SEASONAL, INTER-ANNUAL, AND SPATIAL VARIATION IN RINGED SEAL FEEDING ECOLOGY IN HUDSON BAY ASSESSED THROUGH STABLE ISOTOPE AND FATTY ACID BIOMARKERS

by

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ABSTRACT

Current trends toward warmer air temperatures and longer ice free seasons in Hudson Bay are expected to cause changes in Arctic marine ecosystem dynamics. Ringed seals (*Phoca hispida*) will likely experience changes in levels of predation, competition, and prey availability. The purpose of this thesis was to investigate seasonal, inter-annual, and spatial variation in Hudson Bay ringed seal feeding ecology. Fatty acid composition, $\delta^{15}N$, and $\delta^{13}C$ varied significantly by season, suggesting seasonal changes in foraging habitat and diet. Spatial differences in ringed seal stable isotope ratios occurred between western and eastern Hudson Bay, and there was a strong relationship between spring air temperature and $\delta^{15}N$. Peak $\delta^{15}N$ occurred within a range in spring air temperatures between approximately -5°C and -2°C. I propose that the high $\delta^{15}N$ observed in ringed seals within this temperature range is indicative of relatively greater importance of capelin (*Mallotus villosus*) in the ringed seal diet.

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THESIS FORMAT

This thesis has been prepared as a 'sandwich' style thesis following the guidelines set out by the Faculty of Graduate Studies at the University of Manitoba. Chapter 1 provides introductory information including the overall theme and context of the works. Chapters 2 and 3 are manuscripts which have been submitted for publication in scientific journals. Chapter 4 provides a summary of the major findings and discusses the importance and implications of the findings. Further information on the submitted manuscripts, including the contributions from each co-author, is provided below.

CHAPTER 2. SEASONS OF THE RINGED SEAL: PELAGIC OPEN WATER HYPERPHAGY, BENTHIC FEEDING OVER WINTER, AND SPRING FASTING DURING MOLT

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This manuscript has been submitted to *Wildlife Research* where it has been recommended for publication following minor revisions (4 December 2012). Brent Young and Steve Ferguson conceived and designed the study. Brent Young co-ordinated sample collection, prepared tissue samples for stable isotope analyses and age determination, performed fatty acid analysis, performed statistical analyses, and wrote the manuscript. Steve Ferguson provided guidance and advice throughout the completion of the research and contributed to the manuscript through editorial advice.

CHAPTER 3. USING STABLE ISOTOPES TO UNDERSTAND CHANGES IN RINGED SEAL FORAGING ECOLOGY AS A RESPONSE TO A WARMING ENVIRONMENT

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This manuscript has been submitted to *Marine Mammal Science* where it is in the review process (submitted 19 December 2012). Brent Young and Steve Ferguson conceived and designed the study. Brent Young co-ordinated sample collection, prepared tissue samples for stable isotope analyses and age determination, performed statistical analyses, and wrote the manuscript. Steve Ferguson provided guidance and advice throughout the completion of the research and contributed to the manuscript through editorial advice.

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CHAPTER 1. INTRODUCTION

As a highly abundant, widely distributed, ice-adapted species, the ringed seal (*Phoca hispida*) is considered to be a good indicator for environmental change (Laidre et al. 2008; Vincent-Chambellant 2010). In Hudson Bay, where warmer temperatures and longer open water seasons have already caused ecological changes (Stirling et al. 1999; Gaston et al. 2003; Regehr et al. 2007; Higdon and Ferguson 2009), monitoring of ringed seals will be especially important in the coming years. Long term monitoring of ringed seals provides important information for the effective management of ringed seals as a species, and provides evidence for larger scale ecosystem changes. Although ringed seals in Hudson Bay have received relatively little attention in the past, this thesis contributes to a growing body of knowledge on ringed seal ecology in a changing Hudson Bay.

Ringed seals make use of the sea ice to build sub-nivean lairs where adult females give birth and nurse their young in the early spring, and as a platform on which to bask during the spring molt (McLaren 1958; Smith and Stirling 1975; Smith 1987). The winter and spring is a period of negative energy balance as parturition, lactation, and mating occur over a 3 month period, approximately mid-February to mid-May, followed by the molting season in May and June (Chambellant et al. 2012). The molting period is accompanied by a period of restricted foraging, or fasting, as a significant amount of time is spent basking on the sea ice (McLaren 1958). Kelly et al. (2010) found ringed seals spent approximately 55% of their time out of water during the molt, while Smith and Hammill (1981) observed individuals hauled out for periods lasting more than 40 consecutive hours. Following the re-growth of their fur, ringed seals feed intensely throughout the open water season as they must replenish fat and energy stores in preparation for the coming winter and the following spring. The distinct seasonality of the Hudson Bay environment and of ringed seal life-history imposes seasonal restrictions on ringed seal movements and on their ability to forage as well as the accessibility of particular food items. It is expected that these seasonal restrictions and life history requirements will be reflected in ringed seal diet and energy balance. Over a longer temporal scale, it is expected that environmental conditions have an influence on the distribution and abundance of prey items, and that inter-annual variation in these prey items will also be reflected in ringed seal diet. Long term changes in diet may impact ringed seal survival and reproductive condition and ultimately result in demographic changes to the population abundance and distribution.

While ringed seals are known to feed on a wide variety of prey, Arctic cod (*Boreogadus saida*) has been found to be the dominant prey item throughout much of the circumpolar Arctic (Lowry et al. 1980; Gjertz and Lydersen 1986; Smith 1987; Holst et al. 2001; Labansen et al. 2007, 2011). However, in Hudson Bay, sandlance (*Ammodytes sp.)* and capelin (*Mallotus villosus)* appear to be the preferred prey (Chambellant et al. in press). Based on the diet of thick-billed murres (*Uria lomvia)*, a relatively recent shift from an abundance of Arctic cod to an abundance of sandlance and capelin has occurred in northern Hudson Bay (Gaston et al. 2003). Further shifts, from Arctic to sub-

Arctic and temperate species and ecosystems, are expected as warming continues (Bluhm and Gradinger 2008; Wassmann et al. 2011).

Hudson Bay has already experienced significant warming and increases in the length of the ice-free season (Gagnon and Gough 2005a; Comiso 2006; Stirling and Parkinson 2006; Hocheim and Barber 2010). Between 1971 and 2001, Gagnon and Gough (2005a) reported significant increases in mean annual temperatures for six out of seven weather stations in Hudson Bay, with the greatest increase of 0.75°C/decade occurring at Chesterfield Inlet in western Hudson Bay. However, the use of satellite imagery has revealed even greater warming for offshore areas of Hudson Bay with spring temperatures increasing up to a rate of about 1.2°C/decade from 1981 to 2005 (Serreze and Francis 2006; Stirling and Derocher 2012). Gagnon and Gough (2005a) have also reported significant changes to the length of the open water season in the Hudson Bay/James Bay system, with the greatest changes in breakup and freeze-up dates occurring 1.25 days earlier per year and 0.55 days later per year, respectively. Climate change scenarios predict continued warming and loss of sea ice which will cause significant ecological changes and ice adapted species such as ringed seals and polar bears (*Ursus maritimus*) will face significant challenges in the coming years (Gagnon and Gough 2005b; Stirling 2005; Stirling and Derocher 2012).

I used stable isotope and fatty acid biomarkers to study the variation in ringed seal feeding ecology. As prey items are consumed, isotopic signatures and fatty acids from the prey are incorporated into the tissues of the predator (Kelly 2000; Dalerum and

Angerbjörn 2005; Budge et al. 2006; Iverson 2008). Based on a significant increase in stable nitrogen isotope ratios ($\delta^{15}N$) with each trophic step, $\delta^{15}N$ provides information on trophic positioning (Minagawa and Wada 1984). Stable carbon isotope ratios ($\delta^{13}C$) are only slightly enriched with each trophic step, and provide an indication of the source of carbon, or the foraging habitat (DeNiro and Epstein 1978). Fatty acid composition can provide both qualitative and quantitative information on feeding ecology as individual fatty acids can be associated with different prey items (Budge et al. 2006; Iverson 2008). To quantify the occurrence of specific prey items in the diet using stable isotopes and fatty acids, stable isotope ratios, fatty acid compositions, and species specific calibration coefficients for all prey items are required. The quantitative assessment of specific prey items in the ringed seal diet is beyond the scope of this thesis, though potential changes in important prey items are inferred based on our current knowledge of the Hudson Bay ecosystem and of ringed seal preferred prey.

The purpose of this thesis was to examine seasonal, spatial, and inter-annual patterns in ringed seal feeding ecology in Hudson Bay using stable isotope and fatty acid biomarkers. Chapter 2 focuses on seasonal variation in eastern Hudson Bay using $\delta^{15}N$ and $\delta^{13}C$ stable isotope ratios in muscle and fatty acids in blubber from samples collected over an 18 month period. I expect that ringed seal foraging ecology varies by season, influenced by availability and access to prey related to life history requirements and ice cover. Using samples from both western and eastern Hudson Bay collected over an eight year period, Chapter 3 examines spatial and inter-annual patterns in stable isotope ratios. I expect that ringed seal diet changes from year to year in relation to environmental change, particularly changes in temperature and ice break-up date. Chapter 4 provides a summary of the main conclusions, discusses the implications of the findings, and identifies areas where further research is required.

References

Bluhm BA, Gradinger R (2008) Regional variability in food availability for Arctic marine mammals. Ecological Applications 18:S77–S96

Budge SM, Iverson SJ, Koopman H (2006) Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Marine Mammal Science 22:759–801

Chambellant M, Stirling I, Gough W, Ferguson SH (2012) Temporal variations in Hudson Bay ringed seal (*Phoca hispida*) life-history parameters in relation to environment. Journal of Mammalogy 93:267–281

Chambellant M, Stirling I, Ferguson SH (In Press) Temporal variation in western Hudson Bay ringed seal (*Phoca hispida*) diet in relation to environment. Marine Ecology Progress Series.

Comiso JC (2006) Arctic warming signals from satellite observations. Weather 61:70–76

Dalerum F, Angerbjörn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647–658

DeNiro M, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506

Gagnon AS, Gough WA (2005a) Trends in the Dates of Ice Freeze-up and Breakup over Hudson Bay, Canada. Arctic 58:370–382

Gagnon AS, Gough WA (2005b) Climate change scenarios for the Hudson Bay region: an intermodel comparison. Climatic Change 69:269–297

Gaston AJ, Woo K, Hipfner JM (2003) Trends in Forage Fish Populations in Northern Hudson Bay since 1981, as Determined from the Diet of Nestling Thick-Billed Murres *Uria lomvia*. Arctic 56:227 – 233

Gjertz I, Lydersen C (1986) The ringed seal (*Phoca hispida*) spring diet in northwestern Spitsbergen, Svalbard. Polar Research 4:53–56

Higdon JW, Ferguson SH (2009) Loss of Arctic sea ice causing punctuated change in sightings of killer whales (*Orcinus orca*) over the past century. Ecological Applications 19:1365–1375

Hochheim KP, Barber DG (2010) Atmospheric forcing of sea ice in Hudson Bay during the fall period, 1980–2005. Journal of Geophysical Research 115:1–20

Holst M, Stirling I, Hobson KA (2001) Diet of Ringed Seals (*Phoca hispida*) on the East and West Sides of the North Water Polynya, Northern Baffin Bay. Marine Mammal Science 17:888–908

Iverson SJ (2008) Tracing Aquatic Food Webs Using Fatty Acids: From Qualitative Indicators to Quantitative Determination. In: Kainz M, Brett MT, Arts MT (eds) Lipids in Aquatic Ecosystems. Springer New York, New York, NY, pp 281–307

Kelly BP, Badajos OH, Kunnasranta M, Moran JR, Martinez-Bakker M, Wartzok D, Boveng PL (2010) Seasonal home ranges and fidelity to breeding sites among ringed seals. Polar Biology 33:1095–1109

Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78:1–27

Labansen AL, Lydersen C, Haug T, Kovacs KM (2007) Spring diet of ringed seals (*Phoca hispida*) from northwestern Spitsbergen, Norway. ICES Journal of Marine Science 64:1246–1256

Labansen AL, Lydersen C, Levermann N, Haug T, Kovacs KM (2011) Diet of ringed seals (*Pusa hispida*) from Northeast Greenland. Polar Biology 34:227–234

Laidre KL, Stirling I, Lowry LF, Wiig Ø, Heide-Jørgensen MP, Ferguson SH (2008) Quantifying the sensitivity of Arctic marine mammals to climate-induced habitat change. Ecological Applications 18:S97–S125

Lowry LF, Frost K, Burns JJ (1980) Variability in the Diet of Ringed Seals, *Phoca hispida*, in Alaska. Canadian Journal of Fisheries and Aquatic Sciences 37:2254–2261

McLaren I (1958) The Biology of the Ringed Seal (*Phoca hispida* Schreher) in the Eastern Canadian Arctic. Bulletin of the Fisheries Research Board of Canada 118

Minagawa M, Wada E (1984) Stepwise enrichment of 15N along food chains: Further evidence and the relation between δ^{15}N and animal age. Geochimica et Cosmochimica Acta 48:1135–1140

Regehr E V, Lunn NJ, Amstrup SC, Stirling I (2007) Effects of Earlier Sea Ice Breakup on Survival and Population Size of Polar Bears in Western Hudson Bay. Journal of Wildlife Management 71:2673–2683

Serreze MC, Francis JA (2006) The Arctic on the fast track of change. Weather 61:65–69

Smith TG (1987) The ringed seal, *Phoca hispida*, of the Canadian western Arctic. Canadian Bulletin of Fisheries and Aquatic Sciences 216:1–81

Smith T, Hammill M (1981) Ecology of the ringed seal, *Phoca hispida*, in its fast ice breeding habitat. Canadian Journal of Zoology 59:966–981

Smith TG, Stirling I (1975) The breeding habitat of the ringed seal (*Phoca hispida*). The birth lair and associated structures. Canadian Journal of Zoology 53:1297–1305

Stirling I, Derocher AE (2012) Effects of climate warming on polar bears: a review of the evidence. Global Change Biology 18:2694–2706

Stirling I, Lunn NJ, Iacozza J (1999) Long-term Trends in the Population Ecology of Polar Bears in Western Hudson Bay in Relation to Climatic Change. Arctic 52:294 – 306

Stirling I, Parkinson CL (2006) Possible Effects of Climate Warming on Selected Populations of Polar Bears (*Ursus maritimus*) in the Canadian Arctic. Arctic 59:261-275

Vincent-Chambellant, M. 2010. Ecology of ringed seals (*Phoca hispida*) in western Hudson Bay, Canada. Thesis (Ph.D.) University of Manitoba, Winnipeg, Manitoba.

Wassmann P, Duarte C, Agusti S, Sejr M (2011) Footprints of climate change in the Arctic marine ecosystem. Global Change Biology 17:1235–1249

CHAPTER 2. SEASONS OF THE RINGED SEAL: PELAGIC OPEN WATER HYPERPHAGY, BENTHIC FEEDING OVER WINTER, AND SPRING FASTING DURING MOLT

Abstract

Context. The ringed seal (*Phoca hispida*), a small phocid seal with a circumpolar Arctic distribution and a strong association with sea ice, occurs at the southern limit of its range in Hudson and James Bays: an area that experiences complete ice cover in winter and complete open water in summer. Due to the high seasonal variability in environmental conditions, it is expected that ringed seals experience seasonal changes in diet and foraging habitat that will be reflected in body condition and biomarkers of stable isotopes and fatty acids.

Aims. The purpose of this study was to investigate intra-annual variation in ringed seal feeding habits and body condition.

Methods. Tissue samples and morphological measurements from southeastern Hudson Bay ringed seals were obtained every month during the Inuit subsistence hunt from November 2009 to May 2011 (n=192). Muscle samples were used for $\delta^{15}N$ and $\delta^{13}C$ stable isotope analysis, blubber was used for analysis of fatty acid composition, body weight and sculp weight were used to estimate percent blubber, and lower right canines were used to determine age.

Key results. Fatty acid composition, $\delta^{15}N$, and $\delta^{13}C$ varied significantly by season, suggesting seasonal changes in foraging habitat and diet. Variation in percent blubber indicated poorest body condition occurs following the molting/fasting period, followed by a gradual increase from late summer through fall with the highest body condition occurring in time for freeze-up in December.

Key conclusions. Patterns of $\delta^{13}C$ indicate pelagic feeding during the open water season (Aug. - Dec.) when fat and energy stores are replenished, increased benthic foraging during the period of ice cover (Jan. - May), followed by a period of fasting during the spring molt (June - July). Fatty acid composition suggested seasonal changes in diet that could include increased importance of pelagic fish in the fall during the period of positive energy balance.

Implications. The first continuous collection of ringed seal tissue samples provided a comprehensive seasonal pattern of biomarker composition; baseline data that has important applications for short-term management and ecology studies as well as long-term conservation and monitoring programs.

Introduction

High latitudes are subject to extreme seasonal cycles, which, for marine ecosystems, are often characterized by the presence or absence of sea ice (Walsh 2008). For Arctic marine organisms, seasonal changes in energy requirements and resource availability, which are related to the extreme environmental cycles, require special adaptations. Ice-associated marine mammals are physiologically and behaviourally adapted to predictable periods of positive and negative energy balance (Harington 2008). One of the most well adapted marine mammals to the seasonal extremes of the Arctic is the ringed seal (*Phoca hispida*) (Smith et al. 1991).

Ringed seals use the sea ice as a platform on which to bask in the spring during the annual molt, as territories during mating, and for constructing sub-nivean lairs in which females give birth and nurse a single pup in early spring (McLaren 1958; Smith and Stirling 1975; Smith 1987). Making use of the stable ice environment simplifies the process of parental care and thereby allowing adult females to continue to feed (McLaren 1958; Smith and Hammill 1981). However, adult seals may be somewhat restricted in their movements and food options during the ice season (Kelly et al. 2010). Mating takes place around the time of weaning (Smith 1987; Chambellant et al. 2012), and adult male ringed seals are similarly restricted during this time period as they defend their underwater territory and maintain access to females (Smith and Hammill 1981; Kelly et al. 2010; Yurkowski et al. 2011). The timing of parturition, lactation, and mating is somewhat variable, but, in Hudson Bay, these events generally occur over a 3 month period, roughly mid-February to mid-May (Chambellant et al. 2012), followed by the molting season.

During the molt, ringed seals haul out and bask on the sea ice, spending approximately 55% of their time out of water (Kelly et al. 2010), restricting their time spent foraging (McLaren 1958). Smith and Hammill (1981) observed individual seals hauled out for consecutive periods lasting greater than 40 hours. The basking and molting period lasts from approximately mid-May to mid-July, with a peak in June, and appears to be highly variable among geographic locations and among individuals (McLaren 1958; Finley 1979; Smith and Hammill 1981; Kelly et al. 2010). The degree of fasting that takes place is also likely to be highly variable and is somewhat dependant on ice and weather conditions that would influence the amount of time spent basking (McLaren 1958). Following the molting and fasting period, the open water season is considered an important feeding period for ringed seals as they must replenish fat and energy stores in preparation for the coming winter (McLaren 1958; Smith 1987; Ryg and Oritsland 1991).

Hudson Bay is a seasonally ice covered inland sea in the Canadian Arctic/sub-Arctic. The period of ice cover generally lasts from December to July, with freeze-up usually beginning in October and breakup beginning in May/June (Wang et al. 1994; Gagnon and Gough 2005a). The distinct seasonality of the Hudson Bay environment has resulted in an ecosystem that is dependent on both the open water and ice covered seasons. Recent studies have found a trend toward a longer open water season (Gagnon and Gough 2005a) and climate models predict this trend to continue (Galbraith and Larouche 2011). A longer open water season would likely result in significant environmental and ecological changes (Gagnon and Gough 2005b). Many of these changes are expected to occur in the marine environment with changes in species distribution and abundance (Stirling 2005; Bluhm and Gradinger 2008). Ringed seals are one of the species that may be affected by environmental changes, directly through the alteration of the sea ice habitat on which they rely (Harwood et al. 2000; Ferguson et al. 2005), and indirectly through changes in prey availability (Stirling 2005) and predation (Derocher et al. 2004).

Relatively little is known about ringed seal feeding ecology in Hudson Bay, especially in relation to how seal diet changes on a seasonal basis. Stable isotope and fatty acid biomarkers in conjunction with measures of morphology and body condition are effective tools in the study of feeding ecology (Kelly 2000; Dalerum and Angerbjörn 2005; Budge et al. 2006; Iverson 2008; Newsome et al. 2010). Stable nitrogen isotope ratios ($\delta^{15}N$) provide information related to trophic positioning, based on a significant increase in $\delta^{15}N$ with each trophic step (Minagawa and Wada 1984). In contrast, stable carbon isotope ratios ($\delta^{13}C$) indicate the source of carbon throughout a food chain, based on only a slight enrichment per trophic level (DeNiro and Epstein 1978). Fatty acid analysis provides more detailed information on feeding ecology as individual fatty acids or ratios of fatty acids found in the predator, can be associated with specific prey items or groups of prey items (Budge et al. 2006). The advantage of using biomarkers such as stable isotope ratios and fatty acids is that they incorporate diet information from all prey items consumed over a known period of time (Tieszen et al. 1983; Budge et al. 2006). The time period of the diet represented by stable isotope and fatty acid analyses is dependent on tissue specific turnover rates which are related to metabolic rate. For stable isotope ratios, the length of time reflected ranges from days in blood plasma, to several years or an entire lifetime in metabolically inert tissues such as teeth, claws, or baleen (Hobson and Clark 1992, Newsome et al. 2010).

The primary objective of this study is to determine the seasonal patterns of $\delta^{15}N$ and $\delta^{13}C$ stable isotope ratios in muscle, fatty acid composition of blubber, and body condition in eastern Hudson Bay ringed seals, and to consider how these patterns relate to seasonal changes in feeding ecology.

Methods

Morphological measurements and samples of muscle, blubber, and lower jaw were collected from ringed seals harvested during the Inuit subsistence hunt from Sanikiluaq, NU in the Belcher Islands of eastern Hudson Bay (Figure 2.1) each month from November 2009 until May 2011. Not all types of measurements and tissue samples were obtained or analysed from all 192 harvested seals (Table 2.1). Tissue samples were consistently taken from the mid-dorsal (muscle) and mid-ventral (fat and skin) region of the seal and were kept frozen at -25°C until analysis.

TABLE 2.1: Sample sizes of ringed seal tissues used for $\delta^{15}N$ and $\delta^{13}C$ stable isotope analysis (muscle) and fatty acid analysis (blubber). Samples were collected from Sanikiluaq, NU from November 2009 to May 2011. Samples from different years have been combined by month. A total of 192 seals were harvested over the study period. There were 176 muscle samples available for stable isotope analysis (47 pups, 129 adults/juveniles) and 58 adult/juvenile blubber samples for Fatty Acid analysis.

	Stable Isotope Samples			Fatty Acid Samples	
Month	**Pups**	**Juveniles**	**Adults**	**Juveniles**	**Adults**
January	3	6	5	4	3
February	7	4	1	3	1
March	14	16	4	4	2
April	2	16	16	2	6
May	1	6	8	3	6
June	1	3	5	2	2
July	1	1	7	1	4
August	6	1	3	1	2
September	3	4	3	1	2
October	2	3	4	1	2
November	5	5	2	2	1
December	2	2	4	1	2

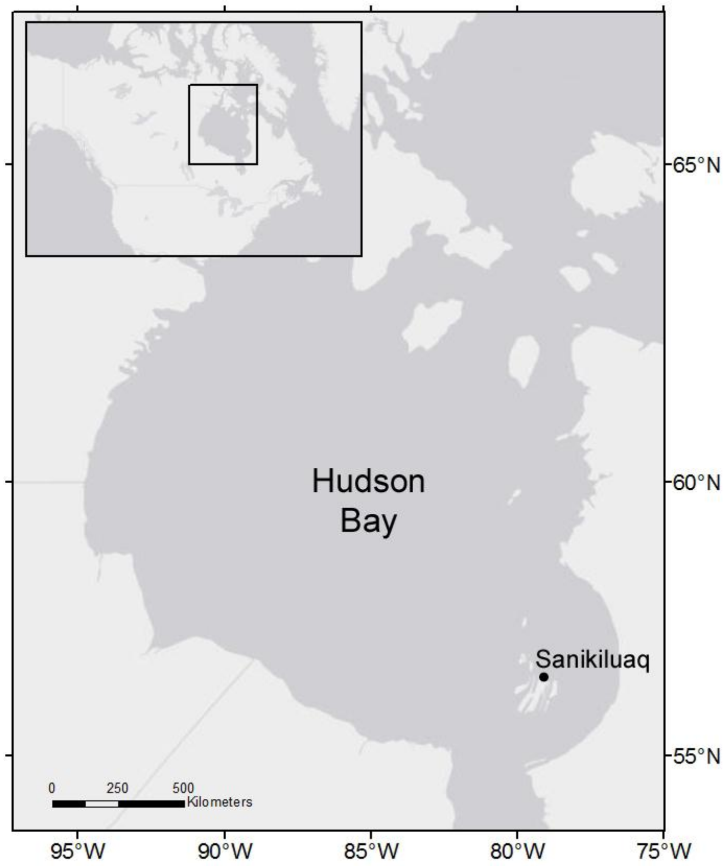

FIGURE 2.1: Ringed seal samples were collected from subsistence harvests by Sanikiluaq Inuit in the waters around the Belcher Islands of eastern Hudson Bay.

Samples of muscle were freeze-dried for approximately 48 hours and then crushed into a fine powder using a mortar and pestle. The samples were sent for $\delta^{15}N$ and $\delta^{13}C$ stable isotope analysis at the Great Lakes Institute for Environmental Research at the University of Windsor. Lipids were removed prior to analysis by mixing twice, for 24 hours, in 5 ml of 2:1 chloroform-methanol solution following Bligh and Dyer (1959). Lipids tend to be depleted in ^{13}C and can cause biased $\delta^{13}C$ values when present in samples (DeNiro and Epstein 1977; Post et al. 2007). To eliminate this bias, it has been generally accepted that lipids should be removed from lipid rich tissues prior to analysis (Lesage et al. 2010). However, it has been found that lipid extraction can bias δ^{15}N results in unpredictable and inconsistent ways (Søreide et al. 2006; Sweeting et al. 2006; Ingram et al. 2007). Ingram et al. (2007) have found the Bligh and Dyer (1959) chloroform-methanol extraction method to have relatively little effect on δ^{15}N, particularly in lipid-rich tissues. Rather than increasing costs by analysing samples separately for δ^{13}C and δ^{15}N, it has been recommended that chloroform-methanol extraction methods be used prior to the analysis of lipid-rich tissues (Ingram et al. 2007; Newsome et al. 2010).

Stable isotope ratios were determined using approximately 0.5 mg samples which were sealed in tin capsules and analyzed on a Thermo Finnigan Delta^{Plus} mass-spectrometer (Thermo Finnigan, San Jose, CA, USA) coupled with an elemental analyzer (Costech, Valencia, CA, USA). Stable isotope ratio values are expressed in parts per thousand (‰) using δ notation as calculated using the following equation:

$$\delta X = \left[\left(R_{Sample}/R_{Standard}\right) - 1\right] \times 1000$$

where X is ^{15}N or ^{13}C, R_{sample} is the ratio (^{15}N/^{14}N or ^{13}C/^{12}C) in the sample, and $R_{standard}$ is the ratio in the standard. The standards for nitrogen and carbon stable isotope analyses are atmospheric nitrogen and Pee Dee belemnite limestone formation, respectively. The analytical precision was determined using the standard deviation of an internal lab standard of fish muscle (n=81, δ^{15}N =0.11‰, δ^{13}C =0.08‰) and National Institute of Standards and Technology (NIST) bovine liver standard 8414 (n=81, δ^{15}N =0.14‰, δ^{13}C

=0.12‰). During sample analysis, NIST standards 8542 and 8549 were each analysed (n=10), and generated values for $\delta^{15}N$ and $\delta^{13}C$ that were within 0.12‰ and 0.01‰ of certified values, respectively.

Subsamples of blubber were taken from the middle of the blubber layer and were freeze-dried for approximately 48 hours. Lipids were extracted following Folch et al. (1957) and fatty acid methyl esters were prepared using sulfuric acid (H_2SO_4) as a catalyst. Gas chromatography was used to identify fatty acids based on retention time. Every tenth sample was analysed in duplicate to ensure consistency in instrument performance and interpretation of chromatograms. Each identified fatty acid is expressed as a percentage of the total fatty acids in the sample. Fatty acids are named using the standard notation of A:Bn-x, where A is the length of the carbon chain, B is the number of double bonds, and n-x is the location of the double bond closest to the terminal methyl group. For example, 16:1n-7 has a chain containing 16 carbon atoms with one double bond occurring at the seventh carbon atom relative to the terminal methyl group.

Teeth were extracted from the lower jaw and canines were sent to Matson's Lab (www.matsonslab.com) in Montana, USA for age determination. Canines were decalcified, cut into thin sections, mounted on microscope slides, and stained. Seal ages were then determined by counting the growth layer groups (GLGs) in the cementum. Seals were separated into three age classes: Pups (<1 year old), juveniles (1-5 years old), and adults (≥6 years old) (McLaren 1958; Holst et al. 1999; Young et al. 2010).

Seals are commonly grouped into age classes because of differences in behaviour, physiology, size, and mating ability. Our decision to use age classes rather than age as a continuous variable is due to limited sample sizes and is influenced by comparability to other studies on ringed seals which use the pup, juvenile, adult age classes (Holst et al. 2001; Young et al. 2010; Yurkowski et al. 2011; Chambellant et al. 2012).

Sex, total body weight, and sculp weight (weight of blubber layer, skin, and fur) were recorded by the hunters at the time of sample collection. Percent blubber was calculated as an indication of seal body condition:

$$Percent\ Blubber = \frac{Sculp\ Weight}{Total\ Body\ Weight} \times 100$$

Our goal was to assess seasonal patterns in foraging and therefore, results from tests for differences in age classes, sex, and year were used to simplify explanatory models. For example, no differences in months from different years resulted in dropping year, and samples from consecutive years were combined by month of collection to increase sample size.

To improve normality, fatty acid data were transformed by calculating the natural log of the ratio of each fatty acid to 18:0 (Budge et al. 2006). Analysis of fatty acid data included the use of Principal Component Analysis (PCA) using the most abundant and variable dietary fatty acids. Dietary fatty acids are those which are predominantly obtained directly from the diet, or which receive large contributions from the diet (‘extended’ dietary fatty acids), as opposed to those which are mainly derived through biosynthesis (see Iverson et al. 2004).

Hierarchical cluster analysis (average linkage, Euclidean distance) was used to determine relationships between months using $\delta^{13}C$, $\delta^{15}N$, PC1, and PC2 as variables. Based on groupings of months in the results from the cluster analysis, distinct time periods, or seasons, were determined for use in further analyses.

For stable isotope data, general linear models (GLM) were used to test for differences between year, season (derived from cluster analysis), age class, and sex. GLMs were also used for analysis of percent blubber data to test for the effects of year, age class, sex, and month. Tukey’s HSD post-hoc test was used to determine significant differences between age classes and seasons or months. Multivariate analysis of variance (MANOVA), using the first two principle components from the PCA, was used to test for differences in fatty acid composition by year, season, age class, and sex.

For all data, normality was assessed using the Shapiro-Wilk normality test. However, for GLMs, when the distribution of the data is not severely different from normal, Type 1 error rate is not drastically affected (Nimon 2012). For fatty acids, Budge et al. (2006) states that, as a result of the proportional nature of the data, fatty acid datasets rarely meet all of the assumptions of the statistical tests used in their analyses. All statistical tests were considered significant at $\alpha=0.05$.

Results

The Shapiro-Wilk normality test indicated that δ^{15}N and percent blubber data were normally distributed while δ^{13}C was not. In this case, transformation of stable isotope data did not improve normality, so untransformed data was used in analyses. The transformation of fatty acid data improved normality, but only resulted in four out of the eleven fatty acids used in analysis having a normal distribution.

A total of 192 ringed seals were harvested from Sanikiluaq, NU over the study period (Table 2.1). There were no differences between the same month from different years for both δ^{15}N (n=174, df=2, F=2.559, P=0.080) and δ^{13}C (n=174, df=2, F=0.390, P=0.678), so years were combined for further analyses. Age class had a significant effect on stable isotope ratios (δ^{15}N: n=174, df=2, F=12.573, P<0.001; δ^{13}C: n=174, df=2, F=9.126, P<0.001), but there were no significant differences between adults and juveniles (Tukey: δ^{15}N: MSE=0.625, df=159, P=0.493; δ^{13}C: MSE=0.390, df=159, P=0.522) or between sexes (δ^{15}N: n=129, df=11,F=0.618, P=0.809; δ^{13}C: n=129, df=11, F=0.898, P=0.546). Therefore, adult and juvenile age classes and sexes were combined for further analyses and are the focus for investigating seasonal patterns of feeding ecology in this study.

A minimum of 70 different fatty acids were identified in all of the adult and juvenile blubber samples and 41 of these occurred with a mean of greater than 0.10% of the total fatty acids identified in the sample. For adult/juvenile blubber samples (n=58), PCA was performed using 11 of the most abundant and variable dietary fatty acids, which represented approximately 85% of the total fatty acids in the samples. The first two principal components accounted for 83% (PC1=47%, PC2=36%) of the variation in the fatty acid composition of blubber.

The results of the hierarchical cluster analysis using $\delta^{13}C$, $\delta^{15}N$, PC1, and PC2 as variables indicated three separate groupings of months (Figure 2.2). June and July (molting/fasting season) formed the most distinct group, while January to May (ice covered season), and August to December (open water season) formed the other two groups. These distinctions were the basis for choosing the seasons to use in further analyses: January to June (ice cover), July (molting/fasting), and August to December (open water) for stable isotope data; and January to May (ice cover), June and July (molting/fasting), and August to December (open water) for fatty acid data. The difference in the seasons used for analyses is a result of June grouping more closely with the ice covered season for stable isotope data, and more closely with the fasting period for fatty acid data.

Season of collection had a significant effect on $\delta^{13}C$ (n=129, df=2, F=31.087 P<0.001), and $\delta^{15}N$ (n=129, df=2, F=5.707, P=0.004). For both $\delta^{13}C$ and $\delta^{15}N$, peak values occurred in samples collected in July following fasting, and lowest values occurred in the open water season (Table 2.2). The difference between the highest and lowest seasonal means was 1.04‰ and 1.64‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively.

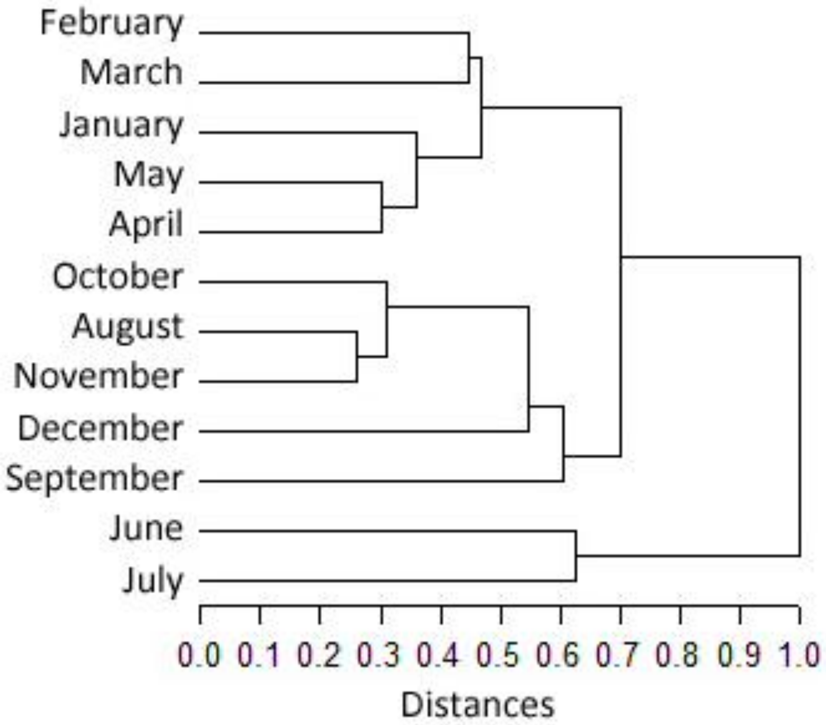

FIGURE 2.2: Hierarchical cluster analysis (average linkage, Euclidean distance) comparing months of the year using stable isotope ratios (muscle $\delta^{15}N$ and $\delta^{13}C$) and fatty acid composition data (PC1 and PC2 from principal component analysis using 11 dietary fatty acids in blubber) from ringed seals harvested from Sanikiluaq, NU from November 2009 to May 2011.

Table 2.2: Seasonal summary of $\delta^{13}C$ and $\delta^{15}N$ values and C:N ratios of adult/juvenile ringed seal muscle from Sanikiluaq, NU collected from November 2009 to May 2011. For $\delta^{13}C$ and $\delta^{15}N$, letters indicate significant differences between seasons, as determined using Tukey's HSD post-hoc test.

	Season	N	Mean ± SE	Range (min, max)
$\delta^{13}C$				
	Ice Cover	91	^{a}-19.22 ± 0.07	-20.51, -16.96
	Fasting	8	^{b}-18.39 ± 0.28	-19.57, -17.21
	Open Water	31	^{a}-20.03 ± 0.10	-20.91, -17.96
$\delta^{15}N$				
	Ice Cover	91	^{a}14.70 ± 0.10	12.37, 16.95
	Fasting	8	^{b}15.41 ± 0.14	14.77, 15.93
	Open Water	31	^{c}14.36 ± 0.14	12.96, 16.88
C:N				
	Ice Cover	91	3.38 ± 0.01	3.25, 3.60
	Fasting	8	3.33 ± 0.03	3.21, 3.52
	Open Water	31	3.36 ± 0.01	3.26, 3.47

MANOVA conducted using the first two principle components indicated no difference between adult and juvenile age classes (λ=0.988, F=0.988, df=2, 55, P=0.379). Year did not have a significant effect (λ=0.972, F=.454, df=2, 31, P=0.639) while sex (λ=0.883, F=3.638, df=2, 55, P=0.033) and season (λ=0.565, F=8.911, df=4, 108, P=0.002) both had significant effects on fatty acid composition. PCA results illustrate the variation among the three seasons with the fasting period having the most distinct fatty acid composition (Figure 2.3, Table 2.3).

There were no differences in percent blubber between years (n=129, df=2, F=0.479, P=0.621), between adults and juveniles (n=129, df=11, F=1.815, P=0.062), or between males and females (n=129, df=11, F=0.516, P=0.888). Month of collection had a significant effect on percent blubber (n=129, df=11, F=4.203, P<0.001) with an approximate 15% change between peak condition in January and lowest condition in June through August (Figure 2.4).

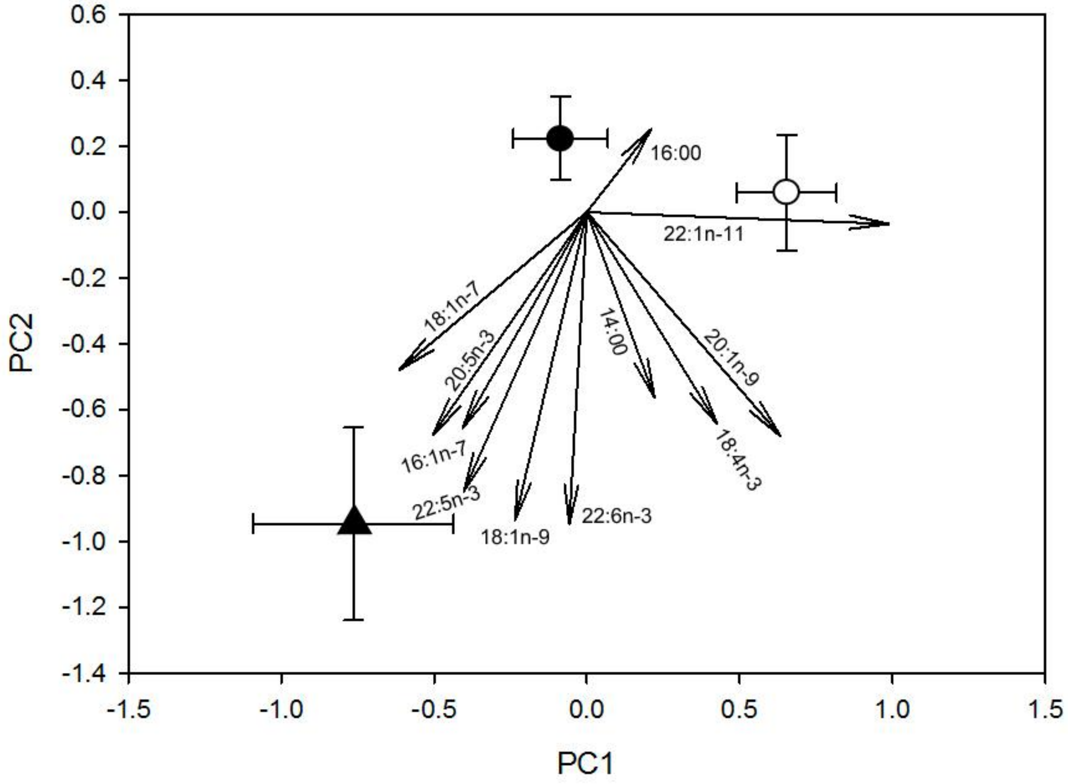

FIGURE 2.3: Principal Component analysis (PCA) was conducted using 11 dietary fatty acids from adult and juvenile ringed seal blubber collected from Sanikiluaq, NU from November 2009 to May 2011. Seasonal variation in fatty acid composition is represented by seasonal mean (± SE) PC1 and PC2 scores from the PCA. Closed circle represents the ice covered season, open circle represents the open water season, and triangle represents the fasting season. Vectors represent the specific fatty acids used in the PCA.

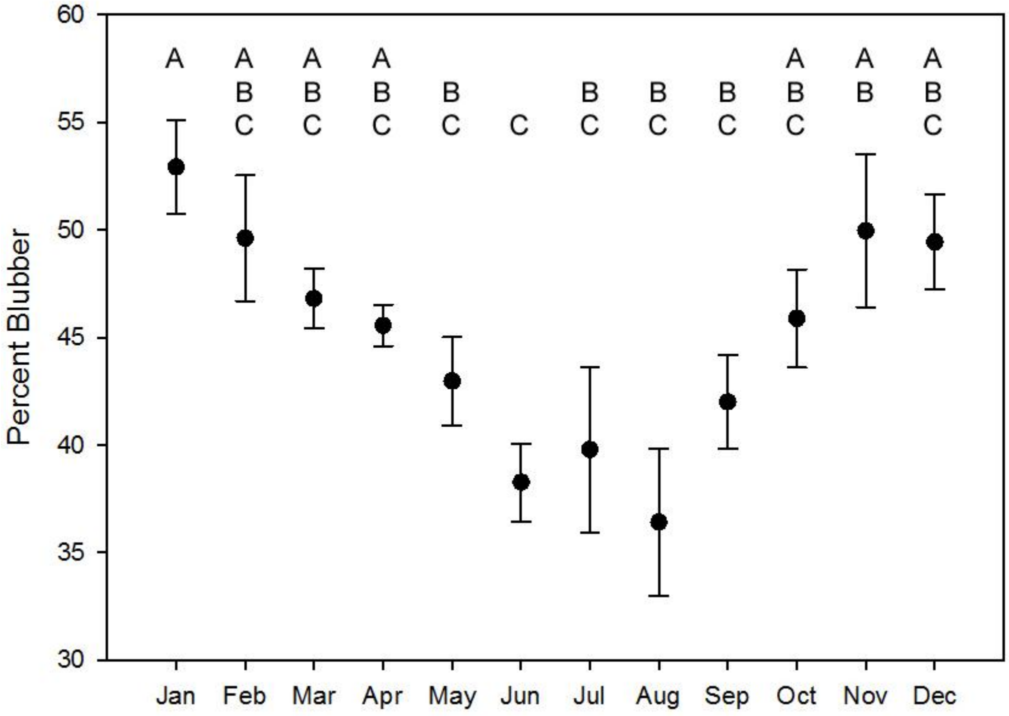

FIGURE 2.4: Monthly variation (mean ± SE) in percent blubber (sculp weight/total body weight x 100) of adult and juvenile ringed seals harvested from Sanikiluaq, NU. Measurements were taken from seals harvested from November 2009 to May 2011 and measurements from different years were combined by month. Letters indicate significant differences between months as determined using Tukey's HSD post-hoc test.

TABLE 2.3: Monthly mean (± SE) fatty acid content (% of total fatty acids) in eastern Hudson Bay ringed seal blubber. Only those fatty acids used in statistical analysis are shown. Monthly mean (± SE) principal component scores are also presented (PC1 and PC2).

	January	February	March	April	May	June	July	August	September	October	November	December
14:0	3.93 ± 0.32	4.21 ± 0.39	4.08 ± 0.28	4.33 ± 0.20	4.11 ± 0.27	3.25 ± 0.28	2.78 ± 0.18	4.23 ± 0.28	4.11 ± 0.40	4.68 ± 0.24	4.74 ± 0.34	4.68 ± 0.16
16:0	8.59 ± 0.68	8.75 ± 0.47	7.46 ± 0.94	9.31 ± 0.80	7.96 ± 0.80	5.11 ± 0.50	4.10 ± 0.46	8.42 ± 0.68	7.25 ± 1.53	10.10 ± 0.76	9.33 ± 0.58	9.49 ± 0.12
16:1n-7	18.78 ± 1.23	19.69 ± 1.63	17.72 ± 1.55	21.24 ± 1.11	17.89 ± 0.76	18.29 ± 1.46	14.78 ± 0.90	17.71 ± 0.86	18.84 ± 1.14	20.89 ± 1.15	17.91 ± 1.96	17.22 ± 0.98
18:1n-9	11.98 ± 0.34	11.65 ± 0.63	12.24 ± 0.53	11.62 ± 0.34	12.48 ± 0.48	14.29 ± 0.53	14.58 ± 0.38	12.52 ± 1.18	14.03 ± 0.53	12.35 ± 0.26	10.62 ± 0.19	10.02 ± 0.08
18:1n-7	5.96 ± 0.67	5.36 ± 0.46	5.03 ± 0.63	6.17 ± 0.37	5.36 ± 0.40	5.18 ± 0.46	4.74 ± 0.39	5.42 ± 0.25	4.82 ± 0.38	6.51 ± 0.28	5.11 ± 0.74	4.40 ± 0.50
18:4n-3	1.63 ± 0.20	1.94 ± 0.23	1.75 ± 0.26	1.28 ± 0.11	1.45 ± 0.17	1.35 ± 0.17	1.24 ± 0.08	1.15 ± 0.10	1.94 ± 0.61	1.16 ± 0.10	2.11 ± 0.52	2.58 ± 0.23
20:1n-9	4.17 ± 0.66	4.76 ± 0.92	4.94 ± 0.83	3.42 ± 0.61	4.43 ± 0.53	3.72 ± 1.25	4.88 ± 0.70	5.67 ± 0.66	3.85 ± 0.61	3.95 ± 0.39	5.04 ± 1.11	5.52 ± 1.00
20:5n-3	10.85 ± 0.70	11.22 ± 0.52	10.52 ± 0.63	11.88 ± 0.62	10.39 ± 0.61	11.10 ± 1.16	9.57 ± 0.92	9.73 ± 0.22	10.85 ± 0.26	11.63 ± 0.87	9.95 ± 1.44	10.32 ± 0.65
22:1n-11	1.13 ± 0.24	1.33 ± 0.40	1.27 ± 0.37	0.71 ± 0.17	0.72 ± 0.12	0.38 ± 0.17	0.43 ± 0.17	1.82 ± 0.71	0.94 ± 0.07	1.33 ± 0.13	2.14 ± 0.74	2.21 ± 0.55
22:5n-3	5.10 ± 0.42	5.22 ± 0.42	5.98 ± 0.80	5.70 ± 0.43	6.49 ± 0.55	7.05 ± 0.54	9.19 ± 0.64	5.85 ± 0.98	5.21 ± 1.06	4.94 ± 0.54	5.41 ± 0.16	4.09 ± 0.21
22:6n-3	13.26 ± 1.12	11.92 ± 0.90	13.78 ± 1.28	11.13 ± 0.97	13.83 ± 0.98	15.75 ± 0.53	17.61 ± 0.58	12.54 ± 0.27	13.35 ± 0.73	9.60 ± 0.37	12.73 ± 1.21	13.70 ± 0.48
PC1	0.12 ± 0.25	0.38 ± 0.34	0.15 ± 0.55	-0.52 ± 0.34	-0.23 ± 0.24	-0.84 ± 0.48	-0.71 ± 0.50	0.59 ± 0.54	0.39 ± 0.19	0.31 ± 0.19	0.83 ± 0.48	1.15 ± 0.36
PC2	0.49 ± 0.19	0.23 ± 0.08	-0.32 ± 0.29	0.52 ± 0.26	0.11 ± 0.32	-0.92 ± 0.34	-0.97 ± 0.49	0.03 ± 0.17	-0.78 ± 0.51	0.54 ± 0.39	0.44 ± 0.20	0.06 ± 0.24

Discussion

The observed changes in percent blubber, as an indication of body condition, over the 12 month period indicate that poorest condition occurs following the molting/fasting period, followed by a gradual increase from late summer through fall with the highest body condition (greatest percent blubber) occurring in time for freeze-up in December (Figure 2.4). High body condition in January declines throughout the period of ice cover. These results are consistent with findings from other studies (McLaren 1958; Smith 1987; Ryg et al. 1990; Goodyear 1999); however, our results are the first to use reasonable sample sizes (ca. 20) for every month of the year.

Increased $\delta^{15}N$ and $\delta^{13}C$ in July correspond to the period of fasting that occurs during the spring molt, roughly during May and June (McLaren 1958; Kelly et al. 2010). Periods of fasting or nutritional stress cause increased stable isotope ratios as fat and energy stores are metabolised in place of consuming prey items (Hobson et al. 1993; Cherel et al. 2005). Turnover rates of stable isotopes in marine mammal muscle have not been determined and estimates for muscle from other species are very limited. For other mammals, estimates of stable isotope turnover in muscle range from $\delta^{13}C$ half-lives of 23.9 days in mice (*Mus musculus*) (MacAvoy et al. 2005) and 27.6 days in gerbil (*Meriones unguienlatus)* (Tieszen et al. 1983) to 151.0 days in steers (*Bos primigenius*) (Bahar et al. 2009) and 178.7 days in alpaca (*Lama pacos*) (Sponheimer et al. 2006). In this study, the difference between the known time period of molting/fasting and the increased stable isotope values observed in July, suggest that it takes roughly 1 month for $\delta^{15}N$ and $\delta^{13}C$ in ringed seal muscle to show noticeable changes in response to fasting.

Fatty acid composition was also affected by fasting. During periods of negative energy balance, fat is not synthesized and fatty acids may be selectively mobilized or retained (Iverson et al. 1995; Budge et al. 2006; Nieminen et al. 2006). The fatty acids 16:0 and 16:1n-7 have been found to be important products of biosynthesis (Iverson et al. 2004; Budge et al. 2006). The relatively low proportions of 16:0 and 16:1n-7 observed in June and July (Table 2.3) are likely a result of fasting, as these fatty acids would not be synthesized during that period. The lower proportion of 20:5n-3 observed during the same period is consistent with other studies that have found selective mobilization of this fatty acid during fasting (Budge et al. 2006; Nieminen et al. 2006). Increased proportions of 22:5n-3 and 22:6n-3 in July suggests that these fatty acids may be selectively retained and could have an important physiological function. Assuming the peaks and troughs in fatty acids in June and July are a result of fasting during the molt, fatty acids in blubber appear to reflect changes in response to fasting at a slightly faster rate than stable isotopes in muscle, but were still thought to occur within a time frame of approximately 1 month. A similar time frame has been estimated for turnover rates of fatty acids in marine mammal blubber. Nordstrom et al. (2008) found a fatty acid turnover rate of 1.5 to 3 months in harbour seal (*Phoca vitulina*) blubber, and Budge et al. (2006) indicated a turnover rate of weeks to months, depending on species and the depth of the blubber layer sampled.

Seasonal changes in ringed seal diet are commonly reported throughout much of the Arctic with differences observed between ice covered and open water periods (Lowry et al. 1980; Weslawski et al. 1994; Thiemann et al. 2007; Labansen et al. 2011; Chambellant et al. In Press). The analysis of δ^{13}C in ringed seal muscle in eastern Hudson Bay indicated higher values for the time period representing the diet during the period of ice cover and lower values during the period of open water (Table 2.2). A similar pattern occurred in δ^{15}N, however, the seasonal shift between the open water and ice cover periods is relatively small and is non-significant (Table 2.2). δ^{13}C isotopic values are considered to be good indicators of the source of a diet, with benthic and inshore habitats being enriched relative to pelagic sources of carbon (Hobson and Welch 1992; France 1995), and ice algae being enriched relative to particulate organic matter or phytoplankton (Hobson et al. 1995). Therefore, the observed shift from high to low δ^{13}C is likely an indication of a shift from a diet originating in ice algae or benthic/inshore habitats during the period of ice cover, to a diet originating in more of a pelagic habitat in the open water season.

The relatively small and non-significant difference between open water and ice cover periods in δ^{15}N does not provide evidence for a seasonal shift in prey items taken by ringed seals. However, it is possible that some dietary change does occur between prey items that have similar δ^{15}N values. Since benthic food webs tend to be enriched in δ^{15}N relative to pelagic food webs (France 1995; Iken et al. 2005), an organism in a pelagic food web could have a lower δ^{15}N than an organism feeding at the same trophic level in the benthic food web. Therefore, it is possible for ringed seals to switch from higher trophic level pelagic prey, to slightly lower trophic level benthic prey, with no significant change in $\delta^{15}N$. In the absence of prey isotope data and/or stomach contents we are unable to discriminate between the alternative explanations.

Many of the processes involved in the incorporation of isotopes into tissues are poorly understood (Jardine et al. 2006). For example, physiological state, growth rate, protein content of diet, and protein quality could all have an effect on $\delta^{15}N$ discrimination factors (Martinez del Rio et al. 2009). In our data, it is possible that physiological differences related to fat loss during the ice cover period and fat growth during the open water period are affecting the way stable isotopes are incorporated into tissues. In addition, changes in environmental variables and variation in carbon and nitrogen uptake by phytoplankton could affect base isotopic levels which are then scaled up the food web (Tamelander et al. 2009; Newsome et al. 2010).

The results of the fatty acid PCA provide evidence in support of a seasonal dietary shift. While the fasting season is distinctly different from the other seasons, we can also see a significant separation between the open water and ice covered periods (Figure 2.3). Seasonal variation in the proportions of individual fatty acids (Table 2.3) provides support for changes in the relative importance of different prey items over the year. For example, higher proportions of 22:1n-11 occurred in seals collected in November and December, followed by a decline in January through July. Studies from other areas have found relatively high amounts of 22:1n-11 in pelagic fish, including capelin (*Mallotus villosus*) and sandlance (*Ammodytes sp.*) (Budge et al. 2002; Iverson et al. 2002;

Andersen et al. 2004). The observed variation in 22:1n-11 in eastern Hudson Bay ringed seals could indicate a seasonal shift in the importance of pelagic fish species such as capelin and sandlance, with a greater importance in the fall (October/November) before freeze-up, and a declining importance over the period of ice cover through to ice breakup in June. This result is consistent with findings from stomach content analysis from eastern Hudson Bay that found capelin, sandlance, and Arctic cod (*Boreogadus saida*) were an important part of ringed seal diet year-round, while capelin had increased importance in fall, and Arctic cod, invertebrates, and other fish species such as sculpins (Cottidae) had increased importance in winter (Data on file).

The observed difference between male and female fatty acid composition could be a result of physiological differences related to reproduction and lactation (Wheatley et al. 2008), and needs to be considered as a possible explanation in addition to the seasonal dietary shift. Low sample size limited statistical analyses for seasonal variation for each sex individually, but inspection of PCA biplots created separately for males and females showed similar patterns between sexes. Investigation into the fatty acid composition of the eastern Hudson Bay prey community, as well as a better understanding of selective mobilization of fatty acids during fasting, is necessary to further understand the meaning of the seasonal variation in ringed seal fatty acid composition.

In summary, the variation in stable isotope ratios and fatty acid composition provide evidence for a seasonal shift in feeding ecology of ringed seals in eastern Hudson Bay. The shift from high to low δ^{13}C suggests a diet originating in ice algae and/or benthic habitats during the period of ice cover, and a diet originating in a more pelagic habitat in the open water season. Changes in fatty acid composition could be a result of seasonal dietary changes, including shifts in the relative importance of specific prey items that may include pelagic fish species such as capelin and sandlance. Unfortunately, the methods used in this study do not allow for the determination of specific prey items or changes in their relative importance, and further research is needed to overcome these limitations.

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References

Andersen S, Lydersen C, Grahl-nielsen O, Kovacs KM (2004) Autumn diet of harbour seals (*Phoca vitulina*) at Prins Karls Forland, Svalbard, assessed via scat and fatty-acid analyses. Canadian Journal of Zoology 82:1230-1245

Bahar B, Moloney AP, Monahan FJ, Harrison SM, Zazzo A, Scrimgeour CM, Begley IS, Schmidt O (2009) Turnover of carbon, nitrogen, and sulfur in bovine longissimus dorsi and psoas major muscles: Implications for isotopic authentication of meat. Journal of Animal Science 87:905–913

Bligh E, Dyer W (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911–917

Bluhm BA, Gradinger R (2008) Regional variability in food availability for Arctic marine mammals. Ecological Applications 18:S77-S96

Budge SM, Iverson SJ, Bowen WD, Ackman RG (2002) Among- and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of St. Lawrence. Canadian Journal of Fisheries and Aquatic Sciences 59:886-898

Budge SM, Iverson SJ, Koopman H (2006) Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Marine Mammal Science 22:759-801

Chambellant M, Stirling I, Gough W, Ferguson SH (2012) Temporal variations in Hudson Bay ringed seal (*Phoca hispida*) life-history parameters in relation to environment. Journal of Mammalogy 93:267–281

Chambellant M, Stirling I, Ferguson SH (In Press) Temporal variation in western Hudson Bay ringed seal (*Phoca hispida*) in relation to environment. Marine Ecology Progress Series.

Cherel Y, Hobson K, Bailleul F, Groscolas R (2005) Nutrition, physiology, and stable isotopes: new information from fasting and molting penguins. Ecology 86:2881-2888

Dalerum F, Angerbjörn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647–658

DeNiro MJ, Epstein S (1977) A mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197**:**261-263

DeNiro M, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495-506

Derocher AE, Lunn NJ, Stirling I (2004) Polar Bears in a Warming Climate. Integrative and Comparative Biology 44:163-176

Ferguson SH, Stirling I, McLoughlin P (2005) Climate Change and Ringed Seal (*Phoca hispida*) Recruitment in Western Hudson Bay. Marine Mammal Science 21:121-135

Finley KJ (1979) Haul-out behaviour and densities of ringed seals (*Phoca hispida*) in the Barrow Strait area, N.W.T. Canadian Journal of Zoology 57:1985–1997

Folch J, Lees M, Sloane Stanley G (1957) A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226:497-509

France R (1995) Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnology and Oceanography 40:1310-1313

Gagnon AS, Gough WA (2005a) Trends in the Dates of Ice Freeze-up and Breakup over Hudson Bay, Canada. Arctic 58:370-382

Gagnon AS, Gough WA (2005b) Climate change scenarios for the Hudson Bay region: an intermodel comparison. Climatic Change 69:269-297

Galbraith PS, Larouche P (2011) Sea-surface temperature in Hudson Bay and Hudson Strait in relation to air temperature and ice cover breakup, 1985 – 2009. Journal of Marine Systems 87:66–78

Gjertz I, Lydersen C (1986) The ringed seal (*Phoca hispida*) spring diet in northwestern Spitsbergen, Svalbard. Polar Research 4:53-56

Goodyear M (1999) Variation in growth and seasonal condition of ringed seal, *Phoca hispida*, from the Canadian Arctic. Thesis (M.Sc.) University of Manitoba, Winnipeg, Manitoba.

Harington C (2008) The evolution of Arctic marine mammals. Ecological Applications 18:S23-40

Harwood L, Smith TG, Melling H (2000) Variation in Reproduction and Body Condition of the Ringed Seal (*Phoca hispida*) in Western Prince Albert Sound, NT, Canada, as assessed Through a Harvest-based Sampling Program. Arctic 53:422-431

Hobson K, Alisauskas R, Clark R (1993) Stable-Nitrogen Isotope Enrichment in Avian Tissues Due to Fasting and Nutritional Stress: Implications for Isotopic Analyses of Diet. Condor 95:388-394

Hobson KA, Ambrose W, Renaud P (1995) Sources of primary production, benthic-pelagic coupling, and trophic relationships within the Northeast Water Polynya: insights from $\delta^{13}C$ and $\delta^{15}N$ analysis. Marine Ecology Progress Series 128:1-10

Hobson K, Clark R (1992) Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. Condor 94:181-188

Hobson KA, Welch H (1992) Determination of trophic relationships within a high Arctic marine food web using δ^{13}C and δ^{15}N analysis. Marine Ecology Progress Series 84:9-18

Holst M, Stirling I, Calvert W (1999) Age structure and reproductive rates of ringed seals (*Phoca hispida*) on the northwestern coast of Hudson Bay in 1991 and 1992. Marine Mammal Science 15:1357-1364

Holst M, Stirling I, Hobson KA (2001) Diet of Ringed Seals (*Phoca hispida*) on the East and West Sides of the North Water Polynya, Northern Baffin Bay. Marine Mammal Science 17:888-908

Iken K, Bluhm BA, Gradinger R (2005) Food web structure in the high Arctic Canada Basin: evidence from δ^{13}C and δ^{15}N analysis. Polar Biology 28:238–249

Ingram T, Matthews B, Harrod C, Stephens T, Grey J, Markel R, Mazumder A (2007) Lipid extraction has little effect on the δ^{15}N of aquatic consumers. Limnology and Oceanography: Methods 5:338–343

Iverson SJ (2008) Tracing Aquatic Food Webs Using Fatty Acids: From Qualitative Indicators to Quantitative Determination. In: Kainz M, Brett MT, Arts MT (eds) Lipids in Aquatic Ecosystems. Springer New York, New York, NY, pp 281–307

Iverson SJ, Field C, Bowen WD, Blanchard W (2004) Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecological Monographs 74:211-235

Iverson SJ, Frost KJ, Lang SL (2002) Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability. Marine Ecology Progress Series 241:161-181

Iverson S, Oftedal O, Bowen W, Boness D, Sampugna J (1995) Prenatal and postnatal transfer of fatty acids from mother to pup in the hooded seal. Journal of Comparative Physiology B 165:1-12

Jardine TD, Kidd KA, Fisk AT (2006) Applications, Considerations, and Sources of Uncertainty When Using Stable Isotope Analysis in Ecotoxicology. Environmental Science & Technology 40:7501–7511

Kelly BP, Badajos OH, Kunnasranta M, Moran JR, Martinez-Bakker M, Wartzok D, Boveng PL (2010) Seasonal home ranges and fidelity to breeding sites among ringed seals. Polar Biology 33:1095-1109

Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78:1–27

Labansen AL, Lydersen C, Haug T, Kovacs KM (2007) Spring diet of ringed seals (*Phoca hispida*) from northwestern Spitsbergen, Norway. ICES Journal of Marine Science 64:1246-1256

Labansen AL, Lydersen C, Levermann N, Haug T, Kovacs KM (2011) Diet of ringed seals (*Pusa hispida*) from Northeast Greenland. Polar Biology 34:227-234

Lesage V, Morin Y, Rioux È, Pomerleau C, Ferguson SH, Pelletier É (2010) Stable isotopes and trace elements as indicators of diet and habitat use in cetaceans: predicting errors related to preservation, lipid extraction, and lipid normalization. Marine Ecology Progress 419:249-265

Lowry LF, Frost K, Burns JJ (1980) Variability in the Diet of Ringed Seals, *Phoca hispida*, in Alaska. Canadian Journal of Fisheries and Aquatic Sciences 37:2254-2261

Macavoy SE, Macko SA, Arneson LS (2005) Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis. Canadian Journal of Zoology 83:631–641

Martínez del Rio C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biological Reviews 84:91–111

McLaren I (1958) The Biology of the Ringed Seal (*Phoca hispida Schreher*) in the Eastern Canadian Arctic. Bulletin of the Fisheries Research Board of Canada 118, pp 97

Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between δ^{15}N and animal age. Geochimica et Cosmochimica Acta 48:1135-1140

Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study marine mammal ecology. Marine Mammal Science 26:509–572

Nieminen P, Käkelä R, Pyykönen T, Mustonen A-mari (2006) Selective fatty acid mobilization in the American mink (*Mustela vison*) during food deprivation. Comparative Biochemistry and Physiology, Part B 145:81-93

Nimon KF (2012) Statistical Assumptions of Substantive Analyses Across the General Linear Model: A Mini-Review. Frontiers in Psychology 3:1–5

Nordstrom C, Wilson L, Iverson SJ, Tollit D (2008) Evaluating quantitative fatty acid signature analysis (QFASA) using harbour seals *Phoca vitulina richardsi* in captive feeding studies. Marine Ecology Progress Series 360:245-263

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–89

Ryg M, Smith TG, Oritsland NA (1990) Seasonal changes in body mass and body composition of ringed seals (*Phoca hispida*) on Svalbard. Canadian Journal of Zoology 68:470–475

Ryg M, Oritsland NA (1991) Estimates of energy expenditure and energy consumption of ringed seals (*Phoca hispida*) throughout the year. Polar Research 10:595-602

Smith TG (1987) The ringed seal, *Phoca hispida*, of the Canadian western Arctic. Canadian Bulletin of Fisheries and Aquatic Sciences 216:1-81

Smith T, Hammill M (1981) Ecology of the ringed seal, *Phoca hispida*, in its fast ice breeding habitat. Canadian Journal of Zoology 59:966-981

Smith TG, Hammill MO, Taugbol G (1991) A Review of the Developmental, Behavioural and Physiological Adaptations of the Ringed Seal, *Phoca hispida*, to Life in the Arctic Winter. Arctic 44:124-131

Smith TG, Stirling I (1975) The breeding habitat of the ringed seal (*Phoca hispida*). The birth lair and associated structures. Canadian Journal of Zoology 53:1297-1305

Søreide J, Tamelander T, Hop H, Hobson K, Johansen I (2006) Sample preparation effects on stable C and N isotope values: a comparison of methods in Arctic marine food web studies. Marine Ecology Progress Series 328:17–28

Sponheimer M, Robinson T, Cerling T, Tegland L, Roeder B, Ayliffe L, Dearing M, Ehleringer J (2006) Turnover of stable carbon isotopes in the muscle, liver, and breath CO_2 of alpacas (*Lama pacos*). Rapid Communications in Mass Spectrometry 20:1395-1399

Stirling I (2005) Reproductive rates of ringed seals and survival of pups in Northwestern Hudson Bay, Canada, 1991-2000. Polar Biology 28:381-387

Sweeting C, Polunin N, Jennings S (2006) Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. Rapid Communications in Mass Spectrometry 20:595–601

Tamelander T, Kivimae C, Bellerby RG, Renaud PE, Kristiansen S (2009) Base-line variations in stable isotope values in an Arctic marine ecosystem: effects of carbon and nitrogen uptake by phytoplankton. Hydrobiologia 630:63–73

Thiemann GW, Iverson SJ, Stirling I (2007) Variability in the blubber fatty acid composition of ringed seals (*Phoca hispida*) across the Canadian Arctic. Marine Mammal Science 23:241-261

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32-37

Walsh JE (2008) Climate of the Arctic marine environment. Ecological Applications 18:S3-22

Wang J, Mysak LA, Ingram RG (1994) Interannual Variability of Sea-Ice Cover in Hudson Bay, Baffin Bay and the Labrador Sea. Atmosphere-Ocean 32:421-447

Weslawski JM, Ryg M, Smith TG, Oritsland NA (1994) Diet of Ringed Seals (*Phoca hispida*) in a Fjord of West Svalbard. Arctic 47:109-114

Wheatley KE, Nichols PD, Hindell MA, Harcourt RG, Bradshaw CJ (2008) Differential Mobilization of Blubber Fatty Acids in Lactating Weddell Seals: Evidence for Selective Use. Physiological and Biochemical Zoology 81:651–662

Young BG, Loseto LL, Ferguson SH (2010) Diet differences among age classes of Arctic seals: evidence from stable isotope and mercury biomarkers. Polar Biology 33:153–162

Yurkowski DJ, Chambellant M, Ferguson SH (2011) Bacular and testicular growth and allometry in the ringed seal (*Pusa hispida*): evidence of polygyny? Journal of Mammalogy 92:803–810

CHAPTER 3. USING STABLE ISOTOPES TO UNDERSTAND CHANGES IN RINGED SEAL FORAGING ECOLOGY AS A RESPONSE TO A WARMING ENVIRONMENT

Abstract

Current trends toward increased temperatures, reduced sea ice extent, and longer open water seasons have resulted in changing Arctic ecosystem dynamics. Expected changes include shifts in distribution and abundance of prey species for seabirds and marine mammals. The purpose of this study was to investigate spatial and inter-annual variation in ringed seal (*Phoca hispida)* feeding ecology in Hudson Bay in relation to environmental variables using stable isotope analysis. Muscle and hair samples from ringed seals harvested during the Inuit subsistence hunt from Arviat, NU and Sanikiluaq, NU were analysed for stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$). Seals from western Hudson Bay (Arviat) had higher $\delta^{15}N$ and lower $\delta^{13}C$ than seals from eastern Hudson Bay (Sanikiluaq). Seal age class and spring air temperature also had significant direct and indirect effects on stable isotope ratios. Peak $\delta^{15}N$ occurred within a range in spring air temperatures, between approximately -5°C and -2°C. This temperature range was characteristic of warm years in western Hudson Bay and cool years in eastern Hudson Bay. We hypothesize that the high $\delta^{15}N$ observed in ringed seals are indicative of relatively greater importance of capelin (*Mallotus villosus*) in the ringed seal diet.

Introduction

Recent warming and reduction of sea ice extent has led to changes in Arctic ecosystem dynamics including shifts in species distribution and abundance (Bluhm and Gradinger 2008; Post et al. 2009; Wassmann et al. 2011). As a subarctic inland sea located at relatively low latitude, Hudson Bay is expected to undergo a high level of environmental and ecological change, earlier than other areas of the Arctic (Gagnon and Gough 2005; Stirling 2005; Parkinson and Cavalieri 2008). In northern Hudson Bay, Gaston et al. (2003) have documented a shift in the dominance of Arctic Cod (*Boreogadus saida*) to capelin (*Mallotus villosus*) in the diet of thick-billed murres (*Uria lomvia)*. Other warming related ecological changes already observed in Hudson Bay include decreased polar bear (*Ursus maritimus*) body condition and survival (Stirling et al. 1999; Regehr et al. 2007), increased presence of killer whales (*Orcinus orca*) (Higdon and Ferguson 2009), and decreased ringed seal (*Phoca hispida*) recruitment (Ferguson et al. 2005).

Due to its high abundance, wide ranging distribution, and adaptations to sea ice, the ringed seal has been identified as a good indicator species for ecological change (Laidre et al. 2008; Vincent-Chambellant 2010). In order to predict and understand long term changes, it is important to understand how species respond to environmental change over shorter time scales. As a high trophic level species known to feed on a variety of prey (Lowry et al. 1980; Weslawski et al. 1994; Holst et al. 2001; Labansen et al. 2011; Chambellant et al. in press), analysis of changes in ringed seal feeding ecology is an effective approach to monitor ecosystem change.

The use of stable isotope analysis provides information on relative changes in feeding ecology as isotopic signatures are passed from prey to predator (Kelly 2000; Dalerum and Angerbjörn 2005; Newsome et al. 2010). Nitrogen stable isotope ratios ($\delta^{15}N$) are subject to trophic enrichment and therefore give information related to trophic positioning (Minagawa and Wada 1984). Carbon stable isotope ratios ($\delta^{13}C$) are only slightly enriched with trophic level and are therefore a better indicator of foraging habitat (DeNiro and Epstein 1978). Stable isotopes are incorporated into different tissues according to tissue specific turnover rates that are related to growth and metabolic rate (Phillips and Eldridge 2006; Newsome et al. 2010). For example, the dietary time period represented in tissues ranges from days in blood plasma, to several years or an entire lifetime in metabolically inert tissues such as teeth, claws, or baleen (Hobson and Clark 1992; Newsome et al. 2010). Monitoring stable isotope ratios over time and in different tissues allows us to determine relative changes in trophic positioning and habitat use.

Through the analysis of stable isotope ratios in tissues, we expect that with warming, ringed seal foraging ecology will change over both short and long time and space scales. The objectives of this study were 1) to identify spatial and temporal trends in $\delta^{15}N$ and $\delta^{13}C$ stable isotope ratios in Hudson Bay ringed seal tissues, 2) relate patterns to environmental variables of ice breakup date and spring air temperature, and 3) interpret isotopic trends in the context of ecological change related to warming.

Methods

Samples of muscle, hair, and lower jaw were collected from ringed seals harvested during the Inuit subsistence hunt from Arviat, NU in western Hudson Bay, and from Sanikiluaq, NU in the Belcher Islands of eastern Hudson Bay (Figure 3.1). Samples were collected from a total of 377 seals (Arviat=122, Sanikiluaq=255) however, not all types of samples were available from all individuals (Table 3.1). Tissue samples were consistently taken from the mid-dorsal (muscle) and mid-ventral (hair) region of the seal and were kept frozen at -25°C until analysis. In Arviat, all samples were collected from September to November each year from 2007 to 2010 while in Sanikiluaq, samples were collected from August to January each year from 2003 to 2010. The minor difference in months of collection between the two areas was not considered to create bias in the analysis since year-round analysis has found no significant difference in $\delta^{15}N$ or $\delta^{13}C$ in eastern Hudson Bay ringed seal muscle from samples collected in August through January (Young and Ferguson, in press).

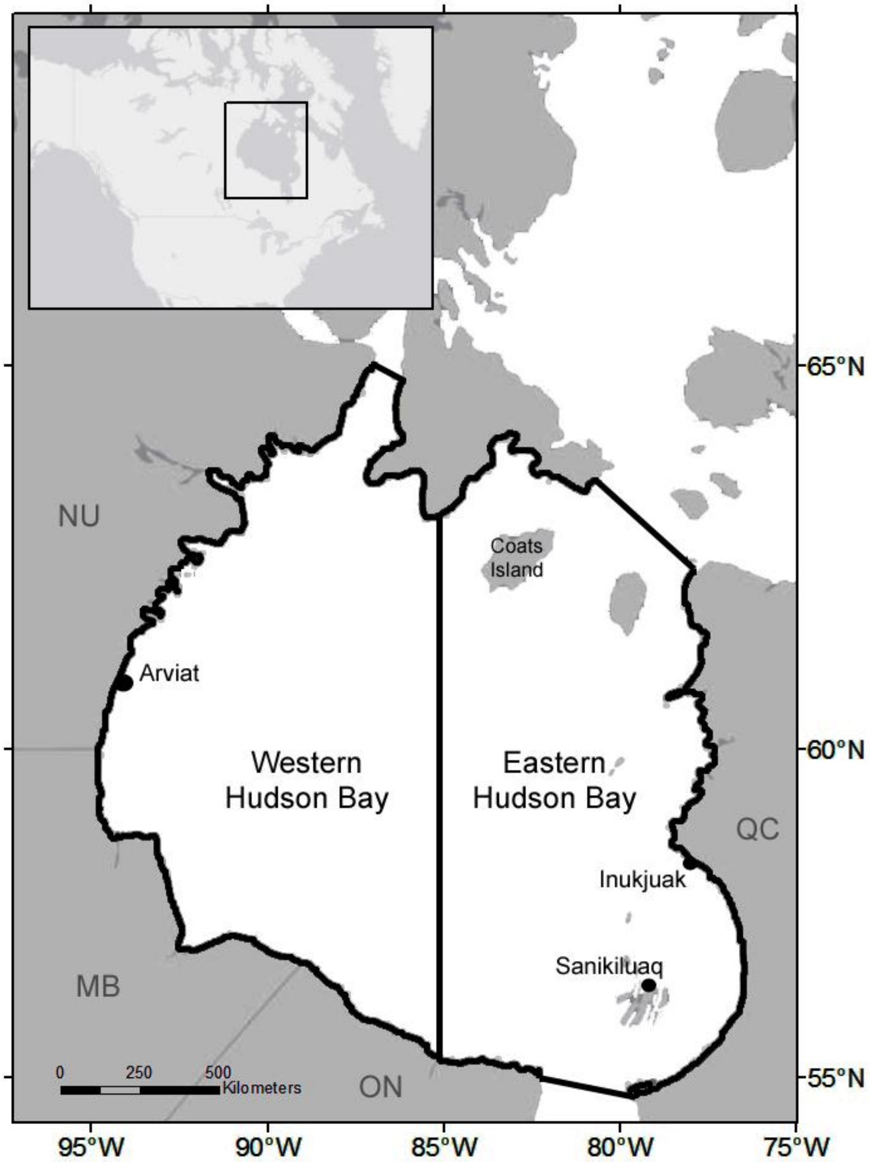

FIGURE 3.1: Ringed seal samples were collected during subsistence harvests from Arviat and Sanikiluaq. Ice breakup dates were determined separately for western and eastern Hudson Bay using approximately 85°W longitude as a boundary.

TABLE 3.1: Ringed seal tissue samples used for $\delta^{15}N$ and $\delta^{13}C$ analysis. Samples were collected from Inuit subsistence harvests from Arviat, NU and Sanikiluaq, NU.

	Muscle				Hair			
	Pups	Juveniles	Adults	Total	Pups	Juveniles	Adults	Total
Arviat								
2007	5	8	17	30	-	-	-	-
2008	10	10	9	29	9	9	9	27
2009	10	10	11	31	10	8	11	29
2010	10	2	20	32	10	2	20	32
Sanikiluaq								
2003	9	10	11	30	-	-	-	-
2004	10	13	7	30	-	-	-	-
2005	10	10	9	29	-	-	-	-
2006	10	10	10	30	-	-	-	-
2007	10	7	9	26	6	8	5	19
2008	10	11	3	24	11	13	9	33
2009	12	11	8	31	13	11	10	34
2010	13	12	16	41	10	21	12	43

Frozen samples of muscle were freeze-dried for approximately 48 hours and then crushed into a fine powder using a mortar and pestle. Hair samples were washed with soap and water to remove any dirt and surface oils, oven dried at approximately 90°C, and homogenized by repeated cutting. The samples were sent for $\delta^{15}N$ and $\delta^{13}C$ analysis at the Great Lakes Institute for Environmental Research at the University of Windsor.

Lipids tend to be depleted in ^{13}C and can cause biased $\delta^{13}C$ values when present in samples (DeNiro and Epstein 1977; Post et al. 2007). To eliminate this bias, lipids are usually removed from lipid rich tissues prior to analysis (Lesage et al. 2010). We removed lipids from muscle samples by mixing twice, for 24 hours, in 5 ml of 2:1

chloroform-methanol solution following Bligh and Dyer (1959). It has been recommended that chloroform-methanol removal methods be used prior to the analysis of lipid-rich tissues (Ingram et al. 2007; Newsome et al. 2010); however, recent studies have found that lipid removal can bias $\delta^{15}N$ results (Sweeting et al. 2006; Søreide et al. 2006; Ingram et al. 2007).

Dry and homogenized muscle and hair samples were weighed to approximately 0.5 mg samples, sealed in tin capsules, and analyzed on a Thermo Finnigan Delta^{Plus} mass-spectrometer (Thermo Finnigan, San Jose, CA, USA) coupled with an elemental analyzer (Costech, Valencia, CA, USA). Stable isotope ratio values are expressed in parts per thousand (‰) using δ notation as calculated using the following equation:

$$\delta X = \left[\left(R_{Sample}/R_{Standard}\right) - 1\right] \times 1000$$

where X is ^{15}N or ^{13}C, R_{sample} is the ratio ($^{15}N/^{14}N$ or $^{13}C/^{12}C$) in the sample, and $R_{standard}$ is the ratio in the standard. The standards for nitrogen and carbon stable isotope analyses are atmospheric nitrogen and Pee Dee belemnite limestone formation, respectively. An internal lab standard of fish muscle (n=81, $\delta^{15}N$ =0.11‰, $\delta^{13}C$ =0.08‰) and National Institute of Standards and Technology (NIST) bovine liver standard 8414 (n=81, $\delta^{15}N$ =0.14‰, $\delta^{13}C$ =0.12‰) were used to determine analytical precision based on standard deviation. In addition, NIST standards 8542 (n=10) and 8549 (n=10) were each included during analysis of seal tissue samples, and generated values for $\delta^{15}N$ and $\delta^{13}C$ that were within 0.12‰ and 0.01‰ of certified values, respectively.

Right canines were removed from the lower jaw and sent to Matson's Lab (www.matsonslab.com) in Montana, USA for age determination. Canines were decalcified, cut into thin sections, mounted on microscope slides, and stained. Seal ages were determined by counting the growth layer groups in the cementum. Seals were separated into three age classes: Pups (<1 year old), juveniles (1-5 years old), and adults (≥6 years old) (McLaren 1958; Holst et al. 1999; Young et al. 2010). Sex was recorded by the hunters at the time of sample collection.

Ice breakup dates were determined following Etkin (1991) and Stirling et al. (1999) using the weekly regional ice analysis charts from the Canadian Ice Service and a grid of 122 points spaced at 1.0° intervals of latitude and longitude. Using the ice analysis charts to determine ice coverage at each of the grid points during the spring breakup season, the breakup date was determined as the date on which the average ice cover was less than or equal to 50% (Stirling et al. 1999). The percentage of ice cover used to define breakup date was chosen to be consistent with previous studies (Stirling et al. 1999). Ice breakup dates were determined separately for both western and eastern Hudson Bay using 85°W longitude as the boundary between east and west (Figure 3.1). The 85°W longitude boundary was considered appropriate for this study as satellite telemetry data indicates significant north-south movements of ringed seals tagged in both eastern and western Hudson Bay, with relatively little movement between east and west (S.H. Ferguson, unpubl data). Air temperatures were obtained from Environment Canada weather stations located at Arviat, NU for western Hudson Bay, and Inukjuak, QC for eastern Hudson Bay. Spring air temperatures were defined as the mean temperature from April 1 to June 30.

Prior to analysis, visual inspection of histograms confirmed that data were approximately normally distributed. Simple linear regression was used to assess the relationship between spring air temperature and breakup date. Paired t-tests were used to compare environmental variables between western and eastern Hudson Bay. General Linear Models (GLM) tested the effects of sex, age class, and the interaction of location and spring air temperature on stable isotope data. Akaike's Information Criterion (AIC) was used to assist in model selection (Anderson et al. 2000).

Results

From 2003 to 2010, mean spring air temperature was 2.8°C warmer in eastern Hudson Bay than western Hudson Bay (t=-6.099, P<0.001) while there was no statistical difference in mean ice breakup date (t=0.167, P=0.872) between the two areas (Table 3.2). Breakup dates had a strong negative relationship with spring air temperatures in both western (R^2=0.58, F=8.109, P=0.029) and eastern Hudson Bay (R^2=0.74, F=17.415, P=0.006).

TABLE 3.2: Ice breakup dates (Julian Day) and spring air temperatures (°C) for western and eastern Hudson Bay, 2003 to 2010. Breakup dates were determined using the weekly regional ice analysis charts from the Canadian Ice Service. Temperatures were obtained from Environment Canada weather stations at Arviat, NU (western Hudson Bay) and Inukjuak, QC (eastern Hudson Bay). Spring air temperatures were defined as the mean temperature from April 1 to June 30.

	Western Hudson Bay		Eastern Hudson Bay	
Year	Breakup Date	Spring Temp. (°C)	Breakup Date	Spring Temp. (°C)
2003	168 (17 Jun)	-3.5	191 (10 Jul)	-3.3
2004	190 (08 Jul)	-7.9	195 (13 Jul)	-4.1
2005	179 (28 Jun)	-2.8	176 (25 Jun)	0.4
2006	173 (22 Jun)	-2.1	165 (14 Jun)	0.4
2007	175 (24 Jun)	-4.6	185 (04 Jul)	-2.7
2008	182 (30 Jun)	-3.8	176 (24 Jun)	0.6
2009	198 (17 Jul)	-6.3	186 (05 Jul)	-2.8
2010	177 (26 Jun)	-3.3	162 (11 Jun)	-0.3

The number of muscle and hair samples used for stable isotope analysis varied by region, age class and year (Table 3.1). C:N ratios ranged from 3.25 to 5.04 (mean=3.46, SE=0.01) in muscle and from 3.08 to 3.58 (mean=3.18, SE=0.004) in hair. Significant interaction effects occurred between location and spring temperature on stable isotope ratios in muscle δ^{15}N (F=63.022, P<0.001, n=359), muscle δ^{13}C (F=80.284, P<0.001, n=360), and hair δ^{13}C (F=58.260, P<0.001, n=184) but not hair δ^{15}N (F=2.741, P=0.100, n=184). Samples collected from Arviat had higher δ^{15}N and lower δ^{13}C than those collected from Sanikiluaq (Figure 3.2). The patterns of inter-annual variation of stable isotope ratios in relation to ice breakup date (Figure 3.3, 3.4) and spring air temperatures (Figure 3.5) were opposite for the east and west study areas: δ^{15}N had a negative relationship with spring temperature in eastern Hudson Bay and a positive relationship in western Hudson Bay.

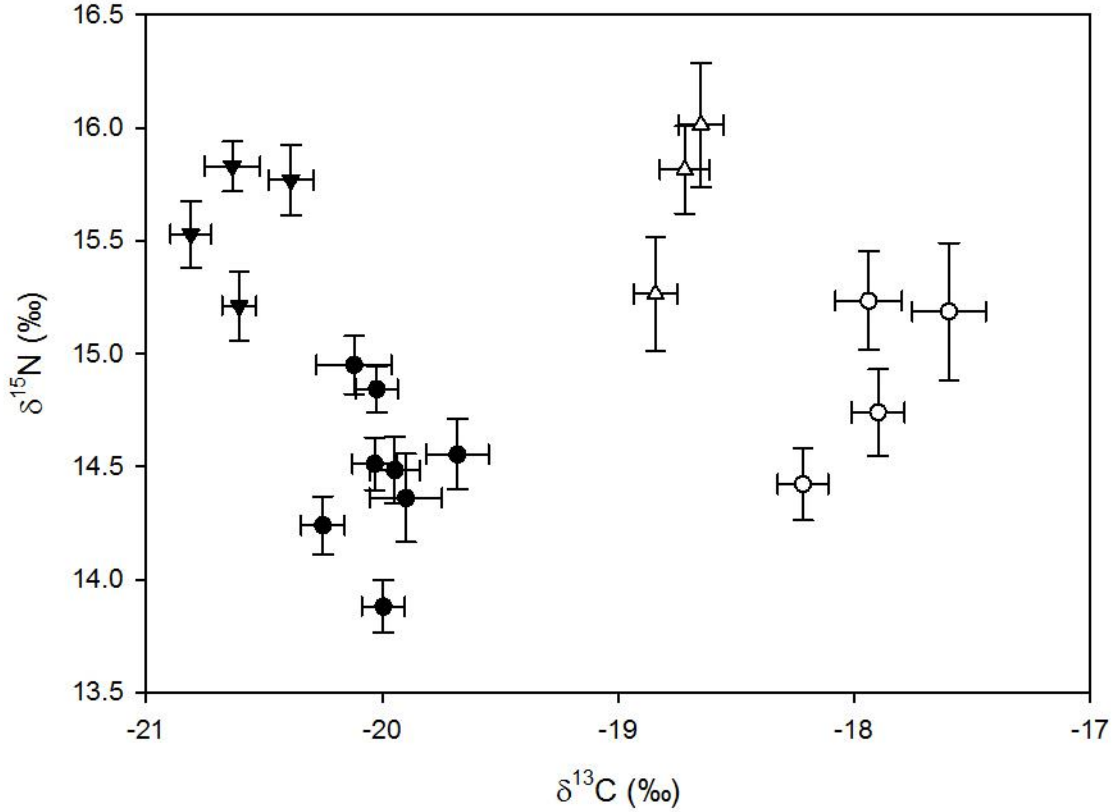

FIGURE 3.2: $\delta^{15}N$ and $\delta^{13}C$ values (mean ± SE) of ringed seal muscle (closed symbols) and hair (open symbols) from western Hudson Bay (WHB, triangles) and eastern Hudson Bay (EHB, circles). Samples are from 2003 to 2010 (EHB muscle), 2007 to 2010 (WHB muscle, EHB Hair), and 2008 to 2010 (WHB hair).

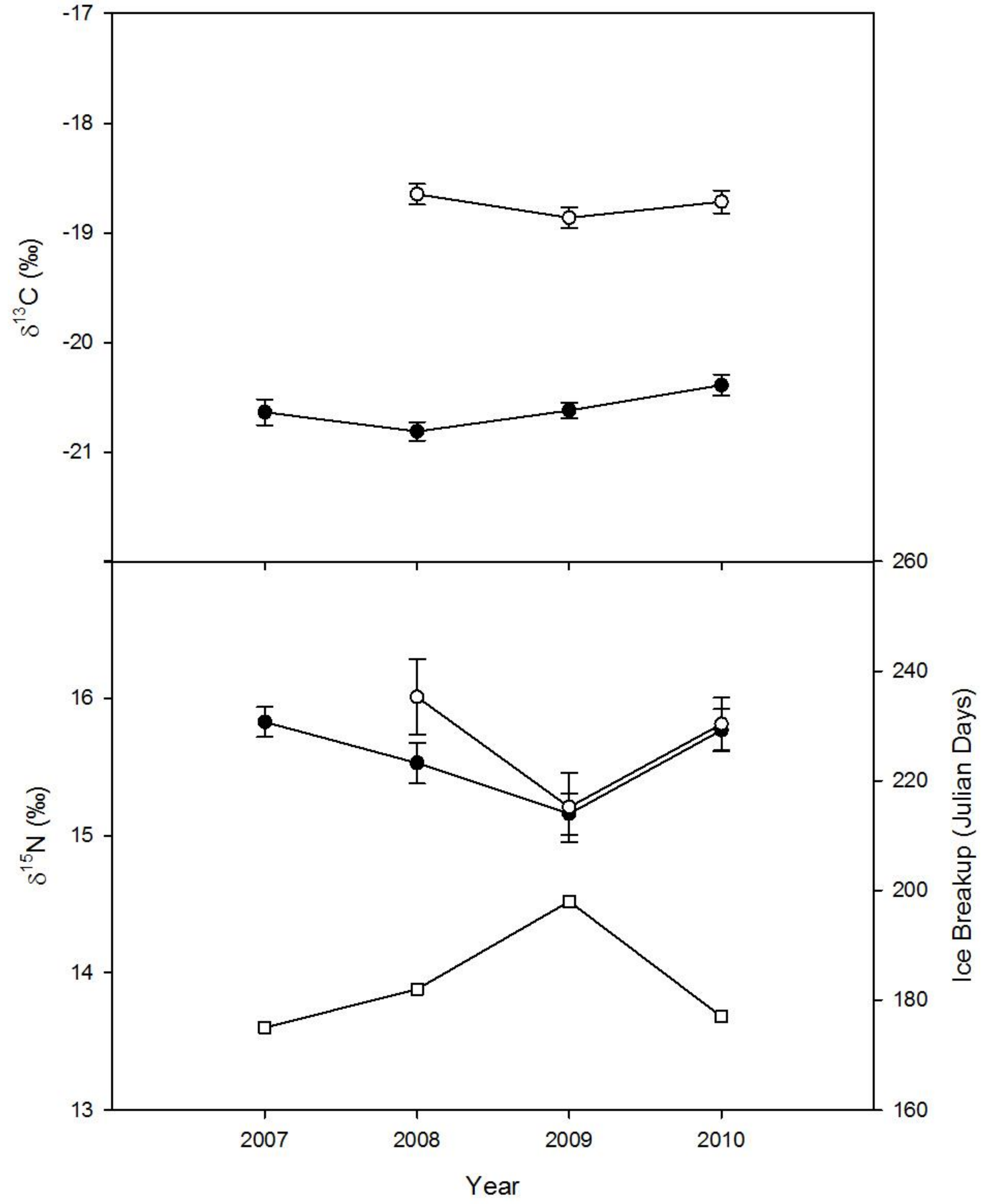

FIGURE 3.3: $\delta^{15}N$ and $\delta^{13}C$ values (mean ± SE) of western Hudson Bay ringed seal muscle (closed circles) and hair (open circles) in relation to ice breakup dates (open squares).

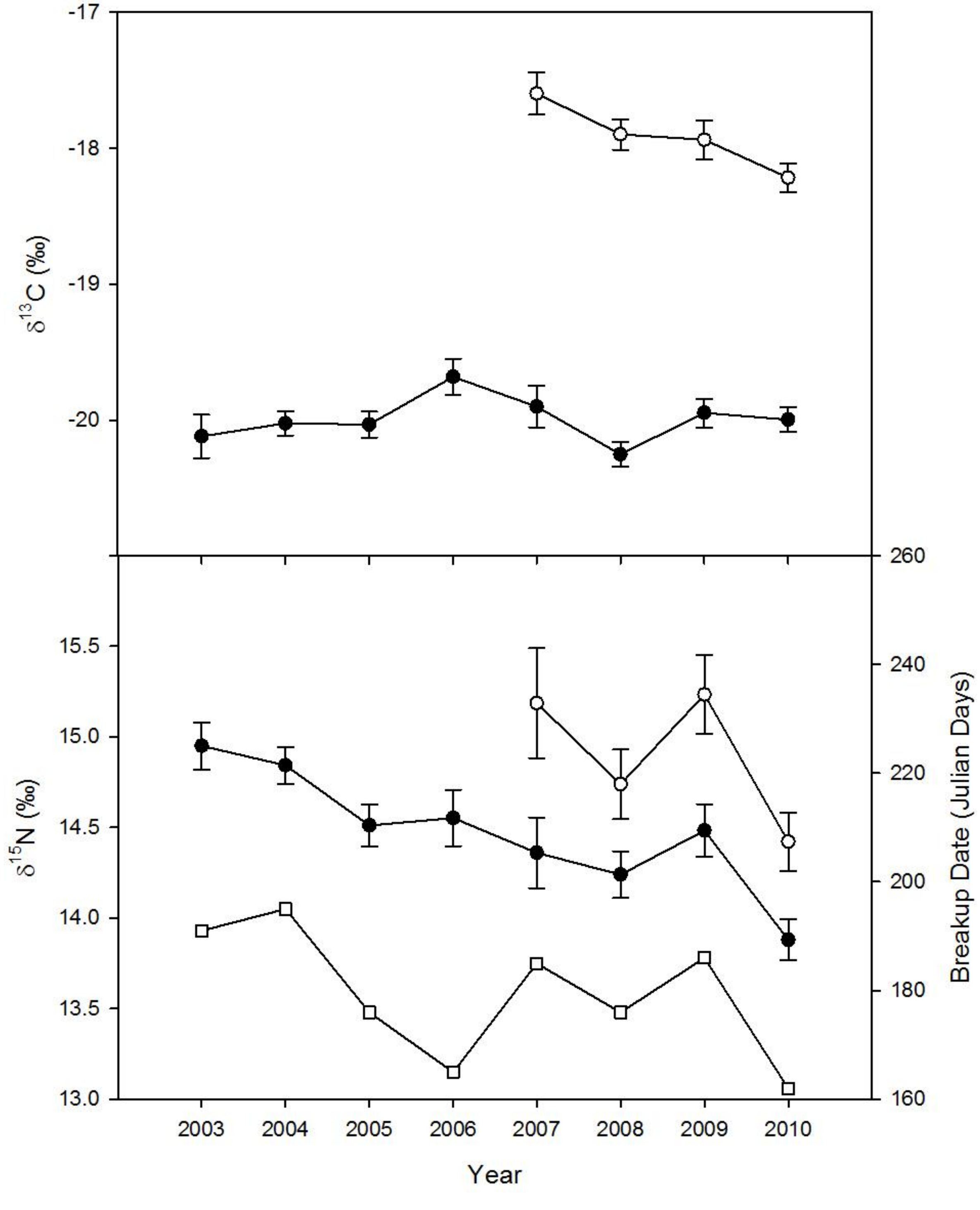

FIGURE 3.4: $\delta^{15}N$ and $\delta^{13}C$ values (mean ± SE) of eastern Hudson Bay ringed seal muscle (closed circles) and hair (open circles) in relation to ice breakup dates (open squares).

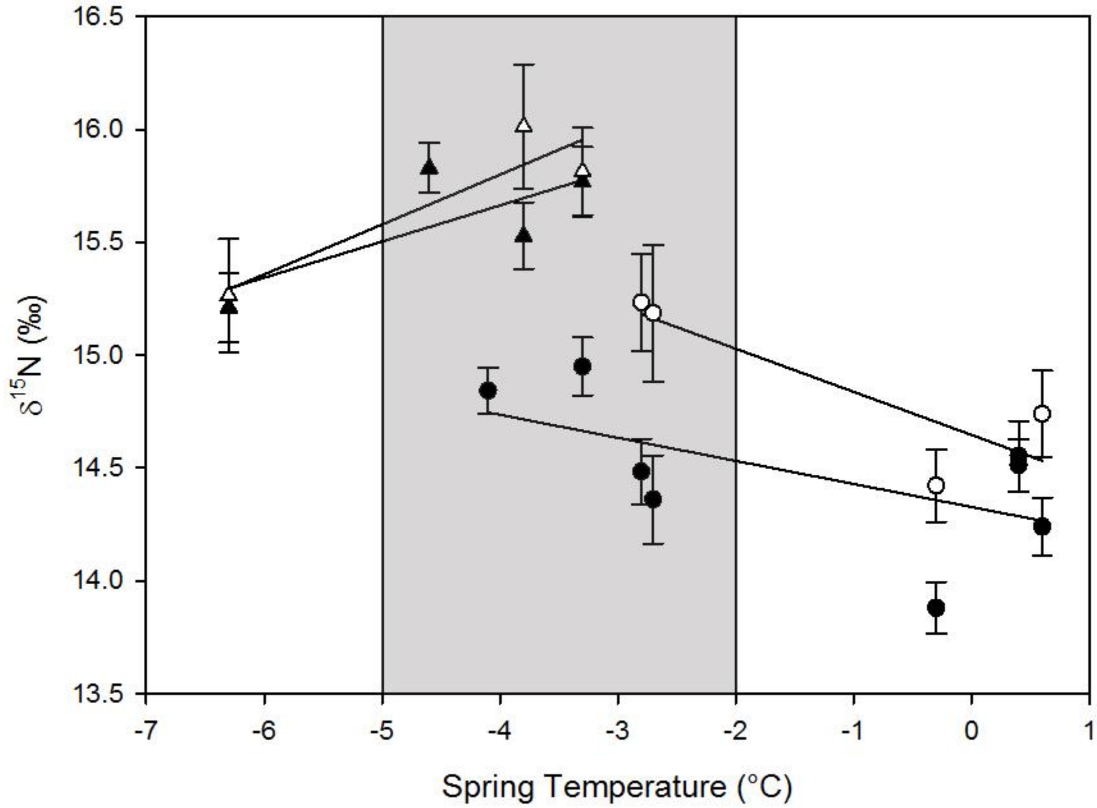

FIGURE 3.5: δ^{15}N values (mean ± SE) of ringed seal muscle (closed symbols) and hair (open symbols) from western Hudson Bay (WHB, triangles) and eastern Hudson Bay (EHB, circles) in relation to spring air temperature. The relationships between δ^{15}N and spring temperature are all significant (WHB muscle: R^2=0.05, P=0.011; WHB Hair: R^2=0.05, P=0.040; EHB muscle: R^2=0.06, P<0.001; EHB Hair: R^2=0.06, P=0.006). The shaded area represents the proposed temperature range for increased capelin in ringed seal diet.

Age class also had a significant effect on stable isotope ratios in muscle δ^{15}N (F=33.63, P<0.001, n=359), muscle δ^{13}C (F=28.59, P<0.001, n=360), and hair δ^{15}N (F=12.04, P<0.001, n=184) but not hair δ^{13}C (n=184, df=2, F=0.10, P=0.901). Where age class differences occurred, adults were most enriched, followed by juveniles, then by pups. Tukey's HSD post-hoc test indicated significant differences between adults/juveniles and pups for muscle $\delta^{15}N$, between all three age classes for muscle $\delta^{13}C$, and between adults and juveniles/pups for hair $\delta^{15}N$. Sex did not affect stable isotope ratios (muscle $\delta^{15}N$: n=359, df=1, F=3.50, P=0.062; muscle $\delta^{13}C$: n=360, df=1, F=0.38, P=0.536; hair $\delta^{15}N$: n=184, df=1, F=0.45, P=0.504; hair $\delta^{13}C$: n=184, df=1, F=1.04, P=0.310).

Since age related difference is a possible confounding factor in the GLMs, roughly equal sample numbers from different age classes were used in analyses. Where sample numbers were skewed in favour of adults or pups for any given year, samples were removed to eliminate any potential bias and GLMs were re-run. In each case, the removal of samples did not change the outcome of analyses and the results presented here are from the complete sample.

Discussion

For both eastern and western Hudson Bay, the patterns of variability in ringed seal $\delta^{15}N$ suggest inter-annual dietary shifts, likely associated with differing prey abundance due to sea ice conditions and water temperature. Although spring air temperature also had a significant effect on ringed seal $\delta^{13}C$, trends were not as pronounced as for $\delta^{15}N$, suggesting variation in spring temperature and breakup date had little influence on differences in ringed seal foraging habitat use. Analysis of ringed seal stomach contents found capelin and sandlance (*Ammodytes sp.*) to be the two most important prey items for ringed seals in eastern Hudson Bay (Appendix A). In western Hudson Bay, ringed seal diet was dominated by sandlance, though the importance of capelin appears to be increasing in recent years (Chambellant et al. in press). We propose that variation in ringed seal $\delta^{15}N$ is largely driven by inter-annual changes in the distribution and abundance of capelin.

Capelin is an important forage fish species that is known to show rapid demographic responses to environmental change. It is predicted that, as ocean temperatures rise, capelin will experience a northward shift in distribution (Rose 2005). Distribution of capelin appears to be limited by temperature at various life stages and spawning is most successful within a narrow range of temperatures, though spawning temperatures appear to vary by region (Winters 1982; Rose 2005). In the waters off Newfoundland, capelin spawning occurs at surface temperatures between 5.5°C and 8.5°C (Templeman 1948; Winters 1982). Although capelin within a given stock are distributed over a range of water temperatures, they tend to be more concentrated in the colder waters within that temperature range (see review by Rose 2005), including in the Barents Sea where adult capelin preferred water temperatures between -1°C and 2°C (Bluhm and Gradinger 2008). However, individuals have also been observed to freeze to death in the cold waters off Iceland (Vilhjálmsson 1994). In contrast, it has been suggested that sandlance distribution and abundance may be more closely linked to depth, habitat quality, and food availability, than to temperature (Scott 1982; Robards et al. 2002).

In western Hudson Bay, capelin did not appear in ringed seal diet prior to 2000 and stomach content analysis has found capelin to be of relatively high importance in spring diet and of little importance in fall diet, at least up until 2006 (Chambellant et al. in press). A recent shift to increased capelin and sandlance in the diet of thick-billed murres in northern Hudson Bay related to warming (Gaston et al. 2003), and high importance of capelin in the diet of western Hudson Bay beluga (*Delphinapterus leucas*) (Kelley et al. 2010) suggest that capelin may be increasing in abundance in this area. As relatively recent arrivals in western Hudson Bay, capelin would be expected to occur in greater abundance in warmer years and result in increased importance in ringed seal diet. Large numbers of capelin occur in the area around the Belcher Islands in southeastern Hudson Bay, possibly a relict group from a population that established during a previous warm period (Dunbar 1983). Recent analysis of ringed seal stomach contents from eastern Hudson Bay found capelin to be more important in ringed seal diet in 2003 and 2004, years with colder spring air temperatures and later ice breakup, compared to 2005 and 2006, years of higher spring air temperature and early ice breakup (Appendix A).

One important difference between the two regions of Hudson Bay is in spring air temperatures. Over the eight year period of this study, the mean spring temperature was 2.8°C higher in eastern Hudson Bay than in western Hudson Bay. While higher $\delta^{15}N$ occurred in years with warmer temperatures in western Hudson Bay and in years with cooler temperatures in eastern Hudson Bay, the warm years in the west and the cool years in the east fall within a similar temperature range (Figure 3.5). We propose that this range in spring air temperature, approximately -5°C to -2°C, coincides with favourable conditions for capelin reproductive success resulting in increased abundance, greater importance in ringed seal diet, and higher ringed seal tissue $\delta^{15}N$. From samples collected near Coats Island in northern Hudson Bay, capelin were found to be enriched in $\delta^{15}N$ (13.83‰) relative to sandlance (12.80‰) (Chambellant et al. in press). As capelin and sandlance appear to make up the bulk of ringed seal diet in Hudson Bay, it would be expected that changes in the relative abundance of these two species would have the greatest influence on ringed seal stable isotope ratios. For example, in the eastern Hudson Bay data for 2003 to 2006, a strong relationship occurs between muscle $\delta^{15}N$ and the importance of capelin, as determined through stomach content analyses (Appendix A).

Possible alternative explanations for the inter-annual variation in ringed seal $\delta^{15}N$ can be considered. The strong relationship between $\delta^{15}N$ in ringed seal tissues and breakup date may be a result of changes to baseline $\delta^{15}N$ related to nitrogen uptake by phytoplankton. In the northern Barents Sea, Tamelander et al. (2009) found a strong positive relationship between phytoplankton $\delta^{15}N$ and bloom progression. In the late-bloom stage, the higher percentage of nitrate uptake by phytoplankton and a $\delta^{15}N$ enriched nitrate pool resulted in higher $\delta^{15}N$ phytoplankton compared to the early-bloom stage (Tamelander et al. 2009; Graham et al. 2010). When ice breaks up early in western Hudson Bay, it is possible that the phytoplankton bloom is able to begin earlier and take advantage of a longer growing season, allowing a higher degree of nitrate uptake causing enriched phytoplankton $\delta^{15}N$. This isotopic enrichment would then be passed up the food web throughout the open water season to be reflected in ringed seal samples collected in the fall harvest. Conversely, when ice breakup is late, it is possible that the phytoplankton bloom does not reach the same degree of nitrate uptake resulting in lower baseline $\delta^{15}N$ for the time period that would be reflected in ringed seal samples collected in the fall harvest. However, this scenario does not take into consideration time delays in expression of the isotopic signal to higher trophic levels and it does not explain the patterns observed in eastern Hudson Bay.

The difference in mean isotope ratios in ringed seal tissues between western and eastern Hudson Bay may be due to differences in baseline isotopic values related to food chain length. Kuzyk et al. (2010) found spatial variability in both $\delta^{13}C$ and $\delta^{15}N$ in Hudson Bay surface sediments with increasing values from south to north and from inshore to offshore areas. These findings are consistent with the observed difference in ringed seal muscle and hair $\delta^{15}N$, which is approximately 1‰ lower in eastern Hudson Bay than in western Hudson Bay. However, inconsistent with patterns in surface sediments, ringed seal $\delta^{13}C$ was slightly higher in the east, and may be an indication of a greater degree of benthic foraging in eastern Hudson Bay (Hobson et al. 1994; France 1995). A more complex food web could also cause higher stable isotope ratios in high trophic level organisms as more trophic linkages results in greater isotopic enrichment. With greater fresh water inputs, south-eastern Hudson Bay has lower and more variable salinity than western Hudson Bay (Saucier et al. 2004). As areas with lower salinity have been associated with lower biodiversity (Cusson et al. 2007; Zettler et al. 2007), it may be expected that eastern Hudson Bay has a less complex food web and fewer trophic linkages resulting in lower $\delta^{15}N$.

In addition to the baseline or food web differences between east and west, eastern Hudson Bay ringed seals are experiencing a long term decline in muscle $\delta^{15}N$ (Figure

3.4) that could be interpreted as a gradual change in diet over time, decreasing baseline stable isotope ratios, or ecosystem changes causing a shift in prey isotope values. Similar long term declines have been observed in other Arctic marine mammals, including a decline in beluga muscle and skin $\delta^{15}N$ in Cumberland Sound (Marcoux et al. in press). Declining trends were also observed in eastern Hudson Bay hair $\delta^{15}N$ and $\delta^{13}C$, though only over a four year period, making it unclear if these results are representative of long term trends.

Significant effects of age class on ringed seal stable isotope ratios and, thus feeding ecology, were found in our study and are consistent with findings from other studies (Bradstreet and Cross 1982; Holst et al. 2001; Labansen et al. 2007; Young et al*.* 2010). Where age differences occur it has been found that pups and immature seals often take a greater proportion of invertebrates while adults feed mainly on fish (Bradstreet and Cross 1982; Holst et al. 2001). With more of a generalist feeding behaviour, pups and juveniles may be better able to adapt to changes in prey availability than adults who appear to specialize on specific prey types and who are more restricted in movements during the mating and pupping season. To assess the relationship between environment and the isotopic trend signal, we need to control for the possible influence of inter-annual differences in population age structure.

The observed differences in muscle and hair for both $\delta^{15}N$ and $\delta^{13}C$ are likely due to differences in the trophic discrimination factors in the different tissues. In captive harp seals (*Pagophilus groenlandicus*), harbour seals (*Phoca vitulina*), and ringed seals, Hobson et al. (1996) found enrichment factors of +2.4‰ and +3.0‰ in $\delta^{15}N$ for muscle and hair, respectively, and +1.3‰ and +2.8‰ in $\delta^{13}C$ for muscle and hair, respectively. Based on different isotopic turnover rates in the two tissues, it is possible that the observed differences in muscle and hair are also partially due to seasonal differences in feeding. Since stable isotopes are incorporated into muscle in the range of several weeks or months (Tieszen et al. 1983; Kurle and Worthy 2002; Dalerum and Angerbjörn 2005; Hobson and Bond 2012), our muscle samples collected in the fall represent diet during the open water period of summer and fall. As a metabolically inert tissue, hair should be representative of diet during the time of hair growth (West et al. 2004, Newsome et al. 2010). Although hair continues to grow through the summer and fall, most hair growth in ringed seals likely occurs in the spring during the molting period. Due to the uncertainty in hair growth rates and differences in trophic discrimination factors, it is difficult to draw conclusions about ringed seal seasonal diet variation based on the comparison between hair and muscle tissue.

Our findings suggest that the importance of capelin in the diet of Hudson Bay ringed seals, indicated by relatively high $\delta^{15}N$, varies in response to environmental changes such as the inter-annual timing of sea ice break-up. Peak $\delta^{15}N$ occurred within a limited range in spring air temperatures, between approximately -5°C and -2°C. This temperature range was characteristic of warm years (early sea ice break-up) in western Hudson Bay and cool years (late sea ice break-up) in eastern Hudson Bay and similar capelin demography may suggest a large-scale capelin population in Hudson Bay. With the warming trend in the Arctic, it is possible that capelin abundance will continue to increase and continue to be an important part of ringed seal diet in Hudson Bay. With continued warming, we are likely to see further ecosystem changes and shifts from Arctic to subarctic or even temperate species and ecosystems (Bluhm and Gradinger 2008; Wassmann et al. 2011). The Hudson Bay marine food web appears to have transitioned to different key pelagic forage fish species: from Arctic cod to sandlance and capelin, and perhaps in the future to rainbow smelt (*Osmerus mordax*) (Franzin et al. 2004). Marine mammals, such as the ice-adapted ringed seal, may be opportunistic and show behavioural plasticity in generalizing their diet. However, continued change will eventually create phenological mismatches in energetic requirement to specific life stages (e.g. food requirements to weaned pups in July) and will possibly contribute to direct competition with more temperate species, such as harbour seals, that may be better adapted to take advantage of recent shifts in the phenology of pelagic fish availability and accessibility. Thus, monitoring of ringed seal stable isotope ratios and stomach contents will be important in tracking future changes to the Hudson Bay food web and assessing potential impacts on upper trophic level species and Inuit communities.

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References

Anderson DR, Burnham KP, Thompson WL (2000) Null hypothesis testing: problems prevalence, and an alternative. Journal of Wildlife Management 64:912-923

Bligh E, Dyer W (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911-917

Bluhm BA, Gradinger R (2008) Regional variability in food availability for Arctic marine mammals. Ecological Applications 18:S77-S96

Bradstreet M, Cross W (1982) Trophic Relationships at High Arctic Ice Edges. Arctic 35:1-12

Chambellant, M., Stirling, I., and Ferguson, S. H. (In Press). Temporal variation in western Hudson Bay ringed seal (*Phoca hispida*) in relation to environment. Marine Ecology Progress Series

Cusson M, Archambault P, Aitken A (2007) Biodiversity of benthic assemblages on the Arctic continental shelf: historical data from Canada. Marine Ecology Progress Series 331:291-304

Dalerum F, Angerbjörn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647-658

DeNiro, M.J. & Epstein, S. 1977. A mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197:261-263.

DeNiro M, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495-506

Dunbar M (1983) A Unique International Polar Year Contribution: Lucien Turner, Capelin, and Climatic Change. Arctic 36:204-205

Etkin, DA (1991) Break-up in Hudson Bay: Its sensitivity to air temperatures and implications for climate warming. Climatological Bulletin 25:21-34

Ferguson SH, Stirling I, McLoughlin P (2005) Climate Change and Ringed Seal (*Phoca Hispida*) Recruitment in Western Hudson Bay. Marine Mammal Science 21:121-135

France R (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Marine Ecology Progress Series 124:307-312

Franzin WG, Barton BA, Remnant RA, Wain DB, Pagel SJ (1994) Range extension, present and potential distribution, and possible effects of rainbow smelt in Hudson Bay drainage waters of northwestern Ontario, Manitoba, and Minnesota. North American Journal of Fisheries Management 14:65–76

Gagnon AS, Gough WA (2005) Climate change scenarios for the Hudson Bay region: an intermodel comparison. Climatic Change 69:269-297

Gaston AJ, Woo K, Hipfner JM (2003) Trends in Forage Fish Populations in Northern Hudson Bay since 1981, as Determined from the Diet of Nestling Thick-Billed Murres *Uria lomvia*. Arctic 56:227-233

Graham BS, Koch PL, Newsome SD, McMahon KW, Aurioles D (2010) Using Isoscapes to Trace the Movements and Foraging Behavior of Top Predators in Oceanic Ecosystems. In: Isoscapes: Understanding Movement, Pattern, and Process on Earth Through Isotope Mapping. pp 299-318

Higdon JW, Ferguson SH (2009) Loss of Arctic sea ice causing punctuated change in sightings of killer whales (*Orcinus orca*) over the past century. Ecological Applications 19:1365-1375

Hobson KA, Bond AL (2012) Extending an indicator: year-round information on seabird trophic ecology from multiple-tissue stable-isotope analyses. Marine Ecology Progress Series 461:233-243

Hobson K, Clark R (1992) Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. Condor 94:181-188

Hobson KA., Piatt JF, Pitocchelli J (1994) Using Stable Isotopes to Determine Seabird Trophic Relationships. The Journal of Animal Ecology 63:786-798

Hobson KA, Schell DM, Renouf D, Noseworthy E (1996) Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Canadian Journal of Fisheries and Aquatic Sciences 53:528-533

Holst M, Stirling I, Calvert W (1999) Age structure and reproductive rates of ringed seals (*Phoca hispida*) on the northwestern coast of Hudson Bay in 1991 and 1992. Marine Mammal Science 15:1357-1364

Holst M, Stirling I, Hobson KA (2001) Diet of Ringed Seals (*Phoca hispida*) on the East and West Sides of the North Water Polynya, Northern Baffin Bay. Marine Mammal Science 17:888–908

Ingram T, Matthews B, Harrod C, Stephens T, Grey J, Markel R, Mazumder A (2007) Lipid extraction has little effect on the δ^{15}N of aquatic consumers. Limnology and Oceanography: Methods 5:338–343

Kelley TC, Loseto LL, Stewart REA, Yurkowski M, Ferguson SH (2010) Importance of Eating Capelin: Unique Dietary Habits of Hudson Bay Beluga. In: A Little Less Arctic: Top Predators in the World’s Largest Northern Inland Sea, Hudson Bay. pp 53-70

Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78:1-27

Kurle C, Worthy G (2002) Stable nitrogen and carbon isotope ratios in multiple tissues of the northern fur seal *Callorhinus ursinus*: implications for dietary and migratory reconstructions. Marine Ecology Progress Series 236:289-300

Kuzyk Z, Macdonald R, Tremblay J, Stern GA (2010) Elemental and stable isotopic constraints on river influence and patterns of nitrogen cycling and biological productivity in Hudson Bay. Continental Shelf Research 30:163-176

Labansen AL, Lydersen C, Haug T, Kovacs KM (2007) Spring diet of ringed seals (*Phoca hispida*) from northwestern Spitsbergen, Norway. ICES Journal of Marine Science 64:1246–1256

Labansen AL, Lydersen C, Levermann N, Haug T, Kovacs KM (2011) Diet of ringed seals (*Pusa hispida*) from Northeast Greenland. Polar Biology 34:227-234

Laidre KL, Stirling I, Lowry LF, Wiig Ø, Heide-Jørgensen MP, Ferguson SH (2008) Quantifying the sensitivity of Arctic marine mammals to climate-induced habitat change. Ecological Applications 18:S97-S125

Lesage V, Morin Y, Rioux È, Pomerleau C, Ferguson SH, Pelletier É (2010) Stable isotopes and trace elements as indicators of diet and habitat use in cetaceans: predicting errors related to preservation, lipid extraction, and lipid normalization. Marine Ecology Progress 419:249-265

Lowry LF, Frost K, Burns JJ (1980) Variability in the Diet of Ringed Seals, *Phoca hispida*, in Alaska. Canadian Journal of Fisheries and Aquatic Sciences 37:2254-2261

Marcoux M, McMeans BC, Fisk AT, Ferguson SH (In Press) Composition and temporal variation in the diet of belugas in Cumberland Sound derived from stable isotopes. Marine Ecology Progress Series

McLaren I (1958) The Biology of the Ringed Seal (*Phoca hispida* Schreher) in the Eastern Canadian Arctic. Bulletin of the Fisheries Research Board of Canada 118

Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between δ^{15}N and animal age. Geochimica et Cosmochimica Acta 48:1135-1140

Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study marine mammal ecology. Marine Mammal Science 26:509-572

Parkinson CL, Cavalieri DJ (2008) Arctic sea ice variability and trends, 1979-2006. Journal of Geophysical Research 113:CO7003

Phillips DL, Eldridge PM (2006) Estimating the timing of diet shifts using stable isotopes. Oecologia 147:195-203

Post E, Forchhammer M, Bret-Harte M, Callaghan T, Christensen T, Elberling B, Fox A, Gilg O, Hik D, Hoye T, Ims R, Jeppesen E, Klein D, Madsen J, McGuire A, Rysgaard S, Schindler D, Stirling I, Tamstorf M, Tyler N, van der Wal R, Welker J, Wookey P, Schmidt N, Aastrup P (2009) Ecological Dynamics Across the Arctic Associated with Recent Climate Change. Science 325:1355-1358

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179-89

Regehr EV, Lunn NJ, Amstrup SC, Stirling I (2007) Effects of Earlier Sea Ice Breakup on Survival and Population Size of Polar Bears in Western Hudson Bay. Journal of Wildlife Management 71:2673-2683

Robards MD, Rose GA, Piatt JF (2002) Growth and abundance of Pacific sand lance, *Ammodytes hexapterus*, under differing oceanographic regimes. Environmental Biology of Fishes 64:429-441

Rose G (2005) Capelin (*Mallotus villosus*) distribution and climate: a sea “canary” for marine ecosystem change. ICES Journal of Marine Science 62:1524-1530

Scott JS (1982) Depth, Temperature and Salinity Preferences of Common Fishes of the Scotian Shelf. Journal of Northwest Atlantic Fishery Science 3:29-39

Stirling I (2005) Reproductive rates of ringed seals and survival of pups in Northwestern Hudson Bay, Canada, 1991-2000. Polar Biology 28:381-387

Stirling I, Lunn NJ, Iacozza J (1999) Long-term Trends in the Population Ecology of Polar Bears in Western Hudson Bay in Relation to Climatic Change. Arctic 52:294-306

Sweeting C, Polunin N, Jennings S (2006) Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. Rapid Communications in Mass Spectrometry 20:595-601

Søreide J, Tamelander T, Hop H, Hobson K, Johansen I (2006) Sample preparation effects on stable C and N isotope values: a comparison of methods in Arctic marine food web studies. Marine Ecology Progress Series 328:17-28

Tamelander T, Kivimae C, Bellerby RG, Renaud PE, Kristiansen S (2009) Base-line variations in stable isotope values in an Arctic marine ecosystem: effects of carbon and nitrogen uptake by phytoplankton. Hydrobiologia 630:63-73

Templeman W (1948) The life history of the capelin *Mallotus villosus* (Müller) in Nefoundland waters. Newfoundland Government Laboratory Research Bulletin 17, 151 pp

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32-37

Vilhjálmsson, H (1994) The Icelandic Capelin Stock: Capelin, *Mallotus villosus* (Müller) in the Iceland-Greenland-Jan Mayen area. Journal of the Marine Research Institute Reykjavik XIII (1) 281 pp

Vincent-Chambellant, M. 2010. Ecology of ringed seals (*Phoca hispida*) in western Hudson Bay, Canada. Thesis (Ph.D.) University of Manitoba, Winnipeg, Manitoba

Wassmann P, Duarte C, Agusti S, Sejr M (2011) Footprints of climate change in the Arctic marine ecosystem. Global Change Biology 17:1235-1249

Weslawski JM, Ryg M, Smith TG, Oritsland NA (1994) Diet of Ringed Seals (*Phoca hispida*) in a Fjord of West Svalbard. Arctic 47:109–114

West A, Ayliffe L, Cerling T, Robinsion T, Karren B, Dearing M, Ehleringer J (2004) Short-term diet changes revealed using stable carbon isotopes in horse tail-hair. Functional Ecology 18:616-624

Winters GH (1982) Life History and Geographical Patterns of Growth in Capelin, *Mallotus villosus*, of the Labrador and Newfoundland Areas. Journal of Northwest Atlantic Fishery Science 3:105–114

Young BG, Ferguson SH (In Press) Seasons of the ringed seal: pelagic open-water hyperphagy, benthic feeding over winter and spring fasting during molt. *Wildlife Research*

Young BG, Loseto LL, Ferguson SH (2010) Diet differences among age classes of Arctic seals: evidence from stable isotope and mercury biomarkers. Polar Biology 33:153-162

Zettler ML, Schiedek D, Bobertz B (2007) Benthic biodiversity indices versus salinity gradient in the southern Baltic Sea. Marine Pollution Bulletin 55:258–270

Appendix A

Stomach contents of ringed seals (*Phoca hispida*) harvested from the Belcher Islands, Nunavut in the fall of 2003 to 2006 were analysed as part of an unpublished contract report for Fisheries and Oceans Canada. Laboratory procedures are described in Vincent-Chambellant (2010) and Chambellant et al. (in press). Analyses included identification of prey in stomachs from 210 ringed seals. Prey groups included Arctic cod (*Boreogadus saida*), capelin (*Mallotus villosus*), cods (Gadidae), sandlance (*Ammodytes sp.*), sculpins (Cottidae), shannies (Stichaeidae), shrimps (Caridae), and other invertebrates. The importance of each prey group was assessed using the index of global importance (*IG*; Moreno and Castro 1995). *IG* is calculated as:

$$IG = \frac{\left((\%P \times FO)^{1/2} + (\%B \times FO)^{1/2}\right)}{2}$$

Where *%P*=percent abundance of prey, *FO* = frequency of occurrence, and *%B* = percent biomass contribution. Results indicate variation in the relative importance of prey groups over the four year period (Table 3.3). Capelin declined from high importance in 2003 and 2004 to lower importance in 2005 and 2006 while Arctic cod, cods, and sculpins showed increased importance in 2005 and/or 2006 compared to 2003 and 2004 (Figure 3.6).

TABLE 3.3: Index of global importance (IG) of prey groups identified in ringed seal stomach contents from the Belcher Islands, NU in the fall of 2003 to 2006. Stomach contents were analysed from a total of 210 ringed seals (2003=92, 2004=51, 2005=33, 2006=34).

Year	Arctic Cod	Capelin	Cods	Sandlance	Sculpins	Shannies	Other Fish	Amphipods	Shrimps	Other Inverts
2003	8.12	73.67	2.32	45.93	0.92	5.70	2.35	13.67	2.00	0.70
2004	3.91	53.28	1.80	27.69	4.99	5.49	6.42	42.33	1.79	13.29
2005	1.21	14.44	22.96	39.34	8.32	3.24	0.00	17.68	0.00	1.43
2006	17.81	23.66	12.90	24.97	10.65	4.47	2.49	23.01	0.00	16.77

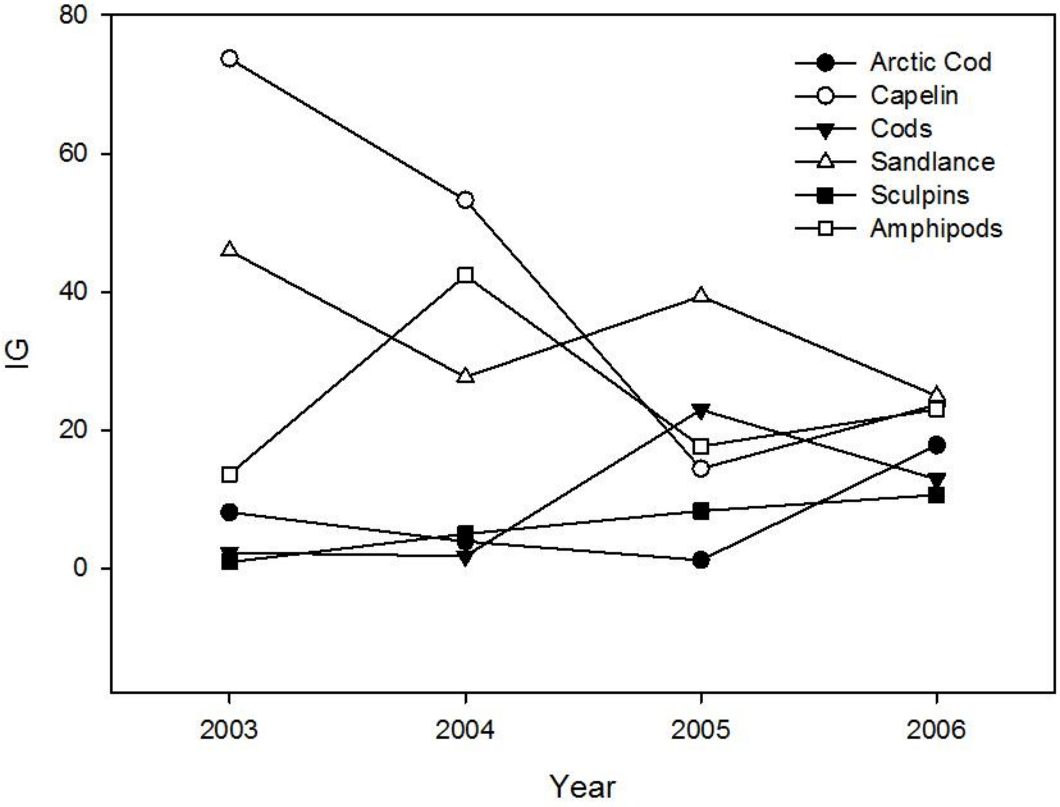

FIGURE 3.6: Variation in the index of global importance (IG) of prey groups identified in ringed seal stomach contents from the Belcher Islands, NU in the fall of 2003 to 2006.

CHAPTER 4. CONCLUSION

Results from this research suggest that foraging ecology of ringed seals (*Phoca hispida*) in Hudson Bay varies seasonally, inter-annually, and spatially in predictable patterns that can help inform harvest management and anticipate future changes that can be used to provide advice on conservation efforts. As ringed seals face challenges related to changes in prey availability, predation pressure, and alteration of their sea-ice habitat, continued monitoring to assess and understand future changes will be important.

For eastern Hudson Bay ringed seals, seasonal variation in fatty acid composition, $\delta^{15}N$, and $\delta^{13}C$ indicated variation in diet and foraging habitat with increased pelagic feeding during the open water season and increased benthic feeding during the period of ice cover. Distinctly different stable isotope ratios and fatty acid composition were observed following the period of fasting during the spring molt. Inter-annual changes in ringed seal stable isotope ratios were closely related to ice breakup date and spring air temperature, suggesting dietary shifts related to changes in the availability and accessibility of important prey species such as capelin (*Mallotus villosus)* and sandlance (*Ammodytes sp.)* in response to environmental variability. As a species which is known to respond quickly to environmental change and which is most successful within a narrow temperature range (Winters 1982; Rose 2005), the relative importance of capelin in the ringed seal diet is hypothesized to be one of the main influences causing the observed isotopic shifts in Hudson Bay ringed seals. Peak $\delta^{15}N$ occurred within a range of spring air temperatures, between approximately -5°C and -2°C: a temperature range which was characteristic of warm years in western Hudson Bay and cool years in eastern Hudson Bay.

The observed seasonal cycles in stable isotope ratios and fatty acid composition of ringed seals have important implications for studies of marine mammal feeding ecology that make use of biomarkers. For example, if samples are collected following a period of fasting or nutritional stress, then stable isotope ratios will be enriched and proportions of individual fatty acids may be either enriched or depleted due to selective mobilisation. The results from this study also provide year-round baseline data on southeastern Hudson Bay ringed seal feeding ecology which will be important for comparisons in future studies as distribution and abundance of prey populations change with changing environmental conditions.

Hudson Bay has already experienced a shift in the relative abundance of species of forage fish from a high abundance of Arctic Cod (*Boreogadus saida*) to increased sandlance and capelin (Gaston et al. 2003). As warming continues, it is possible that the abundance of sub-Arctic species, including capelin and sandlance, will continue to increase and shifts to temperate species may occur (Wassmann et al. 2011). One such invasive temperate forage fish species, the rainbow smelt (*Osmerus mordax*), has already been observed in Hudson Bay (Chambellant et al. in press) where it is expected to thrive (Franzin et al. 1994). Though ringed seals have shown the ability to feed on a variety of prey items (Lowry et al. 1980; Gjertz and Lydersen 1986; Smith 1987; Holst et al. 2001; Labansen et al. 2007, 2011; Chambellant et al. in press) and may be able to adapt to different prey sources, eventually a mismatch may occur where the available prey is not suitable to allow ringed seals to meet their seasonal energetic requirements, or habitat preference may make new prey items unavailable to ringed seals during parturition, mating, and lactation, when adult ringed seals are restricted in their movements. In addition, ringed seals may be faced with higher levels of competition as harbour seals (*Phoca vitulina*) are expected to become more abundant in Hudson Bay (Stirling 2005; Laidre et al. 2008). As a temperate species, harbour seals may be better adapted to take advantage of the changes to the forage fish community of Hudson Bay and cause increased levels of competition, reducing the prey available to ringed seals. If the availability of high quality prey sources becomes limited, ringed seals may become nutritionally stressed resulting in decreased survival, decreased reproductive success, and increased vulnerability to disease.

As warming continues, ringed seals will be affected directly by changes to their ice habitat, as well as indirectly by changes to prey availability, competition, and predation. Recent concerns over these impending changes have resulted in ringed seals being declared a ‘high priority’ candidate species for assessment by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2013). Continued monitoring is required to ensure we have the knowledge necessary for effective conservation of ringed seals in the future. The success of ringed seals is especially important for polar bear (*Ursus maritimus*) populations that rely on ringed seals as the dominant food source (Thiemann et al. 2008), and Inuit communities for whom ringed seals provide subsistence food and are culturally significant.

Future research should continue to monitor all aspects of ringed seal natural history in Hudson Bay, as many of the changes observed here will likely be indicative of coming changes in other areas of the Arctic. Key indicators that would provide early warnings of problems include: (1) age structure data that may indicate decreased survivorship of different age classes; (2) reproductive data that may indicate compromised fertility such as age of first reproduction; and (3) assessment of body condition and health through measurements of body fat, disease, and contaminants. Information obtained from the current and future changes in Hudson Bay can be applied to other regions and allow for effective management of seals and other marine resources. To better understand the significance of spatial and temporal changes in stable isotope and fatty acid biomarkers, a more complete understanding of the Hudson Bay food web is required, and is recommended as another area for future study. This includes data on stable isotope ratios and fatty acid signatures for all potential prey items from a variety of locations to be used for dietary reconstruction studies using stable isotope mixing models and quantitative fatty acid signature analysis.

The work contained in this thesis focuses on changes occurring in lower trophic levels and their effects on ringed seals, though the effect of higher trophic level changes will also be important and should be taken into consideration. For example, how will changing ice conditions effect polar bear predation on seals? Will a shorter period of ice cover reduce predation pressure from polar bears due to a shorter hunting season, or will decreased snow depths be insufficient for effective sub-nivean lairs, leaving pups more susceptible to polar bears, Arctic foxes (*Alopex lagopus*), and avian predators?

The increased presence of killer whales (*Orcinus orca*) is also expected to increase predation pressure and play an important role in Hudson Bay food web dynamics (Higdon and Ferguson 2009).

This thesis contributes important information to a growing knowledge base on ringed seal feeding ecology in Hudson Bay. A good understanding of the current state of ringed seal feeding ecology is important for predicting and understanding future changes in ecosystem dynamics as well as potential threats to ringed seal success, both of which have important management implications. Monitoring ringed seal diet will provide an indication of changes in the composition of the prey community, while tracking seasonal and long term changes in body condition in relation to feeding ecology and biomarkers should help to detect changes to overall health and nutritional stress.

References

Chambellant M, Stirling I, Ferguson SH (In Press) Temporal variation in western Hudson Bay ringed seal (*Phoca hispida*) diet in relation to environment. Marine Ecology Progress Series

COSEWIC (2013) Candidate Wildlife Species. Committee on the Status of Endangered Wildlife in Canada. Web site: http://www.cosewic.gc.ca/eng/sct3/index_e.cfm#5 [accessed 4 February 2013]

Franzin WG, Barton BA, Remnant RA, Wain DB, Pagel SJ (1994) Range estension, present and potential distribution, and possible effects of rainbow smelt in Hudson Bay drainage waters of northwestern Ontario, Manitoba, and Minnesota. North American Journal of Fisheries Management 14:65–76

Gaston AJ, Woo K, Hipfner JM (2003) Trends in Forage Fish Populations in Northern Hudson Bay since 1981, as Determined from the Diet of Nestling Thick-Billed Murres *Uria lomvia*. Arctic 56:227 – 233

Gjertz I, Lydersen C (1986) The ringed seal (*Phoca hispida*) spring diet in northwestern Spitsbergen, Svalbard. Polar Research 4:53–56

Higdon JW, Ferguson SH (2009) Loss of Arctic sea ice causing punctuated change in sightings of killer whales (*Orcinus orca*) over the past century. Ecological Applications 19:1365–1375

Holst M, Stirling I, Hobson KA (2001) Diet of Ringed Seals (*Phoca hispida*) on the East and West Sides of the North Water Polynya, Northern Baffin Bay. Marine Mammal Science 17:888–908

Labansen AL, Lydersen C, Haug T, Kovacs KM (2007) Spring diet of ringed seals (*Phoca hispida*) from northwestern Spitsbergen, Norway. ICES Journal of Marine Science 64:1246–1256

Labansen AL, Lydersen C, Levermann N, Haug T, Kovacs KM (2011) Diet of ringed seals (*Pusa hispida*) from Northeast Greenland. Polar Biology 34:227–234

Laidre KL, Stirling I, Lowry LF, Wiig Ø, Heide-Jørgensen MP, Ferguson SH (2008) Quantifying the sensitivity of Arctic marine mammals to climate-induced habitat change. Ecological Applications 18:S97–S125

Lowry LF, Frost K, Burns JJ (1980) Variability in the Diet of Ringed Seals, *Phoca hispida*, in Alaska. Canadian Journal of Fisheries and Aquatic Sciences 37:2254–2261

Rose G (2005) Capelin (*Mallotus villosus*) distribution and climate: a sea “canary” for marine ecosystem change. ICES Journal of Marine Science 62:1524–1530

Smith TG (1987) The ringed seal, *Phoca hispida*, of the Canadian western Arctic. Canadian Bulletin of Fisheries and Aquatic Sciences 216:1–81

Stirling I (2005) Reproductive rates of ringed seals and survival of pups in Northwestern Hudson Bay, Canada, 1991-2000. Polar Biology 28:381–387

Thiemann GW, Iverson SJ, Stirling I (2008) Polar Bear Diets and Arctic Marine Food Webs: Insights From Fatty Acid Analysis. Ecological Monographs 78:591–613

Wassmann P, Duarte C, Agusti S, Sejr M (2011) Footprints of climate change in the Arctic marine ecosystem. Global Change Biology 17:1235–1249

Winters GH (1982) Life History and Geographical Patterns of Growth in Capelin, *Mallotus villosus*, of the Labrador and Newfoundland Areas. Journal of Northwest Atlantic Fishery Science 3:105–114