem to be entirely age-related *per se* as differences in Hg exposure between age classes was influenced primarily by the generally higher and more variable trophic position proxy ($\delta^{15}N$) observed in adults relative to juveniles. As such, feather $\delta^{15}N$ values were the strongest predictor of feather Hg concentration regardless of age class, and age class differences in blood Hg concentrations were no longer significant once $\delta^{15}N$ values were taken into account. This indicates that individual gentoo penguins feeding, or being fed at, on higher trophic levels may be at greater risk of Hg exposure regardless of their age.

4.3. Conclusions and implications for biomonitoring

The tissue Hg concentrations observed in *Pygoscelis* penguins in this study were comparatively low relative to those found in other studies of seabirds in the Southern Hemisphere (Anderson et al., 2009; Blevin et al., 2013, Carravieri et al., 2013, 2016; Brasso et al., 2015; Carravieri et al., 2016). Hg concentrations in feathers averaged <0.50 ppm and those in blood samples averaged <0.10 ppm. In comparison, adverse effects in terms of reproductive impairment in other aquatic and marine birds have been reported at feather Hg concentrations between 5.0 and 40.0 ppm and blood Hg concentrations >3.0 ppm (Wolfe et al., 1998; Burger and Gochfeld, 2000; Evers et al., 2008). The highest feather and blood Hg concentrations observed in this study from an individual gentoo penguin and were 1.98 ppm and 0.34 ppm, respectively. Thus, it is unlikely that *Pygoscelis* penguins in our study or other areas of the Antarctic (e.g. Brasso et al., 2015) are experiencing negative impacts from Hg exposure.

While the potential for adverse effects due to Hg exposure is currently low in *Pygoscelis* penguins in Antarctica, this study provides important baseline data that will aid in the design and interpretation of future studies seeking to use *Pygoscelis* penguins as biomonitors for Hg availability in the Antarctic marine ecosystem. Specifically this study highlights how inter- and, to a lesser extent, intraspecific variation in foraging habitats and diet influences a species relative exposure to Hg. In addition, our findings indicate that stable isotope analysis alone was not sufficiently robust to identify the differences in penguin foraging ecology that most influenced Hg exposure. It was only by combining isotopic

analyses with data from stomach content analysis that we were able to identify the key differences in foraging habitat and prey selection among *Pygoscelis* penguins acting as strong drivers of Hg exposure. Furthermore, this approach allowed us to substantiate for the first time, the relatively higher risk of Hg exposure for chinstrap penguins foraging in mesopelagic habitats relative to congeners targeting epipelagic or benthic prey species. Therefore, in future studies of Hg exposure in penguins and other seabirds we recommend combining isotopic approaches with other, independent measurers of diets and foraging habitat to help account for the potentially confounding effects of inter and intraspecific variation in foraging ecologies.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.04.097.

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