

MERCURY CONCENTRATIONS IN SOUTHERN BEAUFORT SEA POLAR BEARS: VARIATION BASED ON STABLE ISOTOPES OF CARBON AND NITROGEN

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Abstract—Total Hg concentration was measured in hair and whole blood of 52 adult Southern Beaufort Sea polar bears (*Ursus maritimus*) captured in the spring of 2005. Stable isotopic signatures (i.e., $^{13}\text{C}/^{12}\text{C}$, $\delta^{13}\text{C}$; $^{15}\text{N}/^{14}\text{N}$, $\delta^{15}\text{N}$) in hair and two blood compartments (packed blood cells/clot and serum) were determined to assess the variation of Hg concentrations among polar bears in relation to their feeding ecology and other biological factors. Concentrations of Hg in hair and blood (2.2–23.9 $\mu\text{g/g}$ dry wt and 0.007–0.213 $\mu\text{g/g}$ wet wt, respectively) were within the range of values previously reported for polar bears in Canada and East Greenland. Mercury concentration in hair from females was higher than that in hair from males, and concentration was related to interactions between $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and longitude of capture location. Mercury concentrations in hair were inversely correlated to $\delta^{13}\text{C}$ in hair and blood, suggesting that polar bears with greater total Hg concentrations fed more on pelagic prey, such as ringed seals or beluga whale, than on benthic prey. Variability in Hg concentrations in polar bear hair and blood may be the result of intraspecific or regional variation in prey selection rather than strictly trophic level interactions.

Keywords—Trophic level Arctic food web Arctic marine mammals Biomagnification Bioaccumulation

INTRODUCTION

Contributions from point sources of trace elements, including Hg, in the Alaskan Arctic have been scarcely evaluated. However, nonpoint sources, such as springtime deposition of Hg in the Arctic, have been evaluated [1]. Oil and gas production and municipal solid-waste incineration are additional potential sources [2], but the principal sources of transboundary pollutants to the Alaskan Arctic have been traced to central and western Asia [3]. Because the Alaskan Arctic has the potential to be the recipient of local as well as global Hg contamination, questions arise regarding the fate of this contaminant, especially in wildlife and humans through fish-based food webs.

Concerns about Hg are based mostly on the neurotoxicity of its methylated form (CH_3Hg). Consumption of fish (i.e., piscivory) that accumulate methylmercury (MeHg) in their muscle is the main route of exposure to Hg for organisms higher in the food chain, such as seals and humans [3]. Once MeHg enters the body, it may bioaccumulate by sequestration, be stored in tissues in various forms [4], or be eliminated. For example, a study of rats by Norseth and Clarkson [5] revealed that the intestinal flora in mammals has the capability to demethylate MeHg. After demethylation, the resulting inorganic Hg is accumulated in the liver or excreted in the feces or urine. Whereas Hg in the liver of adult marine mammals is mostly inorganic, Hg in hair, muscle, and blood is mainly in the methylated form [3,5].

Apex predators, such as polar bears (*Ursus maritimus*) of northern Alaska, USA, are long-lived and mostly feed at high trophic levels; therefore, Hg and its effects are of concern, as

are persistent lipophilic chemicals [6]. Observational and chemical feeding ecology data (e.g., biomarkers such as stable isotopes of carbon and nitrogen) indicate that polar bears utilize diverse sources of prey (hunt and scavenge), including a variety of pinnipeds and cetaceans ([7–9], <http://alaska.fws.gov/fisheries/mmm/polarbear/feeding.htm>). The magnitude of this variation and its impact on contaminant exposure to polar bears is still under debate [6,8]. Polar bears in the Southern Beaufort Sea (SBS) feed mainly on ringed seals (*Phoca hispida*) and bearded seals (*Erignathus barbatus*) but have also been reported to hunt spotted seals (*Phoca largha*), beluga whales (*Delphinapterus leucas*), and walrus (*Odobenus rosmarus*) and also scavenge from subsistence-hunted bowhead whale (*Balaena mysticetus*) carcasses found along the northern shore of Alaska [7–9]. Differences in Hg concentrations and forms (total and methylated) in these prey species and across tissues therefore may be responsible for variation in Hg concentrations in polar bears and may determine the degree of bioaccumulation and tissue-specific variation (toxicodistribution) within individual bears.

Studies have shown that naturally occurring variations in the ratio of heavy to light isotopes of carbon ($^{13}\text{C}/^{12}\text{C}$, $\delta^{13}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$, $\delta^{15}\text{N}$) are useful indicators of feeding ecology and can be related to contaminant exposure [10–13]. Higher trophic position can be measured via increased $\delta^{15}\text{N}$ relative to prey items, whereas changes in $\delta^{13}\text{C}$ provide information regarding the location of dietary resources (e.g., terrestrial vs marine, pelagic vs benthic, and regional selection) [12–14]. Different feeding strategies by polar bears have been observed among age and sex cohorts: Larger, more dominant bears consume more energy-rich tissues (i.e., blubber), and smaller, less experienced bears rely more on scavenging (i.e., consumption of viscera, muscle, and bone) [15,16] (S. Amstrup, Senior Polar Bear Scientist, U.S. Geological Survey Alaska Science Center, personal communication). The type of tissue consumed by the polar bear plays a major role in the

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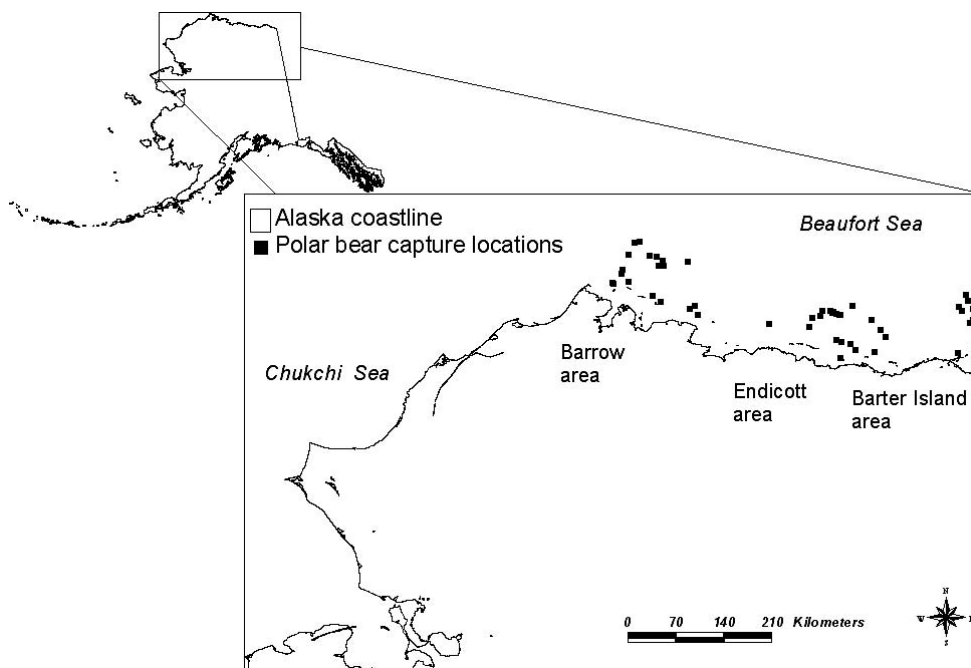


Fig. 1. Polar bear base locations for capture and sampling (Barrow, Endicott, and Barter Island, AK, USA) during the spring of 2005.

amount and type of Hg that will be transferred to the predator because of the toxicodistribution of the different Hg species among tissues. For example, bears that feed mainly on blubber of prey will consume less Hg than bears that feed on muscle: Methylmercury tends to accumulate in muscle, inorganic Hg in organs such as liver [3,4], and blubber contains scant concentrations of Hg. Consumption of liver and kidney therefore results in exposure to the less bioavailable inorganic Hg, whereas consumption of muscle results in exposure to the more bioavailable, bioaccumulative, and toxic MeHg.

Hair samples of mammals are considered to be good indicators of exposure to some contaminants [17]. Hair may be used as a recording filament to observe trace-element history, and this technique has been used successfully to evaluate MeHg exposure and dietary inputs in many species, including polar bears [18–21]. Blood samples (e.g., serum, packed blood cells/clots, and whole blood) can represent recent dietary and contaminant history (from weeks to two or three months previous); hair samples can show the longer-term dietary exposure, dependent on the period of hair growth.

To better understand how diet and other biological variables (i.e., age, sex, and location) may affect exposure to contaminants, we assessed variation in Hg (measured as total Hg [THg]) concentrations of polar bears in the SBS in relation to their feeding ecology as measured using stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) and other biological factors (i.e., age, sex, and capture location). Because elevated Hg concentrations in polar bear tissue can be associated with fish consumption and/or increased trophic level of polar bear prey (i.e., enrichment of $\delta^{15}\text{N}$), we hypothesize that THg concentrations will vary based on the trophic status of the bears, and we provide information regarding the individual's varying dependence on predominantly nonbenthic (e.g., bowhead whale and ringed seals) or more benthic (e.g., bearded seal and walrus) prey (as determined using $\delta^{13}\text{C}$).

MATERIALS AND METHODS

Sample collection

Hair and blood samples were collected from 52 adult polar bears (age, five years or older; 26 males, 26 females) captured from the SBS during the period from March through April of 2005 by the U.S. Geological Survey Ursid Research program (Anchorage, AK) as part of a long-term study of polar bear ecology and population dynamics. Animals were captured from logistics bases at Barrow ($n = 9$), Prudhoe Bay/Endicott ($n = 30$), and Kaktovik/Barter Island ($n = 12$; all AK, USA), and one bear was captured from an unrecorded location near these bases in Alaska (Fig. 1). Polar bears were captured by immobilization with Telazol® (Warner–Lambert)-filled projectile darts fired from a helicopter [22]. Age class and sex were determined for each polar bear at the time of capture, and all animals were given a unique identification number in the form of a lip tattoo and a plastic ear tag. Body mass and capture location (latitude and longitude) were recorded for each bear. Age of each bear was estimated by counting the cementum annuli of a vestigial premolar and ranged from 5 to 28 years for males and 5 to 25 years for females. Details of age estimation and capture procedures, including permitting, are described by Bentzen et al. [8]. Capture procedures were approved by an independent animal care and use committee before the study began (University of Alaska–Fairbanks, Institutional Animal Care and Use Committee, Assurance 04-58).

Hair was sampled from the longest trailing guard hairs at the caudal (palmar) aspect of the forelimb. Blood samples were collected from either the femoral vein or artery into nonadditive and K_3 ethylenediaminetetra-acetic acid Vacutainers™ (BD Vacutainers, Preanalytical Solutions) for stable isotopes and THg analyses, respectively. Blood in nonadditive Vacutainers was prevented from freezing and was centrifuged at 3,500 rpm for 5 min (Triac™ Centrifuge, Clay Adams) within 6 h of collection to separate serum from the packed blood cells

(clotting proteins and blood cells). The serum and pellet were frozen separately for stable isotopic analyses.

Mercury analysis

Hair was rinsed with diluted RBS-35 (contains 0.1–1% NaOH; manufacturer's directions were used for dilution; Pierce Biotechnology) and ultrapure water to eliminate surface oils and other exogenous substances. After rinsing, hair was frozen and freeze-dried to remove water before weighing the individual samples. Hair (0.03–0.05 g) was weighed into a polytetrafluoroethylene-lined digestion vessel of a PerkinElmer Multiwave 3000, where the samples were digested with nitric acid:hydrogen peroxide (3:1, v/v). Digestates were diluted to 20 ml with ultrapure water (Barnstead International). Mercury in a digestate volume of 0.05 ml was reduced with stannous chloride to form Hg^0 , preconcentrated onto a gold trap, and detected by cold-vapor atomic fluorescence spectrometry following a modification of U.S. Environmental Protection Agency Method 1631 (<http://www.epa.gov/waterscience/methods/method/mercury/1631.pdf>) for THg (all Hg species, organic and inorganic) on a Brooks Rand 1630 system (Model III Mercury Detector and Guru™ Software). Whole-blood samples were subsampled, weighed, digested, and analyzed according to the method described above for hair (except for the washing step).

Quality assurance/quality control followed standard laboratory procedures as described by Woshner et al. [14] and included method blanks, method duplicate, standard reference materials, spiked blanks, and spiked samples. Standard reference materials used included a frozen fish homogenate (Lake Superior 1946; National Institute of Standards and Technology), pygmy sperm whale liver homogenate III (Control Material QC03LH3; from the National Institute of Standards and Technology/National Oceanic and Atmospheric Administration interlaboratory comparison exercise), and white-sided dolphin liver homogenate IV (QC04LH4; from the National Institute of Standards and Technology/National Oceanic and Atmospheric Administration interlaboratory comparison exercise). Between two and three standard reference materials were included in each batch of samples digested. Recovery rates for standard reference materials ranged from 88 to 112% for hair analysis and from 121 to 167% for blood analysis. Method detection limit was approximately 25 pg. Data are presented on a wet-weight basis for whole blood and a dry-weight basis for hair as THg. The THg concentrations reported here are considered to be analogous to the concentration of MeHg, because the majority of Hg in hair and blood is in the methylated form.

Stable isotope analysis

Washed hair, serum, and packed blood cell/clot samples were freeze-dried for 24 to 48 h. Freeze-dried samples of 0.3 to 0.5 mg were then weighed using a Sartorius M2P electronic microbalance into small tin capsules and analyzed at the Alaska Stable Isotopes Facility at the University of Alaska–Fairbanks. An elemental analyzer–isotopic ratio mass spectrometer (Costech Elemental Analyzer [ESC 4010] and Finnigan MAT ConFlo III interface with a Delta+XP mass spectrometer) was used. Stable isotopes of carbon and nitrogen determinations were expressed in δ notation as parts per thousand according to the following equation:

$$\delta X = [R_{\text{sample}}/R_{\text{standard}} - 1] \cdot 1,000$$

where $X = {}^{15}\text{N}$ or ${}^{13}\text{C}$ and R = the corresponding ratio ${}^{15}\text{N}/{}^{14}\text{N}$ or ${}^{13}\text{C}/{}^{12}\text{C}$. The standards for ${}^{15}\text{N}$ and ${}^{13}\text{C}$ are atmospheric N_2 (air) and Pee Dee Belemnite standard, respectively. Peptone ($\delta^{15}\text{N} = 7.0$, $\delta^{13}\text{C} = 15.8$; meat-based protein; Sigma Chemical) was used as a working laboratory standard to ensure appropriate quality control and assurance. Duplicate samples differed by less than 0.8‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. The mean of duplicate values were reported for each sample.

Statistics

Statistics were calculated using Sigma Stat® 3.5 and Systat 11 software (Systat Software). The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in serum and $\delta^{13}\text{C}$ in hair from adult polar bears were normally distributed (Kolmogorov–Smirnov normality test). No other parameters (Hg concentrations, age, stable isotopes in packed blood cells, and body mass) were normally distributed. Therefore, either logarithmic or exponential transformations were made for THg and $\delta^{15}\text{N}$ in hair, THg in whole blood, and $\delta^{15}\text{N}$ in packed blood cells/clot for statistical comparisons. We were unable to find a transformation for $\delta^{13}\text{C}$ in packed blood cells that gave a normal distribution; therefore, the data were used as is (no transformation) in statistical analysis.

Groups that were normally distributed were compared using *t* tests and one-way analysis of variance (ANOVA). Mean concentrations of THg in blood (transformed to $\ln[\text{THg blood}]$) for the different capture locations also were assessed through a one-way ANOVA. For comparisons among groups (sex or capture locations) of data not normally distributed, Mann–Whitney rank sum tests were used.

Pearson correlation analyses were performed to identify relationships between independent variables. General linear (simple and multiple) regressions were further performed on variables with a $p < 0.15$ to obtain the strength of the relationships. To avoid autocorrelation, pairs of correlated variables were not used within the same multiple-regression models (e.g., latitude and longitude of capture location). Forward stepwise regressions were used, where $p < 0.15$, to assess the relationship of Hg concentrations and independent variables, including $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (hair, serum, and packed blood cells), age, latitude, and longitude.

RESULTS

Mercury

Concentrations of THg in adult polar bears ranged from 2.2 to 23.9 $\mu\text{g/g}$ (dry wt) for hair and from 0.007 to 0.21 $\mu\text{g/g}$ (wet wt) for whole blood (Table 1). Adult females had a greater mean concentration of THg in hair than adult males (9.0 and 5.37 $\mu\text{g/g}$, respectively; $t = 853.3$, $p = 0.003$). Despite this large range in absolute concentration, values of THg in hair correlated with those in whole blood ($r = 0.46$, $p = 0.011$) (Table 2). No relationship was found between age and THg in hair or blood, or between body mass and THg in blood. A negative correlation was found between THg in hair and body mass ($r = -0.32$, $p = 0.02$) and $\delta^{13}\text{C}$ in hair (Table 2). Because body mass and sex were highly correlated (i.e., polar bears are sexually dimorphic), only sex was used in describing the remaining relationships. A general linear model for \ln hair THg was significantly different for hair $\delta^{13}\text{C}$ ($f = 29.51$, $p < 0.001$), sex ($f = 8.50$, $p = 0.006$), and the interaction term (hair $\delta^{13}\text{C} \times \text{sex}$, $f = 9.11$, $p = 0.004$), and this model further emphasized the greater hair THg in females than males (Fig. 2). Total Hg in hair also related to $\delta^{13}\text{C}$ in packed blood cells

Table 1. Summary statistics of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes and mercury^a

	<i>n</i>	Mean \pm SD	Median	Minimum	Maximum
Hair $\delta^{15}\text{N}$	52	20.5 \pm 0.77 A	20.7	18.0	21.9
♂	26	20.5 \pm 0.88 C	20.7	18.0	21.9
♀	26	20.5 \pm 0.12 C	20.7	18.7	21.4
Packed cells $\delta^{15}\text{N}$	47	19.4 \pm 0.90 AB	19.7	17.0	20.8
♂	22	19.4 \pm 0.97 C	19.7	17.0	20.7
♀	25	19.5 \pm 0.85 C	19.8	17.3	20.8
Serum $\delta^{15}\text{N}$	31	20.9 \pm 0.68 B	21.0	19.7	22.5
♂	14	20.9 \pm 0.63 C	21.1	19.9	21.8
♀	17	20.9 \pm 0.74 C	21.0	19.7	22.5
Hair $\delta^{13}\text{C}$	52	-16.3 \pm 0.55 A	-16.3	-17.3	-14.9
♂	26	-16.3 \pm 0.48 C	-16.3	-17.0	-15.1
♀	26	-16.3 \pm 0.61 C	-16.3	-17.3	-14.9
Packed cells $\delta^{13}\text{C}$	47	-18.7 \pm 0.06 A	-18.9	-19.3	-17.6
♂	22	-18.7 \pm 0.42 C	-18.8	-19.2	-17.6
♀	25	-18.8 \pm 0.46 C	-18.9	-19.3	-17.6
Serum $\delta^{13}\text{C}$	31	-20.0 \pm 0.56 A	-20.1	-21.0	-18.3
♂	14	-19.6 \pm 0.47 C	-19.7	-20.2	-18.3
♀	17	-20.3 \pm 0.37 D	-20.4	-21.0	-19.4
Whole blood Hg ($\mu\text{g/g}$ wet wt)	30	0.07 \pm 0.05 A	0.052	0.007	0.21
♂	13	0.07 \pm 0.03 C	0.065	0.025	0.13
♀	17	0.06 \pm 0.06 C	0.050	0.007	0.21
Hair Hg ($\mu\text{g/g}$ dry wt)	52	7.4 \pm 0.56 B	6.5	2.2	23.9
♂	26	5.7 \pm 1.82 C	5.4	3.1	10.1
♀	26	9.0 \pm 4.9 D	8.7	2.2	23.9

^a Comparisons by tissue matrices (hair, packed cells with clot, and serum) and sex were performed separately for stable isotope and mercury analysis. Different matrices for all adult polar bears were compared using analysis of variance. Differences between male (♂) and female (♀) bears within each matrix were compared using *t* test or a Mann–Whitney rank sum test (for data not normally distributed). Different uppercase letters (A and B) indicate significant differences ($p < 0.05$) among matrices (A and B) and among sex (C and D). SD = standard deviation.

and to $\delta^{13}\text{C}$ in serum (Table 2). No relationship was observed between $\delta^{15}\text{N}$ in serum or packed blood cells and THg in whole blood or between $\delta^{15}\text{N}$ in hair and THg in hair.

No differences were observed in THg concentrations in hair related to capture location (capture base, latitude, and longitude) (Table 2). Mean concentration of THg in blood varied by capture location and was lower for bears captured near Endicott versus those captured near Barrow and Barter Island (ANOVA, $f = 6.334$, $p = 0.006$). Supporting these differences between capture locations is a positive correlation between the latitude of the capture location and THg in blood (Table 2).

Stable isotopes

The range of values for $\delta^{15}\text{N}$ in serum, hair, and packed blood cells/clot was 17.0 to 22.5‰ (Table 1). No significant difference in $\delta^{15}\text{N}$ was found between sexes in any of the matrices examined. Significant differences were found between the mean $\delta^{13}\text{C}$ in serum of males (-19.6‰) and females (-20.3‰; $t = -4.939$, $p = 0.001$). The means for $\delta^{15}\text{N}$ ranked as serum > hair > packed blood cells/clot, with a significant difference existing only between hair and packed blood cells (Kruskal–Wallis one-way ANOVA, pairwise multiple comparison, $p < 0.05$), and means for $\delta^{13}\text{C}$ ranked as serum > packed blood cells/clot > hair, with all pairwise multiple comparisons (Holm–Sidak method) showing statistically significant differences ($f = 570$, $p < 0.001$). Significant positive correlations were observed between $\delta^{15}\text{N}$ in hair and $\delta^{15}\text{N}$ in packed blood cells/clot and between $\delta^{15}\text{N}$ in packed blood cells/clot and $\delta^{15}\text{N}$ in serum. Significant positive correlations also were observed for $\delta^{13}\text{C}$ in packed blood cells/clot and $\delta^{13}\text{C}$ in hair and for $\delta^{13}\text{C}$ in packed blood cells/clot and $\delta^{13}\text{C}$ in serum (Table 2).

Latitude and longitude were strongly correlated ($r = -0.83$, $p < 0.001$, $n = 48$). The latitude increment, because of the

nature of Alaska's coastline, also is indicative of an increase toward Barrow, the westernmost capture location; therefore, longitude (i.e., east-to-west gradient) was used for all remaining analyses, including regressions. A negative correlation, or a decreasing trend toward the east, was observed between $\delta^{15}\text{N}$ in serum and the longitude of the capture (Table 2), but no significant correlations were found between $\delta^{13}\text{C}$ in serum and longitude. A multiple-regression model for THg concentrations in adult male polar bear hair identified $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (in hair) as good predictors of THg. A similar model for females identified $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ (in hair and packed blood cells), and capture location (longitude) as good predictors for THg in hair (Table 3).

DISCUSSION

Mercury concentrations in SBS polar bears

Concentrations of THg in SBS polar bear hair were within the range of those found by Renzoni and Norstrom [23] in bears of the Canadian Arctic (~1.5–9.5 $\mu\text{g/g}$) and those found by Dietz et al. [19] during recent years in East Greenland polar bears (0.79–17.7 $\mu\text{g/g}$). Therefore, our data support those of Born et al. [18], who suggest an east-to-west increase in Hg concentrations in the Greenlandic Arctic (from Svalbard, with ranges of 1.02–4.55 $\mu\text{g/g}$, to the western part of Greenland, with ranges of 4.71–14.19 $\mu\text{g/g}$).

In the present study, a higher average concentration of THg found in hair of females compared with hair of males is inconsistent with the findings of Born et al. [18], who found no differences in hair THg concentrations between male and female polar bears in Greenland. This could reflect differences in prey selection during late spring and summer (the time of hair growth in polar bears), different excretory patterns for Hg, variations in growth of hair between sexes, and/or different

Table 2. Pearson correlations among latitude, longitude, stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) and mercury (Hg) concentrations in adult (age, five years or older) polar bears^a

	exp(Hair $\delta^{15}\text{N}$)	exp(Packed cells $\delta^{15}\text{N}$)	Packed cells $\delta^{13}\text{C}$	Serum $\delta^{15}\text{N}$	Serum $\delta^{13}\text{C}$	ln(Whole-blood Hg)	ln(Hair Hg)
Latitude	0.32 0.03 48	0.23 0.14 43	0.26 0.09 43	0.66 <0.001 28	0.15 0.46 28	0.49 0.008 29	0.16 0.29 48
Longitude	-0.27 0.07 48	-0.16 0.32 43	-0.29 0.06 43	-0.60 <0.001 28	-0.07 0.72 28	-0.20 0.31 29	-0.11 0.46 48
exp(Hair $\delta^{15}\text{N}$)	—	0.32 0.03 47	0.20 0.17 47	0.33 0.07 31	-0.09 0.63 31	0.28 0.13 30	0.20 0.15 52
Hair $\delta^{13}\text{C}$		0.34 0.02 47	0.75 <0.001 47	0.26 0.15 31	0.22 0.24 31	-0.25 0.18 30	-0.65 <0.001 52
Packed cells $\delta^{15}\text{N}$		—	0.45 0.001 47	0.41 0.03 29	0.23 0.23 29	0.22 0.25 30	-0.002 0.99 47
Packed cells $\delta^{13}\text{C}$			—	0.38 0.04 29	0.52 0.004 29	-0.15 0.44 30	-0.49 <0.001 47
Serum $\delta^{13}\text{C}$					—	0.17 0.50 19	-0.48 0.007 31
ln(Whole-blood Hg)						—	0.46 0.01 30

^a Significant correlations are denoted in italics. Age was not significant overall and so was not included in table. Body mass and sex were highly correlated and therefore analyzed separately. *n* = sample size for each correlation; *r* = correlation coefficient; *p* = significance of correlation.

physiological demands between males and females between regions (SBS vs Greenland) or years of sampling (2005 vs 1978–1989). Female polar bears have less body mass than males, a characteristic that could possibly affect their feeding/hunting habits. For example, females may be forced to hunt smaller prey, such as ringed seals (60 kg), because they are unable to handle larger prey species, such as bearded seals and walrus, which can attain weights of more than 300 kg. This relationship is supported by the more depleted $\delta^{13}\text{C}$ found in female as compared to male polar bears, consistent with females feeding more on pelagic-feeding versus benthic-feeding prey species (ringed seals, -18.7‰ ; bearded seals and walrus -17.0‰ , respectively) [24–26] and higher THg concentrations in animals with smaller body mass (in the present study: males, 368.6 ± 87.3 kg; females, 169.1 ± 23.2 kg). Mercury concentrations in the muscle of ringed seals, bearded seals, and walrus are largely unknown, but concentrations of THg in the liver of these prey species (2–143 $\mu\text{g/g}$) are relatively high and dependent on age and location of prey species across the Alaska and Canada regions of the SBS [24,25,27,28]. Female polar bears also may differ in their movement patterns as compared to males to remain in contact with preferred feeding sites and habitats and to avoid conflicts with larger bears (often males), especially when accompanied by dependent young [29]. Thus, females may be forced to scavenge less lipid-rich tissues (e.g., muscle and viscera), which contain greater concentrations of Hg than blubber in pinnipeds [3,4,24,30], from prey in which more energy-rich blubber was removed previously by the bear that made the kill. Elevated THg levels in female polar bears are of concern because of the potential for transfer of Hg burdens to offspring during gestation and lactation (e.g., fetus and neonate are cohorts for concern for Hg toxicosis).

The observed THg hair:blood correlation is an expected

trend, because the THg in hair is not cumulative but, rather, is a representation of the blood THg concentration at the time that follicles are producing hair [17,31], which is during the late spring/early summer for polar bears. Higher concentrations of THg in hair versus blood are related in part to the differences in the dry weight:wet weight concentration of Hg (i.e., blood values are diluted because of greater water content) and different binding properties of Hg (e.g., variations in seleno-Hg and sulfhydryl-Hg interactions) between these tissues. Higher concentrations in hair relative to blood also may reflect the ability of polar bears to transfer much of their circulating MeHg burden to tissues with limited bioavailability (e.g., hair), thus providing an important mode of Hg elimination in mammals that molt yearly, similar to sloughing of skin/epidermis in cetaceans [14].

Total Hg concentrations did not increase with age for hair and blood in SBS polar bears. Differences in THg concentrations among bears therefore may be related more to the toxicodistribution of Hg (i.e., differences in the Hg concentrations and forms in prey species and across tissues) or different feeding habits among bears than to accumulation over time. Accumulation of THg in marine mammals with time has been found to occur mostly as inorganic Hg in the liver or kidney [4,26,30]. The present study, however, focuses on concentrations of THg in hair and blood, which would represent mostly methylated forms of Hg in adult bears. Methylated forms of Hg in the muscle of SBS beluga whales also were found not to increase with age and have been associated with more recent dietary sources of Hg rather than with bioaccumulative processes [32]. Adult polar bears feed at a high trophic level, with a relatively narrow range of $\delta^{15}\text{N}$ values [8]; therefore, bioaccumulation and biomagnification processes for methylated Hg within this species are lower in comparison to the biological variation among individuals (i.e., mass and sex), recent dietary

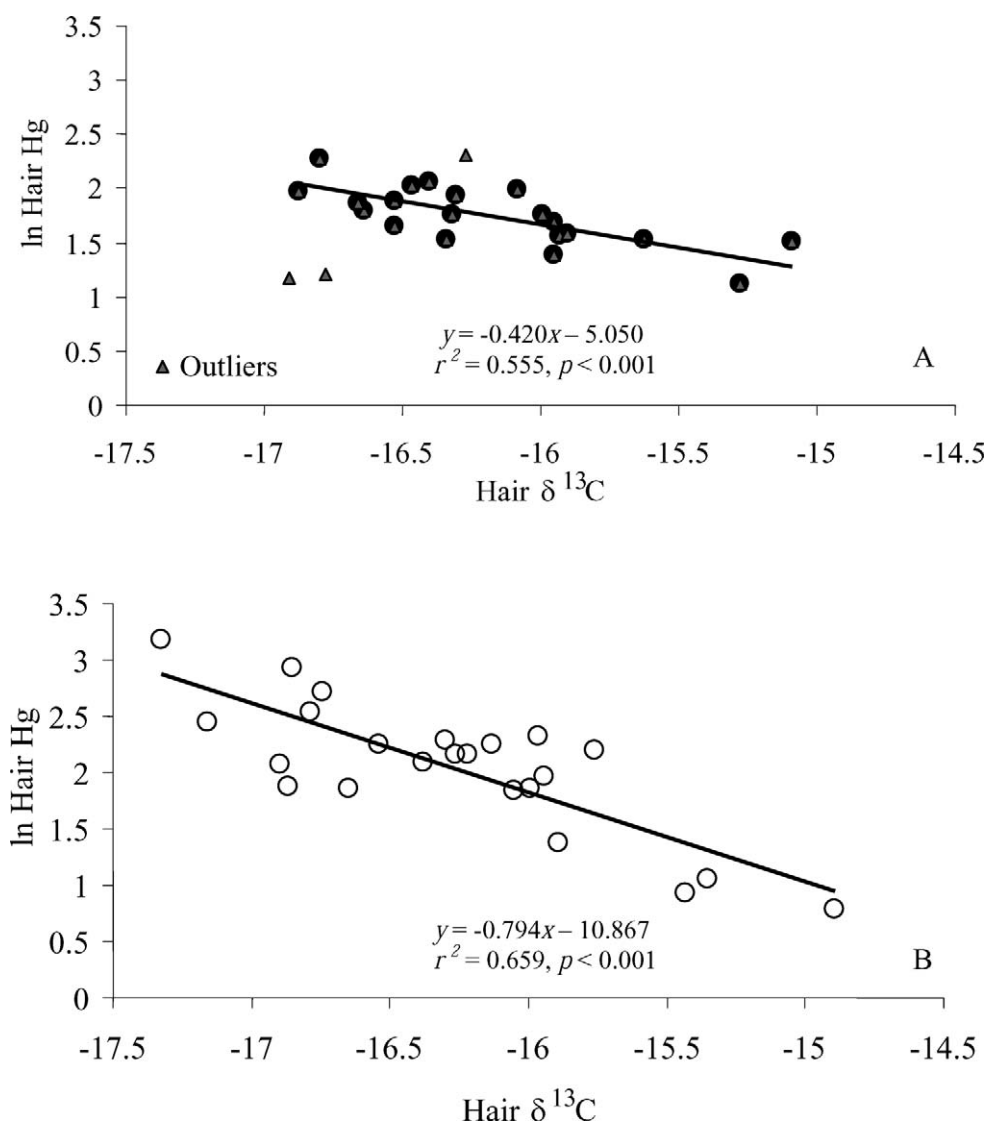


Fig. 2. Mercury concentrations in hair (log transformed, \ln Hg in hair) of adult polar bears as a function of the carbon isotopic signature. The overall general linear model for \ln hair Hg was significantly different for hair $\delta^{13}\text{C}$ ($f = 29.51, p < 0.001$), sex ($f = 8.50, p = 0.006$), and the interaction term (hair $\delta^{13}\text{C} \times$ sex, $f = 9.11, p = 0.004$); therefore, linear regressions are shown separately for males (A; three outliers removed) and females (B).

choice (i.e., prey species and regional selection), and the variation in the toxicodistribution of Hg forms (inorganic vs methylated Hg).

Concentrations of Hg vary with $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$

The range of values for $\delta^{15}\text{N}$ (17.0–22.5‰) in serum, hair, and packed blood cells/clot in the present study were representative of at least one trophic level (3–4‰ [15]) and were

similar to values found by Atwell et al. [33] in polar bear muscle and by Bentzen et al. [8] in polar bear packed blood cells. The lack of differences among sexes for $\delta^{15}\text{N}$ is a finding similar to that of Bentzen et al. [8], indicating feeding at a similar trophic level. The range of $\delta^{15}\text{N}$ in packed blood cells/clot found in the present study was wider than those found by Bentzen et al. (18.1–20.6‰ and 18.3–21.4‰ in 2003 and 2004, respectively [8]), although mean values were very similar

Table 3. Model results for individual variables, including longitude, used in multiple regressions of mercury concentrations in adult (age, five years or older) polar bear tissues^a

Dependent variable	Tissue	$\delta^{15}\text{N}$ ($p(t)$)	$\delta^{13}\text{C}$ ($p(t)$)	Longitude ($p(t)$)	Overall model ($p(f, r^2)$)
Males	Hair Hg	*** (5.44)	*** (-4.98)	NS	*** (13.64, 0.58)
Females	$\ln(\text{Hair Hg})$	*** (-6.28)	*** (-9.20)	# (-1.55)	*** (29.11, 0.82)
Females	$\ln(\text{Hair Hg})$	NS	*** (-5.29)	* (-2.51)	*** (14.14, 0.57)

^a Significant differences designated as follows: NS, not significant; # $p = 0.14$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. Tissue refers to the tissue for which the independent variables $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were analyzed. f = value of continuous distribution probability including all variables; p = significance value; r^2 = coefficient of determination; t = value of t -distribution probability.

(19.5‰ and 19.9‰ vs 19.4‰ in the present study). Bentzen et al. [8] concluded that the 3‰ variation of $\delta^{15}\text{N}$ suggested that polar bears in the SBS feed on prey of varied trophic levels during winter, from ringed seals to bowhead whales. The range of $\delta^{15}\text{N}$ found for polar bears in the present study represents one trophic level above those reported for ringed seals (16.4‰), bearded seals (16.8‰), and beluga whales (16.4‰) [33,34]. Although polar bears also have been reported to feed on walrus and bowhead whales, values of $\delta^{15}\text{N}$ reveal the likelihood that their main prey is seal (ringed and bearded), because walrus (12.8‰) and bowheads (13.4‰) are two trophic levels ($\sim 7\text{‰}$) below polar bears [8]. The lower $\delta^{15}\text{N}$ signature in the packed blood cells/clot of a number of animals, however, suggests that many polar bears also may include lower trophic species, such as bowhead whales during certain times of the year. This observation is consistent with the large number of polar bears remaining on land in fall and early winter in areas where bowhead carcasses are present [10]. Further information regarding seasonal shifts in diet based on stable isotopes in different tissue matrices is warranted but also should include other indices of body condition as well as fat and protein content of the respective tissues studied, because isotopic fractionation rates and routing among tissues are affected by various physiological demands.

An inverse relation exists between THg in hair and $\delta^{13}\text{C}$ in all tissues studied from adult polar bears. Because $\delta^{13}\text{C}$ is not enriched to the same degree as $\delta^{15}\text{N}$ in consumer tissues, $\delta^{13}\text{C}$ is less useful in the determination of trophic level. Variations in $\delta^{13}\text{C}$, however, may provide information about the differences in habitat use and carbon sources among predators [13]. The main prey of polar bears, ringed and bearded seals, are predominantly nonbenthic predators and bottom feeders, respectively [25,35]. Dehn et al. [25] found that bearded seals and walrus were significantly more enriched in $\delta^{13}\text{C}$ than ringed seals. The average hair $\delta^{13}\text{C}$ signature for polar bears was $-16.31\text{‰} \pm 0.55\text{‰}$, similar to that reported by Dehn et al. [25] for walrus. The $\delta^{15}\text{N}$ signature is more than one trophic level above walrus, however, suggesting that prey of a higher trophic level than walrus is an important part of the polar bear diet.

Other species with an isotopic signature similar to that of ringed seals are the beluga whale (*D. leucas*) and the spotted seal (*P. largha*). Dehn et al. [34] found a $\delta^{13}\text{C}$ signature of -18.3 ± 0.9 in spotted seals in Alaska, which also is within the range of values found for ringed seals (-18.5 ± 0.8) in Barrow. In tissues of beluga whales, Wagemann et al. [36] reported a range of 0.09 to 9.25 $\mu\text{g/g}$ (wet wt) of Hg in muscle, a much larger range than for ringed seal muscle (0.05–2.02 $\mu\text{g/g}$). Wagemann et al. [36] also found a range of 0.19 to 1.93 $\mu\text{g/g}$ for Hg in muktuk of beluga whales. Mercury in muscle and muktuk (primarily from the epidermis) is mostly in the methylated form and, thus, is available for bioaccumulation in predators. Dehn et al. [25] reported a Hg range of from 0.01 to 0.09 $\mu\text{g/g}$ in bearded seal muscle and from 0.10 to 0.15 $\mu\text{g/g}$ in spotted seals, with both species showing lower concentrations than beluga whales. Belugas are hunted by polar bears and are harvested by subsistence communities in Alaska and Northwest Territories (Canada), which leave butchered carcasses on land and available to polar bears. In captivity, polar bears have been found to consume $20\% \pm 2\%$ muscle and viscera when allowed to select between muscle and blubber in their diet [37]. Odontocetes have been found to have the greatest proportion of THg burden in muscle, liver, and

muktuk (blubber including epidermis) [38,39], tissues that are known to be consumed by polar bears. If polar bears in the wild behave similarly to those in captivity studied by Best [37] (i.e., consume 20:80, v/v, muscle/viscera:blubber/epidermis), then they are exposed to the consequently high concentrations of MeHg in Odontocete muscle and epidermis, which indicates that beluga whales as well as ringed seals may be considerable sources of Hg to polar bears as compared to walrus and bowhead whales.

In addition to providing information regarding the dietary sources, stable isotopes of carbon also have been found to provide information concerning regional selection of these sources. Regional differences and trends have been observed previously in Hg concentrations and $\delta^{13}\text{C}$ among polar bears throughout the circumpolar Arctic [8,18]. The multiple-regression model for THg concentrations in adult polar bear hair (Table 3) revealed that $\delta^{13}\text{C}$ along with longitude and $\delta^{15}\text{N}$ are good predictors of THg in female polar bears and are moderate predictors for males. These results show the importance of $\delta^{13}\text{C}$ as a regional indicator of habitat use among polar bears and suggest an east-to-west gradient in THg concentrations among adults in the SBS. No direct relationship was found between THg concentrations in hair of polar bears and $\delta^{15}\text{N}$ alone; however, when signatures of $\delta^{15}\text{N}$ were paired with $\delta^{13}\text{C}$ in the respective tissue, the regression model was a good predictor of hair THg among bears. These data confirm the usefulness and importance of considering stable isotopes of carbon and nitrogen in union when assessing interactions between feeding ecology and contaminant exposure. These data are consistent with movements and the distribution of polar bears that gather near the highly productive regions of the SBS of eastern Alaska/western Canada, where multiple prey species (ringed seal, bearded seal, beluga whale, and carcasses of subsistence-harvested bowhead whales) are available throughout the year [8,9,32,40]. Thus, distributions of predators and their prey result in complex interactions between feeding ecology and exposure to Hg that is dependent on tissue and prey species consumed.

Of the 15 bears with the most depleted $\delta^{13}\text{C}$ signature in hair (29% of all polar bears sampled), seven were captured near Endicott, seven near Barter Island, and only one near Barrow. These were the same bears with some of the highest THg concentrations in hair observed during the present study. The trend of an east-to-west $\delta^{13}\text{C}$ gradient across the Chukchi and Beaufort seas is consistent with polar bears sampled in this region during 2003 and 2004 [8]. Bentzen et al. [8] described that variations in $\delta^{13}\text{C}$ were related to differences in hunting locations for individual bears across the Bering, Chukchi, and Beaufort seas. A similar trend of depleted $\delta^{13}\text{C}$ from the Bering and Chukchi seas to the eastern Beaufort Sea was found in baleen plates and muscle of migrating bowhead whales [26,41] as well as in ringed and bearded seals [34]. These data are consistent with a depletion in $\delta^{13}\text{C}$ in fauna near freshwater inputs, such as those found in zooplankton near the Mackenzie River Delta, driven by river-transported organic matter of terrestrial origin [42]. These data suggest that river systems may be important transport routes of Hg and organic matter into the Arctic marine food web, as suggested by Macdonald et al. [43]. Variations in $\delta^{13}\text{C}$ also likely reflect variations in the feeding ecology of males and females (benthic vs pelagic, nearshore vs offshore) or a potential segregation of Chukchi and SBS bears that is more easterly than previously described (near Barrow [44]). However, more data

(and larger sample sizes) are needed regarding movements of individual bears in this region, in concert with behavioral and observational data concerning space use and diet. Therefore, future studies of the contaminant exposure to polar bears should include movement patterns and feeding habitats to identify potential contaminant sources that likely vary by region and season.

CONCLUSION

Total Hg concentrations in hair and whole blood of polar bears of the SBS did not increase with age or $\delta^{15}\text{N}$ alone but, rather, involved interactions between $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and location at capture. The THg concentration in hair was greater in females than in males, which suggests dietary differences between sexes (prey or tissue consumption) or varying physiological demands affecting Hg toxicodistribution. Variability of THg concentrations in polar bears therefore may be caused by intraspecific variation of prey selection by individual bears rather than by strictly trophic level interactions. Sources of this variation include sex, differences in feeding areas (east-to-west gradient for Hg and $\delta^{13}\text{C}$ in the prey), and possibly, individual bear physiology (e.g., sex, reproductive status, denning, and body condition). We suggest hair as a noninvasive matrix to monitor trends in Hg exposure in polar bears across cohorts (e.g., age and sex), years, and regions, including examination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures in tissues with different fractionation rates to provide information on diet incorporating several time periods. Continued studies of the interactions of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures are useful in the determination of trophic status and general prey choice and in better understanding exposure and resultant tissue concentrations of Hg.

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