

Size and Biomagnification: How Habitat Selection Explains Beluga Mercury Levels

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Mercury (Hg) levels in the Beaufort Sea beluga (*Delphinapterus leucas*) population increased during the 1990s; levels have since declined but remain higher than the 1980s. The diet of this beluga population is not well-known, thus it is difficult to assess dietary Hg sources. During the summer, the Beaufort Sea belugas segregate by length, sex, and reproductive status corresponding to habitat use that may result in feeding differences and ultimately Hg uptake. To test this hypothesis, we examine beluga dietary variation using fatty acid profiles and determine which biological variables best predict diet. Relationships between biological variables and fatty acids were further evaluated with stable isotopes and Hg concentrations in liver and muscle. Hg concentrations in muscle were better related to liver $\delta^{15}\text{N}$ than muscle $\delta^{15}\text{N}$. Stable isotopes and fatty acids are compared in their ability to describe dietary Hg processes in beluga. Fatty acids provided support for influences of whale behavior on dietary Hg uptake, whereas stable isotopes inferred tissue Hg metabolic rates. Here, we show beluga length drives diet variability leading to differences in Hg uptake and biomagnification processes dominate beluga Hg levels over Hg bioaccumulation over time.

Introduction

In the 1990s, liver mercury (Hg) levels in the Beaufort Sea beluga (*Delphinapterus leucas*) whale population tripled in comparison with levels in the 1980s (1), and were the highest relative to other Canadian Arctic beluga populations. Although, still higher than the 1980 levels, Hg concentrations have dropped and are now comparable to other Arctic populations (1). Mercury bioaccumulates in organisms over time and biomagnifies at each trophic level, together those processes contribute to high Hg levels in predators such as beluga (2). Little is known about the diet of Beaufort Sea beluga, thus dietary Hg uptake is difficult to model.

Typically in animal diet studies, stomach contents and feces are used to identify diet items and infer dietary

composition. This is not feasible with the Beaufort Sea beluga whales because feces can not be found, and harvested belugas usually have empty stomachs, yet local hunters have observed beluga feeding in their summer grounds (3). Based on relative isotopic fractionation processes, $\delta^{15}\text{N}$ can be used to describe trophic levels (4) and $\delta^{13}\text{C}$ can be used to evaluate food web carbon sources (5). Therefore, tissue stable isotope concentrations can assist in the understanding of diet and can overcome problems associated with conventional diet determination such as over- or under-representation of prey that were recently eaten or quickly digested (6). Similar to stable isotopes, the composition of just over 40 fatty acids can be used to describe diet (7–9). Fatty acids stored in blubber undergo little degradation during digestion and deposition (7). Fatty acid analysis has successfully characterized trophic links among species (10, 11) and determined predator diets in marine and terrestrial ecosystems (11–13) as well as detected the influences of size, sex, and season on predator foraging behavior (14). Krahn et al. (15) incorporated both stable isotopes and fatty acids in the analysis of persistent organic pollutants in killer whales (*Orcinus orca*) and delineated diets and foraging behavior among the North Pacific population. Only stable isotopes have been used to describe beluga diet and Hg levels, thus incorporating fatty acids as a dietary tool may provide new insights into Hg sources.

During the summer, habitat selection among Beaufort Sea belugas differs with length, sex, and reproductive status (16). Habitat segregation may reflect differences in energy requirements and survival strategies that vary with age, sex, size, and reproductive stage (17). For example, in the dimorphic northern elephant seal (*Mirounga angustirostris*), males are up to 10 times the size of females and, as a result, are assumed to require different feeding strategies to maintain their size difference (18). Beaufort Sea beluga are size dimorphic (3); males reach a mean asymptotic length of 4.2 m and the female asymptotic length is approximately 0.5 m less (19). Thus, variation in habitat selection as it relates to size, sex, and reproductive status may support different feeding strategies and dietary composition.

In this study, we determine if beluga dietary composition varies in relation to habitat use and what those effects may have on beluga Hg levels. First, we examined the relationship of biological variables associated with habitat use such as length, sex, age, and harvest site with fatty acids, a proxy for diet. Biological variables that best described diet were then evaluated for relationships with the biomarkers $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ as well as Hg levels in beluga muscle and liver tissues. Finally, stable isotopes and fatty acids were compared as biomarkers to describe dietary Hg processes in beluga.

Materials and Methods

Beluga Tissue Collection. Beluga tissues were sampled from whales harvested at Hendrickson Island located in the shallow Mackenzie delta near the community of Tuktoyaktuk Northwest Territories (NT) and at Browns Harbour near the community of Paulatuk NT (Figure 1). A total of 42 samples were collected, from Tuktoyaktuk (T) in 2004 ($n = 19$) and 2005 ($n = 13$), and at Paulatuk (P) in 2005 ($n = 10$). Sampling occurred within days of one another at each site. Few females were collected from the harvests because hunters generally select for medium to larger sized males (P(f) = 2; T(f) = 7). Whales were significantly younger and shorter at Paulatuk (381 cm \pm 12 se; 18 years \pm 3 se) relative to those harvested at Tuktoyaktuk (407 cm \pm 12 se; 29 years \pm 2 se). One young male (8 years old) from Paulatuk was included in the analysis

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FIGURE 1. Beluga summer region and harvest sites. Hendrickson Island was the harvest location for Tuktoyaktuk, and Browns Harbour is the harvest location for Paulatuk, both communities are located in Northwest Territories Canada.

that may have been sexually immature (20). Beluga tissues were sampled and frozen on site in a portable freezer at -20°C and shipped to Fisheries and Oceans Canada in Winnipeg for analysis. Blubber was sampled with the skin attached distally and muscle proximally. Muscle and liver tissues were selected for Hg and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ determination. Ages were determined from a thin section of a tooth by counting individual growth layer groups in the dentine (21).

Fatty Acid Extraction. The inner blubber layer was used for fatty acid analysis because it is the most metabolically active and represents the most recent deposit of fatty acids from diet (22). The blubber sample was taken after slicing and discarding the outer surface of the tissue. Lipids were extracted from 0.5 g duplicate samples using 2:1 chloroform-methanol containing 0.01% BHT (v/v/w) to avoid oxidation. This method was modified from Folch et al., (23). The lipid phase was collected, washed, and filtered through anhydrous sodium sulfate and evaporated under nitrogen to obtain the total lipid. The extracted lipid was used to prepare the fatty acid methyl esters by transesterification with Hilditch reagent (0.5 N H_2SO_4 in methanol). The samples were heated for 1 h at 100°C . Fatty acid methyl ester samples were analyzed using gas chromatography (Hewlett Packer HP series 6890) with a mass spectrometer detector (Hewlett-Packard 5973). Inlet temperature set at 250°C and the following temperature program was used: start at 153°C for 2 min, then ramp up at $2.3^{\circ}\text{C min}^{-1}$, hold at 174°C for 0.2 min and ramp up at $2.5^{\circ}\text{C min}^{-1}$ and hold at 220°C for 3 min, as described previously (10). A silica column (30 m \times 0.25 mm i.d.) coated with 50% cyanopropyl polysiloxane (0.25 μm film thickness; J&W DB-23) was used. Helium was the carrier gas that was equipped with an oxygen scrubber. Up to 66 fatty acid methyl esters were identified according with verification of ion mass spectroscopy and known standard mixtures (Nu Check Prep.). Fatty acids were integrated and expressed as a mass percent of the total fatty acids. Fatty acid identifications were checked on all chromatograms and reintegrated if necessary. Each fatty acid was described using the shorthand nomenclature of A:Bn-X, where A represents the number of carbon atoms, B the number of double bonds, and X the position of the double bond closest to the terminal methyl group.

Carbon and Nitrogen Isotope Fractionation Analysis. Nitrogen isotope determination was performed on dried homogenized subsamples of beluga liver and muscle. Lipids were removed for the carbon isotope determination using a chloroform/methanol extraction and then dried for analysis. Carbon and nitrogen isotopic analyses were accomplished by continuous flow, ion-ratio, mass spectrometry (CF-IRMS)

using a GV-Instruments IsoPrime attached to a peripheral, temperature-controlled, EuroVector elemental analyzer (EA) (University of Winnipeg Isotope Laboratory, UWIL). One-mg samples were loaded into tin capsules and placed in the EA autosampler along with internally calibrated carbon/nitrogen standards. Carbon and nitrogen isotope results are expressed using standard delta (δ) notation in units of *per mil* (‰). The delta values of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) represent deviations from a standard such as

$$\delta_{\text{sample}} \text{‰} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where R is the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratio in the sample and the standard. The standards used for carbon and nitrogen isotopic analyses are Vienna PeeDee Belemnite (VPDB) and IAEA-N-1 (IAEA, Vienna), respectively. Analytical precision, determined from the analysis of duplicate samples of every fifth sample, was $\pm 0.19\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.13\text{‰}$ for $\delta^{15}\text{N}$. Accuracy was obtained through the analysis of laboratory standards used for calibration of results.

Total Mercury Analysis. Beluga muscle and liver tissues were analyzed for Total Hg (THg). The majority of Hg in beluga liver is inorganic, whereas the majority in muscle is methylmercury (MeHg) (24). Sub samples were taken from partially thawed tissue after slicing away the outer surface. All samples were weighed to approximately 0.15 g for THg analysis. Samples were digested with a hydrochloric/nitric acid mixture (Aqua Regia) heated to 90°C . The digest was analyzed for THg by Cold Vapor Atomic Absorption spectroscopy (CVAAS) (25). The detection limit was 0.005 $\mu\text{g/g}$. Certified standard reference materials (CRM 2976, TORT-2, DOLT-2) were analyzed in duplicate in every run. Recovery within 10% of the certified values was used as a batch validation for samples. Average differences in duplicates for beluga are 5%.

Data Analysis. Fatty acids were measured as a percent of the total and were log transformed before all statistical analyses. Although 65 fatty acids were identified, only the fatty acids known to biotransfer from prey to predator were evaluated, we used 40 of the 41 described by ref 8. Multivariate analyses were carried out using SYN-TAX Ordination 2000 (Budapest, Hungary). Systat 11 (Systat Software Inc., 2004, San Jose, CA) was used for univariate statistical analysis, and SASVersion 8 (SAS Institute Inc., Cary, NC) was used to calculate the Akaike information criteria (AIC).

A principle component analysis (PCA) with a covariance matrix was used to examine the beluga fatty acid variation among individuals. We examined which of the biological variables best explained variation in beluga fatty acids by regressing the biological variables with the PCA scores from axis one and two, with the expectation of comparable results. Biological variables that best related to the fatty acids, described by PCA axes were examined for predictive relationships on Hg and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ in liver and muscle tissues. Differences in Hg concentrations and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ between beluga harvested at Paulatuk and Tuktoyaktuk were evaluated using an analysis of variance and analysis of covariance to test for length and age interactions. To evaluate the relationships of length and age as predictors of Hg and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ in beluga, regressions were used. Seasonal diet differences were evaluated by regressing individual stable isotopes for liver and muscle, if diet was consistent over seasons we would expect a high correlation despite differences in turnover rates (26).

The best biological variable to describe Hg or $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ in muscle and liver tissue was selected using the lowest AIC value (27). To examine which individual or combination of diet biomarkers (i.e., $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and fatty acids described by PCA axis one and two scores) best explained Hg levels in beluga tissues the Akaike differences (Δ_i), and normalized Akaike weights were calculated (w_i) to select the best variable

or variables. A Δ AIC of zero and up to two were considered to be the most important model variables (28).

Results and Discussion

Beluga Fatty Acid Profile and Biological Variables. Fatty acids prevalent in belugas included the saturated fatty acids 14:0, 16:0, and 18:0, the monounsaturates 16:1 (n-9), 18:1 (n-9), 20:1 (n-9) and 22:1 (n-9) and the essential polyunsaturates 20:5 (n-3) and 22:6 (n-3). High levels of those fatty acids were similar to beluga near Svalbard (29), and other marine mammals such as pinnipeds (11). Individual belugas varied in fatty acid profiles (Figure 2a). Seventy-four percent of the variance in fatty acid profiles was explained by the first and second PCA axes (50%; 24%). The fatty acids explaining the beluga distribution along axis one included the long chain C₂₀ and C₂₂ monounsaturates and the essential polyunsaturated eicosapentaenoic acid (20:5 (n-3)) (Figure 2b).

Beluga length and harvest site were important variables describing the fatty acid profiles. PCA axis one scores were best correlated to beluga length ($r = 0.46$; $P = 0.002$) and axis two best related to harvest site ($r = 0.47$; $P = 0.002$). Length and harvest site were correlated ($r = -0.31$; $P = 0.043$) because whales harvested from Paulatuk were generally shorter than those harvested from Tuktoyaktuk. Age was not as strongly related to the first or second PCA axis ($r = 0.32$; -0.05 , $P = 0.04$; 0.8 , axes one and two, respectively), and sex was the weakest biological variable to predict beluga fatty acid profiles ($r = -0.14$; -0.09 , $P = 0.4$; 0.6 , axes one and two, respectively). Beluga length and age were not significantly related ($r^2 = 0.08$; $P = 0.068$) because whales evaluated here (with the exception of one 8 year old) were adults, and have reached their asymptotic length (Figure 3). Since whales were in the asymptotic part of the Gompertz growth curve (30), Hg tissue trends were evaluated below without confounding age and length effects.

The predominance of length over age as a predictor of diet described by fatty acids shows that beluga diet composition is a function of size and, to a lesser extent, age. The relationship of beluga diet with length likely reflects specific resource use and habitat selection that relates to size requirements (17). Habitat segregation among male belugas was described by size (16) and was thought to relate to size maintenance as described by the foraging selection hypothesis whereby foraging more or on higher quality food resulted in differential habitat use (31, 32). Here variation in fatty acids with length provides support that belugas selecting different habitats also had different dietary compositions.

Unlike the findings in ref 16, where sex was an important predictor of habitat use and segregation, here sex did not appear to influence diet, likely due to our small sample size of females ($n = 9$). Often sex is an important variable explaining diet variation, particularly in dimorphic mammals (32–34). The use of fatty acids as a diet proxy should not have hindered our analysis because they have discriminated among sexes (14).

Trends in Nitrogen and Carbon Isotope Fractionation. The importance of length, harvest site, and age as factors describing diet were further examined in relation to $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Muscle and liver $\delta^{15}\text{N}$ were best described by beluga length (Table 1). The positive relationship of $\delta^{15}\text{N}$ with length supports the fatty acid results, and shows a gradient of diet composition with beluga size whereby dietary trophic level increases with size. Liver and muscle $\delta^{13}\text{C}$ related most strongly with age, but were only significant for liver (Table 1). Thus, length effects on diet composition were only supported by $\delta^{15}\text{N}$. Differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ among the harvest sites occurred in muscle $\delta^{15}\text{N}$ and liver $\delta^{13}\text{C}$ (Figure 4), and there were no interactions with age or length.

Higher liver $\delta^{15}\text{N}$ in the larger Tuktoyaktuk whales suggest they are feeding at higher trophic levels than the shorter

Paulatuk whales. The variation in $\delta^{15}\text{N}$ with length supports size mediated diet variation, yet the difference among sites was below 3‰, suggesting feeding variation occurred within a trophic level (4, 35). The lack of a length relationship with beluga $\delta^{13}\text{C}$, and a weak relationship with age suggests different processes are driving $\delta^{13}\text{C}$. Differences in liver $\delta^{13}\text{C}$ among the harvest sites suggest that beluga harvested at Tuktoyaktuk fed more pelagically, whereas higher $\delta^{13}\text{C}$ in Paulatuk beluga reflect terrigenous sources associated with near shore feeding (5, 36). Regional feeding differences are supported by the habitat use observations, whereby the smaller sized whales remained closer to the mainland coastline (16, 37). On the other hand, the depleted $\delta^{13}\text{C}$ from the Mackenzie Delta may have also influenced $\delta^{13}\text{C}$ to be lower in whales harvested at Tuktoyaktuk. The overall weak relationship between tissue $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and length and harvest site indicate that stable isotopes were not as effective as fatty acids when testing for the influences related to habitat use. Nonetheless, stable isotopes provided additional information regarding trophic level and regional feeding patterns.

Consequences of Feeding Behavior on Mercury Levels. Exploring the biological variables driving Beaufort Sea beluga diet provided new insight to Hg accumulation processes in liver and muscle tissues. Unlike fatty acid results, length was not as important as age for describing Hg concentrations in liver (Table 1; Figure 5a). Beluga age has successfully been used to describe a linear relationship with liver Hg concentrations here, and in previous studies (1, 38). In contrast, Hg concentrations in muscle tissue were best explained by length (Table 1). An exponential relationship with length had a slightly better fit than a linear relationship (Table 1). Nevertheless adult belugas showed an increase in muscle Hg with length (ca. $1.02 \mu\text{g g}^{-1} \text{m}^{-1}$) (Figure 5b), and not age ($r^2 = 0.13$; $P = 0.02$). Mercury concentrations in liver and muscle were significantly higher in beluga harvested from Tuktoyaktuk ($31.4 \pm 5 \mu\text{g/g}_{\text{liver}}$; $1.24 \pm 0.1 \mu\text{g/g}_{\text{muscle}}$) relative to those at Paulatuk ($4.5 \pm 7.7 \mu\text{g/g}_{\text{liver}}$; $0.5 \pm 0.1 \mu\text{g/g}_{\text{muscle}}$). Despite length and age site differences, there was no interaction of Hg concentrations among sites with length or age, supporting similar Hg accumulation rates and processes between sites.

The linear increase in liver Hg concentrations with age supports the additive processes of Hg bioaccumulation over time. Beluga liver tissue accumulates Hg over time because MeHg, the predominant form in muscle tissue, is demethylated to inorganic Hg creating a biologically unavailable complex with selenium in liver as a detoxification mechanism (1, 38, 39). The similar liver Hg accumulation rates among the two harvest sites indicate comparable rates of MeHg demethylation across beluga age ranges, which concurs with previous mammal studies (40).

The length and muscle Hg relationship indicates that larger belugas are either feeding at higher trophic levels or in different food webs with higher Hg sources as hypothesized in (41). The $\delta^{15}\text{N}$ results reported here support the former. The half-life of Hg in muscle (as MeHg) in mammals is relatively quick (e.g., $t_{1/2}$ 12 days in rabbits (42)) suggesting muscle Hg levels reflect recent dietary Hg sources. The relationship of muscle Hg with beluga length, instead of age, suggests muscle Hg concentrations reflect dietary Hg uptake and to a lesser extent bioaccumulation over time. This observation is supported by the lack of Hg accumulation with age in ringed seal muscle (43) that further suggests this relationship can be applied to other marine mammals. We acknowledge that Hg will accumulate in beluga muscle with time, but it appears that diet and the process of Hg biomagnification up food webs is the predominant factor explaining Hg levels in muscle and the overall beluga body burden. Thus, muscle tissue is a better indicator of dietary Hg sources and the processes of Hg biomagnification driven

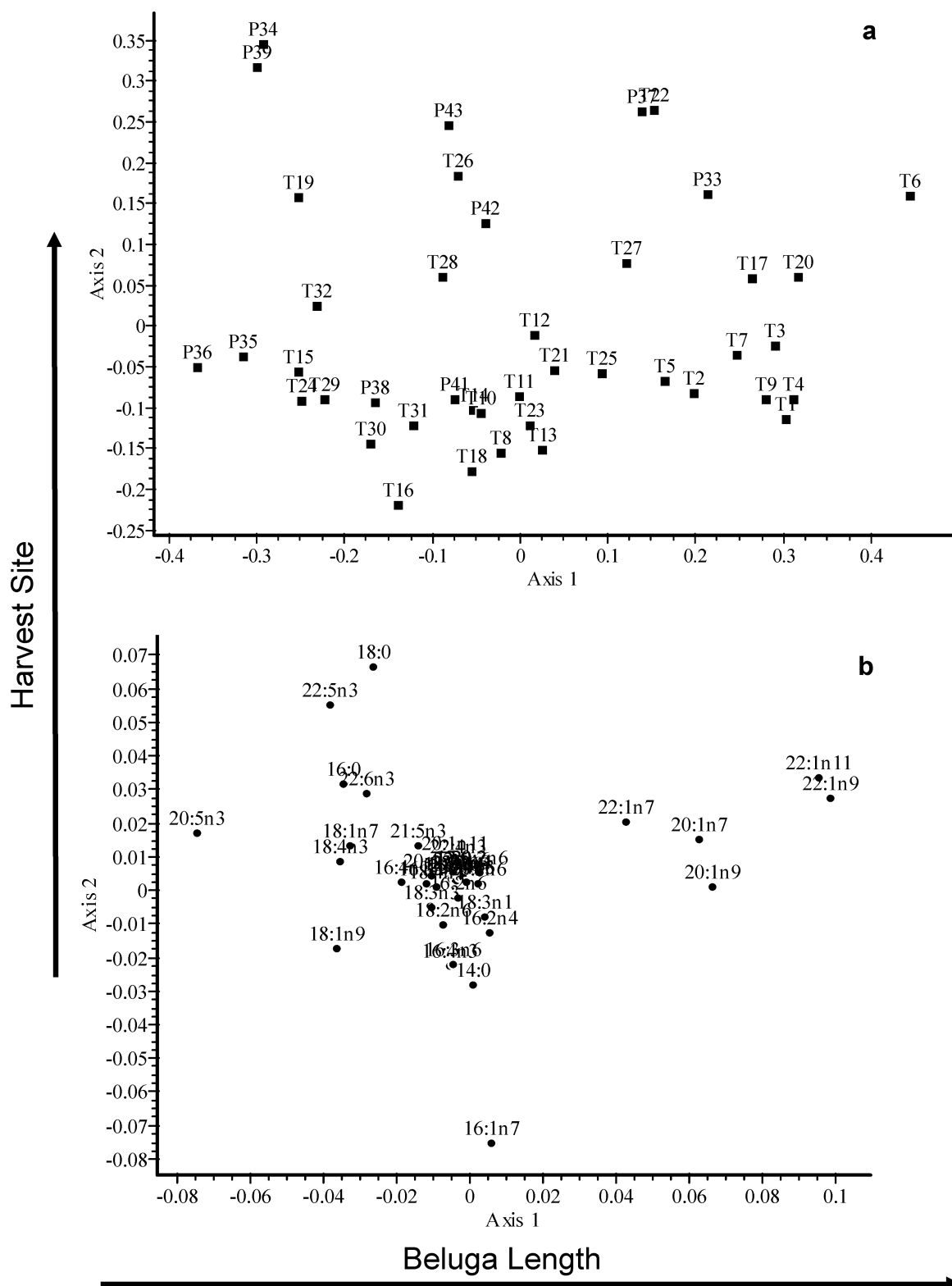


FIGURE 2. Principle component analysis of the 40 dietary fatty acids in 42 beluga (numbered squares), explaining 75% of the variance (axis 1: 50%; axis 2: 24%). 2a. Beluga plot, belugas harvested at Tuktoyaktuk (T), and harvested at Paulatuk (P). 2b. Variable scores plot of the 40 dietary fatty acids. The positions of the fatty acids describe the position of belugas on beluga plot, and the location of the fatty acids indicates the importance, those in the center have the lowest importance relative to those furthest from the origin.

by food web structure predict muscle levels. Since Hg concentrations in muscle better reflect dietary sources, it can be used as a biomarker for feeding preferences among beluga size ranges. Mercury in beluga skin was not presented here but has strong linear relationship with muscle Hg (38). Beluga skin is similar to fur or hair in mammals, representing

the Hg body burden (40); thus, we support the use of skin biopsy samples to estimate the dietary Hg loads.

Diet Biomarkers and Mercury. Stable isotopes and fatty acids were important biomarkers describing beluga Hg concentrations (Table 2). Mercury levels in muscle were best described by the first axis of the fatty acid PCA and liver $\delta^{15}\text{N}$

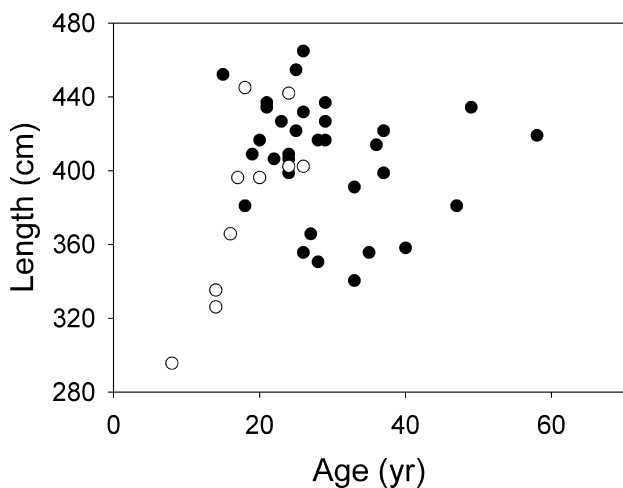


FIGURE 3. Age (years) and length (cm) relationship in beluga harvested at Tuktoyaktuk (closed symbols) and Paulatuk (open symbols). Note all whales with the exception of one 8 year old are over the asymptotic growth length (19).

TABLE 1. Best Fit Regressions, Where Variables Were Selected by Lowest Akaike Information Criteria (AIC) value^a

best fit regressions dependant*response	R ²	P _{value}
(1) stable isotopes*biological indice		
Log ₁₀ Liver δ ¹⁵ N * Log ₁₀ Length	0.135	0.017
Log ₁₀ Muscle δ ¹⁵ N * Log ₁₀ Length	0.196	0.003
Log ₁₀ Liver δ ¹³ C * Log ₁₀ Age	0.121	0.024
Log ₁₀ Muscle δ ¹³ C * Age	0.062	0.112
(2) mercury*biological indice		
Log ₁₀ Liver Hg * Log ₁₀ Age	0.491	<0.001
Log ₁₀ Muscle Hg * Length ²	0.356	<0.001
Log ₁₀ Muscle Hg * Length	0.355	<0.001
(3) mercury* stable isotopes		
Log ₁₀ Liver Hg * Log ₁₀ Muscle δ ¹⁵ N	0.223	0.02
Log ₁₀ Muscle Hg * Log ₁₀ Liver δ ¹⁵ N	0.359	<0.001

^a (1) Beluga tissue stable isotopes and their best fit regressions with either length or age, (2) Total mercury (Hg) in beluga liver and muscle best fit regressions with age and length, and (3) Hg in liver and muscle best described by stable isotopes in liver and muscle tissues.

values (Table 2). Liver Hg levels were also explained by the fatty acid PCA axis 1 scores and liver δ¹⁵N values, in addition to liver δ¹³C. The best individual variables to explain liver Hg was the fatty acid PCA axis 1 score, whereas liver δ¹⁵N values best explained muscle Hg concentrations (Table 2). These relationships provide information relating to biochemical processes and relative metabolic rates in beluga. For example, positive relationships between muscle Hg and liver δ¹⁵N support similar metabolic turnover rates. Protein turnover rates in liver are relatively fast (e.g., humans 15–54% day⁻¹ (44)), and Hg in the form of MeHg has been shown to be transported quickly from muscle to liver in some animals (e.g., t_{1/2} 12 days in rabbits (42)). Both metrics support quick metabolic processes and their association supports harmonious rates. In addition, results support that the Hg turnover rates in muscle are much faster than muscle δ¹⁵N turnover rates, thus we suggest the use of liver δ¹⁵N to reflect the trophic level of a recent dietary Hg sources rather than muscle δ¹⁵N.

The weak δ¹³C relationships with both the biological variables and beluga Hg levels suggest that carbon turn over rates in beluga tissues are different than Hg in tissues. δ¹³C

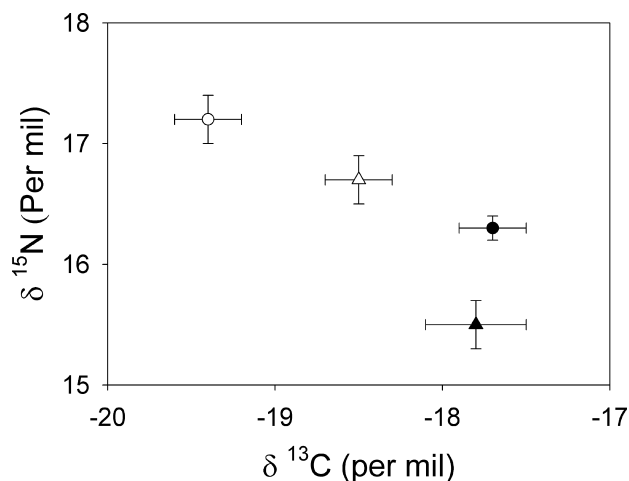


FIGURE 4. Mean δ¹⁵N and δ¹³C (±SE) for beluga liver (open symbols) and muscle (closed symbols) tissues collected from Tuktoyaktuk (circles) and Paulatuk (triangles) Northwest Territories, Canada.

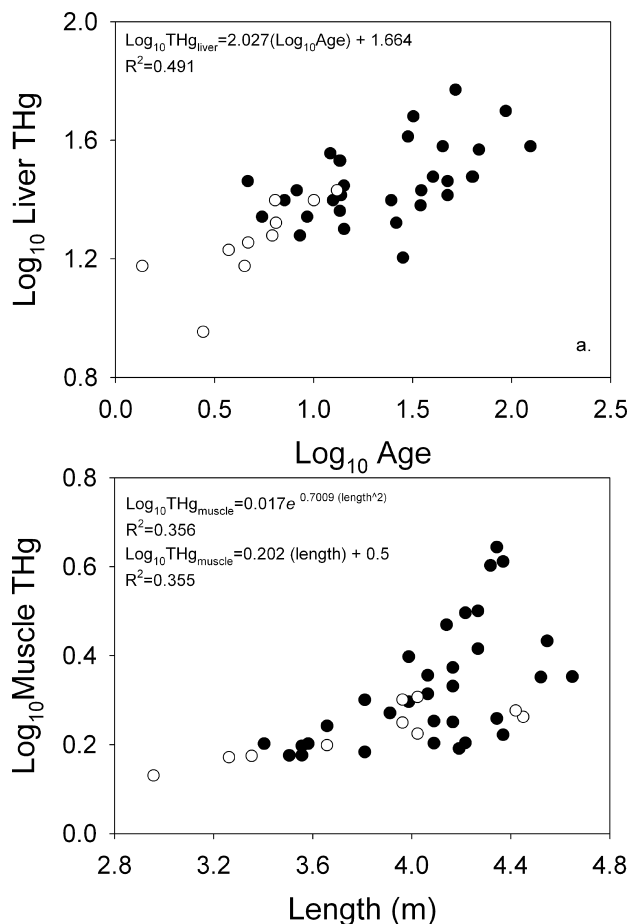


FIGURE 5. Best fit regression for mercury in beluga tissue with biological correlates as determined by Akaike's Information criterion (AIC) (Table 2). Beluga harvested from Paulatuk (open symbols) and Tuktoyaktuk whales (closed symbols). 5a. Total mercury (THg) in liver (log transformed) was best described by a linear relationship with age (log transformed) demonstrating accumulation over time 5b. Total mercury (THg) in muscle (log transformed) was best described by an exponential relationship with length (m) demonstrating (Table 2).

may have a longer turn over rate than protein (δ¹⁵N) in liver and muscle, suggesting levels measured here reflect a diet from a different feeding period relative to Hg and δ¹⁵N values. δ¹³C in liver and muscle are more similar to δ¹³C in prey

TABLE 2. Comparison of Best Fit Regressions to Describe Beluga Mercury in Liver and Muscle Using the Lowest ΔAIC_c Regression with the Diet Biomarkers Stable Isotopes in Liver and Muscle and Fatty Acid PCA Scores from First and Second Axes.

best fit regressions dependant*response	R ²	P value	AIC _c ^a	$\Delta_i AIC_c^b$	W _i ^c
log liver Hg					
*PCA1, log ₁₀ liver $\delta^{15}N$, log ₁₀ liver $\delta^{13}C$	0.53	<0.001	-82.824	0.000	0.133
*PCA1, log ₁₀ liver $\delta^{13}C$, log ₁₀ muscle $\delta^{15}N$, log ₁₀ muscle $\delta^{13}C$	0.55	<0.001	-82.041	0.783	0.090
*PCA1	0.39	<0.001	-76.286	6.538	0.005
log muscle Hg					
*PCA1, Log ₁₀ liver $\delta^{15}N$	0.43	<0.001	-185.788	0.000	0.217
*PCA1, log ₁₀ liver $\delta^{15}N$, log ₁₀ liver $\delta^{13}C$	0.46	<0.001	-184.977	0.812	0.145
*log ₁₀ liver $\delta^{15}N$	0.35	<0.001	-183.592	2.196	0.072

^a AIC_c = second order Akaike information criteria (AIC = $n \log(\sigma^2) + 2K$) bias adjusted AIC for small sample size = AIC + $2K(K + 1)/(n - K - 1)$ where K is the total number of estimated regression parameters including σ^2 (no intercept) and n is sample size. ^b Δ_i = AIC differences computed as AIC_i-AIC_{min}. ^c $w_i = \exp(-1/2\Delta_i)/\sum \exp(-1/2\Delta_i)$.

items measured in the Bering Sea (45) relative to $\delta^{13}C$ in Beaufort Sea prey (41). Given that the Beaufort Sea beluga population spend the winter in the Bering Sea, and $\delta^{13}C$ enrichment is usually near 1‰, it appears that $\delta^{13}C$ values reflect their winter diet. In support of seasonal feeding differences, we found a weak relationship between muscle $\delta^{13}C$ and liver $\delta^{13}C$ (Pearson correlation) and to a lesser extent muscle and liver $\delta^{15}N$ ($r = 0.1, 0.5; p = 0.7, 0.002; \delta^{13}C, \delta^{15}N$ respectively). The weak correlations differ from the strong relationships found for organisms with a consistent seasonal diet in their isotopic values (e.g., ref 26).

Here we use fatty acids and stable isotopes as diet biomarkers to evaluate differences in diet composition and those consequences on beluga Hg concentrations. Fatty acid analysis in concert with the analysis of biological variables provided support for previously hypothesized variation in diet among beluga driven by differences in habitat use (41). The analysis of fatty acids provided motivation for the investigation of length effects on Hg uptake. For the first time we demonstrated that Hg levels in beluga muscle reflects biomagnification processes rather than bioaccumulation over time. Beluga length defines the summer habitat use that likely reflects specific energy requirements (16), and in this study length best described diet composition and muscle Hg levels. Therefore, differences in habitat use among beluga did result in different diets and dietary Hg sources.

Diet biomarker comparison between fatty acids and stable isotopes revealed that behavioral influences on dietary Hg uptake are better described by fatty acid analysis, whereas $\delta^{15}N$ and $\delta^{13}C$ provided information pertinent to trophic feeding and energy sources in addition to tissue Hg uptake processes. Together, stable isotopes and fatty acids were strong predictors of beluga Hg concentrations. Thus, stable isotopes, particularly $\delta^{15}N$, and fatty acids are in general agreement, proving both are beneficial when describing recent dietary Hg uptake, but different enough to provide greater information together than alone.

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Note Added after ASAP Publication

There was an error in the caption of Figure 4 in the version published ASAP May 3, 2008; the corrected version published ASAP May 29, 2008.

Literature Cited

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