THE INTERCONNECTEDNESS OF DIET, PHYSIOLOGY, AND PHYSICAL CONDITION IN BELUGA WHALES AS A SENTINEL SPECIES FOR ENVIRONMENTAL CHANGE IN THE BEAUFORT SEA ECOSYSTEM

by

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Abstract

Arctic ecosystems are changing at an alarming rate, with the Arctic Ocean predicted to be summer sea ice free within the next few decades. Beluga whales (*Delphinapterus leucas*) are the most abundant Arctic odontocete, exhibiting a circumpolar distribution and a strong association to sea ice, and are thus a sentinel species for the effects of climate change. The vulnerability of belugas to changing environmental conditions will depend on their adaptive capacity and resilience to changes in the prey base. The overall objective of my thesis was to examine the potential effects of prey shifts due to changing environmental conditions on Beaufort Sea beluga whales by examining relationships among body condition, dietary tracers, and physiology. Differences in lipid content and carbonates in the tissues of beluga and their potential prey affected both carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios, which could lead to incorrect ecological interpretations. Inter-annual variation in blubber fatty acid signatures and liver $\delta^{13}C$ and $\delta^{15}N$ values in beluga whales may be related to annual differences in environmental conditions and abundances of Arctic cod (*Boreogadus saida*). To establish an effective approach for identifying prey, I used Bayesian mixing model Fatty Acid Source Tracking Algorithm in R (FASTAR) to reconstruct the known diets of two captive beluga whales using fatty acid signatures. FASTAR was then used to reconstruct the offshore diets of Beaufort Sea belugas. Although diets varied annually, Arctic cod and capelin (*Mallotus villosus*), two pelagic species with high lipid content, were identified as the main prey of belugas. Finally, I examined physiological limits and the relationships between body condition and physiological parameters pertaining to oxygen storage capacity in belugas. Males had higher oxygen stores than females due to larger body size and higher hemoglobin concentrations. Body condition indices positively correlated with myoglobin and hemoglobin concentrations, and hematocrit,

resulting in lower calculated aerobic dive limits in whales with lower body condition. Overall, climate-induced prey shifts that reduce fitness will lead to lower oxygen stores in belugas, a potential positive feedback mechanism. The interconnectedness of diet, body condition, and physiology should be a conservation priority to monitor the long-term effects of climate change on belugas and other Arctic marine mammals.

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General Introduction

Beluga whales (*Delphinapterus leucas*) are the most abundant Arctic odontocete and a potential indicator species for the effects of climate change (Tynan and DeMaster 1997; Laidre 2008; Moore and Huntington 2008; Laidre et al. 2015). The eastern Beaufort Sea is one of the largest populations, with an estimated 32,453 to 39,258 individuals (Allen and Angliss 2014) and has an annual migration from the Bering Sea to the eastern Beaufort Sea (Richard et al. 2001; Harwood and Smith 2002). Upon arrival in early July, belugas enter the waters of the Mackenzie River estuary to give birth to calves, nurse, and moult (Harwood and Smith 2002). As the belugas leave the estuary they segregate into habitat use-groups based on sex and size-specific energetic requirements: nursing females with young calves select open-water habitat close to the mainland in the Amundsen Gulf, large males select offshore pack-ice habitat, and medium-sized males and females with older calves use ice-edge habitat (Loseto et al. 2006).

Recently, a decline in the growth rates of individuals over a twenty-year period has been identified within the beluga population, along with reductions in body condition in other marine mammals, seabirds, and fish species in the Beaufort Sea ecosystem, hypothesized to be the result of an ecosystem regime shift (Harwood et al. 2014; Harwood et al. 2015). Loss of sea ice and warming ocean temperatures are predicted to decrease the abundance of Arctic cod (*Boreogadus saida*) through the northward expansion of subarctic competitors (Michel et al. 2012; Falardeau et al. 2014; McNicholl et al. 2016). Arctic cod is one of the most energy-dense prey in the Arctic and an important forage fish to many top predators including Beaufort Sea belugas (Loseto et al. 2009; Harter et al. 2013). The decline in individual growth rates in beluga whales is hypothesized to be the result of prey shifts, and the long-term effects on the population remains unknown.

Since 2000, scientists have partnered with Inuvialuit communities in a long-standing community-based monitoring program to better understand beluga health in the Inuvialuit Settlement Region, specifically on Hendrickson Island (Harwood and Smith 2002). Data collected from harvested whales include biological metrics (e.g. size, age, sex), contaminants (e.g. mercury, PCBs), ecological tracers of diet (e.g. stable isotopes, fatty acids) and nutritional indicators (e.g. hormones, vitamins, lipids). This program has expanded to other hunting camps across the Inuvialuit Settlement Region, both within and outside of the Mackenzie Estuary (e.g. Brown's Harbour, East Whitefish, and Kendall Island). This partnership between Inuvialuit hunters, scientists, community members, and co-management boards has produced the largest and longest-running database of harvested beluga whales in Canada.

As a participant in the beluga health monitoring program, the overall objective of my thesis was to examine the potential effects of prey shifts due to changing environmental conditions on Beaufort Sea beluga whales by examining relationships among physical condition, dietary tracers, and physiology. If changes in prey species adversely affect body condition, they may also affect physiological plasticity, and ultimately survival of belugas in Arctic marine ecosystems. To address my overall objectives, my thesis examined the following sub-objectives: (1) studying the effect of sample preparation and lipid removal and acidification on nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotope ratios in different prey species of beluga whales; (2) examining inter-annual variation in body condition and ecological tracers of diet in belugas in relation to environmental conditions; (3) developing an effective approach to reconstruct the diets of beluga whales using fatty acid signatures; (4) identifying inter-annual variation in offshore prey of beluga whales using fatty acid signatures and stable isotope ratios; and (5) examining physiological parameters pertaining to oxygen storage capacity in Beaufort Sea

beluga whales and their potential relationships with indices of body condition. All chapters are written for publication and stand alone; thus, there is some redundancy in information in their introductions and methodologies. **Chapters 1 and 3** are method development papers that are used to support research in **Chapters 2 and 4. Chapter 5** incorporated information and analyses conducted in **Chapters 2 and 4.**

One of the challenges of using stable isotope ratios for diet reconstruction is that differences in lipid content can influence $\delta^{13}C$. Lipids are 2 to 8‰ depleted in ^{13}C compared to other compounds, as a result, differences in lipid content among predators and prey can lead to incorrect interpretations of food web structure (DeNiro and Epstein 1977; Monson and Hayes 1982; Peterson and Fry 1987). This consideration is particularly important for polar species as they are typically lipid rich relative to species from lower latitudes (Falk-Petersen et al. 2000; Kattner and Hagen 2009). Furthermore, calcium carbonate in the exoskeleton of invertebrates can be a source of positive $\delta^{13}C$ bias (Soreide et al. 2006; Schlacher and Connolly 2014). My objectives in **Chapter 1** were to determine if lipid content and calcium carbonate were a potential source of variation in $\delta^{13}C$ values in beluga tissues and their potential prey. The $\delta^{13}C$ and $\delta^{15}N$ values of beluga tissues, capelin (*Mallotus villosus*), and five invertebrate species were examined before and after lipid removal and carbonate acidification. To quantify the effects of sample preparation methods, I examined differences in isotopic niche breadth and dispersion metrics among treatments, since these methods are commonly used for ecological interpretations of stable isotope data. The lipid and carbonate correction models for $\delta^{13}C$ values produced from this chapter were applied in the subsequent chapters to identify the prey of beluga whales.

In order to better understand the long-term decline in individual growth rates of beluga whales, it is important to understand the causal factors associated with health and physical

condition of beluga whales. **Chapter 2** examines inter-annual variability in body condition and ecological tracers of diet (fatty acid signatures and stable isotope ratios) within the Beaufort Sea beluga population. The first objective of **Chapter 2** was to establish a body condition index based on the residuals of fitted linear models of maximum girth and blubber thickness. Next, I examined inter-annual variation in body condition and dietary tracers in beluga and their relationships to habitat-use and concurrent environmental conditions. Most long chain and polyunsaturated fatty acid signatures from the inner blubber of marine mammals are directly transferred from prey to predator, and can be used to infer diet (Budge et al. 2006). Findings on variability in diet and body condition indices in beluga whales were used in my subsequent chapters to identify prey linkages and relationships between body condition and physiology.

The application of Bayesian statistics has improved estimates of predator diets by calculating a probability distribution of prey contributions (Moore and Semmens 2008; Galloway et al. 2015; Stock and Semmens 2016). Aquarium studies of predators with known diets are valuable for testing these models. Bayesian mixing models may also be a potential solution for determining the diets of marine mammals in circumstances in which calibration coefficients are not available, by using fatty acids transferred directly through diet and undergoing little modification through metabolism (Iverson et al. 2004). In **Chapter 3**, I used Fatty Acid Source Tracking Algorithm in R (FASTAR), a Bayesian mixing model (Galloway et al. 2015), to reconstruct the known diets of two captive beluga whales from the Vancouver Aquarium. Since many studies employ qualitative multivariate analysis to compare the fatty acid signatures of predators with potential prey, I compared the effectiveness and limitations of qualitative versus Bayesian analyses for reconstructing the diets of marine mammals. The methodological approach

verified in this chapter was used in **Chapter 4** to reconstruct the offshore diets of Beaufort Sea beluga whales.

Comparisons of fatty acid profiles have suggested Arctic cod are an important prey species for belugas in the coastal and offshore ecosystems (Loseto et al. 2009). However, this study used mostly coastal species and did not include fish collected below depths of 100 m. Furthermore, Bayesian mixing models can provide proportional diet estimates and account for uncertainty associated with multiple prey sources (Moore and Semmens 2008). In **Chapter 4**, the Bayesian mixing model FASTAR was used to identify the contribution of demersal and pelagic offshore prey to Beaufort Sea beluga whales using fatty acid signatures and stable isotope ratios. This chapter was conducted in collaboration with the Beaufort Regional Environmental Assessment (BREA) program, the first comprehensive baseline study of marine fish diversity in the Canadian Beaufort Sea. I reconstructed the diets of beluga whales using the fatty acid and stable isotope signatures of the most abundant demersal and pelagic fish and invertebrate species in the BREA survey [Arctic cod, Greenland halibut (*Reinhardtius hippoglossoides*), Adolf's eelpout (*Lycodes adolfi*), Arctic staghorn sculpin (*Gymnocanthus tricuspis*), Canadian eelpout (*Lycodes polaris*), stout eelblenny (*Anisarchus medius*), kelp snailfish (*Liparis tunicatus*), Arctic alligatorfish (*Aspidophoroides olrikii*), isopods (*Saduria sabini*), green shrimp (*Argis dentata*), circumpolar eualid (*Eualus gaimardii*), polar shrimp (*Sclerocrangon ferox*), octopus (*Cirroteuthis muelleri*), and capelin (*Mallotus villosus*)].

Beluga whales are well adapted to deep diving and prolonged underwater submergence, which is important for not only for navigating heavy ice conditions and foraging for demersal prey, but for evading predators such as killer whales (*Orcinus orca*; Ridgway et al. 1984). The most frequent dives depths performed by Beaufort Sea beluga whales were between 700 to 900

metres deep, lasting 15 to 20 minutes; however, only male belugas ventured into areas deeper than 600 metres (Richard et al. 1997). The deep dives performed were "v-shaped", with the deepest dive measured at 1160 metres and lasting 25 minutes (Richard et al. 1997; Richard et al. 1998). The purpose of these deep dives are unknown, but are hypothesized for foraging purposes in deep-water feeding areas (Harwood and Smith 2002), orientation by acoustic reckoning (Richard et al. 1998), and locating possible breathing holes in pack ice (Richard et al. 1997). Diving capacity of marine mammals increases with body size, due to an increase in overall muscle oxygen stores and a decrease in mass-specific metabolic rate (Kooyman 1989; Noren and Williams 2000). In **Chapter 5**, I examined whether differences in foraging ability between sexes and size classes in beluga whales may be explained by physiological parameters pertaining to oxygen storage capacity, such as myoglobin concentrations and proton buffering capacity of the *longissimus dorsi* muscle, hemoglobin concentrations and hematocrit in whole blood, as well as spleen mass. I also examined relationships between the body condition of beluga whales and these physiological attributes, and their relationship to underwater submergence times. Physiological limits and constraints are often overlooked, but may be important considerations for wildlife conservation and management.

Overall, this thesis examines the relationships between physiology, diet, and body condition to better understand the impacts of environmental change on beluga whales, which is essential for evaluating their adaptive capacity and resilience (Williams et al. 2008). By examining inter-annual variability in prey and developing an approach to identify and estimate beluga diets, future monitoring efforts may be able to more effectively reconstruct the diets of marine mammals and detect climate-induced prey shifts. Understanding the linkages between prey and environmental conditions with body condition of beluga whales may help to better

predict the flexibility of beluga whales and Arctic marine mammals to changing prey regimes. Finally, the relationship between body condition and oxygen storage capacity in beluga whales may demonstrate how changing environmental conditions affect physiology, which could impair the ability of belugas to survive amongst sea ice and forage for their optimal prey. As a long-lived Arctic marine mammal that may be highly specialized to the Arctic marine environment, environmental change may pressure beluga whales to adjust their foraging behaviours and migration routes. Due to their wide range and use of different habitats, Beaufort Sea beluga whales are also considered a sentinel species for the Beaufort Sea ecosystem. Additionally, as a marine top predator exhibiting a circumpolar distribution, a comprehensive study of the diverse effects of climate change on beluga whales may provide valuable information on the effects of environmental change and linkages to the Beaufort Sea ecosystem, Arctic marine mammals and vertebrates, and other Arctic marine systems.

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Chapter 1:

Lipid removal and acidification affect nitrogen and carbon stable isotope ratios of beluga whales (*Delphinapterus leucas*) and their potential prey species in the Beaufort Sea ecosystem

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Dr. Lisa Loseto provided funding for stable isotope analysis and comments throughout the writing process.

Dr. Jim Roth provided comments throughout the writing process.

Emily Choy designed the research project, prepared all samples for stable isotope analysis (which were determined by the University of Waterloo Environmental Isotopes Laboratory), assisted with sampling of beluga tissues, analyzed and interpreted the data, produced all figures and tables, and wrote the manuscript.

Samples were collected by the Beaufort Regional Environmental Assessment Marine Fishes Project and beluga monitors as part of the Beluga Health Monitoring Project at Fisheries and Oceans Canada.

Abstract

Carbon and nitrogen stable isotope ratios are ecological tracers that can provide insights into the diets of marine mammals. As a generalist predator, beluga whales (*Delphinapterus leucas*) consume a variety of prey; however, differences in lipid content and the presence of inorganic carbon in prey may cause variability in the $\delta^{13}C$ signal that is not related to food sources. We examined the effects of carbonate and/or lipid removal in beluga muscle and liver tissues and potential prey and tested whether the C:N ratio was a valid indicator of lipid content. The C:N ratio was a good predictor of the change in $\delta^{13}C$ after lipid removal in capelin (*Mallotus villosus*), octopus (*Cirroteuthis muelleri*), green shrimp (*Argis dentata*), and circumpolar eualid (*Eualus gaimardii*). Despite relatively low C:N ratios, lipid removal significantly increased $\delta^{13}C$ values but also affected $\delta^{15}N$. Removal of carbonates from invertebrate samples significantly decreased $\delta^{13}C$ values and had variable effects on $\delta^{15}N$. Overall, the variability in $\delta^{13}C$ within a species decreased after removing lipids and carbonates. Variability in $\delta^{15}N$ did not change for species requiring only lipid removal, but increased after acidification. We also evaluated the effect of these sample preparation methods on niche dispersion metrics. After lipid and carbonate treatments, centroid locations differed significantly in all species except beluga muscle, and niche breadth and mean distance to the centroid decreased. Failure to remove lipids and carbonates for $\delta^{13}C$ values may lead to incorrect interpretations for isotopic niche, which may have major ecological implications, such as predicting the impacts of invasive species or determining the dietary linkages of beluga whales.

Keywords: carbon and nitrogen stable isotope signatures, lipid removal, carbonates, acidification, beluga whales, Beaufort Sea, marine invertebrates, capelin, octopus, decapods, isopod, isotopic niche, niche dispersion metrics

Introduction

Ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotopes can provide insights into the structure of marine food webs (e.g. Boecklen et al. 2011). Nitrogen stable isotope values ($\delta^{15}N$) typically increase 3 to 5‰ with every trophic transfer and can indicate trophic position in a food web, whereas carbon stable isotope values ($\delta^{13}C$) vary with differences in baseline primary producers, usually increasing between 0 to 1‰ per trophic level (Peterson and Fry 1987). Stable isotope ratios can also provide extremely valuable dietary information for marine mammals in which behavioural and feeding observations are difficult to obtain (Ryan et al. 2014; Matley et al. 2015). As a generalist predator, beluga whales (*Delphinapterus leucas*) reportedly feed on both pelagic and benthic invertebrate and fish species (Loseto et al. 2008; Loseto et al. 2009; Marcoux et al. 2012; Quakenbush et al. 2014). However, differences in lipid content among different prey and the influence of inorganic carbon on the $\delta^{13}C$ signal may be a source of variability and uncertainty, which can lead to incorrect interpretations of food web structure. Lipids are reported to be between 2 to 8‰ depleted in ^{13}C compared to other compounds due to fractionation during lipid synthesis (DeNiro and Epstein 1977; Monson and Hayes 1982; Peterson and Fry 1987). Inorganic carbon, such as calcium carbonate ($CaCO_3$) in exoskeletons, can also be a source of positive $\delta^{13}C$ bias in marine invertebrates (Soreide et al. 2006; Schlacher and Connolly 2014).

C:N has been reported as a good measure of lipid content in aquatic organisms, and it is recommended that aquatic organisms with C:N ratios greater than 3.5 (5% lipid content) have

lipids extracted prior to analysis (Post et al. 2007). However, significant lipid effects have been documented in Arctic marine zooplankton despite lower (~3 to 4) C:N ratios (Pomerleau et al. 2014), and data on the effects of lipids and carbonates on $\delta^{13}C$ and $\delta^{15}N$ values from other Arctic marine invertebrates and fish are insufficient or lacking. Our objectives were to examine the effects of lipid removal and acidification on $\delta^{15}N$ and $\delta^{13}C$ values in different potential prey species of beluga whales. We have analyzed beluga muscle and liver tissues along with six potential Arctic prey species [green shrimp (*Argis dentata*), circumpolar eualid (*Eualus gaimardii*), polar shrimp (*Sclerocrangon ferox*), a marine isopod (*Saduria sabini*), Müller's cirroctopod (*Cirroteuthis muelleri*), and capelin (*Mallotus villosus*)] before and after carbonate and/or lipid removal. As the C:N ratio is a predictor of lipid content, we examined the relationship between C:N and % lipid as well as the change in $\delta^{13}C$ after lipid extraction to evaluate whether C:N ratios can be used to correct for lipids and carbonates in marine organisms. In our efforts to better quantify the effects of sample preparation methods on potential prey in the beluga whale food web, we examined the sensitivity of isotopic niche metrics, such as isotopic niche breadth (Layman et al. 2007a; Jackson et al. 2011) and dispersion metrics (Turner et al. 2010). Niche metrics have broad applications to the field of ecology, such as quantifying the interactions of globally widespread invasive species (Jackson et al. 2012) and assessing the impacts of ecosystem fragmentation to top predators (Layman et al. 2007b). By comparing the effect of lipid and carbonate removal on isotopic niche metrics, we have demonstrated that failing to perform these pre-treatment methods may influence the interpretation of an organism's ecology.

Methods

Muscle (*longissimus dorsi*) and liver samples from 69 beluga whales were collected from Inuvialuit hunting camps at Hendrickson Island, Brown's Harbour, and Kendall Island in the Inuvialuit Settlement Region, Northwest Territories, Canada from July 6th to 24th 2011 and June 30th to August 6th 2012 (Appendix 1.1; Figure 1.1). Samples were frozen at -20˚C in portable freezers on site and shipped to the Freshwater Institute at Fisheries and Oceans Canada in Winnipeg for processing.

Marine invertebrates (i.e. three shrimp species, one isopod, and octopus) were collected in 2012 using an Atlantic Western IIA benthic fishing trawl as part of the Beaufort Regional Environmental Assessment Program (Appendix 1.2; Table 1.1). Green shrimp and polar shrimp were both collected at the same transect (TBS; Figure 1.1) on August 26th 2012 at 200 m (station 3) and 350 m (station 4) depths, whereas circumpolar eualid and isopod samples were both collected at transect KUG from August 13th to 15th at 40 m (station 1) and 350 m (station 4) depths. Octopi were collected at depths between 500 to 1000 m along the four different transects (GRY, KUG, TBS, DAL; Figure 1.1). Capelin samples were collected using a 3 m beam trawl at station BPT-03 close to Darnley Bay on August 6th 2013.

Stable isotope analysis

Four subsets of samples were analyzed for carbon and nitrogen stable isotope ratios in order to determine the effects of the respective treatments: bulk untreated samples for all species, acid-treated, and acid-treated-lipid extracted samples for invertebrates with exoskeletons, and lipid-extracted samples for capelin, octopus, and beluga tissues. All samples were freeze dried for at least 48 hours prior to analysis and homogenized using a mortar and pestle at the

Freshwater Institute. Sample preparation and analysis of C and N stable isotope ratios were conducted at the University of Waterloo Environmental Isotopes Laboratory. First, bulk $\delta^{13}C$ and $\delta^{15}N$ values were measured as a control for all species prior to any treatments. For invertebrates with an exoskeleton (three shrimp and one isopod species), carbonates were removed from approximately 100 to 200 mg of tissue using a 0.5N HCl (~5%) treatment and heated to 50-60°C for approximately 90 minutes. The pH was then checked using pH indicator strips (pHydrion®, Micro Essential Laboratory, New York) and if not acidic, water was decanted and more acid added for another 60 minutes. Once the pH remained acidic, three cycles of decanting and washing with de-ionized water was performed to remove all leftover salts. Once the samples were neutralized, they were decanted, freeze-dried for approximately 48 hours, and analyzed for stable isotope ratios as our subset of acid-treated tissues. Next, the acid-treated samples, along with the untreated beluga, octopus, and capelin tissues were lipid extracted. Approximately 100 to 200 mg of tissues were treated at room temperature using a 2:1 chloroform-methanol wash, left standing for 30 minutes, centrifuged for 5 minutes, and the supernatant discarded. The extraction procedure was repeated three times and the residue was oven dried for 24 hours at 80°C (Folch et al. 1957; Bligh and Dyer 1959). Percent lipid was measured separately at the Freshwater Institute for invertebrate and fish tissues using a 2:1 chloroform-methanol extraction (Folch et al. 1957) and reported in dry weight. As % lipid was measured during fatty acid analysis, we did not acquire % lipid for beluga muscle or liver tissues as blubber was used instead for fatty acids.

The analysis of solid materials for $\delta^{13}C$ and $\delta^{15}N$ measurements was conducted through combustion conversion of sample material to gas through a 4010 Elemental Analyzer (Costech Instruments, Italy) coupled to a Delta Plus XL (Thermo-Finnigan, Germany) continuous flow

isotope ratio mass spectrometer. Stable isotope ratios are expressed in delta (δ) notation in per mil (‰), and were calculated against known certified elemental standard materials (Vienna Pee Dee Belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$), following:

$\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard})-1] \times 1000$

where R is the ratio of the heavy to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$).

Data quality control was monitored and corrections made using an array of international reference material and in-house standards that were calibrated using certified international reference materials (i.e. IAEA-N1 + N2, IAEA-CH3 + CH6, USGS-41 + 41). National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) standard 1577B (bovine liver) was used as a post-correction check throughout the analysis, with approximately 20% of the total sample number run as standards or reference materials. Standards and reference materials were run to ensure precision and accuracy, with an analytical error of 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$ required for reportable data. Every eighth sample was run in duplicate.

Data analysis

First, we used paired t-tests and a one-way analysis of variance (ANOVA) to compare the change in $\delta^{13}C$, $\delta^{15}N$, and the C:N ratio for each species before and after their respective treatments. Next, we used regression models to examine the relationship of the C:N ratio with % lipid and the change in $\delta^{13}C$ ($\Delta^{13}C$) following acidification and /or lipid extraction. As non-linear models have been found to best fit the relationship between lipid content and the C:N ratio for lipid-rich samples and marine mammal tissues (Logan et al. 2008; Lesage et al. 2010), we compared both linear and logarithmic models. We have provided linear normalization equations as both models produced similar results and met assumptions. To examine the potential consequences of not removing lipids and carbonates on trophic niche, we compared niche

breadth and dispersion metrics within individual species using bulk $\delta^{15}N$ but before and after treatments for $\delta^{13}C$. We used the Stable Isotope Bayesian Ellipses in R (SIBER) tools (Jackson et al. 2011) in the package Stable Isotope Analysis in R (SIAR 4.2.2; Parnell and Jackson 2013) to create posterior probability distributions of the area of the standard ellipses (i.e. isotopic niche breadth) (Layman et al. 2007a; Jackson et al. 2011). The standard ellipse area (SEA) characterizes spatial variability in the stable isotope data as a two-dimensional standard deviation, governed by the covariance matrix between $\delta^{13}C$ and $\delta^{15}N$ values and containing approximately 40% of the data. Using SIBER, for each species, we calculated the proportion of the Bayesian estimate of standard ellipse area (SEA_b) that was smaller prior to sample treatments (Parnell and Jackson 2013). Niche dispersion metrics such as location of the centroid (LOC), mean Euclidean distance to centroid (CD, an indicator of species spacing), and mean nearest-neighbour distance (NND, a measure of overall species density) were calculated and hypotheses tested using null distributions generated by a residual permutation procedure (RPP; Turner et al. 2010). The LOC, CD, and NND were considered significantly different if the difference between them was significantly greater than zero. After comparing niche breadth and dispersion metrics between individual species, comparisons were made for the whole community before and after sample preparation methods. All statistical tests were performed using R version 3.2.2 (R Core Team 2015). RPP tests were conducted using R code available from an online supplement provided by Turner et al. (2010). Regression graphs were created using Sigmaplot Version 12.0 (Systat Software, San Jose, CA).

Results

Carbon to nitrogen ratio and percent lipid content

C:N ratios in all of our species were relatively low, ranging from 3.4 to 5.5. Variability in lipid content was greater than C:N ratios for all species (Table 1.1). The % lipid ranged from 6.7% in isopod to 31.3% in capelin muscle. Lipid content was positively related to C:N ratios in capelin ($F_{1,15}$=6.6, r^2=0.26, p=0.021), green shrimp ($F_{1,13}$=5.3, r^2=0.24, p=0.038), and octopus tissues ($F_{1,13}$=23.6, r^2=0.62, p=0.0003; Figure 1.2). The relationship between % lipid and C:N ratio was not significant in isopod (p=0.056), polar shrimp (p=0.085), or the circumpolar eualid (p=0.436).

Effect of acidification and lipid removal on $\delta^{13}C$ and $\delta^{15}N$ in marine invertebrates

The $\delta^{13}C$ values of all marine invertebrate species decreased significantly after acidification (paired t tests, p<0.0001; Table 1.1). For example, in isopods $\delta^{13}C$ decreased from -19.8±0.4‰ to -23.2±0.3‰, a difference of 3.4‰ (t_{13}=8.9, p<0.0001). Although acidification did not affect $\delta^{15}N$ values in green shrimp or polar shrimp, post treatment $\delta^{15}N$ values increased in the circumpolar eualid ($\Delta^{15}N$ = 0.8‰, t_{13}=-8.2, p<0.0001) and decreased in isopods ($\Delta^{15}N$ =-0.9‰, t_{13}=2.4, p=0.03). Following lipid removal, $\delta^{13}C$ values increased in beluga muscle (paired t-test, t_{68}=-7.1, p<0.0001) and liver (t_{67}=-44.0, p<0.0001), and $\delta^{15}N$ values also increased in muscle (t_{68}=-5.9, p<0.0001) and liver (t_{67}=-4.5, p<0.0001). Likewise, $\delta^{13}C$ and $\delta^{15}N$ values increased after lipid extraction in octopus ($\delta^{13}C$: t_{14}=-10.3, p<0.0001; $\delta^{15}N$: t_{14}=-3.3, p=0.005) and capelin tissues ($\delta^{13}C$: t_{16}=-6.7, p<0.0001; $\delta^{15}N$: t_{16}=-6.0, p<0.0001). $\delta^{13}C$ values differed significantly among treatments for isopods (ANOVA, $F_{2,39}$=26.3, p<0.0001), circumpolar eualid ($F_{2,39}$=26.1, p<0.0001), green shrimp ($F_{2,42}$=44.2, p<0.0001), and polar shrimp ($F_{2,42}$=31.5, p<0.0001). $\delta^{13}C_{bulk}$ values were higher than $\delta^{13}C$ in acidified ($\delta^{13}C_A$) (Tukey HSD post hoc test,

p<0.0001) and $\delta^{13}C$ in acidified and lipid extracted ($\delta^{13}C_{ALE}$) samples (p<0.0001); but there was no difference between $\delta^{13}C_A$ and $\delta^{13}C_{ALE}$ for isopods (p=0.77) and polar shrimp (p=0.05). $\delta^{13}C_{bulk}$ were higher than $\delta^{13}C_A$ and $\delta^{13}C_{ALE}$ for circumpolar eualid (A: p<0.0001; ALE: p=0.01) and green shrimp (A: p<0.0001; ALE: p=0.001); however, $\delta^{13}C_{ALE}$ were also higher than $\delta^{13}C_A$ values for circumpolar eualid (p=0.0004) and green shrimp (p<0.0001). $\delta^{15}N$ values differed significantly among treatments for isopods ($F_{2,39}$=7.1, p=0.002), and circumpolar eualid ($F_{2,39}$=6.6, p=0.003), but were similar for green shrimp ($F_{2,42}$=0.7, p=0.50) and polar shrimp ($F_{2,42}$=0.7, p=0.49). $\delta^{15}N_{bulk}$ values were higher than $\delta^{15}N_{ALE}$ (p=0.002), but there was no difference between $\delta^{15}N_A$ and $\delta^{15}N_{bulk}$ (p=0.4), or $\delta^{15}N_A$ and $\delta^{15}N_{ALE}$ (p=0.05) for isopods. For circumpolar eualid, $\delta^{15}N_{bulk}$ values were lower than $\delta^{15}N_A$ (p=0.03) and $\delta^{15}N_{ALE}$ (p=0.004), but there was no difference between $\delta^{15}N_A$ and $\delta^{15}N_{ALE}$ (p=0.7).

The change in $\delta^{13}C$ *after lipid removal and acidification in relation to bulk C:N ratio and* $\delta^{13}C$

The relationship between C:N ratio and the change in $\delta^{13}C$ after lipid extraction ($\delta^{13}C_{LE}$ - $\delta^{13}C_{bulk}$= $\Delta^{13}C_{LE\text{-}bulk}$) was species-specific. All marine organisms without exoskeletons demonstrated significant positive relationship between bulk C:N and $\Delta^{13}C_{LE\text{-}bulk}$ (Figure 1.3). The relationship between $\Delta^{13}C_{LE\text{-}bulk}$ and C:N ratios was positive in capelin ($F_{1,15}$=339.6, r^2=0.96, p<0.0001) and octopus ($F_{1,13}$=49.7, r^2=0.78, p<0.0001). Although weak, the relationship was also positive in beluga whale muscle ($F_{1,67}$=4.3, r^2=0.05, p=0.043) and liver ($F_{1,66}$=10.3, r^2=0.12, p=0.002). There was also a positive relationship between C:N ratio and the difference in $\delta^{13}C$ after acidification and lipid removal in green shrimp ($F_{1,13}$=51.3, r^2=0.78, p<0.0001), and circumpolar eualid ($F_{1,12}$=18.0, r^2=0.57, p=0.001). There was no relationship between the C:N ratio and $\Delta^{13}C_{LE\text{-}bulk}$ in isopods ($F_{1,12}$=0.001, r^2=-0.08, p=0.98) and polar shrimp ($F_{1,13}$=2.51,

r^2=0.1, p=0.14). Variability in $\delta^{13}C$ values declined after lipid extraction and carbonate removal, with the exception of beluga tissues and circumpolar eualid, which remained similar (Table 1.1). Specifically, green shrimp and polar shrimp were both collected at the same transect and had the same mean $\delta^{13}C$ of -22.1±0.1 ‰ after acidification and lipid removal. Isopods (mean $\delta^{13}C_{ALE}$=-22.9±0.2 ‰) and circumpolar eualid (mean $\delta^{13}C_{ALE}$=-22.7±0.2‰) were also collected at the same transect.

The difference in $\delta^{15}N$ values ($\Delta^{15}N$) between bulk and lipid extracted or acidified and lipid extracted samples was unrelated to bulk C:N in octopus, capelin, beluga tissues, circumpolar eualid, isopods, and polar shrimp. However, bulk C:N ratios were positively related to $\Delta^{15}N$ in green shrimp ($F_{1,13}$=7.4, r^2 =0.31, p=0.018).

To examine potential correction factors for $\delta^{13}C$, we also examined the relationship between bulk $\delta^{13}C$ with the final $\delta^{13}C$ after the respective treatments (Figure 1.4). The relationship between bulk and $\delta^{13}C$ after lipid extraction was positive for beluga muscle ($F_{1,67}$=425.7, r^2=0.86, p<0.0001), liver ($F_{1,66}$=244.1, r^2=0.78, p<0.0001), and octopus tissues ($F_{1,13}$=53.6, r^2=0.79, p<0.0001). There was also a weak relationship between bulk and $\delta^{13}C_{ALE}$ for circumpolar eualid ($F_{1,12}$=5.2, r^2=0.24, p=0.042). However, there was no relationship between $\delta^{13}C_{bulk}$ and $\delta^{13}C_{ALE}$ values in isopods ($F_{1,12}$=0.8, r^2=-0.01, p=0.40), green shrimp ($F_{1,13}$=4.1, r^2=0.18, p=0.065), and polar shrimp ($F_{1,13}$=0.8, r^2=-0.02, p=0.40), or $\delta^{13}C_{bulk}$ and $\delta^{13}C_{LE}$ values in capelin ($F_{1,15}$=1.9, r^2=0.05, p=0.19).

Effects of carbonate and lipid removal on niche metrics

After lipid and carbonate removal, there was a 95.4% probability that the Bayesian estimate of the standard ellipse area was smaller in isopods (Figure 1.5A). There was also a high

probability that the ellipses were also smaller in green shrimp (Bayesian P=0.94) and polar shrimp (P=0.85) after acidification and lipid removal, and in capelin (P=0.95) and octopus (P=0.87) after the removal of lipids (Figure 1.5). The ellipse areas did not differ before and after sample treatments for circumpolar eualid (P=0.49), beluga muscle (P=0.50) or liver tissues (P=0.38). Testing with RPP and Hotelling's T^2 revealed that Euclidean distances between centroids differed significantly before and after treatments for isopod (distance=3.06; p=0.001; Hotelling's T^2=58.35, p<0.0001), octopus (distance=2.25; p=0.001; Hotelling's T^2=20.62, p=0.0005), green shrimp (distance=0.83; p=0.001; Hotelling's T^2=16.74, p=0.002), polar shrimp (distance=1.51; p=0.001; Hotelling's T^2=39.27, p<0.0001), capelin (distance=1.32; p=0.001, Hotelling's T^2=57.26, p<0.0001), circumpolar eualid (distance=0.94; p=0.003; Hotelling's T^2=13.09, p=0.006), and beluga liver (distance=1.50, p=0.001; Hotelling's T^2=226.99, p<0.0001), but remained the same for muscle (distance=0.19, p=0.14; Hotelling's T^2=4.60, p=0.11) (Table 1.2). Mean distance to the centroid decreased after treatments for all species except beluga, and was significant for green shrimp ($|CD_1\text{-}CD_2|$= 0.33, p=0.005) and capelin ($|CD_1\text{-}CD_2|$= 0.31, p=0.002); therefore, individuals within a species occupied more isotopic space before sample treatments. For comparisons for the overall community, there was a 100% probability that the Bayesian estimate of standard ellipse area was smaller after sample treatment methods. Hotelling's T^2 revealed that differences in the Euclidean distance between the centroids was nearly significant (distance=0.35, p=0.12; Hotelling's T^2= 6.03, p=0.05); however there was no significant difference in niche dispersion metrics ($|CD_1\text{-}CD_2|$= 0.07, p=0.62; ($|MNN_1\text{-}MNN_2|$= 0.03, p=0.12) before and after sample treatments.

Discussion

Carbon to nitrogen ratio and percent lipid content

The C:N ratio was a predictor of lipid content in capelin, octopus, and green shrimp, but not in the other shrimp and isopod species. Although some studies have shown a strong relationship between C:N ratio and lipid content (e.g. Sweeting et al. 2006), other studies of marine zooplankton, fish, and aquatic invertebrates have questioned the ability of the C:N ratio to serve as a proxy for % lipid (e.g. Kiljunen et al. 2006; Kidd et al. 2011; Pomerleau et al. 2014). Kilijunen et al. (2006) further recommended that the C:N ratio not be used for lipid normalization in aquatic invertebrates. Previous studies have found bulk C:N ratio is not a good indicator of lipid content in marine mammal tissues (Wilson et al. 2014; Yurkowski et al. 2014). Although we did not have % lipid values for our beluga tissues, Yurkowski et al. (2014) found that liver samples had higher and more variable lipid content (13.9±3.6%) than muscle (9.3±1.1%). Across several Arctic marine mammal species, the C:N ratio was positively related to lipid content in liver, but not muscle tissues (Yurkowski et al. 2014).

Effect of acidification on $\delta^{13}C$ and $\delta^{15}N$ in marine invertebrates

Acidification led to a significant decrease in $\delta^{13}C$ values in all invertebrate species, suggesting that variable amounts of inorganic carbon among species could be a potential source of bias. In a previous study on Arctic invertebrates, acidification caused a depletion in ^{13}C in an Arctic mollusk, but did not affect values in marine zooplankton (Pomerleau et al. 2014). However, acidification decreased $\delta^{13}C$ and $\delta^{15}N$ in whole body tissues of crayfish (Stenroth et al. 2006), ice amphipod (*Gammarus wilkitzkii*), and krill (*Thysanoessa inermis*) (Soreide et al. 2006). The presence of carbonates had a significant effect on several species of Antarctic

invertebrates, and acidification was recommended prior to stable isotope analysis for marine invertebrates (Jacob et al. 2005). We also found variable effects of acidification on $\delta^{15}N$ values, which may be the result of rinsing the acidified sample with distilled water (Jacob et al. 2005).

Lipid removal and acidification normalization in beluga whales and potential prey

Lipid extraction significantly increased $\delta^{13}C$ values in all species; however, it also significantly increased $\delta^{15}N$ values. Previous studies have found lipid extraction increased $\delta^{13}C$ and $\delta^{15}N$ values in marine mammal and fish tissues (Sotiropoulos et al. 2004; Murry et al. 2006; Sweeting et al. 2006; Logan and Lutcavage 2008; Mintenbeck et al. 2008; Lesage et al. 2010; Ryan et al. 2012; Yurkowski et al. 2014) likely due to a loss of proteins (i.e. amino acids) and nitrogen-containing lipids from the lipid matrix (e.g. Sotiropoulos et al. 2004; Sweeting et al. 2006; Logan and Lutcavage 2008; Svensson et al. 2016). Chemical extraction methods may also be a source of variation in $\delta^{13}C$ and $\delta^{15}N$ values (Logan and Lutcavage 2008; Elliott and Elliott 2016). Lipid extraction and carbonate removal elevated $\delta^{13}C$ values in Arctic marine zooplankton (Pomerleau et al. 2014), whereas the combined effect decreased $\delta^{13}C$ values in our isopod and shrimp species. We expected within species variability in $\delta^{13}C$ to be higher in more motile species such as beluga and capelin, as well as species that were collected across several trawling stations, such as the octopi. After sample treatment methods, octopi still had the highest variability in $\delta^{13}C$, but capelin and beluga tissues were within the range of the other invertebrates. Benthic invertebrates had similar $\delta^{13}C$ values after acidification and lipid extraction, which was expected since they are likely feeding from the same carbon pool.

Yurkowski et al. (2014) found the best lipid normalization models to be species-specific between $\delta^{13}C_{bulk}$ in relation to $\Delta^{13}C_{LE\text{-}bulk}$ (Lesage et al. 2010) and $C{:}N_{bulk}$ in relation to $\Delta^{13}C_{LE\text{-}bulk}$ (Ehrich et al. 2011) for liver and muscle tissues across several Arctic marine mammal

species. Yurkowksi et al. (2014) further found that predicting $\Delta^{13}C_{LE\text{-}bulk}$ based on $C{:}N_{bulk}$ to be the best fit for beluga based on a sample size of approximately 30 individuals. However, based on our results, we recommend lipid normalization based on $\delta^{13}C_{bulk}$ in relation to $\delta^{13}C_{LE}$ similar to Lesage et al. (2010) for muscle and liver tissues. In other studies, no relationship was found between the C:N ratio and $\Delta^{13}C_{LE\text{-}bulk}$ in skin and blubber tissues of balaenopterid whales (Ryan et al. 2012) and skin tissues of bottlenose dolphins (*Tursiops truncatus*) (Wilson et al. 2014). Ryan et al. (2012) recommended against lipid normalization models for $\delta^{13}C$ for balaenopterid skin or blubber tissues, as models underestimated the change in $\delta^{13}C$ due to lipid extraction or overestimated this change at higher C:N ratios greater than 4.5. As seen in other studies (Ryan et al. 2012; Wilson et al. 2014), the effectiveness of our correction equation may weaken in muscle or liver tissues with lipid contents higher than 9 to 13%, or with C:N ratios higher than 3.4 to 4.5.

Effects of carbonate and lipid removal on niche metrics

We have demonstrated that failure to remove carbonates and lipids prior to the analyses of stable isotopes can affect isotopic niche breadth and dispersion metrics. The niche breadth and centroid positions of samples with stronger relationships between lipid content and the C:N ratio seemed to be most affected. Bulk samples had greater within species variability in $\delta^{13}C$ values, occupying a larger isotopic space and niche breadth. Broader niche breadth and isotopic spacing due to lack of sample treatment may lead to overestimates of diet diversity. Interestingly, with the exception of niche breadth, differences in dispersion metrics were not detected at the community level. Further investigations into the overlap of standard ellipses among species may reveal that species relationships within the community change after lipid and carbonate removal are performed prior to stable isotope analysis. In summary, improper sample treatment for stable isotopes may lead to incorrect interpretations for isotopic niche breadth and space, which may

have major implications for predicting the impacts of invasive species, species loss on marine ecosystems, or determine the dietary linkages of marine predators.

In conclusion, we have found that despite low C:N ratios, inorganic carbon and lipid content are a potential source of bias for $\delta^{13}C$ when examining the trophic linkages of different species in Arctic marine food webs. However, relationships among C:N and $\Delta^{13}C$ and lipid content vary by species. As lipid and carbonate may also affect $\delta^{15}N$, we support the recommendations that $\delta^{13}C$ and $\delta^{15}N$ be analyzed separately (Sotiropoulos et al. 2004; Soreide et al. 2006; Lesage et al. 2010; Ryan et al. 2012). Finally, failure to perform sample pre-treatments such as lipid removal may lead to inaccurate interpretations of food web dynamics, such as positions in isotopic space and potentially niche overlap, which are important consideration for wildlife conservation and management. We acknowledge that lipid removal and carbonate acidification is an expensive and time consuming process; therefore, we recommend caution and consideration when examining the prey linkages of very different species. If lipid extraction and carbonate removal of tissues for $\delta^{13}C$ is not possible, we recommend that species-specific correction equations are used to normalize for the effects of lipids and carbonates.

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Table 1.1. Percent lipid and carbon and nitrogen stable isotopes values (mean ± 1 standard error) for untreated (bulk), acidified (A), and lipid extracted (LE) samples for beluga, capelin (muscle), octopus (mantle) tissues, and marine invertebrate whole body tissues.

Species	***n***	**% Lipid**	**$\delta^{13}C_{bulk}$**	**$\delta^{15}N_{bulk}$**	**$C:N_{bulk}$**	**$\delta^{13}C_{A}$**	**$\delta^{15}N_{A}$**	**$C:N_{A}$**	**$\delta^{13}C_{LE}$**	**$\delta^{15}N_{LE}$**	**$C:N_{LE}$**
Beluga muscle	69	9.3±1.1[a]	-19.0±0.1	17.2±0.1	3.4±0.0				-18.8±0.1	17.4±0.1	3.1±0.0
Beluga liver	68	13.9±3.6[a]	-20.9±0.1	18.1±0.1	4.5±0.0				-19.4±0.1	18.3±0.1	3.4±0.0
Octopus	15	17.3±2.9	-23.8±0.4	13.8±0.3	5.5±0.5				-21.6±0.3	14.8±0.3	3.7±0.1
Capelin	17	31.3±1.5	-24.4±0.2	14.7±0.1	4.1±0.2				-23.0±0.1	15.1±0.1	3.3±0.0
Green shrimp	15	9.7±1.4	-21.2±0.2	15.1±0.1	4.0±0.1	-23.3±0.2	15.1±0.3	5.5±0.3	-22.1±0.1	15.4±0.3	4.2±0.1
Circumpolar eualid	14	11.9±1.0	-21.8±0.2	14.9±0.2	4.0±0.1	-24.1±0.2	15.7±0.2	5.6±0.3	-22.7±0.2	15.9±0.2	4.1±0.0
Isopod	14	6.7±1.0	-19.8±0.4	12.2±0.3	5.0±0.2	-23.2±0.3	11.3±0.6	5.2±0.2	-22.9±0.2	9.4±0.6	5.0±0.1
Polar shrimp	15	8.8±1.5	-20.6±0.2	15.4±0.2	3.7±0.1	-22.8±0.2	15.0±0.5	5.1±0.2	-22.1±0.1	14.6±0.6	4.4±0.2

a values from Yurkowski et al 2014.

Table 1.2. Isotopic niche metrics for all species for untreated (bulk) and treated (carbonate and/or lipid removed) samples (bulk $\delta^{15}N$ values were used in all metrics). The estimated standard ellipse area (SEA) represents the isotopic niche breadth. The location of the centroid (LOC) indicated where the niche is centred in isotopic space. The mean distance to the centroid (CD) and mean nearest neighbour distances are measures of isotopic dispersion. Bold values indicate significant difference between species post-treatment. Community includes all species and tissues as one group.

Species	**Bulk**				**Treated**			
	SEA	**LOC**	**CD**	**NND**	**SEA**	**LOC**	**CD**	**NND**
Isopod	**4.98**	**-19.81, 12.24**	1.67	0.69	**2.25**	**-22.87, 12.24**	1.14	0.49
Octopus	6.41	**-23.82, 13.84**	1.69	0.72	4.07	**-21.57, 13.84**	1.25	0.66
Green shrimp	1.15	**-21.24, 15.13**	**0.82**	**0.37**	0.37	**-22.08, 15.13**	**0.49**	**0.19**
Polar shrimp	1.43	**-20.62, 15.39**	0.91	0.40	0.82	**-22.13, 15.39**	0.64	0.36
Capelin	0.70	**-24.35, 14.74**	**0.68**	0.27	0.25	**-23.03, 14.74**	**0.37**	0.16
Circumpolar eualid	1.22	**-21.79, 14.93**	0.88	0.42	1.22	**-22.73, 14.93**	0.74	0.35
Beluga muscle	0.80	-18.95, 17.18	0.69	0.15	0.79	-18.76, 17.18	0.69	0.14
Beluga liver	0.92	**-20.88, 18.08**	0.65	0.15	0.88	**-19.38, 18.08**	0.65	0.16
Community	10.21	-20.74, 16.35	2.32	0.20	6.84	-20.39, 16.35	2.39	0.17

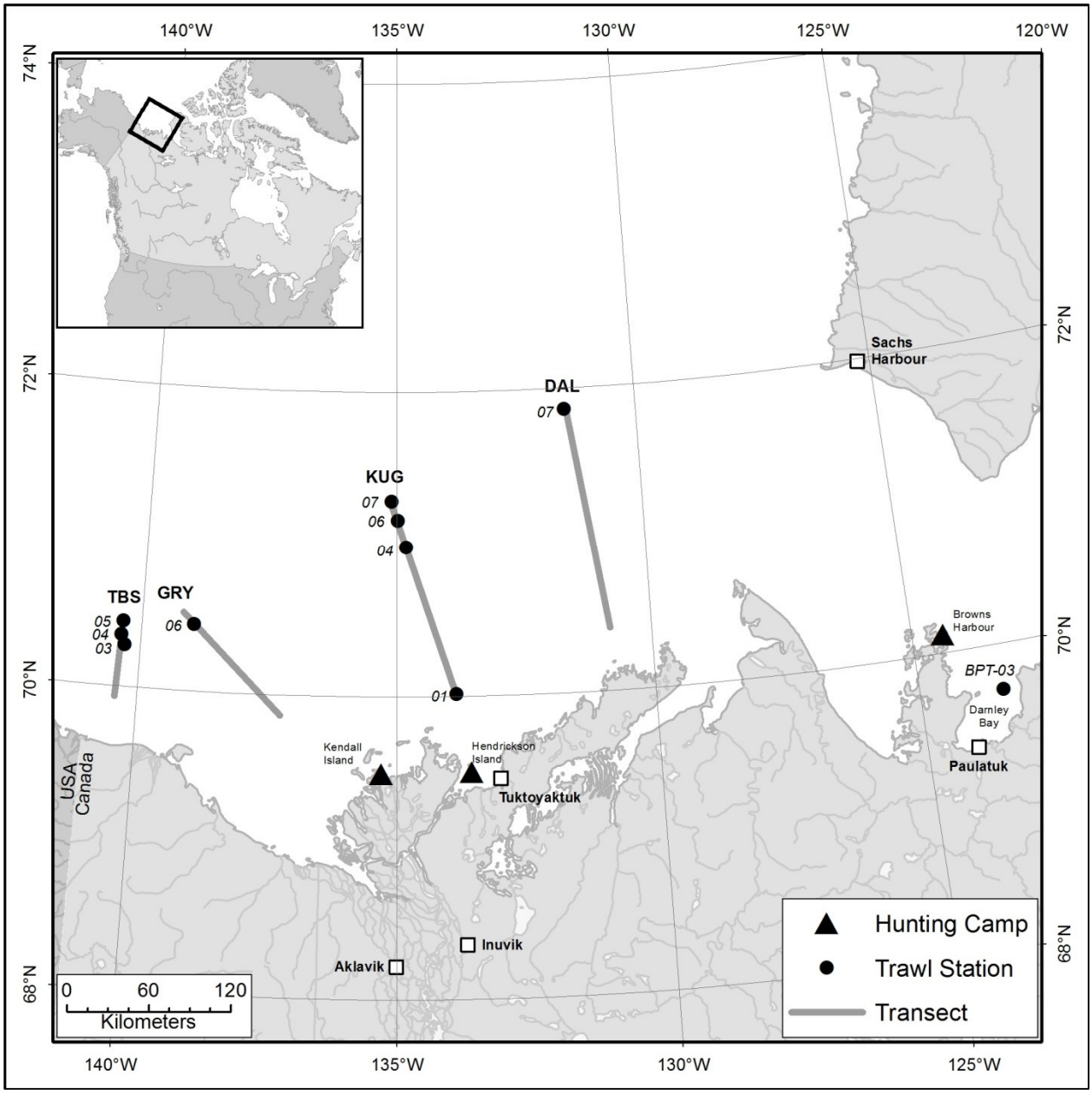

Figure 1.1. Study area in the Beaufort Sea ecosystem, including sample collection sites for beluga whale tissues at traditional Inuvialuit hunting camps in the Inuvialuit Settlement Region, Inuvik, Northwest Territories, Canada, and numbered trawling stations for sampling invertebrates and capelin from the Beaufort Environmental Assessment Program. Labelled transects are: transboundary-transect (TBS), Garry Island (GRY), Kugmallit Bay (KUG), Dalhousie (DAL), and Bennett Point (BPT).

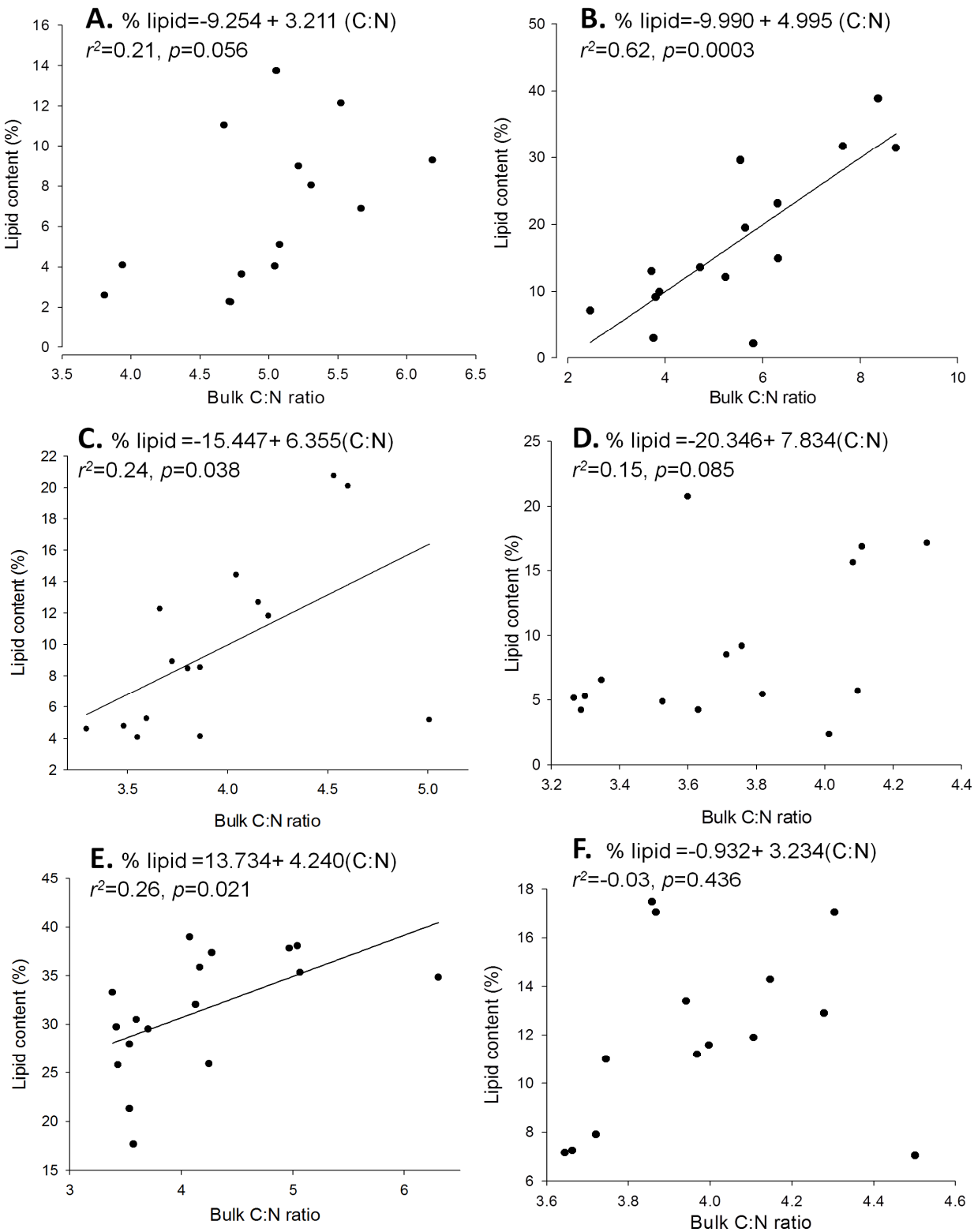

Figure 1.2. Relationships between C:N and % lipid in A) isopod (n = 14), B) octopus (n = 15), C) green shrimp (n = 15), D) polar shrimp (n = 15), E) capelin (n = 17), and F) circumpolar eualid (n = 14).

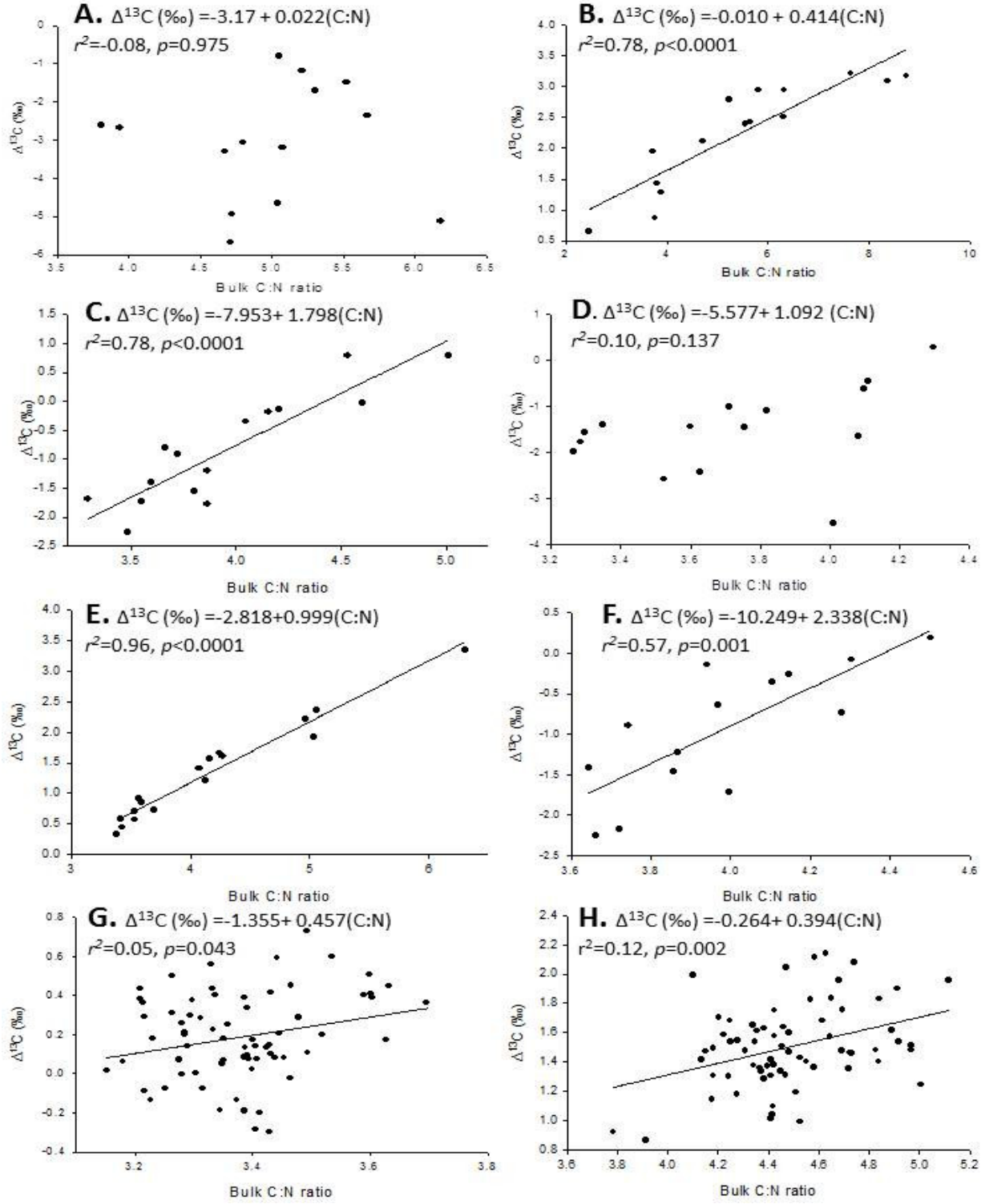

Figure 1.3. Relationships between C:N and the change in $\delta^{13}C$ after lipid extraction ($\Delta^{13}C$) in A) isopod (*n* =14), B) octopus (*n* =15), C) green shrimp (*n* =15), D) polar shrimp (*n* =15), E) capelin (*n* =17), F) circumpolar eualid (*n* =14), G) beluga whale muscle (*n* =69), and H) beluga whale liver (*n* =68). Isopod (A), green shrimp (C), polar shrimp (D), and circumpolar eualid (F) samples were acidified prior to lipid removal.

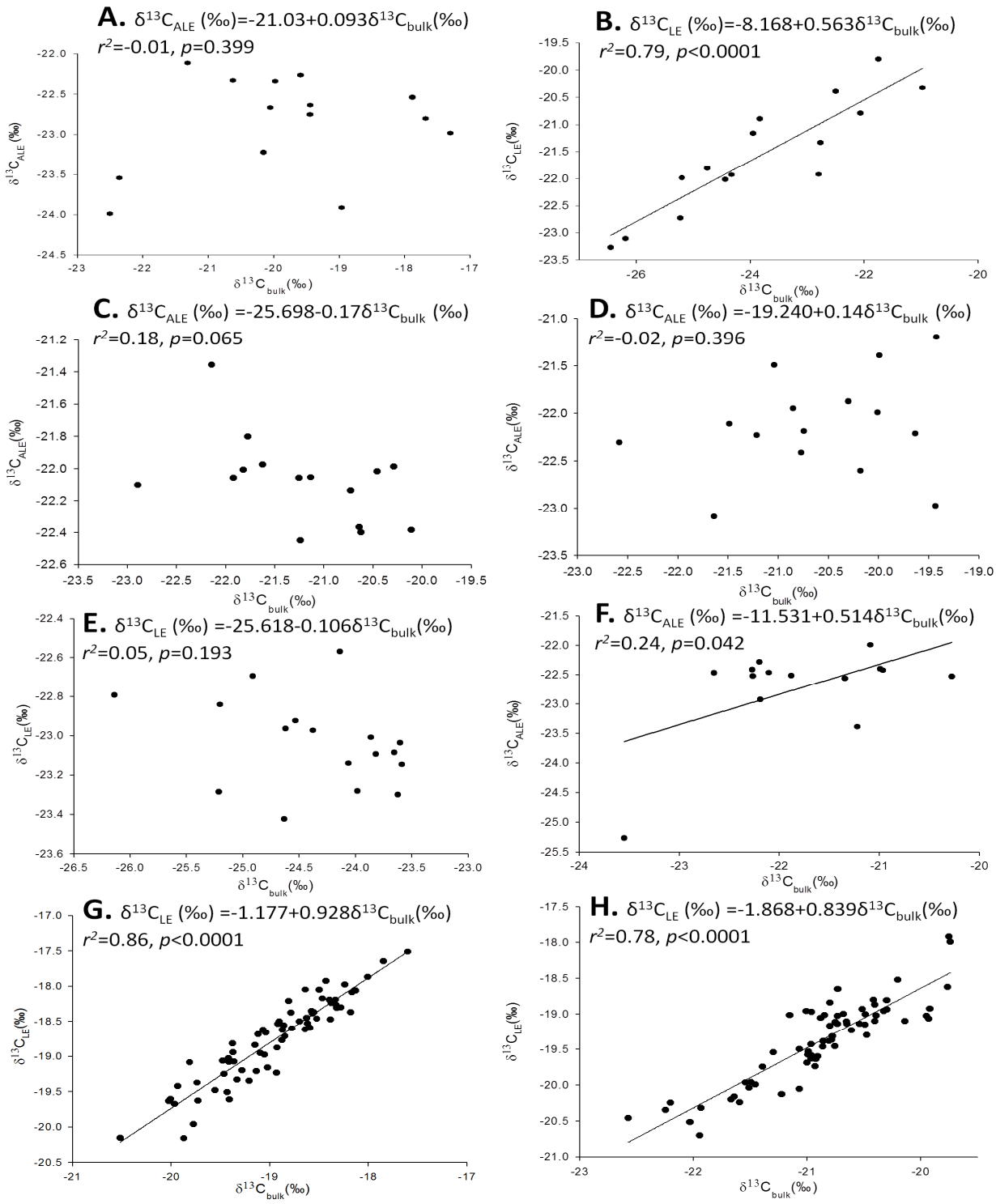

Figure 1.4. Relationships between bulk $\delta^{13}C$ and $\delta^{13}C$ after lipid extraction ($\delta^{13}C_{LE}$) or after acidification and lipid extraction ($\delta^{13}C_{ALE}$) in A) isopod (n =14), B) octopus (n =15), C) green shrimp (n =15), D) polar shrimp (n =14), E) capelin (n =17), F) circumpolar eualid (n=14), G) beluga whale muscle (n =69), and H) beluga whale liver (n =68).

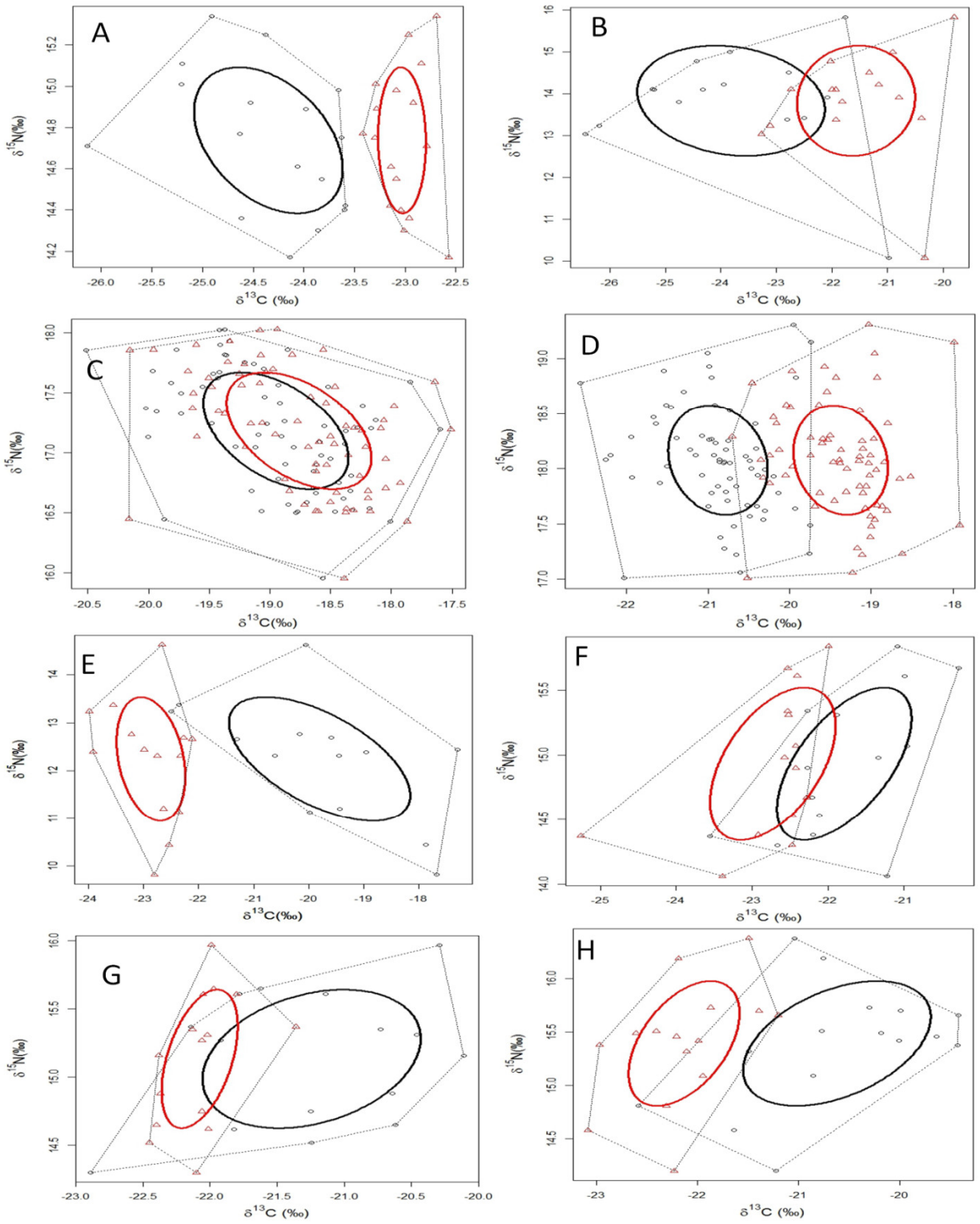

Figure 1.5. Isotopic niches (standard ellipse and convex hull) before (black) and after (red) lipid removal for A) capelin (*n* =17), B) octopus (*n* =15), C) beluga whale muscle (*n* =69), and D) liver (*n* =68), and after (red) lipid extraction and carbonate acidification for E) isopod (*n* =14), F) circumpolar eualid (*n* =14), G) green shrimp (*n* =15), and H) polar shrimp (*n* =15). Bulk $\delta^{15}N$ values were used in all metrics.

Chapter 2:

Inter-annual variations in environmental conditions affect prey and body condition of eastern Beaufort Sea beluga whales (*Delphinapterus leucas*)

Paper in press in Marine Ecology Progress Series

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Contributions:

Dr. Lisa Loseto provided funding for laboratory analysis and field work and comments throughout the writing process.

Dr. Jim Roth provided comments throughout the writing process.

Bruno Rosenberg provided technical support and expertise for fatty acid analysis using gas chromatography as well as quality assurance and control.

Emily Choy designed the study, assisted with sample collection extracted inner, middle, and outer blubber layers for fatty acid signatures, completed chromatography on all samples, analyzed the data, produced all figures and tables, and wrote the manuscript.

Abstract

Declines in individual growth rates in eastern Beaufort Sea (EBS) beluga whales (*Delphinapterus leucas*) over the past 20 years are hypothesized to be the result of changing environmental conditions. To better understand short-term variation in diet, we examined inter-annual variations in body condition indices, fatty acid, and stable isotope signatures in EBS beluga whales in relation to environmental conditions. We also examined if differences in dietary tracers in beluga whales reflect sex and size-based habitat selection. During a warm year anomaly (2012), belugas demonstrated greater overlap in dietary tracers among sex-and-size classes, whereas greater differences occurred during years with greater sea ice extent in the Mackenzie Shelf (2013 and 2014). Body condition indices (maximum girth and blubber thickness) were highest in belugas in 2011 and 2012 and lowest in 2014. Total *Calanus* markers 20:1n-9 and 22:1n-11 contributed the most to annual variability and had the lowest proportions in small male and females in 2014, a year that coincided with low Arctic cod (*Boreogadus saida*) biomass. Age and year were the strongest predictors of fatty acids and $\delta^{13}C$ values, whereas length influenced $\delta^{15}N$ values in beluga whales, possibly a reflection of larger whales diving to greater depths to feed on Arctic cod. Annual variability in sea ice conditions and prey availability may be associated with inter-annual variation in dietary tracers and condition in beluga whales. As Arctic marine ecosystems are currently undergoing rapid change, understanding the factors causing inter-annual variation in diet should be a conservation priority for this population.

Introduction

As the most abundant Arctic odontocete with a circumpolar distribution, beluga whales (*Delphinapterus leucas*) are a potential indicator species for Arctic climate change (Tynan and DeMaster 1997; Laidre 2008; Moore and Huntington 2008; Laidre et al. 2015). The eastern Beaufort Sea (EBS) beluga whale population is one of Canada's largest, with an estimated population of 40,000 individuals (Allen and Angliss 2014). Departing from Alaska in April, the EBS beluga stock arrives in the Canadian Beaufort Sea in May to late June where parturition, nursing, and possibly moulting take place near the waters of the Mackenzie River estuary. By September, whales return to their wintering grounds in the Bering Sea (Richard et al. 2001; Harwood and Smith 2002). Habitat selection of the EBS beluga population is separated by sex, size, and reproductive status during the open water season (Richard et al. 2001; Loseto et al. 2006). Large males select offshore pack ice habitat and have a larger summer range than females, whereas smaller males and females with young calves select coastal habitat (Loseto et al. 2006). Tagging data revealed that in July, males travelled to offshore habitats of the Canadian Beaufort Sea, Arctic Ocean, and Viscount Melville Sound, whereas females travelled between Amundsen Gulf and the Mackenzie River estuary or the continental shelf (Richard et al. 2001).

Recently, there has been concern over the decline in body condition in marine mammals, seabirds, and forage fish species in the Beaufort Sea (Harwood et al. 2012; Harwood et al. 2014; Divoky et al. 2015; Harwood et al. 2015). EBS beluga whales harvested by Inuvialuit subsistence hunters significantly declined in body size-at-age from 1989 to 2008 (Harwood et al. 2014). Male belugas also experienced a decline in blubber thickness from 2000 to 2007, with the thinnest blubber in 2005, a year that coincided with poor body condition in ringed seals (*Phoca*

hispida) and polar bears (*Ursus maritimus*) (Harwood et al. 2014). Declines in body condition are believed to be caused by climate-induced ecosystem shifts that may have resulted in reduced availability of prey (Harwood et al. 2014). Fatty acid signatures in blubber from whales sampled in 2004 and 2005 revealed their diet to be primarily Arctic cod (*Boreogadus saida*) (Loseto et al. 2009), the most abundant fish in the Canadian Beaufort Sea (Benoit et al. 2008). However, growth and physical condition of Arctic cod is compromised by increasing temperature, making cod vulnerable to climate change (Laurel et al. 2016). In addition, the loss of sea ice has led to the northward range expansion of temperature and subarctic species, such as Pacific sand lance (*Ammodytes hexapterus*), that have been recently detected in the Beaufort Sea and are predicted to displace Arctic cod (Falardeau et al. 2014).

In response to the observed decline in body condition, monitoring of beluga diet has increased to determine the vulnerability of the population to future environmental change. Whales harvested by Inuvialuit subsistence hunters typically have empty stomachs (Harwood and Smith 2002) and observations of feeding behaviours are difficult to obtain; therefore, ecological tracers that are transferred from prey to predator, such as fatty acids and carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios can provide useful information on the diets of marine mammals (Falk-Petersen et al. 2004; Iverson et al. 2004; Budge et al. 2006; Newsome et al. 2010). Most essential fatty acids such as long-chained monounsaturates (MUFAs) and polyunsaturates (PUFA) are obtained from an organism's diet, with the exception of a few long-chained MUFAs and PUFAs (e.g. 22:5n-3, 18:1n-11) that can be biosynthesized due to metabolism of other fatty acids (Iverson et al. 2004). Short-chained fatty acids (< 14 carbons) in blubber are produced by *de novo* synthesis and are not incorporated from prey, as they are immediately oxidized in the liver (Budge et al. 2006). Likewise, stable isotope ratios of predators

reflect the stable isotope ratios of their prey (Hobson et al. 1996; Parnell et al. 2013; Phillips et al. 2014). $\delta^{15}N$ values typically increase 3 to 5‰ with every trophic transfer whereas $\delta^{13}C$ values varies with differences in baseline primary producers, increasing approximately 0 to 1‰ per trophic transfer (Peterson and Fry 1987). The combination of fatty acid signatures and stable isotope ratios is a powerful technique for the interpretation of trophic linkages in aquatic ecosystems (El-Sabaawi et al. 2009).

To investigate if shifts in diet and body condition are linked to changing environmental conditions, we examined inter-annual variation in fatty acid signatures and stable isotope ratios of Beaufort Sea beluga whales from 2011 to 2014. As sea ice conditions influence the habitat and range of beluga whales (Heide-Jørgensen et al. 2010; Hornby et al. 2016), we first examined whether variability in diet in beluga whales was influenced by habitat selection. Using sex and size-based habitat groups defined in Loseto et al. (2006) and (2008b), we predicted that dietary tracers found in large males would differ from small males and females due to differences in habitat use. We also predicted that annual differences in habitat selection due to changing environmental conditions would be reflected in dietary tracers. Our next objective was to assess body condition indices in beluga whales using morphometrics from harvested whales, to examine if higher body condition in whales corresponds with favourable environmental conditions. Finally, we investigated biological factors influencing fatty acid composition and stable isotope ratios in beluga whales. As Arctic marine ecosystems are undergoing rapid change, our goal was to increase our understanding of inter-annual variations in beluga diet in response to environmental conditions, as well as to establish a baseline for monitoring the response of belugas to environmental changes.

Methods

Sample collection

Blubber and liver samples were collected from 26 female and 151 male adult beluga whales harvested at Inuvialuit hunting camps in July to early August 2011-2014 at Hendrickson Island, Brown's Harbour, Kendall Island, and East Whitefish in the Inuvialuit Settlement Region, Northwest Territories, Canada (Figure 2.1). Sex, body length, maximum half girth (indicated by the dorsal ridge to the approximate ventral midline), and axillary blubber thickness were recorded for each specimen. Age was estimated by counting growth layer groups, in which one growth layer group (comprised of a dark and light layer) equals one year, from teeth collected from lower jaws (Stewart et al. 2006). Blubber samples (approximately 10 cm by 10 cm, throughout the entire blubber depth) were removed from the mid thoracic region near the front flipper. All individuals had empty stomachs. Samples were frozen at -20˚C in portable freezers and shipped to Fisheries and Oceans Canada in Winnipeg for laboratory analysis.

Fatty acid extraction

Blubber was divided into three equal layers: inner, middle, and outer. Total lipid content was extracted from 0.5 g of blubber using chloroform: methanol (2:1 v/v/w) containing 0.01% butylated hydroxytoluene and 0.7% NaCl, for a final proportion of chloroform/methanol/water of 8:4:3 following a method modified from Folch et al. (1957) as described in Budge et al. (2006). Fatty acid methyl esters (FAME) were prepared using the lipid extract through transesterification using 1.5 mL of dichloromethane and Hilditch reagent (0.5 N H_2SO_4 in dry methanol) and incubated for 1 h at 100°C. The purified FAME was dissolved in hexane.

Gas chromatography was performed using an Agilent Technologies 7890N coupled to a Flame Ionization Detector. Run procedures are described in detail in Giraldo et al. (2016). Briefly, FAME samples were analyzed using gas chromatography (Hewlett Packer HP series 6890) with a mass spectrometer detector (Hewlett-Packard 5973). Fatty acid standards were obtained from Supelco (37 component FAME mix; Sigma-Aldrich Canada Co., Oakville, Ontario) and Nuchek (54 component mix GLC-463; Nu-Chek Prep, Inc., Elysian, Minnesota, USA). Fatty acids that were not present in the Supelco standard were quantified using response factors for fatty acids of similar chain length and retention time. A total of 72 fatty acids were identified by retention time based on Supelco and Nuchek standards and reported as the percentage of the total fatty acids. Dietary fatty acids identified by Iverson et al. (2004) are best represented in the inner blubber layer (Koopman et al. 2002) and were used for analysis. Thirty fatty acids identified by Iverson et al. (2004) as having dietary origins and mean proportions greater than <0.1% were kept for analyses.

Stable isotope analysis

Liver samples were freeze-dried for at least 48 hours and analyzed for C and N stable isotope ratios at the University of Waterloo Environmental Isotopes Laboratory as described in Chapter 1. Samples for 2014 were analyzed at the Freshwater Institute biotracers lab as described in Rosenberg et al. (2015). To ensure data were comparable, 10 liver samples were compared for inter-laboratory variability and reported to have a mean difference of 0.07 ‰ for $\delta^{13}C$ and 0.30 ‰ for $\delta^{15}N$. The standard deviations of the liver samples between laboratories were reported to be 0.05 ‰ for $\delta^{13}C$ and 0.22 ‰ for $\delta^{15}N$ (Rosenberg et al. 2015).

As lipids significantly affect both $\delta^{13}C$ and $\delta^{15}N$ values in beluga tissues, a lipid correction model ($\delta^{13}C_{extracted} = -1.868+0.839\times\delta^{13}C_{bulk}$ for liver; Chapter 1) was used on bulk $\delta^{13}C$ values.

Statistical analyses

Body condition

Using an approach similar to George et al. (2015) for bowhead whales (*Balaena mysticetus*), we developed two body condition indices based on maximum girth and blubber thickness. Each body condition index was computed from the residuals of the most parsimonious model based on AICc with length, age, and sex as factors using the package nlme for linear models (Pinheiro et al. 2015) in R 3.2.5 (R Core Team 2016). Models were assessed for multicollinearity using a variance inflation factor (<2.5) for each predictor, normality of residuals using a Shapiro-Wilk test, and serial autocorrelation of standard residuals using a Durbin-Watson test. Variables were log-transformed when appropriate to meet assumptions of normality. Difference in body condition indices among years were tested using one-way analyses of variance (ANOVA) followed by post-hoc Tukey HSD (Honestly Significant Difference) tests.

Dietary tracers

Although dietary tracers can provide insights into the diets of free-ranging cetaceans, the relative turnover rates of fatty acids in blubber and stable isotope ratios have not been quantified in belugas or other cetaceans (Newsome et al. 2010). Turnover of fatty acids in blubber are approximately 1.5 to 3 months in newly weaned harbour seals (*Phoca vitulina*) (Nordstrom et al. 2008); however, significant changes in fatty acids during prey-switching experiments have been detected after 14 days in harp seals (*Phoca groenlandica*) (Kirsch et al. 2000). Stable isotope

signatures in liver typically have the fastest turnover rate relative to other tissues in mammals, ranging from a few days in small rodents to 37.3 days in alpacas (*Lama pacos*) (Tieszen et al. 1983; Arneson et al. 2006; Sponheimer et al. 2006; Miller et al. 2008; DeMots et al. 2010). Therefore, we assumed the relative turnover rate of fatty acids in the inner blubber and stable isotope ratios in the liver to be approximately two to five weeks, and indicative of the spring-summer diet of Beaufort Sea belugas.

Fatty acid signatures

To determine if different fatty acid signatures reflected differences in habitat-use, we divided males into three size classes defined in Loseto et al. (2008): small males (<3.8 m) that use coastal habitat, medium-sized males that use mixed sea-ice (3.8-4.2 m), and large males that select pack ice (>4.2 m). We kept females as one class due to small sample size (n = 26). Non-parametric multivariate analyses were performed using Plymouth routines in multivariate ecological research (PRIMER) v.7.0 (Clarke and Gorley 2015) and PERMANOVA + (Anderson et al. 2008). We ran a 2-factor permutational multivariate analysis of variance (PERMANOVA) to investigate the variation in fatty acid composition by year and sex-and size-class, followed by post hoc pairwise tests. PERMANOVA partitions variation of multivariate data in an ANOVA design using permutational methods (Anderson et al. 2008). PERMANOVA is not affected by violations in normality, but is sensitive to dispersion of multivariate data. We did not include the single female sampled in 2011 since there is no dispersion for a group with a sample size of one (Anderson et al. 2008). Fatty acids among beluga whales (n = 175) were homogeneously dispersed by year (PERMDISP, $F_{3,171} = 0.79$, $p = 0.60$) and sex-and-size class ($F_{3,171} = 1.28$, $p = 0.37$). PERMANOVA tests used Euclidean distance, fixed factors and Type III sums of squares,

and significance was determined using 9999 unrestricted permutations of the raw data and Monte Carlo (MC) generated p-values when the number of unique permutations was <100. To identify the influential fatty acids contributing to dissimilarities between sex-and-size classes and year, we performed a two-way similarity percentage routine (SIMPER) analysis. SIMPER first tabulates fatty acid contributions to the average similarity of individuals within each groups followed by the average dissimilarity (Clarke et al. 2014; Clarke and Gorley 2015). We designated a cut-off from the dominant fatty acids that characterized up to 80% of dissimilarities.

We used distance-based linear models (DISTLM) to examine the variation of fatty acid signatures explained by biological factors and year. DISTLM partitions variation of a multivariate dataset according to a multiple regression model. Unlike PERMANOVA, DISTLM allows predictor variables to fit individually or together, and allows for the testing of significance of continuous predictor variables. Parsimonious models can be built using model selection criteria (Anderson et al. 2008). Fatty acids were log (x+1) transformed to increase the weighing of fatty acids found in lower proportions. Predictor variables included continuous (age, length, girth, blubber thickness) and categorical variables (sex, and year) coded as binary variables. Missing values were inputted into the worksheet of the predictor variables using an expectation maximum likelihood algorithm (Clarke and Gorley 2015). To control for scaling and sexual dimorphism, predictor variables were normalized within each sex by subtracting the value of each variable by their mean and dividing by their standard deviation. A draftsmen plot was used to check assumptions of multicollinearity between predictor variables, revealing an $r < 0.65$ for every pair-wise comparison. We used a step-wise selection procedure with 9999 permutations and using adjusted R^2 as the selection criterion. The full model was visualized using distance-based redundancy analysis (dbRDA) ordination, which shows the percentage of fitted variation

of the model explained by the first two axes and the remaining unexplained variation. Vector overlays displayed the multiple partial correlations of significant predictor variables with the dbRDA axes.

Stable isotope ratios

A 2-factor PERMANOVA was also run on $\delta^{13}C$ and $\delta^{15}N$ values to investigate the effect of year and sex-and-size class, followed by post-hoc pairwise tests. Due to violation of homogeneity of dispersion for stable isotope values, $\delta^{13}C$ and $\delta^{15}N$ values were log-transformed (using x+40 for $\delta^{13}C$ and x+1 for $\delta^{15}N$ to make all values non-negative). Based on the significant predictor variables of the PERMANOVA, we examined the influence of sex-and-size class and year on isotopic niche breadth using Stable Isotope Bayesian Ellipses in R (SIBER) tools (Jackson et al. 2011) in the package Stable Isotope Analysis in R (SIAR 4.2.2; Parnell and Jackson 2013). Stable isotope data were visualized using the standard ellipse areas ($‰^2$) corrected for small sample size, which characterized spatial variability in $\delta^{13}C$ and $\delta^{15}N$ ratios in two dimensional space and encompassed approximately 40% of the data (Jackson et al. 2011). Using pairwise comparisons between year and sex-and-size class, we calculated the overlap area between ellipses followed by the percentage of overlap for each standard ellipse area. We also compared standard ellipse areas by calculating the probability that the posterior distribution of one ellipse was smaller than another, using Bayesian inference based on Markov chain Monte Carlo simulations using 10^4 posterior draws (Layman et al. 2007; Jackson et al. 2011) using the script provided by Parnell and Jackson (2013).

We assessed the influence of biological variables and year on $\delta^{13}C$ and $\delta^{15}N$ values using the package nlme for linear models (Pinheiro et al. 2015) following the same approach we used for body condition indices. Kruskal-Wallis rank sum test was selected as a non-parametric

comparison followed by Dunn's test for post hoc multiple comparisons tests. Significance was judged at $\alpha = 0.05$ for all statistical procedures. All univariate analysis were conducted using R 3.2.5 (R Core Team 2016).

Results

Annual differences in body condition indices

The most parsimonious models included log [length] + sex × age as predictors for log [maximum girth] ($F_{4,154} = 48.77$, $r_{adj}^2 = 0.55$, $p < 0.01$) and length + sex + age for blubber thickness ($F_{2,156} = 13.01$, $r_{adj}^2 = 0.13$, $p < 0.01$; Appendix 2.1). In our models for body condition, maximum girth was significantly influenced by length ($t = 5.97$, $p < 0.01$) and the interaction sex×age ($t = 2.80$, $p < 0.01$), whereas only length ($t = 4.99$, $p < 0.01$) was a significant factor for blubber thickness (Table 2.1). The body condition index for maximum girth differed among years (ANOVA, $F_{3,155} = 6.64$, $p < 0.01$). Residual maximum girth was higher in 2012 than in 2011 (*Tukey HSD test,* $t = 2.80$, $p = 0.03$), 2013 ($t = -3.53$, $p < 0.01$), and 2014 ($t = -3.96$, $p < 0.001$) (Figure 2.2). The body condition index for blubber thickness also differed among years ($F_{3,155} = 4.97$, $p < 0.01$), and was lowest in 2014 compared to 2011 ($t = -3.46$, $p < 0.01$) and 2012 ($t = -2.90$, $p = 0.02$).

Inter-annual variation and biological factors affecting fatty acid signatures

Fatty acid signatures of EBS beluga whales ($n = 175$) varied by year (Two-way PERMANOVA, *Pseudo-*$F_{3,160} = 6.07$, $p < 0.01$) and sex-and-size class (*Pseudo-*$F_{3,160} = 7.87$, $p < 0.01$), with a significant year × sex-and-size class interaction (*Pseudo-*$F_{8,160} = 2.00$, $p = 0.01$). Pairwise comparisons of the year × sex-and-size class interaction demonstrated that there was a

significant difference in fatty acids between large and small males in 2011 (t = 1.66, p = 0.03), 2013 (t = 3.61, p < 0.01) and 2014 (t = 2.21, p = 0.02), but not in 2012 (t = 0.24, p = 1.00). There was also a significant difference between large males and females in 2013 (t = 2.25, p < 0.01) and 2014 (t = 2.67, p < 0.01), but not in 2012 (t = 1.00, p = 0.39). Medium-sized males were also different from small males in 2013 (t = 2.74, p = 0.01), and 2014 (t = 3.82, p < 0.01), but not in 2012 (t = 0.85, p = 0.54) and 2011 (t = 1.03, p = 0.38). There was also a significant difference between large and medium-sized males (t = 1.82, p = 0.03) and small males and females (t = 2.24, p = 0.04) in 2013, and medium-sized males and females (t = 3.58, p < 0.01) in 2014. In 2012, fatty acid compositions did not differ among sex-and-size classes with the exception of females and medium-sized males (t = 2.00, p < 0.01). For comparisons of fatty acid signatures between years, there was a significant difference between 2012 and 2013 for large (t = 1.97, p < 0.01) and medium-sized (t = 2.18, p < 0.01) males. Fatty acid signatures were different between 2012 and 2014 for small males (t = 2.66, p_{MC} = 0.02) and females (t = 2.79, p < 0.01). Fatty acids of females were different between 2013 and 2014 (t = 2.76, p < 0.01), and for small males between 2011 and 2014 (t = 2.62, p < 0.01).

Across all years and size classes, 14 fatty acids comprised between 83.2 to 90.5 % of the total fatty acids (Figure 2.3). 16:1n-7 was the predominant fatty acids in small males in 2013 (19.7 %) and 2014 (18.1 %) and was lowest in medium-sized males in 2012 (10.6 %). 20:1n-9 and 22:1n-11 were predominant in large males in 2013 (13.8 and 13.5 %) and 2011 (12.9 and 13.1 %) and were lowest in females (8.5 and 6.9 %) and males (9.6 and 6.9 %) in 2014. 16:1n-7, 22:1n-11, and 20:1n-9 contributed the most to dissimilarities among sex-and-size classes, accounting for 61.1 to 76.4 % (2-way SIMPER; Appendix 2.2). 16:1n-7 accounted for the most differences between all size classes except large males and females, for which 22:1n-11

contributed to most of the differences (25.1 %). 16:1n-7 and 22:1n-11 accounted for the most inter-annual variation (36.6 to 54.5 %), with 20:1n-9, 18:1n-9, 20:1n-11, 22:6n-3, and 16:0 also contributing to differences between years.

Among environmental variables, the DISTLM analysis revealed that age (9.4 %) explained most of the total variation. Year (7.9 %), girth (7.7 %), length (5.7 %), and sex (4.6 %) followed as the next most important variables (Appendix 2.3). Blubber thickness was the only variable tested that was not significant (p = 0.62). The first two dbRDA axes accounted for 82.5 % of the variability of the fitted model and 19.8 % of the variation of the entire fatty acid dataset (Figure 2.4). The first axis explained 67.9 % of the variability of the fitted model and 16.3 % of the total variation in fatty acids. Age (r = -0.56), maximum girth (r = -0.44), length (r = -0.36), and males (r = 0.30) were negatively correlated to the first axis whereas year 2014 (r = 0.36) and females (r = 0.30) were positively correlated. On the second axis, years 2012 (r = -0.59) and 2011 (r = -0.24) had the strongest negative correlations whereas 2014 (r = 0.50), age (r = 0.44), and 2013 (r = 0.34) had the strongest positive correlations. Along the first axis, there was a sex-and-size class gradient, with large male belugas located at one extreme and small males and females at the opposite. The results of the sequential step-wise test using adjusted R^2 as a selection criterion procedure for each data set are shown in Table 2.2. The cumulative variation explained by the full model was 23.9 % with an adjusted R^2 of 0.21.

Inter-annual variation and biological factors affecting stable isotope ratios

Stable isotope signatures among beluga whales (n = 169) differed by year ($Pseudo\text{-}F_{3,154}$ = 3.30, $p <$ 0.01) and sex-and-size class ($Pseudo\text{-}F_{3,154}$ = 4.11, $p <$ 0.01). Unlike fatty acids, there was no significant interaction ($Pseudo\text{-}F_{8,154}$ = 1.45, p = 0.13). Post hoc pairwise tests

revealed that stable isotope signatures between large and small males (t = 2.31, p = 0.01), and medium and small males (t = 2.01, p = 0.02) were significantly different, as well as for large males and females (t = 2.37, p = 0.01), and medium-sized males and females (t = 2.36, p = 0.01), but not small males and females (t = 1.60, p = 0.09) or medium and large-sized males (t = 1.45, p = 0.13). Between years, pairwise tests showed that 2011 and 2013 (t = 2.72, $p < 0.01$), 2012 and 2013 (t = 2.02, p = 0.02) and 2013 and 2014 (t = 2.53, p = 0.01) had significantly different stable isotope ratios, but not 2011 and 2012 (t = 0.67, p = 0.64), 2011 and 2014 (t = 1.29, p = 0.19), and 2012 and 2014 (t = 0.74, p = 0.55).

Isotopic niche breadth estimates for the beluga population by year were 2011 = 0.54 ‰^{2}, 2012 = 0.81 ‰^{2}, 2013 = 0.60 ‰^{2} and 2014 = 1.01 ‰^{2} (Figure 2.5). The standard ellipse area was largest in 2014, displaying the most overlap with 2011 (84.7 %), 2012 (64.0 %) and 2013 (86.4 %). 2012 had the second largest standard ellipse area and overlapped with 2011 (51.9 %), 2013 (67.4 %), and 2014 (51.5 %). There was no overlap between ellipses in 2011 and 2013. The standard ellipse area was smaller in 2011 (Bayesian P = 0.90), 2012, (P = 0.87), and 2013 (P = 0.99) than 2014. The standard ellipse area was also smaller in 2013 than 2012 (P = 0.91). Isotopic niche breadth estimates by sex-and-size class were small males = 0.52 ‰^{2}, medium males = 0.75 ‰^{2}, large males = 1.25 ‰^{2}, and female = 0.75 ‰^{2}. The standard ellipse area of large males displayed the greatest overlap with medium-sized males (94.5 %; Figure 2.5). The ellipse of small males overlapped least with large males (25.2 %), and the ellipse of females also demonstrated the lowest overlap with large males (33.8 %). The standard ellipse area was smaller in small males (P = 0.92), medium-sized males (P = 1.00), and females (P = 0.88) than large males.

The most parsimonious model included age + year ($F_{4,154} = 9.99$, $r_{adj}^2 = 0.19$, $p < 0.01$) as predictors for log [$\delta^{13}C+40$] and length+ age ($F_{2,156} = 13.34$, $r_{adj}^2 = 0.14$, $p < 0.01$) for $\delta^{15}N$ in liver (Appendix 2.1). $\delta^{15}N$ values increased with log[length] (Table 2.3; $t = 5.13$, $p < 0.0001$), but had no relationship with age ($t = 0.11$, $p = 0.91$). $\delta^{13}C$ values decreased with age ($r_{adj}^2 = 0.08$, $p < 0.01$) and differed by year (Kruskal-Wallis test, $\chi^2 = 33.83$, df = 3, $p < 0.01$), with values highest in 2011 and lowest in 2013 among all years; there was no difference between 2012 and 2014 (Dunn's test; Figure 2.6).

Discussion

Body condition indices based on blubber thickness and maximum girth varied annually, and appeared to be highest during years with greater open water conditions and lower sea ice extent. The body condition index for girth was highest in 2012 relative to all years, whereas the index for blubber was highest in 2011 and 2012. During the study period, the largest loss of sea ice in the Western Arctic occurred in 2012, with record lows occurring in June and September (Perovich et al. 2012). Sea ice extent rebounded in 2013 though was lower in 2014 than 2013, but still higher than 2012 and 2011 (Perovich et al. 2011; Perovich et al. 2013; Perovich et al. 2014). Open water conditions may be favorable to beluga whales, similar to bowhead whales in the Beaufort Sea (George et al. 2015). Long-term temporal trends showed an increase in fall body condition in bowhead whales with the reduction of sea ice in the Beaufort Sea, along with a significant correlation between summer open water conditions and body condition (George et al. 2015). Although both condition indices followed similar trends in belugas, maximum girth may be better suited as a condition index because it was a significant predictor of fatty acids signatures. Girth also incorporates changes in thickness in the hypothermal layer, muscle mass,

and visceral fat (George et al. 2015). Similarly, maximum girth was recommended as a body condition index for belugas greater than 290 cm from the St. Lawrence River, since it was positively correlated with the scaled mass index, whereas blubber thickness was not (Larrat 2014). Because blubber has an important function in thermoregulation (Worthy and Edwards 1990; Dunkin et al. 2005), belugas and other Arctic cetaceans may avoid using energy reserves from blubber, instead utilizing energy reserves in muscle and other tissues (Koopman 2001; Koopman et al. 2002; Irvine et al. 2017)

Fatty acid signatures varied according to year, age, and sex-and-size class. Beluga whales demonstrated the greatest overlap in diet among sex-and-size classes in 2012. In all years except 2012, small males and females differed in their fatty acids from large males. Females and small males did not differ in their fatty acid signatures, nor did medium-sized males and large males (with the exception of 2013), supporting the observation they share similar habitats (Loseto et al. 2006). Overall, there were greater differences in fatty acid signatures among sex-and-size classes in 2013 and 2014 in comparison to years 2011 and 2012. Our findings of dietary overlap among sex-and-size classes in 2012 support the findings by Hornby et al. (2016), in which decreased sea ice extent in June 2012 allowed EBS belugas access to a wider range of habitats over the continental shelf, whereas beluga habitat use was restricted in 2013 due to heavier ice conditions (Hornby et al. 2016). Aerial surveys conducted in June 2012 and 2013 found EBS beluga whales to be most associated with light ice conditions, but contrasting spring conditions resulted in the selection of different levels of habitat variables between years (Hornby et al. 2016). Open water conditions and low sea ice extent have resulted in the range expansion of other beluga populations, such as belugas from West Greenland which expanded their habitat range during years with low sea ice coverage and early ice break-up (Heide-Jørgensen et al. 2010).

Although 16:1n-7 was the predominant fatty acid accounting for differences between years and individual belugas, 20:1n-9 and 22:1n-11 combined contributed to the greatest dissimilarities between years and sex-and-size classes. Fatty acids 20:1n-9 and 22:1n-11 are synthesized *de novo* by *Calanus* copepods, which are consumed by Arctic cod (Falk-Petersen et al. 2009), the main species of prey fish of EBS beluga whales (Loseto et al. 2009; Quakenbush et al. 2014). Therefore, dissimilarities in 20:1n-9 and 22:1n-11 may be related to differences in Arctic cod consumption among whales or *Calanus* consumption by cod. Acoustic surveys conducted in the Canadian Beaufort Sea and Mackenzie Shelf from 2010 to 2014 revealed that the integrated biomass and abundance of Arctic cod decreased with delays in ice break up dates and decreases in spring-summer sea surface temperature (Geoffroy 2016). Mean standard length of Arctic cod as well as the proportions of age-2 cod in the mesopelagic layer (100 to 500 m) were significantly lower in 2014 than in 2013 and 2012. Although the highest biomass of Arctic cod in the mesopelagic layer was measured in 2012 (Geoffroy 2016), the highest relative abundance of Arctic cod was observed in 2013 across all stations and depths (Majewski et al. 2016). Additionally, Geoffroy (2016) reported the biomass of Arctic cod in 2014 to be unable to sustain the energetic requirements of ringed seals (*Pusa hispida*) and beluga whales, a year that coincides with our findings of low body condition. The proportions of 20:1n-9 and 22:1n-11 were lowest in small males and females in 2014, which may be related to lower consumption of Arctic cod. Large males had the highest proportions of 22:1n-11 and 20:1n-9 and likely had greater access to Arctic cod at greater depths (Richard et al. 2001). Although a diatom marker (Dalsgaard et al. 2003), 16:1n-7 can be synthesized in marine mammals as a result of Δ9 desaturase enzyme activity (Iverson 2009); however, without access to the fatty acid signatures of prey, we can only speculate on the function of 16:1n-7 in beluga whales.

Stable isotope ratios supported fatty acid signatures, reflecting differences in habitat selection between certain sex-and-size classes. Age and year most influenced $\delta^{13}C$ values; whereas $\delta^{15}N$ was most influenced by length, similar to previous findings by Loseto et al. (2008a). Stable isotope ratios of beluga whales may be influenced by the variation in the values of their main prey, Arctic cod. Arctic cod in the Canadian Beaufort Sea demonstrate a $\delta^{15}N$ and $\delta^{13}C$ gradient with depth (Stasko et al. 2016). Cod collected from the lower shelf (750-1000 m, mean $\delta^{15}N$, $\delta^{13}C$: 14.57, -23.41 ‰) and upper slope (350-500 m, 14.07, -23.59 ‰) have higher $\delta^{15}N$ and $\delta^{13}C$ values and are larger in body size than those from the nearshore shelf (18-50 m; 12.57. -24.04 ‰; Stasko et al. 2016). Therefore, larger beluga whales may dive to greater depths to feed on larger Arctic cod from the lower shelf than smaller whales, resulting in higher $\delta^{15}N$ values. Though $\delta^{15}N$ may be indicative of the trophic position of beluga prey, the $\delta^{13}C$ may also reflect differences in feeding sites. $\delta^{13}C$ values were higher in adult versus subadult bowhead whales, and were hypothesized to be the result of older whales feeding on ^{13}C-enriched prey from the Bering-Chukchi Sea and younger whales feeding on ^{13}C-depleted prey from the western Beaufort (Schell et al. 1989). Arctic cod from the Canadian Beaufort Sea have lower $\delta^{13}C$ values than those from the Bering-Chukchi Sea (-20.3 ‰; Hoekstra et al. 2002). As our whales have recently migrated from the Bering Chukchi Sea, perhaps the $\delta^{13}C$-age relationship is indicative of older whales arriving earlier to the Canadian Beaufort Sea relative to younger whales, or differential feeding areas. Comparisons of isotopic niche breadth also supported differences in habitat use, with large males having the widest isotopic niche breadth, likely due to their larger summer habitat range and ability to exploit a higher diversity in habitats (Richard et al. 2001). Likewise, lack of an isotopic niche overlap between 2011 and 2013 suggest beluga were feeding from different areas. $\delta^{13}C$ values decreased with year from 2011 until 2013, but were not

different between years 2012 and 2014. Belugas had greater access to multiple habitats in 2012, which may be reflected by wider range in $\delta^{13}C$ from their prey. As belugas from 2012 and 2014 had the greatest contrast in fatty acid signatures, the wider range in $\delta^{13}C$ for those years may reflect different influences. Since Arctic cod were at their lowest abundance in 2014, belugas may have been more opportunistic in their prey, resulting in a greater range in stable isotope values and niche breadth.

Implications for future monitoring of changing environmental conditions

Recent declines in individual body size of EBS beluga whales have been identified over a twenty year timescale and are hypothesized to be due to changes in the prey base (Harwood et al. 2014). Our results show that there is high inter-annual variability in diet and body condition over short time periods. To understand whether declines in body size are ongoing and reflect an ecosystem change, we must first understand the factors that affect short term variability. Annual variation in sea ice extent and biomass fluctuations of Arctic cod may be linked to inter-annual changes in diet and body condition of beluga whales. The year 2012 experienced the largest loss of Arctic sea ice, resulting in beluga whales having greater access to multiple habitats and displaying greater overlap in diet among sex-and-size classes than other years. Differences in proportions of *Calanus* markers 20:1n-9 and 22:1n-11 contributed the most to variability in fatty acid signatures within the EBS beluga population. High levels of *Calanus* 20 and 22 monounsaturates levels have been linked to improved body condition in pinnipeds (Kirsch et al. 2000; Falk-Petersen et al. 2004; Falk-Petersen et al. 2009a). Therefore, 20:1n-9 and 22:1n-11 may be effective indicators for prey abundance, body condition, and overall ecosystem changes. Lower proportions of *Calanus* markers in small males and females in 2014 suggest less

consumption of Arctic cod, and therefore, small or young males and females may be most sensitive to environmental changes as documented in other species such as polar bears (*Ursus maritimus*) (Molnár et al. 2010; Rode et al. 2010; Stirling and Derocher 2012). Our results support the conclusions of Laidre et al. (2008), which identify beluga whales as a moderately sensitive marine mammal with high flexibility to changes in sea ice and diet. However, as a long lived species with a low reproductive rate, climate induced effects on beluga fitness may take a long period of time to become detectable within the population (Gilg et al. 2012).

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Table 2.1. Multiple linear regression models for body conditon indices based on maximum girth (*n*=159) and blubber thickness (*n*=158) for eastern Beaufort Sea beluga whales. Results are presented for the most parsimonious model based on AIC_c.

Dependent	Predictor	Value	t	p
Log [Maximum Girth] (cm)	Intercept	0.36	0.50	0.62
	Sex	-0.11	-1.31	0.19
	Age	0.00	0.28	0.78
	Log[Length]	0.72	5.97	<0.01
	Sex × Age	6.00×10^{-3}	2.80	0.01
Blubber Thickness (cm)	Intercept	-3.37	-1.35	0.18
	Age	-0.04	-1.92	0.06
	Length	-0.03	4.99	<0.01

Table 2.2. The percentage of variation and cumulative variation of fatty acid signatures attributed to explanatory variables in a distance-based linear model sequential step-wise tests using adjusted R^2 selection criterion for fatty acids in eastern Beaufort Sea beluga whales. Res.df= residual degrees of freedom; regr.df= regression degrees of freedom.

Variable	Adj R	SS (trace)	Pseudo-F	p	Variation (%)	Cumulative Variation (%)	res.df	regr.df
Age	0.09	13.43	17.88	<0.01	9.37	9..37	173	2
Year	0.16	11.71	5.61	<0.01	8.17	17.54	170	5
Sex	0.20	6.11	9.22	<0.01	4.26	21..80	169	6
Girth	0.21	2.19	3.35	0.02	1.53	23.33	168	7
Length	0.21	0.88	1.35	0.23	0.62	23.95	167	8

Table 2.3. Multiple linear regression models for factors influencing $\delta^{13}C$ and $\delta^{15}N$ values in liver tissues of eastern Beaufort Sea beluga whales (*n*=169). Results are presented for the most parsimonious model based on AIC_c.

Dependent variable	Predictor	Value	t	p
$\delta^{15}N$	Intercept	15.04	24.9	<0.01
	Length	7.60×10^{-3}	5.13	<0.01
	Age	5.25×10^{-4}	0.11	0.91
Log($\delta^{13}C$+ 40)	Intercept	3.05	422.79	0
	Age	-6.00×10^{-4}	-2.93	<0.01
	Year2012	-1.30×10^{-2}	-1.94	0.05
	Year2013	-2.70×10^{-2}	-4.15	0
	Year2014	-7.0×10^{-3}	-1.08	0.28

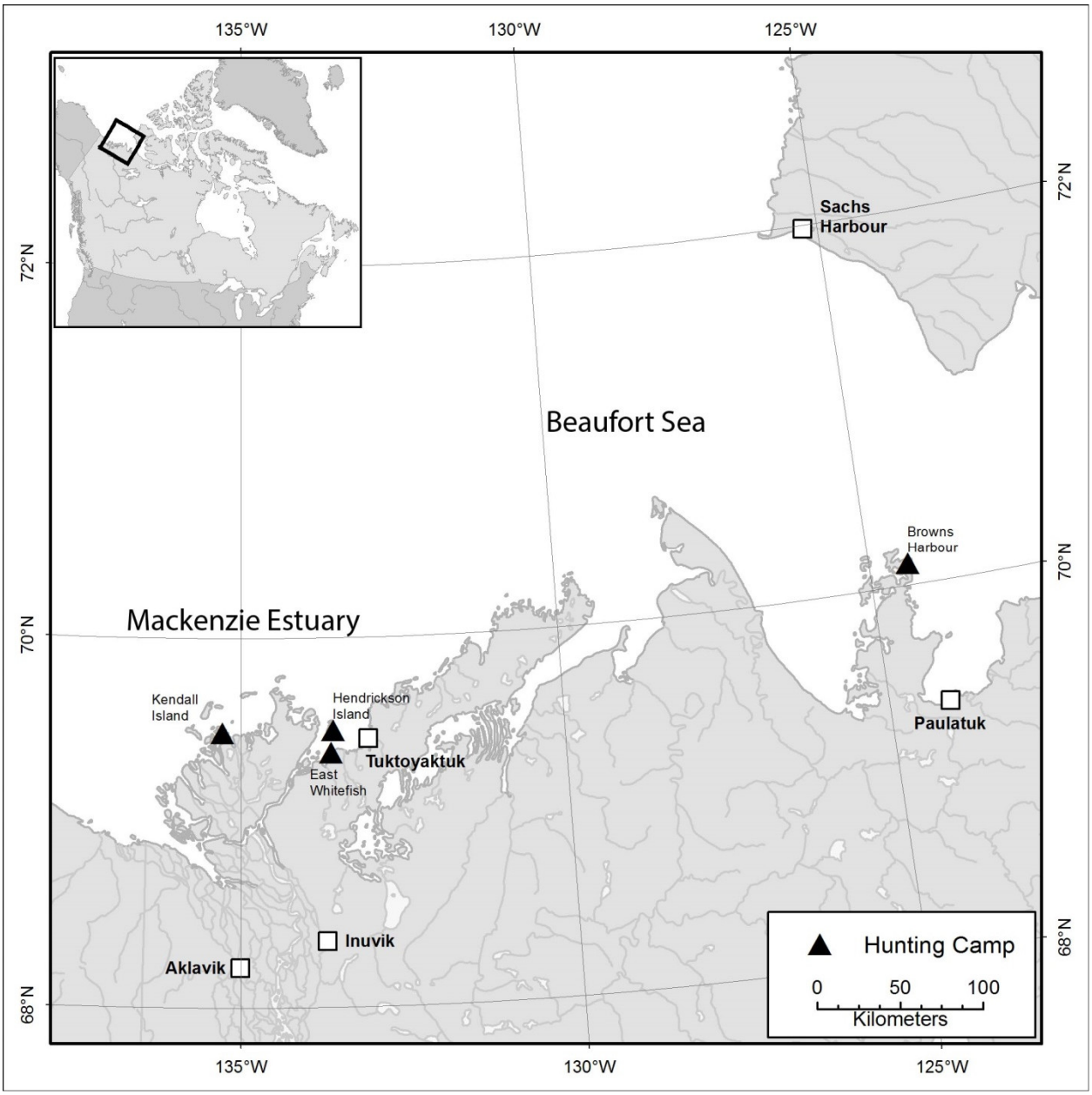

Figure 2.1. Sample collection sites for beluga whale tissues at traditional Inuvialuit hunting camps (triangles) located in the Inuvialuit Settlement Region, Northwest Territories, Canada.

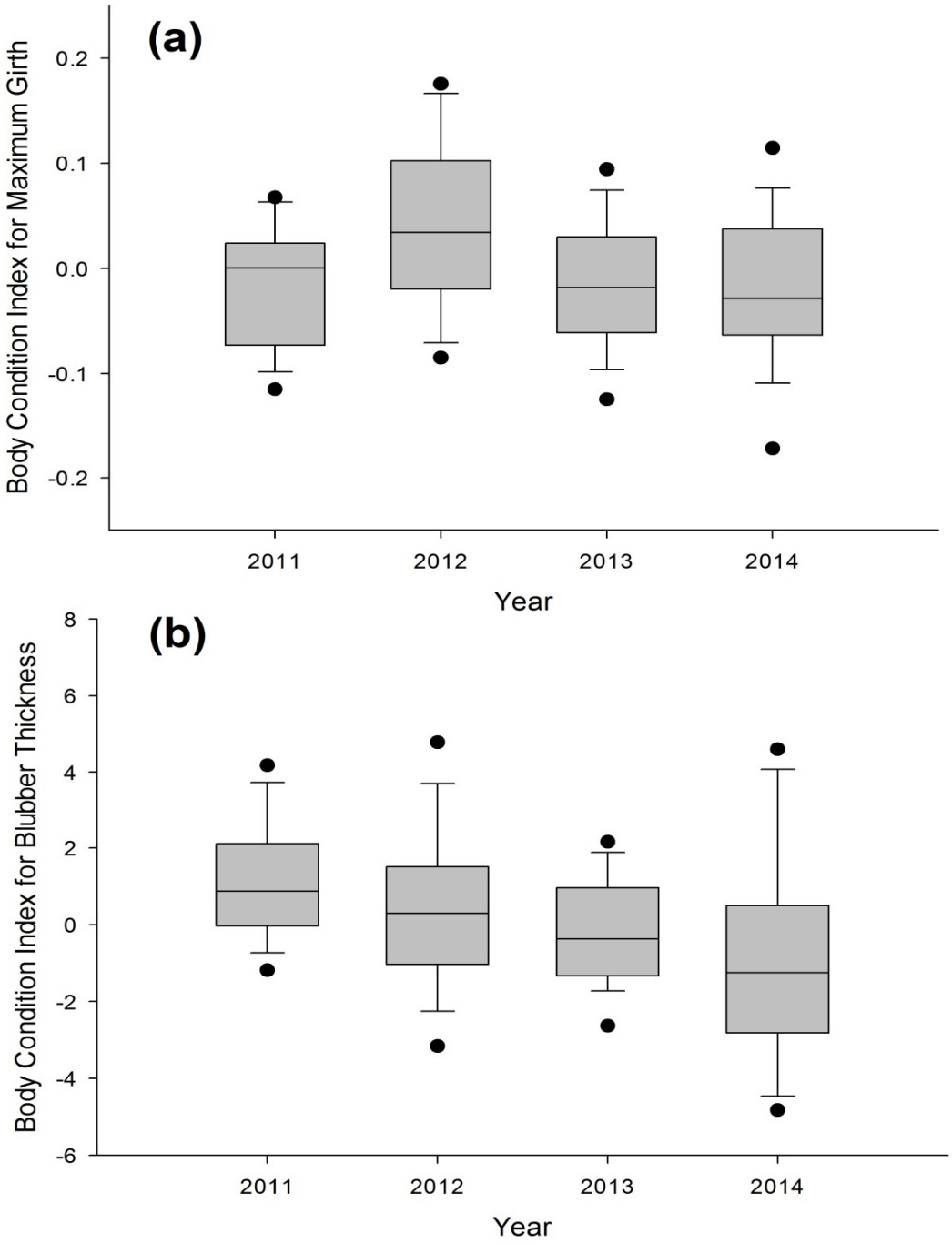

Figure 2.2. Box plots displaying medians and quartiles of body condition indices based on (a) maximum girth and (b) blubber thickness for eastern Beaufort Sea beluga whales (*n*=159). Black dots represent the 5 and 95% percentiles**.**

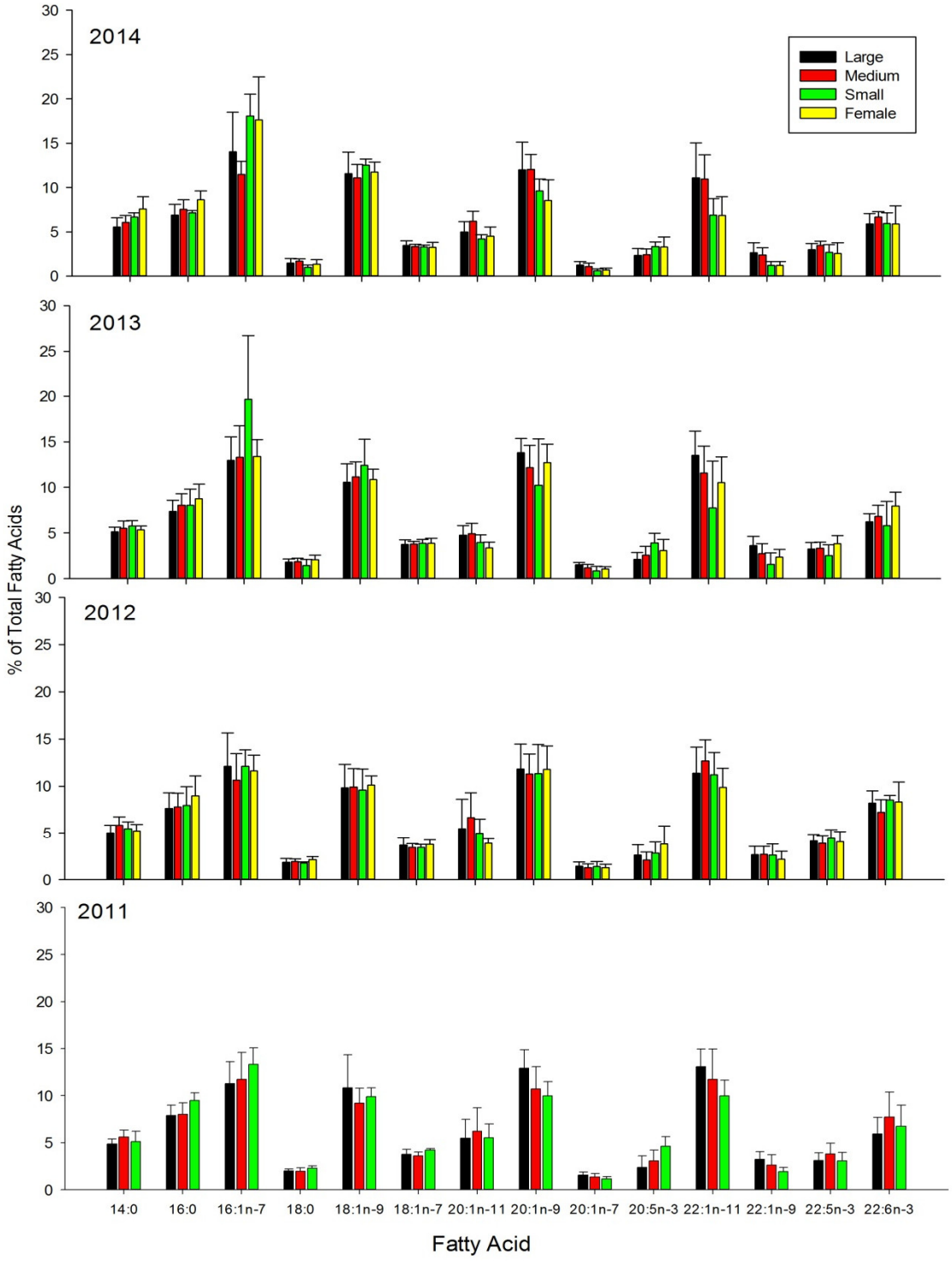

Figure 2.3. Annual mean percentages (%) ± 1 standard deviation of fatty acids in eastern Beaufort Sea beluga whales (*n*=176) according to habitat class. Only dietary fatty acids that contribute to more than 1% of the total percent fatty acids are shown. Small, medium, and large size-classes refer to males only.

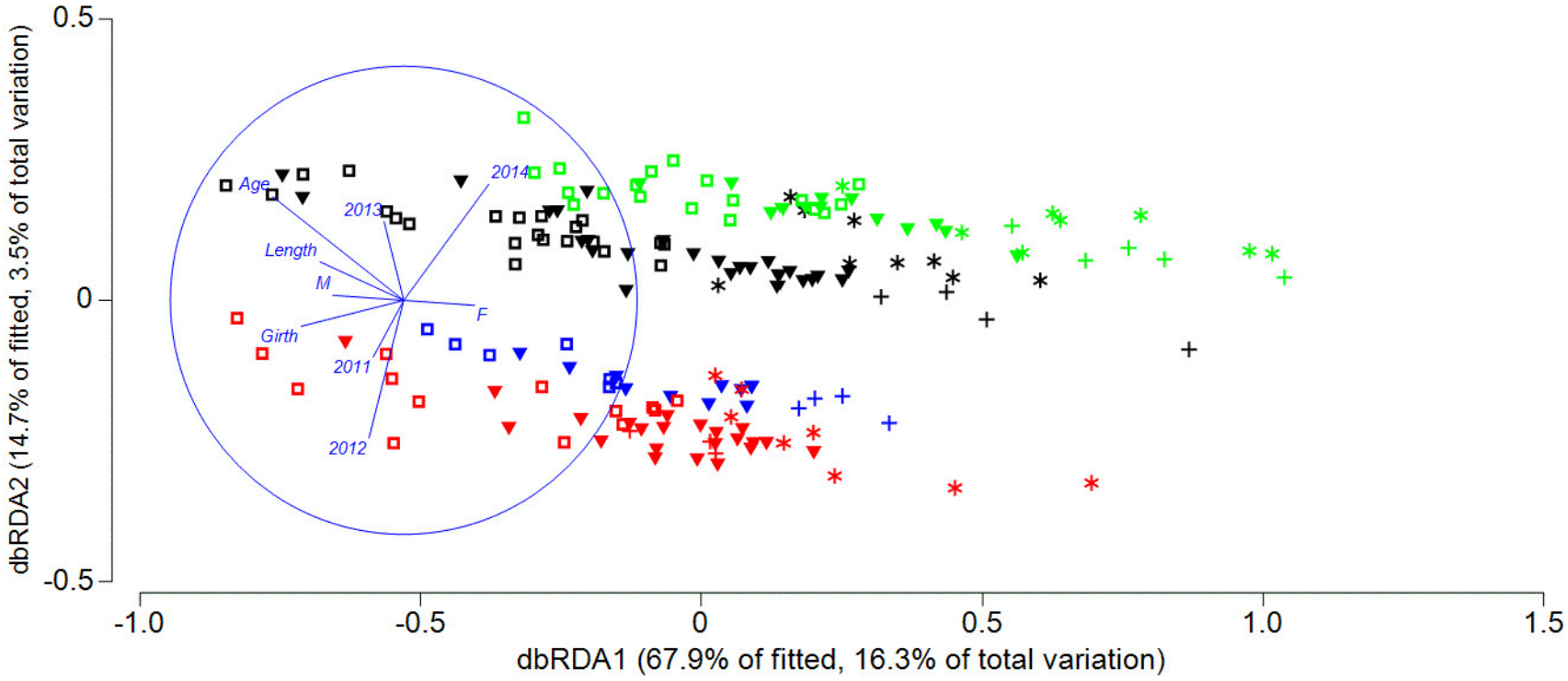

Figure 2.4. Biplot of distance-based redundancy analysis (dbRDA) relating the variation of fatty acid signatures in eastern Beaufort Sea beluga whales explained by predictor variables based on the full distance-based linear model. Vectors overlays demonstrate the strength of the relationship of significant predictor variables (multiple partial correlations r>0.2) with the dbRDA axes. Large males (M) are represented by open squares, medium males by closed triangles, small males by crosses, and females (F) by asterisks. The year 2011 is indicated by blue, 2012 by red, 2013 by black, and 2014 by green.

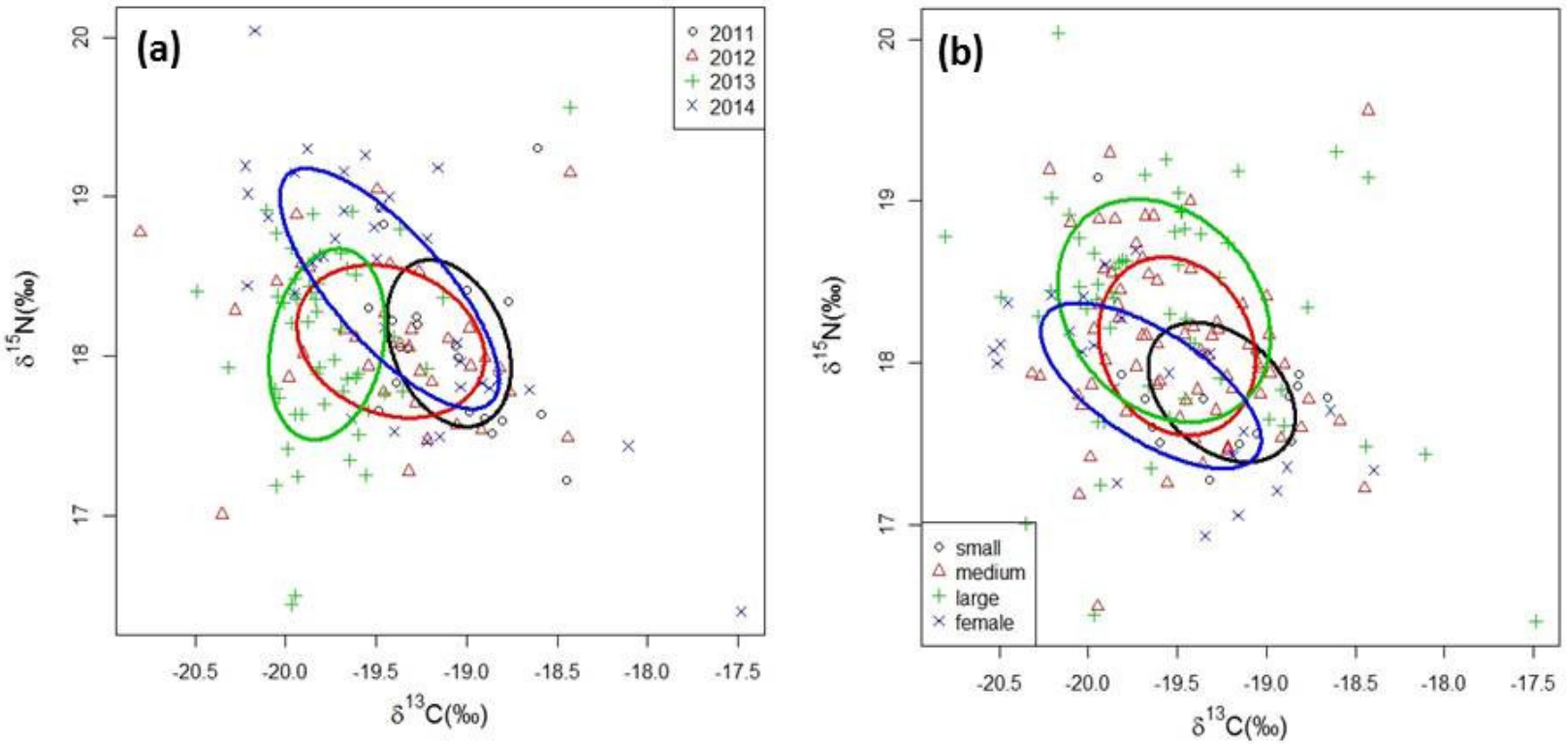

Figure 2.5. Isotopic niche breadth based on the standard ellipse area for liver samples from eastern Beaufort Sea beluga whales across different years (males only) (a) and sex-and-size classes (b). Small, medium, and large size-classes refer to males only.

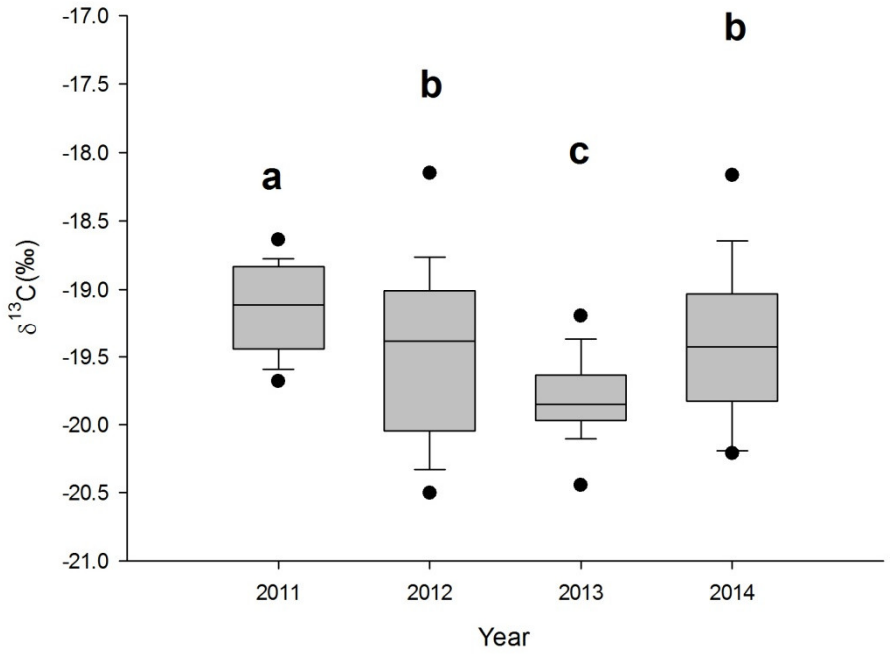

Figure 2.6. Boxplot of $\delta^{13}C$ ratios of beluga liver tissue by year (n = 169). Boxes with the same letters are not statistically different (α=0.05) according to a Dunn's test. Black dots represent the 5 and 95% percentiles**.**

Chapter 3:

A Bayesian mixing model approach to reconstruct the diets of beluga whales using fatty acid signatures

Chapter Three: Manuscript Summary

Paper in review in Ecosphere

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Contributions:

Dr. Lisa Loseto provided funding for analysis and comments throughout the writing process.

Dr. Jim Roth provided comments throughout the writing process.

Brian Sheehan and Dr. Martin Haulena collected samples from the Vancouver Aquarium and provided insights during the writing process.

Bruno Rosenberg provided technical support and expertise for fatty acid and stable isotope analysis using gas chromatography as well as quality assurance and control.

Emily Choy designed the research, completed chromatography on all samples, analysed the data, produced all figures and tables, and wrote the manuscript.

Abstract

Arctic marine environments are undergoing significant ecosystem changes, such as the northward migration and expansion of subarctic species. Beluga whales (*Delphinapterus leucas*) are a potential indicator species for climate change. Whether belugas are resilient to prey changes remains uncertain, partly due to a lack of dietary information. Bayesian mixing models are promising tools to evaluate the diets of marine mammals using fatty acid signatures. Additionally, aquarium-based studies can be used to verify the effectiveness of these tools for application to wild populations. We examined the ability of fatty acid signatures to reconstruct the diets of captive belugas fed mostly Pacific herring (*Clupea harengus pallasi*), and in smaller proportions capelin (*Mallotus villosus*) and squid (*Doryteuthis opalescens*) using a combination of qualitative analysis and a Bayesian mixing model. Qualitative analysis signatures using non-metric multidimensional scaling revealed fatty acid signatures of belugas to be more similar to herring and Chinook salmon (*Oncorhynchus tshawytscha*) than capelin and squid. The Bayesian mixing model "Fatty Acid Source Tracking Algorithm in R" identified herring as the dominant prey. Dietary estimates for herring were most similar to the actual proportions when the mixing model used fatty acids that were the most influential at differentiating diet. When we compared the fatty acids of captive beluga whales to individuals from a wild Arctic beluga population, differences in signatures were primarily influenced by 18:1n-9, 16:0, and 20:1n-9, likely reflecting differences in diet. This is the first study to apply a Bayesian mixing model to reconstruct the diets of two mammals using fatty acid signatures. We recommend caution when using these models with prey that have high within-species variability, as well as prey that do not differ in fatty acid signatures. The application of Bayesian mixing models is promising to monitor the effects of climate-induced prey shifts on free-ranging Arctic beluga whales.

Key words: Beluga whales, fatty acid signatures, dietary tracers, Bayesian statistics, mixing model, squid, capelin, Pacific herring, Chinook salmon

Introduction

Arctic marine environments are undergoing significant ecosystem changes, such as the northward migration and expansion of subarctic species (Michel et al. 2012). Due to their unique life history traits and specialized adaptations, Arctic vertebrates may be unable to readily adapt to rapid prey shifts (Gilg et al. 2012). Beluga whales (*Delphinapterus leucas*) are one of three cetacean species endemic to the Arctic (Kovacs et al. 2011), although they are also found in the subarctic, such as the Gulf of St. Lawrence and Cook Inlet (Laidre 2008). With 29 management stocks with a circumpolar distribution, they are the most abundant Arctic odontocete and a potential indicator species for climate change (Tynan and DeMaster 1997; Laidre 2008; Moore and Huntington 2008; Laidre et al. 2015). The effects of climate change on beluga whales and other ice-associated marine mammals are uncertain due to lack of information on diet, health, and body condition indices (Moore and Huntington 2008). Arctic beluga whales are opportunistic predators, and feed on several different species of marine invertebrates and fish. Stomach contents from 62 individuals from the eastern Beaufort Sea (EBS) beluga stock harvested in Alaska during their spring migration revealed their consumption of 16 species of invertebrates and 8 species of fish (Quakenbush et al. 2014). However, stomach contents are highly biased towards hard indigestible parts and reveal only a snapshot of a predator's diet (Bowen and Iverson 2012). Since climate-induced ecosystem shifts have led to the range expansions of subarctic species to northern latitudes (Michel et al. 2012), a more thorough understanding of the diets of beluga whales and other Arctic marine mammals is critical to monitor the long term effects of climate change on Arctic marine ecosystems.

Fatty acid signatures can be used as dietary tracers to identify linkages between marine mammals and prey (Iverson and Frost 1997b; Thiemann et al. 2008b; Budge et al. 2008; Loseto et al. 2009). Fatty acid signatures, the main components of lipids, consist of a chain of carbon molecules with a terminal carboxyl group and methyl group (Budge et al. 2006). In the marine environment, long chain and essential fatty acids are transferred from prey to marine mammals, and are stored in the metabolically active inner layer of blubber (Iverson et al. 2004; Koopman 2007). However, as a result of metabolism of certain fatty acids, such as shortening of long chain fatty acids to shorter chain (e.g. 22:1n-11 to 18:1; Cooper et al. 2006), fatty acids of predators are never a direct match to their prey (Iverson et al. 2004). Studies of captive pinnipeds fed a monotypic diet have allowed for the calculation of calibration coefficients to correct for the effects of metabolism on fatty acids (Iverson et al. 2004; Nordstrom et al. 2008). Unfortunately, feeding trials are difficult to conduct on marine mammals, and there have been no captive studies on the effects of metabolism or the ability of fatty acids to reconstruct the diets of cetaceans. Since cetaceans and pinnipeds have different digestive systems, such as complex versus simple stomachs due to very different evolutionary histories (Berta et al. 2015), the calibration coefficients for seal blubber may not be representative of fatty acid fractionation in beluga whales.

Through the use of calibration coefficients to correct for metabolism, quantitative fatty acid signature analysis has been used to predict the diets of marine mammals, including seals (Budge et al. 2004; Nordstrom et al. 2008) and polar bears (*Ursus maritimus*) (Thiemann et al. 2008a). Recently, Bayesian mixing models have also been used to estimate the proportional contributions of prey toward a predator's diet using fatty acid signatures. Fatty Acid Source Tracking Algorithm in R (FASTAR), a Bayesian mixing model, has been successfully used to

estimate prey contributions to the diets of isopods, zooplankton, and *Daphnia* spp. (Galloway et al. 2014a; Galloway et al. 2014b; Galloway and Winder 2015). One advantage of using fatty acid signatures in Bayesian mixing models is that over 70 tracers can be utilized; therefore, the issue of an undetermined model can be avoided for consumers that feed on several prey species (Brett 2014; Galloway et al. 2015; Brett et al. 2016). Bayesian mixing models also account for uncertainty associated with multiple prey sources and fractionation by calculating a probability distribution of prey contributions (Moore and Semmens 2008). Therefore, Bayesian mixing models may be a solution for determining the diets of marine mammals in circumstances in which calibration coefficients are not available, by using approximately 40 fatty acids transferred through diet that have been identified as undergoing little modification through metabolism (Iverson et al. 2004).

In this study, we used fatty acid signatures to reconstruct the known diets of two captive beluga whales at the Vancouver Aquarium. An adult and juvenile female beluga whale were both fed a consistent daily diet of Pacific herring (*Clupea harengus pallasi*), capelin (*Mallotus villosus*), and opalescent inshore squid (*Doryteuthis opalescens*). Capelin, herring, and squid have all been identified as important prey of wild Arctic beluga whale populations (Kelley et al. 2010; Marcoux et al. 2012; Quakenbush et al. 2014). As beluga whales are generalists that feed on fish and invertebrates, our captive diet represents the mixed-species diets of free-ranging populations. Our overall goal was to develop an approach to estimate the diets of captive beluga whales with a known diet using fatty acid signatures, which could be applied to wild populations and potentially other marine mammals. Our specific objectives were to (1) determine if prey species can be distinguished based on their fatty acid signatures; (2) determine if fatty acid signatures of beluga whales are influenced by their prey; (3) test if a Bayesian mixing model can

accurately predict the dietary composition of captive beluga whales using fatty acid signatures, and finally (4) compare the fatty acid signatures of captive whales to individuals from a wild Arctic population, to provide insights into the fatty acids influenced by diet for future monitoring purposes.

Methods

Diet schedule

Two unrelated female beluga whales (an adult and a juvenile) housed at the Vancouver Aquarium were fed a consistent diet of capelin, opalescent inshore squid and Pacific herring from July 4 to August 5, 2012 (Table 3.1; adult) and from July 27th to September 14th 2011 (juvenile). These diets were part of their regular feeding routine, and were representative of the long term diets of the belugas. Daily dietary intake (mass and calories) was recorded by the Vancouver Aquarium throughout their lives. Additionally, the juvenile was nursing, and her mother had also consumed a consistent diet of capelin, squid, and herring. The juvenile's mother had also consumed a daily intake of 1.7 kg of Chinook salmon (*Oncorhynchus tshawytscha*) for approximately 18 weeks until August 6, 2011. Unexpectedly, the juvenile beluga whale passed away on September 15th 2011 at 3 years old due to congestive heart failure and the adult passed away on Monday August 6th 2012 at 46 years old due to adenocarcinoma. Due to their illnesses, both whales reduced their appetite immediately prior to death; however, the juvenile was observed nursing two days before her passing.

Sample Collection

Immediately after death, full depth blubber samples were collected and frozen at -20˚C and shipped to the Freshwater Institute in Winnipeg, Canada. Whole body subsamples of capelin

[*n*=4; from Atlantic Ocean Food and Agriculture Organization of the United Nations (FAO) Area 21, Northwest Atlantic Fisheries Organization (NAFO) subdivisions 4R, 3K, 3L, provided by Beothic Fish processors ltd], opalescent inshore squid (*n*=2; FAO Area 77, Tomich Bros Fish Co Inc), Pacific herring (*n*=3; Pacific Ocean FAO Zone 67, Scanner Enterprises) and Chinook salmon (*Oncorhynchus tshawytscha*; *n*=2; provided by Walcan SSV ltd), the same source used by the Vancouver Aquarium, were also frozen and shipped.

Additionally, blubber subsamples from the eastern Beaufort Sea beluga population were acquired to compare their fatty acid signatures with the captive beluga whales. Blubber was collected from 7 female adult beluga whales harvested at Inuvialuit hunting camps in July to early August 2011-2012 at Hendrickson Island, Brown's Harbour, and Kendall Island, in the Inuvialuit Settlement Region, Northwest Territories, Canada (Chapter 2). Each blubber sample was removed through its entire depth from the mid thoracic region of the animal. Samples were frozen at -20˚C in portable freezers and shipped to Fisheries and Oceans Canada in Winnipeg for laboratory analysis.

Fatty acid extraction

Detailed methodology of fatty acid extraction and run procedures are available in Giraldo et al. (2016). Following length and mass measurements, prey samples were whole-body homogenized prior to fatty acid analysis (Appendix 3.1). Lipids were extracted from 0.5 g of tissue using 2:1 chloroform: methanol containing 0.01% butylated hydroxytoluene (BHT) using a method modified from Folch et al. (1957) as described in Budge et al. (2006). Percent lipid was determined gravimetrically and recorded in wet weight. The extracted lipid was used to prepare the fatty acid methyl esters by transesterification with Hilditch reagent (0.5 N H_2SO_4 in dry methanol). 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). Fatty acid standards were obtained from Supelco (37 component FAME mix) and Nuchek (54 component mix GLC-463) and were used to verify the retention times of fatty acid peaks.

Data Analyses

As fat content can affect the digestibility and transfer of fatty acid from prey to marine predators (Iverson et al. 2004; Trumble and Castellini 2005), we first compared the % lipid among our prey species using a one-way analysis of variance (ANOVA). Fatty acid signatures were visualized using non-metric multidimensional scaling (NMDS) based on Euclidean distances using the package Vegan (Oksanen et al. 2016). We used 34 fatty acids identified as dietary by Iverson et al. 2004 and with mean percentages above 0.1 %. NMDS uses rank order based on a distance matrix and maps compositional dissimilarities in a two dimensional configuration (Zuur et al. 2007; Oksanen 2015). To determine if we could distinguish between prey species based on fatty acids, we used a 1-way permutational multivariate analysis of variance (PERMANOVA) followed by post-hoc pairwise tests. PERMANOVA tests used Euclidean distance, fixed factors, and Type III sums of squares, with significance determined using 9999 unrestricted permutations of the raw data. We used PERMDISP to confirm that the fatty acid data met the assumption of homogeneity of multivariate dispersion for our PEMANOVA. As the number of unique permutations was <200, we used Monte Carlo (MC) generated p-values for post hoc comparisons (Anderson et al. 2008). Multivariate significance tests were performed using PRIMER v.7.0 and PERMANOVA+ whereas univariate tests were conducted using R 3.2.5 (R Core Team 2016). Based on species groups defined by PERMANOVA, a one-way similarity percentage routine (SIMPER) was used to identify which

fatty acids were contributing most to dissimilarities among prey. SIMPER first tabulates fatty acid contributions to the average similarity of individuals within each groups followed by the average dissimilarity (Clarke et al. 2014; Clarke and Gorley 2015). We designated a cut-off of fatty acids that characterized up to 80% of dissimilarities. We re-ran the same statistical procedures to compare the fatty acid signatures between the captive and wild beluga whales.

Fatty acid mixing model

Using the 34 dietary fatty acids and those identified by SIMPER as most influential, we used the Bayesian mixing model FASTAR to calculate the proportional contribution of each prey species to beluga diet (Galloway et al. 2015). FASTAR used Dirichlet priors (α=1). Posterior distributions were estimated using Markov Chain Monte Carlo chains using 100,000 iterations with a 50,000 iteration burn-in and a thinning rate of 50. Using the R script provided by Galloway et al. (2015), we used the fatty acid percentages of the adult and juvenile belugas as "consumers". Instead of a resource library based on feeding trials of the predator fed 100% of a specific prey, we inputted the average percentage and standard deviation of fatty acids of each of the prey as "sources" within the model. In addition to fatty acids, we ran the model using 7 fatty acid trophic markers (FATMs) of marine ecosystems: 16:1n-7/16:0, 18:1n-9/18:1n-7, 16PUFA, 18PUFA, 20:5n-3/22:6n-3, $\sum$20:1 (20:1n-11 and 20:1n-9) and $\sum$22:1 (22:1n-11 and 22:1n-9) (Dalsgaard et al. 2003; Falk-Petersen et al. 2004). Primary producers are characterized by specific patterns of FATMs that are transferred conservatively. FATMs can discern dietary sources in marine mammals (Iverson et al. 1997b; Dalsgaard et al. 2003). FASTAR was run in R version 3.2.5 (R Core Team 2016).

Results

Fatty acid signatures in predator and prey

Thirteen fatty acids comprised 88.6 to 89.4% of the total fatty acids of both captive beluga and prey species (Figure 3.1). 18:1n-9 (mean 28.2 % of the total fatty acids) was the main fatty acid in both captive belugas, followed by 16:0 (15.7 %) and 16:1n-7 (9.4 %). Similarly, 18:1n-9 (22.2 and 28.8 %) followed by 16:0 (21.0 and 17.3 %) were the dominant fatty acids in herring and salmon. 22:6n-3 had the highest proportions in capelin (17.1 %) and squid (31.1 %), followed by 16:0 (12.7 %) in capelin and 20:5n-3 (17.7 %) in squid. Lipid content differed among prey (1-way ANOVA, $F_{3,7}$= 34.73, p = 0.0001). Herring had higher lipid content (Appendix 3.1; mean = 9.7%) than capelin (mean = 2.0%; Tukey post-hoc test, p = 0.001) and squid (2.6%; p = 0.005). Salmon (mean = 13%) had a higher lipid content than capelin (p = 0.0002) and squid (p = 0.0008). Lipid content did not differ between squid and capelin (p = 0.97) or herring and salmon (p = 0.15).

The adult and juvenile beluga whales had similar fatty acid patterns based on the NMDS ordination (Figure 3.2). The fatty acid composition of beluga whales was closer in two-dimensional space to Pacific herring and salmon than capelin and squid, suggesting greater similarities between whales to herring and salmon. Fatty acid signatures among prey groups met the assumption of homogeneity of dispersion (PERMDISP, $F_{3,7}$ = 6.05, p = 0.21). Fatty acid signatures differed significantly among prey (PERMANOVA, *Pseudo*-$F_{3,7}$ = 10.97, p = 0.0003). Post-hoc pairwise tests demonstrated significant differences in fatty acid signatures between capelin and herring (t=2.80, p_{MC} = 0.010), and salmon (t = 3.19, p_{MC} = 0.008), but not capelin and squid (t = 2.23, p_{MC} = 0.09). Herring and salmon (t = 3.02, p_{MC} = 0.020), herring and squid

(t = 6.32, p_{MC} = 0.004), and salmon and squid (t = 16.4, p_{MC} = 0.003) also had different fatty acid signatures.

The SIMPER analysis revealed that nine fatty acids contributed to 80 % of the dissimilarities among prey and beluga whales: 14:0, 16:0, 16:1n-7, 18:1n-9, 18:2n-6, 20:1n-9, 20:5n-3, 22:1n-11, and 22:6n-3 (Table 3.2). 18:1n-9 contributed to most of the dissimilarities in fatty acid signatures between salmon and capelin (44.9 %), as well as salmon and squid (40.6 %). 18:1n-9 also contributed to 39.4 % of the differences between herring and capelin. 18:2n-6 contributed to 31.3 % of the differences between herring and salmon, whereas 22:6n-3 contributed to most of the differences between squid and capelin (36.8 %), as well as salmon and herring (49.5 %). 18:1n-9, 18:2n-6, and 20:5n-3 contributed to most of the differences in fatty acid signatures between beluga whales and their prey.

Actual proportions vs. mixing model estimates

Throughout their daily feeding regimes, herring was the predominant prey for both juvenile [median proportion= 0.51 of total diet (kg)] and adult (0.74) whales (Table 3.3), with the highest weekly percent contributions to dietary intake (Table 3.1). Capelin was the second most consumed prey for the juvenile (0.42) and adult (0.15), although the adult had consumed more squid (21 %) during the first two weeks of the experimental period. The mother of the juvenile had consumed a consistent long-term diet composed of herring (69 %), capelin (18 %), and squid (13 %). For all three fatty acid datasets, FASTAR predicted herring as a dominant prey item for both captive beluga whales. When we used 34 fatty acid tracers in the model, herring was predicted as the dominant prey for the adult beluga whale (median 0.52 of the diet; Table 3.3; Appendix 3.2) followed by capelin (0.34) and squid (0.13). When fatty acid signatures were reduced to the nine identified by SIMPER, the diet breakdown changed to 0.67 herring, 0.34

capelin, and 0.008 squid. Using the 7 FATM, the dietary breakdown was 0.74 herring, 0.23 capelin, and 0.02 squid. For the juvenile whale, FASTAR predicted the dietary breakdown using 34 fatty acid signatures to be 0.46 capelin, 0.36 herring, 0.08 squid, and 0.11 salmon (Table 3.3; Appendix 3.3). Using the nine fatty acid data set, the breakdown changed to 0.38 capelin, 0.40 herring, 0.004 squid, and 0.10 salmon. FASTAR predictions using the seven FATMs also supported herring as the dominant prey, with a breakdown of 0.31 capelin, 0.62 herring, 0.04 squid, and 0.02 salmon.

Comparisons of captive belugas with wild populations

Fatty acid signatures between captive and wild beluga whales met the assumption of homogeneity of multivariate dispersion ($F_{1,9}$ = 3.88, p = 0.24). Wild and captive beluga whales had significantly different fatty acid profiles (PERMANOVA, *Pseudo-*$F_{1,9}$ = 31.6, p_{MC} = 0.0001). The mean percentage of 18:1n-9 and 16:0 in the blubber of captive whales (28.2 and 15.7 %, respectively) was higher than in wild beluga whales (9.8 and 8.8 %, respectively). Other fatty acids including 16:1n-7 (11.8 vs. 9.4 %), 20:1n-9 (11.8 vs. 4.0 %), 22:1n-11 (10 vs. 4.8 %) and 22:6n-3 (8.4 vs. 4.8 %) were also higher in wild belugas than captive whales (Figure 3.3). SIMPER analysis revealed that 18:1n-9, 20:1n-9, and 16:0, contributed to 63.2 %, 12.2 %, and 9.5 % of the dissimilarities in fatty acid signatures between captive and wild beluga whales.

Discussion

Fatty acid signatures in predator and prey

The predominant fatty acids in herring and salmon, 18:1n-9 and 16:0, had the highest proportions in the captive beluga whales. However, a third major fatty acid (18:2n-6) contributed to dissimilarities between salmon and beluga. Farmed salmon consume higher levels

of plant-based oils, leading to higher levels of 18:2n-6, a major constituent of corn, rapeseed, and soybeans (Megdal et al. 2009). As a result, higher proportions of 18:2n-6 can be used to distinguish farmed salmon from wild populations, and differentiated the fatty acid signatures of the farmed Chinook salmon from the other prey species and belugas. Wild Pacific herring mainly feed on various species of crustaceans that vary seasonally and inter-annually (Hill et al. 2015). Additionally, 18:1n-9 is known to be significantly higher in non-spawning Pacific herring compared to spawning fish (Huynh et al. 2007). Although 18:1n-9 and 16:0 were highest in captive beluga whale blubber, 20:5n-3 was low, despite having the third highest proportion in herring. 20:5n-3 also contributed the most to dissimilarities between the fatty acid signatures of herring and beluga. 20:5n-3 can be metabolized to 22:5n-3 in marine mammals(Budge et al. 2004), which may have led to lower proportions in the beluga whales.

We were able to differentiate between all prey based on fatty acid signatures, except squid and capelin. Squid and capelin had 22:6n-3 as their dominant fatty acid followed by 16:0, which are both dinoflagellate fatty acid markers (Dalsgaard et al. 2003). Capelin off Newfoundland feed primarily on copepods *Calanus finmerchicus* and *Calanus glacialis* (Dalpadado and Mowbray 2013); however, capelin from NAFO division 3L also feed on krill *Thysanoessa raschii* (Gerasimova 1994). As the capelin came from three different catch areas, the high within-species variability may be due to differences in their prey, such as copepods and krill. Opalescent inshore squid also have a diverse diet, feeding on euphausiids, crustaceans, copepods, fish, and other cephalopods, including cannibalism (Zeidberg 2013).

Qualitative analysis using NMDS ordination revealed fatty acid patterns in beluga whales to be most similar to herring and salmon, suggesting they were key prey. As the stress, an indicator of fit, was 0.03, the plot is an excellent representation of the fatty acid signatures in a

two-dimensional configuration with no prospect of misinterpretation (Zuur et al. 2007; Clarke et al. 2014).Yet, according to the daily feeding schedules of both beluga whales, Pacific herring was the dominant prey and salmon was absent from the diet. Fatty acids from salmon may have been incorporated into the juvenile whale's fatty acid signatures while nursing. On the other hand, the adult whale had last consumed salmon almost one year (September 27, 2011) before the experimental period. The fatty acid composition of salmon and beluga whales may have appeared similar to one another in the NMDS plot as a result of the data being forced into two dimensions. Therefore, interpretations of predator diets based on qualitative analysis of fatty acid signatures may be misleading, as species with some similarities in fatty acids that are not part of the predator's diet may be perceived as important prey.

Mixing model diet estimates

The FASTAR model estimates for the captive adult beluga correctly identified herring as the primary prey of the adult beluga whale; however, the model was unable to accurately estimate the relative contributions of squid and capelin (Table 3.3). High within-species variability in fatty acids may have affected the ability to distinguish between squid and capelin. The mixing model may also be less accurate when predicting the proportions of minor prey, as seen in the squid estimate for both whales. The turnover rate of fatty acid signatures in blubber may have also affected dietary proportions, as capelin replaced squid as the second dominant prey source during week 3. Blubber fatty acid signatures are estimated to reflect diet assimilated between 1.5 to 3 months (Nordstrom et al. 2008), but changes in fatty acids during prey switching experiments can be detected after 2 weeks (Kirsch et al. 2000). Although we are unable to specify the exact timeframe, we hypothesize that the blubber fatty acids incorporated

prey consumed beyond a 2-week timeframe, as both belugas were eating close to 100% herring in the weeks prior to death.

For the diet of the juvenile beluga, nine fatty acids and seven fatty acid trophic markers, identified herring as the dominant prey, but with 34 fatty acids, capelin was identified as the main prey source. The juvenile whale was fed fairly similar proportions (on a per mass basis) of capelin (0.42 to 0.45 per week) and herring (0.50 to 0.54), which was consistent throughout most of her life. We cannot account or quantify the effect of nursing on the juvenile beluga's fatty acid profile. Fatty acid signatures from Chinook salmon may have been transferred to the juvenile through milk, as fatty acids in milk represent the dietary history of the mother (Iverson et al. 1997a). Previous studies have found the fatty acid signatures from the inner blubber layer of older calves (1 year) to be more similar to their mother's blubber than their mother's milk (Birkeland et al. 2005). Nursing beluga whale calves are hypothesized to have higher proportions of 18:1n-9 than their mothers due to a more rapid Δ-9 desaturate system (Birkeland et al. 2005). In our study, proportions of 18:1n-9 and 16:1n-7 were higher in the juvenile than the adult although the whales were not related.

Model performance improved using the nine fatty acids identified by SIMPER, which better reflected the proportions of prey by mass fed to the whales. In other studies, the performance of fatty acid Bayesian mixing models significantly improved from two to seven tracers, with additional tracers demonstrating only a slight effect on performance (Brett et al. 2016). Overall, Bayesian mixing models perform better when the effect of trophic modification of fatty acids is accounted for (Brett et al. 2016). Previous studies of FASTAR have used a prey resource library, based on the fatty acid signatures of the predators fed 100% of one prey (Galloway et al. 2014a; Galloway et al. 2015), whereas we used the fatty acid compositions of

the actual prey. We were unable to calculate calibration coefficients as belugas were not fed a monotypic diet (Nordstrom et al. 2008).

Another factor that may have affected the deposition of fatty acid signatures was the fat content of prey. Prey species with higher fat content are more digestible (Trumble and Castellini 2005), and contribute more to a predator's fatty acid signature (Iverson et al. 2004). Therefore, higher fat content may have influenced the higher proportions of fatty acids from herring to the fatty acid signatures of the belugas. Although we selected the fatty acids that undergo little modification from prey to marine mammals (Iverson et al. 2004; Tollit et al. 2010), metabolism may have affected the fatty acids of the beluga whales. Certain fatty acids, specifically 16 and 18 saturates and monounsaturates, may be elevated in marine mammals due to biosynthesis via excess dietary amino acids (Iverson et al. 2004). Δ9 desaturase enzyme activity of 14:0, 16:0 and 18 saturates to produce 14:1n-5, 16:1n-7, and 18:1n-9, and peroxisomal shortening of 22:1 and 20:1 to their 18 carbon isomers, may have modified the fatty acid profiles of the whales (Iverson 2009). As a result, proportions of fatty acids such as 16:1n-7 and 18:1n-9 tend to be higher in predators than their prey (Iverson et al. 2004).

The illness of the whales and their reduced consumption prior to death may be confounding factors that affected the composition of blubber fatty acids. In previous studies on the fatty acid composition in the blubber of emaciated harbor porpoises (*Phocoena phocoena*), selective mobilization of fatty acids led to a decrease in 22:6n-3, 20:1n-9, 20:5n-3, 22:1n-11, and 18:2n-6 levels and an increase in endogenous fatty acids in blubber relative to 'normal' porpoises (Koopman 2001). As a result, the fatty acid composition of the inner blubber of starved whales resembled the outer blubber layer, with fatty acids 14:1n-5, iso-15:0, and iso-16:0 comprising over one percent of the total fatty acids (Koopman 2001). Considering that fatty acids

transferred through diet contributed to the highest percent composition in both captive whales, and the percent of endogenous fatty acid such as 14:1n-5, iso-15:0, and iso-16:0 was less than 0.5 percent of total fatty acids and similar to wild EBS beluga whales, the reduction in appetite experienced by the captive whales during their illnesses may not have induced the mobilization of blubber fatty acids as energy reserves. In addition, although the adult female reduced her consumption gradually beginning in week two (Table 3.1), she was feeding up until the week of her death, and the juvenile was observed nursing two days before her passing.

Another potential useful application of FASTAR is the ability to identify prey species that are not consumed or less important to the diet of the predator. This ability may be useful in scenarios in which the predator has been observed to feed on a variety of species, such as with free-ranging beluga whales. In our study, salmon and squid were identified as minor prey sources with little influence on the fatty acid profiles of the beluga whales.

Comparisons to wild beluga whales

The fatty acid signatures of captive beluga whales were significantly different from the wild EBS beluga population, likely due to differences in diet and feeding behaviour. Higher proportions of 18:1n-9 and 16:0 in captive whales reflect the fatty acids of their main prey, Pacific herring (53-68% of their diet). Higher proportions of 20:1n-9 and 22:1n-11 in EBS beluga whales likely reflect their dominant prey, Arctic cod and other pelagic fish species (Loseto et al. 2009). Arctic cod (*Boreogadus saida*) primarily feed on *Calanus* copepods, which synthesize 20 and 22 monounsaturates (Falk-Petersen et al. 2009). Other factors such as seasonality and reproduction may have also contributed to differences in fatty acid signatures between whales, as the EBS beluga whales were sampled in July.

Conclusions

Bayesian mixing models, such as FASTAR, are a promising tool to estimate the diet of wild marine mammals using fatty acid signatures. As the effects of metabolism are difficult to account for, we recommend using fatty acid signatures known to be transferred through diet and identified by SIMPER as influential in distinguishing prey. Running different sets of fatty acid markers, such as FATMs and fatty acids identified by SIMPER, may help to understand variance of the model estimates. We recommend caution when using these models with prey that have high within-species variability, as well as prey that do not differ in fatty acid signatures. In these cases, prey species may need to be grouped into a broader classification category, such as genus, family, or ecosystem type. We also caution against the use of qualitative analysis alone to infer predator diets using fatty acids. As seen in our NMDS ordination, the fatty acid signatures in whales were most similar to Pacific herring but also Chinook salmon, a prey item not directly consumed by the whales. However, qualitative analysis through NMDS or principal component analysis is useful as an initial test to identify prey with overlapping fatty acid signatures, high variability, and other issues that may need to be resolved before using mixing models. Finally, the application of Bayesian mixing models to identify predator diets is promising to monitor the effects of climate induced prey shifts on free-ranging Arctic beluga whales. Recently, a decline in growth rates of individual EBS beluga whales was identified over a thirty year time period, hypothesized to be the result of prey changes (Harwood et al. 2014). Predicting the diets of beluga whales using fatty acids and Bayesian mixing models may help to identify the potential underlying causes that have led to the decline in growth rates of EBS beluga whales, and may also be useful tools for long term monitoring on the effects of climate change on Arctic marine mammals.

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Table 3.1. Weekly dietary proportions in mass and total percentage of a juvenile and an adult female beluga whale.

Whale	Week	Herring		Capelin		Squid	
		kg	% of Diet	kg	% of Diet	kg	% of Diet
Juvenile	Week 1	14.1	50.2	12.6	44.9	1.4	5.0
	Week 2	14.7	51.2	12.6	43.9	1.4	4.9
	Week 3	15.9	53.2	12.6	42.1	1.4	4.7
	Week 4	16.1	53.5	12.6	41.9	1.4	4.7
	Week 5	9.2	53.5	7.2	41.9	0.8	4.7
	Week 6	0.8	100	0	0	0	0
	Week 7	1.4	63.6	0.8	36.4	0	0
Adult	Week 1	46.9	60.9	14	18.2	16.1	20.9
	Week 2	40	64.4	9.2	14.8	12.9	20.8
	Week 3	50.4	75.0	10.2	15.2	6.6	9.8
	Week 4	46.9	94.0	1.9	3.8	1.1	2.2
	Week 5	9.5	100	0	0	0	0

Table 3.2. Percentages of fatty acids contributing to the overall dissimilarities in fatty acid signatures among the blubber of two captive beluga whale and their prey items as determined by similarity percentages routine analysis (SIMPER).

Comparison	14:0	16:0	16:1n-7	18:1n-9	18:2n-6	20:1n-9	20:5n-3	22:1n-11	22:6n-3	Total (%)
Capelin vs. herring				39.4		14.0		14.0	16.9	84.2
Capelin vs. salmon				44.9		10.1		10.9	16.0	81.9
Herring vs. salmon				28.3	31.3		21.0			80.6
Capelin vs. squid		8.7				13.7		26.2	36.9	85.5
Herring vs. squid				38.5					49.5	88.0
Salmon vs. squid				40.6					40.0	80.7
Capelin vs. adult beluga				45.9		8.7	12.2		20.7	87.5
Herring vs. adult beluga		13.2		23.1			37.6		10.3	84.2
Squid vs. adult beluga				35.3			14.8		42.0	92.1
Capelin vs. juvenile beluga				49.0			10.2	10.2	17.4	86.8
Herring vs. juvenile beluga		14.5		32.8			31.4		7.6	86.3
Salmon vs. juvenile beluga	5.8		21.2		57.0					84.1
Squid vs. juvenile beluga				39.8			13.6		38.7	92.1

Table 3.3. Actual diet proportions and FASTAR estimates of the prey contributions to an adult and juvenile beluga whales using 34 and 9 fatty acids (FAs), and 7 fatty acid trophic markers (FATM). Data represent the median (50%) and the Bayesian credible interval in parentheses (5^{th} and 95^{th} percentile).

	Adult				Juvenile			
	Actual	**34 FAs**	**9 FAs**	**7 FATM**	**Actual**	**34 FAs**	**9 FAs**	**7 FATM**
Capelin	0.16 (0.13-0.18)	0.34 (0.31-0.37)	0.33 (0.27-0.38)	0.23 (0.15-0.33)	0.42 (0.36-0.48)	0.46 (0.42-0.54)	0.38 (0.32-0.44)	0.31(0.23-0.40)
Herring	0.74 (0.66-0.82)	0.52 (0.50-0.56)	0.67 (0.62-0.72)	0.74 (0.63-0.83)	0.51 (0.44-0.59)	0.36 (0.32-0.39)	0.49 (0.46-0.59)	0.62 (0.52-0.72)
Squid	0.1 (0.07-0.13)	0.13 (0.12-0.15)	0.008 (0.003-0.02)	0.02 (0.02-0.08)	0.05 (0.04-0.05)	0.08 (0.3-9.6)	0.004 (0.00-0.002)	0.04 (0-0.11)
Salmon	NA	NA	NA	NA	NA	0.11 (9.3-12.8)	0.1 (0.07-0.12)	0.02 (0-0.06)

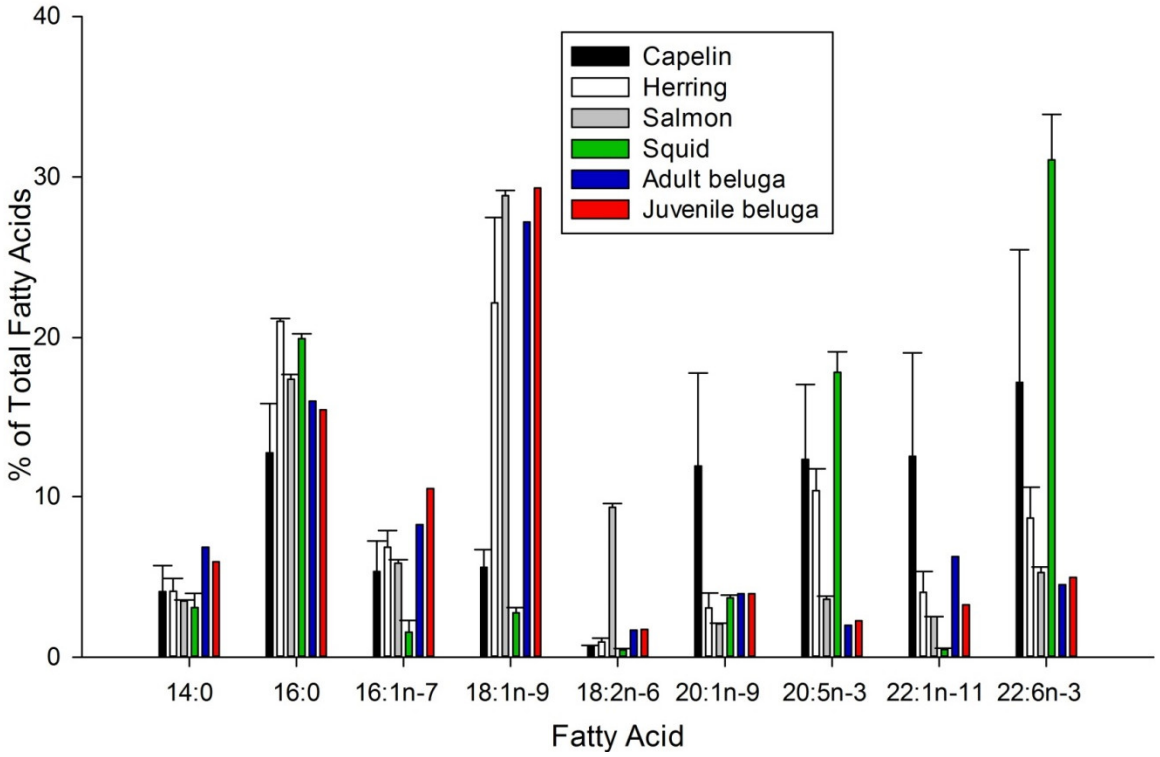

Figure 3.1. Mean percentage of fatty acids (plus 1 standard deviation) from the inner blubber of an adult and juvenile beluga whale and prey species in their diet. Only dietary fatty acids that contribute to more than 1% of the total percent fatty acids are shown.

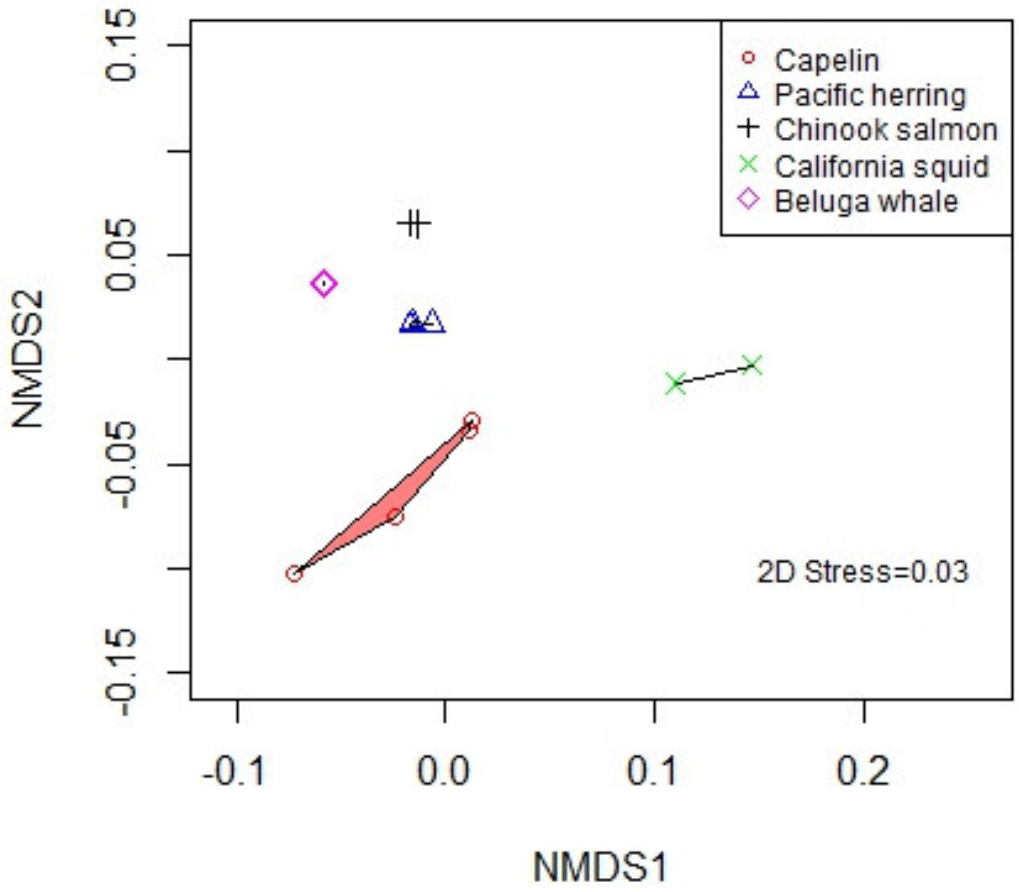

Figure 3.2. Non-metric multidimensional scaling (NMDS) ordination with excellent goodness of fit (stress=0.03) based on the total percentage of 34 fatty acids of two captive beluga whales and their prey (capelin, Pacific herring, Chinook salmon, and California squid). Orthogonal polygons define each group.

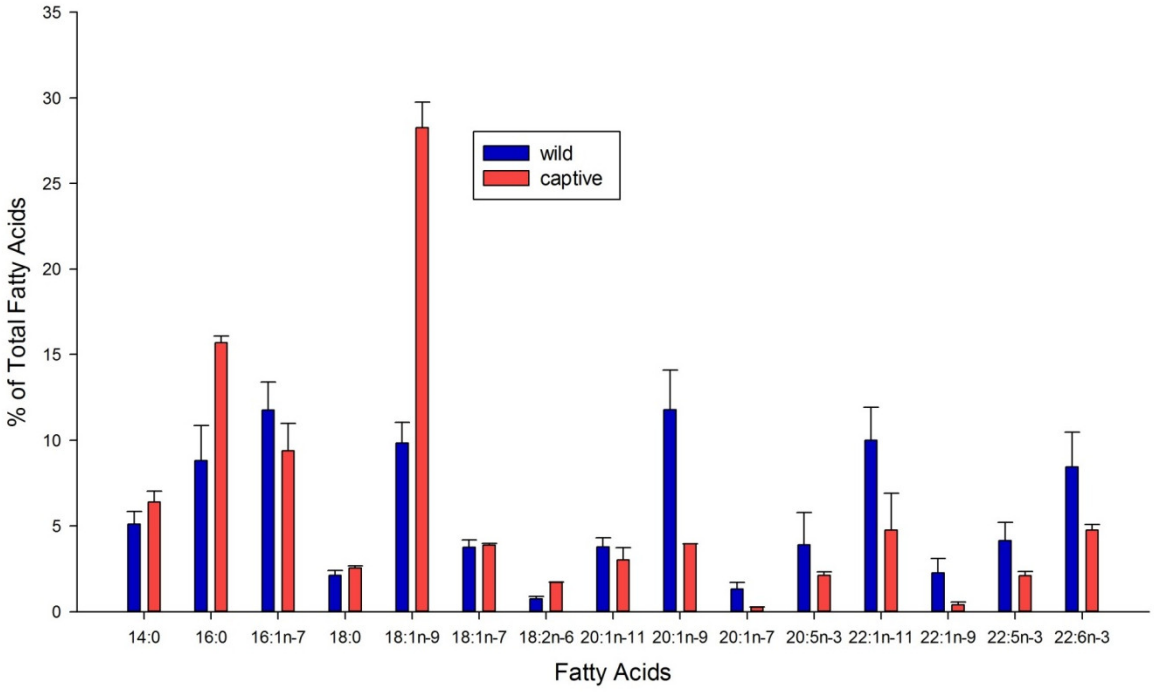

Figure 3.3. Mean percentage of fatty acids (plus 1 standard deviation) within captive (*n*=2) and wild (*n*=9) female beluga whale blubber samples. Only dietary fatty acids that contribute to more than 1% of the total percent fatty acids are shown.

Chapter 4:

Bayesian analysis of eastern Beaufort Sea beluga whale inter-annual diet: insights on climate change effects.

Chapter Four: Manuscript Summary

Manuscript in preparation

Citation:

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Contributions:

Dr. Carolina Giraldo completed chromatography on the fatty acid signatures on fish and provided comments on the manuscript.

Bruno Rosenberg provided technical support and expertise for fatty acid analysis using gas chromatography as well as quality assurance and control.

Dr. Jim Roth provided comments throughout the writing process.

Andy Majewski coordinated the BREA fish survey program of which the fish and invertebrate samples were obtained.

Ashley Stasko, Dr. Heidi Swanson, and Dr. Michael Power provided stable isotope ratios of fish samples and provided comments on the manuscript.

Dr. Lisa Loseto provided funding for stable isotope analysis and comments throughout the writing process.

Dr. Jim Reist is the principal investigator for the BREA project and provided funding.

Emily Choy designed the study, prepared all invertebrate samples for stable isotope analysis and fatty acid signatures, extracted fatty acids from beluga tissues, completed chromatography on invertebrate and beluga samples, analyzed the data, produced all figures and tables, and wrote the manuscript.

Abstract

As a top predator with an Arctic circumpolar distribution, beluga whales (*Delphinapterus leucas*) are an indicator species for the effects of Arctic climate change. The eastern Beaufort Sea beluga whale population, one of Canada's largest, has experienced a twenty year decline in individual growth rates, hypothesized to be the result of climate-induced prey shifts. We used fatty acid signatures and stable isotope ratios coupled with Bayesian statistics to reconstruct the diets of beluga whales and identify food web linkages useful for evaluating this hypothesis. Correspondence analysis revealed similarities between beluga whale fatty acid signatures and lipid rich pelagic fish species, such as capelin (*Mallotus villosus*), Arctic cod (*Boreogadus saida*), and Greenland halibut (*Reinhardtius hippoglossoides*). Individual diet estimates obtained from a Bayesian mixing model identified Arctic cod and capelin as the dominant prey, but indicated beluga whales also consumed decapods (*Argis dentata*, *Eualus gaimardii*, and *Sclerocrangon ferox*) and octopus (*Cirroteuthis muelleri*). Diet estimates varied among individuals and year, with belugas consuming the greatest prey diversity in 2014, consuming the highest median proportion of Arctic cod and capelin in 2013, and the highest median proportion of decapods in 2012. Among-year dietary variations may reflect environmental conditions, relative prey abundances or a combination of the two. As capelin and other subarctic fish species expand their ranges northwards, understanding how inter-annual variations in beluga diet reflect environmental conditions will allow better predictions of the long-term effects and conservation implications of beluga whales shifting from an Arctic cod to a capelin or decapod-dominated diet.

Introduction

Arctic ecosystems are currently undergoing rapid change, with the Arctic Ocean predicted to be free of summer sea ice within the next few decades (Stroeve et al. 2007; Wang and Overland 2012). Sea ice decline and warming ocean temperatures have facilitated the northward migration and expansion of temperate marine species that may have significant impacts on the structure of existing food webs (Michel et al. 2012). Arctic vertebrates that are highly specialized for living in Arctic marine environments are particularly vulnerable to climate change (Laidre et al. 2008; Williams et al. 2011); unfortunately, the impacts are difficult to predict due to knowledge gaps in species ecology. Specialist species will be most vulnerable and may be unable to adapt to rapid changes in the distribution, quality, and species of available prey (Gilg et al. 2012). As the Arctic Ocean is coupled to both the Pacific and Atlantic Ocean, monitoring changes to Arctic marine ecosystems is important for predicting the global effects of climate change (Michel et al. 2012).

The eastern Beaufort Sea (EBS) beluga whale (*Delphinapterus leucas*) population is one of Canada's largest, with an estimated 40,000 individuals (Allen and Angliss 2014). The EBS beluga population arrives in the Canadian Beaufort Sea in late May to early June, with calving and nursing occurring in early July near the Mackenzie Estuary. The beluga whales spend the summer feeding in the Canadian Beaufort Sea and Amundsen Gulf before migrating in September to their winter grounds in the eastern Bering and Chukchi Sea (Richard et al. 2001; Harwood and Smith 2002). Although beluga whales are opportunistic predators that feed on a wide range of fish and invertebrate species (Seaman et al. 1982; Quakenbush et al. 2014), the EBS population is thought to specialize on Arctic cod (*Boreogadus saida*) (Loseto et al. 2009),

which is also the most abundant fish species in the Canadian Beaufort Sea (Benoit et al. 2008; Geoffroy et al. 2011).

Recent studies on the EBS beluga whale population have revealed a decline in individual growth rate over a twenty-year period, which is hypothesized to be the result of prey shifts due to changing environmental conditions (Harwood et al. 2014; Harwood et al. 2015). Arctic cod is a sea ice-associated fish that is vulnerable to climate change (Laurel et al. 2016). Declining sea ice extent and warming ocean temperatures have facilitated the northward migration and expansion of temperate species, such as Pacific sand lance (*Ammodytes hexapterus*) and capelin (*Mallotus villosus*), that may displace Arctic cod (Falardeau et al. 2014; McNicholl et al. 2016). As Arctic cod is one of the most energy-dense Arctic forage fish and is estimated to supply 75% of the energy transfer between plankton and vertebrates, the disappearance or northward displacement of Arctic cod could have major impacts on beluga whales as well as many other Arctic marine top predators (Welch et al. 1992; Welch et al. 1993; Crawford and Jorgenson 1996; Harter et al. 2013).

Ecological tracers such as fatty acid and carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios can provide valuable information on ecosystem changes as well as dietary linkages of marine mammals (Iverson et al. 2004; Budge et al. 2006; Newsome et al. 2010). Several long-chain (> 14 carbons) monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) are transferred conservatively from prey to the blubber of marine mammals (Iverson et al. 2004; Budge et al. 2006). These fatty acids cannot be synthesized by vertebrates and are thus representative of marine food web structure: 20:1 (n11, n9) and 22:1 (n11, n9) are synthesized by *Calanus* copepods (Falk-Petersen et al. 1987), 18PUFAs and 22:6n-3 by dinoflagellates, and 16:1n-7 and 20:5n-3 by diatoms (Dalsgaard et al. 2003). Stable isotope ratios also provide

insights into diet and trophic structure. $\delta^{13}C$ values vary with the source of baseline primary production (e.g., benthic vs. pelagic sources) and increase approximately 0 to 1 ‰ with every trophic transfer, whereas $\delta^{15}N$ is indicative of trophic position, increasing approximately 3 to 5 ‰ between trophic levels (Peterson and Fry 1987; France 1995).

Bayesian mixing models are powerful tools for the reconstruction of predator diets (Moore and Semmens 2008; Parnell et al. 2013; Galloway et al. 2015). An advantage of using fatty acids in Bayesian mixing models is that over 70 tracers can be utilized; therefore, the issue of an undetermined model (in which prey sources outnumber tracers) can be avoided for generalists that feed on several prey species (Brett 2014; Galloway et al. 2015; Brett et al. 2016). Bayesian mixing models also account for uncertainty associated with multiple prey sources and fractionation by estimating a probability distribution of prey contributions (Moore and Semmens 2008). Recently, the Fatty Acid Source Tracking Algorithm in R (FASTAR), a Bayesian mixing model, was used to reconstruct the diets of two captive beluga whales using fatty acids transferred conservatively through diet (Chapter 3). Since many fatty acids are species-specific and have been shown to reflect ecosystem changes, such as shifts to a diet dominated by benthic prey in bearded seals (*Erignathus barbatus*) from the northern Bering Sea (Cooper et al. 2009), FASTAR may be able to provide dietary estimates and detect recent changes to beluga diets.

The overall objective of our study was to examine inter-annual variation in prey of beluga whales using fatty acid signatures and stable isotope ratios. In collaboration with the Beaufort Regional Environmental Assessment Marine Fishes Project (BREA MFP) survey, we examined the relative contributions of nine fish and five invertebrate species collected from different depths (20 to 1000 m) and habitats to the diets of beluga whales. Our first objective was to determine if prey species differed based on lipid content, to support the hypothesis that beluga

whales prefer high energy density prey. Our next objective was to differentiate among prey species using fatty acid signatures and stable isotope ratios. Finally, we used the Bayesian mixing model FASTAR to reconstruct the diets of beluga whales sampled from 2011 to 2014 using fatty acid signatures and stable isotope ratios. Understanding inter-annual variation in diet provides insights into the flexibility of the eastern Beaufort Sea beluga population to adapt to long-term prey shifts predicted by the northward migration of subarctic fish and invertebrate species. This study also demonstrates the effectiveness of the "multi-dimensional marker approach" by using fatty acid and stable isotope ratios to study trophic dynamics.

Methods

Sample collection

Blubber and liver samples were collected from adult beluga whales (n = 171) harvested at Inuvialuit beluga hunting camps from July to early August between the years 2011 to 2014 at Hendrickson Island, Brown's Harbour, Kendall Island, and East Whitefish in the Inuvialuit Settlement Region, Northwest Territories, Canada (Figure 4.1). Details of sample collection for beluga whales are available in Chapter 2. Samples were frozen at -20˚C in portable freezers on site and shipped to Fisheries and Oceans Canada in Winnipeg for laboratory analysis.

Nine fish and five macroinvertebrate species were collected as potential prey from August 6 to September 3 2012 as part of the BREA MFP. As habitat range varies with body size in beluga whales (Richard et al. 2001; Loseto et al. 2006; Chapter 2), prey were selected from different transects, sampling stations and depths to reflect the potential spatial variability in feeding. Trawling was conducted at 26 stations across four transects in the Canadian Beaufort Sea in 2012 (Figure 4.1; Table 4.1). Arctic cod, Greenland halibut (*Reinhardtius*

hippoglossoides), Adolf's eelpout (*Lycodes adolfi*), Arctic staghorn sculpin (*Gymnocanthus tricuspis*), Canadian eelpout (*Lycodes polaris*), stout eelblenny (*Anisarchus medius*), kelp snailfish (*Liparis tunicatus*), and Arctic alligatorfish (*Aspidophoroides olrikii*), isopods (*Saduria sabini*), green shrimp (*Argis dentata*), circumpolar eualid (*Eualus gaimardii gaimardii*), polar shrimp (*Sclerocrangon ferox*), and octopus (*Cirroteuthis muelleri*) were collected on board the *F/V Frosti* using a modified Atlantic Western IIA benthic otter trawl (mesh sizes 90 and 130 mm) or a 3 m benthic beam trawl (mesh sizes 45, 70.5, 100, and 155 mm). Capelin samples were collected using a 3 m beam trawl at Bennett Point (BPT-03) on August 6th 2013. Fish were sorted and identified on board to the species level and measured to the nearest 0.1 mm. All fish species had standard lengths greater than 100 mm except for Arctic alligatorfish, which had a maximum standard length of 64 mm. All samples were immediately frozen at -20˚C until processing.

Fatty acid extraction

Fish and invertebrate samples were whole-body homogenized (fish cut in half lengthwise), homogenized in a Retsch GM200 grinder in a semi-frozen state, freeze-dried and then re-frozen and stored at -80˚C until fatty acid analysis (Giraldo et al. 2016). Detailed methodology for fatty acid extraction for fish and invertebrates are outlined in Giraldo et al. (2016) and in Chapter 2 for beluga whales. In brief, lipids were extracted from 0.5 g of tissue with a 2:1 chloroform: methanol solution containing 0.01% butylated hydroxytoluene (BHT) using a method modified from Folch et al. (1957) and used in Budge et al. (2006). Percent lipid was determined gravimetrically and recorded in dry weight (g). The extracted lipid was used to prepare the fatty acid methyl esters by transesterification with Hilditch reagent (0.5 N H_2SO_4 in

dry methanol). 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). Fatty acid standards were obtained from Supelco (37 component FAME mix) and Nuchek (54 component mix GLC-463) and were used to verify the retention times of fatty acid peaks. 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. A total of 73 fatty acids were examined for peaks.

Stable isotope analysis

Liver tissue samples (approximately 0.5 g) for beluga whales, whole body tissues for invertebrates, and dorsal muscle tissues for fish were freeze-dried for at least 48 hours and analyzed for C and N stable isotope ratios at the University of Waterloo Environmental Isotopes Laboratory. Full methodologies are described in Giraldo et al. (2016), Stasko et al. (2016), and Chapter 1. Stable isotope ratios are expressed in delta (δ) notation in per mil (‰), and were calculated against known certified elemental standard materials (Vienna Pee Dee Belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$; Craig 1957; Mariotti 1983), following:

$\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard})-1] \times 1000$

where R is the ratio of the heavy to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$).

Data quality control was monitored and corrections made using international reference material and in-house standards that were cross-calibrated using certified international reference materials (i.e. IAEA-N1 + N2, IAEA-CH3 + CH6, USGS-41 + 41), with an analytical error of 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$ required for reportable data. National Institute of Standards

and Technology (NIST; Gaithersburg, MD, USA) standard 1577B (bovine liver) was used as a post-correction check throughout the analysis, with approximately 20% of the total sample number standards or reference materials. Every eighth sample was run in duplicate.

Since differences in lipids and carbonates have a significant effect on the interpretation of $\delta^{13}C$ values among species, invertebrate tissues were treated for lipids and carbonates (for species with exoskeletons) following methodologies described in Chapter 1. For beluga liver tissues, a lipid correction model ($\delta^{13}C_{extracted} = -1.868+0.839 \times \delta^{13}C_{bulk}$) derived in Chapter 1 was used to correct bulk $\delta^{13}C$ values.

Bulk $\delta^{13}C$ values in fish were corrected using Post et al. (2007) lipid normalization model for aquatic animals based on C:N ratios: $\Delta^{13}C = -3.32 +0.99 \times C:N$. Bulk untreated samples were used for $\delta^{15}N$ values for all species. In order to better visualize potential prey items in stable isotope biplots and estimate prey proportions in the mixing model, stable isotope ratios for beluga whales were corrected for trophic enrichment using trophic modification factors for killer whale (*Orcinus orca*) liver [$\Delta^{15}N = 2.92$ (without delipidation), $\Delta^{13}C = 1.27$ (after delipidation)] (Caut et al. 2011).

Data analyses

A one-way ANOVA was used to examine differences in % lipid among species. Percentage data were square-root transformed for analysis to meet assumptions of normality of residuals and homogeneity of variance. We used 32 fatty acids identified as dietary by Iverson et al. (2004) and with mean percentages above 0.1 % of the total fatty acid signatures. Correspondence analysis was performed on untransformed data to compare fatty acid signatures among all species using the package “ade4” (Chessel et al. 2004) and visualized using “ggord”. Correspondence is an exploratory technique that calculates a chi-square (inertia) distance matrix

to define the relationship between individuals and fatty acids, and is visualized in two-dimensional space (Greenacre and Primicerio 2013).

To determine if we could distinguish prey using biotracers, we compared fatty acid signatures and stable isotope ratios of each species separately using one-way permutational multivariate ANOVA (PERMANOVA) and analysis of similarities (ANOSIM; one-way test) tests followed by post-hoc pairwise tests. Each procedure tests different properties of the data; the null hypothesis of PERMANOVA is that centroids of the groups as defined in the chosen distance measure are equivalent, whereas the null hypothesis for ANOSIM is that the average of the ranks of within-group distances is greater or equal to the average of the ranks between group distances (Anderson and Walsh 2013). In cases where the results of both tests are not significant and R static of ANOSIM is small, the effect of the factor is weak. PERMANOVA and ANOSIM used Euclidean distance, fixed factors and Type III sums of squares, and significance was determined using 9999 unrestricted permutations of the raw data and Monte Carlo generated p-values when the number of unique permutations was < 200. The *a priori* significance level was $\alpha = 0.05$ for all statistical procedures. PERMANOVA and ANOSIM are not affected by violations in normality, but may be sensitive to dispersion of multivariate data (although differences in dispersion are not substantial enough to inflate the error rates of PERMANOVA; Anderson et al. 2008). Therefore, non-metric multidimensional scaling (NMDS) was performed to interpret the nature of differences detected in ANOSIM and PERMANOVA (Anderson and Walsh 2013). NMDS uses rank order based on a distance matrix and maps compositional dissimilarities in a two dimensional configuration (Zuur et al. 2007; Oksanen 2015). Fatty acid signatures were visualized using NMDS based on Euclidean distances using the package Vegan (Oksanen et al. 2016).

A one-way similarity percentage routine (SIMPER) was used to identify which fatty acids contributed most to dissimilarities among prey. SIMPER first tabulates fatty acid contributions to the average similarity of individuals within each group followed by the average dissimilarity (Clarke et al. 2014; Clarke and Gorley 2015). We designated a cut-off of fatty acids that characterized up to 80% of dissimilarities. Multivariate significance tests were performed using PRIMER v.7.0 and PERMANOVA+.

Dietary estimates using a Bayesian mixing model

Fatty acids in the inner blubber layer and stable isotope ratios from liver tissues were assumed to be most representative of early summer and spring diets. Although the relative turnover rates of fatty acid signatures or stable isotope ratios have not been quantified in cetaceans, the turnover rate of fatty acids is estimated at 1.5 to 3 months in blubber (Nordstrom et al. 2008); however, changes in diet have been detected after 14 days (Kirsch et al. 2000). Stable isotope ratios in liver have faster turnover rates relative to other tissues and are representative of recent diet (a few days in small rodents to approximately 37.3 days in alpacas (*Lama pacos*) Tieszen et al. 1983; Arneson et al. 2006; Sponheimer et al. 2006; Miller et al. 2008; DeMots et al. 2010).

We applied the Bayesian mixing model "FASTAR" to the 32 dietary fatty acids and estimated the proportional contribution of each prey species to beluga diets (Galloway et al. 2015). FASTAR uses Dirichlet priors ($\alpha = 1$). Posterior probability distributions were estimated using Markov Chain Monte Carlo chains and 100,000 iterations with a 50,000 iteration burn-in and a thinning rate of 50. Using the R script provided by Galloway et al. (2015), we used the fatty acid percentages of belugas as "consumers". Instead of a resource library based on feeding

trials of the predator fed 100% of a specific prey, we inputted the average percentage and standard deviation of fatty acids for each prey as "sources" within the model. Individual species and prey that did not differ based on fatty acid signatures using PERMANOVA and ANOSIM tests and were ecologically similar were pooled together as sources. Due to the high variability in fatty acid signatures within the EBS beluga population (Chapter 2), we examined the dietary estimates of individual beluga whales. Only dietary estimates with unique solutions were reported. Estimates with bimodal distributions were removed due to lack of convergence. FASTAR was run in R version 3.2.5 (R Core Team 2016).

Results

Lipid content and dietary tracers of beluga prey

Prey species differed in lipid content (one-way ANOVA, $F_{13, 276} = 34.76$, $p < 0.01$), with Arctic cod (mean ± standard deviation: 33.4 ±10.7 %, $n = 45$), Greenland halibut (33.2 ± 7.8 %, $n = 54$), and capelin (31.3 ± 6.1 %, $n = 17$) having the highest mean lipid content (Figure 4.2). Benthic invertebrates and specifically isopods (6.7 ± 3.9 %, $n = 14$) had the lowest lipid content of all species. Of the 73 fatty acids identified, 17 comprised 88.3 to 92.1% of the total fatty acid composition in beluga and their 14 potential prey species (Table 4.2). 16:1n-7 was the dominant fatty acid in all species except green and polar shrimp, octopus, Greenland halibut, and kelp snailfish. 18:1n-9 was highest in Greenland halibut and kelp snailfish and was second highest in proportion in Adolf's eelpout. 20:5n-3 was highest in green and polar shrimp and had the second highest proportion in circumpolar eualid and Canadian eelpout. 16:0 and 22:6n-3 were the dominant fatty acids in octopus. 22:1 and 20:1 comprised over 20% of the fatty acid composition in Arctic cod, capelin, and Greenland halibut. The first axis of the correspondence analysis

explained 50.1 % of the variation in fatty acid signatures among species whereas axis 2 explained 14.4 %, for a total of 64.5 % (Figure 4.3). PUFAs such as 20:4n-6 and 22:3n-3 were found more to the left side of axis 1 whereas MUFAs like 20:1n-11 and 22:1n-11 were located at the opposite end.

The stable isotope ratios of pelagic species such as Arctic cod and capelin had relatively lower $\delta^{13}C$ values than benthic fish species, with the exception of kelp snailfish and stout eelblenny (Figure 4.4). However, $\delta^{13}C$ values of Greenland halibut were more similar to Arctic staghorn sculpin, Arctic alligatorfish, Canadian eelpout, and Adolf's eelpout. The $\delta^{13}C$ and $\delta^{15}N$ values of all decapod species overlapped in isotopic space, but differed from isopods and octopus. After correction with the trophic enrichment factor, the mean $\delta^{13}C$ and $\delta^{15}N$ values of beluga whales overlapped in isotopic space with Arctic staghorn sculpin, Arctic alligatorfish, and Greenland halibut.

Differences in dietary tracers among prey species

The fatty acids of fish and invertebrates differed among species (PERMANOVA; 32 fatty acids, $Pseudo\text{-}F_{13} = 42.4$, $p < 0.01$). All pairwise species comparisons were significantly different ($p \leq 0.03$) except between isopod and Canadian eelpout ($t = 1.66$, $p = 0.09$) and green and polar shrimp ($t = 0.95$, $p = 0.35$). A one-way ANOSIM also supported fatty acid compositional differences among species (ANOSIM, Global $R = 0.73$, $p < 0.01$). Pairwise tests indicated significant differences between species ($p \leq 0.03$) except Arctic cod and capelin ($R = -0.01$, $p = 0.51$), and green and polar shrimp ($R = 0.04$, $p = 0.17$). The NMDS plot confirmed similarities in fatty composition found in PERMANOVA and ANOSIM tests: the fatty acid composition of capelin completely overlapped with Arctic cod, and all decapod species closely overlapped with

each other (Figure 4.5). The fatty acid composition of beluga whales overlapped with Arctic cod, capelin, and Greenland halibut in two-dimensional space, suggesting greater similarities.

Eleven fatty acids contributed to 80% of the dissimilarities among species: 16:1n-7, 18:1n-9, 18:1n-7, 20:1n-9, 20:1n-7, 20:4n-6, 20:5n-3, 22:1n-11, 22:1n-9, and 22:6n-3 (SIMPER; Appendix 4.1). 16:1n-7 contributed to dissimilarities in fatty acid signatures between most species, specifically isopods (44.2 to 76.5 % of dissimilarities in fatty acid signatures among species), Canadian eelpout (23.5 to 68.7 %), Arctic staghorn sculpin (13.3 to 64.0 %), Adolf's eelpout (17.2 to 76.2 %), octopus (11.7 to 63.6 %) and stout eelblenny (4.7 to 74.0 %). 20:5n-3 was also a major contributor to dissimilarities among fatty acid signatures between species, specifically green shrimp (16.6 to 43.4 %), polar shrimp (11.4 to 35.9 %), and circumpolar eualid (14.0 to 34.6 %), as well as 22:6n-3 in octopus (11.8 to 30.4 %). 22:1n-11 contributed to most dissimilarities in fatty acid signatures between Arctic cod (14.8 to 32.0 %) and capelin (18.8 to 32.9 %) with other prey, whereas 18:1n-9 contributed to most dissimilarities in Greenland halibut (6.6 to 51.2 %) versus Arctic cod (43.5%), capelin (51.2%), and octopus (19.7%).

The stable isotope ratios of potential prey also differed among species (PERMANOVA, *Pseudo-*F_{13} = 52.78, $p < 0.01$; ANOSIM, *Global* $R = 0.53$, $p < 0.01$). Most pairwise comparisons were significantly different (PERMANOVA $p \leq 0.01$; ANOSIM $p \leq 0.04$) except between Arctic alligatorfish and Arctic staghorn sculpin (PERMANOVA pairwise test $t = 1.62$, $p = 0.08$; ANOSIM $R = 0.06$, $p = 0.08$), between Arctic alligatorfish and Greenland halibut ($t = 1.61$, $p = 0.09$; $R = 0.09$, $p = 0.07$), between Canadian eelpout and octopus ($t = 0.60$, $p = 0.74$; $R = 0.02$, $p = 0.25$), and between green shrimp and polar shrimp ($t = 1.08$, $p = 0.30$; $R = 0.01$, $p = 0.30$). In one of the two tests, pairwise differences were not found between: circumpolar eualid and capelin ($t = 1.39$, $p = 0.15$), Arctic cod and capelin ($R = -0.06$, p = 0.80), Greenland halibut and

polar shrimp (R = 0.10, p = 0.10), kelp snailfish and stout eelblenny (R = 0.06, p = 0.10), and circumpolar eualid and polar shrimp (R = 0.06, p = 0.08).

Mixing model estimates

Although the fatty acid signatures of circumpolar eualid were significantly different between green (t = 2.04, p = 0.03; R = 0.14, p = 0.03) and polar shrimp (t = 2.12, p = 0.02; R = 0.15, p = 0.02), these differences were small relative to differences between other species (Figure 4.5). Due to similarities in fatty acid signatures, Arctic cod and capelin were identified as a group and decapod species were grouped together, for a total of 11 distinct prey groups. The FASTAR mixing model provided dietary estimates for 60 of the 171 individual beluga whales; prey estimates were removed for the remaining 111 belugas due to lack of convergence. Across all years, 51 of the 60 whales fed on Arctic cod and capelin, with median diet proportions ranging from 0.35 to 0.98 (Appendix 4.2). The next most common prey were decapods, with median estimates ranging from 0.01 to 0.62 in 29 beluga whales. Octopus was also a prey species commonly consumed by the whales (median = 0.01 to 0.97; n = 16 whales). Diets differed among years; for individual diet estimates for 2011, the most common prey item was Arctic cod and capelin (median 0.38-0.96; n= 6 whales; Figure 4.6). Other prey consumed included decapods (0.18-0.57; n= 2), kelp snailfish (0.58-0.81; n=2), and octopus (0.97; n=1). Of 13 whales in 2012, seven consumed a combination of decapods (0.43-0.62) and Arctic cod and capelin (0.35-0.47). The remaining 2012 belugas consumed Arctic cod and capelin or octopus. In 2013, 26 out of 27 individuals consumed Arctic cod and capelin (median: 0.40-0.96). Other prey consumed by whales included decapods and octopus. In 2014, nine out of 13 individual

consumed Arctic cod and capelin (median: 0.35-0.97). Other prey in 2014 included decapods, octopus, Arctic staghorn sculpin, Canadian eelpout, Adolf's eelpout, and kelp snailfish.

Discussion

Pelagic fish had higher lipid content than benthic fish species and invertebrates, and therefore, may be a higher quality prey to beluga whales and other marine mammals. Pelagic fish tend to have higher and more variable lipid content than demersal species due to differences in energy allocation by attaining reproductive maturity at a smaller size and allocating more energy for storage and reproduction (Anthony et al. 2000; Litzow et al. 2006). Since prey items were sampled in August to early September, seasonality may have affected the lipid content results for species such as capelin, which have higher lipid content during the winter (Lawson et al. 1998). In addition to possessing higher lipid contents, pelagic species had the highest levels of *Calanus* fatty acid markers. *Calanus* copepods convert low-energy carbohydrates and proteins produced by phytoplankton and ice algae to high-energy wax esters such as 20:1 and 22:1 fatty alcohols and acids, with the energy content of lipids maximized by increasing chain length (Falk-Petersen et al. 2009). The relative proportion of essential fatty acids such as eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) was highest in decapod species, approximately 2 to 15 % higher than all other species.

The $\delta^{13}C$ values of most benthic fish, octopus, and Greenland halibut overlapped in isotopic space and were higher relative to Arctic cod, capelin, and kelp snailfish. Greenland halibut is considered an integrator of benthic and pelagic food webs in the Canadian Beaufort Sea (Stasko et al. 2016), which may explain their similarity in $\delta^{13}C$ values to benthic species. As a shelf species, the $\delta^{13}C$ values of kelp snailfish may be influenced by the $\delta^{13}C$ depleted and

terrestrial- derived organic inputs of the Mackenzie Shelf (Dunton et al. 2006; Dunton et al. 2012). Capelin was the only prey collected from Darnley Bay, which may have also been influenced by terrestrial subsidies. Stable C and N isotope ratios of Arctic cod in the Canadian Beaufort vary with depth, with cod collected from the lower shelf (750-1000 m, mean $\delta^{15}N$, $\delta^{13}C$: 14.57, -23.41 ‰, respectively) having higher $\delta^{15}N$ and $\delta^{13}C$ values than those from the nearshore shelf (18-50 m; 12.57. -24.04 ‰, respectively; Stasko et al. 2016). Since cod were collected in the upper shelf, their $\delta^{13}C$ and $\delta^{15}N$ values would be lower than deeper dwelling cod preferred by large male belugas (Loseto et al. 2009). In terms of effectiveness, fatty acid signatures were better able to differentiate among prey with more consistency than stable isotope ratios, likely because fatty acids offer a larger suite of markers. Our results support previous reports that stable isotope ratios are not as sensitive as fatty acids for detecting fine scale differences, capturing subtle dietary shifts, or inter-annual variability in marine species (El-Sabaawi et al. 2009).

Results of the FASTAR Bayesian mixing model identified Arctic cod and capelin as the dominant prey of beluga whales. Decapods and octopus were also important prey. Our estimates are supported by stomach contents collected from 62 EBS beluga whales during their spring migration from April to June 1983 to 2003 at Point Hope and Little Diomede, Alaska, in which decapods followed by octopus, and Arctic cod were identified as the dominant prey (Quakenbush et al. 2014). In Quakenbush et al. 2014, most individuals (92%) consumed invertebrates, with 66% of stomachs containing invertebrate species exclusively. Decapods were the most common prey [60% frequency of occurrence (FO) in beluga stomachs] item followed by cephalopods (52% FO). Arctic cod was the predominant prey fish, accounting for 82% of all fish species consumed by whales with a 21% frequency of occurrence. Overall, the stomach contents

revealed Beaufort Sea belugas consumed eight fish and 16 invertebrate species, with high variability among individuals, and thus are generalists in comparison to other Alaskan beluga populations (Quakenbush et al. 2014). According to our diet estimates, beluga whales mostly consumed prey occurring at slope (200 to 500 m) and lower shelf (750 to 1000 m) habitats. Although the Arctic cod in this study were collected between 18 to 76 m (Table 4.1), the highest catch biomass of Arctic cod across all transects and depths occurred between 350 and 500 m in 2012 (Majewski et al. 2016b) and 200 to 400 m from 2006 to 2012 (Geoffroy et al. 2016); therefore, belugas were likely consuming cod from depths ranging from 200 to 500 m.

Bayesian diet estimates of beluga varied annually from 2011 to 2014. The highest median estimates of decapod consumption by belugas was in 2012, a warm year anomaly with the largest loss of sea ice observed in the Western Arctic (Perovich et al. 2012). In the Barents Sea, krill (*Thysanoessa* spp.) biomass increased with warming sea temperatures, but also encountered increased predation pressure from capelin (Eriksen and Dalpadado 2011; Michel et al. 2012). Therefore, warming ocean temperatures and reduced sea ice may result in an increase in beluga predation on decapod species. Median diet estimates of Arctic cod and capelin were highest in 2013, and belugas from 2014 had the greatest prey diversity, consuming seven species. During the BREA survey, the percent relative abundances of Arctic cod in the Canadian Beaufort Sea was highest in 2013 and lowest in 2014 across all stations and depths (Majewski et al. 2016a). As a result of the lower abundance of Arctic cod, the relative abundance of *Cottidae* and *Zoarcidae* was higher in 2014 than previous years along with other taxonomic groups (Majewski et al. 2016a). Acoustic surveys from 2010 to 2014 in the Canadian Beaufort Sea also found the biomass of Arctic cod to be lowest in 2014 (Geoffroy 2016). Additionally, beluga whales in 2014 had the lowest levels of *Calanus* fatty acid markers and body condition indices (Chapter 2).

Therefore, beluga whales in 2014 may have had a more opportunistic diet, feeding on a greater diversity of prey due to the lower availability of Arctic cod.

Although we were unable to distinguish between capelin and Arctic cod based on fatty acid signatures, it is likely that beluga primarily consumed Arctic cod based on its ubiquitous distribution and abundance in the Beaufort Sea. Arctic cod are the most abundant fish species in the Canadian Beaufort Sea, occurring in all habitats, transects, and station depths sampled by the BREA MFP survey (Majewski et al. 2016b). On the other hand, the range of capelin was more restricted; capelin were only captured in the Amundsen Gulf and Darnley Bay in 2013 (Majewski et al. 2016a). Although Arctic cod and capelin were identified as the dominant prey, the $\delta^{13}C$ values of beluga did not match our Arctic cod or capelin samples. Since the belugas had recently migrated, it is possible that $\delta^{13}C$ values of liver still reflect prey from the Bering Sea. $\delta^{13}C$ values of Arctic cod collected from the Alaskan Bering Sea (mean=-20.3‰; Hoekstra et al. 2002) are higher relative to cod from the Canadian Beaufort Sea (mean range = -23.41 to -24.04‰; Stasko et al. 2016). In bowhead whales (*Balaena mysticetus*), oscillations in the $\delta^{13}C$ values in baleen plates reflect geographic variations in $\delta^{13}C$ of prey consumed along their migration route from the Bering Sea to their summer grounds in Canadian Beaufort Sea (Schell et al. 1989). This discrepancy may have also affected our mixing model, since FASTAR was unable to find unique solutions for all whales. The lack of convergence may be associated with our use of 11 prey sources and therefore, the existence of multiple solutions for source proportions (Phillips and Gregg 2003; Phillips et al. 2014). It is also possible that the ecological tracers of prey in 2012 (with the exception of capelin) were not representative of prey from other years.

Capelin and Arctic cod share the same dietary niche in the Canadian Beaufort Sea, and are predicted to expand into the offshore with reductions in sea ice (Hop and Gjøsæter 2013; McNicholl et al. 2016). The replacement of Arctic cod with capelin is not predicted to impact energy flow since the two species are of similar size and energy content, with capelin being an important forage fish to several cetacean, seal, and seabird species (Carscadden et al. 2001; Kelley et al. 2010; Hop and Gjøsæter 2013). However, limitations to the expansion and success of capelin in the Arctic include the lack of antifreeze proteins, which have resulted in mass mortalities (Hop and Gjøsæter 2013). Accordingly, stock collapses of capelin in the Barents Sea impacted the body condition, distribution, and reproductive success of various marine mammal, seabird, and fish predators (Gjøsæter et al. 2009). The severity of the impact capelin crashes had on predators depended on the availability of alternative prey sources. Additionally, prey switches from Arctic cod to capelin were believed to be partly responsible for lower nestling growth rates in Brünnich's guillemot in the Canadian Arctic (*Uria lomvia*) due to the smaller body size of capelin relative to cod (Gaston et al. 2005). Therefore, although capelin may provide the same energy density as Arctic cod, its inclination for population crashes and smaller body mass may make capelin an unreliable prey source to beluga whales and other marine predators in the Beaufort Sea ecosystem.

Conclusions and implications for future monitoring and conservation

In the Canadian Beaufort Sea, beluga whales primarily consume a pelagic diet of lipid-rich fish. Dietary estimates of eastern Beaufort Sea beluga whales displayed high inter-annual and intra-populational variation. Bayesian analysis identified Arctic cod and capelin as the main prey, but belugas also consumed decapods and octopus. The reduction in growth rate of EBS beluga whales over time (Harwood et al. 2014) could be related to the northward range

expansion of capelin or other subarctic competitors of Arctic cod as a result of climate shifts. In addition, climate shifts are predicted to restructure marine ecosystems, with the replacement of lipid-rich to lipid-poor fish species that have low concentrations of essential fatty acids (Litzow et al. 2006). As capelin and other subarctic species expand their ranges northwards, understanding inter-annual variations in diet in response to environmental conditions, and in particular, the long-term effects of beluga whales shifting from an Arctic cod to a capelin or a decapod-dominated diet, should be a priority for monitoring the health and resilience of this population.

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Table 4.1. Sample size (*n*), catch depth minimum and maximum, and transects of fish and invertebrate species collected as potential prey of eastern Beaufort Sea beluga whales. Transects are: transboundary-transect (TBS), Garry Island (GRY), Kugmallit Bay (KUG), Dalhousie (DAL), and Bennett Point (BPT).

Species	*n*	Depth (m)		Transects
		Min	Max	
Arctic alligatorfish	19	40	75	DAL, GRY, KUG, TBS
Arctic cod	46	18	76	DAL, GRY, KUG, TBS
Arctic staghorn sculpin	19	17	75	DAL, KUG
Adolf's eelpout	31	750	1000	DAL, GRY,
Canadian eelpout	15	17	350	DAL, GRY, KUG
Capelin	16	125	125	BPT
Circumpolar eualid	16	350	350	KUG
Green shrimp	15	200	200	TBS
Greenland halibut	53	350	1000	DAL, GRY, KUG
Isopod	15	40	40	KUG
Kelp snailfish	46	500	850	GRY, TBS
Octopus	15	500	1000	DAL, GRY, KUG, TBS
Polar shrimp	15	350	350	TBS
Stout eelblenny	24	40	40	KUG

Table 4.2. Mean percent fatty acid signatures of potential prey species of beluga whales. Only fatty acids that contribute to more than 1% of the total percent fatty acids are shown. Values are given as mean ± 1 standard deviation.

	Isopod	Octopus	Circumpolar eualid	Capelin	Arctic cod	Arctic staghorn sculpin	Canadian eelpout	Adolf's eelpout	Greenland halibut	Kelp snailfish	Stout eelblenny	Arctic Alligatorfish	Green shrimp	Polar shrimp
14:0	1.62 ± 0.8	2.17 ± 1.1	2.01 ± 0.6	6.28 ± 0.5	3.77 ± 0.7	3.13 ± 0.8	3.69 ± 0.9	1.95 ± 0.7	3.51 ±0.4	2.77 ±0.4	3.86 ± 0.6	1.94 ± 0.5	1.39 ± 0.4	1.22 ± 0.2
16:0	12.86 ± 1.7	13.51 ± 2.5	13.15 ± 2.1	10.79 ± 1.1	11.86 ± 2.4	14.72 ± 1.3	13.28 ± 1.7	12.86 ± 1.8	12.01 ± 2.5	10.51 ± 1.4	15.29 ± 1.0	14.51 ± 1.0	13.71 ± 2.3	14.39 ± 2.1
16:1n-7	33.39 ± 13.0	9.82 ± 6.3	17.18 ± 5.1	18.02 ± 2.3	17.39 ± 2.8	21.49 ± 7.8	27.27 ±8.2	16.27 ± 9.1	14.40 ±1.4	9.52 ± 2.4	17.98 ± 4.4	17.76 ± 4.7	14.25 ± 5.1	14.04 ± 4.1
18:0	2.24 ± 1.0	3.53 ± 0.9	2.14 ± 0.5	0.73 ± 0.1	1.46 ± 0.6	2.93 ± 0.6	2.42 ± 0.4	2.75 ± 1.1	2.33 ± 0.2	1.94 ± 0.6	2.99 ± 0.3	4.16 ± 0.8	1.61 ± 0.3	1.88 ± 0.4
18:1n-9	8.15 ± 3.7	8.93 ± 5.7	7.89 ± 1.1	5.89 ± 0.9	5.95 ± 1.6	10.98 ± 2.1	7.99 ± 1.4	13.22 ± 2.1	16.50 ± 2.3	13.23 ± 2.5	11.74 ± 1.1	11.28 ± 2.4	6.41 ± 1.9	6.85 ± 2.0
18:1n-7	8.09 ± 2.5	10.21 ± 4.8	10.34 ±0.9	2.68 ± 0.2	4.04 ± 1.1	9.95 ± 1.1	8.59 ± 1.0	7.51 ± 1.0	4.82 ± 0.4	6.46 ± 0.9	7.07 ± 0.9	11.38 ± 1.5	11.70 ± 0.7	12.51 ± 1.0
18:2n-6	0.73 ± 0.2	0.22 ± 0.1	0.73 ± 0.1	1.12 ± 0.2	0.53 ± 0.1	0.70 ± 0.2	0.61 ± 0.1	0.93 ± 0.2	0.67 ± 0.1	0.88 ± 0.1	0.95 ± 0.1	0.59 ± 0.2	0.64 ± 0.1	0.52 ± 0.1
20:1n-11	0.75 ±0.4	1.02 ± 0.6	0.64 ± 0.2	0.79 ± 0.2	0.87 ± 0.3	0.46 ± 0.4	0.19 ± 0.1	0.83 ± 0.3	0.93 ± 0.2	2.01 ± 0.7	0.40 ± 0.1	1.16 ± 0.4	0.95 ± 0.5	0.65 ± 0.5
20:1n-9	0.65 ± 0.2	9.92 ± 2.5	1.87 ± 0.7	13.02 ± 1.6	10.27 ± 4.4	1.59 ± 0.7	1.05 ± 0.3	3.84 ± 1.2	12.14 ± 2.4	10.05 ± 2.2	1.25 ± 0.4	0.97 ± 0.2	0.61 ± 0.2	0.66 ± 0.4
20:1n-7	2.03 ± 1.0	1.56 ± 0.8	1.31 ± 0.5	2.00 ± 0.7	3.12 ± 3.3	1.23 ± 0.3	1.13 ± 0.3	1.51 ± 0.4	1.39 ± 1.5	1.58 ± 0.4	1.12 ± 0.2	4.93 ± 2.2	1.35 ± 0.6	1.02 ± 0.9
20:4n-6	3.54 ± 1.9	2.82 ± 2.6	1.96 ± 0.5	0.21 ± 0.7	0.30 ± 0.2	2.30 ± 0.9	2.22 ± 0.8	3.94 ± 1.9	0.58 ± 0.1	0.85 ± 0.3	1.49 ± 0.3	2.22 ± 0.7	2.53 ± 0.5	2.45 ± 0.8
20:5n-3	10.24 ± 5.4	9.95 ± 7.8	16.68 ± 4.1	5.42 ± 1.1	8.19 ± 2.2	10.27 ± 3.5	14.1 ± 4.5	9.86 ± 1.2	5.18 ± 0.7	10.55 ± 2.7	12.29 ± 1.1	9.23 ± 1.6	19.90 ± 3.6	18.81 ± 2.5
22:1n-11	0.41 ± 0.3	2.18 ± 1.8	1.46 ± 0.9	13.23 ± 2.4	11.40 ± 4.4	0.43 ± 0.2	0.30 ± 0.2	1.37 ± 0.6	6.95 ± 1.5	2.78 ± 1.4	0.63 ± 0.3	0.10 ± 0.1	0.16 ± 0.1	0.26 ± 0.3
22:1n-9	0.16 ± 0.1	1.91 ± 0.9	0.46 ± 0.3	2.08 ± 0.4	3.82 ± 2.4	0.48 ± 0.2	0.23 ± 0.1	0.77 ± 0.2	1.92 ±0.3	1.35 ± 0.4	0.42 ± 0.1	0.31 ± 0.1	0.14 ± 0.1	0.16 ± 0.1
22:5n-3	0.77 ± 0.4	0.53 ± 0.4	1.73 ± 0.5	0.65 ± 0.1	0.80 ± 0.3	1.80 ± 0.5	0.79 ± 0.4	1.12 ± 0.4	1.10 ± 0.2	0.78 ± 0.1	1.01 ± 0.2	1.63 ± 0.6	3.03 ± 0.5	3.01 ± 0.5
22:6n-3	4.18 ± 2.9	10.25 ± 10.1	10.19 ± 3.3	6.56 ± 2.4	6.03 ± 3.0	7.46 ± 2.8	5.24 ± 1.7	9.77 ± 4.0	6.82 ± 1.3	12.18 ± 3.4	10.48 ± 1.4	5.96 ± 2.6	10.14 ± 3.7	12.25 ± 3.5

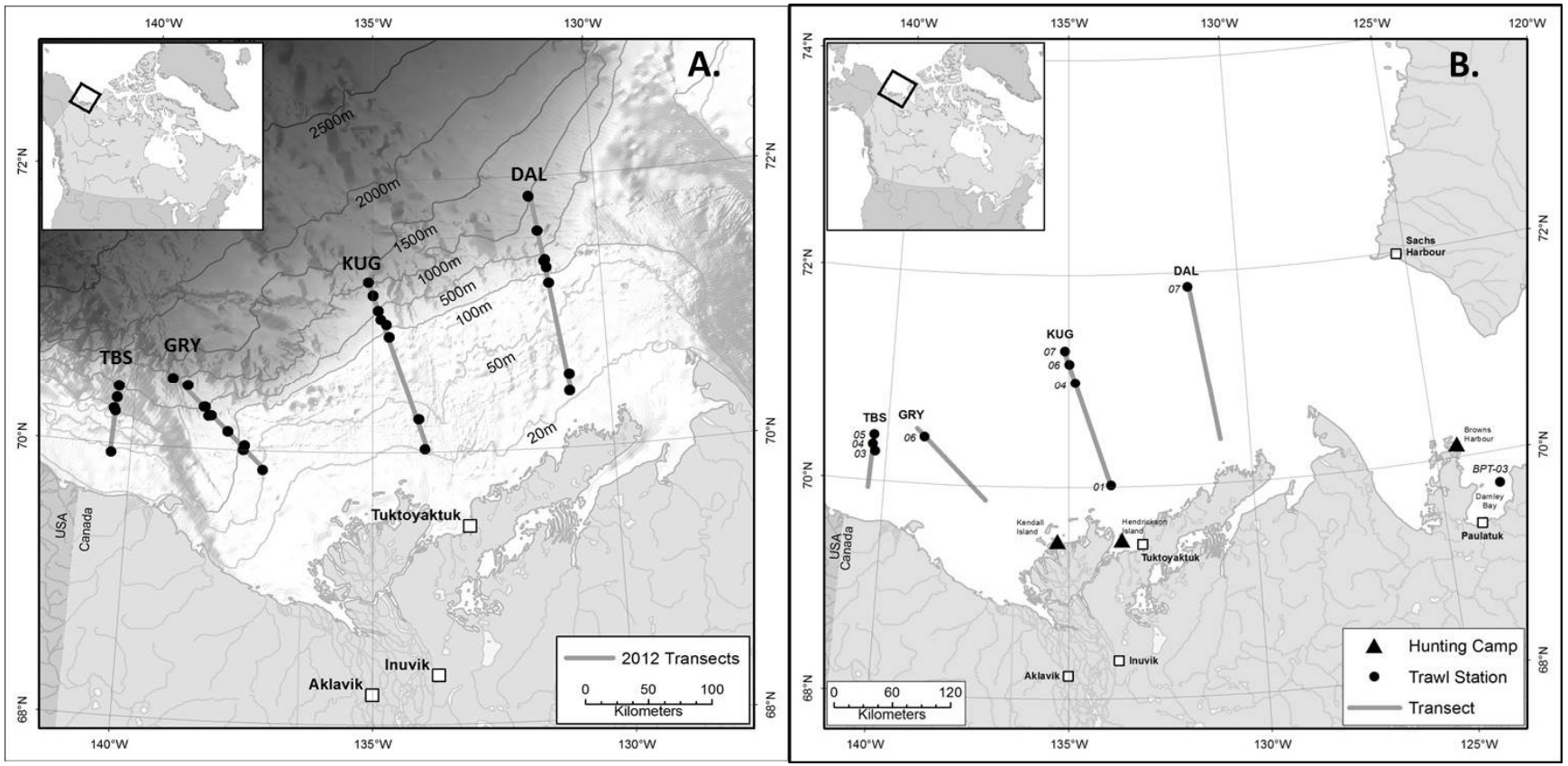

Figure 4.1. Study area in the Beaufort Sea ecosystem, including trawling stations for collection of fish species (A) as well as invertebrate species and capelin (B) from the Beaufort Regional Environmental Assessment Marine Fishes Program. Labelled transects are: transboundary-transect (TBS), Garry Island (GRY), Kugmallit Bay (KUG), Dalhousie (DAL), and Bennett Point (BPT). Beluga whale tissues were collected at traditional Inuvialuit hunting camps in the Inuvialuit Settlement Region, Northwest Territories, Canada.

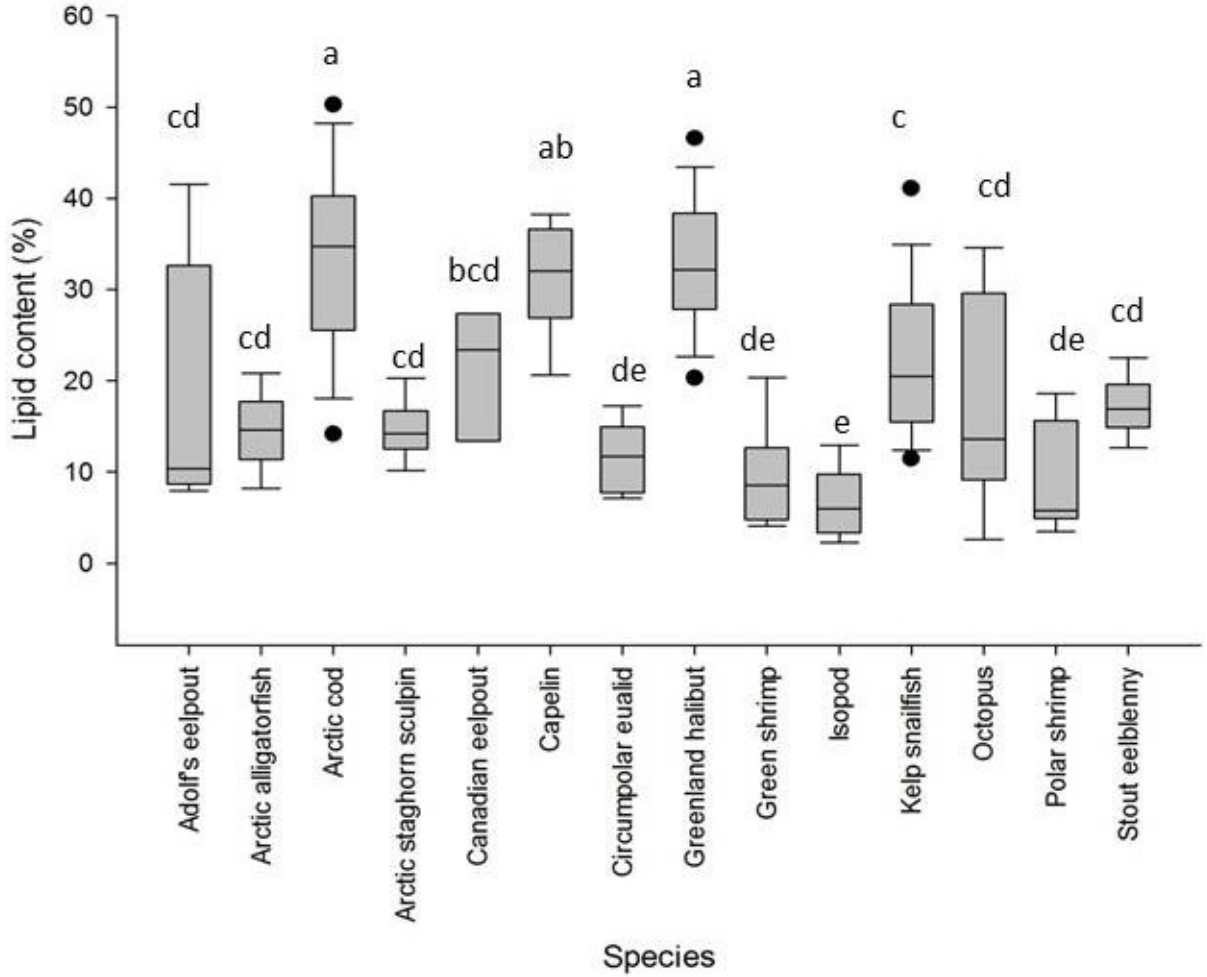

Figure 4.2. Boxplot of percentage lipid content of potential prey species of Beaufort Sea beluga whales. Boxes with the same letters are not statistically different (α=0.05) according to a Tukey HSD post-hoc test. Error bars define 10th and 90th percentiles. Black dots represent the 5 and 95% percentiles.

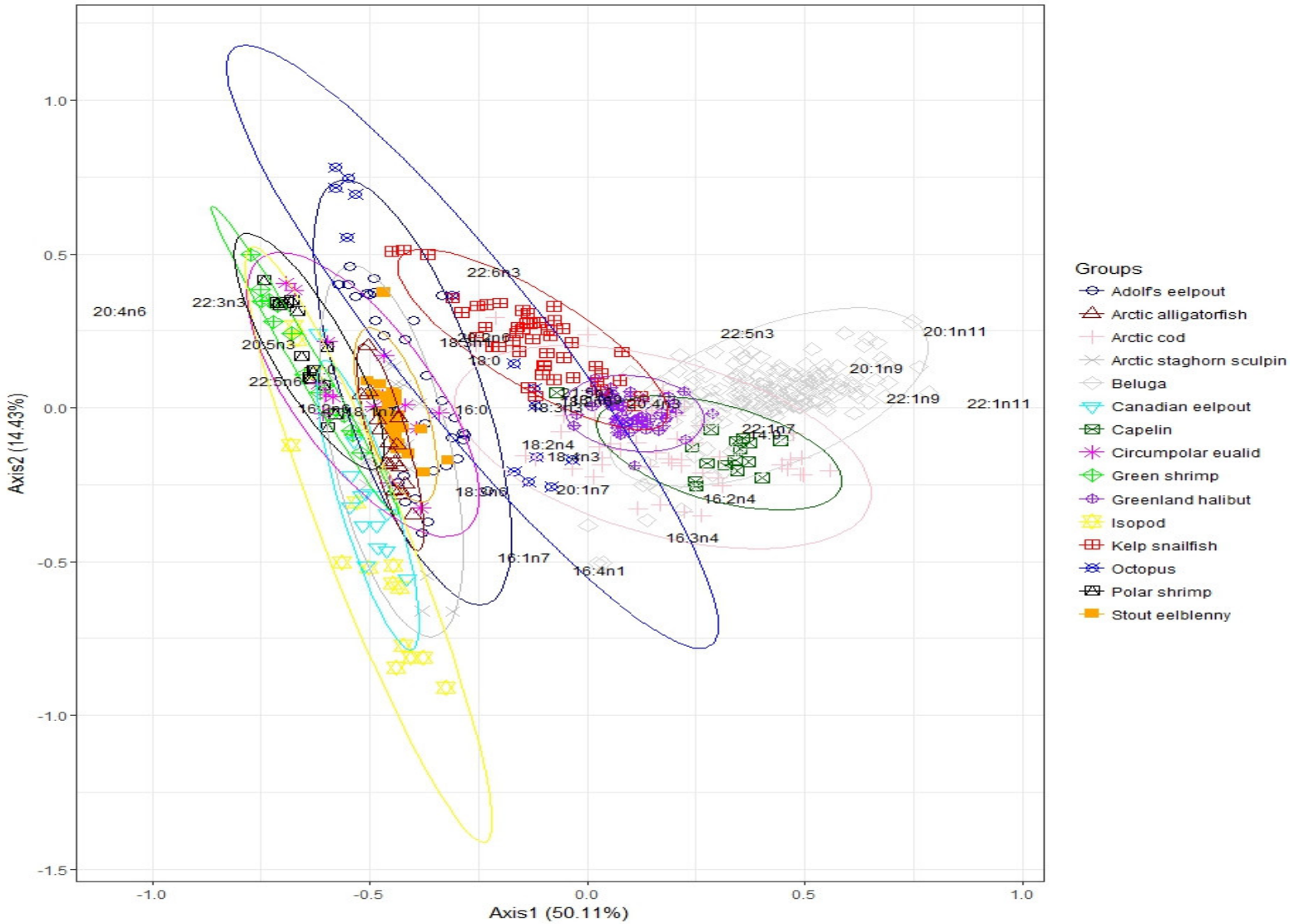

Figure 4.3. Biplot of correspondence analysis of fatty acid profiles from the inner blubber of Beaufort Sea beluga whales (*n*=178) and their potential prey species. The ellipses show the 95% confidence regions for the mean for each species.

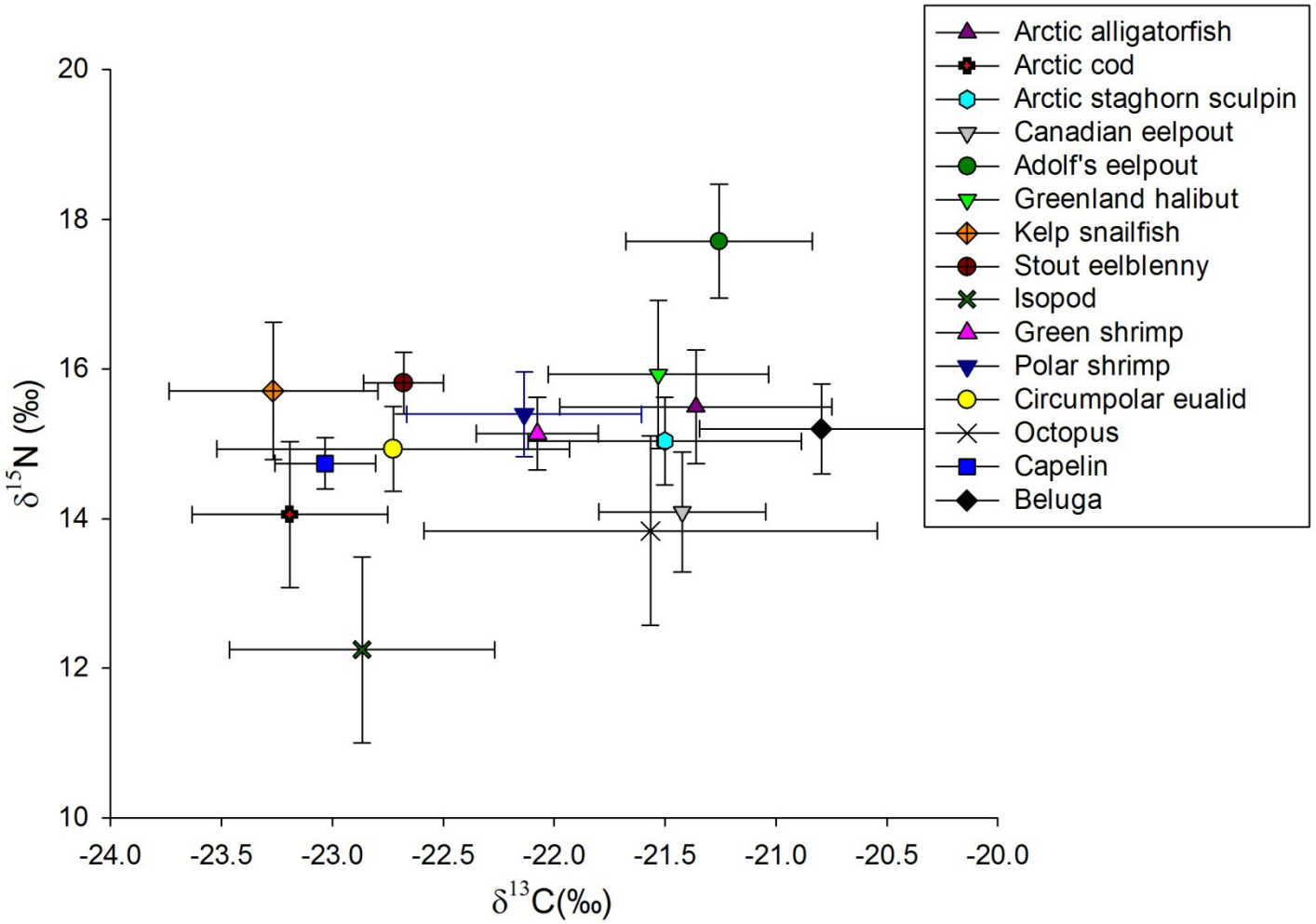

Figure 4.4. Mean carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of beluga whale liver tissues and potential prey species with ± 1 standard deviation error bars, corrected using a trophic enrichment factor (TEF) from killer whales (Caut et al. 2011).

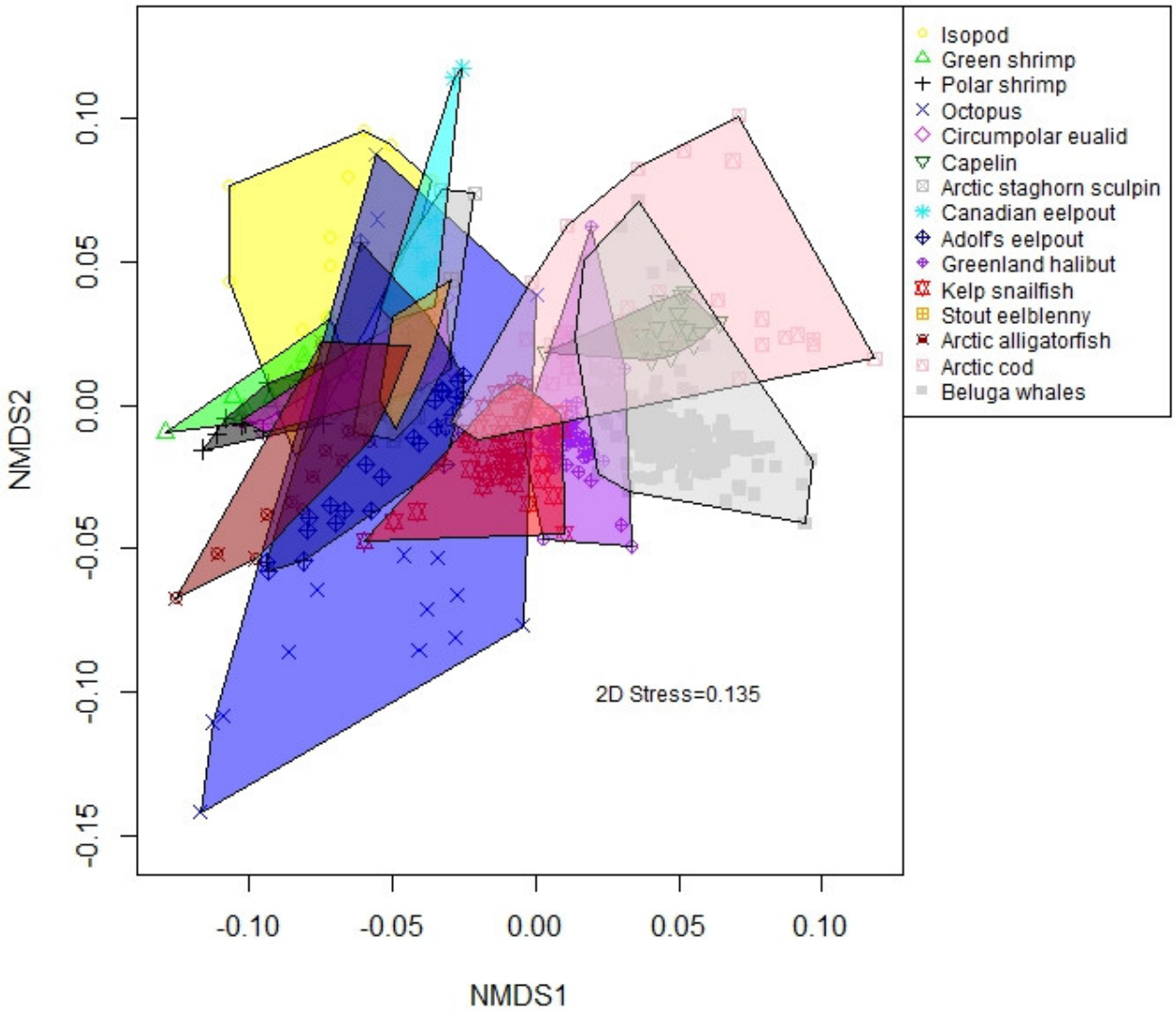

Figure 4.5. Non-metric multidimensional scaling (NMDS) ordination with goodness of fit (stress=0.14) based on the percentage of 32 fatty acids from eastern Beaufort Sea beluga whales and their potential prey. Orthogonal polygons define each group.

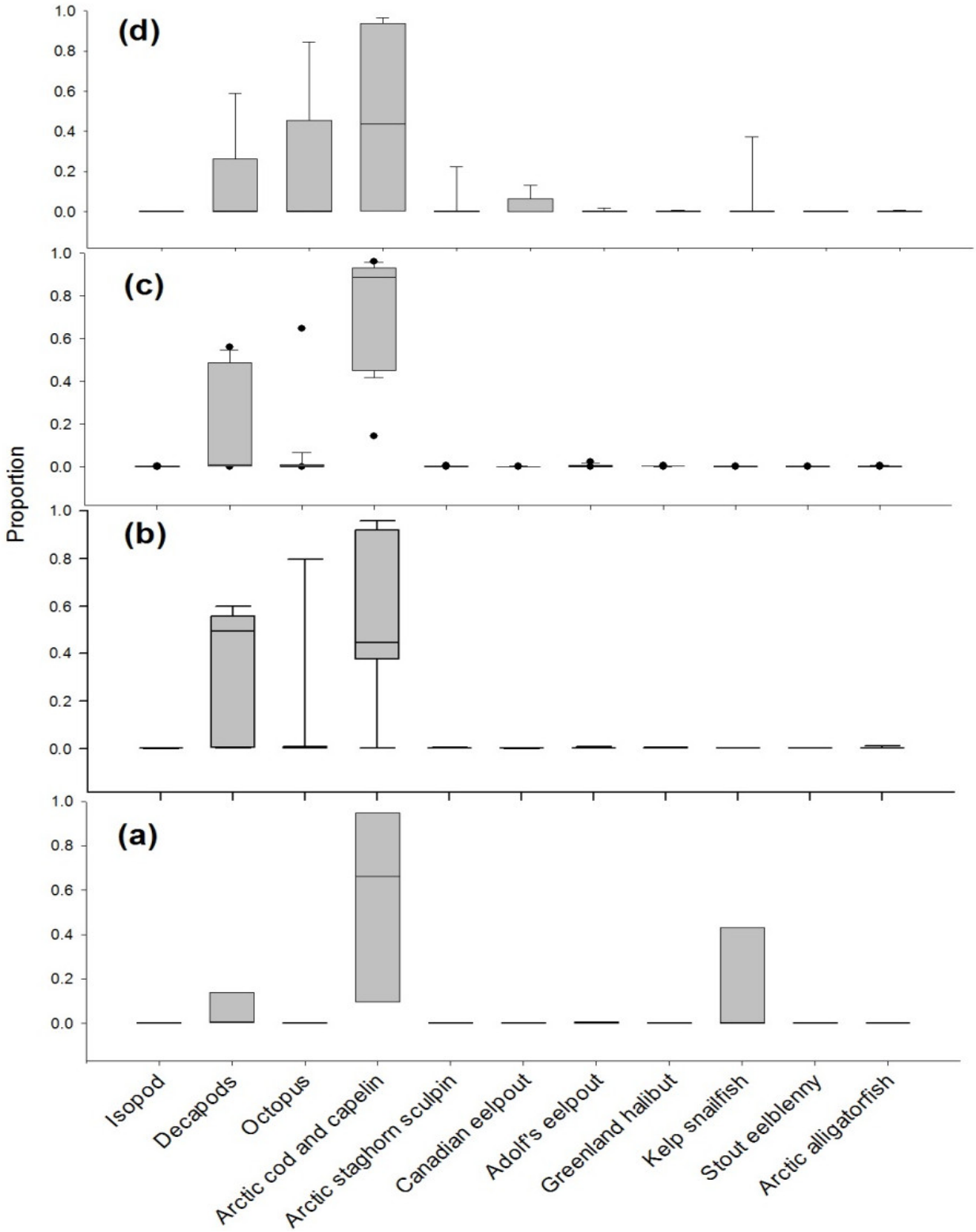

Figure 4.6. Boxplots of median diet estimates for 60 eastern Beaufort Sea beluga whales from 2011 to 2014 (a to d) using stable isotope trophic enrichment factors based on killer whales (Caut et al. 2011). Error bars define 10th and 90th percentiles. Black dots represent the 5 and 95% percentiles.

Chapter 5:

Declining body condition is associated with reduced oxygen storage capacity and estimated aerobic dive limits in beluga whales (*Delphinapterus leucas*)

Chapter Five: Manuscript Summary

Manuscript in preparation.

Citation:

Choy ES, Campbell KL, Berenbrink M, Roth JD, Loseto LL. Body condition affects oxygen storage capacity and aerobic dive limits in Beaufort Sea beluga whales. [in preparation]

Contributions:

Dr. Kevin Campbell provided guidance for myoglobin analysis, muscle buffering capacity, and myoglobin protein sequencing and provided comments throughout the writing process.

Dr. Michael Berenbrink provided the spectral deconvolution algorithm to correct myoglobin concentrations and provided comments throughout the writing process.

Dr. Lisa Loseto provided funding for field work and comments throughout the writing process.

Dr. Jim Roth provided comments throughout the writing process.

Emily Choy designed the study, collected samples from Kendall Island, analyzed all samples for myoglobin, muscle buffering capacity, hemoglobin, hematocrit, and myoglobin RNA, analyzed the data, produced all figures and tables, and wrote the manuscript.

Abstract

Arctic marine ecosystems are currently undergoing rapid environmental changes, and long-lived Arctic vertebrates with low reproductive rates are particularly vulnerable. Over the past 20 years, individual growth rates of beluga whales (*Delphinapterus leucas*) have declined, suggesting that this species may be an indicator of the physiological response of Arctic marine mammals to environmental change; however, scarcity of physiological data makes it difficult to gauge the adaptive capacity and resilience of the species. To address this shortcoming, we explored relationships between body condition and physiological parameters pertaining to oxygen storage capacity in 77 beluga whales from the eastern Beaufort Sea population. Mass specific total oxygen storage capacity averaged 62.6 mL kg^{-1}, while muscle myoglobin concentrations averaged 77.9 mg g^{-1} [83.9 mg g^{-1} using the Reynafarje (1963) method], one of the highest values reported for marine mammals. Males had higher total body oxygen stores than females due to larger body sizes and higher hemoglobin concentrations, consistent with their deeper foraging dives. In addition, blood hematocrit and hemoglobin concentrations, muscle myoglobin concentrations, and calculated aerobic dive limits (cADL) were positively related to indices of body condition. Consequently, environmental changes that negatively impact condition appear to be linked to decreases in breath-hold endurance, which may affect foraging efficiency and the ability to evade predators or escape ice entrapments. Importantly, the relationship between body condition and oxygen storage capacity may represent a positive feedback mechanism in beluga whales, in which environmental changes resulting in decreased body condition impair foraging efficiency, leading to further reductions in condition through diminished prey acquisition and/or increased foraging efforts.

Introduction

Arctic marine ecosystems are undergoing rapid change, with the Arctic Ocean predicted to be free of summer sea ice within the next few decades (Stroeve et al. 2007; Wang and Overland 2012). Long-lived Arctic vertebrates with low reproductive rates are particularly vulnerable, having evolved specialized behavioural, physiological, and morphological adaptations that have enabled their survival in Arctic environments (Gilg et al. 2012). The sensitivity of a species to climate change is assessed based on its adaptive capacity and resilience to environmental perturbations, which is determined by physiological limits, ecological traits, and genetic diversity (Williams et al. 2008; Huey et al. 2012). Unfortunately, for most wild populations there is a scarcity of information on physiological traits to evaluate phenotypic plasticity in order to predict a species' response to climate change (Williams et al. 2008; Hetem et al. 2014). This is important since animals that routinely operate at their maximum physiological capacity may be unable to compensate for declines in prey availability and environmental fluctuations (Costa et al. 2001)

Beluga whales (*Delphinapterus leucas*) exhibit a circumpolar distribution and are the most abundant Arctic species of toothed whales (Odontoceti), and are thus a potential indicator species for the response of Arctic marine mammals to climate change (Tynan and DeMaster 1997; Laidre 2008; Moore and Huntington 2008; Laidre et al. 2015). There are over 150,000 beluga whales worldwide (Jefferson et al. 2012), with approximately 40,000 individuals belonging to the eastern Beaufort Sea beluga population, one of Canada's largest (Allen and Angliss 2014). Habitat use of Beaufort Sea beluga whales is associated with sea ice and differs by size, sex, and reproductive status; large males use permanent pack ice in the Canadian Arctic Archipelago whereas small males and females select coastal and open-water habitat, cycling

between the Amundsen Gulf and Mackenzie Delta (Richard et al. 2001; Loseto et al. 2006). Differences in foraging strategies exist between sexes, as only male belugas venture into areas deeper than 600 m and have been documented to dive to over 500 m in Viscount Melville Sound and the Canadian Basin (Richard et al. 2001). The purpose of these deep dives is unknown, but is hypothesized to be for finding breathing holes in heavy ice pack (Richard et al. 1997), orientation by acoustic reckoning (Richard et al. 1998), or for foraging in deep-water feeding areas (Harwood and Smith 2002).

Recently, a decline in the growth rate of individuals has been observed in the beluga population over a twenty-year period, a trend hypothesized to have arisen from long-term environmental change (Harwood et al. 2014; Harwood et al. 2015). Loss of sea ice has been associated with reductions in body condition in ringed seals, *Pusa hispida* (Ferguson et al. 2017), polar bears, *Ursus maritimus* (Stirling and Derocher 2012), and mortality events in walrus, *Odobenus rosmarus* (Fischbach et al. 2009). Reductions in sea ice will not only affect habitat use of whales but has also facilitated the northward migration of temperate prey species that may displace Arctic cod, *Boreogadus saida* (Falardeau et al. 2014; McNicholl et al. 2016). As a result, beluga whales may have to adopt new foraging strategies to accommodate shifts in preferred (or traditional) prey abundance. Reductions in sea ice may also result in increases in predation pressure as well as in human activity. Already, an increase in killer whale (*Orcinus orca*) sightings corresponding to sea ice loss has been reported across the eastern Canadian Arctic (Higdon and Ferguson 2009). As a long-lived species reaching ages beyond 60 years old with a low reproductive rate, beluga whales may not be able to readily adapt to the challenges induced by climate change. Greater information on the flexibility of beluga whales for

physiological and behavioural adjustments is critical to understand their response to prey shifts and sea ice declines.

Emaciation in starved harbour porpoises (*Phocoena phocoena*) has been associated with declines in muscle mass in addition to blubber stores (Stegall et al. 1999; Koopman 2001). As muscle and body size influence the oxygen storage capacity of odontocetes (Noren and Williams 2000), the overall objective of our study was to estimate the capacity and partitioning of oxygen stores in Beaufort Sea beluga whales to determine physiological limits and examine their relationship with indices of body condition. We hypothesized that declines in body condition may have adverse physiological effects, such as lower oxygen storage capacity that may affect breath hold endurance and foraging efficiency. To address this question, we measured blood hematocrit and hemoglobin concentrations, and myoglobin concentrations in the major locomotory *longissimus dorsi* muscle, and assessed their relationships with two indices of body condition. We also determined indices of aerobic and anaerobic potential (lung, blood, and muscle oxygen storage capacity, and proton buffering capacity of *longissimus dorsi*, respectively) to establish whether the ability of males to perform deeper (and hence longer) foraging dives is associated with one or both of these parameters. A second objective was to examine the potential role of the spleen in augmenting blood oxygen stores in belugas. The spleen acts as a reservoir for red blood cells, contracting during diving to increase blood oxygen capacity, and the size of this organ has been linked to diving capacity in phocid seals (Cabanac et al. 1997; Cabanac 2002).We hypothesized that a larger spleen size may be associated with an increase in blood oxygen stores during diving bouts through higher circulating hematocrit and hemoglobin concentrations. Understanding the physiological limits and constraints of beluga whales will be useful for identifying individuals within the population that are most vulnerable to

environmental change, as well as for future conservation efforts directed at other marine mammals.

Methods

Sample collection

Samples were collected from 77 adult beluga whales (♀ = 20, ♂ = 57) harvested at Inuvialuit hunting camps in July to early August 2012-2014 at Hendrickson Island (69°50N,133°58'W), Kendall Island (69°49'N, -135°29'W), and East Whitefish (69°22'N, 133°37'W) in the Inuvialuit Settlement Region, Northwest Territories, Canada (Figure 5.1). Sex, standard length (straight line measurement from the tip of the rostrum to the fluke notch; Sergeant and Brodie 1969), maximum half girth (measured from the dorsal ridge to the approximate ventral midline), and fluke span (linear distance between fluke tips) were recorded for each specimen. Age was estimated by counting growth layer groups from teeth collected from lower jaws, in which one growth layer group (comprised of a dark and light layer) equals one year (Stewart et al. 2006). Teeth were cut and growth layer groups from the longitudinal midline sections were counted in three blind replicates by one reader using a binocular microscope.

Blood and tissue sample collection and analysis

Sixty whole blood samples (~2 mL) were collected from the carotid artery for hematocrit and hemoglobin determination. Hematocrit was determined on-site by centrifuging whole blood samples in 32 mm capillary tubes in duplicate for five minutes at 11 000 rpm using a micro-hematocrit centrifuge (SpinCrit, Brown, Indianapolis, IN, USA). Approximately 10 g of *longissimus dorsi* muscle from the dorsal ridge area of 75 individuals was also collected. All samples were placed in cryovials and immediately frozen in dryshippers containing vaporized

liquid nitrogen, and stored at cryogenic temperature (ca. -150°C) until analysis at Fisheries and Oceans Canada's Freshwater Institute and the University of Manitoba in Winnipeg, Canada. Whole blood was stored without an additive, but samples were well-mixed prior to freezing to ensure no clumping. A follow-up comparison was completed to determine if blood samples treated with heparin exhibited different hemoglobin concentrations than untreated samples from the same individuals (paired T-test, $n = 25$). Due to a dryshipper failure, myoglobin samples from 18 belugas briefly thawed but were immediately frozen at -20°C for 2 weeks before being stored at -80°C until analysis. We subsequently used this incident as an opportunity to examine the effects of storage conditions on muscle myoglobin concentration (i.e. test for differences between these individuals and the 57 specimens continuously stored at cryogenic temperatures). Whole spleens from 69 whales were removed and weighed to the nearest 0.1 g using a portable field balance (Ohaus compact Series CS2000). Visual inspection of the spleens revealed they were largely devoid of blood, suggesting they were contracted (Cabanac et al. 1997; Cabanac 2002).

Whole-blood samples were thawed, well mixed, and then analyzed for hemoglobin concentration (Hemoglobin Colorimetric Assay Kit, Cayman Chemical) according to the manufacturer's instructions. Briefly, in the presence of heme, the detection reagent reacts to create a by-product which absorbs between 560 to 590 nm. Therefore, an absorbance scan (560 to 590 nm in 1 nm steps) was conducted using a Biotek Synergy HT Multi-Mode Microplate Reader. Bovine hemoglobin (H2000-1G) provided by Sigma-Alrich was used to verify absorbance of peaks. All samples were run in triplicate.

Myoglobin analysis was performed using methods modified from Reynafarje (1963) and Noren and Williams (2000). Frozen muscle samples (ca. 0.5 g) were quickly minced, weighed to

the nearest 0.1 mg on an analytical balance (Mettler model AJ100), and placed directly into 15 mL glass mortars immersed in an ice bath. Five mL of chilled low ionic strength buffer (40 mM phosphate, pH = 6.6) were added and the samples homogenized for 1-2 minutes on ice using a hand drill with a tissue grinder. The remaining volume of buffer was added directly to the falcon tube for a final buffer-to-tissue ratio of 79.25 mL per g^{-1} wet tissue. Samples were centrifuged at 4°C and 28 000 g for 50 minutes. 10 mL of clear supernatant was then transferred to a round-bottom glass flask, which was rotated and bubbled at room temperature with pure CO for 8 minutes. Sodium dithionite (~0.02 g) was then added to ensure complete heme reduction and the bubbled solution rotated for 2 more minutes. An absorbance scan (500 to 700 nm in 1 nm steps) was conducted on ~2 mL of supernatant in a glass cuvette using an Ultrospec 70 spectrophotometer (Biochrom Ltd, Cambridge, UK). Peaks were verified using a myoglobin standard from equine muscle (M0630-1G, Sigma-Aldrich). To account for potential differences in percent water content among samples, water content was determined gravimetrically by measuring the difference in mass after tissues were oven-dried at 70°C for 24 hours. Myoglobin concentrations were subsequently calculated following Reynafarje (1963) and corrected to a water content of 75%. All samples were run in duplicate.

Myoglobin concentration determination using spectral deconvolution

Spectral deconvolution has been shown to improve the accuracy of myoglobin concentration determinations by separating additive peak components using a modified algorithm for heme proteins (Masuda et al. 2008). We thus employed a non-linear, iterative curve-fitting algorithm (Völkel and Berenbrink 2000) using SigmaPlot 12.0 software that used the optical spectra (500 and 700 nm) of known concentrations of pure carbonyl myoglobin, carbonyl hemoglobin, and reduced cytochrome C to assess their contributions to the measured

spectra of the diluted CO-equilibrated and reduced tissue extracts. Pure carbonyl myoglobin was obtained by reducing a small quantity of crystalline horse skeletal muscle metmyoglobin (Sigma M0630) with dithionite in extraction buffer that was equilibrated with CO. Carbonyl hemoglobin was obtained by lysing a few drops of human blood from a finger prick in 3 volumes of water followed by further dilution in extraction buffer. After centrifugation, the clear supernatant was equilibrated with CO. The concentrations of these standard solutions were obtained using extinction coefficients of 14.7 and 13.4 cm^{-1} mM^{-1} at 540 nm for myoglobin and hemoglobin, respectively (Masuda et al. 2008). The spectrum of a 1 mM solution of reduced horse skeletal muscle cytochrome C [which does not bind CO at physiological pH (Butt and Keilin 1962)], in a 1 cm path length cuvette from 500-700 nm was interpolated from data in Margoliash and Frohwirt (1959). A spectrum of diluted milk was used to mimic the sloping baseline absorption spectra of samples where some protein precipitation seemed to have occurred. The measured millimolar concentrations in the cuvette were converted to mg g^{-1} wet muscle (corrected to 75% water content) using the dilution factor of 20 ml g^{-1} wet muscle during extraction and the assumed relative molecular mass of myoglobin and hemoglobin subunits of 17000 g mol^{-1}, as in Reynafarje (1963).

Muscle buffering capacity

The buffering capacity of *longissimus dorsi* was determined following the procedures of Castellini and Somero (1981). Buffering capacity (β) is defined as the μmoles of base needed to change the pH of the homogenate by one pH unit per gram wet weight of muscle tissue (equivalent to 1 Slyke; Van Slyke 1922). Samples of frozen muscle (ca. 0.5 g) were quickly minced, placed in chilled 15 mL glass mortars, and homogenized in 10.0 mL of 0.15 M NaCl using a pestle and hand drill over ice. Sample homogenates were placed in a falcon tube and

equilibrated to 37°C in a water bath. The initial pH of the homogenate was recorded using an Accumet Basic AB 15 (Fisher Scientific) pH meter equipped with an Accumet 13-620-96 Micro glass combination pH electrode (Fisher Scientific). 40 µL aliquots of 0.2 M NaOH were sequentially added to the sample, the sample mixed, and the pH recorded (per aliquot) until a pH change of 1 unit had been observed (between pH 6 and 7). All samples were run in duplicate.

Body condition index

We determined the body condition index for maximum half girth using an approach similar to George et al. (2015) for bowhead whales (*Balaena mysticetus*), as the residuals of the best fitting model with length, age, and sex as predictors and using the corrected Akaike's information criterion (AICc) as selection criterion (Chapter 2). In addition, we used girth to length ratio as it is commonly used as a body condition index in other marine mammals (Trites and Jonker 2000; Sato et al. 2002).

Total body oxygen stores and calculated aerobic dive limits

Since the relationship between body mass and length for beluga whales is consistent across populations (Doidge 1990), we estimated the total body mass (kg) for males and females using allometric relationships for eastern Hudson Bay belugas determined by Doidge (1990):

$$\text{Mass}_{\text{females}}\ (\text{kg}) = 10^{-3.96} \times \text{length}\ (\text{cm})^{1.08} \times \text{maximum girth}\ (\text{cm})^{1.71} \quad \textbf{(1)}$$

$$\text{Mass}_{\text{males}}\ (\text{kg}) = 10^{-4.33} \times \text{length}\ (\text{cm})^{2.46} \times \text{maximum girth}\ (\text{cm})^{0.36} \quad \textbf{(2)}$$

Oxygen storage capacity was calculated as the total volume of usable oxygen stored in the lungs, blood, and muscle tissues (Kooyman 1989; Ponganis 2011). Oxygen stores in the lungs were estimated based on total lung capacity (TLC). TLC was calculated from body mass and the allometric equation for marine mammals (Kooyman 1973; Piscitelli et al. 2013):

$$\text{TLC}\ (\text{L}) = 0.135 \times \text{mass}\ (\text{kg})^{0.92} \quad \textbf{(3)}$$

As cetaceans inspire immediately before diving (Ponganis 2011), diving lung volume was assumed to equal TLC, and exploitable lung O_2 stores calculated by multiplying this value by an alveolar oxygen extraction efficiency of 15% that assumes a fractional oxygen concentration of 0.20 upon submergence and a value of 0.05 at the end of the dive (Kooyman 1973; Kooyman 1989; Ponganis 2011):

Lung O_2 stores (L) = TLC × 0.15 **(4)**

Muscle O_2 stores were calculated based on the equation:

Muscle O_2 stores (L) = [mass (kg) × 0.159] × [(myoglobin (g $100g^{-1}$) × 0.00134 (L O_2 g^{-1})] **(5)**

where 0.159 is the proportion of muscle mass in beluga whales (Sergeant and Brodie 1969) and 0.00134 is the oxygen binding capacity of myoglobin (L O_2 g^{-1}) (Kooyman 1989).

To calculate blood oxygen stores, blood volume (BV) was first estimated based on the equation:

BV (mL kg^{-1}) = 813 × hemoglobin (g mL^{-1}) – 38.6 (Snyder 1983; Noren and Suydam 2016) **(6)**

Total blood oxygen stores were determined assuming an initial arterial oxygen saturation of 95% and final arterial saturation of 20%, and an initial venous oxygen saturation that is 5 volume % (5 mL O_2 dL^{-1}) less than the initial arterial oxygen saturation and a final venous oxygen content of zero (Ponganis 2011). We also assumed 0.00134 L O_2 g^{-1} to be the oxygen binding capacity of hemoglobin (Kooyman 1989), and 0.33 and 0.67 as the estimated proportions of arterial and venous blood (Lenfant 1970):

Arterial O_2 (L) = [0.33 BV (mL kg^{-1}) × mass (kg)] × Hb (g mL^{-1}) × 0.00134 (L O_2 g Hb^{-1}) × (0.95 – 0.20 saturation) **(7)**

Venous O_2 (L) = [0.67 BV (mL kg^{-1}) × mass (kg)] × (arterial O_2 content – 5 vol %) **(8)**

To determine the calculated aerobic dive limits (cADL) for diving and swimming, we used two different oxygen consumption rate estimates. For deep dives, we employed 2× basal metabolic

rate (Kleiber 1975) as this has been suggested as the best approximate of diving metabolic rate (DMR) for several diving vertebrates including odontocetes (Noren et al. 2002; Noren and Suydam 2016):

$$DMR\ (mL\ O_2\ kg^{-1}\ min^{-1}) = 2 \times 10.13\ mL\ O_2\ min^{-1} \times mass\ (kg)^{-0.25} \quad \textbf{(9)}$$

Horizontal under-ice swimming metabolic rates (SwMR) were estimated using the total cost of transport (COT_{TOT}) equation of Williams (1999), which was determined using pinnipeds and cetaceans undergoing transit swimming at a steady rate while submerged for brief intervals. To estimate SwMR, COT_{TOT} was multiplied by the mean speed of migration of 85.0 m min^{-1} (5.1 km h^{-1}) for the Beaufort Sea beluga population (Richard et al. 2001):

$$SwMR,\ (mL\ O_2\ kg^{-1}\ min^{-1}) = 32.95\ mL\ O_2\ min^{-1} \times mass\ (kg)^{-0.29} \quad \textbf{(10)}$$

Using equations 9 and 10, cADLs were estimated as follows:

$$cADL_{dive}\ (min) = Total\ mass\ specific\ O_2\ stores\ (mL\ kg^{-1}) / DMR\ (mL\ O_2\ kg^{-1}\ min^{-1}) \quad \textbf{(11)}$$

$$cADL_{swim}\ (min) = Total\ mass\ specific\ O_2\ stores\ (mL\ kg^{-1}) / SwMR\ (mL\ O_2\ kg^{-1}\ min^{-1}) \quad \textbf{(12)}$$

We also evaluated the association between body condition and oxygen storage capacity, maximum dive depths, and durations. We used our cADLs for males and females with maximum, median, and minimum body condition indices to estimate the maximum depths of V-shaped and square-profile dives, the most common dives types observed in belugas (Martin and Smith 1992; Richard et al. 1997; Martin et al. 1998; Martin and Smith 1999). To calculate the maximum dive depth, $cADL_{dive}$ was multiplied by a maximum diving velocity of 1.97 m s^{-1} based on the maximum average ascent and descent rates recorded in High Arctic beluga whales from Martin and Smith (1992; descent: 2.20 m s^{-1}, ascent: 1.84 m s^{-1}), and Martin and Smith (1999; descent: 1.85 m s^{-1}, ascent: 1.97 m s^{-1}), and converted to metabolic rate assuming a caloric

equivalent of 2.01 x 10^4 J L^{-1} O_2 (Williams et al. 2011). Maximum velocities were used since both ascent and descent rates increase with dive depth (Heide-Jørgensen et al. 1998; Martin and Smith 1999; Hauser et al. 2015). To calculate dive depth, we assumed whales dove at a 30º angle based on previous approximations and assumptions for belugas (Martin and Smith 1992; Martin and Smith 1999). For square-profile dives, we allocated 50% of the $cADL_{dive}$ for bottom travel, since optimal foraging theory predicts that diving animals should maximize foraging time at depth and minimize depth transit times (Thompson and Fedak 2001; Hanuise et al. 2013). The maximum distance for travel under sea ice to locate breathing holes was also estimated using the mean speed of migration (5.1 km h^{-1}) of Beaufort Sea belugas (Richard et al. 2001) multiplied by $cADL_{swim}$. Published data on depth and dive durations for Beaufort Sea beluga whales (Richard et al. 1998) were included for comparison.

Myoglobin protein sequence determination

Due to the very high muscle myoglobin concentrations measured in this study (see Results), the coding sequence of the beluga myoglobin gene was determined in order to calculate the myoglobin net surface charge (Z_{Mb}) (Mirceta et al. 2013). Total RNA was extracted from approximately 50 mg of frozen muscle homogenate from a 33 year old adult male following the TRIzol method (Invitrogen Life Technologies Limited, Carlsbad, California). RNAs were dissolved in 50 µL of RNAse free water and checked for concentration and quality using a NanoDrop (2000c, Thermo Scientific, Wilminton, DE) and stored at -20°C until use.

Real time reverse transcriptase polymerase chain reactions (RT-PCR) were performed using SuperScript II RT by Invitrogen (Carlsbad, California) to create first strand cDNA using one Taq DNA polymerase (New England Biolabs) according to the supplied protocol using cycling parameters and conditions modified from Mirceta et al. (2013) and outlined in Appendix

5.1. The primers used to amplify and sequence the protein coding region of the myoglobin were (in the 5' to 3' direction): cetacean_Mb_forward (AGCTGTCGGAGCCAGGAYAC) and cetacean_Mb_reverse (GCCYCTCACAAACAAAGCAGG). Resulting amplicons (603 bp) were purified and extracted using the GeneJET Gel Extraction Kit (Thermo Scientific). Amplicons were prepared using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Life Technologies Limited, Warrington, UK) according to the manufacturer's instructions and sequenced using an Applied Biosystems 3130 Genetic Analyzer (Foster City, CA). The ZR DNA Sequencing Clean-up^{TM} Kit (Zymo Research) was used to purify the sequence prior to genetic analysis. Obtained sequences were aligned using Sequencher 5.1 software (Gene Codes Corporation, Ann Arbor, MI), the primary sequence deduced, and Z_{Mb} calculated following Mirceta et al. (2013).

Statistical analyses

Multiple linear regression models were used to assess the relationships among body condition indices, sex, age, and mass on hemoglobin concentrations, hematocrit, myoglobin concentrations, buffering capacity, spleen mass, and cADLs using the package nlme for linear models (Pinheiro et al. 2015). In addition, spleen mass was also included as a predictor variable in the models for hemoglobin concentration and hematocrit. Since mass was used to directly calculate oxygen stores and diving metabolic rates, it was not included as a predictor for cADL. Model selection was based on AICc. Two sample t-tests were used to assess differences in biochemical parameters and oxygen storage capacity between sexes. When assumptions were not met, the Wilcoxon-Mann-Whitney test was used. Plots of residuals and the Shapiro-Wilk test were used to ensure that the assumptions for normality, linearity, and homogeneity of variance

were met. Models were also assessed for multicollinearity using variance inflation factors (VIF<2.5) for each predictor. Significance was judged at $\alpha = 0.05$ for all statistical procedures. All statistical analysis were conducted using in R 3.2.5 (R Core Team 2016). Data are reported as mean ± 1 standard deviation.

Results

Sample treatment and storage

Myoglobin concentrations calculated from 57 individuals following the method of Reynafarje (1963) (Table 5.1; 83.9 ± 6.4 mg g^{-1}) were higher than those determined using spectral deconvolution (77.9 ± 5.4 mg g^{-1}) (paired t-test, $t_{56} = 22.10$, $p < 0.0001$). Mean muscle water content was 73.6 ± 1.7%; however, correction of myoglobin tissues to 75% water content did not affect total myoglobin concentration (paired t-test, $t_{56} = -0.87$, $p = 0.39$). Myoglobin concentrations generated using spectral deconvolution and corrected to 75% water content were used for further analyses with biological parameters and oxygen storage calculations.

Myoglobin concentrations determined via the spectral deconvolution algorithm from tissues stored for 2 weeks at -20°C (70.6 ± 7.6 mg g^{-1}, $n = 18$) were significantly lower than samples immediately stored at cryogenic temperatures (77.9 ± 5.4 mg g^{-1}, $n = 57$; two-sample t-test, $t_{73} = -4.53$, $p < 0.0001$). As a result, only samples that had been continuously stored at cryogenic temperatures were used in subsequent myoglobin and buffering capacity calculations. By contrast, blood samples that were frozen with (22.8 ± 3.2 g dL^{-1}, $n = 25$) or without heparin (22.8 ± 3.4 g dL^{-1}, $n = 25$) did not differ (paired t-test, $t_{24} = 0.21$, $p = 0.84$).

Oxygen storage capacity and maximum dive durations

Muscle buffering capacity ($n = 57$) in *longissimus dorsi* averaged 73.6 ± 5.0 Slykes (Table 5.1) and increased with myoglobin concentration ($F_{1,55} = 13.96$, $r^2 = 0.19$, $p = 0.0004$).

For whole blood (n = 60), hemoglobin concentrations averaged 23.0 ± 3.2 g dL^{-1} while hematocrit averaged 58.7 ± 8.8%. Hemoglobin concentration was significantly related to blood hematocrit ($F_{1,58}$ = 92.5, r_{adj}^2 = 0.61, p < 0.0001).

Total oxygen storage capacity of beluga whales averaged 55.8 ± 19.7 L (mass-specific oxygen stores: 62.6 ± 11.5 mL O_2 kg^{-1}), with greater oxygen stores in blood (Figure 5.2; mean 32.4 ± 14.1 L or 53.0 ± 9.7% of total oxygen stores) relative to muscle (14.4 ± 4.0 L; 27.7 ± 5.7%) and lungs (10.1 ± 2.4 L; 19.6 ± 4.0%). Due to a greater body mass, males (n=57) had significantly higher lung (two sample t-test, t_{75} = -8.82, p < 0.0001), blood (t_{58} = -4.81, p < 0.0001), and muscle (t_{55} = -7.15, p < 0.0001) oxygen stores than females (n= 20), and hence higher total body oxygen stores (65.2 ± 18.3 L) than females (36.5 ± 10.7 L; t_{43} = -4.69, p < 0.0001; Figure 5.2). Females had higher mass-specific oxygen stores than males in their lungs (Wilcoxon-Mann-Whitney test, Z = 6.03, p <0.0001) and blood (t_{58} = -2.19, p = 0.033), but did not differ in muscle (t_{55} = -0.07, p = 0.94) or total mass-specific oxygen stores (t_{43} = -1.46, p = 0.15). Similarly, males and females did not differ in the proportion of oxygen distributed in lungs (Wilcoxon-Mann-Whitney test, Z = 1.83, p = 0.063), blood (t_{43} = -1.60, p = 0.12), and muscle (t_{43} = 1.50, p = 0.14).

The cADLs of beluga whales were higher in males than females for both swimming (14.2 ± 2.8 min vs. 11.2 ± 2.2 min; t_{43} = -3.15, p = 0.003) and diving (17.5 ± 3.4 min vs. 14.1 ± 2.8 min; t_{43} = -2.96, p = 0.005). Males had lower estimated mass-specific diving metabolic rates (3.7 ± 0.2 mL O_2 kg^{-1} min^{-1} vs. 4.1 ± 0.2 mL O_2 kg^{-1} min^{-1}; Z = 6.03, p < 0.0001) and swimming metabolic rates (4.5 ± 0.3 mL O_2 kg^{-1} min^{-1} vs. 5.2 ± 0.3 mL O_2 kg^{-1} min^{-1}; Z = 6.03, p < 0.0001) than females.

Effects of age, sex, body mass, and condition on physiological parameters and oxygen stores

The model of best fit for maximum half girth was sex + age + length (Appendix 5.2; $F_{3,60}$ = 21.9, r_{adj}^2 = 0.50, p < 0.0001), with length (t = 4.58, p < 0.0001), age (t = 3.42, p = 0.001), and sex (t = 2.48, p = 0.016) as significant predictors (Table 5.3). Using the residuals as a body condition index (BCI) along with the other biological predictors, the model of best fit for hematocrit was BCI + spleen mass (Appendix 5.2; $F_{2,42}$ = 7.07, r_{adj}^2 = 0.22, p = 0.002), whereas that for hemoglobin concentration was BCI + spleen mass + age × sex ($F_{5,39}$ = 4.62, r_{adj}^2 = 0.29, p = 0.002). Hemoglobin concentration significantly increased with body condition (Figure 5.3; Table 5.3; t = 2.91, p = 0.0006) with a significant sex × age interaction (t = 2.22, p = 0.032), and decreased with age (t = -2.37, p = 0.023). Myoglobin concentration also increased with body condition (Figure 5.3; $F_{1,45}$ = 6.32, r_{adj}^2 = 0.10, p = 0.016). The model of best fit for cADL was BCI + age × sex for both swimming (Table 5.3; $F_{4,31}$ = 8.87, r_{adj}^2 = 0.47, p < 0.0001) and diving ($F_{4,31}$ = 8.32, r_{adj}^2 = 0.46, p = 0.0001). cADL increased with body condition (Figure 5.3D) and due to a significant age × sex interaction, decreased with age in females (r_{adj}^2 = 0.89, p = 0.0008).

The relationships between physiological parameters pertaining to oxygen stores with body condition were also supported by linear models fit with girth to length (GL) ratios. The model of best fit for hematocrit was GL + age + spleen mass ($F_{3,41}$ = 5.50, r_{adj}^2 = 0.24, p = 0.003) and for hemoglobin concentration was GL + spleen mass + age × sex ($F_{2,39}$ = 4.73, r_{adj}^2 = 0.30, p = 0.0018). Both blood hematocrit and hemoglobin concentration increased with GL ratio, but hemoglobin was also significantly predicted by age (Table 5.4; t = -3.37, p = 0.0017), sex (t = -2.05, p = 0.047), and age × sex (t = 2.55, p = 0.014). The best model for myoglobin concentration was GL + age ($F_{2,44}$ = 4.19, r_{adj}^2 = 0.12, p = 0.022), with myoglobin increasing

with GL (t = 2.75, p = 0.009). $cADL_{dive}$ and $cADL_{swim}$ were best fitted by GL + age × sex, with durations increasing with GL and decreasing with age in females (Table 5.3).

Both BCI and GL ratio did not affect *longissimus dorsi* muscle proton buffering capacity or spleen mass. Log buffering capacity was best fitted by mass ($F_{1,55}$ = 1.39, r_{adj}^2 = 0.01, p = 0.24). Log spleen mass was best fitted by age (t = -2.28, p = 0.027) + mass (t = 1.89, p = 0.064; $F_{2,54}$ = 4.74, r_{adj}^2 = 0.12, p = 0.013) (Figure 5.4). Although the spleen mass of males (202.1 ± 98.0 g, n = 51) was larger than females (134.9 ± 69.6 g, n = 18), spleens comprised the same percentage of total body mass for both sexes (~0.02 %).

Based on our empirical estimates, a male beluga whale with the observed minimum BCI could perform square-profile (303 m; Table 5.5) and V-shaped (605 m) dives at approximately half the maximum depth of the whale with the maximum body condition (square: 614 m; V-shaped: 1227 m, respectively), despite only an approximately 100 kg (10%) difference in body mass. The whale with the highest BCI also was estimated to travel twice as far (1657 m) under sea ice as the whale with the lowest BCI (814 m). Although males were predicted generally to have greater dive performance and to travel under sea ice farther than females, the female with the highest BCI had higher cADLs (swimming = 13.1 min; diving 16.3 min) than the male with the lowest BCI (9.6 and 11.8 min, respectively), despite being ~140 kg lighter (Table 5.5).

Discussion

With a mean concentration of 77.9 ± 5.4 mg g^{-1} [83.9 ±6.4 mg g^{-1} using Reynafarje (1963) method], beluga whales have some of the highest myoglobin concentrations reported in marine mammals (Kooyman and Ponganis 1998; Noren and Williams 2000; Ponganis 2011; Mirceta et al. 2013). Myoglobin concentrations in our study are notably higher than previous measurements for beluga whales (mean ± 1 standard error: 34.4 ± 0.39 mg g^{-1}, Noren and Williams 2000; 69.1

± 0.35 mg g^{-1}, Noren and Suydam 2016), possibly because our samples were stored immediately at cryogenic temperatures. Indeed, even a brief thaw followed by -20°C storage was enough to lower myoglobin concentrations by ~10%. Buffering capacities (73.6 ± 5.0 Slykes) from our belugas were lower than those measured in adults from the Chukchi population (mean ± 1 standard error: 84.3 ± 1.4 Slykes; Noren and Suydam 2016), but similar to previous measurements for beluga whales (74.2 ± 0.4 Slykes; Noren 2004). Myoglobin concentration in the *longissimus dorsi* of narwhals (*Monodon monoceros*) was measured at 78.7 ± 1.7 mg g^{-1} (Williams et al. 2011), which is similar to our values for beluga whales. The similarity in myoglobin concentration between the two species may be expected since the myoglobin primary sequence of beluga whales only differs from narwhals at a single site (47Lys→Arg) and thus has the same net protein surface charge and hence maximal predicted concentration (Mirceta et al. 2013). A recently published coding sequence of the beluga myoglobin gene (Genbank: KT191276.1) precisely matched our sequence.

Mass-specific oxygen stores of our belugas (62.6 ± 11.5 mL O_2 kg^{-1}) were higher than previous estimates of captive belugas (51.0 to 51.9 mL O_2 kg^{-1}; Shaffer et al. 1997; Noren et al. 2012) and belugas from the Chukchi Sea population (approximately 50 mL O_2 kg^{-1}; Noren and Suydam 2016), but similar to other deep diving delphinoid cetaceans such as the short-finned pilot whale, *Globicephala macrorhynchus* (68.3 mL O_2 kg^{-1}; Velten et al. 2013). In addition, the partitioning of oxygen stores in beluga whales (Fig. 5.2) is similar to other prolonged deep diving marine mammals such as Weddell seals (*Leptonychotes weddellii*), and sperm whales (*Physeter macrocephalus*), with most oxygen stored in blood (Kooyman and Ponganis 1998; Villegas-Amtmann and Costa 2010).

Total body oxygen storage capacity of belugas (55.8 ± 19.7 L) was lower than in narwhals (74.5 L; Williams et al. 2011); however, muscle stores in narwhals were calculated based on the fractional muscle mass (0.36; Goforth 1986) of bottlenose dolphins (*Tursiops truncatus*), which likely inflated the values since Arctic cetaceans have a greater proportion of adipose tissue than tropical species. Nonetheless, as Arctic marine mammals, the high oxygen storage capacity of belugas and narwhals may be an adaptation to sea ice. Submergence for long durations under sea ice would be particularly important for Beaufort Sea beluga whales during their winter migration (Richard et al. 2001) as well as for evading predators (Asselin et al. 2011) and searching for breathing holes to avoid ice entrapments (Heide-Jørgensen et al. 2014). Therefore, we conclude that beluga diving physiology is more similar to that of narwhals than previously appreciated, and that their specialized physiology for prolonged diving and navigating ice habitat make them particularly sensitive to climate change.

Our high myoglobin concentrations and oxygen storage capacities are supported by historical telemetry data on beluga diving capacity. According to telemetry data, the deepest and longest dive of Beaufort Sea belugas were to 1160 m and 25 minutes by a male in 1995, with the most frequent dives performed at 700 to 900 metres depths and lasting 15 to 20 minutes (Richard et al. 1997), which is within the range of the $cADL_{dive}$ of our whales (16.8 ± 3.5 min; range 9.8 to 24.0 min, n = 45) and estimated V-shaped dive depths (858 ± 180 m; range 502 to 1227 m, n = 45). Empirically derived ADLs based on blood lactate levels (9 to 10 min) during diving as well as cADLs (8 to 10 min) for swimming in two trained belugas (Shaffer et al. 1997) were lower than dive durations of wild Arctic belugas. Our cADLs for males ($cADL_{dive}$: 17.5± 3.4 min, $cADL_{swim}$: 14.2± 2.8 min) and females ($cADL_{dive}$: 14.1± 2.8 min, $cADL_{swim}$: 11.2± 2.2 min) were higher than those calculated for Chukchi Sea belugas ($cADL_{dive}$ males: 13.5 min; females 12.6

min; Noren and Suydam 2016), possibly due to higher myoglobin concentrations measured in Beaufort Sea belugas and slight difference in the calculations of total body oxygen stores. Buffering capacity was significantly related to myoglobin concentrations in belugas, as also found for bottlenose dolphins (Noren 2004). Although buffering capacity is considered to play only a role in a small fraction of marine mammal dives (Castellini and Somero 1981), the relatively high values for belugas may be particularly important in extending dive time during stressful events (e.g. evading predators, searching for breathing holes).

Our results reveal that sex and age affect blood oxygen concentrations in beluga whales, but not their muscle oxygen concentrations. Skeletal muscle myoglobin concentrations in beluga whale calves are reported to reach adult levels at approximately 14 months (Noren and Suydam 2016), which may explain the lack of relationship found between age and muscle myoglobin (and buffering capacity) in adult whales. By contrast, male beluga whales had higher hemoglobin concentrations than females, and due to their larger size had greater oxygen stores in blood, muscle, and lungs, resulting in an overall greater oxygen storage capacity. Captive beluga males also had significantly higher hematocrit and hemoglobin levels than females, which were also found to decrease with age (Norman et al. 2013). Therefore, differences in foraging depths between sexes are predominantly due to higher cADLs in males than females because of larger body sizes and higher blood oxygen stores. Another factor affecting metabolic rate and hematology is season, with captive whales having higher metabolic demands and lower red blood cell counts during the winter months (Norman et al. 2013; George and Noonan 2014). Since our whales were sampled during summer, our measurements may not reflect physiological parameters and oxygen storage capacities of whales during winter in the Bering Sea.

Based on our study, spleen size was not related to male/female differences or overall diving ability in beluga whales. Although the models of best fit for hematocrit and hemoglobin concentrations included spleen mass, it was not a significant predictor. The percent of body mass of the spleen in beluga whales is also similar to other cetaceans (0.02%), and therefore, it likely does not serve in a significant blood storage role (Cowan and Smith 1999; Berta et al. 2015). Accessory spleens have been hypothesized to serve as extra reservoirs to increase blood oxygen capacity during diving in some cetaceans (e Silva et al. 2014). Although not common, one beluga that was sampled had seven, albeit very small, accessory spleens.

Perhaps the most significant finding of our study was that body condition indices affected hematocrit and hemoglobin and myoglobin concentrations, and hence affected cADLs in beluga whales beyond what would have been anticipated from the associated decrease in body mass alone. As a result, whales in poorer body condition are predicted to have lower oxygen stores, which may compromise diving durations and foraging efficiency. Harbour porpoises lose epaxial muscle mass during starvation, hypothesized to be the result of protein catabolism and dehydration (Stegall et al. 1999; Koopman 2001). A body condition index based on maximum girth incorporates muscle mass (George et al. 2015); therefore, observed declines in myoglobin content with body condition may be associated with catabolism of lean muscle tissues. Considering there has been a twenty year decline in growth of individual body size as well as observed declines in blubber thickness (Harwood et al. 2014; Harwood et al. 2015), there may also be ongoing changes in the dive depths and/or breath hold endurance of beluga whales. Pacific beluga whales target dive depths that correspond with the peak abundance of Arctic cod (200 to 300 m; Hauser et al. 2015) but Beaufort Sea beluga whales also forage on decapods and octopus, which are found at depths between 200 to 1000 m (Quakenbush et al. 2014; Chapter 4).

Our estimates of maximum dive depths suggest that belugas with the lowest BCI are able to allocate less time to foraging (5.9-7.3 min) and only attain maximum depths between 303 to 375 m for square-profile foraging dives relative to those with the highest BCI (8.1-12.0 min; 417-614 m, Table 5.5). Because Arctic cod display a size-class gradient with depth, with peak biomass in the Canadian Beaufort Sea occurring between 350 m and 500 m (Majewski et al. 2016), beluga whales in poor body condition may not be able to attain the depths of the largest or greatest biomass of prey, and may be forced to feed on smaller fish, leading to reduced caloric consumption. Of note, males demonstrated a larger range in BCI than females, and hence their diving ecology may be more heavily impacted by climate change. Belugas in better physical condition may fare better under stressful circumstances such as evading predators or ice entrapments, as they are predicted to swim up to 2 times farther than whales with the lowest BCI values (Table 5.5).

Conclusions

Arctic beluga whales are specialized for prolonged deep diving and navigating under sea ice, and exhibit one of the highest myoglobin concentrations measured in marine mammals. The physiological profile of beluga whales as long-duration divers is supported by historical satellite telemetry data. The high oxygen storage capacity of belugas is similar to narwhals, the beluga's closest relative and only other surviving member of the family Monodontidae (Sergeant and Brodie 1969), and presumably is also a specialization to sea ice (Williams et al. 2011). Differences in foraging ability and habitat use between sexes appear to be due to higher oxygen storage capacity and cADLs in males as a result of larger body sizes and hemoglobin concentrations. Notably, belugas of both sexes in better physical condition may perform better under stressful circumstances (i.e. exhibit lower mortality) such as evading predators or ice

entrapments. The relationships between indices of body condition and myoglobin, hemoglobin, hematocrit, and cADLs, raises concerns that declines in prey quality that reduce fitness may result in a decrease in oxygen storage capacity and foraging ability. Considering the influence on oxygen storage capacity, the observed decline in growth rate of individual body size as well as diminishing sea ice may have a confounding effect on beluga whales, and should be investigated in future monitoring efforts. The relationship between body condition and oxygen storage capacity may represent a positive feedback mechanism in beluga whales, in which environmental changes resulting in decreased body condition impair diving ability leading to further reductions in condition through diminished prey consumption and/or increased foraging efforts, and a heightened mortality risk due to predation and ice entrapment.

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Table 5.1. Biological, morphometric, and physiological parameters of oxygen storage capacity (mean ± 1 standard deviation) for male (*n* =57) and female (*n* =20) Beaufort Sea beluga whales. 'Corrected' refers to myoglobin concentrations that have been determined using spectral deconvolution.

Sex	Age (years)	Length (cm)	Mass (kg)	Fineness ratio	Fluke Span (cm)	Myoglobin [mg/g]	Myoglobin [mg/g] (corrected)	Buffering Capacity (Slykes)	Hemoglobin [g/dl]	Hematocrit (%)	Spleen Mass (g)
F	41.8± 11.7	372.1± 23.9	599.8±124.3	5.7±0.4	88.0 ±12.4	83.2±7.9	77.8± 5.8	72.4±6.0	21.2±3.4	55.0±7.3	134.9±69.6
M	28.8±8.2	419.6 ±26.2	952.7±164.7	5.7±0.5	99.1±14.6	84.1±5.8	77.9± 5.3	74.0±4.6	23.4±3.0	59.6±8.9	202.1±98.0
All	31.5±10.4	407.2± 33.0	861.1±219.3	5.7±0.5	96.2±14.8	83.9±6.4	77.9±5.4	73.6±5.0	23.0±3.2	58.7±8.8	184.6±95.7

Table 5.2. Multiple linear regression model results for a body condition index based on maximum girth in Beaufort Sea beluga whales. Results are presented for the most parsimonious model based on AIC_c.

Dependent	Predictor	Value	*t*	*p*
Girth (cm)	Intercept	4.00	0.24	0.81
	Age	0.48	3.42	0.001
	Sex	10.33	2.48	0.016
	Length	0.21	4.58	<0.0001

Table 5.3 Multiple linear regression model results for the effects of biological variables including body condition index (BCI) on oxygen storage parameters and calculated aerobic dive limits (cADL) in Beaufort Sea beluga whales. Results are presented for the most parsimonious model based on AIC_c.

Dependent	**Predictor**	**Value**	***t***	***p***
Hemoglobin [g/dl]	Intercept	28.88	8.91	<0.0001
	BCI	0.13	2.91	0.0006
	Spleen mass	-5.00×10^{-03}	-1.27	0.21
	Age	-0.17	-2.37	0.023
	Sex	-5.06	-1.45	0.16
	Age×Sex	0.20	2.22	0.032
Hematocrit (%)	Intercept	58.03	21.13	<0.0001
	BCI	0.48	3.66	0.0007
	Spleen mass	5.00×10^{-03}	0.41	0.69
Myoglobin [mg/g]	Intercept	77.81	104.36	0.000
	BCI	0.19	-2.51	0.016
Log[buffering capacity(Slykes)]	Intercept	4.26	123.40	<0.0001
	Mass	4.53×10^{-05}	1.18	0.24
Log[spleen mass (g)]	Intercept	5.08	16.44	0.000
	Age	-0.01	-2.28	0.027
	Mass	5.00×10^{-04}	1.89	0.064
Log[$cADL_{dive}$ (min)]	Intercept	3.06	17.33	<0.0001
	BCI	0.01	3.65	0.001
	Age	-0.01	-2.55	0.016
	Sex	-0.32	-1.57	0.13
	Age×Sex	0.014	2.62	0.014
Log[$cADL_{swim}$ (min)]	Intercept	2.83	15.91	<0.0001
	BCI	0.01	3.71	0.0008
	Age	-0.01	-2.53	0.017
	Sex	-0.32	-1.57	0.13
	Age×Sex	0.015	2.71	0.01

Table 5.4. Multiple linear regression model results for the effects of biological variables including girth to length ratio (GL) on oxygen storage parameters and calculated aerobic dive limits (cADL) in Beaufort Sea beluga whales. Results are presented for the most parsimonious model based on AIC_c.

Dependent	**Predictor**	**Value**	***t***	***p***
Hemoglobin [g/dl]	Intercept	16.72	3.08	0.004
	GL	55.58	2.98	0.005
	Age	-0.25	-3.37	0.002
	Sex	-7.05	-2.05	0.047
	Spleen mass	-0.01	-1.20	0.24
	Age×Sex	0.23	2.55	0.014
Hematocrit (%)	Intercept	5.26	0.38	0.70
	GL	205.96	3.96	0.0003
	Age	-0.14	-1.14	0.26
	Spleen mass	0.01	0.40	0.69
Myoglobin [mg/g]	Intercept	58.16	7.12	<0.0001
	GL	85.69	2.75	0.009
	Age	-0.13	-1.74	0.09
Log[$cADL_{dive}$ (min)]	Intercept	2.22	6.82	<0.0001
	GL	3.89	3.39	0.002
	Age	-0.02	-3.63	0.001
	Sex	-0.46	-2.25	0.032
	Age×Sex	0.02	2.97	0.005
Log[$cADL_{swim}$ (min)]	Intercept	1.97	6.01	<0.0001
	GL	3.95	3.40	0.002
	Age	-0.02	-3.61	0.001
	Sex	-0.47	-2.25	0.032
	Age×Sex	0.02	3.06	0.005

Table 5.5. Estimates for maximum dive depths for common dive types of beluga whales and calculated aerobic dive limits (cADL) for swimming and diving of beluga whales at different indices of body condition (BCI). Dive depths were assumed to be performed at maximum vertical transit speeds of 1.97 ms^{-1} and at 30° angles. Foraging time was estimated at 50% of the cADL for diving based on optimal foraging theory. Population estimates for dive depths and max ADL were from Richard et al. (1997). The maximum swimming distance under sea ice in order to locate breathing holes was estimated by multiplying the $cADL_{swim}$ as the maximum breath hold endurance (minutes) by the mean speed of migration (85.2 m min^{-1}) (Richard et al. 2001).

	BCI	**Mass (kg)**	**cADL (min)**		**V-shaped dive**	**Square profile dive**		**Max swimming distance under sea ice (m)**
			Swimming (min)	Diving (min)	Max depth (m)	Foraging time (min)	Max depth (m)	
Population estimates	N/A		N/A	25	700-900	5-8	15-600	N/A
Male	28.17	1091.7	19.5	24.0	1227	12.0	614	1657
	-1.40	851.5	14.1	17.5	898	8.8	449	1201
	-23.13	991.2	9.6	11.8	605	5.9	303	814
Female	9.87	852.9	13.1	16.3	833	8.1	417	1114
	-1.06	709.5	9.6	12.0	502	6.0	307	816
	-10.49	514.1	11.6	14.7	751	7.3	375	984

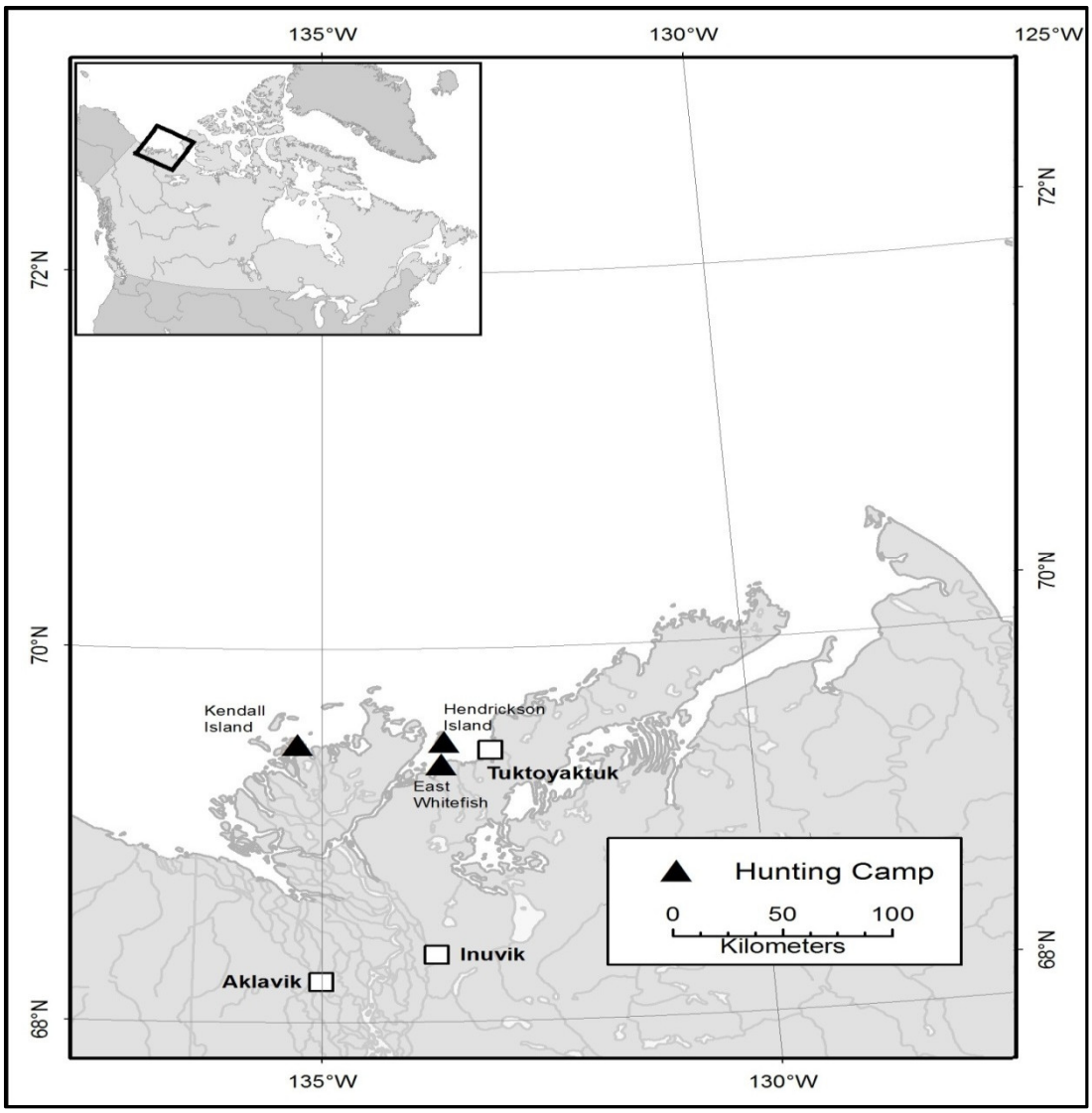

Figure 5.1. Sample collection sites for beluga whale tissues at traditional Inuvialuit hunting camps (triangles) located in the Inuvialuit Settlement Region, Northwest Territories, Canada.

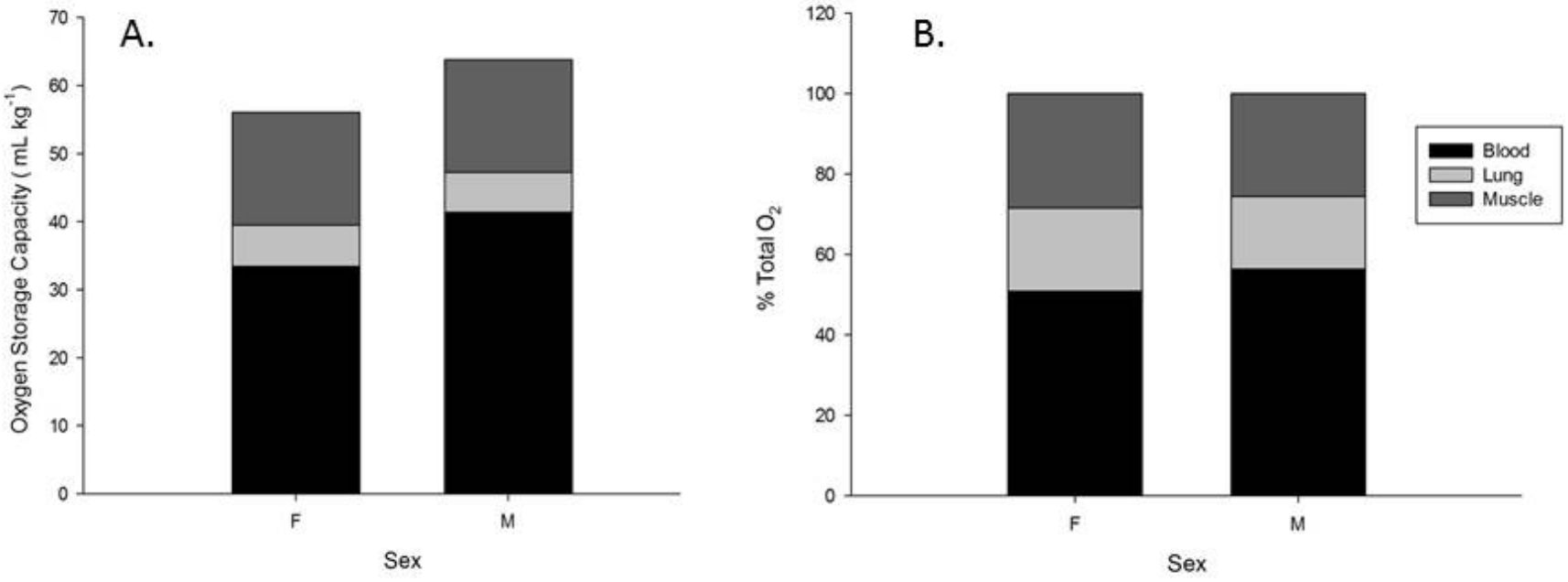

Figure 5.2. Comparison of oxygen storage capacity and distribution in male (M; *n* = 35) and female (F*; n* = 10) beluga whales.

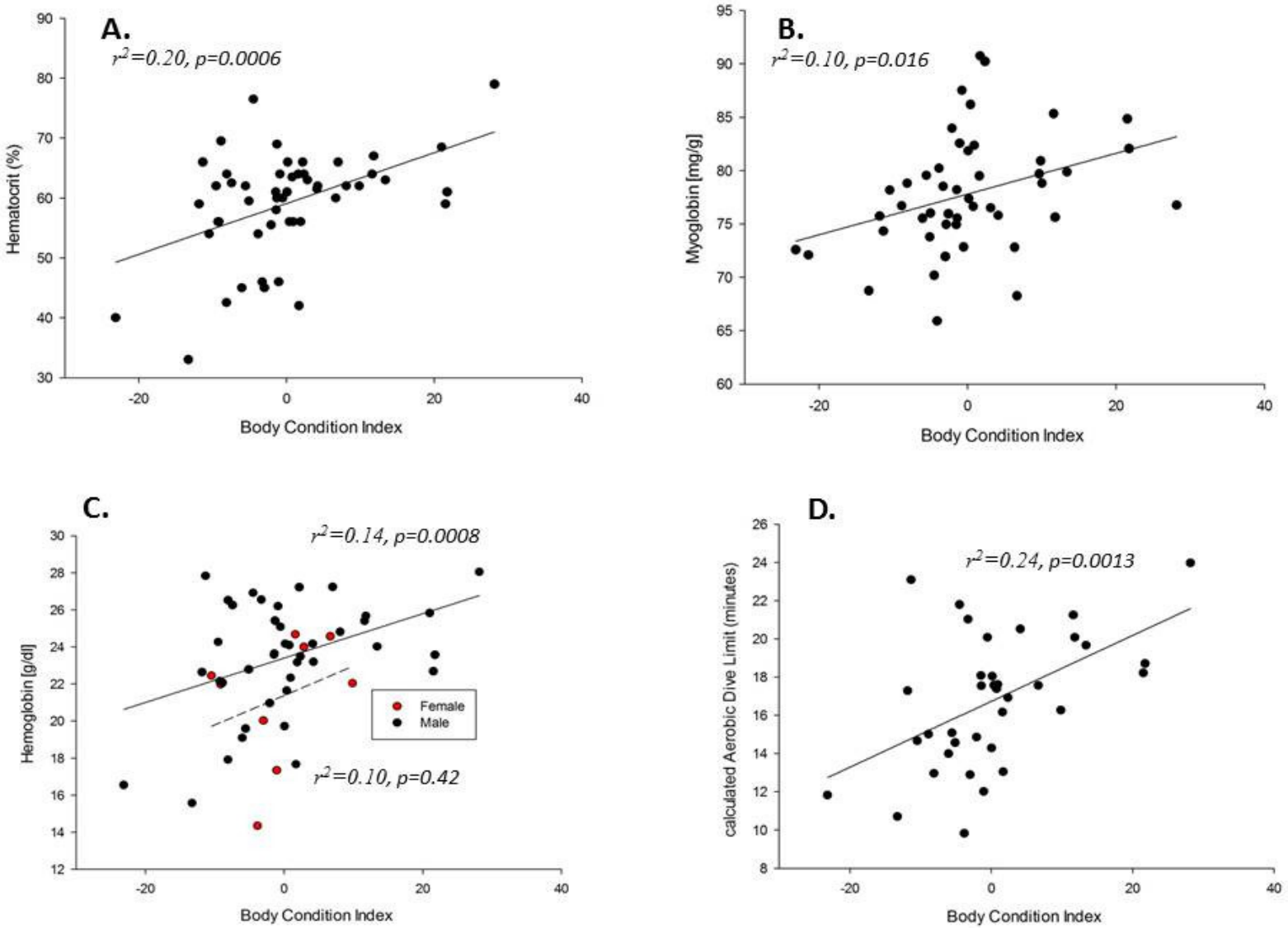

Figure 5.3. Relationships between a body condition index based on maximum girth and physiological parameters pertaining to oxygen storage capacity, including blood hematocrit (A), myoglobin concentrations (B), hemoglobin concentrations (C) and calculated aerobic dive limits (D) in Beaufort Sea beluga whales.

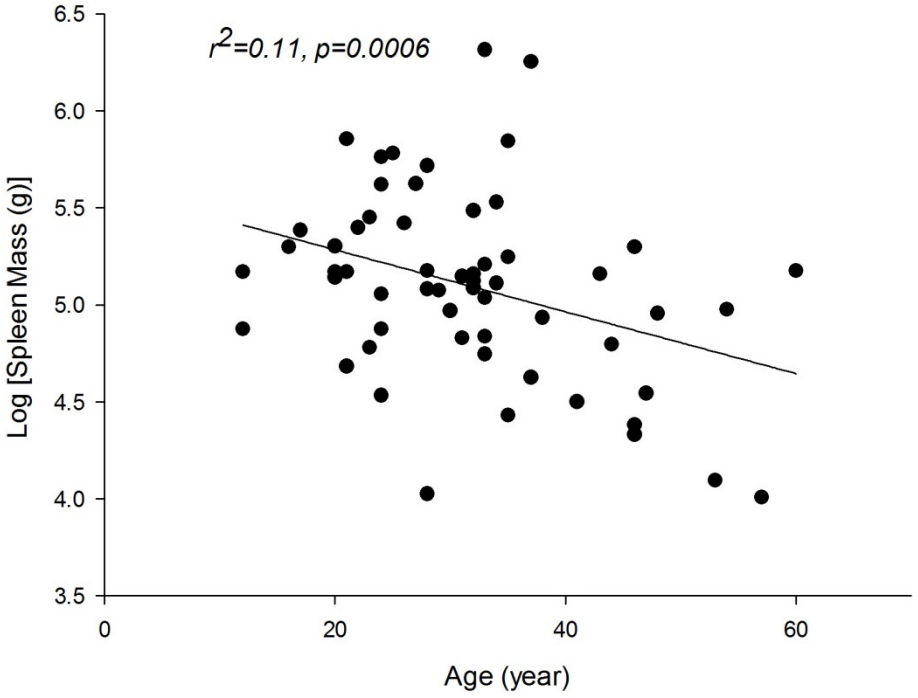

Figure 5.4. The relationship between log[spleen mass (g)] and age for Beaufort Sea beluga whales (n = 57).

Conclusions

Information on feeding ecology, physiology, and physical condition is logistically difficult to obtain on beluga whales and other Arctic marine mammals, but is imperative for assessing their vulnerability to climate change. This thesis provides comprehensive data on the interconnectedness of diet, physical condition, environmental conditions, and physiology in Beaufort Sea beluga whales, and thus is a distinct contribution to our understanding of the response(s) of Arctic vertebrates to climate change. Beluga whales that are in better condition have higher oxygen stores, allowing for prolonged duration dives and underwater submergence, which is critical for navigating sea ice and surviving Arctic marine ecosystems. Individuals that are less fit have shorter dives and may be unable to forage efficiently for their preferred prey. The main prey of belugas is Arctic cod, which display a size-depth gradient (Geoffroy 2016), but they also feed on decapods and octopi. My research also highlights some of the challenges of using fatty acid signatures and stable isotope ratios to identify dietary linkages in predators. In ecological studies, these tracers are often applied to wild populations without extensive method development or adequate testing through empirical studies, which may lead to inaccurate interpretations.

Chapter 1 highlights the importance of sample preparation methods on stable isotope ratios. Although novel tools are available for measuring ecological niche breadth and metrics (Turner et al. 2010; Jackson et al. 2011; Layman et al. 2012) and estimating predator diets (Moore and Semmens 2008; Parnell et al. 2013), the value of their interpretation is compromised if there is no consensus on sample treatment methods among different studies. Lipids and carbonates affect $\delta^{13}C$ and $\delta^{15}N$ values of beluga whales and their potential prey. The discrepancies between these treatments were apparent using estimates of isotopic niche breadth

and overlap, which are valuable tools with several ecological applications, such as assessing the impacts of invasive species (Jackson et al. 2012). As a result, $\delta^{13}C$ values were corrected for lipid content in my subsequent chapters.

In **Chapter 2,** my research demonstrated that body condition and dietary tracers in beluga whales displayed inter-annual variation that may be driven by environmental conditions and prey fluctuations. During this study, the greatest loss of sea ice observed in the western Arctic occurred in 2012 (Perovich et al. 2012). Coincidentally, dietary tracers among sex-and-size classes overlapped in 2012, but displayed greater differences in 2013, supporting the findings that sea ice extent and spring conditions influenced the distribution and habitat-use of beluga whales in 2012 and 2013 (Hornby et al. 2016). In addition, the biomass of Arctic cod, the primary prey of beluga whales, was lowest in 2014 (Geoffroy 2016). Incidentally, body condition of beluga whales based on maximum girth was highest in 2012 and lowest in 2014. Open-water conditions and low sea ice extent are favourable to body condition indices in bowhead whales (George et al. 2015), one of the three cetaceans (including belugas and narwhals) that are endemic to the Arctic. *Calanus* markers 20:1n-9 and 22:1n-11, the predominant fatty acids in Arctic cod, exhibited the most inter-annual variation in blubber, supporting the hypothesis that fluctuations in Arctic cod biomass influenced fatty acid signatures in beluga whales.

In **Chapter 3**, the Bayesian mixing model Fatty Acid Source Tracking Algorithm in R (FASTAR) was able to predict herring as the dominant prey in two captive beluga whales. However, **Chapter 3** also highlights the challenges of using fatty acid signatures to identify predator diets. Using qualitative multivariate analysis, the fatty acid signatures of belugas were more similar to herring and salmon than capelin and squid; however, salmon was not directly

consumed by either whale. High within-species variability and low sample sizes also affected diet estimates, by causing difficulties in differentiating prey based on fatty acid signatures. The accuracy of the Bayesian estimates relative to the actual dietary proportions supports the application of Bayesian mixing models as promising tools to reconstruct the diets of Arctic beluga whales and other free-ranging marine mammals.

Based on the results from **Chapter 3**, I applied Bayesian mixing model "FASTAR" to identify the offshore diets of Beaufort Sea beluga whales in **Chapter 4**. To examine the importance of prey quality and the hypothesis that belugas prefer lipid-rich prey, lipid content was measured and compared among different prey. Qualitative multivariate analysis and Bayesian mixing model FASTAR were used to assess the potential prey of individual whales using fatty acids signatures and $\delta^{13}C$ and $\delta^{15}N$ values. Beluga whales fed on pelagic species with relatively high lipid content, such as Arctic cod, capelin, and Greenland halibut. However, the $\delta^{13}C$ values in beluga whales were more similar to benthic than pelagic species. Discrepancies in $\delta^{13}C$ values may be due to the whales feeding on Arctic cod in the Bering Sea during their spring migration. FASTAR identified Arctic cod and capelin as the dominant prey of beluga whales, with decapods and octopus also identified as common prey. Diet estimates varied by year, with a greater diversity in beluga diet in 2014, coinciding with a lower abundance and biomass of Arctic cod. These diet estimates are supported by stomach contents of Beaufort Sea beluga whales during their spring migration from Alaska (Quakenbush et al. 2014).

Chapter 5 examined the relationship between body condition and physiology in Beaufort Sea beluga whales. Beluga whales have one of the highest myoglobin concentrations measured in marine mammals, supporting their physiological profile as deep divers with the ability for prolonged underwater submergence. Males had higher oxygen storage capacities than females

due to their larger body size and higher hemoglobin concentrations, which explains differences in dive depths recorded between sexes (Richard et al. 1997; Richard et al. 1998). Intriguingly, positive correlations between body condition and physiological parameters pertaining to oxygen storage capacity suggest belugas that are in better physical condition have greater diving ability. Considering that body size and individual growth rates of beluga whales have declined over twenty-year period, oxygen storage capacity and aerobic dive limits may have also decreased as a function of body mass. As a result, the depths of foraging dives and distances travelled under-ice to locate breathing holes may have also been compromised in the EBS beluga population.

Overall, the consequences of climate change on Beaufort Sea beluga whales remain difficult to predict due to the effects of several confounding factors. Increases in open-water conditions due to declining sea ice will allow greater access to habitats and feeding areas for the beluga population, which will favour physical condition, but will also lead to the northward expansion of subarctic competitors that will adversely affect Arctic cod. Although capelin may serve as an alternative prey source and was indistinguishable from Arctic cod based on fatty acid signatures, collapses of capelin stocks have negatively impacts on marine predators (Gjøsæter et al. 2009) and thus, capelin may not be a sustainable prey source for beluga whales. Therefore, although increases in open-water conditions may increase habitat-use and improve physical condition of whales, considering the long-term declines in beluga growth rates, declines in Arctic cod and erratic annual sea ice conditions may be having an opposite effect.

Although the underlying factors causing the decline in individual growth rates are unknown, body condition affects the physiology of beluga whales. Declines in body mass are hypothesized to be a universal response to climate change in several species and have been documented in other large mammals as a result of lower metabolic requirements due to warmer

temperatures (Gardner et al. 2011; Hetem et al. 2014). These declines in body size-at-age are also hypothesized to be the result of mammals breeding at earlier ages and thereby allocating energy requirements to reproduction rather than growth in response to warming temperatures (Hetem et al. 2014). In belugas, body size and condition both impact oxygen storages and the ability of whales to forage at greater depths for longer durations. Possibly, the physiological specializations of belugas are adaptations to sea ice, and with the unprecedented loss of sea ice occurring in the Arctic, there is less pressure for high oxygen stores and prolonged underwater submergence. As a result, declines in oxygen storage capacity may be an adaptive response of belugas to changing environmental conditions associated with declines in body size and condition, resulting in shorter foraging dives and causing belugas to shift their prey selection from species such as Arctic cod, octopus, and decapods, from the Beaufort Sea's lower and upper slope to species residing at shallower depths, such as Arctic alligatorfish, Arctic staghorn sculpin, juvenile Arctic cod, stout eelblenny, and isopods. Everything considered, long-term monitoring will reveal whether the response of belugas whales to climate change is resilience, adaptation, or extinction.

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Appendix 1.1 Individual sample data for beluga muscle and liver tissues for bulk and lipid extracted (LE) stable isotope treatments.

Sample ID	Muscle			Liver			Muscle			Liver		
	$C:N_{bulk}$	$\delta^{13}C_{bulk}$	$\delta^{15}N_{bulk}$	$C:N_{bulk}$	$\delta^{13}C_{bulk}$	$\delta^{15}N_{bulk}$	$C:N_{LE}$	$\delta^{13}C_{LE}$	$\delta^{15}N_{LE}$	$C:N_{LE}$	$\delta^{13}C_{LE}$	$\delta^{15}N_{LE}$
Beluga 01	3.26	-18.43	16.75	4.18	-20.30	17.62	3.15	-17.93	16.59	3.46	-18.81	17.80
Beluga 02	3.33	-19.37	17.82	4.13	-20.99	18.93	3.16	-18.82	17.6	3.72	-19.57	18.80
Beluga 03	3.44	-18.64	16.7	4.25	-20.40	17.66	3.1	-18.05	16.99	3.42	-18.87	17.59
Beluga 04	3.3	-19.04	17.47	4.35	-20.96	18.83	3.3	-18.66	17.05	3.46	-19.43	18.56
Beluga 05	3.36	-18.87	16.9	4.27	-20.47	18.00	3.08	-18.62	17.03	3.47	-19.30	18.18
Beluga 06	3.23	-18.57	15.95	4.55	-20.86	18.06	3.16	-18.39	17.2	3.67	-19.46	18.32
Beluga 07	3.21	-19.12	17.05	4.18	-20.91	18.23	3.04	-18.68	17.39	3.45	-19.60	18.68
Beluga 08	3.63	-18.62	16.66	4.46	-20.75	18.25	3.22	-18.45	17.2	3.43	-19.11	18.56
Beluga 09	3.49	-19.81	17.58	4.73	-20.99	18.26	3.04	-19.08	17.95	3.69	-19.53	18.19
Beluga 10	3.43	-18.34	16.68	4.35	-20.42	18.41	3.03	-18.19	17.01	3.46	-18.80	18.34
Beluga 11	3.29	-18.01	16.43	4.18	-19.76	17.23	3.23	-17.87	17	3.19	-18.62	17.76
Beluga 12	3.26	-19.15	16.68	4.47	-21.00	17.66	3.23	-18.84	17.3	3.52	-19.69	18.48
Beluga 13	3.4	-18.78	16.51	4.45	-20.49	18.07	3.29	-18.60	16.58	3.63	-19.15	18.01
Beluga 14	3.63	-19.07	16.51	4.64	-21.07	18.30	3.13	-18.62	17.37	3.87	-19.50	18.10
Beluga 15	3.21	-18.92	17.42	4.52	-20.81	18.05	3.2	-18.54	16.99	3.62	-19.38	18.28
Beluga 16	3.22	-18.47	16.51	4.57	-20.88	17.84	3.14	-18.17	17.4	3.80	-19.06	18.15
Beluga 17	3.15	-18.32	16.52	3.91	-19.93	17.64	3.05	-18.31	16.92	3.50	-19.07	18.03
Beluga 18	3.29	-18.86	17.86	3.78	-19.95	19.31	3.25	-18.56	17.65	3.28	-19.03	19.32
Beluga 19	3.39	-19.41	18.02	4.42	-21.54	18.89	3.12	-19.08	18.25	3.39	-19.97	18.81
Beluga 20	3.39	-17.60	17.19	4.65	-19.75	17.49	3.15	-17.51	17.86	3.26	-17.92	18.46
Beluga 21	3.41	-19.41	17.9	N/A	N/A	N/A	3.23	-19.61	17.5	N/A	N/A	N/A
Beluga 22	3.39	-18.38	17.18	4.83	-20.49	17.57	3.17	-18.24	17.05	3.57	-19.01	17.92
Beluga 23	3.35	-18.55	17.22	4.37	-20.30	17.99	3.18	-18.37	17.16	3.35	-18.94	18.02
Beluga 24	3.6	-20.03	17.37	5.01	-21.94	18.29	3.19	-19.64	17.65	3.63	-20.70	18.73
Beluga 25	3.45	-18.17	17.2	4.62	-20.20	17.93	3.16	-18.09	17.72	3.40	-18.52	18.07
Beluga 26	3.6	-18.78	16.5	4.84	-20.85	17.38	3.18	-18.38	17	3.28	-19.01	17.96
Beluga 27	3.35	-18.93	16.78	4.42	-20.61	17.06	3.16	-18.88	17.05	3.38	-19.23	17.59
Beluga 28	3.43	-19.48	17.25	4.41	-21.07	17.94	3.12	-19.06	17.99	3.41	-20.05	18.33
Beluga 29	3.59	-20.01	17.13	4.89	-21.93	17.92	3.22	-19.60	17.23	3.56	-20.32	18.26
Beluga 30	3.35	-18.13	17.27	5.12	-20.80	18.06	3.14	-18.06	17.82	3.45	-18.84	18.48
Beluga 31	3.4	-19.09	17.15	4.92	-21.50	18.02	3.1	-18.95	17.47	3.46	-19.96	18.73
Beluga 32	3.29	-18.39	16.62	4.72	-21.59	17.87	3.08	-18.19	16.64	3.53	-20.24	18.30
Beluga 33	3.39	-18.18	16.54	4.41	-20.14	17.78	3.07	-18.37	17.28	3.47	-19.10	17.85
Beluga 34	3.39	-18.62	16.9	4.58	-20.32	17.54	3.17	-18.53	17.04	3.52	-18.96	17.82
Beluga 35	3.34	-19.77	17.86	4.45	-20.65	17.84	3.16	-19.96	17.89	3.27	-19.14	17.95
Beluga 36	3.44	-19.28	17.05	4.34	-21.39	18.28	3.07	-19.20	17.7	3.30	-19.74	18.84
Beluga 37	3.46	-18.28	17.21	4.31	-20.86	18.08	3.2	-18.30	17.52	3.28	-19.39	18.86
Beluga 38	3.37	-19.02	17.24	4.74	-20.73	17.91	3.24	-19.16	17.36	3.35	-18.65	18.14
Beluga 39	3.28	-18.24	17.39	4.38	-20.92	18.58	3.18	-17.98	17.5	3.35	-19.64	18.31

Beluga 40	3.41	-19.56	17.55	4.40	-20.39	17.94	3.13	-19.48	17.96	3.19	-19.02	18.43
Beluga 41	3.42	-18.85	16.57	4.69	-21.51	18.58	3.18	-18.71	16.94	3.65	-20.04	18.73
Beluga 42	3.49	-18.88	17.26	4.41	-20.78	18.17	3.12	-18.77	17.72	3.54	-19.37	17.85
Beluga 43	3.31	-19.13	17.74	4.63	-21.15	18.12	3.09	-19.21	18.01	3.35	-19.01	18.61
Beluga 44	3.39	-19.42	17.67	4.42	-21.22	18.17	3.12	-19.03	18.29	3.38	-20.13	18.76
Beluga 45	3.28	-18.59	17.09	4.72	-21.45	18.56	3.11	-18.59	17.27	3.53	-19.99	18.50
Beluga 46	3.4	-19.87	16.45	4.48	-20.73	18.53	3.25	-20.16	16.48	3.33	-19.14	18.58
Beluga 47	3.43	-19.73	17.5	4.97	-22.03	17.01	3.09	-19.63	17.86	3.76	-20.52	16.90
Beluga 48	3.48	-19.96	17.68	4.97	-21.64	18.41	3.07	-19.68	18.33	3.50	-20.16	18.45
Beluga 49	3.33	-19.37	18.03	4.58	-22.57	18.78	3.2	-18.94	18.36	3.42	-20.46	18.37
Beluga 50	3.7	-20.52	17.86	4.68	-22.20	18.12	3.06	-20.16	18.29	3.58	-20.24	18.24
Beluga 51	3.43	-18.93	17.57	4.91	-22.25	18.08	3.11	-19.23	17.78	3.50	-20.35	18.69
Beluga 52	3.52	-18.70	16.59	4.84	-20.54	18.11	3.19	-18.50	17.15	3.40	-19.14	18.29
Beluga 53	3.4	-18.64	16.84	4.22	-20.52	17.67	3.05	-18.62	17	3.52	-18.93	17.57
Beluga 54	3.18	-18.52	17.55	4.53	-19.92	18.83	2.99	-18.46	17.9	3.37	-18.93	17.73
Beluga 55	3.31	-19.36	17.81	4.48	-20.78	17.79	3.17	-19.08	17.79	3.37	-19.31	17.90
Beluga 56	3.25	-19.43	17.63	4.47	-21.01	19.05	3.2	-19.51	17.15	3.26	-18.96	19.19
Beluga 57	3.39	-19.05	17.7	4.15	-21.67	18.47	3.21	-18.98	17.55	3.55	-20.20	18.59
Beluga 58	3.45	-17.85	17.59	4.70	-21.30	18.70	3.2	-17.64	17.56	3.35	-19.54	19.20
Beluga 59	3.21	-19.73	17.33	4.42	-19.74	19.15	3.13	-19.37	17.93	3.40	-17.99	18.86
Beluga 60	3.28	-19.46	17.66	4.24	-20.76	17.71	3.1	-19.25	17.81	3.33	-19.45	18.67
Beluga 61	3.34	-18.91	17.14	4.41	-20.41	18.18	3.15	-18.50	16.59	3.29	-19.10	17.90
Beluga 62	3.22	-18.38	17.07	4.10	-20.96	18.27	3.16	-18.47	16.95	3.24	-18.97	18.41
Beluga 63	3.23	-19.21	17.76	4.34	-20.96	18.27	3.17	-19.35	17.95	3.48	-19.59	18.21
Beluga 64	3.3	-19.33	17.93	4.37	-20.96	17.78	3.18	-19.33	17.81	3.33	-19.63	18.09
Beluga 65	3.6	-19.93	17.34	4.51	-20.93	18.12	3.15	-19.42	17.66	3.56	-19.74	18.27
Beluga 66	3.54	-18.81	17.05	4.20	-20.73	18.05	3.14	-18.21	17.53	3.25	-19.03	17.63
Beluga 67	3.33	-18.58	16.98	4.38	-20.80	17.28	3.14	-18.35	16.85	3.24	-19.17	17.71
Beluga 68	3.47	-18.50	16.95	4.28	-20.65	17.22	3.09	-18.05	17.27	3.29	-19.11	17.75
Beluga 69	3.27	-18.33	16.81	4.25	-20.68	17.48	3.11	-18.26	17.18	3.15	-19.00	18.06

Appendix 1.2. Individual sample data for capelin and invertebrate species for bulk, acidified (A), and lipid extracted (LE) stable isotope treatments. Lipid extracted samples for isopod, green shrimp, polar shrimp, and circumpolar eualid tissues were acidified prior to lipid removal.

Sample ID	Lipid %	$C:N_{bulk}$	$\delta^{13}C_{bulk}$	$\delta^{15}N_{bulk}$	$C:N_{A}$	$\delta^{13}C_{A}$	$\delta^{15}N_{A}$	$C:N_{LE}$	$\delta^{13}C_{LE}$	$\delta^{15}N_{LE}$
Isopod 01	4.10	3.94	-19.59	12.69	3.81	-22.11	13.76	4.42	-22.27	10.50
Isopod 02	2.30	4.71	-17.30	12.44	3.95	-22.63	13.90	4.70	-22.99	10.68
Isopod 03	11.05	4.67	-19.45	12.31	5.20	-22.39	9.95	5.08	-22.75	8.11
Isopod 04	8.05	5.30	-20.62	12.31	5.05	-22.21	11.55	4.83	-22.33	9.77
Isopod 05	2.60	3.81	-20.05	14.63	4.27	-22.85	13.96	4.35	-22.67	12.50
Isopod 06	13.75	5.05	-21.31	12.66	5.59	-22.60	13.43	4.50	-22.12	11.48
Isopod 07	4.05	5.04	-17.88	10.44	5.66	-22.93	7.01	5.82	-22.54	5.15
Isopod 08	12.15	5.52	-22.50	13.24	6.46	-25.30	9.78	5.10	-23.99	9.94
Isopod 09	9.00	5.21	-22.36	13.37	5.67	-25.02	13.23	4.45	-23.55	12.71
Isopod 10	3.65	4.80	-20.16	12.76	4.79	-24.18	12.62	4.75	-23.22	10.75
Isopod 11	2.26	4.72	-18.96	12.39	4.77	-24.18	11.29	5.12	-23.92	9.02
Isopod 12	5.10	5.08	-19.44	11.20	5.08	-22.43	9.30	5.78	-22.64	4.68
Isopod 13	6.90	5.67	-19.98	11.13	5.77	-22.23	9.39	5.10	-22.35	8.10
Isopod 14	9.30	6.18	-17.68	9.82	6.12	-23.17	8.42	5.42	-22.80	7.73
Green shrimp 01	5.16	5.01	-22.89	14.30	8.83	-24.73	15.35	4.37	-22.10	15.59
Green shrimp 02	11.80	4.20	-21.92	15.27	6.69	-24.14	15.66	4.20	-22.06	15.44
Green shrimp 03	20.75	4.53	-22.14	15.37	5.97	-23.16	16.01	4.03	-21.36	16.68
Green shrimp 04	4.76	3.48	-20.11	15.16	4.58	-22.73	14.10	4.28	-22.38	14.06
Green shrimp 05	14.40	4.04	-21.62	15.65	5.24	-23.14	16.32	4.12	-21.97	16.46
Green shrimp 06	8.45	3.80	-20.46	15.31	4.82	-23.10	15.73	3.90	-22.02	15.99
Green shrimp 07	4.05	3.55	-20.64	14.88	4.77	-22.80	14.38	4.28	-22.37	15.16
Green shrimp 08	8.52	3.86	-21.24	14.52	5.34	-23.83	13.96	4.48	-22.45	14.09
Green shrimp 09	12.67	4.15	-21.82	14.62	5.87	-23.64	14.99	4.18	-22.01	15.21
Green shrimp 10	5.24	3.60	-20.73	15.35	4.71	-22.95	15.90	4.02	-22.13	15.76
Green shrimp 11	20.10	4.60	-21.78	15.61	6.79	-23.75	15.71	4.34	-21.80	16.88
Green shrimp 12	4.10	3.86	-20.62	14.65	5.17	-23.40	13.63	4.52	-22.40	13.61
Green shrimp 13	12.25	3.66	-21.25	14.75	4.83	-23.30	13.65	3.93	-22.06	15.14
Green shrimp 14	8.90	3.72	-21.14	15.61	4.79	-22.80	14.28	4.09	-22.05	14.88
Green shrimp 15	4.58	3.30	-20.29	15.97	4.04	-22.13	16.56	3.74	-21.99	16.50
Polar shrimp 01	5.75	4.10	-21.49	15.32	5.69	-23.60	15.00	4.33	-22.11	14.93
Polar shrimp 02	5.19	3.27	-20.01	15.42	4.40	-22.46	16.82	3.95	-21.99	16.73
Polar shrimp 03	6.57	3.35	-19.99	15.70	4.53	-22.22	16.69	3.86	-21.39	16.52
Polar shrimp 04	4.24	3.29	-19.42	15.66	4.03	-20.87	16.76	3.85	-21.20	16.21
Polar shrimp 05	5.48	3.82	-20.85	15.09	4.88	-23.01	15.79	4.02	-21.95	15.67
Polar shrimp 06	8.55	3.71	-21.22	14.20	5.00	-23.28	15.23	4.04	-22.23	15.31
Polar shrimp 07	16.85	4.11	-21.04	16.38	5.67	-22.56	15.94	4.20	-21.49	16.52
Polar shrimp 08	5.32	3.30	-20.30	15.73	3.90	-21.96	17.14	3.74	-21.87	16.92
Polar shrimp 09	2.38	4.01	-19.43	15.38	5.78	-23.07	10.19	5.93	-22.97	9.65
Polar shrimp 10	4.25	3.63	-20.18	15.49	4.69	-22.89	16.28	4.44	-22.61	14.94

Polar shrimp 11	4.90	3.52	-19.64	15.46	5.20	-22.34	12.53	5.20	-22.21	11.29
Polar shrimp 12	20.75	3.60	-20.75	16.19	5.22	-23.27	15.24	4.40	-22.19	14.84
Polar shrimp 13	9.21	3.76	-21.64	14.58	4.91	-23.57	13.61	4.58	-23.09	13.06
Polar shrimp 14	17.14	4.30	-22.59	14.81	6.51	-24.54	14.38	4.09	-22.31	15.02
Polar shrimp 15	15.63	4.08	-20.77	15.51	5.48	-22.54	12.87	5.08	-22.41	11.97
Circumpolar eualid 01	11.00	3.74	-21.09	15.84	4.75	-23.23	16.98	3.87	-21.99	17.44
Circumpolar eualid 02	7.90	3.72	-21.22	14.06	5.12	-24.18	14.63	4.43	-23.39	13.78
Circumpolar eualid 03	7.25	3.66	-20.28	15.67	5.09	-23.34	16.42	4.29	-22.53	15.81
Circumpolar eualid 04	7.05	4.50	-22.66	14.30	8.88	-25.07	15.54	4.10	-22.47	16.28
Circumpolar eualid 05	13.40	3.94	-22.27	14.90	6.04	-24.61	14.98	3.87	-22.42	15.94
Circumpolar eualid 06	11.90	4.11	-22.11	14.53	5.13	-23.29	15.63	3.85	-22.46	16.38
Circumpolar eualid 07	11.19	3.97	-21.88	15.31	5.26	-23.42	16.23	4.08	-22.52	16.52
Circumpolar eualid 08	7.16	3.64	-20.99	15.61	4.71	-22.99	16.64	3.95	-22.40	16.72
Circumpolar eualid 09	11.58	4.00	-23.55	14.37	5.39	-26.18	15.11	3.97	-25.26	15.30
Circumpolar eualid 10	14.29	4.15	-22.27	15.34	5.79	-23.70	16.00	3.97	-22.53	16.46
Circumpolar eualid 11	17.05	3.87	-21.34	14.98	5.41	-23.82	15.30	4.22	-22.57	15.51
Circumpolar eualid 12	17.48	3.86	-20.96	15.07	5.40	-24.17	15.93	4.09	-22.42	16.17
Circumpolar eualid 13	12.90	4.28	-22.19	14.38	5.94	-24.29	14.75	4.17	-22.92	14.79
Circumpolar eualid 14	17.05	4.30	-22.20	14.67	6.00	-24.51	15.24	3.94	-22.28	15.60
Octopus 01	31.75	7.64	-25.20	14.10				4.12	-21.99	14.59
Octopus 02	14.94	6.31	-24.75	13.81				4.05	-21.81	15.28
Octopus 03	9.89	3.88	-22.07	13.91				3.21	-20.79	13.74
Octopus 04	12.15	5.23	-23.95	14.22				3.81	-21.16	15.10
Octopus 05	23.14	6.31	-25.23	14.11				4.06	-22.73	13.99
Octopus 06	19.47	5.64	-24.44	14.78				4.04	-22.02	14.42
Octopus 07	7.14	2.46	-20.98	10.08				3.14	-20.33	13.18
Octopus 08	2.92	3.76	-22.80	13.38				3.45	-21.92	14.61
Octopus 09	38.89	8.37	-26.19	13.24				4.11	-23.11	13.76
Octopus 10	9.17	3.80	-22.77	14.51				3.43	-21.33	15.71
Octopus 11	13.60	4.71	-22.50	13.42				3.67	-20.39	17.23
Octopus 12	29.60	5.54	-24.33	14.10				3.83	-21.93	15.04
Octopus 13	31.48	8.73	-26.45	13.04				4.02	-23.27	13.67
Octopus 14	2.16	5.81	-23.83	15.00				3.50	-20.90	15.04
Octopus 15	13.05	3.71	-21.75	15.83				3.47	-19.80	16.97
Capelin 01	38.08	5.04	-25.21	15.01				3.47	-23.29	15.61
Capelin 02	21.32	3.53	-23.60	14.40				3.30	-23.04	14.83
Capelin 03	32.04	4.13	-24.63	14.77				3.44	-23.42	15.18
Capelin 04	39.00	4.07	-24.38	15.25				3.36	-22.97	15.35
Capelin 05	25.78	3.43	-23.59	14.42				3.24	-23.15	14.75
Capelin 06	29.75	3.42	-23.66	14.98				3.28	-23.09	15.11
Capelin 07	37.38	4.27	-24.53	14.92				3.30	-22.92	15.30
Capelin 08	27.96	3.54	-23.98	14.89				3.30	-23.28	15.27
Capelin 09	37.83	4.97	-24.91	15.34				3.34	-22.69	16.00

Capelin 10	33.29	3.38	-23.63	14.75	3.30	-23.30	14.60
Capelin 11	30.50	3.59	-23.86	14.30	3.27	-23.01	14.85
Capelin 12	35.35	5.06	-25.20	15.11	3.30	-22.84	15.42
Capelin 13	25.90	4.25	-24.62	14.36	3.31	-22.96	14.97
Capelin 14	29.52	3.70	-23.82	14.55	3.27	-23.09	14.98
Capelin 15	34.85	6.30	-26.14	14.71	3.23	-22.79	14.89
Capelin 16	35.86	4.16	-24.14	14.17	3.26	-22.57	15.25
Capelin 17	17.69	3.57	-24.06	14.61	3.27	-23.14	14.88

Appendix 2.1. Multiple linear regression models for biological variables on body condition morphometrics and stable isotope ratios in beluga whales (n =159) and their corresponding AIC_c values. $\Delta_i AIC_c$ is the difference between AIC_c for the current model and the minimum of AIC_c among all the models.

Morphometric	Model	AIC_c	Akaike weights (w_i)	$\Delta_i AIC_c$
Log(Girth)	Sex×Age+log (Length)	-321.03	0	0.61
	Sex×Age+Sex×log (Length)	-319.02	2.01	0.22
	Sex×Age+ Sex×log (Length)+log (Length)×Age	-317.2	3.83	0.09
	Sex×Age×log (Length)	-316.02	5.01	0.05
	Sex+ Age + log (Length)	-315.26	5.77	0.03
Blubber	Length+Age	721.59	0	0.53
	Sex+Age+Length	723.14	1.55	0.24
	Sex×Length+Age	724.49	2.9	0.12
	Sex×Age+Age×Length	726.46	4.87	0.05
	Sex×Age×Length	726.7	5.11	0.04
	Sex×Age+Sex×Length+Length×Age	728.57	6.98	0.02
$\delta^{15}N$	Length + Age	278.81	0	0.38
	Length × Age	280.93	2.12	0.13
	Length×Age+Sex	281.45	2.64	0.10
	Length×Age×Sex	285.27	6.46	0.01
	Length	289.43	10.62	0.00
Log ($\delta^{13}C$+40)	Age+Year	-721.78	0	0.82
	Age×Year	-718.16	3.62	0.13
	Age×Year+Sex	-715.27	6.51	0.03
	Age×Year+Sex×Age	-713.07	8.71	0.01
	Age	-705.10	16.68	0.00

Appendix 2.2. Percentages (%) of fatty acids contributing to the overall dissimilarities in fatty acid signatures among beluga whales between sex-and-size classes and years produced by a two-factor similarity percentages routine analysis (SIMPER). Small, medium, and large size-classes refer to males only.

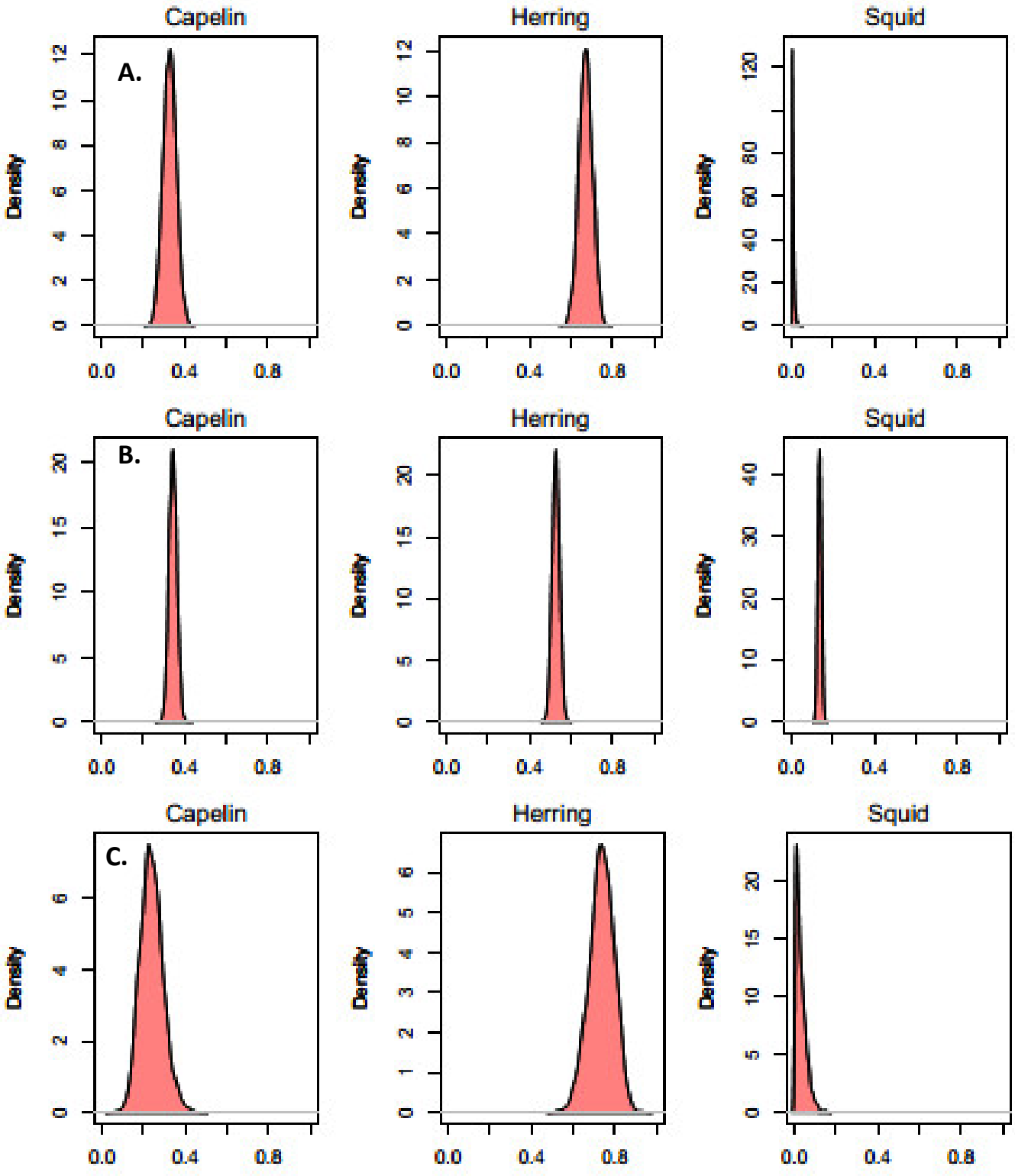
Comparison	16:1n-7	22:1n-11	20:1n-9	18:1n-9	16:0	22:6n-3	20:1n-11	Total
Large vs. small	35.0	26.2	15.2	6.9				83.3
Large vs. medium	24.8	25.1	14.5	6.0	6.2	5.3		81.9
Large vs. female	21.2	39.9	13.6	5.6				80.3
Female vs. medium	26.2	21.4	13.6		6.0	6.3	9.1	82.6
Female vs. small	39.5	17.0	15.9	5.3		6.9		84.6
Medium vs. small	39.9	21.2	13.6	5.6				80.3
2011 vs. 2012	19.3	17.3	12.3	11.1		10.2	14.6	84.8
2011 vs. 2013	25.5	18.5	13.4	12.8		8.3	8.4	86.9
2011 vs. 2014	22.6	22.1	12.1	14.7		7.2	7.2	85.9
2012 vs. 2013	27.3	18.1	13.6	9.6			11.4	80.0
2012 vs. 2014	26.7	19.3	12.9	10.9		6.2	9.3	85.3
2013 vs. 2014	28.4	26.1	14.8	9.3	3.9			82.5

Appendix 2.3. Results of the distance-based linear models (DISTLM) marginal tests, quantifying the relative contribution (proportion of variance) and significance levels of variables explaining fatty acid patterns in the blubber of eastern Beaufort Sea beluga whales (*n*=175).

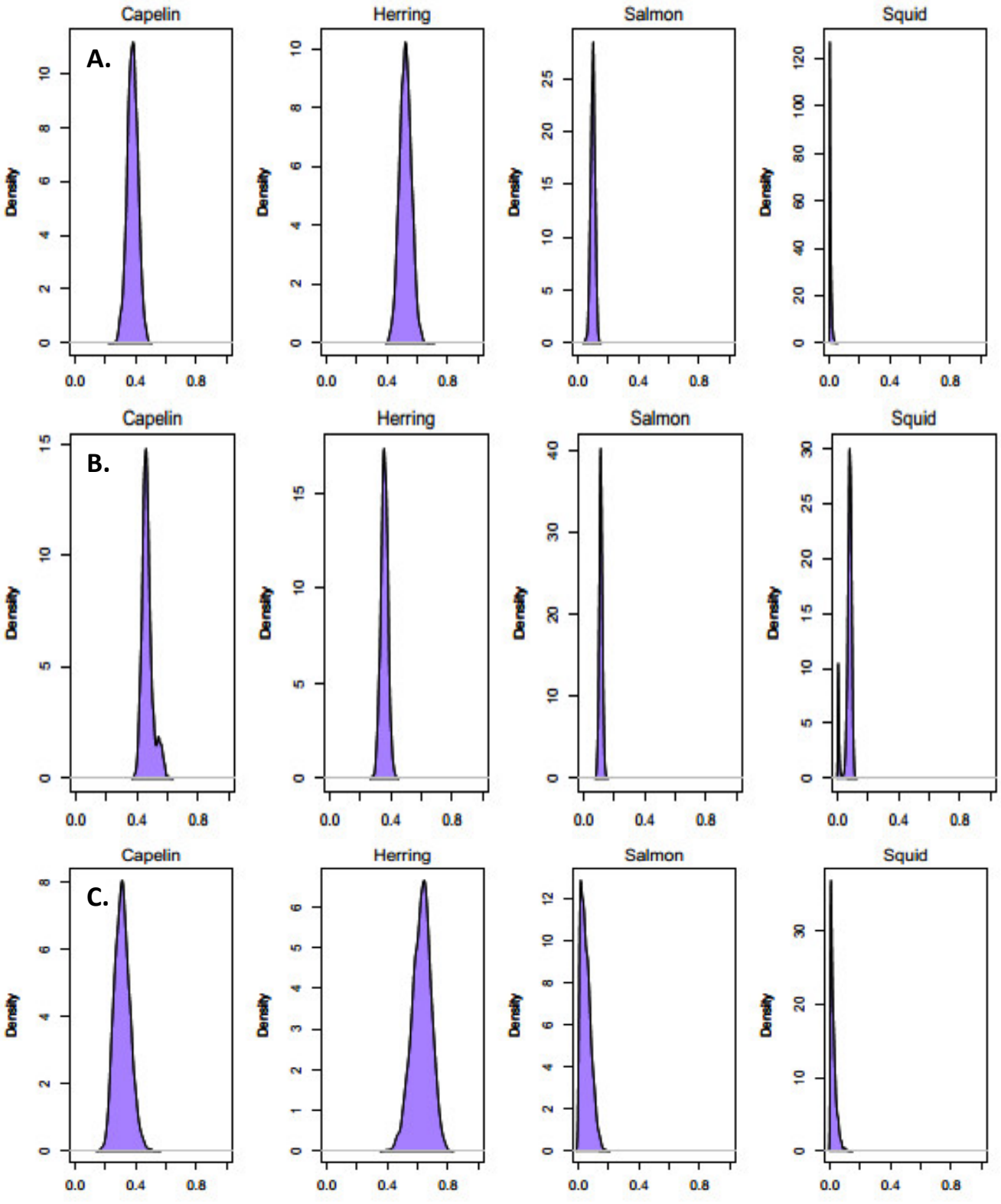
Variable	Sstrace	*Pseudo-F*	*p*	Variance (%)	res.df	reg.dr
Age	13.43	17.88	<0.01	9.37	173	2
Year	11.35	4.90	<0.01	7.92	171	4
Maximum Girth	11.04	14.43	<0.01	7.70	173	2
Length	8.19	10.49	<0.01	5.72	173	2
Sex	6.63	8.39	<0.01	4.62	173	2
Blubber Thickness	0.52	0.62	0.62	0.36	173	2

Appendix 3.1. Length, mass, and percent lipid content (wet weight) of prey fed to two beluga whales at the Vancouver Aquarium. Salmon was not directly consumed by either beluga, but was fed to the mother of the nursing juvenile.

Individual	Mass	Length	Lipid
Capelin 1	24.78	16	3.3
Capelin 2	20.31	15	2.2
Capelin 3	20.73	15	1.1
Capelin 4	21.49	15	1.6
Herring 1	61.36	18	7.2
Herring 2	52.89	19	10.9
Herring 3	86.23	22	11
Salmon 1	290.91	28	14
Salmon 2	310.73	29	12
Squid 1	NA	NA	1.9
Squid 2	NA	NA	3.3



Appendix 3.2. Fatty Acid Source Tracking Algorithm in R (FASTAR) predictions of prey proportions fed to an adult beluga whale based on 9 (A) and 34 (B) fatty acid signatures, as well as 7 fatty acid trophic markers (C).



Appendix 3.3. Fatty Acid Source Tracking Algorithm in R (FASTAR) estimates of prey proportions fed to a juvenile beluga whale based on 9 (A), and 34 (B) fatty acid signatures, as well as 7 fatty acid trophic markers (C).

Appendix 4.1. Percentages (%) of fatty acids contributing to 80% of the dissimilarities in fatty acid signatures between potential prey of Beaufort Sea beluga whales produced by similarity percentages routine analysis (SIMPER).

Species1	Species 2	16:0	16:1n-7	18:1n-9	18:1n-7	20:1n-9	20:1n-7	20:4n-6	20:5n-3	22:1n-11	22:1n-9	22:6n-3	Total
Isopod	Green shrimp		68.44						16.62				85.06
Isopod	Polar shrimp		67.68						13.14				80.82
Isopod	Circumpolar eualid		70.39						13.3				83.69
Isopod	Octopus		63.57			7.76						11.83	83.16
Isopod	Arctic staghorn sculpin		74.12						8.05				82.17
Isopod	Kelp snailfish		70.79			8.97						8.02	87.78
Isopod	Stout eelblenny		73.98									8.84	82.82
Isopod	Arctic alligatorfish		76.45						5.49				81.94
Isopod	Adolf's eelpout		76.18									7.79	83.97
Isopod	Canadian Eelpout		68.71						16.18				84.89
Isopod	Arctic cod		50.42			13.26				16.63			80.31
Isopod	Capelin		44.17			17.17				18.75			80.09
Isopod	Greenland halibut		57.05	9.58		15.07							81.7
Green shrimp	Polar shrimp	8.48	36.52						18.2			24.52	87.72
Green shrimp	Circumpolar eualid		39.24						26.06			14.74	80.04
Green shrimp	Octopus		13.82			16.57			30.95			19.17	80.51
Green shrimp	Arctic staghorn sculpin		40.68	8.64					35.23				84.55
Green shrimp	Adolf's eelpout		29.76	15.24					32.06			7.52	84.58
Green shrimp	Kelp snailfish		11.82	14.19	6.86	22.87			26.97				82.71
Green shrimp	Stout eelblenny		25.73	15.01	9.55				32.22				82.51
Green shrimp	Arctic alligatorfish		19.67	11.12					43.44			12.59	86.82
Green shrimp	Canadian Eelpout		63.25						16.01			9.94	89.2
Green shrimp	Capelin				10.29	19.9			29.14	22.36			81.69
Green shrimp	Arctic cod		7		9.38	17.65			25.09	22.79			81.91
Green shrimp	Greenland halibut			16.91	7.02	20.61			35.02	7.17			86.73
Polar shrimp	Circumpolar eualid	7.16	37.02						19.43			18.94	82.55
Polar shrimp	Octopus		13.38	7.42		17.48			27.11			21.03	86.42

Polar shrimp	Adolf’s eelpout		30.04	14.51					26.03			9.68	80.26
Polar shrimp	Canadian Eelpout		61.07						11.39			15.14	87.6
Polar shrimp	Arctic staghorn sculpin		41.04						28.95			13.24	83.23
Polar shrimp	Kelp snailfish		11.06	13.49	10.25	24.77			21.68				81.25
Polar shrimp	Arctic alligatorfish			10.33	18.06				35.9			20.45	84.74
Polar shrimp	Stout eelblenny		24.76	14.42	15.61				24.78			8.09	87.66
Polar shrimp	Arctic cod				11.88	17.81			19.84	22.89		9.41	81.83
Polar shrimp	Greenland halibut			16.03	9.49	21.6			30.2	7.39			84.71
Polar shrimp	Capelin				12.72	20.17			24.22	22.56		6.38	86.05
Circumpolar eualid	Octopus		23.2			14.25			23.81			21.1	82.36
Circumpolar eualid	Arctic staghorn sculpin		44.11						30.08			10.89	85.08
Circumpolar eualid	Arctic alligatorfish		21.52	8.46					34.57			16.06	80.61
Circumpolar eualid	Canadian Eelpout		64.08						14.03			12.57	90.68
Circumpolar eualid	Kelp snailfish		27.3	11		22.14			18.57			7.79	86.8
Circumpolar eualid	Stout eelblenny		30.96	12.17	8.57				25.62			8.55	85.87
Circumpolar eualid	Adolf’s eelpout		39.84	12.79					23.96			9.58	86.17
Circumpolar eualid	Arctic cod				8.84	19.01			19.68	25.07		7.66	80.26
Circumpolar eualid	Greenland halibut		7.03	16.59		23			30.62	6.83			84.07
Circumpolar eualid	Capelin				10.23	21.87			24.72	24.88			81.7
Octopus	Arctic alligatorfish		24.05	8.3		17.1			12.08			23.82	85.35
Octopus	Kelp snailfish		11.67	15.3	9.91				18.01			30.36	85.25
Octopus	Adolf’s eelpout		32.47	10.96		9			12.08			22.55	87.06
Octopus	Canadian Eelpout		50.48			10.53			11.71			15.29	88.01
Octopus	Arctic staghorn sculpin		39.68			12.99			11.99			18.94	83.6
Octopus	Stout eelblenny		25.84	8.45		17.18			13.63			20.53	85.63
Octopus	Arctic cod		17.41	7.18	10.31				11.22	18.18		20.75	85.05
Octopus	Capelin		17.3		12.38				12.64	20.71		18.14	81.17
Octopus	Greenland halibut		12.58	19.71	10.63				17.15			22.9	82.97
Arctic staghorn sculpin	Arctic alligatorfish		54.58				10.7		9.04			9.28	83.6

Arctic staghorn sculpin	Stout eelblenny		59.25						11.47			12.27	82.99
Arctic staghorn sculpin	Kelp snailfish	5.13	49.99			18.55						9.9	83.57
Arctic staghorn sculpin	Adolf's eelpout		64.02	5.2					5.18			10.77	85.17
Arctic staghorn sculpin	Canadian Eelpout		62.12						18.36				80.48
Arctic staghorn sculpin	Capelin		13.32		9.67	23.88			6.53	30.31			83.71
Arctic staghorn sculpin	Greenland halibut		26.53	9.58		28.37			9.25	10.84			84.57
Arctic staghorn sculpin	Arctic cod		17.04	6.56	7.71	19.53			4.35	28.77			83.96
Canadian Eelpout	Adolf's eelpout		64.16						9.32			9.28	82.76
Canadian Eelpout	Kelp snailfish		59.44			13.27						9.6	82.31
Canadian Eelpout	Arctic alligatorfish		58.72				6.34		15.18				80.24
Canadian Eelpout	Stout eelblenny		65.08						9.13			12.36	86.57
Canadian Eelpout	Greenland halibut		36.64	12.58		20.43			15.73				85.38
Canadian Eelpout	Capelin		23.53			22.3			14.61	26.39			86.83
Canadian Eelpout	Arctic cod		29.6			18.17			10.32	24.83			82.92
Adolf's eelpout	Kelp snailfish		49.36			16.73		4.96				12.1	83.15
Adolf's eelpout	Arctic alligatorfish		46.06	6	8.06		7.18					15.95	83.25
Adolf's eelpout	Stout eelblenny	5.73	58.28					5.5	4.84			10.04	84.39
Adolf's eelpout	Greenland halibut		27.87	6.59		24.64			7.69	10.93		8.28	86
Adolf's eelpout	Capelin		17.15	11.45		17.08				28.38		6.01	80.07
Adolf's eelpout	Arctic cod		19.63	13.08		13.42				26.14		8.29	80.56
Stout eelblenny	Kelp snailfish	9.91	37.05			31.86						6.21	85.03
Stout eelblenny	Arctic alligatorfish		27.68		14.97		13.24		9.04			19.96	84.89
Stout eelblenny	Greenland halibut		10.06	8.8		37.71			15.83	12.79			85.19
Stout eelblenny	Capelin		4.68	7.27		28.32			9.93	32.93			83.13
Stout eelblenny	Arctic cod		6.37	8.83		23.78			5.43	31.96		7.22	83.59
Kelp snailfish	Arctic alligatorfish	5.39	26.96		7.75	24.89						16.04	81.03
Kelp snailfish	Greenland halibut		16.57	11.65					19.38	11.5		22.12	81.22
Kelp snailfish	Capelin		20.83	15.33					8.7	29.38		12.19	86.43
Kelp snailfish	Arctic cod		19.87	16.23		6.14				25.02		15.24	82.5
Arctic alligatorfish	Greenland halibut		9.15	10.13	12.19	35.03			5.18	13.2			84.88

Arctic alligatorfish	Capelin		4.54	6.21	13.75	26.03				31.33			81.86
Arctic alligatorfish	Arctic cod		6.21	7.77	12.27	22.53				31.33			80.11
Greenland halibut	Capelin	3.8	8.5	51.18						20.23			83.71
Greenland halibut	Arctic cod		6.83	43.47		10.08	5.85			14.83			81.06

Appendix 4.2. Fatty Acid Source Tracking Algorithm in R (FASTAR) results summary of the prey contributions to individual beluga whales (*n*=60) using 32 fatty acids signatures and $\delta^{13}C$ and $\delta^{15}N$ values based on trophic enrichment factors from Caut et al. (2011). Data represent the median (50%) and Bayesian credible interval (BCI; 5th and 95th percentile).

	Isopod		Decapods		Octopus		Arctic cod and capelin		Arctic staghorn sculpin		Canadian eelpout		Adolf's eelpout		Greenland halibut		Kelp snailfish		Stout eelblenny		Arctic alligatorfish	
Year	0.5	90 BCI	0.5	90 BCI	0.5	90 BCI	0.5	90 BCI	0.5	90 BCI	0.5	90 BCI	0.5	90 BCI	0.5	90 BCI	0.5	90 BCI	0.5	90 BCI	0.5	90 BCI
2011	0.002	0.0002-0.009	0.569	0.523-0.612	0.003	0.0002-0.011	0.394	0.353-0.439	0.003	0.0002-0.010	0.002	0.0001-0.009	0.002	0.0001-0.01	0.005	0.0004-0.0200	0.003	0.0002-0.0160	0.002	0.0002-0.0099	0.002	0.0002-0.0100
2011	0.0006	0.000-0.002	0.182	0.164-0.199	0.0005	0.000-0.002	0.0009	0.000-0.004	0.0006	0.000-0.003	0.0007	0.000-0.003	0.0006	0.000-0.002	0.0007	0.000-0.003	0.81	0.792-0.828	0.0006	0.000-0.003	0.0006	0.000-0.003
2011	0.003	0.0002-0.013	0.008	0.0006-0.034	0.003	0.0003-0.0145	0.929	0.899-0.956	0.006	0.0004-0.028	0.003	0.0003-0.015	0.009	0.0007-0.031	0.005	0.0004-0.019	0.004	0.0003-0.017	0.003	0.0003-0.013	0.005	0.0004-0.023
2011	0.002	0.0001-0.008	0.008	0.0004-0.038	0.002	0.0001-0.010	0.38	0.309-0.450	0.003	0.0002-0.016	0.001	0.000-0.006	0.007	0.0005-0.023	0.004	0.0003-0.015	0.575	0.506-0.645	0.002	0.0001-0.007	0.003	0.0002-0.011
2011	0.002	0.0002-0.009	0.005	0.0004-0.021	0.002	0.0001-0.007	0.963	0.940-0.978	0.003	0.0002-0.013	0.002	0.0001-0.009	0.003	0.0002-0.012	0.003	0.0002-0.012	0.002	0.0002-0.011	0.002	0.0002-0.009	0.003	0.0002-0.014
2011	0.003	0.0002-0.012	0.005	0.0003-0.020	0.003	0.0002-0.010	0.944	0.915-0.967	0.005	0.0003-0.019	0.003	0.0003-0.014	0.004	0.0003-0.0145	0.005	0.0003-0.021	0.004	0.0004-0.020	0.003	0.0002-0.014	0.006	0.0005-0.025
2011	0.003	0.0002-0.012	0.005	0.0004-0.023	0.003	0.0002-0.012	0.951	0.926-0.972	0.004	0.0003-0.017	0.002	0.0002-0.010	0.006	0.0004-0.024	0.003	0.0002-0.012	0.003	0.0002-0.012	0.002	0.0002-0.011	0.003	0.0003-0.015
2011	0.002	0.0001-0.007	0.002	0.0001-0.008	0.969	0.949-0.982	0.003	0.0002-0.014	0.002	0.0001-0.009	0.003	0.0003-0.014	0.002	0.000-0.007	0.002	0.0002-0.010	0.002	0.0002-0.009	0.002	0.0001-0.008	0.002	0.0001-0.009
2012	0.001	0.000-0.006	0.622	0.587-0.658	0.002	0.0002-0.008	0.353	0.319-0.389	0.002	0.000-0.007	0.002	0.0001-0.006	0.002	0.000-0.008	0.003	0.0003-0.012	0.002	0.0002-0.011	0.002	0.0001-0.007	0.002	0.000-0.007
2012	0.002	0.0001-0.007	0.565	0.517-0.607	0.002	0.0002-0.010	0.403	0.362-0.453	0.002	0.0002-0.008	0.002	0.0002-0.007	0.002	0.0002-0.010	0.004	0.0003-0.017	0.004	0.0002-0.015	0.002	0.0001-0.008	0.002	0.0002-0.010
2012	0.002	0.0002-0.010	0.562	0.504-0.615	0.002	0.346-0.458	0.4	0.0002-0.013	0.003	0.0002-0.015	0.003	0.0002-0.010	0.002	0.0002-0.010	0.005	0.0004-0.020	0.004	0.0003-0.017	0.003	0.0002-0.011	0.003	0.0002-0.011
2012	0.002	0.0002-0.008	0.546	0.497-0.592	0.002	0.0002-0.010	0.422	0.375-0.471	0.002	0.0002-0.009	0.002	0.0001-0.008	0.002	0.0002-0.010	0.004	0.0002-0.016	0.003	0.0003-0.014	0.002	0.0002-0.008	0.002	0.0001-0.008
2012	0.002	0.000-0.007	0.529	0.474-0.575	0.002	0.0002-0.009	0.444	0.397-0.498	0.002	0.0002-0.008	0.001	0.0001-0.006	0.002	0.0000-0.009	0.003	0.0003-0.014	0.003	0.0003-0.013	0.002	0.0001-0.008	0.002	0.0002-0.008

2012	0.002	0.0002-0.009	0.508	0.448-0.561	0.002	0.0001-0.011	0.456	0.401-0.517	0.003	0.0002-0.011	0.002	0.0002-0.010	0.002	0.0002-0.010	0.005	0.0004-0.020	0.004	0.0003-0.017	0.002	0.0002-0.010	0.002	0.0002-0.010
2012	0.002	0.0002-0.009	0.492	0.435-0.546	0.002	0.001-0.010	0.469	0.416-0.529	0.002	0.0002-0.011	0.002	0.0002-0.009	0.003	0.0002-0.012	0.005	0.0004-0.022	0.004	0.0004-0.019	0.002	0.0002-0.010	0.003	0.0002-0.011
2012	0.002	0.0002-0.008	0.426	0.372-0.480	0.543	0.491-0.595	0.003	0.002-0.014	0.002	0.0002-0.009	0.002	0.0002-0.008	0.002	0.0002-0.010	0.003	0.0002-0.012	0.003	0.0002-0.013	0.002	0.0002-0.009	0.002	0.0002-0.009
2012	0.002	0.0002-0.008	0.012	0.0005-0.065	0.002	0.0002-0.009	0.938	0.905-0.963	0.003	0.0002-0.017	0.002	0.0001-0.007	0.013	0.0006-0.037	0.003	0.0002-0.012	0.002	0.0002-0.012	0.002	0.0001-0.009	0.003	0.0002-0.013
2012	0.002	0.0002-0.011	0.008	0.0007-0.032	0.004	0.0004-0.017	0.945	0.917-0.967	0.004	0.0002-0.016	0.002	0.0002-0.009	0.005	0.0004-0.020	0.003	0.0003-0.014	0.004	0.0003-0.015	0.003	0.0002-0.011	0.004	0.0003-0.018
2012	0.004	0.0003-0.016	0.007	0.0006-0.032	0.013	0.001-0.027	0.901	0.859-0.938	0.006	0.0005-0.025	0.004	0.0002-0.016	0.006	0.0004-0.023	0.005	0.0003-0.021	0.005	0.0003-0.026	0.005	0.0004-0.019	0.018	0.002-0.071
2012	0.002	0.0002-0.008	0.004	0.0004-0.018	0.002	0.0001-0.008	0.965	0.945-0.980	0.003	0.0002-0.012	0.002	0.0001-0.007	0.003	0.0003-0.013	0.003	0.0002-0.010	0.002	0.0002-0.010	0.002	0.0001-0.008	0.003	0.0002-0.012
2012	0.002	0.0002-0.008	0.002	0.0001-0.009	0.965	0.938-0.980	0.003	0.0003-0.014	0.003	0.0002-0.010	0.005	0.0003-0.028	0.002	0.0001-0.007	0.002	0.0002-0.010	0.002	0.0002-0.010	0.002	0.0002-0.011	0.002	0.0002-0.008
2013	0.002	0.0002-0.008	0.564	0.510-0.612	0.002	0.0001-0.010	0.401	0.354-0.455	0.002	0.0001-0.009	0.002	0.0001-0.009	0.002	0.0001-0.010	0.004	0.0004-0.019	0.004	0.0003-0.018	0.002	0.0002-0.009	0.002	0.0002-0.010
2013	0.001	0.0000-0.005	0.559	0.523-0.592	0.002	0.0002-0.006	0.423	0.390-0.460	0.001	0.0000-0.005	0.001	0.0000-0.004	0.001	0.0001-0.006	0.002	0.0001-0.008	0.002	0.0001-0.008	0.001	0.0001-0.006	0.001	0.0001-0.006
2013	0.0001	0.0000-0.006	0.541	0.494-0.584	0.0002	0.0001-0.008	0.435	0.393-0.483	0.002	0.0002-0.006	0.001	0.0001-0.005	0.002	0.0000-0.008	0.003	0.0002-0.011	0.003	0.0002-0.011	0.001	0.0001-0.006	0.002	0.0002-0.007
2013	0.001	0.0001-0.006	0.535	0.491-0.576	0.002	0.0002-0.008	0.443	0.403-0.487	0.001	0.0001-0.006	0.001	0.0001-0.005	0.002	0.0001-0.007	0.003	0.0002-0.01	0.002	0.0002-0.010	0.001	0.0000-0.006	0.001	0.0000-0.007
2013	0.002	0.0002-0.008	0.513	0.452-0.563	0.002	0.0002-0.010	0.454	0.404-0.516	0.002	0.0000-0.008	0.002	0.0001-0.007	0.002	0.0002-0.010	0.004	0.0003-0.017	0.004	0.0003-0.017	0.002	0.0002-0.009	0.002	0.0002-0.010
2013	0.002	0.0002-0.010	0.509	0.450-0.565	0.003	0.0002-0.011	0.445	0.387-0.507	0.003	0.0003-0.014	0.003	0.0002-0.013	0.003	0.0002-0.012	0.007	0.0006-0.029	0.005	0.0004-0.020	0.003	0.0002-0.013	0.003	0.0003-0.013
2013	0.002	0.0001-0.008	0.482	0.413-0.537	0.002	0.0001-0.010	0.487	0.432-0.558	0.002	0.0002-0.009	0.002	0.0001-0.009	0.002	0.0002-0.009	0.004	0.0002-0.016	0.004	0.0003-0.016	0.002	0.0002-0.009	0.002	0.0003-0.010
2013	0.002	0.0001-0.007	0.476	0.382-0.537	0.002	0.0001-0.008	0.497	0.437-0.592	0.002	0.0001-0.008	0.002	0.0001-0.007	0.002	0.0001-0.008	0.003	0.0002-0.013	0.003	0.0002-0.013	0.002	0.0001-0.008	0.009	0.0002-0.009
2013	0.002	0.0001-0.007	0.466	0.386-0.527	0.002	0.0002-0.009	0.504	0.444-0.585	0.002	0.0001-0.008	0.002	0.0001-0.008	0.002	0.0001-0.009	0.004	0.0003-0.015	0.003	0.0002-0.014	0.002	0.0001-0.008	0.002	0.0002-0.009
2013	0.003	0.0002-0.014	0.040	0.002-0.101	0.011	0.001-0.037	0.879	0.833-0.917	0.005	0.0004-0.025	0.002	0.0001-0.010	0.012	0.0005-0.051	0.006	0.0004-0.025	0.006	0.0005-0.026	0.003	0.0002-0.015	0.006	0.0005-0.029
2013	0.003	0.0002-0.011	0.018	0.001-0.076	0.007	0.0006-0.027	0.920	0.879-0.951	0.004	0.0003-0.021	0.002	0.0002-0.009	0.009	0.0005-0.033	0.004	0.0002-0.015	0.003	0.0002-0.015	0.003	0.0002-0.012	0.005	0.0004-0.022
2013	0.003	0.0003-0.013	0.018	0.001-0.063	0.006	0.0003-0.024	0.919	0.883-0.918	0.005	0.0004-0.024	0.003	0.0002-0.011	0.008	0.0002-0.016	0.004	0.0002-0.016	0.004	0.0003-0.017	0.003	0.0002-0.013	0.006	0.0005-0.026
2013	0.003	0.0002-0.014	0.013	0.0009-0.053	0.003	0.0002-0.012	0.910	0.873-0.940	0.008	0.0005-0.040	0.003	0.0003-0.014	0.014	0.001-0.040	0.006	0.0005-0.025	0.005	0.0003-0.021	0.004	0.0003-0.016	0.007	0.0006-0.031
2013	0.003	0.0002-0.014	0.012	0.001-0.049	0.017	0.001-0.044	0.908	0.872-0.940	0.005	0.0004-0.022	0.003	0.0002-0.012	0.009	0.0008-0.031	0.006	0.0003-0.024	0.005	0.0003-0.024	0.004	0.0003-0.015	0.006	0.0005-0.027
2013	0.002	0.0002-0.010	0.01	0.0007-0.044	0.005	0.0005-0.020	0.941	0.908-0.965	0.004	0.0003-0.017	0.002	0.0002-0.009	0.006	0.0004-0.024	0.003	0.0002-0.014	0.003	0.0002-0.013	0.003	0.0002-0.011	0.004	0.0003-0.018

2013	0.003	0.0002-0.011	0.007	0.0004-0.041	0.077	0.040-0.105	0.869	0.835-0.900	0.004	0.0002-0.018	0.002	0.0002-0.009	0.006	0.0004-0.031	0.004	0.0003-0.016	0.004	0.0003-0.016	0.003	0.0002-0.012	0.005	0.0003-0.021
2013	0.003	0.0002-0.012	0.007	0.0003-0.035	0.062	0.028-0.091	0.879	0.844-0.912	0.005	0.0003-0.019	0.002	0.0002-0.009	0.006	0.0004-0.029	0.005	0.0004-0.020	0.004	0.0003-0.019	0.003	0.0002-0.013	0.006	0.0005-0.026
2013	0.002	0.0001-0.008	0.006	0.0004-0.048	0.006	0.0005-0.025	0.938	0.912-0.961	0.002	0.0002-0.012	0.001	0.0001-0.006	0.021	0.001-0.042	0.002	0.0002-0.010	0.002	0.0002-0.010	0.002	0.0002-0.007	0.003	0.0002-0.011
2013	0.002	0.0001-0.009	0.005	0.0004-0.052	0.01	0.0009-0.044	0.928	0.899-0.952	0.003	0.0002-0.012	0.002	0.0001-0.007	0.025	0.001-0.046	0.003	0.0002-0.011	0.002	0.0002-0.010	0.002	0.0001-0.008	0.003	0.0002-0.011
2013	0.002	0.0001-0.008	0.005	0.0003-0.027	0.004	0.0003-0.017	0.955	0.932-0.974	0.002	0.0002-0.011	0.001	0.0001-0.006	0.010	0.0005-0.031	0.002	0.0001-0.008	0.002	0.0002-0.009	0.002	0.0001-0.007	0.002	0.0001-0.011
2013	0.002	0.0002-0.011	0.005	0.0004-0.022	0.011	0.001-0.035	0.944	0.915-0.966	0.003	0.0002-0.014	0.002	0.0001-0.009	0.005	0.0004-0.019	0.003	0.0002-0.014	0.003	0.0003-0.014	0.003	0.0002-0.011	0.004	0.0003-0.017
2013	0.002	0.0001-0.007	0.004	0.0003-0.018	0.005	0.0003-0.021	0.961	0.938-0.978	0.002	0.0002-0.010	0.001	0.0001-0.005	0.006	0.0004-0.027	0.002	0.0001-0.008	0.002	0.0001-0.008	0.002	0.0001-0.007	0.002	0.0002-0.009
2013	0.003	0.0002-0.012	0.004	0.0002-0.016	0.062	0.034-0.090	0.894	0.861-0.925	0.003	0.0002-0.015	0.002	0.0001-0.009	0.004	0.0003-0.018	0.004	0.0003-0.019	0.005	0.0004-0.020	0.003	0.0002-0.012	0.003	0.0002-0.014
2013	0.002	0.0002-0.009	0.004	0.0002-0.019	0.002	0.0001-0.010	0.962	0.941-0.979	0.003	0.0002-0.014	0.002	0.0002-0.008	0.004	0.0002-0.017	0.002	0.0001-0.009	0.002	0.0002-0.011	0.002	0.0001-0.008	0.003	0.0002-0.013
2013	0.002	0.0002-0.009	0.004	0.0003-0.018	0.059	0.029-0.088	0.905	0.873-0.936	0.003	0.0002-0.012	0.002	0.0001-0.008	0.003	0.0002-0.014	0.003	0.0003-0.013	0.003	0.0002-0.013	0.002	0.0002-0.009	0.003	0.0003-0.013
2013	0.002	0.0002-0.009	0.002	0.0001-0.009	0.955	0.910-0.976	0.005	0.0004-0.025	0.003	0.0002-0.012	0.007	0.0004-0.055	0.002	0.0002-0.008	0.003	0.0002-0.011	0.003	0.0002-0.011	0.003	0.0002-0.011	0.002	0.0002-0.010
2014	0.001	0.0001-0.006	0.623	0.0001-0.008	0.002	0.0001-0.008	0.352	0.316-0.392	0.002	0.0001-0.008	0.002	0.0002-0.007	0.002	0.0001-0.008	0.003	0.0003-0.013	0.003	0.0001-0.011	0.002	0.0000-0.008	0.002	0.0001-0.008
2014	0.001	0.0001-0.006	0.541	0.486-0.582	0.002	0.0002-0.007	0.438	0.396-0.493	0.002	0.0001-0.007	0.002	0.0000-0.007	0.001	0.0001-0.007	0.002	0.0000-0.011	0.002	0.0002-0.010	0.001	0.0000-0.006	0.002	0.0001-0.007
2014	0.002	0.0001-0.008	0.523	0.462-0.577	0.002	0.0002-0.011	0.438	0.384-0.500	0.002	0.0001-0.011	0.002	0.0002-0.010	0.003	0.0003-0.012	0.006	0.0004-0.025	0.005	0.0004-0.019	0.002	0.0002-0.011	0.003	0.0002-0.011
2014	0.002	0.0002-0.008	0.006	0.0004-0.055	0.003	0.0002-0.012	0.938	0.911-0.959	0.003	0.0002-0.013	0.001	0.0001-0.007	0.025	0.001-0.045	0.002	0.0002-0.009	0.002	0.0002-0.010	0.002	0.0001-0.008	0.003	0.0002-0.011
2014	0.003	0.0004-0.014	0.005	0.0004-0.022	0.004	0.0003-0.017	0.935	0.904-0.960	0.005	0.0003-0.022	0.004	0.0003-0.015	0.006	0.0004-0.021	0.005	0.0004-0.022	0.005	0.0003-0.022	0.003	0.0002-0.015	0.006	0.0005-0.024
2014	0.003	0.0002-0.011	0.005	0.0004-0.021	0.002	0.0001-0.007	0.948	0.921-0.969	0.004	0.0003-0.019	0.003	0.0002-0.011	0.005	0.0003-0.019	0.005	0.0003-0.019	0.004	0.0003-0.019	0.003	0.0002-0.012	0.004	0.0003-0.020
2014	0.004	0.0003-0.015	0.004	0.0003-0.017	0.003	0.0002-0.012	0.932	0.897-0.960	0.005	0.0004-0.020	0.004	0.0003-0.016	0.005	0.0004-0.019	0.007	0.0006-0.032	0.006	0.0005-0.027	0.004	0.0003-0.018	0.006	0.0005-0.026
2014	0.002	0.0002-0.010	0.004	0.0004-0.020	0.079	0.050-0.106	0.882	0.852-0.910	0.003	0.0002-0.013	0.002	0.0001-0.008	0.004	0.0003-0.020	0.003	0.0002-0.014	0.003	0.0001-0.010	0.002	0.0001-0.010	0.003	0.0003-0.013
2014	0.002	0.0002-0.009	0.002	0.0002-0.011	0.829	0.789-0.870	0.005	0.0003-0.022	0.003	0.0002-0.013	0.134	0.094-0.170	0.002	0.0000-0.008	0.003	0.0002-0.013	0.003	0.0002-0.014	0.002	0.0001-0.010	0.003	0.0003-0.012
2014	0.002	0.0001-0.007	0.002	0.0001-0.008	0.840	0.805-0.879	0.003	0.0002-0.014	0.002	0.0002-0.009	0.133	0.095-0.166	0.001	0.0002-0.007	0.002	0.0002-0.020	0.002	0.0002-0.010	0.002	0.0001-0.008	0.002	0.0001-0.009
2014	0.001	0.0000-0.004	0.002	0.0001-0.008	0.001	0.0000-0.005	0.979	0.960-0.989	0.001	0.0000-0.005	0.0009	0.0000-0.004	0.004	0.0002-0.025	0.001	0.0000-0.005	0.001	0.0000-0.005	0.001	0.0000-0.004	0.001	0.0000-0.005
2014	0.002	0.0002-0.008	0.002	0.0001-0.008	0.847	0.810-0.938	0.004	0.0003-0.018	0.002	0.0002-0.010	0.125	0.020-0.160	0.002	0.0001-0.007	0.003	0.0002-0.011	0.002	0.0002-0.010	0.002	0.0002-0.008	0.002	0.0001-0.009

Appendix 5.1. PCR conditions used to amplify the beluga myoglobin gene from cDNA

	Enzyme used	Reaction mix	Cycle conditions
cDNA amplification	*Taq* DNA polymerase (5U/ uL)	9.1 uL 5X PCR Buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl] 36.4 uL 10mM dNTP Mix 3.64 uL Forward primer (10uM) 3.64 uL Reverse primer (10uM) 1.82 uL *Taq* DNA polymerase (5U/ uL) 9.1 uL cDNA from first-strand reaction 123.76 uL nuclease free water	Denaturation: 94°C for 30 s 30 cycles: 4°C for 30 s 58 – 50°C gradient for 30 s 68°C for 1 min final extension: 68°C for 5 min Hold: 4°C

Appendix 5.2. Multiple linear regression models for the body condition index (BCI) based on maximum girth as well as biological variables including BCI and girth to length ratio (GL) on physiological parameters of oxygen storage capacity and their corresponding AIC_c values. $\Delta_i AIC_c$ is the difference between AIC_c for the current model and the minimum of AIC_c among all the models.

Tissue	Model	AIC_c	Akaike weights (w_i)	$\Delta_i AIC_c$
Girth (cm)	Age+Sex+Length	478.23	0.48	0
	Age×Sex+Length	479.04	0.32	0.81
	Age×Sex+Sex×Length	480.8	0.13	2.57
	Age×Sex+Age×Length+Sex×Length	482.99	0.04	4.76
	Age×Sex×Length	485.5	0.01	7.27
Hemoglobin [g/dl]	BCI+Spleen Mass+Age×Sex	232.27	0.58	0
	BCI+Spleen Mass+Age+Sex	234.81	0.16	2.54
	BCI+Spleen Mass+Age	235.27	0.13	3.00
	BCI+Spleen Mass	235.83	0.10	3.56
	BCI×Spleen Mass	238.18	0.03	5.91
	BCI	261.09	3.2×10^{-07}	28.82
Hematocrit (%)	BCI+Spleen Mass	324.67	0.40	0
	BCI+Spleen Mass+Sex	325.67	0.24	1.00
	BCI×Spleen Mass	326.59	0.15	1.92
	BCI+Spleen Mass+Age	327.17	0.11	2.50
	BCI+Spleen Mass×Sex	327.52	0.10	2.85
	BCI	360.81	5.64×10^{-09}	36.14
Myoglobin [mg/g]	BCI	291.14	0.56	0
	BCI+Sex	292.97	0.22	1.83
	BCI+ Mass+Sex	293.78	0.15	2.64
	BCI+Mass+Age+Sex	296.41	0.04	5.27
	BCI×Age+Sex+Mass	297.54	0.02	6.40
	BCI× Age × Sex × Mass	306.35	2.8×10^{-04}	15.21
Log[buffering capacity(Slykes)]	Mass	-140.61	0.41	0
	Sex	-140.54	0.40	0.07
	Sex+Mass	-138.51	0.14	2.10
	Sex×Mass	-136.34	0.05	4.27
	Sex+Mass+Age	-110.24	1.04×10^{-07}	30.37
	BCI×Age × Sex × Mass	-82.22	8.60×10^{-14}	58.39
Log[spleen mass (g)]	Age+Mass	75.36	0.55	0
	Age	76.69	0.28	1.33
	BCI+Age+Mass	77.6	0.18	2.24
	BCI+Age+Sex+Mass	80.1	0.05	4.74
	BCI+Age×Sex+Mass	82.49	0.02	7.13
	BCI× Age × Sex × Mass	111.91	6.34×10^{-9}	36.55
Log[$cADL_{dive}$ (min)]	BCI+Age×Sex	-20.81	0.82	0

	BCI+Age+Sex	-16.53	0.10	4.28
	BCI+Spleen Mass+Age×Sex	-15.57	0.06	5.24
	BCI×Spleen Mass+Age×Sex	-13.11	0.02	7.7
	BCI×Age+BCI×Spleen Mass+Age×Sex	-10.4	4.51×10^{-3}	10.41
	BCI×Age × Sex × Spleen Mass	26.84	3.69×10^{-11}	47.65
Log[cADL$_{swim}$(min)]	BCI+Age×Sex	-20.26	0.84	0
	BCI+Age+Sex	-15.49	0.08	4.77
	BCI+Spleen Mass+Age×Sex	-15	0.06	5.26
	BCI×Spleen Mass+Age×Sex	-12.56	0.02	7.7
	BCI×Age+BCI×Spleen Mass+Age×Sex	-9.77	4.43×10^{-3}	10.49
	BCI×Age × Sex × Spleen Mass	27.56	3.47×10^{-11}	47.82
Hemoglobin [g/dl]	GL+Age×Sex+Mass+Spleen Mass	231.87	0.79	0
	GL+Age×Sex+Mass×Sex+Spleen Mass	235.15	0.15	3.28
	GL×Spleen Mass+Age×Sex+Mass×Sex	237.57	0.05	5.70
	GL×Spleen Mass+Age×Sex+Mass×Sex+Spleen Mass×Mass	240.27	0.01	8.40
	GL×Mass+Age×Sex+Mass×Sex+Spleen Mass×GL+Spleen Mass×Mass	243.88	0.002	12.01
	GL+Age×Sex+Spleen Mass	257.94	1.72×10^{-6}	26.07
	GL×Age×Mass×Sex×Spleen Mass	312.26	2.75×10^{-18}	80.39
Hematocrit (%)	GL+Age+Spleen Mass	325.05	0.71	0
	GL+Age+Spleen Mass+Sex	327.6	0.20	2.55
	GL+Age+Mass+Spleen Mass+Sex	330.42	0.05	5.37
	GL+Age+Mass+Spleen Mass×Sex	331.35	0.03	6.3
	GL+Age+Mass×Sex+Spleen Mass×Sex	332.5	0.02	7.45
	GL+Age	360.76	1.24×10^{-8}	35.71
	GL×Age×Mass×Sex×Spleen Mass	412.35	7.81×10^{-20}	87.3
Myoglobin [mg/g]	GL+Age	291.63	0.64	0
	GL+Mass+Age	293.58	0.24	1.95
	GL+Mass+Age+Sex	296.15	0.07	4.52
	GL+Mass+Age×Sex	297.61	0.03	5.98
	GL×Age+Mass+Age×Sex	298.44	0.02	6.81
	GL×Age+Age×Mass+Age×Sex	320.56	3.34×10^{-7}	28.93
Log[buffering capacity(Slykes)]	Mass	-140.61	0.41	0
	Sex	-140.54	0.40	0.07
	Sex+Mass	-138.51	0.14	2.10
	Sex×Mass	-136.34	0.05	4.27
	Sex+Mass+Age	-110.24	1.04×10^{-7}	30.37
	GL×Age × Sex × Mass	-83.78	1.88×10^{-13}	56.83
Log [spleen mass (g)]	Age+Mass	75.36	0.51	75.36
	Age	76.69	0.26	76.69
	GL+Age+Mass	77.65	0.16	77.65
	GL+Age+Sex+Mass	80.12	0.05	80.12

	GL+Age×Sex+Mass	82.5	0.01	82.5
	GL× Age × Sex × Mass	109.36	2.12×10^{-8}	109.36
Log[cADL$_{dive}$ (min)]	GL+Age×Sex	-19.28	0.88	0
	GL+Spleen.Mass+Age×Sex	-13.66	0.05	5.62
	GL+Age+Sex	-13.15	0.04	6.13
	GL× Spleen.Mass+Age×Sex	-11.84	0.02	7.44
	GL× Age+GL× Spleen.Mass+Age×Sex	-9.7	7.30×10^{-3}	9.58
	GL× Age× Sex× Spleen.Mass	25.17	1.95×10^{-10}	44.45
Log[cADL$_{swim}$(min)]	GL+Age×Sex	-18.44	0.89	0
	GL+Spleen.Mass+Age×Sex	-12.79	0.052	5.65
	GL+Age+Sex	-11.82	0.03	6.62
	GL× Spleen.Mass+Age×Sex	-10.93	0.02	7.51
	GL× Age+GL× Spleen.Mass+Age× Sex	-8.74	6.95×10^{-3}	9.7
	GL× Age× Sex× Spleen.Mass	26.22	1.78×10^{-10}	44.66