Beaufort Sea Beluga Whales: an Ecological Approach to Examining Diet and Dietary Sources of Mercury

by

Lisa Lucia Loseto

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements of the degree of

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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DOCTOR OF PHILOSOPHY

Abstract

Mercury (Hg) levels in the Beaufort Sea beluga *(Delphinapterus leucas)* population have been increasing since the 1990's. Ultimately, it is the Hg content of prey that determines beluga Hg levels. However, the Beaufort Sea beluga diet is not understood, and little is known about the food webs and Hg sources in their summer habitat. Using satellite telemetry data, the Beaufort Sea Beluga were found to segregate into habitat use groups, by length, sex and reproductive status. Segregation of habitat use lead to the hypothesis that beluga may feed differently, explaining Hg dietary sources. Mercury levels were measured in three food webs in the western Canadian Arctic to assess their dietary Hg contribution. Results revealed that potential beluga prey had variable Hg concentrations. With the use of the diet biomarkers stable isotopes and fatty acids in both prey items and beluga, the beluga diet was evaluated by incorporating beluga biological variables such as age, length, sex and harvest location. Here, we show the factors driving beluga diet variability lead to differences in dietary Hg uptake. Diet variability within the Beaufort Sea beluga was evident, whereby larger beluga appeared to feed predominantly on offshore arctic cod (*Boreogadus saida*), and medium and smaller sized beluga incorporated more near-shore fish collected from the Mackenzie shelf. The variation in beluga diet was supported by the differences in Hg concentrations and $\delta^{15}N$ values in both prey and beluga. For the first time, we demonstrate that food web Hg biomagnification processes drive beluga muscle Hg levels, rather than Hg bioaccumulation over time. This conclusion revealed that dietary Hg levels varied with habitat use, where the shelf was low source of Hg and the

offshore was a high source. Therefore, incorporating beluga habitat use along with food web complexity was important in determining the factors driving beluga Hg levels. These observations lend support to the possibility that the high levels of Hg leaving the Mackenzie River only become bioavailable for food web uptake once entering offshore habitats.

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Table of Contents

List of Figures

Lists of Tables

Thesis Format and Manuscript Claims

The thesis is presented in a manuscript format. Chapters 2, 3, 4 and 5 and written in a manuscript style containing an Abstract, Introduction, Materials and Methods, Results and Discussion and Conclusions. Chapter 1 introduces the overall theme of the thesis and Chapter 6 synthesizes and concludes the major findings of all the papers.

Chapter 2: **Loseto, L.L.** Richard, P., Stern, G.A., Orr, J., Ferguson, H. 2006. *Segregation of Beaufort Sea beluga whales during the open-water season.* Canadian Journal of Zoology, 84: 1743-1751. Lisa Loseto analyzed beluga tracking data previously collected (1993-1997), and wrote the manuscript with the guidance of the co-authors.

Chapter 3: **Loseto, L.L.** Stern, G.A., Deibel, D., Connelly, T.L., Prokopowicz, A., Fortier, L., Ferguson, H. *Linking Habitat and Feeding Behaviour to Mercury Exposure in Beaufort Sea Beluga Whales.* This manuscript was accepted on June 13 2007 by the Journal of Marine Systems. Lisa Loseto collected and analyzed mercury in a multitude of species comprising several food webs in the eastern Beaufort Sea. Co-authors participated in sample collections of various organisms. Lisa Loseto wrote the manuscript with the participation of the co-authors.

Chapter 4: **Loseto, L.L.** Stern, G.A., Ferguson, H. *Beluga Mercury levels defined by Habitat Use and Size revealed by Fatty Acids and Stable Isotopes.* This manuscript is in preparation for submission to Environmental Science and Technology on September 20 2007. Lisa Loseto co-ordinated the beluga tissue sample collection, and analyzed samples in the laboratory with summer student assistance. Lisa Loseto ran all statistical analyses and wrote the manuscript under the supervision of her supervisors.

Chapter 5: **Loseto, L.L.** Stern, G.A., Deibel, D., Gemmill, B., Connelly, T.L., Prokpowicz, A., Fortier, L, Ferguson, H. *Beaufort Sea Beluga Diet described by Fatty Acid Signature Analysis and Insights to Arctic cod Distribution.* Lisa Loseto with the assistance of B. Gemmill, the extracted in species listed in chapter 3 for fatty acid analysis. Lisa Loseto analyzed the data and ran all statistics and was the lead author of the manuscript.

1.0 General Introduction

1.1 Concern over Mercury in Beaufort Sea Beluga

Mercury (Hg) in the form of methyl mercury (MeHg) is a serious contaminant of concern because it is a neurotoxin that concentrates in kidneys, liver, the central nervous system and particularly targets the brain because it can cross the blood brain barrier. Therefore, Hg is a high priority to federal departments such as the Department of Indian and Northern Affairs, Health Canada and the World Health Organization (WHO 1990, NCP 2002).

Over the last decade there has been concern over the high levels of Hg in the Beaufort Sea beluga *(Delphinapterus leucas)* whale population that summers in the eastern Beaufort Sea and Mackenzie Delta region in the western Canadian Arctic (Figure 1.1). In the 1990's, liver Hg levels in this beluga population tripled in comparison to 1980 levels (Lockhart et al. 2005), and were the highest relative to other Canadian Arctic beluga populations. Although still higher than 1980 levels, Hg concentrations have dropped and are now comparable to other Arctic populations (Lockhart et al. 2005). Elevated Hg levels represent a risk to beluga, and to predators of beluga such as polar bears and Inuvialuit subsistence hunters, who harvest them in the summer (Usher 2002).

In response to the concern over high Hg levels in Beaufort Sea beluga whales, a workshop was held in 2002 at the Fisheries & Oceans Canada in Winnipeg, that was organized by the Fisheries Joint Management Committee; a land-claim based co-management organization from the Inuvialuit Settlement Region (Rosenberg 2003).

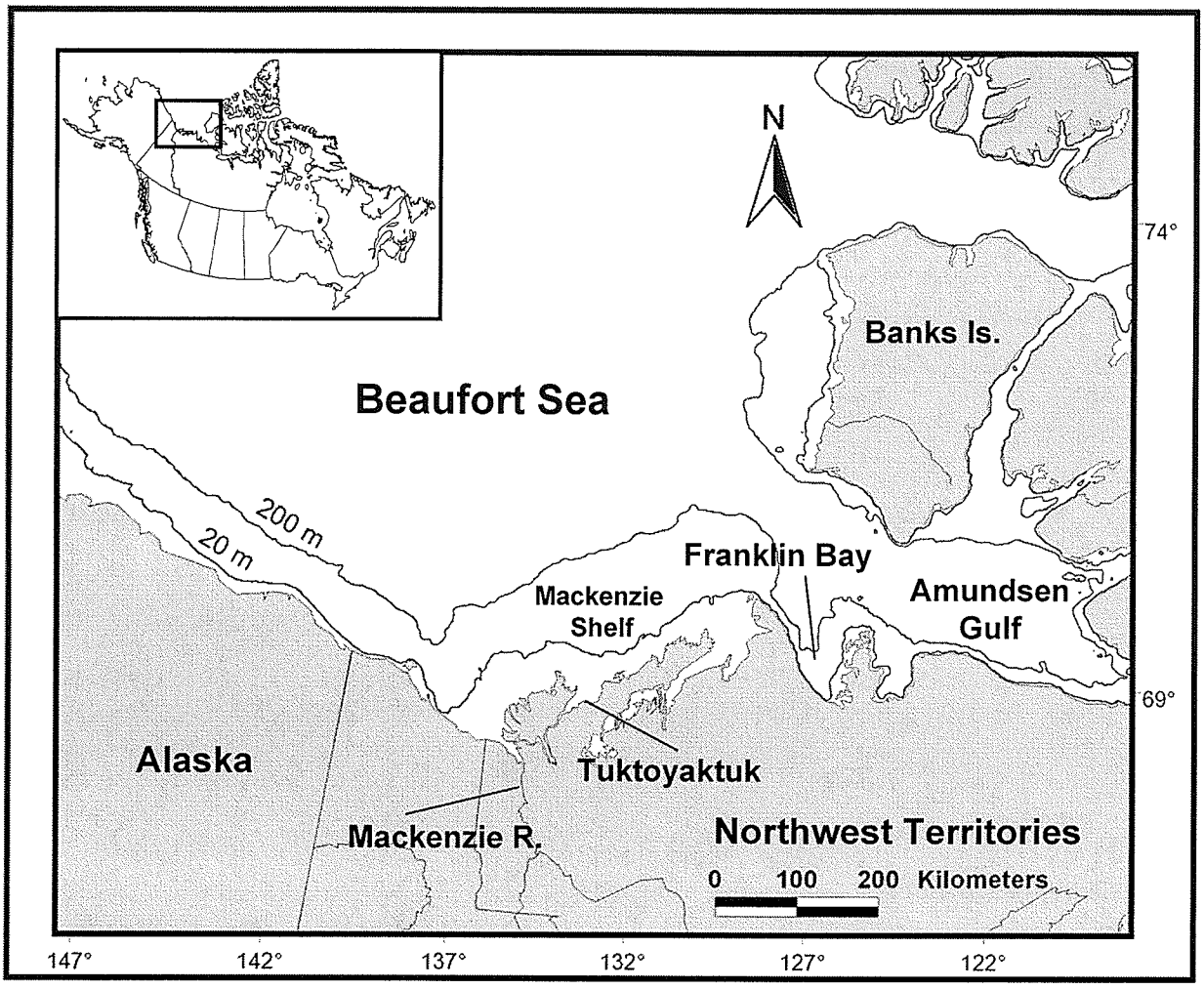

Figure 1.1 Study area of the eastern Beaufort Sea, where the Beaufort Sea Beluga travel to in the summer

The workshop recommended several research programs be carried out to understand the causes of the high Hg levels measured in the 1990's, and assist with future decision making (Rosenberg 2003). Proposed research programs included measuring Hg sources from the Mackenzie River, creation of beluga Hg physiological models, and the evaluation of beluga diet sources of Hg along with movement patterns (Rosenberg 2003). Due to present socio-economical and climate pressures in the Canadian western Arctic, understanding these processes are necessary to provide information for future planning and ocean conservation management.

This thesis addresses the dietary sources of Hg to the Beaufort Sea beluga whale population in order to evaluate processes contributing to present Hg levels.

1.2 Mercury Processes in Biota

Dietary Hg sources, rather than exposure in the surrounding environment (e.g. air or water) is the dominant source pathway for Hg to organisms (Mathers and Johansen 1985, Kelly et al. 1997). Hg can exist as various organic metal species. Unlike inorganic Hg, it is difficult to eliminate MeHg from biological systems, which results in high levels in organisms (Watras and Bloom 1992). Contaminant pathways to biota can be ascribed by three processes: bioconcentration, bioaccumulation, and biomagnification. These processes are briefly discussed below to provide the reader with background on approaches used in this thesis. There are various versions, and interpretations of the equations presented below that stem from specific applications to particular contaminants and their unique physical-chemical properties. The equations presented here were chosen for there applications to Hg in this thesis.

Bioconcentration describes the absorption or uptake of a contaminant from the surrounding environment (air or water). The degree to which a contaminant will concentrate in an organism is expressed as a bioconcentration factor (BCF):

BCF = [contaminant in tissue]/[contaminant exposure in environment] (1)

The bioconcentration factor is based on the hydrophobicity of a compound and thus describes the movement from water or organic matter into tissue. BCFs are typically used in aquatic species such as invertebrates and fish, when evaluating the octanol-water partition coefficient (K_{ow}) of organic contaminants. Bioconcentration factor is not commonly used to evaluate Hg in higher-level organisms because the process of bioaccumulation and biomagnifications via diet and food web processes dominate levels in organisms (Kelly et al. 1997).

Bioaccumulation describes the increase in concentration of Hg in an organism over time. It is a dynamic process that summarizes the uptake, storage, metabolism and excretion of a compound or contaminant (Arnot and Gobas 2004). For a contaminant to bioaccumulate in an organism, the net uptake and storage must be greater than the organism's ability to break down and excrete the contaminant, enabling accumulation over time. Several methods of calculating the bioaccumulation factor (BAF) are described in Arnot and Gobas (2004).

Biomagnification describes the ability of a contaminant such MeHg to become increasingly concentrated at successively higher trophic levels, thus biomagnification takes the bioaccumulation processes into account. Mercury bioaccumulates in an organism over time, and then is transferred to a higher trophic level in a multiplicative or exponential transfer. Biomagnification is well demonstrated in aquatic ecosystems

where food chains are often longer than terrestrial food webs, which results in high Hg levels in the top consumers relative to water. The low MeHg levels in the air and water are deceiving because once MeHg enters microscopic organisms concentrations can biomagnify as much as a million times up a food web, reaching dangerously toxic levels. Methyl mercury increases in proportion to total Hg (THg) up the food chain from 10% in the water to 30% in zooplanton to 95% in fish (Watras and Bloom 1992). $\delta^{15}N$ measurements are used to estimate Hg biomagnification factors in a foodweb because it similarly becomes enriched at each trophic level (Cabana and Rasmussen 1994). Given that Hg can biomagnify differently at each trophic level, biomagnification factors (BMFs) can be used to describe the transfer of Hg between prey and predator using the formula below (Gobas and Morrison 2000, Fisk et al. 2001):

$$BMF = (Hg_{predator}/Hg_{prey})/(TL_{predator}/TL_{prey}) \quad (2)$$

This formula requires an estimate of the trophic level (TL) of both predator and prey. In marine systems this has been carried out by normalizing $\delta^{15}N$ to *Calanus* spp. $\delta^{15}N$ values, assuming that *Calanus* represents a consumer that feeds only on phytoplankton in a marine system (Fisk et al. 2001), with the following equation:

$$TL_{consumer} = 2.0 + (\delta^{15}N_{consumer} - \delta^{15}N_{Calanus})/3.8 \quad (3)$$

The denominator of 3.8 represents the nitrogen isotopic fractionation from one trophic level to the next (Hobson and Welch 1992). Using $\delta^{15}N$ instead of trophic levels reduces the need to know the enrichment factor of nitrogen or to assume a TL of 2.0 for pooled *Calanus* spp.. Thus, replacing the trophic levels in equation (2) used in Dehn et al., (2006) with the raw $\delta^{15}N$ values as follows may be more accurate:

$$BMF = (Hg_{predator}/Hg_{prey})/(\delta^{15}N_{predator}/\delta^{15}N_{prey}). \quad (4)$$

This formula does not allow for several prey species to be incorporated into the predator diet. Multi-prey mixing models might assist with this problem if stable isotope enrichment factors from prey to predator are known (e.g. (Phillips and Gregg 2003)). Biomagnification is the process by which Hg is biotransferred from one trophic level to the next, and the process from the bottom of the food web to the top can be quantified by calculating the food web magnification factor (FWMF), also known as the trophic magnification factor (TMF). This can be determined from the food web slope for Hg concentrations in organisms and their associated $\delta^{15}N$. Reporting the coefficient of the slope, or using the following formula (where the b = slope) have commonly been used to describe the trophic-level transfer of contaminants up a food web (e.g.(Fisk et al. 2001, Power et al. 2002):

$$\text{FMWF} = e^{b} \tag{5}$$

This approach has been used in several arctic studies to describe both Hg and persistent organic pollutant trophic level transfer (Fisk et al. 2001, Campbell et al. 2005, Dehn et al. 2006).

1.3 Predator Diet Items and Dietary Mercury levels

Determining biomagnification factors between predator and prey as well as food web magnification factors has provided insight into contaminant transfer. In the case of organic contaminants such as PCB's, these calculations have revealed information about contaminant metabolism and detoxification (e.g. Fisk et al., 2001). In situations where the food web is not fully understood and/or the predator of interest has a diet that is variable, these approaches may not be suitable. Because diet is the main vector of Hg transfer to higher-level predators such as beluga, two broad factors should be

considered: their diet preferences and the Hg levels in their prey items. Figure 1.2 lists the variables that can influence predator feeding preferences and Hg levels in diet items. It is not known to what extent these factors effect the interpretation or calculation of Hg trophic-level transfer to top predators. Therefore, the objective of this thesis was to examine dietary sources of Hg to Beaufort Sea beluga whales by incorporating predator feeding patterns and food web dynamics.

Calculating the Hg biotransfer might be better assessed if the relative importance of prey was known and incorporated into the FWMF calculation. To effectively measure the trophic level transfer of Hg to a predator, it is important to consider energy or biomass transfer within a food web. Due to the remoteness and spatial extent of the Arctic marine food web, little is known about the dynamics of this food web. In addition to considering food web Hg processes, the complexity of the predator population and feeding strategies should also be evaluated to effectively characterize dietary sources of Hg. The effects of animal feeding behaviour can be considered by measuring the influences of sex, size, age and reproductive state, as they will likely impact contaminant levels (Borga et al. 2004). According to the foraging theory that states that an organism must consume the most amount of energy while expending the least, the large spatial extent of the Beaufort Sea beluga range supports that they are feeding on several food webs (Krivan and Eisner 2003). Therefore, there is a need to examine both predator dietary preferences and food web dynamics when determining processes of Hg transfer to predators. The examination of predator and environmental variables to understand the trophic transfer of persistent organochlorine contaminants was highlighted in Borga et al., (2004).

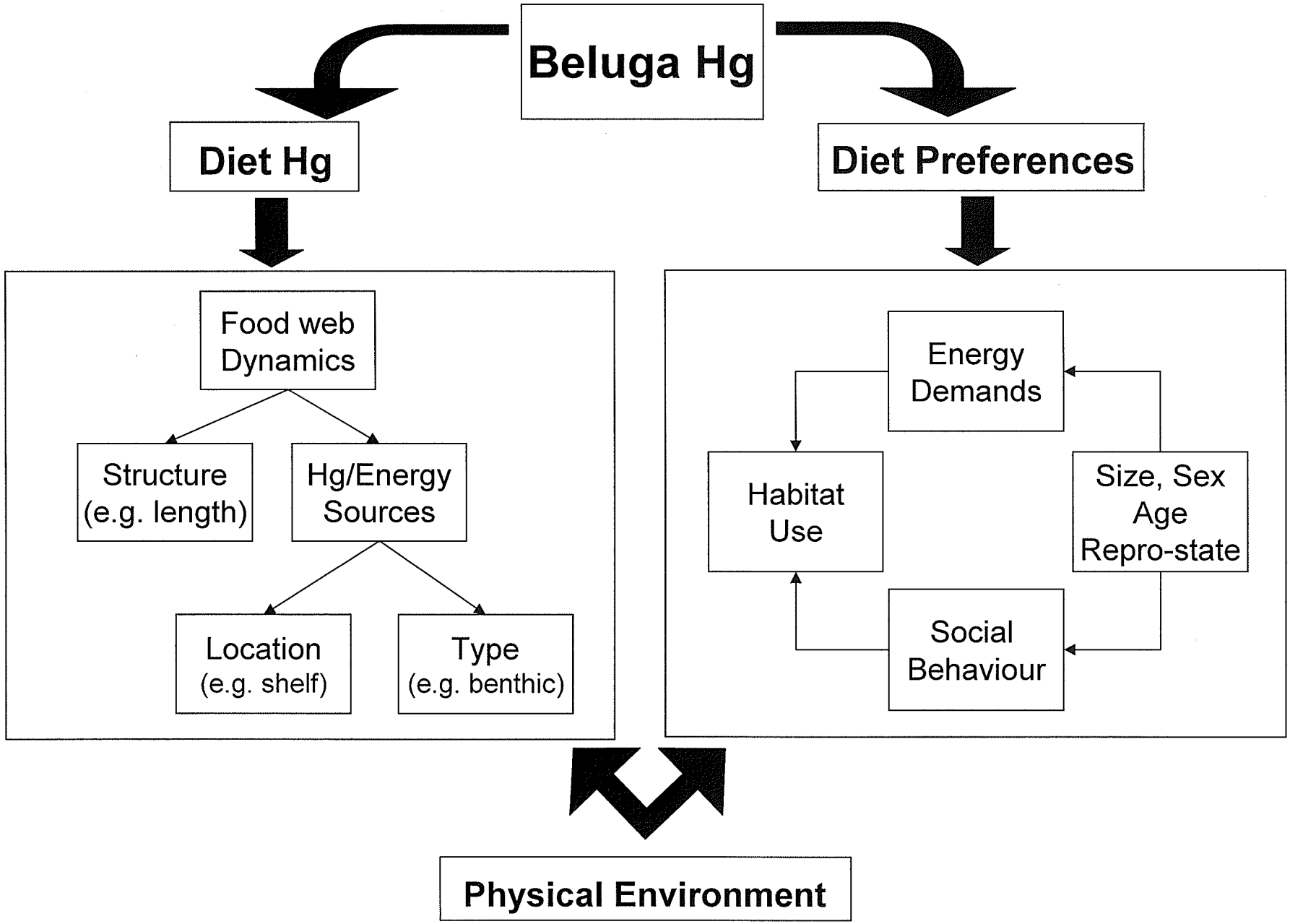

Figure 1.2 Influences on beluga mercury (Hg) uptake incorporating dietary preferences and food web dynamics driving prey Hg levels

1.3.1 Food web Complexity and Mercury Dynamics

Food webs describe the feeding relationships between species in a community and represent the transfer of energy and material, such as contaminants, from one species to another. An organism within a food web occupies a trophic-level position based on what it is feeding on. Food webs differ among ecosystems and can be described by the number of trophic levels, species diversity, diet linkages between organisms, and overall structure, all of which describe the food web dynamics as well as transfer of energy and contaminants.

Food web dynamics can be governed by bottom-up processes via a resource-based regulation of higher trophic levels, as well as top-down processes of predator-based regulation of lower trophic levels (Lindeman 1942, Slobodkin 1960). In addition, processes in the center of the food web can also alter energy flow and dynamics (Carpenter et al. 1996). The food web structure, in particular length (number of trophic levels), is an important factor controlling Hg levels in top predators because a longer food web allows Hg to biomagnify to a greater extent (Cabana and Rasmussen 1994).

Within aquatic systems there may be several food web pathways with different originating energy sources that may ultimately affect Hg levels in top predators. For example, benthic food webs are comprised of organisms at the bottom of a body of water obtaining energy and carbon sources from detritus, whereas the pelagic food web is composed of organisms living within the water column, obtaining energy from phytoplankton. A top predator within this system may feed on several organisms that derive their energy from either the benthic or pelagic food webs (Vander Zanden and Vadeboncoeur 2002). Food web pathways within an ecosystem can vary in contaminant

loads in relation to carbon and energy sources and/or regional inputs at the beginning of the food web. Analysis of DDT food web biomagnification in a lake with a pelagic and benthic food web revealed pelagic food webs accumulated more DDT than the benthic food web (Kidd et al. 2001). This demonstrates the effect of food web complexity on biomagnification of contaminants to top predators.

In the early summer, Beaufort Sea beluga whales travel to the eastern Beaufort Sea which hosts several food web pathways that likely have unique energy and contaminant sources. These pathways include the pelagic and benthic environments as well as a shelf region. The Mackenzie shelf receives significant freshwater input from the Mackenzie River that has the fourth largest discharge in the world (Millot et al. 2003). As a result, the Mackenzie shelf is a low saline environment and creates an estuarine habitat for organisms and food webs to obtain energy sources from riverine inputs (Parson et al. 1989). Beaufort Sea belugas utilize this habitat every summer, similar to other beluga populations that congregate in estuaries for reasons that are not fully known (Smith et al. 1994, Richard et al. 2001b). The Mackenzie River was found to have some of the highest Hg outputs relative to other Arctic river systems (Leitch et al. 2007). Thus, within the summering region of Beaufort Sea beluga, there are several food webs beginning with different energy sources that may also introduce different Hg concentrations to organisms associated with different food web pathways.

1.3.2 Predator Intrinsic Factors

Organism energy requirements and survival strategies vary with age, sex, size, and reproductive stage (Stevick et al. 2002). The foraging selection hypothesis relates to size dimorphism, whereby maintaining a larger body mass is achieved by foraging

more or on higher quality food (Clutton-Brock et al. 1982, Conradt 1998). This hypothesis can be broadened to consider the unique energy demands of pregnant or lactating females (Loudon 1985). Variability in energy requirements within a species may be manifested by differences in feeding behaviour and habitat use. Larger individuals within a species may choose to feed at higher trophic levels or on prey to meet their larger energy needs (Bowyer 2004), thus exposing these larger individuals to higher dietary contaminant loads (Cabana and Rasmussen 1994, Arnot and Gobas 2004).

Many factors surrounding energetics contribute to resource selection, feeding preferences and prey selection (Belovsky et al. 1989, Bowyer and Kie 2004). Previous contaminant studies have tested for the effects of sex and age in marine mammals (Dehn et al. 2005, Lockhart et al. 2005), and fish (McIntyre and Beauchamp 2007) on contaminant loads. Evaluating the effects of size on contaminant loads are more challenging to examine in birds and mammals because after they reach maturity they do not continue to grow (as fish typically do). Previous studies have illustrated that Hg concentrations in top predators may vary as a result of diversity in foraging behaviour (Atwell et al. 1998). Differences in feeding behaviour and energy requirements have been observed to influence stable isotope concentrations (Tucker et al. 2007). Thus, differences in foraging behaviour within a species can have measurable variation in Hg levels and other biochemical diet indicators.

Beluga whales are one of the most abundant odontocetes (toothed whales) in arctic waters (Brodie 1989); as such, they likely play an important role within regional food web dynamics. Odontocetes feed at higher trophic levels (e.g. fish) compared to

baleen whales that feed on zooplankton. Some beluga populations, such as the Alaskan Cook Inlet, the High Arctic Cumberland Sound and the Gulf of St. Lawrence populations have small home ranges or short migration routes (Richard et al. 2001a, Hobbs et al. 2005) thus, their diet sources of Hg can be associated within a common feeding region. On the other hand, the Beaufort Sea beluga whale population undergoes one of the longest migrations; travelling to the Bering Sea in the winter and to the eastern Beaufort Sea and Amundsen Gulf in the summer (Richard et al. 2001b). This large home range incorporates two different ocean bodies and their associated ecosystems, which increases the seasonal and regional complexity of diet Hg sources.

Arctic cod (*Boreogadus saida*) is thought to be the dominant forage species for some beluga populations (Seaman et al. 1982, Welch et al. 1993, Dahl et al. 2000). Feeding on arctic cod is likely habitat-specific because arctic cod are not available to more southern populations (e.g. Gulf of St. Lawrence belugas). Belugas have been described as generalist feeders because a variety of fish and invertebrate species have been found in stomach contents. For example, Greenland beluga whales were found to have the following stomach contents: redfish (*Sebastes marinus*), halibut (*Reinhardtius hippoglossoides*), shrimp (*Pandalus borealis*) in addition to squid beaks (Heide-Jorgensen 1994). Other observations show Pacific salmon (*Oncorhynchus* spp.) are important food items to the Alaskan beluga populations (Bristol Bay, Cook Inlet) (Frost and Lowry 1990). These observations suggest beluga feeding may be population-specific and vary with habitat, seasonal prey abundance and diet preferences.

Beluga whales are size dimorphic with males being larger than females on average (Harwood and Smith 2002) whereby males reach a mean asymptotic length of

4.2 metres and the female asymptotic length is approximately 0.5 metres less (Luque and Ferguson 2006). Observations of habitat-segregation by sex and age classes have been reported for populations that summer in estuaries of the Canadian High Arctic and eastern Hudson Bay (Smith et al. 1994). Dimorphism and differences in habitat use within a population suggest that beluga whale diet and foraging behaviour may vary among individuals in relation to specific energetic needs. Therefore, dietary Hg intake may vary with habitat use and energetic demands.

1.3.3 Underlying Physical Forcing

The physical environment influences bottom-up and top-down processes by providing suitable habitat as well as limiting resources. Previous food web studies have shown that physical environmental forces can drive food web dynamics (Tynan and DeMaster 1997). Seasonality in the Arctic is different from temperate latitudes where primary production is more evenly distributed throughout the year. Production in marine systems is governed by the marked changes in light and sea ice cover limiting production and energy to top predators (Mundy et al. 2007). Contaminant studies need to incorporate these processes into the overall pathways that effect contaminant levels if they hope to predict future scenarios under climate change. Shifts in climate indirectly affected Bering Sea marine mammal populations through changes in ocean up welling that altered the distribution and abundance of their preferred prey (Benson and Trites 2002).

Change in the physical environment will impact resource availability, likely altering the predator feeding behaviour and habitat use (Manly et al. 1993). For example, beluga may respond to changes in the distribution of open-water and the

location of the ice edge due to resource availability, predation, and/or reproductive state (Barber et al. 2001). Alterations in the physical environment over space and time will influence prey availability, highlighting the need to understand food webs to be able to predict how they will respond to disturbance. The arctic environment is sensitive to changes in climate (ACIA 2005), which will likely result in positive and negative feed backs to abiotic and biotic Hg processes (AMAP 2005).

The physical environment may also impact abiotic pathways of Hg to the arctic that in turn can also alter biotic processes (Macdonald 2006). For example the magnitude of Arctic Oscillations (AO) may influence the source transport of Hg via atmospheric and oceanic currents (AMAP 2005). For example, the changes in ocean and atmospheric patterns can result in the transport of Hg from a high source area to the Arctic; however, if the Hg is not methylated to the MeHg form then it cannot enter the food web to magnify to high concentrations to predators. Therefore, an increase in Hg to the Arctic environment is only a concern to higher-level organisms if it is readily methylated (Macdonald et al. 2005).

Recent warming in the western Canadian Arctic has reduced sea ice extent and concentration over the last 20 years (Serreze et al. 2007). Depletion of sea ice may impact the ecosystem stability by disrupting the prey and predator dynamics that are closely tied to sea ice. During the summer, some male belugas spend time in the relatively ice-rich Arctic Archipelago, while most females remain close to the mainland shoreline and in the relatively ice free Amundsen Gulf (Barber et al. 2001, Richard et al. 2001b). Barber et al. (2001) suggested that sea ice and bathymetry are important habitat features to consider when investigating beluga habitat use. Here, it is suggested

that the physical environment must be examined in relation to food web processes especially with respect to the higher-order predators, as their habitat use is likely tied to feeding. Thus, a reduction in sea ice may increase beluga stress from energy exertion as a result of increased search time for preferred prey associated with sea ice.

1.4 Thesis Approach and Objectives

Due to the biomagnifying properties of MeHg, Hg concentrations in predators such as beluga, largely reflect the Hg levels in their diet (Mathers and Johansen 1985). Little is known about the Beaufort Sea beluga diet, thus dietary Hg sources cannot be accounted for. Typically in animal diet studies, stomach contents and feces are used to identify diet items. This is not feasible with the Beaufort Sea beluga because harvested whales often have empty stomachs, and feces cannot be found; possibly a result of their quick digestive rates (approx. 4hr) (Kastelein et al. 1994). Despite this the Beaufort Sea beluga have been observed feeding during the summer by local hunters (Harwood and Smith 2002).

Advancements in diet biomarkers such as stable isotopes and fatty acids, have reduced the need to collect stomach contents to determine animal diets (e.g.(Hobson and Welch 1992, Iverson 1993). Based on relative isotopic fractionation processes, $\delta^{15}N$ can be used to describe trophic levels (DeNiro and Epstein 1981, Cabana and Rasmussen 1994) and $\delta^{13}C$ can be used to evaluate carbon sources (France 1995). Fatty acids can be used to examine diet because they undergo little degradation during digestion and are stored in the predator adipose tissue. In addition to the use of diet biomarkers, predator movement data as it relates to habitat use and foraging behaviour

has also provided important information about diet preferences (Le Boeuf et al. 2000, Baechler et al. 2002). In this study the combination of biochemical and telemetry data will provide a better understanding of beluga diet and diet sources of Hg. This thesis examines beluga Hg levels using four approaches that incorporate biological, physical and ecological forcing on beluga diet preferences and Hg levels in prey items.

In chapter two, beluga habitat use is examined to understand how belugas use the surrounding physical environment. Resource selection function models are employed to measure habitat selection under the conditions of changing habitat availability (Manly et al. 1993, Arthur et al. 1996). Beluga habitat is described by sea ice cover concentrations, bathymetry and distance from coastlines. Satellite tracking data, in combination with habitat type are used to execute the resource selection model. Results from this chapter provide motivation to examine and incorporate behavioural consequences of habitat use on dietary Hg uptake in the following chapters.

In chapter three, the importance of food web dynamics and Hg sources are addressed in the analysis of trophic level transfer of Hg. Three food webs within the beluga summering region are examined to determine Hg levels in possible diet items. This chapter uses findings from the habitat use analysis (Chapter 2) to create beluga feeding groups and pair them with food webs that best fit their habitat use. With the use of Hg concentrations and nitrogen stable isotopes, beluga Hg BMFs are calculated and used as a tool to increase our understanding of beluga diet. This is the first study to measure Hg in pelagic, estuarine and epibenthic organisms from the eastern Beaufort Sea.

Findings from the habitat use analysis provided a basis to initiate sampling at an additional beluga sampling site within the Inuvialuit region to better capture influences of habitat use on diet preferences. Thus, chapter four also uses results from Chapter 2 as justification to test for differences in diet associated with habitat use, and further examines if those factors predict dietary Hg uptake. Fatty acids are used as diet biomarkers to test which beluga biological variables (i.e. length, age, sex and harvest site) best describe diet. Beluga biological variables that best described diet were examined for similar trends in stable isotopes and Hg levels in beluga tissues. Results from the fatty acid analysis reveal the importance of biomagnification processes on beluga Hg levels, as well as providing new information about Hg uptake. Stable isotope diet biomarkers provide support for metabolic and turnover rates of Hg as well as links to beluga feeding in the Bering Sea.

In chapter five, the beluga food web pairings hypothesized in chapter three are evaluated using fatty acid signature analysis. First, we determined if the prey items collected can be discriminated from one another based on their ecology using multivariate analyses. The relative importance of prey items to beluga diet is assessed, and beluga size ranges and habitat use are incorporated to understand their role on dietary preferences and ultimately Hg uptake. Fatty acid analysis results are compared with Hg and stable nitrogen isotope values from chapters 3 and 4.

Overall, the objective of this thesis and therefore the next four chapters is to examine predator and food web complexity to better understand the processes involved in determining beluga Hg levels attributed to diet sources.

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2.0 Segregation of Beaufort Sea beluga whales during the open-water season

Abstract

Population segregation by habitat use occurs because energy requirements and survival strategies vary with age, sex, size, and reproductive stage. From late summer to early fall in 1993, 1995 and 1997, relative length (age), sex, and reproductive status of satellite-tagged beluga whales (*Delphinapterus leucas*) in the eastern Beaufort Sea, were tested for habitat segregation. We used (1) resource selection function models to evaluate how belugas used areas of varying sea ice concentration and shelf habitats and (2) distance analysis to measure the selection of areas varying in distance to mainland and island coastlines. Resource selection functions and distance analysis established that habitat selection differed with length, sex, and reproductive status of whales: (1) females with calves and smaller males selected open water habitats near the mainland; (2) large males selected closed sea ice cover, in and near the Arctic Archipelago; and (3) smaller males and two females with calves (not newborn) selected habitat near the ice edge. The segregation of habitat use according to sex, age and reproductive status relates to the different resources required at different life stages and may represent characteristics of beluga social structure. We discuss our results in context of two common sexual segregation hypotheses and conclude that summer habitat segregation of belugas reflects differences in foraging ecology, risk of predation, and reproduction.

2.1 Introduction

Population segregation by habitat use occurs because energy requirements and survival strategies vary with age, sex, size, and reproductive stage. Sexual segregation can be defined as the selection of different habitats or locations by gender (Conradt 1998). Some studies have found sexual segregation to be driven by body size dimorphism (Ruckstuhl and Neuhaus 2002, Bowyer 2004), where males and females within a species have different resource demands related to size. Differences in reproductive condition may also lead to segregation (Stevick et al. 2002). Sexual segregation has been documented in sexually dimorphic mammals (Ralls 1977, Bonenfant et al. 2004, Bowyer 2004) including marine mammals (Stewart 1997, Austin et al. 2006). For example, in the dimorphic northern elephant seal (*Mirounga angustirostris*), males are up to ten times the size of females and, as a result, are assumed to require different feeding strategies, resources, and habitat use to maintain this size difference (Le Boeuf et al. 2000). Among cetaceans, sexual segregation occurs in both mysticetes (e.g., Atlantic humpback whale (*Megaptera novaesngliae*), (Stevick et al. 2003) and odontocetes (e.g., bottlenose dolphin (*Tursiops truncatus*)(Conner et al. 1999).

Beluga whales (*Delphinapterus leucas*) are sexually size dimorphic, with males being larger than females, on average (Harwood and Smith 2002) whereby males reach a mean asymptotic length of 4.2 metres and the female asymptotic length is approximately 0.5 metres less (Luque and Ferguson 2006). Observations of segregation by sex and age classes have been reported for populations that summer in estuaries of the Canadian High Arctic and eastern Hudson Bay (Smith et al. 1994). However, no quantitative assessments accompanied these observations. Smith et al., (1994) observed

adult nursing females and older female offspring nearshore in the estuaries, while large males formed large pods (10 to 15 individuals) that spent little time in the estuaries.

Every spring the eastern Beaufort Sea beluga population migrates from the Bering Sea to summer in the eastern Beaufort Sea. The summer harvest of Beaufort beluga whales by communities of the Inuvialuit Settlement Region (Northwest Territories, Canada) is an important component of the Inuvialuit subsistence lifestyle (Usher 2002). The Beaufort beluga whale population is one of the world's largest, with a minimum estimate of 20,000 individuals (Harwood et al. 1996). At present there is no indication of population decline.

During the summer, some males spend time north of the Mackenzie Delta, in the Arctic Archipelago, and most females remain close to the mainland shoreline and in Amundsen Gulf (Richard et al. 2001). Why this population segregates by summering habitat and how factors such as resource use and availability, predator avoidance, and foraging behaviour affect habitat selection are unknown. Barber et al. (2001) suggested that sea ice and bathymetry are important habitat features to consider when investigating beluga habitat use. Recent changes in climate have affected sea ice characteristics, including sea ice thickness (Yu et al. 2004), extent (Serreze et al. 2007), and concentration (Barber and Hanesiak 2004), necessitating an understanding of sea ice as a habitat resource for beluga whales to evaluate possible impacts of environmental change.

Here we examine the segregation of Beaufort beluga whales by summer habitat using satellite tracking data collected in 1993, 1995 and 1997. We consider how Beaufort beluga whales selectively use habitat relative to sex, age, and reproductive

status according to 1) resource selection functions (Arthur et al. 1996) of sea ice concentration and shelf habitat and 2) distance to island and mainland coastlines.

2.2 Materials and Methods

2.2.1 Tagging and satellite telemetry

Three, thirteen, and eight beluga whales were captured in the Mackenzie Delta, Northwest Territories, Canada (Figure 2.1), and tagged with satellite transmitters in 1993 (10-19 July), 1995 (03-16 July 16) and 1997 (26 July-1 August), respectively (see Table 2.1) (Richard et al. 2001). Whales were captured and tagged using techniques approved by the Department of Fisheries & Oceans Canada Animal Care Committee, described in Orr et al., (2001). Briefly, a whale is selected and herded to shallow waters (approx. 2 m depth) by boats. A seine net is then deployed that encircles the whale, and people in zodiac boats remove the whale from the net and secure it using tail ropes and a hoop net. After the whale is secured, size measurements are taken, and satellite-linked transmitter tags are placed. The tags are positioned onto the dorsal ridge with surgical nylon pins (6 mm diameter) that are fastened with washers secured through the skin and blubber of the dorsal ridge (Orr et al. 2001).

Deployed tags transmit location data when the whale surfaces, exposing the tag antenna to satellites in the area (Richard et al. 2001). Transmissions from tags were received by ARGOS satellites that estimate the precision of the point location of transmission and provide a quality index termed location class. All location classes were used to derive beluga tracks after filtering to remove outliers (Richard et al. 2001). We filtered low quality locations having errors greater than 1 km using a filter

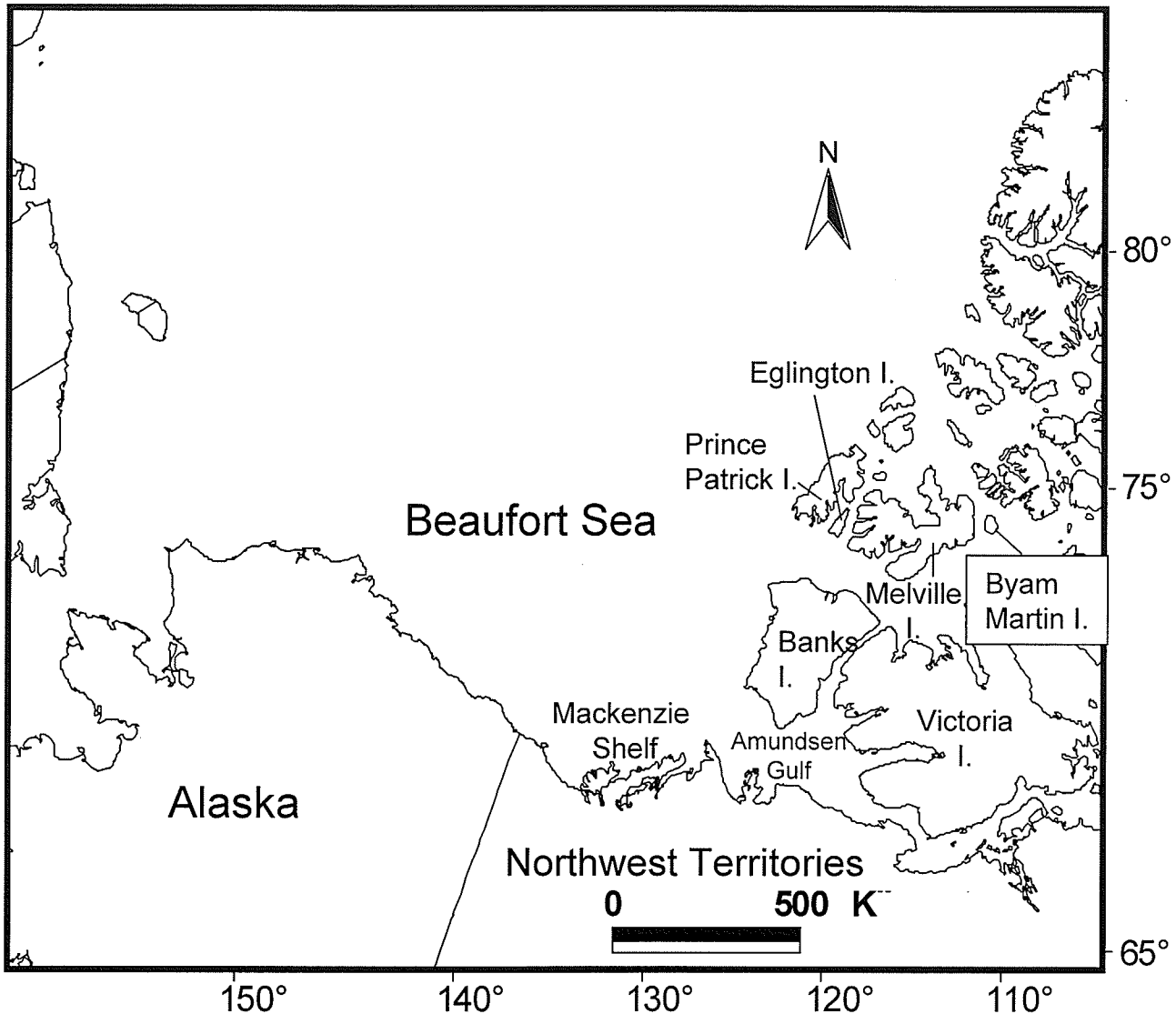

Figure 2.1. Area used by Beaufort beluga whales (*Delphinapterus leucas*) during late summer and early fall. Beluga capture occurred in the Mackenzie Delta during July in 1993, 1995, and 1997.

Table 2.1 Resource selection functions (b_j values) for beluga whales (*Delphinapterus leucas*) captured in 1993, 1995, and 1997 in the eastern Beaufort Sea, Canada (M=male, F=female, Fc=female with calf; superscripts a, b, c correspond with the MANOVA groups: juvenile, female with calf and adult).

							Habitat Category b_j values			
Year	Tag ID	Sex	Adult Length (cm)	Calf length (cm)	Number of days transmitted	Offshore open water	Mixed ice	Closed ice	Shallow open water	Cluster groups
1993	17002	M[c]	457		11	0.12	0.32	0.53	0.037	2
	17005	M[c]	442		61	0	0.71	0.29	0	2
	17009	F[a]	302		14	0.66	0	0	0.34	1
1995	17001	M[c]	427		27	0.52	0.17	0.31	0	3
	17002	M[a]	404		28	0.72	0	0	0.28	1
	17003	M[c]	432		17	0.02	0.2	0.75	0.04	2
	17004	M[a]	373		24	0.5	0.04	0.14	0.31	1
	17005	M[a]	353		23	0.42	0.2	0.16	0.23	1
	17007	F[c]	373		26	0.38	0.34	0	0.27	1
	17008	Fc[b]	361	182 [1]	9	0.64	0	N/A [3]	0.36	1
	8754	Fc[b]	363	217	23	0.35	0	N/A	0.65	1
	17010	M[a]	399		26	0.35	0.39	0.21	0.04	3
	17011	M[a]	402		25	0.22	0.25	0.46	0.07	2
	17012	M[a]	404		30	0.15	0.32	0.31	0.22	3
	17013	M[a]	402		30	0.13	0.31	0.52	0.03	2
	17014	Fc[b]	340	223	30	0.18	0.36	0.23	0.23	3
1997	2118	Fc[b]	374	? [2]	27	0.34	0.14	0.51	0	3
	10692	Fc[b]	338	243	33	0.55	0.19	0	0.25	1
	10693	M[a]	395		48	0.41	0.11	0.34	0.14	3
	8754	M[a]	405		50	0.3	0.26	0.33	0.11	3
	8755	M[a]	400		58	0.64	0.08	0	0.28	1
	25846	M[a]	374		46	0.56	0.16	0.19	0.08	1
	8757	M[a]	379		37	0.35	0.14	0.39	0.11	3
	8758	M[c]	421		46	0.3	0.08	0.54	0.09	3

[1] neonate

[2] length unknown

[3] Habitat categories not available for use

based on swim speeds calculated using good quality locations (less than 1 km error). All available daily locations (n = 9565) from the filtering were then reduced to median daily locations for each animal (n = 1099).

2.2.2 Habitat classification

Four habitat categories were chosen based on sea ice cover and water depth for the three years (1993, 1995, and 1997) and 2 months (July and August) of tracking. Weekly composite sea ice charts were obtained from Environment Canada's Canadian Ice Service (http://ice-glaces.ec.gc.ca) as digital layers and imported into ArcView 3.3® (Environmental Systems Research Institute, Inc., Redlands, California). These charts provide weekly polygons of sea ice cover categories (in tenths) within the study area. Based on previous findings, whereby belugas from several populations spent the majority of their time in either open water or closed ice (Barber et al. 2001), we grouped ice cover into three categories: open water (sea ice concentrations of 0/10 to 1/10), mixed ice (sea ice concentrations of 2/10 to 8/10), and closed ice concentrations, (sea ice concentrations 9/10 to 10/10). In addition, we classified shelf and non-shelf habitat according to a 200m water depth threshold (Macdonald et al. 1989). Combining our sea ice categories with shelf categories resulted in four habitat groupings: open water - off shelf (hereafter, offshore open water), mixed ice - off shelf (mixed ice), closed ice-off shelf (closed ice), and open water-shelf (shallow open water). Little or no ice was present in the shelf zone over the duration of the study, so the habitat groupings mixed ice-shelf and closed ice-shelf were not considered.

2.2.3. Resource selection function analysis

Resource selection function analysis provides a means to evaluate if the use of habitats by wildlife is selective, thus demonstrating a preference for a particular habitat and the resources associated with it (Manly et al. 1993). Selection is defined as the use of particular habitat types more often than would be expected if all habitat types were used randomly and in proportion to their availability (Johnson 1980). Resource selection indices are calculated for each habitat category from the ratio of percent used to percent available: the entire set of indices (ratios) is collectively termed the "resource selection function" (Manly et al. 1993). The resource selection function estimates the probability a habitat category will be selected relative to the probability of selecting other habitats based on availability.

Resource selection function analysis is problematic where habitat changes temporally (e.g., seasonal sea ice) or where animals undertake long-distance movements resulting in different mixtures of habitat available over time and space (Manly et al. 1993). Arthur et al. (1996) developed an iterative method for the estimation of the maximum likelihood of the resource selection function to control for changing availability. This method has been used successfully to evaluate habitat use at the seasonal scale in polar bears (*Ursus maritimus*) (Ferguson et al. 2000) as well as at the finer scale of daily habitat selection in woodland caribou (*Rangifer tarandus caribou*) (Rettie and Messier 2000).

Habitat use was defined for each individual beluga daily median location by assigning 100% to the habitat at that location, and 0% to the other habitat categories. Habitat availability was defined by the percentage of each habitat category inside a buffer circle around the median location for the previous day for the individual beluga

(Figure 2.2). The buffer circle radius (137.2 km) was determined by calculating the 95th percentile of daily distance travelled for all individuals and was used for all point location-buffers, as recommended by Rettie and Messier (2000). Within the buffer any area occupied by land was removed from the proportion of habitats available. For each animal, maximum likelihood estimates of the habitat selection function were obtained using the iteration method proposed by Arthur et al. (1996). The selection indices were calculated as follows:

$$w_k = \frac{\sum_{i=1}^{D} o_{ik}}{\sum_{i=1}^{D} \frac{A_{ik}}{\sum_{j=1}^{H} A_{ij} b_j}}, \qquad (1)$$

$$b_k = \frac{w_k}{\sum_{J=1}^{H} w_j}, \qquad (2)$$

where k is the habitat category from the set $j = 1$ to H, O_{ik} is the proportion of habitat type k used on day i (either 0 or 1), A_{ik} is the proportion of habitat type (k) available on day i, b_k is the estimated selection index for habitat type k, and D is the number of days the animal was located. The value 0.25 (or equal probability of use for four habitat categories, 1/4) was used for all b_j in the first step of the iteration. Next, equation 2 was used to calculate the new values for b_j and the process was repeated. Values for selection indices were then standardized by returning the b_j indices into the first equation to continue the iteration. The iteration stopped when values of b_j stabilized, i.e.: $b_j = w_j$ for all j.

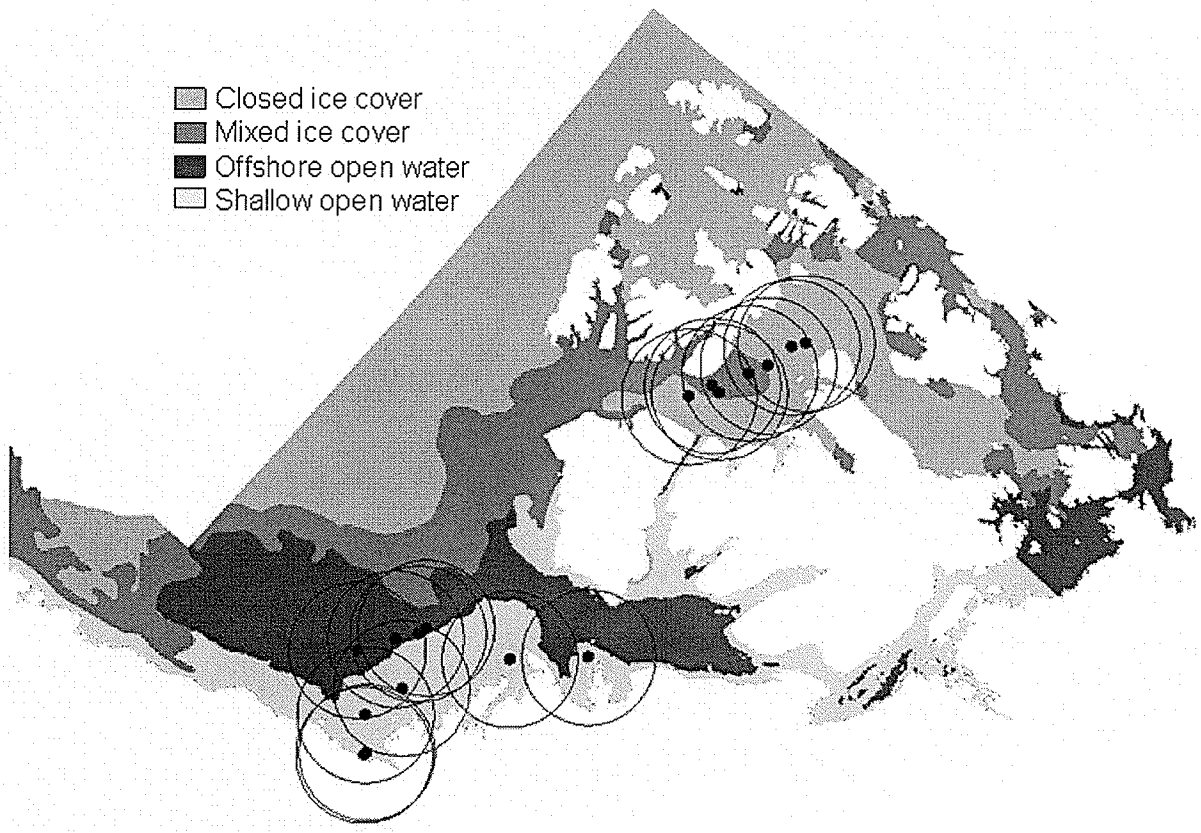

Figure 2.2. Example habitat map (week of Aug 15, 1995) used to determine habitat use and availability of four habitat types: Offshore open water, Mixed ice cover, Closed ice cover, and Shallow open water. Median daily locations (• demarcates used) and buffers (radius = 95th percentile of daily distance travelled) indicates habitat available to whales (see Materials and Methods).

2.2.4 Distance analysis

Distances to the mainland and island coastlines were calculated to provide an additional confirmatory test of the results of the resource selection function. First, median daily locations for whales in all three years were combined to estimate home range using the kernel 95% home range method of the Animal Movement extension to ArcView Extension (Worton 1989, Hooge and Eichenlaub 1997). Within this home range, 1099 random points (equal to the number of actual locations) were generated. The shortest distance to the coastline of the mainland and the shortest distance to the coastline of one of the six islands (Banks, Melville, Victoria, Prince Patrick, Eglington and Byam Martin) (Figure 2.1) were determined for all randomly generated and actual point location.

2.2.5 Statistical analysis

We used a multivariate analysis of variance to determine if habitat use was significantly different from random. Multiple dependent variables were synthetic values derived from the differences between the selection indices (b_j) for the habitat types (e.g. b_j mixed ice - b_j closed ice = synthetic variable) and the mean vector of contrasts was compared to a vector of 0 (Arthur et al. 1996). The use of synthetic variables allows for the comparison of habitat use relative to availability between habitat pairs (Arthur et al. 1996). When habitat use was significantly different from random, habitat categories were ranked according to the mean selection indices (b_j) and compared using univariate paired *t* tests (Arthur et al. 1996).

We tested for effects of gender and year whales were tagged on habitat use with a multivariate analysis of variance. Next, we tested for the effects of length (proxy for age) and reproductive status along with years. We reorganized the age and reproductive status combinations into three categories: 1) females with calves, 2) juveniles and 3) adults. Adult males were defined as whales greater than the asymptotic length of 420 cm (Luque and Ferguson 2006). Two females without calves were present: the one larger (373cm) than those with calves was placed into the adult category, and the female at 302cm was classified as a juvenile based on models of individual growth rates for the Beaufort Sea population (Luque and Ferguson 2006). The effects of age-reproductive class and year were assessed with multivariate analysis of variance as described above.

Cluster analysis was used to objectively group individual whales based on similarity of habitat selection indices (b_j) (PROC CLUSTER, SAS® version 8, SAS Institute Inc., Cary, North Carolina). The complete cluster method was the unpaired group mean and the distance measure was the Euclidean distance (Romesburg 1984). Groups were defined based on discontinuities in the F values (SAS Institute Inc. 1989). Analysis of variance was used to test for differences among the groups identified by the cluster analysis.

As a secondary assessment of the resource selection results, we tested for differences in distances to the mainland and islands among the groups identified using cluster analysis. Differences between group locations and random point locations were tested by analysis of variance with Dunnetts post hoc test (Sokal and Rohlf 1980).

2.3 Results

2.3.1 Habitat selection indices

Beluga habitat use was not random because resource selection indices of the four habitat types were not equally probable (i.e. resource selection indices b_j for habitat were different from 0.25) (Table 2.1). Habitat selection was significantly different from random ($F = {}_{16.98}$, df = 3, 19, $P < 0.01$). Offshore open water was used most often ($\bar{x}\ b_j = 0.37$, $\sigma = 0.04$), more than mixed ice and shallow open water ($P = 0.02$; $P < 0.001$). The second most selected habitat category was closed ice ($\bar{x} = 0.28$, $\sigma = 0.04$), which was used significantly more than shallow open water ($P = 0.048$).

2.3.2 Grouping according to sex, length and reproductive class

According to the multivariate analysis of variance males, and females did not select habitat differently from one another, and there was no significant difference in habitat selection among the three years (Table 2.2). In the second analysis, habitat use differed for age (length) and reproductive groups (Table 2.2). Due to these differences in habitat selection, we used a cluster analysis to objectively group belugas into more meaningful groups.

Three groups of belugas were identified by the cluster analysis (Tables 2.3 and 2.4). A discriminant function analysis described dispersion among the three groups (Figure 2.3). Factor 1 explained 94 % of the variation and was principally explained by the closed ice habitat. In the first cluster, both offshore and shallow open water habitat categories were used more often than expected ($\bar{x}\ b_j$ values = 0.55 and 0.28, respectively; Table 2.4). This group was composed of ten individuals: five females

Table 2.2 Results from multivariate analysis of variance of habitat selection indices (four habitats) for 24 beluga whales from the eastern Beaufort Sea: (1) effects of year, gender and interaction; and (2) effects of year, and age-reproductive class.

Tests among habitat section indices and explanatory factors	***df***	F_{ratio}	P_{value}
Test 1			
Years	8, 26	1.217	0.33
Gender	4, 13	1.641	0.22
Interaction	8, 26	1.66	0.16
Test 2			
Age-reproductive class	8, 28	2.756	0.02
Year	8, 28	1.048	0.43

Table 2.3 Number and length of beluga whales captured from the eastern Beaufort Sea according to sex and reproductive class. Groupings based on similarity of resource selection functions (see Methods).

	Number of whales (length range in cm)		
Cluster Groups	**Males**	**Females with calves**	**Females without calves**
1 (open water)	5 (353-404)	3 (338-363)	2 (302-373)
2 (closed ice)	5 (402-457)	0	0
3 (ice edge)	7 (379-427)	2 (340-374)	0

Table 2.4 Beluga whales clustered into three groups based on habitat selection by sex and length ($F_{8,32} = 7.6$, $P < 0.0001$)

	Habitat Categories (b_j values)				**Distance (Km)**	
Cluster Groups	Off shore open water	Mixed ice	Closed ice	Shallow open water	Mainland	Islands
Group 1 (open water)	0.55 (0.04)	0.12 (0.04)	0.04 (0.02)	0.28 (0.03)	264 (10)	824 (23)
Group 2 (closed ice)	0.1 (0.04)	0.34 (0.07)	0.53 (0.06)	0.04 (0.01)	402 (16)	306 (36)
Group 3 (ice edge)	0.35 (0.04)	0.21 (0.03)	0.34 (0.04)	0.1 (0.03)	255 (10)	308 (23)
Random control (distance analysis)					424 (7)	752 (16)

Note: Values are means with SE in parentheses. Distance to the mainland and island coastlines differed among the three groups and a control group (random points) (mainland: $F_{3,2176} = 86.6$, $P < 0.001$; island: $F_{3,2176} = 114.7$, $P < 0.0001$)

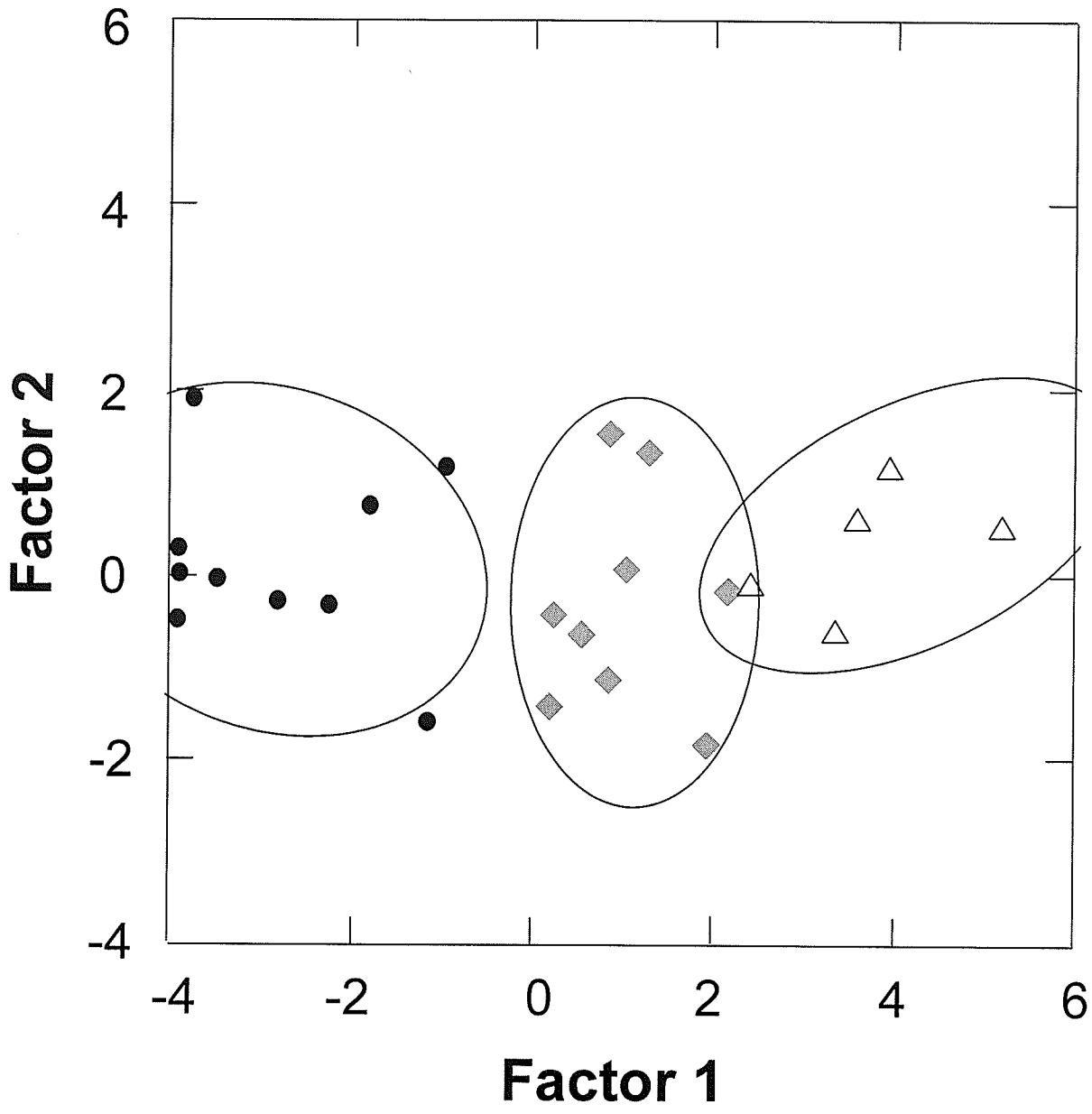

Figure 2.3 Results of discriminant function analysis of beluga selection indices for four habitat categories demonstrate groupings derived by the cluster analysis. First group selected open water (closed circle symbol); second group selected closed ice (triangle symbol); and third group selected ice edge habitat (diamond symbol).

(three with calves) and five males in the smaller length range (353 to 404 cm; Table 2.3). The second cluster selected the closed ice habitat, followed by the mixed ice habitat ($\bar{x}\ b_j = 0.53; 0.34$). This group was made up of five males; three were the largest of all the tagged whales. The final cluster selected the offshore open water ($\bar{x}\ b_j = 0.35$) and closed ice habitats ($\bar{x}\ b_j = 0.34$) more than expected (Table 2.4). Examination of movement patterns illustrated that belugas followed the ice edge. This third group was composed of two females with calves and seven males that ranged in length from 379 to 427 cm (Table 2.3).

2.3.3 Distance analysis

Analyses of variance tests for the distance to the mainland and island coastlines revealed the three cluster groups and the control group (random point locations were significantly different (Table 2.4). Post hoc tests revealed whales that selected heavy ice concentration were the farthest from the mainland and closest to the islands, and their distances were not different from distances to the mainland coastline generated from random points. Whales that selected open water and ice edge habitats were closer to the mainland coast and were not significantly different from one another (Table 2.4). Whales farthest from the islands were those that selected open water were, and revealed that distances were not different from those generated from random points (Table 2.4). Whales that selected ice edge habitat and those that selected heavy ice concentration had similar distances to the island coastlines.

2.4 Discussion

Habitat selection by beluga whales during the open-water season was affected by age and reproductive status, not individually but in concert. Sexual segregation refers to different habitat use by each sex (Conradt 1998); however, here three groups emerged that were not defined by sex alone. Sexual segregation into three groups occurs in other odontocetes such as northern bottlenose whales (*Hyperoodon ampullatus*) (Gowans et al. 2001). In some cases sexual segregation results from complex social structures; for example, in sperm whales (*Physeter macrocephalus*), females, calves and immature whales form long-term, multi-year associations (Christal and Whitehead 2001). In contrast, adult male sperm whales are often solitary and form temporary loose aggregations (Whitehead and Weilgart 2000). Intra-species segregation can be a consequence of different requirements within the population that vary over space and time (Stevick et al. 2002). Here, resource selection functions quantified habitat use and subsequent analysis revealed segregation, but why belugas segregate and what specific habitat attributes were selected is unclear. Two common explanations for sexual segregation are the predation risk hypothesis and forage selection hypothesis, which are presented here to assist in the interpretation of the observed Beaufort beluga whale segregation.

2.4.1 Sexual segregation hypotheses

The predation risk hypothesis postulates that predator avoidance behaviour by reproductive females will result in the geographic segregation of sexes by habitat use (Main et al. 1996). Predators of Beaufort beluga whales include humans, polar bears,

and killer whales (*Orcinus orca*). There is also the potential for infanticide (calf killing) by adult beluga males, which occurs in some marine mammals such as bottlenose dolphins (*Tursiops sp.*) (Patternson et al. 1998) and polar bears (Derocher and Wiig 1999). The basis of the predation risk hypothesis is habitat selection to avoid mortality by predation, but in a more general framework the hypothesis could be expanded to include abiotic causes of mortality, such as drowning caused by ice entrapment or increased risk to mortal injury from sea ice.

The forage selection hypothesis relates to size dimorphism and maintaining a larger body mass by foraging more or on different-quality food resulting in differential habitat use (Clutton-Brock et al. 1982, Conradt 1998). This hypothesis in its simplest form addresses energy requirements related to size, but could be broadened to include the unique energy demands of pregnant or lactating females (Loudon 1985). Next, we discuss our findings of beluga segregation into three groups those in open water near mainland, those in ice-covered areas in and near the Arctic Archipelago, and those selecting ice edge habitat in the context of these two general hypotheses.

2.4.2 Habitat selection: Open water near mainland

The selection of open-water habitat near the mainland shore by females with calves and small males, lends support to the predation risk hypothesis. Females invest two to three years of traveling with their offspring (Caron and Smith 1990) likely avoiding habitats associated with predation and mortality risk. Use of open-water regions would reduce the risk of polar bear predation and avoid the risk of ice entrapment. Shallow open water could provide refuge from killer whale predation

because the large size of these predators would inhibit their mobility in shallow waters. Physiological constraints of calves such as lung capacity or dive depth limitations may also restrain movement into regions of extensive sea ice cover. The smaller males in this group are likely immature whales that may also be avoiding mortality risks associated with sea ice, or aggression by large males (Scott et al. 2005). On the other hand, they may be part of a social unit with the females and calves, not having made a transition to adult male behaviour and associated habitat use. Segregation of females with calves along and immature whales from adult males is common in marine mammals, such as sperm whales (Christal and Whitehead 2001). If this group represents a social unit, perhaps the immature males are participating in the protection of offspring, which is thought to be an important factor in the formation of whale social groups (Whitehead 2003).

2.4.3 Habitat selection: Ice-covered areas in and near the Canadian Arctic Archipelago

This group consisted of large males, including the three largest, that selected closed ice and mixed ice habitat, closest to island coastlines. Spatial and temporal segregation of older adult males from females and immature whales occurs in belugas (Smith et al. 1994) and other odontocetes, such as bottlenose whales (Gowans et al. 2001). It is unknown whether large beluga males travel together as a unit or remain solitary during the summer, similar to adult male sperm whales (Whitehead and Weilgart 2000). Habitats selected by large male belugas have both predation and ice entrapment risks, thus segregation is not explained by mortality or predation avoidance.

In the summer, closed or mixed ice often overlies deeper areas of the Beaufort Sea and the Arctic Archipelago. Since these males were the closest to the island coastlines of the Arctic Archipelago, likely either ice cover or deep water was being selected. If habitats are selected for feeding, then belugas could be feeding on arctic cod (*Boreogadus saida*) under the ice (Gradinger and Bluhm 2004), or they may be feeding on the rich benthic food available from the deep-water environment. Therefore use of these high-risk habitats likely offers energy benefits to support the greater growth and biomass necessary for the development of dimorphism, a scenario that supports the forage selection hypothesis. Adult males from the Chukchi Sea beluga whale population also enter heavy sea ice over deep sea regions, for reasons that are unclear (Suydam et al. 2001). If the sea ice provides an important feeding resource, then the predicted loss of ice cover with climate change may affect the energy requirements of large adult males.

2.4.4 Habitat selection: Ice edge

The third beluga group selected the ice edge regions (offshore open water, closed ice cover). This group was close to both mainland and island coastlines, suggesting habitat use was not related to coastlines. Ice edge areas are highly productive zones providing seasonal food for marine birds and mammals (Moore et al. 2000, Harwood and Smith 2002). Therefore, this habitat is likely selected for feeding, supporting the forage selection hypothesis of segregation. The seven males in this group were generally smaller than those selecting ice cover, and larger than immature males selecting open-water habitats. Perhaps these males have passed an age or

maturity threshold that enables them to leave their maternal groups and protective habitats to explore more productive regions. They may represent an intermediate stage such as subadult males that segregate from their maternal groups, and remain separate from large adult males, due to social structure or physiological demands. Formation of subadult male social groups occurs in northern bottlenose whales and some bottlenose dolphins (Smolker et al. 1992, Gowans et al. 2001), and in sperm whales subadult males form loose associations or remain solitary (Christal et al. 1998).

Two females with calves were also present in this group. This does not necessarily suggest that subadult males and females form semi-permanent social groups. Selection of ice edge habitats by these females is likely risky relative to selection of open-water habitats; however, their calves were not newborns, so susceptibility to predators may be reduced. These females may represent a segment of the population with older calves that are ready to begin interacting with males again and are prepared to mate during winter. Without tracking data of the calves, it is not known if they still accompanied their mothers once leaving the estuary of capture. Also, we cannot assess whether these females consistently used ice edges from year to year regardless of reproductive stage.

2.5 Conclusions

The two segregation hypotheses evaluated are not mutually exclusive (Bleich et al. 1997), and our data were inadequate to conclusively test between the two. Our results illuminate the factors responsible for beluga segregation based on habitat selection. Beluga habitat segregation according to age and reproductive class demonstrates the different resource needs of individuals and the various habitats that

can provide these resources. Our results provide the first quantitative report of habitat segregation that may represent beluga social structure. Previous observations of beluga sexual segregation in northern Canada (Smith et al. 1994) and Greenland (Heide-Jorgensen and Lockyer 2001) did not quantify the segregation relative to habitat selection. The lack of beluga segregation and social structure studies is largely due to the difficultly in observing Arctic marine mammals. In addition, little is known about beluga life stages, body growth dynamics, and maternal development, resulting in the difficulty in identifying age structure and reproductive status. Our study demonstrates that protecting beluga whale habitat requires understanding the needs of various age, sex and reproductive groups and the different habitats they select.

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3.0 Linking Mercury Exposure to Habitat Use and Feeding Behaviour in Beaufort Sea Beluga Whales

Abstract

Mercury (Hg) levels in the Beaufort Sea beluga population have been increasing since the 1990's. Ultimately, it is the Hg content of prey that determines beluga Hg levels. However, the Beaufort Sea beluga diet is not understood, and little is known about the food web Hg sources in the summer habitat. During the summer they segregate into social groups based on habitat use leading to the hypothesis that they may feed in different food webs explaining Hg dietary sources. Methyl mercury (MeHg) and total mercury (THg) levels were measured in the estuarine-shelf, Amundsen Gulf and epibenthic food webs in the western Canadian Arctic collected during the Canadian Arctic Shelf Exchange Study (CASES) to assess their dietary Hg contribution. To our knowledge, this study is the first study to report MeHg levels in estuarine fish and epibenthic invertebrates from the Arctic Ocean. Although the Mackenzie River is a large source of Hg, the estuarine-shelf prey items had the lowest MeHg levels, ranging from 0.1 to 0.27 ug/g dry weight (dw) in arctic cisco (*Coregonus autumnalis)* and saffron cod (*Eleginus gracilis*) respectively. Highest MeHg levels occurred in fourhorn sculpin (*Myoxocephalus quadricornis*) (0.5 ug/g dw) from the epibenthic food web. Beluga hypothesized to feed in the epibenthic and Amundsen Gulf food webs had the highest Hg levels matching with high Hg levels in associated food webs, and estuarine-shelf belugas had the lowest Hg levels (2.6 ug/g dw), corresponding with the low food web Hg levels, supporting the variation in dietary Hg uptake. The trophic level transfer of Hg was similar among the food webs, highlighting the importance of Hg sources at the bottom of the food web as well as food web length.

We propose that future biomagnification studies incorporate predator behaviour with food web structure in the evaluation of dietary Hg sources.

3.1 Introduction

Mercury (Hg) in the form of methyl mercury (MeHg) bioaccumulates in organisms over time, and biomagnifies at each trophic level (Morel et al. 1998). As a result, Hg concentrations at trace levels in water can reach toxic levels in top predators. For example, high Hg levels have been found in the Beaufort Sea beluga whale *(Delphinapterus leucas)* population that spends summers in the eastern Beaufort Sea and Mackenzie Delta region of the Canadian western Arctic. Elevated Hg levels represent a risk to beluga and predators of beluga, such as polar bears and Inuvialuit subsistence hunters. In the 1990's, liver Hg levels in this beluga population tripled in comparison to 1980 levels (Lockhart et al. 2005b), and were the highest relative to other Canadian Arctic beluga populations. Although, still higher than the 1980 levels, Hg concentrations have dropped and are now comparable to other Arctic populations (Lockhart et al. 2005b).

Due to the biomagnifying properties of MeHg, Hg concentrations in predators such as beluga largely reflect the Hg levels in their diet (Mathers and Johansen 1985). Little is known about the Beaufort Sea beluga diet. Thus, dietary Hg sources cannot be accounted for. Typically in animal diet studies, stomach contents and feces are used to identify diet items. This is not feasible with the Beaufort Sea beluga because harvested whales often have empty stomachs, and feces cannot be found, yet local hunters have observed beluga feeding during the summer (Harwood and Smith 2002).

Stable isotopes are useful diet biomarkers that provide information about animal feeding preferences and can overcome problems associated with conventional diet determination such as over-or under-representation of prey that were recently eaten or quickly digested (Tollit et al. 1997). Stable isotopes have been used in previous studies to examine diet and trophic-level transfer of Hg (Atwell et al. 1998, Dehn et al. 2006b). At higher trophic levels, variability in top predator Hg was observed and attributed to diversity in foraging behaviour resulting from intra-species differences in sex or size, in addition to variation in seasonal prey abundance (Atwell et al. 1998). Atwell et al., (1998) found Hg levels in top predator muscle tissue largely reflected diet and to a lesser extent Hg bioaccumulation with age, supporting the need to understand dietary sources of Hg and biomagnification.

The Canadian Arctic Shelf Exchange Study (CASES) provided an opportunity to sample regions where the Beaufort Sea belugas spend summer, such as the eastern Beaufort Sea, Amundsen Gulf, Franklin Bay and the Mackenzie Delta. Habitat use of Beaufort Sea beluga differs with length, sex and reproductive status likely reflecting social structure (Chapter 2). Variation in beluga habitat selection suggests diet may differ, which can result in differing dietary Hg uptake. Due to the diversity of possible Beaufort Sea beluga prey, a variety of food items, as well as lower trophic level organisms, were collected during the CASES expedition.

Here, we propose an approach to determine beluga prey and resulting Hg levels that incorporates beluga behaviour and the complexity of the eastern Beaufort Sea ecosystem. First, we examine Hg levels in organisms in three food webs in the beluga summering range to establish if dietary sources of Hg differ. Second, we determine if

Hg levels differ among beluga feeding groups defined a priori by their length, sex and reproductive status. Finally, beluga feeding groups were paired with food webs that best fit their habitat use, and using Hg and stable isotopes, beluga Hg biomagnification factors (BMFs) were calculated and used as a tool to increase our understanding of beluga feeding.

3.2 Material and Methods

3.2.1 Sample Collection

Food web samples were collected during 2002, 2003, 2004 and 2006 from the CCGS *Amundsen*, *Nahidik* and *Pierre Radisson* in the Western Arctic region, encompassing the Mackenzie Delta, Amundsen Gulf, Franklin Bay, and the eastern Beaufort Sea (Figure 3.1). All biota samples were frozen at -20°C after collection and shipped to Fisheries & Oceans Canada in Winnipeg where they were stored at -20°C until analysis.

3.2.1.1 Zooplankton

Calanus spp. and *Themisto libellula* were collected from the CCGS *Amunsden* during CASES from September 2003 to September 2004. The one-year sampling expedition included a fall survey covering the entire study area, an over-wintering of the ship in Franklin Bay, and a summer/autumn survey of the Amundsen Gulf and Mackenzie Shelf (Figure 3.1). During the open-water season, integrated vertical tows were taken with a double tucker trawl (200 μm mesh and 500 μm mesh) and depth-stratified samples were taken with a Hydrobios multi-net (200 μm mesh). During the

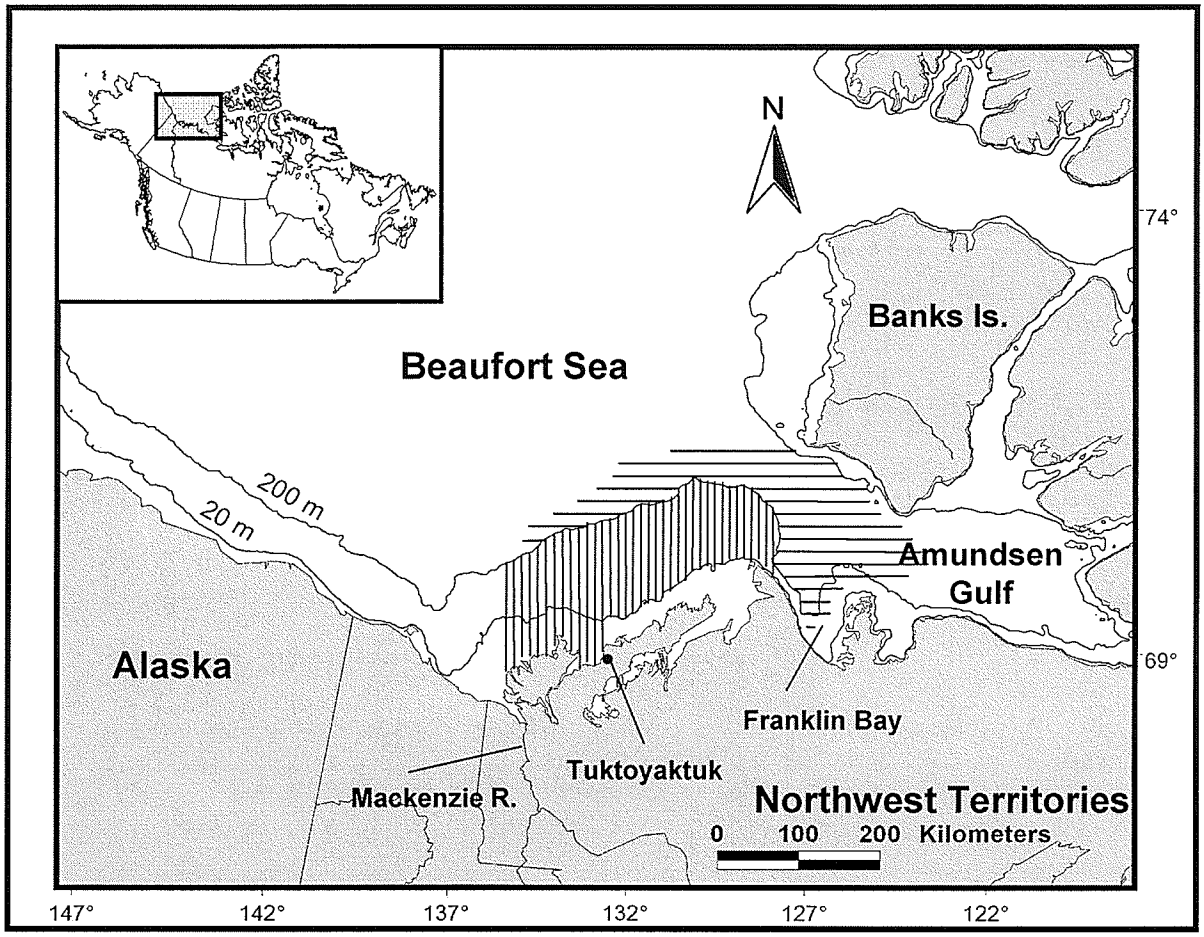

Figure 3.1. Study Area: Amundsen Gulf, Franklin Bay, Mackenzie Delta, eastern Beaufort Sea. The 20m isobath north of the Mackenzie outflow is where estuarine fish were collected, whereas the 200m isobath separates the Mackenzie shelf from the eastern Beaufort Sea. The short vertical lines represent the estuarine-shelf food web collection region, and the horizontal lines represents the Amundsen Gulf food web region. Epibenthic food web organisms were collected from the epibenthos of both regions. Tuktoyaktuk, Northwest Territories, is the location of the beluga harvest and tissue sample collection.

over-wintering period we sampled under the sea ice using a horizontal double tucker trawl (200 μm mesh and 500 μm mesh).

Calanus spp. were picked from the samples and placed into plastic (Whirlpak®) bags and frozen at -20°C. Additional *T. libellula* samples were collected during the 2002 fall cruise of the CCGS *Pierre Radisson*. *T. libellula* were identified, sexed, measured, staged and dried at 60°C. Mercury analysis was completed for *T. libellula* collected in 2002 and 2003/2004, and stable isotope analyses were completed only for 2002 samples. Zooplankton samples from the shallow Mackenzie Delta region (< 20 m depth) were collected from the CCGS *Nahidik* in the late summer of 2003 and 2004. Zooplankton were not sorted prior to being placed into plastic (Whirlpak®) bags and frozen at -20°C. Analysis of relative species abundance revealed that the two dominant zooplankton species were omnivorous *Pseudocalanus spp.* and *Calanus glacialis* (65% and 26% respectively).

3.2.1.2 Epibenthic Invertebrates

Epibenthic animals were collected in the fall of 2003 and spring/summer of 2004 with a modified MACER-GIROQ sled that collected organisms living directly on and within 60 cm of the seafloor (Choe and Deibel 2000). The sled was equipped with a 500-μm net, a partially closed cod end, and a door that opened when the sled was on the sea floor and closed while in the water column. As soon as the sled came on board, the contents of the cod end were gently rinsed into coolers. Cooler contents were then rinsed with surface water to remove mud from the samples. The decapods *Eualus* spp. and *Bythocaris* spp. (which will be referred to as 'shrimp'), the amphipods *Anonyx* spp.

and *Acanthostepheia malmgreni,* as well as four pooled mysid genera (*Psuedamma* spp., *Erythrops* spp., *Mysis* spp., *Michthyops* spp.) were picked from the samples and identified onboard the ship.

3.2.1.3 Fish

Arctic cod (*Boreogadus saida*) were collected from March 15 to May 27 2004. Most of the samples were collected in Franklin Bay using a 90-m long gill net set vertically from the bottom (i.e., 230 m), where most fish captured were in the lower 100m of the net. In addition, arctic cod were collected in September 2006 from the CCGS *Nahidik* between 10 and 100 metre depths north of the Mackenzie River outflow. Only adult arctic cod were selected for analysis (length > 110 mm).

Fish in the brackish water of the Mackenzie Delta were collected from the shoreline out to the 20m isobath via community-based sampling programs and on board the CCGS *Nahidik* using gill nets. Species collected near the shoreline from community based sampling included rainbow smelt (*Osmerus mordax)*, arctic cisco (*Coregonus autumnalis),* least cisco (*Coregonus sardinella)*, and species collected from the CCGS *Nahidik* included pacific herring *(Clupea palasii)*, and saffron cod (*Eleginus gracilis*). Fish characteristic of the epibenthic habitat, such as the starry flounder *(Platichthys stellatus),* arctic flounder (*Pleuronectes glacialis)* and fourhorn sculpin (*Myoxocephalus quadricornis*) were also collected in this region using gill nets from shore and the CCGS *Nahidik.* For our study, all fish species will be referred to by their common names. Only adult fish were used (> 200 mm).

3.2.1.5 Beluga

Beluga tissue samples were collected from Tuktoyaktuk harvests in 2004 (n = 26). Twenty-one belugas were male, and five were female, of which two were in their first trimester of pregnancy and three were lactating, suggesting they had calves in attendance. Muscle tissue was selected for Hg analysis because unlike liver tissue which contains largely elemental Hg, 97 - 100 % of the THg in muscle is MeHg (Wagemann et al. 1998). More importantly, muscle may better reflect dietary sources of Hg, whereas liver is a site of MeHg demethylation (Wagemann et al. 1998). Ages were determined from a thin section of a tooth by counting growth layer groups in the dentine (Stewart et al. 2006).

3.2.2 Food Webs: Estuarine-Shelf, Amundsen Gulf, Epibenthic

Organisms were placed into an estuarine-shelf, Amundsen Gulf or epibenthic food web group based on collection regions and their feeding ecology. The estuarine-shelf food web combined samples that represented an estuarine environment influenced by the brackish water of the Mackenzie River (samples collected in the delta < 20m depth) and samples from deeper regions of the delta, reaching the shelf break (20 to 200m depth). Although processes occurring within the estuary may be different than in the deeper shelf waters, these areas were combined due to the important influence of the Mackenzie River on energy and Hg sources to the local food web (Leitch et al. 2007). Samples collected in the shallow estuary region included rainbow smelt, saffron cod, pacific herring, least cisco, arctic cisco and zooplankton (65% *Pseudocalanus spp.*, and 26% *Calanus glacialis*). The estuarine habitat is diverse, offering a large gradient of salinity, temperature and depth. This food web includes first level consumers and planktivorours fish followed by beluga. Samples collected in deeper shelf waters

included the first level consumer *Calanus* spp., the predator *T. libellula* and arctic cod (Figure 3.1).

The Amundsen Gulf food web represents an off-shelf, pelagic ecosystem. This food web is characterized by *Calanus* spp., *T. libellula* and arctic cod collected from Franklin Bay, Amundsen Gulf and Eastern Beaufort Sea (Figure 3.1). Thus, this food web is similar in structure to the shelf, beginning with copepods, and then carnivorous macrozooplankton *T.libellula*, followed by planktivours fish that may feed on both zooplankton or just one, followed by beluga the top predator.

Lastly, the epibenthic food web included invertebrates and fish from the near-bottom environment throughout the CASES study region. Sculpin, starry and arctic flounder were collected in the estuary, but were placed within the epibenthic food web because their food Hg sources are specific to the near-bottom environment and differ from the estuarine group. The four epibenthic invertebrates included shrimp, two amphipod species and mysids (see above). We were unable to clearly identify a first consumer level (i.e. herbivores) in this food web, and selected the mysids because many species are known to feed on fresh phytodetritus shortly after it sinks to the bottom (Richoux et al. 2004).

3.2.3 Beluga Feeding Groups and Food Web Pairing

Animal length, age, sex and body condition should be considered when examining predator Hg levels because they influence feeding behaviour (Atwell et al. 1998, Dehn et al. 2006b). Based on sex, length, reproductive status, satellite tagged (1993-1997), Beaufort Sea beluga segregated during summer into three separate

habitats that differed in sea ice concentration, bathymetry and distance from the coast (Chapter 2). Three beluga habitat groups were defined: 1) shallow open-water near the mainland was selected by females with and without calves and by small males (< 4 m); 2) the sea ice edge was selected by medium length males (3.8 - 4.3 m) and a few females (>3.4 m) without neonates; and 3) heavy sea ice concentrations in deep, offshore waters were selected by the largest males (4 - 4.6 m). This type of habitat segregation suggests a complex beluga social structure and indicates where the Beaufort Sea belugas are feeding during the summer season in the western Arctic.

Length of beluga among the three habitat groups overlapped. Thus, Beaufort Sea beluga growth curves (Luque and Ferguson 2006) were used to define differences (Table 3.1). For females, the adult asymptotic length of 3.7 m was used to demarcate smaller females (with and without calves) using shallow open-water and larger animals selecting the sea ice edge (Table 3.1). The maximum length of the smallest males selecting the shallow open water habitat was based on the mid-point on the growth curve between juveniles and adults (3.8 m). Lastly, the adult asymptotic length of 4.2 m was used to separate large whales selecting heavy sea ice concentrations from smaller males selecting the sea ice edge (Table 3.1).

We paired the beluga feeding groups with hypothetical, habitat-specific food webs according to their spatial segregation, i.e.: 1) beluga using shallow, open-water habitats likely use the Mackenzie Delta extensively and were therefore paired with the estuarine-shelf food web; 2) beluga selecting the ice edge are likely feeding on the sea ice associated arctic cod (Gradinger and Bluhm 2004) and/or in pelagic food webs, thus we paired them with the Amundsen Gulf food web that included organisms collected

Table 3.1. Lengths of female and male belugas in the three habitat use groups (Chapter 2). Number of whales from 2004 and lengths used to create feeding groups based on growth length curves (Luque and Ferguson, 2006). Beluga feeding groups were matched with food webs

	Length Boundaries		Number of whales (length range in m)		
Habitat Use Groups	**Males**	**Females**	**Males**	**Females**	**Feeding Groups**
Shallow open water	< 3.8 m	<3.7	3 (3.51 - 3.56)	4 (3.4 - 3.76)	Estuarine-Shelf
Sea ice edge	3.8 - 4.2	>3.7	8 (3.89 - 4.16)	1 (3.81)	Amundsen Gulf
Closed sea ice	> 4.2	n/a	10 (4.22 - 4.55)	0	Epibenthic

offshore in the pelagic environment; and 3) beluga selecting high levels of sea ice concentration do so in deep waters where they dive up to 800 m (Richard et al. 1997), likely feeding benthically, and therefore were paired with the epibenthic food web. The assigned feeding groups are an oversimplification of the temporal and spatial complexities involved in beluga movement and seasonal feeding preferences. However, we consider this approach of pairing whale groups with habitat-specific food webs an important development in evaluating Hg dietary sources.

3.2.4 Total and Methyl Mercury Extraction and Analysis

Beluga muscle and fish muscle tissue were subsampled for MeHg and THg analysis. Whole individual *T. libellula* were dried at 60°C and those weighing < 0.01g were pooled by station and age class before analysis. Freeze-dried shrimp and amphipods were analyzed individually when enough biomass was available, otherwise similar small mysids were pooled by station before analysis. Generally, ten whole dried *Calanus* spp. were pooled by sample station for analysis. Due to low biomass for *Calanus* spp. and *T.libellula* collected from the shelf region, they were not analyzed for MeHg, and values presented in figures are back calculated based on the percent MeHg found in Amundsen Gulf *Calanus* spp. and *T. libelulla*. All samples were weighed to approx 0.15g for THg analysis. Samples were digested with a hydrochloric/nitric acid mixture (Aqua Regia) heated to 90°C. The digested samples were analyzed for THg by Cold Vapour Atomic Absorption spectroscopy (CVAAS) (Armstrong and Uthe 1971). The detection limit was 0.005 ug/g.

For MeHg analysis, beluga and fish muscle tissue samples were extracted according to the procedure of Uthe et al. (1972). Wet tissue samples were homogenized

in an acidic bromide and copper sulphate solution. This was extracted with toluene and partitioned into a thoisulfate solution where the addition of potassium iodide allowed back-extraction into toluene. The extract was analyzed on a gas chromatograph with an electron capture detector. Due to the low biomass and unknown range of MeHg levels in invertebrate organisms a modified method was used that required less mass. The extraction was completed using a dichloromethane phase rather than toluene (Cai 2000). Samples were analyzed by capillary gas chromotography - atomic fluorescence spectrometry at the University of Ottawa (D. Lean).

Certified standard reference materials (CRM 2976, TORT-2, DOLT-2) were analyzed in duplicate in every run. Recovery within ten percent of the certified values was used as a batch validation for samples. Average differences in duplicates for beluga, fish muscle tissue and *Calanus* spp. was five, five and ten percent respectively.

3.2.5 Stable Isotope Analysis and Food Web Calculations

Based on relative isotopic fractionation processes, $\delta^{15}N$ can be used to describe trophic levels (Hobson and Welch 1992) and $\delta^{13}C$ can be used to evaluate food web carbon sources (France 1995). To prepare for stable isotope analysis, beluga muscle tissue and whole *Calanus* spp. and epibenthic invertebrates were freeze dried, while fish muscle tissue samples and individual whole *T. libellula* were oven dried at 60°C. Carbon and nitrogen isotopic analyses on the muscle (protein) were accomplished by continuous flow, isotopic ratio mass spectrometry (CF-IRMS) using a GV-Instruments® IsoPrime attached to a peripheral, temperature-controlled, EuroVector® elemental analyzer (EA) (University of Winnipeg Isotope Laboratory, *UWIL*). One-mg

samples were loaded into tin capsules and placed in the EA auto-sampler along with internally calibrated carbon/nitrogen standards. Carbon and nitrogen isotope results are expressed using standard delta (δ) notation in units of *per mil* (‰). The delta values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) represent deviations from a standard such as

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

where R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio in the sample and the standard. The standards used for carbon and nitrogen isotopic analyses are Vienna PeeDee Belemnite (VPDB) and IAEA-N-1 (IAEA, Vienna), respectively. Analytical precision, determined from the analysis of duplicate samples of every fifth sample, was ± 0.15 ‰ for $\delta^{13}C$ and ± 0.175 ‰ for $\delta^{15}N$. Accuracy was obtained through the analysis of laboratory standards used for calibration of results.

To estimate Hg biomagnification factors in a food web, the slope determined from logarithmically transformed THg concentrations against $\delta^{15}N$ or trophic level is used (e.g. Campbell et al., 2005). Given that Hg can biomagnify differently at each trophic level, biomagnification factors (BMFs) are often used to describe the transfer of Hg between prey and predator using the formula below:

$$BMF = (Hg_{predator}/Hg_{prey})/(TL_{predator}/TL_{prey}) \quad (2)$$

This formula requires an estimate of the trophic level (TL) of both predator and prey. This is typically done by normalizing $\delta^{15}N$ to *Calanus* spp. $\delta^{15}N$ values, assuming that *Calanus* represents a consumer that feeds only on phytoplankton (Fisk et al. 2001), with the following equation:

$$TL_{consumer} = 2.0 + (\delta^{15}N_{consumer} - \delta^{15}N_{Calanus})/3.8 \quad (3)$$

The denominator of 3.8 represents the nitrogen isotopic fractionation from one trophic level to the next based on the whole food web (Hobson and Welch 1992). We argue that using 3.8 or any other number for the nitrogen fractionation value may bias determination of the true trophic levels because we do not fully understand the nitrogen fractionation process in the food webs investigated here. Therefore we calculated the biomagnification factor by replacing the trophic levels in equation (2) with the raw $\delta^{15}N$ values as follows:

$$BMF = (Hg_{predator}/Hg_{prey})/(\delta^{15}N_{predator}/\delta^{15}N_{prey}). \quad (4)$$

Using $\delta^{15}N$ instead of trophic levels reduces the need to know the enrichment factor of nitrogen or to assume a TL of 2.0 for pooled *Calanus* spp.

3.2.6 Statistical Analysis

Differences in mean values of MeHg, THg and stable isotopes among fish species and invertebrates were determined with an analysis of variance followed by pair-wise *a posteriori* Tukey tests. Comparisons between shelf and Amundsen Gulf *Calanus* spp., *T. libellula* and arctic cod THg, and stable isotopes were analyzed individually with paired t-tests. To test if collection site affected arctic cod Hg levels, an analysis of covariance was used to control for age. A general linear model was used to assess the effects of length, age and sex on Hg levels in beluga muscle. Differences among beluga feeding groups in length, age, THg, MeHg concentrations and stable isotopes was tested with an analysis of variance, followed by pair-wise Tukey tests. Analysis of covariance was employed to evaluate differences in slopes among the three food webs and between MeHg and THg. All statistical tests were carried out using

Systat 11® (Systat Software Inc., 2004, San Jose, CA). All data is presented as mean +/- standard error and all significant statistical tests are $P < 0.05$.

3.3. Results

3.3.1 Methyl and Total Mercury in Three Food webs

In fish muscle, MeHg averaged 88% of the THg, ranging from 78% in arctic cisco to 94% in least cisco (Table 3.2). In shrimp, MeHg averaged 72% of THg and ranged from 65 to 80%, whereas *Anonyx* spp. ranged considerably from 12 to 90% MeHg. MeHg in *T. libellula* represented 71 to 100% of THg, and *Calanus* spp. had the lowest percent of THg present as MeHg (34 ± 5%). The proportion of MeHg in *Calanus* spp. did not differ significantly between the shelf and Amundsen Gulf food webs.

Estuarine fish had mean MeHg levels ranging from 0.1 ug/g dw in pacific herring to 0.27 ug/g dw in saffron cod. Pacific herring, arctic cod, arctic cisco, least cisco and rainbow smelt had similar MeHg, THg and $\delta^{15}N$ values, that were lower than concentrations in saffron cod ($P < 0.01$) (Table 3.2; Figure 3.2). Pacific herring $\delta^{13}C$ values (-23.7 ± 0.2 ‰) were lower than those for arctic cod and least cisco (Figure 3.2). Mean MeHg and THg concentrations in beluga prey items, normalized by $\delta^{15}N$ values, reveal that the estuarine-shelf beluga prey had half the Hg of the Amundsen Gulf and epibenthic beluga prey ($\delta^{15}N$ normalized values not shown). Zooplankton from the near shore estuary had higher, but not significantly different THg levels than did *Calanus* spp. collected in the deeper shelf water.

Table 3.2 Summary of mean (± standard error) MeHg, THg, stable isotopes and morphometrics of organisms collected from three food webs in the eastern Beaufort Sea. "N" is the number of samples taken for THg/stable isotopes/MeHg. Fish age data was obtained from otoliths (see Methods). Age data was not available for sculpin.

Organisms (code)	MeHg (ug/g dw)	THg (ug/g dw)	Del ^{15}N (‰)	Del ^{13}C (‰)	N	% MeHg	Length (mm)	Age
Estuarine-Shelf								
Zooplankton (EZP)	0.010 ± 0.001	0.035 ± 0.005	9.1 ± 0.2	-25.6 ± 0.3	8/2/0	28*		
Calanus spp. (CAL)	0.007 ± 0.001	0.025 ± 0.003	9.6 ± 0.6	-25.6 ± 0.2	8/5/3	28.0		
T. libellula (TLB)	0.066 ± 0.002	0.087 ± 0.007	9.4 ± 0.2	-26.0 ± 0.2	40/28/0	75*		
pacific herring (PHR)	0.111 ± 0.003	0.123 ± 0.005	12.4 ± 0.5	-23.7 ± 0.2	29/28/5	90.2	207.1 ± 2.0	5.1 ± 0.6
arctic cisco (ACS)	0.104 ± 0.003	0.133 ± 0.009	12.3 ± 0.1	-22.9 ± 0.1	30/15/5	78.0	311.5 ± 6.0	5.6 ± 0.5
least cisco (LCS)	0.125 ± 0.009	0.133 ± 0.009	12.3 ± 0.4	-22.2 ± 0.4	20/10/5	94.1	285.4 ± 8.3	7.6 ± 0.8
raimbow smelt (RST)	0.163 ± 0.009	0.181 ± 0.028	13.1 ± 0.3	-22.9 ± 0.1	10/10/5	89.9	203.6 ± 15.9	6.1 ± 0.9
saffron cod (SCD)	0.273 ± 0.008	0.308 ± 0.029	13.7 ± 0.2	-22.8 ± 0.3	20/20/5	88.8	280.1 ± 10.7	5.8 ± 0.5
arctic cod (ACD)	0.158 ± 0.008	0.163 ± 0.016	13.1 ± 0.2	-22.3 ± 0.3	20/20/5	97.2	143.3 ± 3.1	2.6 ± 0.2
Amundsen Gulf								
Calanus spp. (CAL)	0.011 ± 0.001	0.032 ± 0.002	9.4 ± 0.2	-25.1 ± 0.2	33/32/5	34.3		
T. libellula (TLB)	0.095 ± 0.019	0.127 ± 0.019	10.3 ± 0.2	-26.1 ± 0.2	57/28/4	75.0		
arctic cod (ACD)	0.301 ± 0.023	0.377 ± 0.029	14.7 ± 0.2	-22.0 ± 0.2	60/39/20	79.9	158.3 ± 3.4	3.4 ± 0.13
Epibenthic								
mysids (MYS)	0.032 ± 0.013	0.081 ± 0.009	10.9 ± 0.4	-23.0 ± 0.4	15/15/4	39.5		
shrimp (SHP)	0.228 ± 0.026	0.316 ± 0.061	13.5 ± 0.2	-21.5 ± 0.7	14/3/7	72.3		
Anonyx spp. (ANX)	0.160 ± 0.083	0.291 ± 0.050	11.9 ± 0.3	-21.4 ± 0.0	6/6/6	55.0		
A. malmgremi (AMI)	0.052 ± 0.028	0.116 ± 0.011	12.7 ± 0.1	-21.2 ± 0.4	3/3/2	45.0		
arctic flounder (AFR)	0.234 ± 0.055	0.255 ± 0.044	11.6 ± 0.2	-23.6 ± 0.2	9/9/5	91.9	226.4 ± 10.6	9.1 ± 0.6
starry flounder (SFR)	0.226 ± 0.090	0.277 ± 0.073	11.5 ± 0.2	-24.4 ± 0.5	11/11/5	81.5	246.8 ± 13.5	9.0 ± 1.2
sculpin (SCP)	0.474 ± 0.046	0.587 ± 0.076	16.1 ± 0.9	-23.0 ± 0.7	5/2/5	80.7	219.8 ± 32.3	

*MeHg concentrations back calculated based on levels in the same species in the Amundsen Gulf food web

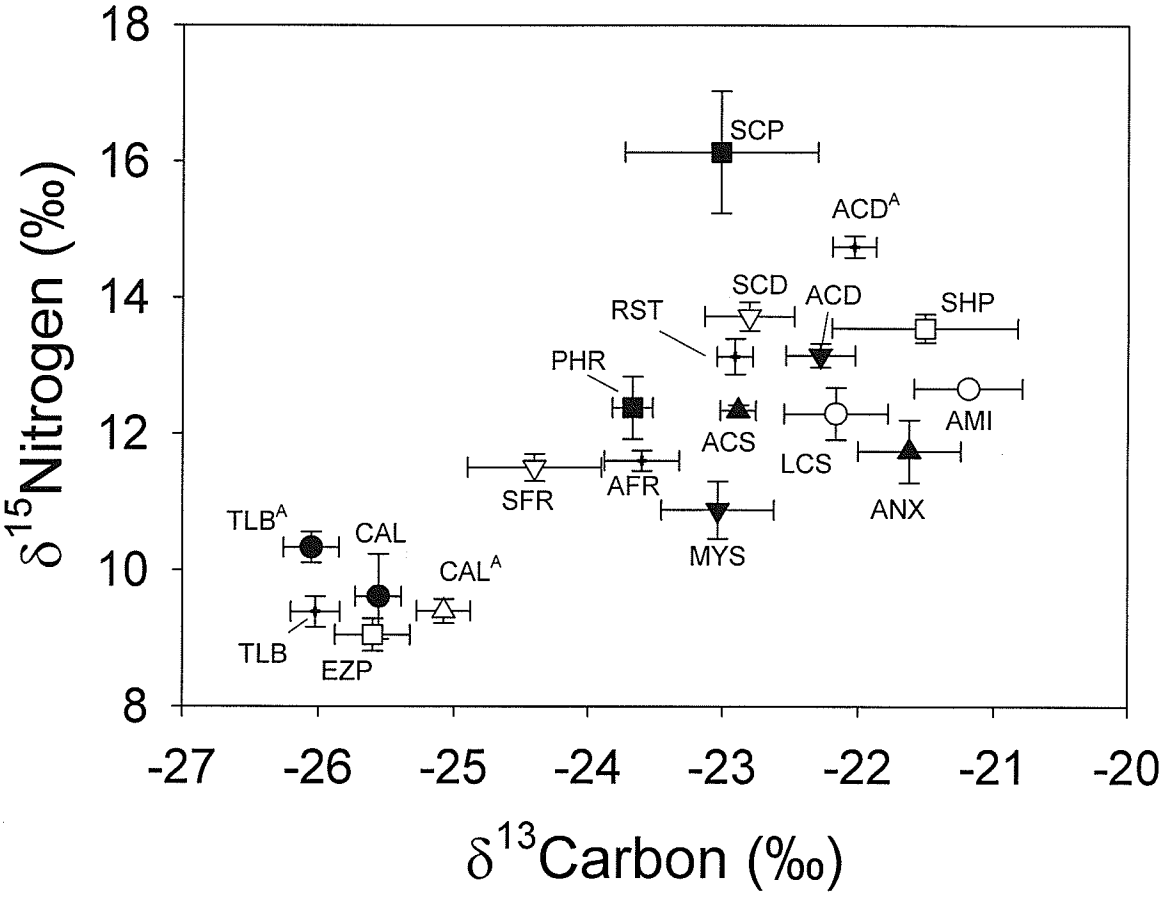

Figure 3.2. Stable isotope values for $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) (± SE) in organisms from the estuarine-shelf, Amundsen Gulf and epibenthic food webs. For species abbreviations see Table 3.1. Superscript "A" represents Amundsen Gulf prey.

Amundsen Gulf arctic cod had higher MeHg, THg and $\delta^{15}N$ levels than estuarine-shelf fish species ($P < 0.01$), but THg and MeHg concentrations were not significantly higher than levels in saffron cod (Table 3.2). Although Amundsen Gulf and shelf arctic cod were the same length, those from the shelf were younger and had lower THg, MeHg and $\delta^{15}N$ values ($P < 0.05$), yet $\delta^{13}C$ values were similar (Figure 3.2). THg differences among the Amundsen Gulf and shelf arctic cod were not significant when controlled for age ($P > 0.05$).

Within the Amundsen Gulf food web, *Calanus* spp. collected from the eastern Beaufort Sea near the Mackenzie Delta shelf break had the highest mean THg concentrations (0.055 ug/g dw ± 0.005; $P < 0.05$) relative to those collected in Franklin Bay (0.026 ug/g dw ± 0.002) and the Amundsen Gulf (0.026 ug/g dw ± 0.003). Total Hg in *T. libellula* did not differ among the two years sampled ($P > 0.05$), thus samples from both years were combined. *T. libellula* from Amundsen Gulf had significantly higher THg levels than did shelf *T. libellula*, yet the trophic increase in Hg levels relative to *Calanus* spp. did not correspond with a trophic enrichment in $\delta^{15}N$ (e.g. 3‰) (Table 3.2).

Prey in the epibenthic food web had high MeHg and THg levels, in some cases invertebrate MeHg concentrations exceeded estuarine-shelf fish levels (Table 3.2). Sculpin had the highest Hg, and the $\delta^{15}N$ value was similar to values in beluga. Stomach content analysis of sculpin revealed a diet of amphipods and small fish supporting the high Hg and $\delta^{15}N$ values. Among invertebrates, shrimp had the highest MeHg, THg and $\delta^{15}N$ levels of potential prey items (Table 3.2).

3.3.2 Mercury Levels of Beluga Feeding Groups

In beluga muscle, 99% of the THg was present as MeHg. Beluga length was the best predictor of MeHg concentrations in muscle ($P < 0.01$), whereas sex and age did not have significant effects ($P > 0.05$). Sex was correlated with length ($r = -0.62$; $P < 0.01$), with females shorter than males. Beluga feeding groups were significantly different in length, but not age, and beluga length and age had a weak correlation ($r = 0.2$; $P > 0.1$). The longest whales were not the oldest whales, and mean ages in beluga feeding groups were within several years of one another (Table 3.3).

Concentrations of THg, MeHg and $\delta^{15}N$ values were significantly different among the beluga feeding groups ($P < 0.05$) (Table 3.3). Estuarine-shelf beluga had the lowest MeHg and THg levels that were significantly lower than levels of the Amundsen Gulf and epibenthic beluga groups (Table 3.3). The longest beluga whales that we hypothesized to feed epibenthically had the highest Hg and MeHg levels, but not significantly higher than levels of the Amundsen Gulf group. The $\delta^{15}N$ values did not follow the Hg and beluga length trend because highest $\delta^{15}N$ values were not associated with largest beluga with highest Hg levels (Table 3.3). $\delta^{13}C$ did not differ among the three whale groups.

Table 3.3 Mean and standard error of MeHg, THg (ug/g dw), and stable isotopes ($\delta^{15}N$ ‰, $\delta^{13}C$ ‰) in beluga muscle tissue. Beaufort Sea belugas partitioned into three groups based on length and sex parameters associated with habitat use. The three groups are referred to by their hypothesized feeding behaviour as follows: shallow open water as the estuarine-shelf group; sea-ice edge as the Amundsen Gulf group; and heavy ice concentration as the epibenthic group. Significant differences from a posterior Tukey tests are designated by different letters ('a' and 'b' symbols).

Beluga Feeding Groups	n (f)	MeHg (µg/g dw)	THg (µg/g dw)	$\delta^{15}N$(‰)	$\delta^{13}C$(‰)	Length (m)	Age
Estuarine-Shelf	7 (4)	2.59 ± 0.73^{a}	2.56 ± 0.8^{a}	16.2 ± 0.3^{a}	-18.6 ± 0.3^{a}	3.55 ± 0.1^{a}	28.0 ± 2.9^{a}
Amundsen Gulf	9 (1)	4.42 ± 0.64^{b}	4.41 ± 0.7^{b}	16.9 ± 0.2^{b}	-18.6 ± 0.2^{a}	4.08 ± 0.1^{b}	31.9 ± 2.6^{a}
Epibenthic	10 (0)	6.03 ± 0.61^{b}	6.53 ± 0.7^{b}	16.6 ± 0.2^{ab}	-18.4 ± 0.2^{a}	4.33 ± 0.1^{c}	26.1 ± 2.4^{a}
F-ratio		7.95	8.45	4.44	0.51	96.88	1.38
P value		0.002	0.002	0.023	0.600	<0.001	0.300

3.3.3 Trophic-Level Transfer of Methyl and Total Mercury

MeHg and THg trophic magnification slopes did not differ between the three food webs ($P > 0.1$, Figure 3.3). In all three food webs, MeHg biomagnification slopes were greater than the THg slopes, and within each food web MeHg and THg slopes did not differ ($P > 0.1$). The Amundsen Gulf food web had the highest rate of MeHg biomagnification (Figure 3.3b). The MeHg slope in the estuary-shelf food web was influenced by the low $\delta^{15}N$ in *T. libellula* (Figure 3.3a). The range in $\delta^{15}N$ values across similar Hg concentrations in the epibenthic food web resulted in a shorter food web length and greater unexplained variance in Hg values than for the other two habitats (Figure 3.3c).

A large difference between the THg and MeHg BMFs was present at the lower end of the food web (Figure 3.3) that converged at higher trophic levels. This was due to the increase in the percent of MeHg up the food web. Calculated BMFs from prey to beluga were higher for MeHg than THg because it is the form that biomagnifies (Table 3.4). BMFs from estuarine-shelf fish to beluga ranged from 8 in saffron cod to 19 in arctic cisco (Table 3.4). Arctic cod BMFs from the Amundsen Gulf food web fell within the range for estuarine-shelf fish to beluga (Table 3.4). The MeHg BMFs from potential epibenthic prey items to beluga ranged considerably from 10.8 in sculpin to 88.5 in a small amphipod (*A. malmgreni*) (Table 3.4).

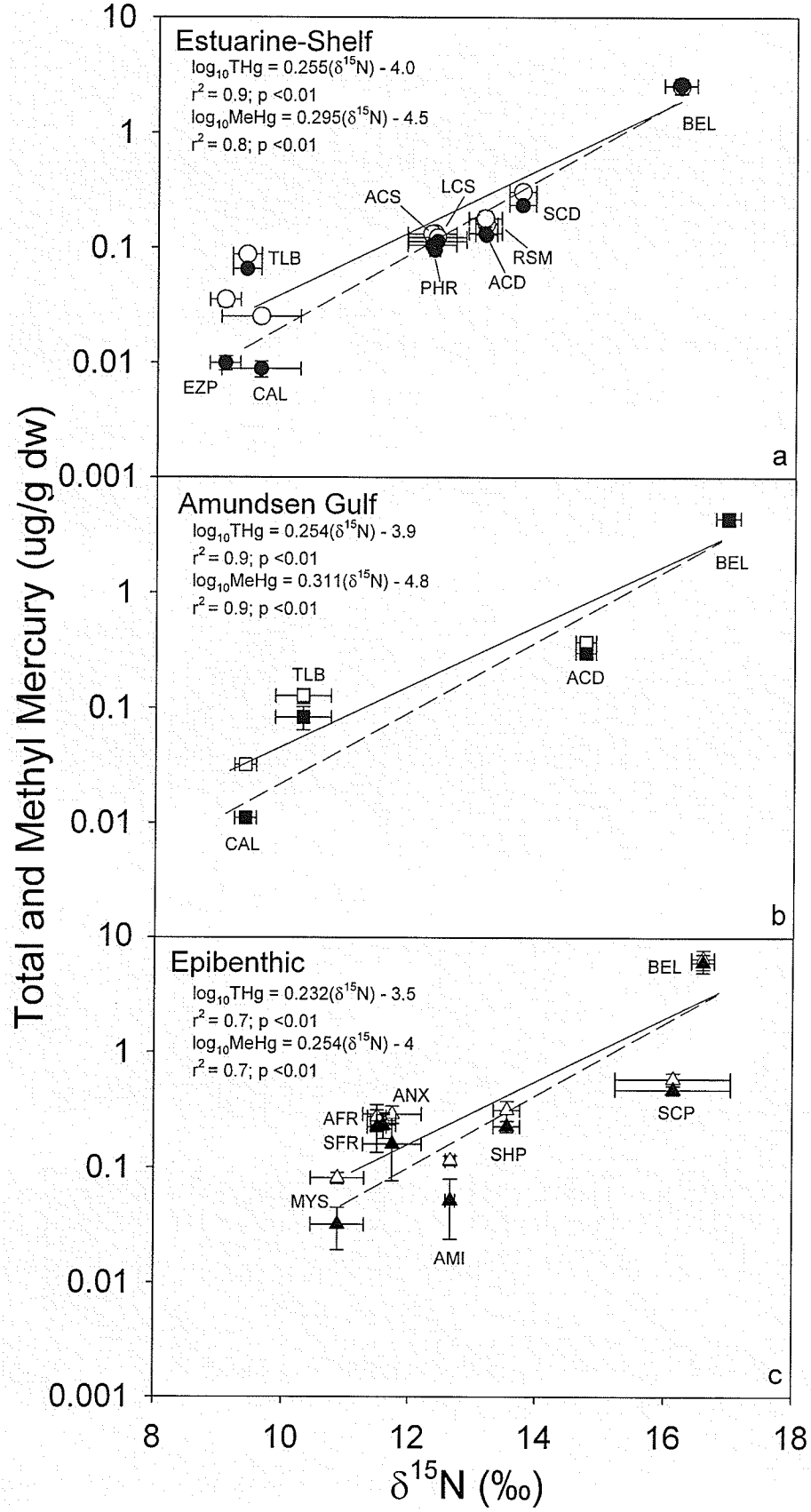

Figure 3.3. Biomagnification of MeHg and THg in three food webs: a) Estuarine-shelf, b) Amundsen Gulf and c) Epibenthic. Mean ±SE of log transformed MeHg (dashed line) and THg (solid line) (ug/g dw) versus $\delta^{15}N$ depicting potential trophic relationships of species. For species abbreviations see Table 3.1.

Table 3.4 Total and methyl mercury biomagnification factors (BMFs) in three food webs measured between beluga and potential prey items within their paired groups (see equ. 4). Refer to Table 3.2 for species abbreviations.

Biomagnification Factors (BMF's)		
	Beluga/Prey MeHg	**Beluga/Prey THg**
Estuarine-shelf		
ACS	19.00	14.64
LCS	15.74	14.64
RST	12.75	11.48
SCD	8.03	7.06
PHR	17.86	15.91
ACD	13.32	12.79
Average	*14.45*	*12.76*
Amundsen Gulf		
ACD	12.77	10.20
Epibenthic		
SHP	39.08	16.87
ANX	26.67	15.88
AMI	88.50	43.10
AFR	18.01	20.07
SFR	18.51	16.32
SCP	10.88	10.81
Average	*22.63*	*15.99*

3.4. Discussion

3.4.1 Methyl and Total Mercury in Three Food Webs

The importance of determining beluga diet and foraging behaviour is demonstrated by the variability of Hg concentrations among food webs in the western Arctic. The percent of MeHg in arctic cod and beluga are consistent with previous studies (e.g. arctic cod 100% in Campbell et al. 2005; 97% beluga in Wagemann et al. 1998). To our knowledge, this study is the first to report MeHg levels in estuarine fish and epibenthic invertebrates collected from the Arctic Ocean. The percentages of MeHg for the estuarine fish are similar to levels typically measured in fish (Morel et al. 1998), and THg in pacific herring and least cisco are within the range reported during the 1990's (Lockhart et al. 2005a). Shrimp MeHg content was in the lower range reported in Barents Sea shrimp (ca. 77-100%) (Joiris et al. 1997). Generally, the high percent MeHg in shrimp, *Anonyx* and *T. libellula* is comparable to fish and thus, negates the possibility of using the MeHg:THg ratio as an indicator of piscivory in belugas as was suggested for seals (Dehn et al. 2005). The low percentage of MeHg in *Calanus* spp. is common for suspension-feeding zooplankton (Watras and Bloom 1992) and comparable to *Calanus* spp. in the Beaufort Sea (Stern and Macdonald 2005) and in the Northwater Polynya (Campbell et al. 2005).

Despite high elemental Hg and MeHg output from the Mackenzie River plume (Leitch et al. 2007), fish in the estuary-shelf habitat (excluding saffron cod) had the lowest Hg concentrations. The diet of the estuary-shelf fish species is not fully known, and although they are almost twice the size of Amundsen Gulf arctic cod their low $\delta^{15}N$ and Hg levels suggest that they prey on lower trophic level organisms. *Calanus* spp.,

T.libellula and arctic cod collections from the Amundsen Gulf and Mackenzie shelf provided a common means for spatial comparison of food web Hg levels. Mercury levels in Amundsen Gulf *Calanus* spp. are comparable to previous reports (Stern and Macdonald 2005), whereas Hg in *T.libellula* were higher than reported in Campbell et al., (2005). Arctic cod from the Amundsen Gulf food web had higher Hg levels than did others in the same size range reported in the literature (Atwell et al. 1998, Campbell et al. 2005, Stern and Macdonald 2005). Hg levels in shelf arctic cod were similar to previous literature reports. Although differences in Amundsen Gulf and shelf arctic cod Hg were probably driven by age, their dominant prey items *Calanus* spp. and *T. libelulla* (Lonne and Gulliksen 1989) demonstrated spatial differences in Hg and $\delta^{15}N$ levels, with higher values in the Amundsen Gulf, Franklin Bay and eastern Beaufort Sea region. The higher Hg and $\delta^{15}N$ in Amundsen Gulf *T.libellula* slightly lengthened this portion of the food web possibly causing Amundsen Gulf arctic cod to have higher values. Given that arctic cod in the Amundsen Gulf are older, they may predominantly feed on the macrozooplankton *T.libellula* than on *Calanus* spp.; thus, bioaccumulation with age in addition to food web structure may drive the regional differences in arctic cod Hg levels. Alternatively, regional differences in Hg may have caused the differences observed.

High Hg and relatively lower $\delta^{15}N$ in several epibenthic organisms suggests different Hg uptake or trophic-level transfer processes in the epibenthic food web. Epibenthic species feed in or near sediments, where Hg methylation occurs (Bloom et al. 1999), exposing them to 10^6 times higher Hg levels than those feeding in the water column (Morel et al. 1998). Mobile epibenthic animals have access to organic matter

falling through the water column, re-suspended from the benthos and deposited on the sea floor. The quality of organic material may be highly variable and the heterogeneous nature of available food sources complicates interpretation of Hg sources and trophic transfer. Dead or previously processed organic material may contain high MeHg or elemental Hg that can increase Hg flux to these organisms, and start the food web at a higher Hg level (Lindberg and Harris 1974). This may explain the high and variable Hg levels among the epibenthic invertebrates observed here and in previous studies (Lawrence and Mason 2001).

High Hg outputs from the Mackenzie Delta (Leitch et al. 2007) did not result in highest Hg concentrations in the estuarine-shelf food web, yet Hg from the Mackenzie River was found to be an important source to the Beaufort Sea marine food web (Stern and Macdonald 2005). Those findings, and the high Hg levels in the Amundsen Gulf food web (that includes the eastern Beaufort Sea), suggests that Hg outputs from the Mackenzie River may only become bioavailable after leaving the delta and entering the Eastern Beaufort Sea region. The majority of MeHg leaves the Mackenzie River in a dissolved phase, whereas element Hg is bound to particulate (Leitch et al., 2007), influencing mobility and food web uptake dynamics that require further investigation.

3.4.2 Variability in Beluga Mercury Levels

Variation in Hg among the beluga feeding groups may be explained by differing Hg levels in their diets in the various habitats. The lack of a relationship between beluga age and muscle Hg concurs with previous marine mammal observations that muscle Hg concentrations largely reflect Hg biomagnification through diet, and to a lesser extent by bioaccumulation over time (Wagemann et al. 1990, Atwell et al. 1998).

However, liver tissue not examined here does have a strong positive relationship with age due to the continuous demethylation of MeHg and accumulation of mercuric selenide (Wagemann et al. 1998). The beluga length and muscle Hg relationship supports our use of length data to create habitat-specific beluga feeding groups to evaluate Hg exposure through diet. The weak relationship between age and length was due the beluga largely being over the asymptotic length of the Gompertz curve (Luque and Ferguson 2006).

Low Hg and $\delta^{15}N$ levels in the estuary-shelf beluga feeding group corresponded with the low Hg levels in the estuarine-shelf food web, supporting this pairing. Mercury levels in the estuarine-shelf beluga group were similar to Hg muscle levels in Point Lay, Alaska, beluga (ca. 1.1 ug/g ww THg) (Dehn et al. 2006a). Fifty percent of belugas in the estuarine-shelf group were females, which may confound interpretation of dietary Hg effects because Hg elimination during birthing may decrease Hg levels in mothers. However, lactating females had higher Hg levels than did the other females, indicating minimal effect of birthing on muscle Hg levels, as has been found in previous studies (Lockhart et al. 2005b).

The lack of a significant Hg difference between the epibenthic and Amundsen Gulf beluga groups may be a result of the similarly high Hg levels found in the two food webs. Consequently, differentiating between food web Hg contributions to these two beluga groups and assessing Hg uptake differences in diet is not feasible given the commonly high Hg in both beluga and prey. Mercury levels in the epibenthic beluga group was similar to Beaufort Sea beluga levels in 2001 (Lockhart et al. 2005b), and $\delta^{15}N$ were similar to Alaskan beluga populations (Dehn et al. 2006a).

Although food webs differed structurally, the differences in $\delta^{15}N$ values among the three beluga groups were within a range of 3‰, suggesting that they all fed at a similar trophic level, or at a combination of trophic levels (Cabana and Rasmussen 1994). Highest Hg levels in the epibenthic beluga group did not associate with the highest $\delta^{15}N$ values, suggesting food web Hg sources associated with the sediment interface may be more important in driving Hg levels than food web length.

3.4.3 Biomagnification in three Arctic food webs

Similar food web biomagnification slopes showed that Hg biomagnification processes did not differ among food webs. It also demonstrates the importance of Hg, and specifically MeHg concentrations at the bottom of the food web, as well as food web length. If the between-trophic-level transfer of Hg remains consistent upward through a food web, then the initial Hg concentration at the bottom of the web will constrain Hg levels in top predators. In addition, lengthening the food web by increasing the number of trophic levels would result in higher concentrations of Hg in top predators (Cabana and Rasmussen 1994).

Total Hg biomagnification slopes were comparable to, but slightly higher than those reported in previous Arctic aquatic studies (e.g. slopes: 0.197 in Campbell et al., 2005; 0.2 in Atwell et al., 1998; 0.19 in (Power et al. 2002). If Hg biomagnification is relatively constant over space and time, then we can use BMFs as tools to test how well the beluga social groups pair with their habitat-specific potential prey.

Methyl mercury BMFs are more relevant than THg BMFs because it is the MeHg fraction that biomagnifies up a food web. To our knowledge this study reports

the first MeHg BMFs to beluga. Thus, we are unable to draw comparisons to previous work. Total Hg BMFs from arctic cod to beluga in this study were comparable to the THg BMF value of 10 calculated for Lancaster Sound beluga feeding on arctic cod using $\delta^{15}N$ and THg levels in Atwell et al., (1998). The similar BMF values provide support for our Amundsen Gulf-beluga food web pairing. Despite significant differences in THg concentrations between estuary-shelf fish and Amundsen Gulf arctic cod, their THg BMFs to beluga were comparable. This suggests that the Hg trophic transfer from fish to beluga is predictable and supports our estuarine-shelf pairing. However, the THg BMF for sculpin was also comparable (ca. 10), but is not likely important in the beluga diet given the similar $\delta^{15}N$ values. High BMFs for items such as *A. malmgremi* suggest they are not important to beluga diet. The overall higher BMFs in the epibenthic food web may result from their association with high Hg environments (e.g. sediments and detritus), or perhaps trophic-level transfer processes from crustaceans to beluga is different as compared with fish to beluga. For example, different energy content among diet items may alter the total amount of food consumed and influence the resulting Hg biomagnifications and calculated BMF values. We recommend these diet factors be considered in future food web and biomagnification studies of Hg.

3.5 Conclusions

Here we documented Hg variability within three food webs in the Canadian Western Arctic where Beaufort Sea beluga spend their summer. Hg levels in organisms locally exposed to high levels of Hg in the Mackenzie River outflow had the lowest Hg

concentrations; however, the higher Hg levels in biota collected from the Amundsen Gulf, Franklin Bay and eastern Beaufort Sea may suggest that Hg from the Mackenzie River may only become available for biological uptake in offshore environments. On the other hand, food web structure may also be an important factor influencing beluga Hg levels. The Amundsen Gulf food web length may have caused Hg levels to be higher in arctic cod and beluga relative to the estuarine-shelf, whereas the epibenthic association with high Hg level environments such as sediment and detritus likely resulted in high Hg levels. These findings, along with similar Hg trophic level transfer slopes among food webs, suggest that future research should focus on MeHg uptake processes at the bottom of the food web and food web structure.

This study demonstrated the importance of incorporating predator habitat selection when examining Hg uptake processes from prey. By incorporating information on potential beluga prey in food webs associated with their habitat use, we found beluga Hg levels to correspond with prey Hg levels. Using BMFs from prey to beluga provided insight for beluga-food web pairing and for examining potential Hg diet sources and trophic level transfer. Beluga diet was not directly determined in this study, therefore we suggest future studies incorporate dietary biomarkers such as fatty acids signatures and compound-specific stable isotope analyses to confirm beluga prey items. Finally, we propose that future studies evaluating contaminants in predators should incorporate foraging habitat selection and subsequent foraging characteristics into their analysis.

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4.0 Beluga Mercury levels described by Habitat Use revealed with Fatty Acids and Stable Isotopes

Abstract

Mercury (Hg) levels in the Beaufort Sea beluga *(Delphinapterus leucas)* population increased during the 1990's; levels have since declined but remain higher than the 1980's. The diet of this beluga population is not well known, thus it is difficult to assess dietary Hg sources. During the summer, the Beaufort Sea belugas segregate by length, sex and reproductive status corresponding to habitat use that may result in dietary differences and ultimately influence Hg uptake. To test this hypothesis, we examined beluga dietary variation using fatty acid signature analysis and determine which biological variables best predicted diet. The relationships of the beluga biological variables and fatty acids were evaluated with stable isotopes and Hg levels. Stable isotopes and fatty acids were compared in their ability to describe dietary Hg processes in beluga. Fatty acids provided support for dietary differences on Hg uptake, whereas stable isotopes inferred tissue Hg metabolic rates. Here, we show beluga length drives diet variability leading to differences in Hg uptake. For the first time, we demonstrate that food web Hg biomagnification processes drive muscle Hg levels more so than Hg bioaccumulation over time. In addition, we show that Hg concentrations in muscle describing diet sources relate better to liver $\delta^{15}N$, rather than muscle $\delta^{15}N$ that has a long turn over rate.

4.1 Introduction

In the 1990's, mercury (Hg) levels in the livers of the Beaufort Sea beluga whale *(Delphinapterus leucas)* population tripled in comparison with levels in the 1980's (Lockhart et al. 2005), and were the highest relative to other Canadian Arctic populations. Although, still higher than the 1980 levels, Hg concentrations have dropped and are now comparable to other Arctic populations (Lockhart et al. 2005). Elevated Hg levels present a risk to predators of beluga such as polar bears and Inuvialuit subsistence harvesters. Mercury bioaccumulates in organisms over time and biomagnifies at each trophic level, together those processes contribute to high Hg levels in predators such as beluga (Morel et al. 1998). Little is known about the diet of Beaufort Sea beluga and, thus, Hg dietary uptake is difficult to model.

Typically in animal diet studies, stomach contents and feces are used to identify diet items and infer dietary preferences. This is not feasible with the Beaufort Sea beluga whales because feces can not be found and harvested belugas usually have empty stomachs, yet local hunters have observed beluga feeding in their summer grounds (Harwood and Smith 2002). Based on relative isotopic fractionation processes, $\delta^{15}N$ can be used to describe trophic levels (Hobson and Welch 1992) and $\delta^{13}C$ can be used to evaluate food web carbon sources (France 1995). Therefore, tissue stable isotope concentrations can assist in the understanding of diet and can overcome problems associated with conventional diet determination such as over-or under-representation of prey that were recently eaten or quickly digested (Tollit et al. 1997). Similar to stable isotopes, fatty acids do not require the recovery of stomach contents and can also be used as a proxy for the recent diet of a predator (Iverson 1993, Kirsch et

al. 2000). Fatty acids stored in blubber undergo little to no degradation during digestion and deposition (Iverson 1993). Fatty acid analysis has successfully characterized trophic links among species (Budge et al. 2002, Iverson et al. 1997) and determined predator diets in marine and terrestrial ecosystems (Iverson et al. 1997, Iverson et al. 2001, Bradshaw et al. 2003) as well as detected the influences of size, sex and season on predator foraging behaviour (Beck et al. 2007). Krahn et al., (2007) incorporated both diet biomarkers in the analysis of persistent organic pollutants in killer whales (*Orcinus orca*) and delineated diets and foraging behaviour among the North Pacific population. Only stable isotopes have been evaluated with respect to Hg and diet, thus incorporating fatty acids as a tool may provide new insights to beluga Hg uptake.

During the summer, Beaufort Sea beluga habitat use differs with length, sex and reproductive status (Chapter 2). Habitat segregation may reflect differences in energy requirements and survival strategies that vary with age, sex, size, and reproductive stage (Bowyer 2004). For example, in the dimorphic northern elephant seal (*Mirounga angustirostris*), males are up to ten times the size of females and as a result are assumed to require different feeding strategies, resources, and habitat use to maintain their size difference (Le Boeuf et al. 2000). Beaufort Sea beluga are size dimorphic (Harwood and Smith 2002), whereby males reach a mean asymptotic length of 4.2 metres and the female asymptotic length is approximately 0.5 metres less (Luque and Ferguson 2006). Variation in beluga habitat selection suggests that they may have different diets and foraging behaviour (Chapter 2).

In this study we use fatty acid signature analysis to examine if beluga habitat use reflects feeding variation that may result in different dietary Hg sources. Fatty acids

are used as a proxy for diet to test which biological variables associated with habitat use such as length, sex, age, and harvest site, best predict diet. Relationships observed with fatty acids and the biological variables are then evaluated in beluga muscle and liver tissue values of stable isotopes and Hg. Finally, stable isotopes and fatty acids are compared as biomarkers to describe dietary Hg processes in beluga.

4.2 Materials and Methods

4.2.1 Beluga tissue collection

Beluga tissues were sampled from whales harvested at Hendrickson Island located in the shallow Mackenzie delta near the community of Tuktoyaktuk Northwest Territories (NT) and at Browns Harbour near the community of Paulatuk NT (Figure 4.1). A total of 42 samples were collected, from Tuktoyaktuk (T) in 2004 (n = 19) and 2005 (n =13), and at Paulatuk (P) in 2005 (n = 10). Sampling occurred within days of one another at each site. Few females were collected from the harvests because hunters generally select for medium to larger sized males (Table 4.1). One young male (8 years old) from Paulatuk was included in the analysis that may have been sexually immature (Robeck et al. 2005). Beluga tissues were sampled and frozen on site in a portable freezer at -20°C and shipped to Fisheries & Oceans Canada in Winnipeg for analysis. Blubber was sampled with the skin attached distally and muscle proximally. Muscle and liver tissues were selected for Hg, and nitrogen and carbon stable isotope analysis. Ages were determined from a thin section of a tooth by counting growth layer groups in the dentine (Stewart et al. 2006).

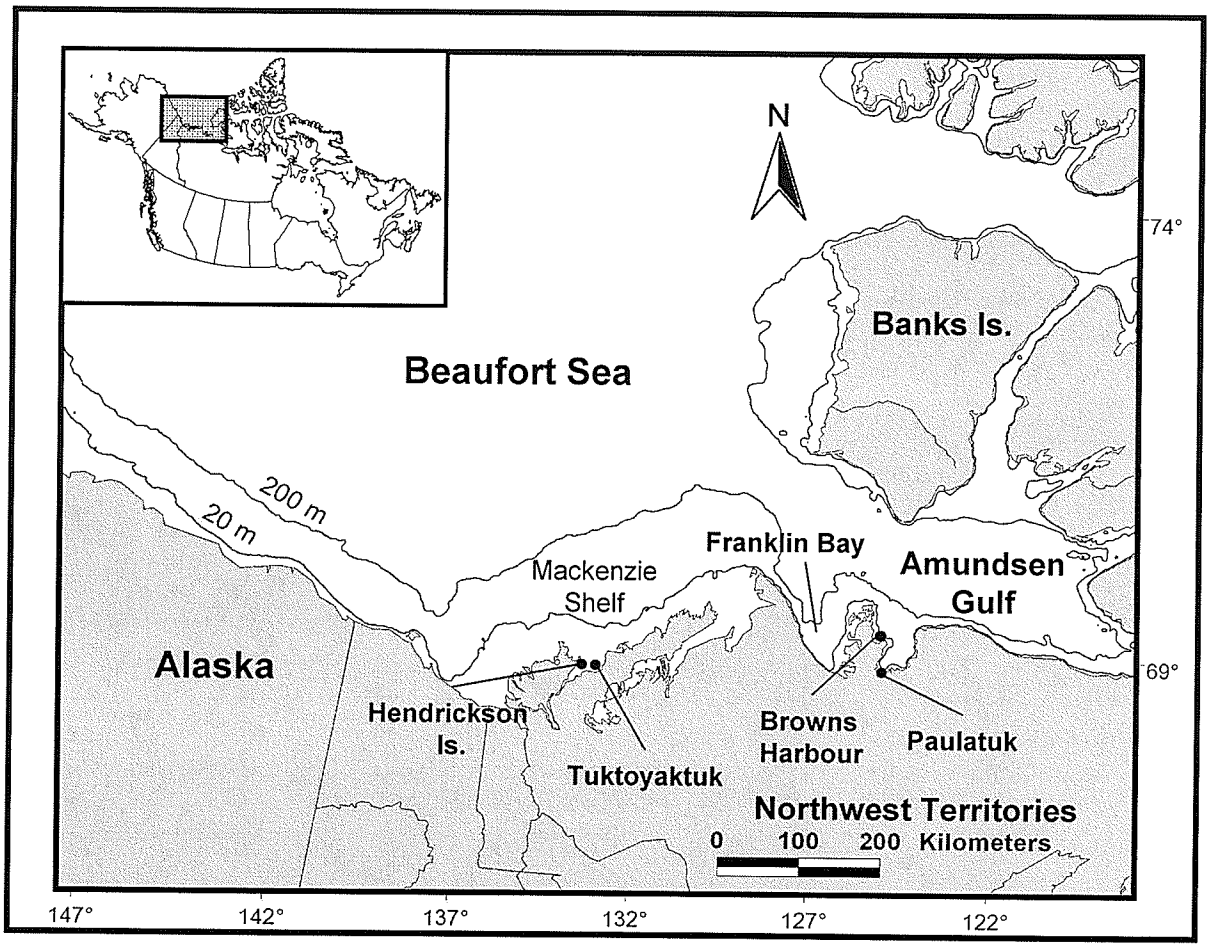

Figure 4.1 Beluga summer region and harvest sites. Hendrickson Island is the harvest location for the community of Tuktoyaktuk, and Browns Harbour is the harvest location for the community of Paulatuk Northwest Territories, Canada. Beaufort Sea beluga whales summer in eastern Beaufort Sea region.

Table 4.1 Summary of beluga collected at Paulatuk and Tuktoyaktuk NT, Canada. Mean total mercury in muscle and liver (ug/g ww) and $\delta^{15}N$ and $\delta^{13}C$ (per mil) in beluga liver and muscle. ANOVA performed on variables to test for mean differences and ANCOVAs found variable interaction with length or age.

	Harvest Site		ANOVA	
	Tuktoyaktuk	Paulatuk	F_{ratio}	P_{value}
Sample Size	32	10		
no. females	7	2		
Length (cm)	407.3 ± 5.8	380.7 ± 11.6	4.38	0.043
Age	29.20 ± 1.7	18.10 ± 2.8	18.05	<0.0001
THg Muscle	1.24 ± 0.1	0.50 ± 0.1	4.88	0.033
THg Liver	31.40 ± 5.0	4.50 ± 7.7	26.46	<0.0001
$\delta^{15}N$ Muscle	16.30 ± 0.1	15.52 ± 0.2	11.16	0.002
$\delta^{15}N$ Liver	17.20 ± 0.2	16.68 ± 0.2	3.00	0.091
$\delta^{13}C$ Liver	-19.40 ± 0.2	-18.45 ± 0.2	8.13	0.007
$\delta^{13}C$ Muscle	-17.74 ± 0.2	-17.84 ± 0.3	0.10	0.753

4.2.2 Fatty acid extraction

The inner beluga blubber layer was used for fatty acid analysis because it is the most metabolically active and represents the most recent deposit of fatty acids from diet (Koopman et al. 2002). The blubber sample was taken after slicing and discarding the outer surface of the tissue. Lipids were extracted from 0.5 g duplicate samples using 2:1 chloroform-methanol containing 0.01%BHT (v/v/w) to avoid oxidation. This method was modified from Folch et al., (1957). The lipid phase was collected, washed, and filtered through anhydrous sodium sulphate and evaporated under nitrogen to obtain the total lipid. The extracted lipid was used to prepare the fatty acid methyl esters by transesterfication with Hilditch reagent (0.5 N H_2SO_4 in methanol). The samples were heated for 1hr at 100°C. Fatty acid methyl ester samples were analyzed using gas chromatography (Hewlett Packer HP Series 6890) with a mass spectrometer detector (Hewlett Packard 5973). Inlet temperature set at 250°C and the following temperature program was used: start at 153°C for 2 min, then ramp up at 2.3°C min^{-1}, hold at 174°C for 0.2 min and ramp up at 2.5°C min^{-1} and hold at 220°C for 3 min, as described previously (Budge et al. 2002). A silica column (30m x 0.25mm ID) coated with 50% cyanopropyl polysiloxane (0.25um film thickness; J&W DB-23) was used. Helium was the carrier gas that was equipped with an oxygen scrubber. Up to 66 fatty acid methyl esters were identified according with verification of ion mass spectroscopy and known standard mixtures (Nu Check Prep.). Fatty acids were integrated and expressed as a mass percent of the total fatty acids. Fatty acid identifications were checked on all chromatograms and reintegrated if necessary. Each fatty acid was described using the

shorthand nomenclature of A:Bn-X, where A represents the number of carbon atoms, B the number of double bonds, and X the position of the double bond closest to the terminal methyl group.

4.2.3 Stable isotope Analysis

Stable nitrogen isotope analysis was performed on dried homogenized subsamples of beluga liver and muscle. Carbon isotope analysis was performed on tissues that were removed from lipids using a chloroform/methanol extraction and then dried for analysis. Carbon and nitrogen isotopic analyses were accomplished by continuous flow, isotope, mass spectrometry (CF-IRMS) using a GV-Instruments® IsoPrime attached to a peripheral, temperature-controlled, EuroVector® elemental analyzer (EA) (University of Winnipeg Isotope Laboratory, *UWIL*). One-mg samples were loaded into tin capsules and placed in the EA auto-sampler along with internally calibrated carbon/nitrogen standards. Carbon and nitrogen isotope results are expressed using standard delta (δ) notation in units of *per mil* (‰). The delta values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) represent deviations from a standard such as

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

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

Accuracy was obtained through the analysis of laboratory standards used for calibration of results.

4.2.4 Total Mercury analysis

Beluga muscle and liver tissues were analyzed for Total Hg (THg). The majority of Hg in beluga liver is elemental, whereas the majority in muscle is methylmercury (MeHg) (Lockhart et al. 2005). Subsamples were taken from partially thawed tissue after slicing away the outer surface. All samples were weighed to approximately 0.15g for THg analysis. Samples were digested with a hydrochloric/nitric acid mixture (Aqua Regia) heated to 90°C. The digest was analyzed for THg by Cold Vapour Atomic Absorption spectroscopy (CVAAS) (Armstrong and Uthe 1971). The detection limit was 0.005 ug/g. Certified standard reference materials (CRM 2976, TORT-2, DOLT-2) were analyzed in duplicate in every run. Recovery within ten percent of the certified values was used as a batch validation for samples. Average differences in duplicates for beluga are five percent.

4.2.5 Data Analysis

Fatty acids were measured as a percent of the total number of fatty acids identified and were log transformed before all statistical analyses. Although 65 fatty acids were identified, only the fatty acids known to biotransfer from prey to predator were evaluated. We used 40 of the 41 described by Iverson et al., (2004). Multivariate analyses were carried out using SYN-TAX© Ordination 2000 (Budapest, Hungary). Systat 11® (Systat Software Inc., 2004, San Jose, CA) was used for univariate statistical

analysis and SAS®Version 8 (SAS Institute Inc., Cary, North Carolina) used to calculate the Akaike information criteria (AIC).

A principle component analysis (PCA) with a covariance matrix was used to examine the beluga fatty acid variation among individuals. We examined which of the biological variables best explained variation in beluga fatty acids by regressing the biological variables with the PCA scores from axis one and two, with the expectation of comparable results.

The strongest biological variables predicting the fatty acids in the PCA analysis were examined for predictive relationships on Hg and stable isotopes in liver and muscle tissues. Differences in Hg concentrations and stable isotope values between beluga harvested at Paulatuk and Tuktoyaktuk were evaluated using an analysis of variance and analysis of covariance to test for length and age interactions. To evaluate the relationships of length and age as predictors of Hg and stable isotopes in beluga, regressions were used. The best individual variable to describe Hg or isotopic value was selected using the lowest AIC value (Burnham and Anderson 2002). To examine which individual or combination of diet biomarkers (e.i. stable isotopes and fatty acids described by PCA axis 1, 2 scores) best explained Hg levels in beluga tissues the Akaike differences (Δ_i) and normalized Akaike weights were calculated (w_i) to select the best variable or variables. A ΔAIC of zero and up to two were considered to be the most important model variables (Ferguson et al. 2006).

4.3 Results and Discussion

4.3.1 Beluga Fatty Acid Profile and Biological Variables

Fatty acids prevalent in beluga included the saturated fatty acids 14:0, 16:0 and 18:0, and monounsaturates 16:1(n-9), 18:1(n-9), 20:1(n-9) and 22:1(n-9) and the essential polyunsaturates 20:5(n-3) and 22:6(n-3). The predominance of those fatty acids were similar to beluga near Svalbard (Dahl et al. 2000), and other marine mammals such as pinnipeds (Iverson et al. 1997). Individual beluga appeared to vary in fatty acid profiles (Figure 4.2a). Seventy-four percent of the variance in beluga fatty acid profiles were explained in the PCA (axis 1: 50%; axis 2: 24%). The fatty acids explaining the beluga distribution along axis one included the long chain C_{20} and C_{22} monounsaturates and the essential polyunsaturated eicosapentaenoic acid (20:5(n-3)) (Figure 4.2b).

The length and harvest site were important variables describing beluga fatty acid profiles. PCA axis one scores were best correlated to beluga length ($r = 0.46$; $P = 0.002$) and axis two related to harvest site ($r = 0.47$; $P = 0.002$). Length and harvest site were related ($r = -0.31$; $P = 0.043$), because whales harvested from Paulatuk were generally shorter than those harvested from Tuktoyaktuk (Table 4.1). Age was not as strongly related to the first or second PCA axis ($r = 0.32$; -0.05, $P = 0.04$; 0.8, axis 1; 2 respectively) and sex was the weakest biological variable to predict beluga fatty acid profiles ($r = -0.14$; -0.09, $P = 0.4$; 0.6, axis 1; 2 respectively). Beluga length and age were not significantly related ($r^2 = 0.08$; $P = 0.068$), because whales evaluated here (with the exception of one 8yr old) were adults, and have reached their asymptotic length (Figure 4.3). Since whales were in the asymptotic part of the Gompertz growth

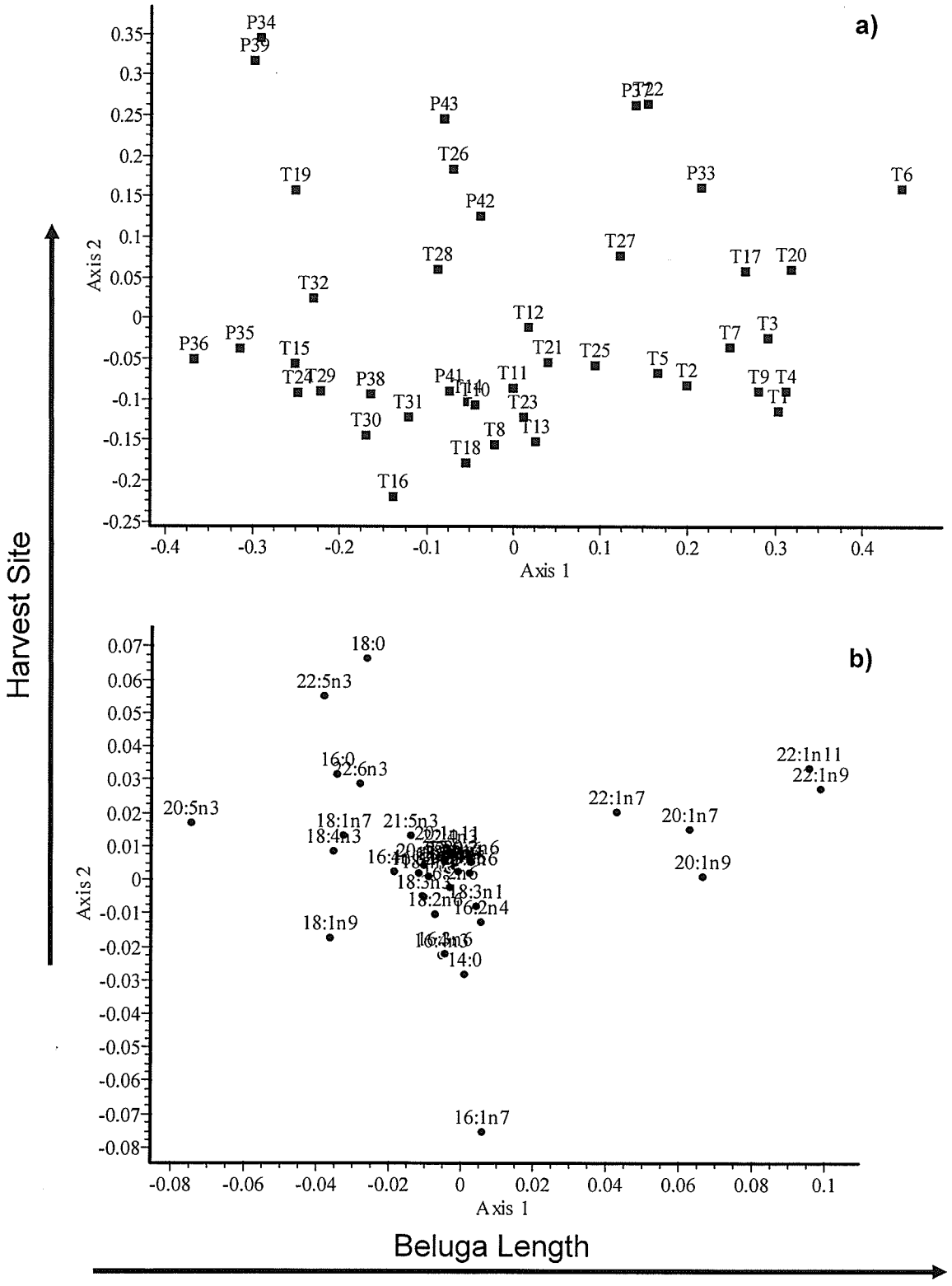

Figure 4.2. Principle component analysis of the 40 dietary fatty acids in 42 beluga, explaining 75% of the variance (axis 1: 53%: axis 2: 22%). **2a.** Species plot, belugas labelled with T were harvested at Tuktoyaktuk, and belugas labelled with P were harvested at Paulatuk. **2b.** Variable scores plot of fatty acids. The fatty acid positions describe the position of prey on the species plot, and the location of the fatty acids indicates importance; those in the centre have the lowest importance relative to those furthest from the origin.

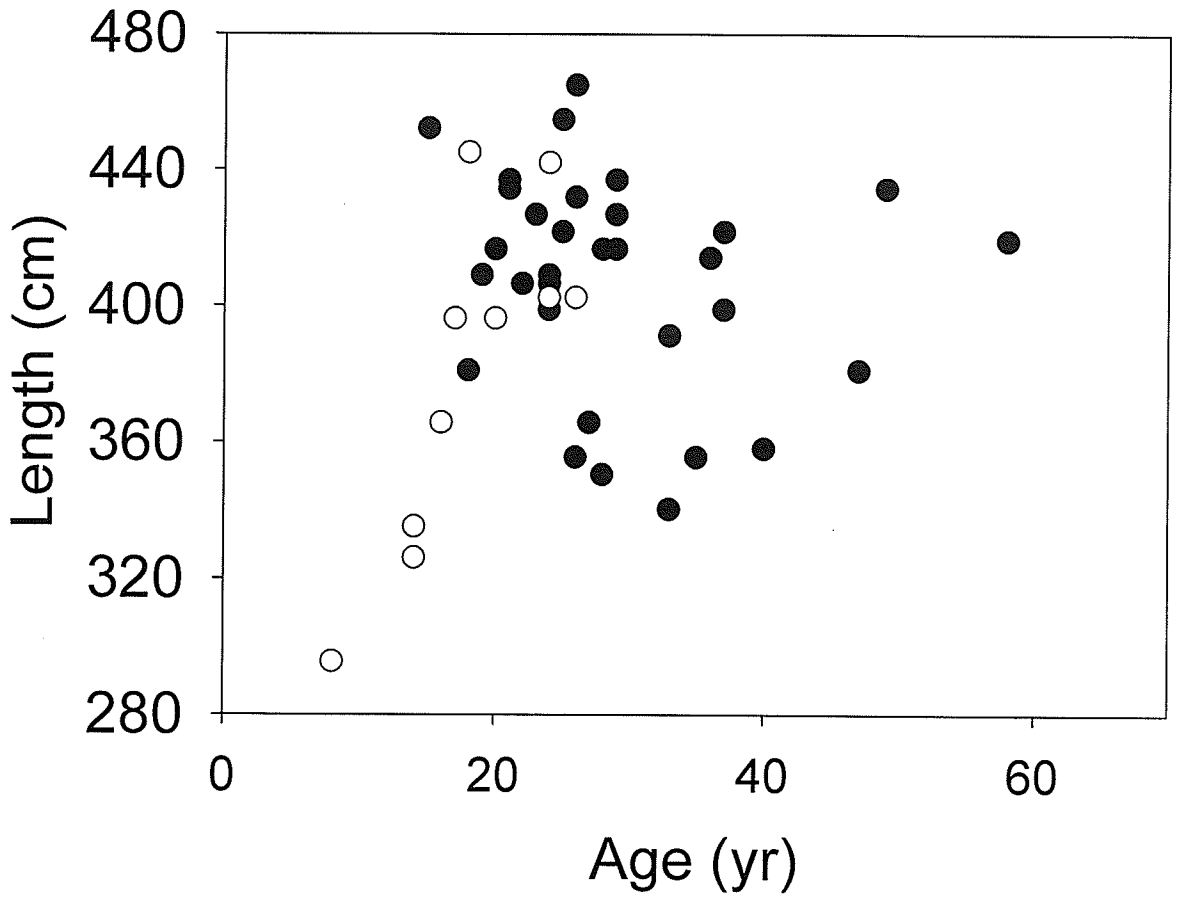

Figure 4.3. Age (years) and length (cm) relationship in beluga harvested at Tuktoyaktuk (closed symbols) and Paulatuk (open symbols). Note all whales with the exception of one 8year old are over the asymptotic growth length (Luque and Ferguson 2005).

curve (Windsor 1932) Hg tissue trends were evaluated below without confounding age and length effects.

The biological variables length and harvest site selected to represent habitat use of sea ice and bathymetry (Chapter 2) were found to be important predictor variables of beluga diet. The predominance of length over age as a predictor of diet described by fatty acids, supports that prey preference is a function of size, and to a lesser extent age, which provides new information on the structure of beluga social groups. Explanations of diet variations with length can be attributed differences in resource use and habitat selection (Bowyer 2004), which may be described by habitat use segregation hypotheses. The predation risk hypothesis postulates that predator avoidance behaviour by reproductive females and young will result in the geographic segregation of sexes in habitat use (Main et al. 1996). On the other hand, the foraging selection hypothesis relates to size dimorphism and maintaining a larger body mass by foraging more or on higher quality food will result in differences of habitat use (Clutton-Brock et al. 1982, Conradt 1998). Although the hypotheses are not mutually exclusive, they offer explanations for length influences on dietary preferences and ultimately Hg dietary sources. A third hypothesis that has been refuted in terrestrial ecosystems (Bowyer 2004) suggests habitat segregation results from interference competition, whereby the selection of different resources occurs to reduce intra-species competition (Stewart et al. 2002).

Unlike findings in Chapter 2, where sex was an important predictor of habitat use, here sex did not appear to influence diet. Often sex is an important variable explaining diet variation, particularly in dimorphic mammals (Clutton-Brock et al.

1982, Loudon 1985, Boyd et al. 1997). The use of fatty acids as a diet proxy should not have hindered our analysis because it has found differences between sexes (Beck et al. 2007). Our low sample size of females (n=9) may have reduced the ability to detect dietary differences between sexes.

4.3.2 Trends in stable isotope levels

Fatty acid analysis revealed the importance of length, harvest site and age in describing diet; thus, those variables were evaluated for trends with tissue stable isotope ratios. $\delta^{15}N$ in liver and muscle were best described by beluga length (Table 4.2). The positive relationship of $\delta^{15}N$ with length supports the fatty acid results, and shows that larger whales are feeding at higher trophic levels than smaller whales, demonstrating a gradient of feeding preferences associated with size. Ratios of liver and muscle $\delta^{13}C$ related most strongly with age, but were only significant for liver (Table 4.2). Thus, it appears that length effects on dietary preferences were only supported by $\delta^{15}N$ tissue values. Differences in stable isotopes among the beluga harvest sites were measured in muscle $\delta^{15}N$ and liver $\delta^{13}C$ values (Table 4.1). The tissue differences among sites did not have an interaction with age or length differences. Based on the stable isotope results alone, it would be difficult to conclude that diet differed among beluga from Tuktoyaktuk and Paulatuk because only two of the four tissue isotopic values differed.

Higher $\delta^{15}N$ liver values in the larger Tuktoyaktuk whales suggest they are feeding at higher trophic levels than the shorter Paulatuk whales. The variation in $\delta^{15}N$ with length supports size mediated diet variation, yet the difference between sites was below 3‰, suggesting dietary variation occurred within a trophic level (Hobson and

Table 4.2. Best fit regressions, where variables were selected by lowest AIC.
a) tissue stable isotopes best described by age and length relationships,
b) Hg in beluga liver and muscle best described by age or length,
and c) Hg in beluga liver and muscle best described by stable isotopes

Best Fit Regressions Dependant*Response	R^2	P_{value}
1) Stable Isotopes*Biological Indice		
Log_{10}Liver $\delta^{15}N$ * Log_{10}Length	0.135	0.017
Log_{10}Muscle $\delta^{15}N$ * Log_{10}Length	0.196	0.003
Log_{10}Liver $\delta^{13}C$* Log_{10}Age	0.121	0.024
Log_{10}Muscle $\delta^{13}C$*Age	0.062	0.112
2) Mercury*Biological Indice		
Log_{10}Liver Hg * Log_{10}Age	0.491	< 0.001
Log_{10}Muscle Hg*Length^2	0.356	< 0.001
Log_{10}Muscle Hg*Length	0.355	< 0.001
3) Mercury* Stable Isotopes		
Log_{10}Liver Hg* Log_{10}Muscle $\delta^{15}N$	0.223	0.02
Log_{10}Muscle Hg* Log_{10}Liver $\delta^{15}N$	0.359	< 0.001

Welch 1992, Cabana and Rasmussen 1994). The lack of a length relationship with $\delta^{13}C$ tissue values, and a weak relationship with age suggests different processes are governing $\delta^{13}C$ ratios. Differences in liver $\delta^{13}C$ among the harvest sites suggested that the beluga harvested at Tuktoyaktuk fed more pelagically, whereas higher $\delta^{13}C$ values in Paulatuk beluga reflect terrigenous sources suggesting near shore feeding (Meyers 1994, France 1995). The hypothesized regional feeding differences are supported by the habitat use observations, whereby the smaller-sized whales remained closer to the mainland coastline (Chapter 2) (Richard et al. 2001). The overall weakness between tissue stable isotope and beluga biological variables indicate that stable isotopes were not as effective as fatty acids when testing for the influences of biological variables. Nevertheless, stable isotopes provided additional information regarding trophic level and regional feeding patterns.

4.3.3 Consequences of Diet on Tissue Mercury

Exploring the biological variables driving Beaufort Sea beluga diet provided new insight to Hg accumulation processes in liver and muscle tissues. Unlike fatty acid results, length was not as important as age in describing Hg concentrations in liver (Table 4.2; Figure 4.4). Beluga age has successfully been used to describe a linear relationship with liver Hg concentrations here, and in previous studies (Wagemann et al. 1998, Lockhart et al. 2005). In contrast, Hg concentrations in muscle tissue were best explained by length, rather than age (Table 4.2). An exponential relationship with length had a slightly better fit than a linear relationship (Table 4.2). Given the relatively low r-square value that reflects the relationship at the higher Hg levels and length, it is difficult to select the exponential relationship as a better descriptor than a linear

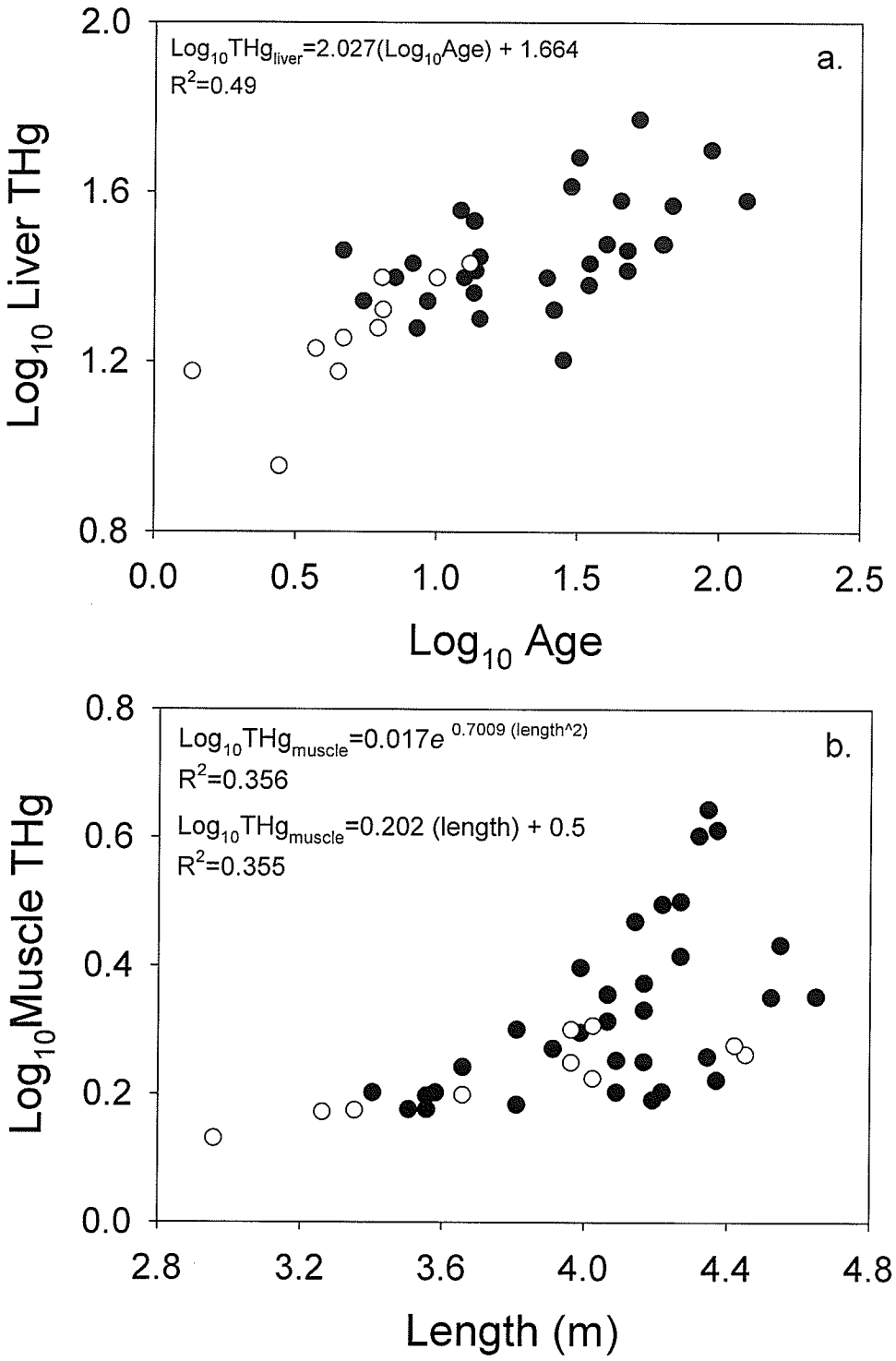

Figure 4.4. Best fit regression for mercury in beluga tissues with biological correlates as determined by Akaike's Information criterion (AIC). Age, length and tissue total mercury (THg) values $\log_{10}$ tranformed from two harvest sites Paulatuk (open symbols) and Tuktoyaktuk (closed symbols). **a.** Liver THg best described by beluga age; **b.** Muscle THg best described by beluga length.

relationship with length (Figure 4.4b). However, adult beluga that differ in length and diet also show an increase in muscle Hg with length (ca. 1.02 $\mu g\ g^{-1}m^{-1}$) (Figure 4.4b) but not age ($r^2 = 0.13$; $P = 0.02$). Mercury concentrations in liver and muscle tissues were different among the two harvest sites (Table 4.1). Despite length and age site differences, there was no interaction of Hg concentrations among sites with length or age, supporting similar Hg accumulation processes between sites.

The linear increase in beluga liver Hg concentrations with age supports the additive processes of Hg bioaccumulation over time. Beluga liver tissue accumulates Hg over time because it demethylates MeHg, the predominant form in muscle tissue, to elemental Hg creating a biologically unavailable complex with selenium as a means of detoxification (Farris et al. 1993, Wagemann et al. 1998, Lockhart et al. 2005). Despite differences in age at the two harvest sites, liver Hg bioaccumulation rates were similar, indicating comparable rates of liver demethylation across beluga size ranges. This result concurs with previous mammal studies (Young et al. 2001). The rates of Hg accumulation were much lower than previously reported for this population (Lockhart et al. 2005).

The length and muscle Hg relationship indicates that larger beluga are either feeding at higher trophic levels or in different food webs with higher Hg sources as hypothesized in Chapter 3. The $\delta^{15}N$ results reported here support the former. The half-life of Hg in muscle (as MeHg) in mammals is relatively quick (e.g. $t_{1/2}$ =12 days in rabbits Petersson et al. 1991), suggesting muscle Hg levels reflect recent dietary Hg sources. The relationship of muscle Hg with beluga length, instead of age, suggests muscle Hg concentrations reflect dietary Hg uptake and to a lesser extent

bioaccumulation over time. This observation is supported by the lack of Hg accumulation with age in ringed seal muscle (Atwell et al. 1998), and suggests this relationship can be applied to other marine mammals. We acknowledge that Hg will accumulate in beluga muscle with time, but it appears that diet and the process of Hg food web biomagnification are the predominant factors explaining Hg levels in muscle. Thus, muscle tissue is a better indicator of dietary Hg reflecting biomagnification processes. Because Hg concentrations in muscle better reflect dietary sources, it can be used as a biomarker for feeding preferences among beluga size ranges. Mercury in beluga skin was not presented here but has strong linear relationship with muscle Hg (Wagemann et al. 1998). Beluga skin is similar to fur or hair in mammals, reflecting the Hg body burden (Young et al. 2001); thus, we support the use of skin biopsy samples to estimate the dietary Hg loads.

4.3.4 Biomarkers and Mercury

Stable isotopes and fatty acids were both important biomarkers describing beluga Hg concentrations (Table 4.3). Mercury levels in muscle were best described by the first axis of the fatty acid PCA and liver $\delta^{15}N$ values (Table 4.3). Liver Hg levels were also explained by the fatty acid PCA axis 1 scores and liver $\delta^{15}N$ values, in addition to liver $\delta^{13}C$. The best individual variables to explain liver Hg was the fatty acid PCA axis 1 score, whereas liver $\delta^{15}N$ values best explained muscle Hg concentrations (Table 4.3). These relationships provide information relating to biochemical processes and relative metabolic rates in beluga. For example, positive relationships between muscle Hg and liver $\delta^{15}N$ may support similar metabolic turnover rates. Protein turnover rates in liver are relatively fast (e.g. humans 15-54% d^{-1} Welle

Table 4.3. Comparison of best fit regressions to describe beluga mercury in liver and muscle using the lowest ΔAIC_c regression with the diet biomarkers stable isotopes in liver and muscle and fatty acid PCA scores from first and second axis

Best Fit Regressions Dependant*Response	R^2	P_{value}	AIC_c [1]	$\Delta_i AIC_c$ [2]	w_i [3]
Log_{10}Liver Hg					
*PCA1 Liverδ^{15}N Liverδ^{13}C	0.53	< 0.001	-82.824	0.000	0.133
*PCA1 Liverδ^{13}C Muscleδ^{15}N Muscleδ^{13}C	0.55	< 0.001	-82.041	0.783	0.090
*PCA1	0.39	< 0.001	-76.286	6.538	0.005
Log_{10}Muscle Hg					
*PCA1, Liverδ^{15}N	0.43	< 0.001	-185.788	0.000	0.217
*PCA1 Liverδ^{15}N Liverδ^{13}C	0.46	< 0.001	-184.977	0.812	0.145
*Liverδ^{15}N	0.35	< 0.001	-183.592	2.196	0.072

[1] AIC_c = second order Akaike information criteria ($AIC = n \log(\sigma^2) + 2K$) bias adjusted AIC for small sample size = AIC + (2K(K+1/(n-K-1) where K is the total number of estimated regression parameters including σ^2 (no intercept) and n is sample size.

[2] Δ_i = AIC differences computed as $AIC_i - AIC_{min}$

[3] $w_i = \exp(-1/2D_i)/S\exp(-1/2D_r)$

1999), and Hg in the form of MeHg has been shown to be transported relatively quickly from muscle to liver in some animals (e.g. $t_{1/2}$ 12 days in rabbits Petersson et al. 1991). Both metrics support quick metabolic processes and their association may support harmonious rates. Therefore, Hg concentrations in muscle are best described by liver $\delta^{15}N$ rather than muscle $\delta^{15}N$, reflecting a recent diet and the relative diet associated trophic level.

The weak $\delta^{13}C$ relationships with both the biological variables and beluga Hg levels suggest that carbon turn over rates in beluga tissues are different than Hg in tissues. $\delta^{13}C$ may have a longer turn over rate than protein ($\delta^{15}N$) in liver and muscle, suggesting concentrations measured here reflect a diet from a different feeding period relative to Hg and $\delta^{15}N$ values. $\delta^{13}C$ values in liver and muscle are more similar to $\delta^{13}C$ in prey items measured in the Bering Sea (Hobson et al. 1997) relative to $\delta^{13}C$ in Beaufort Sea prey (Chapter 3). Given that the Beaufort Sea beluga population spend the winter in the Bering Sea, and $\delta^{13}C$ enrichment is usually near 1‰ it appears that the carbon isotopes reflect their winter diet.

4.5 Conclusions

Here we use fatty acids and stable isotopes as diet biomarkers to evaluate the consequences of feeding variation on beluga Hg concentrations. Fatty acid signature analysis in concert with the redundancy analysis of biological variables provided support for previously hypothesized feeding variation among beluga driven by differences in habitat use (Chapter 2, 3). The analysis of fatty acids provided motivation for the investigation of length effects on diet and Hg uptake. For the first time we demonstrated that beluga muscle Hg levels reflect food web Hg biomagnification processes rather than bioaccumulation over time which is best described in liver Hg levels. Beluga length defines summer habitat use likely associated with energy requirements causing differences in diet. Here we show these processes lead to differences in dietary Hg uptake. Diet biomarker comparison between fatty acids and stable isotopes revealed that behavioural influences on dietary Hg uptake are better described by fatty acid analysis. Stable isotopes provide information pertinent to trophic feeding and energy sources in addition to tissue Hg uptake processes. Together, stable isotopes and fatty acids were strong predictors of beluga Hg concentrations. Thus, stable isotopes, particularly $\delta^{15}N$, and fatty acids are in general agreement, proving both are beneficial when describing dietary Hg uptake, but different enough to provide greater information together than alone.

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5.0 Beaufort Sea Beluga Diet described by Fatty Acid Signature Analysis and Insights into Arctic cod Distribution

ABSTRACT

Beaufort Sea beluga whales *(Delphinapterus leucas)* are numerous top carnivores in the eastern Beaufort Sea and likely influence top down trophic dynamics. The specific diet composition of this beluga population is not known. Therefore, to evaluate beluga diet, potential prey items were collected in the beluga summer region and discriminated from one another using multivariate analysis of fatty acids. Fatty acids effectively partitioned prey items into groups associated with their habitat and ecology. Next, the relative contribution of various prey items to beluga diet was investigated as a function of body size. Beluga appeared to feed predominantly on pelagic fish, particularly, Arctic cod and Pacific herring. However, diet varied as a function of body size, whereby larger beluga preferred offshore Arctic cod, and medium and smaller sized beluga preferred Arctic cod collected from the Mackenzie shelf as well as other nearshore fish species. Juvenile beluga had a more diverse fatty acid composition than larger whales suggesting a more complex diet, likely caused by inexperience and/or diving limitations. Beluga habitat use matched feeding on different Arctic cod and provided new information about Arctic cod occurrence and behaviour. The observed relationships among beluga diet, body size and habitat use were supported by mercury and stable isotopes measurements we have made in previous studies.

5.1 Introduction

Beluga (*Delphinapterus leucas*) are one of the most abundant odontocetes in Arctic waters (Brodie 1989), and thus likely play an important role in food web structure and function; influencing prey abundance and food web dynamics. Arctic cod (*Boreogadus saida*) is thought to be an important forage species for some beluga populations (Seaman et al. 1982, Welch et al. 1993, Dahl et al. 2000); however, redfish (*Sebastes marinus*), halibut (*Reinhardtius hippoglossoides*), and shrimp (*Pandalus borealis*) were found to be important to Greenlandic beluga (Heide-Jorgensen 1994), and Pacific salmon (*Oncorhynchus* spp.) are important food items for Alaskan beluga populations (Frost and Lowry 1981). These observations suggest beluga diet may be population specific and vary with habitat, seasonal prey abundance and diet preferences.

During the summer, Beaufort Sea beluga segregate into various habitats defined by differences in coverage of sea ice and bathymetry (Chapter 2). Those beluga that select ice edge habitats may feed preferentially on Arctic cod (Chapter 2, 3) which is an important species in the low diversity Arctic food web (Frost and Lowry 1981, Bradstreet and Cross 1992, Welch et al. 1992) that is associated with sea ice (Lonne and Gulliksen 1989, Gradinger and Bluhm 2004). Warming in the western Canadian Arctic where the Beaufort Sea beluga spend summer, has resulted in declining sea ice extent (Serreze et al. 2007). Depletion of sea ice may impact the ecosystem stability by disrupting the predator-prey dynamics that are closely coupled to the sea ice habitat.

The diet of Beaufort Sea belugas is not well known because harvested animals usually have empty stomachs, and feces cannot be found; yet, local hunters have

observed beluga feeding in the Mackenzie River estuary (Harwood and Smith 2002). Advancements in the use of biomarkers such as stable isotopes and fatty acids have reduced the need to collect stomach contents to determine animal diets (e.g.(Hobson and Welch 1992, Iverson 1993). For example, diet biomarkers can overcome biases inherent to stomach content analyses including the over-or under-representation of prey that were recently eaten or quickly digested (Tollit et al. 1997). Recent analysis of fatty acids in Beaufort Sea beluga revealed that diet varied in relation to body size (Chapter 4) describes beluga habitat selection (Chapter 2). However, specific diet items of Beaufort Sea beluga have not yet been investigated with fatty acids.

Stable isotopes are less useful for determining beluga diet because they represent integrated values from several prey species that have similar isotopic values (Chapter 3). The use of fatty acids as biomarkers may assist with differentiation among prey types because 41 individual fatty acids are biotransferred from prey to predator many of which undergo little degradation during digestion that are then stored in the blubber tissue of the predator (Iverson 1993, Iverson et al. 2004). Fatty acid signature analysis has successfully characterized trophic links within and among species (Iverson et al. 1997, Budge et al. 2002, Stevens et al. 2004a, 2004b, Richoux et al. 2005) as well as determined predator diets in marine and terrestrial mammals (Iverson et al. 2001, Bradshaw et al. 2003).

The Beaufort Sea beluga population summers where several coastal, anadromous and marine fish occur, in addition to invertebrates and bottom-feeding fish. In a previous paper, we examined the trophic transfer of mercury from prey to beluga by relating beluga diet to habitat use and to habitat-specific food webs (Chapter 3).

Habitat use by differed sized whales 1) smaller-sized beluga that use shallow, open-water habitats were paired with an estuarine-shelf food web from the Mackenzie River delta; 2) medium-sized adult beluga that select ice edge habitats were paired with a pelagic food web that included Arctic cod as the main prey species; and 3) largest male belugas that select heavy sea ice concentrations over deep waters were paired with the epibenthic food web. Although, these three defined feeding groups are a simplification of the temporal and spatial complexities involved in beluga movement and seasonally changing feeding behaviour, the results provided insight to beluga diet which may be further explored with fatty acid analysis. The importance of habitat use and body size for beluga feeding preferences was supported by the fatty acid analysis (Chapter 4).

In this study, we evaluate the beluga and food web pairings in Chapter 3 using fatty acid signature analysis of Beaufort Sea beluga and their potential prey. First, we determined if prey items collected in the beluga summering region can be discriminated from one another using multivariate analysis of fatty acid profiles. Next, we assessed the relative importance of various prey items to beluga diet across a beluga body size range that reflects variable habitat use.

5.2 Materials and Methods

5.2.1 Sample collection

5.2.1.1 Beluga

Beluga tissue samples were collected during local harvests at Hendrickson Island near the community of Tuktoyaktuk, and at Browns Harbour near the community of Paulatuk, in the Inuvialuit Settlement Region, Northwest Territories Canada (Figure 5.1). A total of 43 samples were collected in July, from Tuktoyaktuk in 2004 (n = 19)

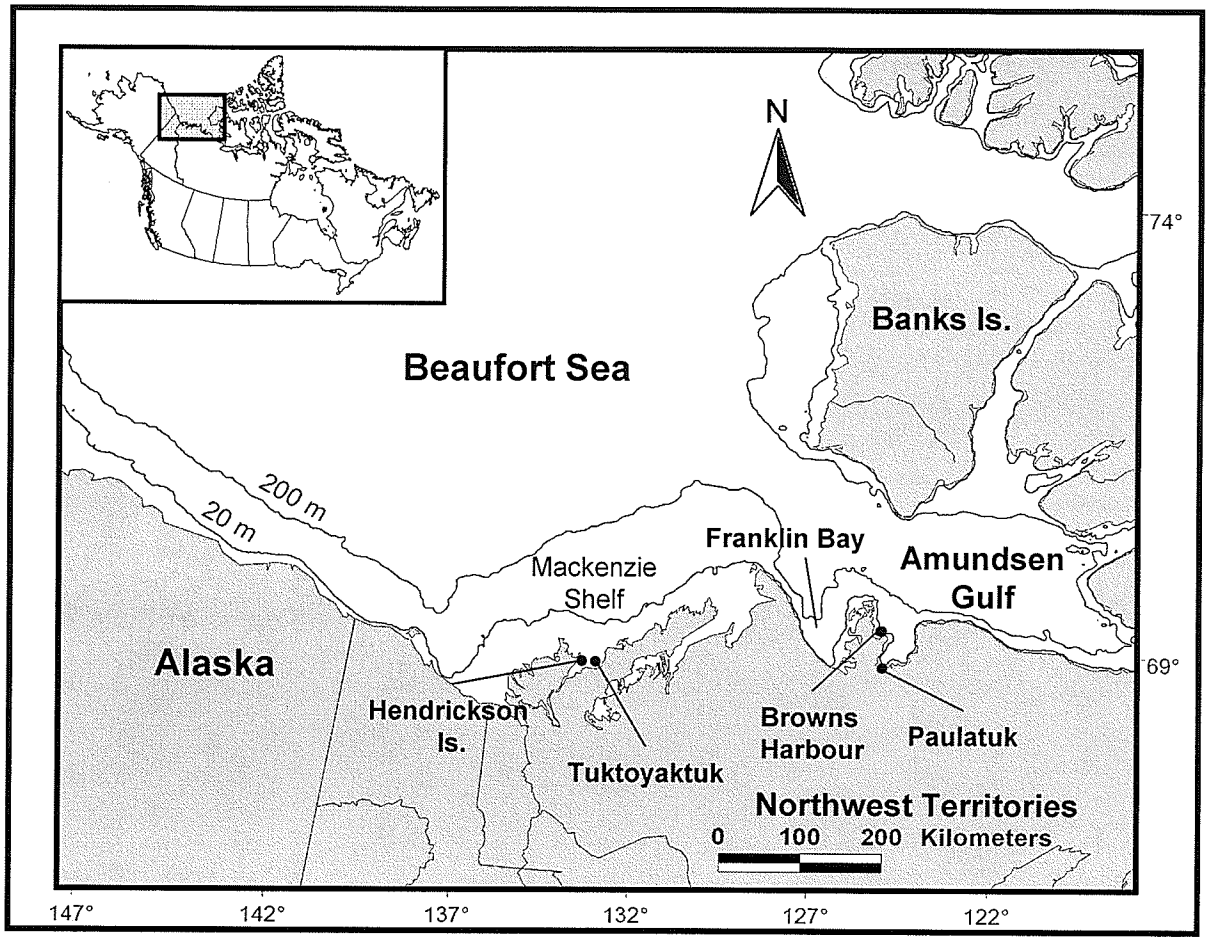

Figure 5.1. Study area of beluga and food web sampling regions: Amundsen Gulf, Franklin Bay, Mackenzie Delta, eastern Beaufort Sea. The 20m isobath north of the Mackenzie outflow is where estuarine fish were collected, whereas the 200m isobath separates the Mackenzie shelf from the eastern Beaufort Sea. Hendrickson Island is the harvest location for the community of Tuktoyaktuk, and Browns Harbour is the harvest location for the community of Paulatuk Northwest Territories, Canada.

and 2005 (n =13) and from Paulatuk in 2005 (n = 11). Two of the youngest beluga were collected from Paulatuk, one of which was a young of the year (1.9 m) and the other was eight years old and sexually immature (Robeck et al. 2005). All these belugas with the exception of the one year old were evaluated for mercury, fatty acids and stable isotopes in (Chapter 4). Few females were collected from the harvests because hunters generally select for medium to larger sized males (n = 9). Beluga blubber was collected for fatty acid analysis as described in Chapter 4. Samples were frozen at -20°C on site, stored in portable freezers and shipped to Fisheries & Oceans Canada in Winnipeg for analysis. Ages of beluga were determined from a thin section of a tooth by counting growth layer groups in the dentine (Stewart et al. 2006).

5.2.1.2 Prey items

Prey samples were collected in the summer and fall of 2004 to 2005 from the eastern Beaufort Sea, the Mackenzie River Delta, Franklin Bay and Amundsen Gulf (Figure 5.1). A summary of prey mercury concentrations, stable isotopes and morphometrics is provided in Table 3.2 in Chapter 3 Abbreviations for the names of prey presented in this paper can be found in Table 5.1.

Arctic cod were collected from March 15 to May 27 2004. Most of the fish were collected in Franklin Bay using a 90-m long gill net set vertically from the bottom (i.e., 230 m). Most of the fish were captured in the lower 100m of the net and represent offshelf Arctic cod. In addition, Arctic cod were collected in September 2006 from the CCGS *Nahidik* between 10 and 100 metre depths north of the Mackenzie River

Table 5.1 Short hand used to describe prey items collected in the eastern Beaufort Sea in figures

Prey item	short hand
Pacific herring	PHR
Arctic cisco	ACS
Least cisco	LCS
Rainbow smelt	RST
Saffron cod	SCD
Arctic cod (offshore)	ACD
Arctic cod (shelf)	SACD
Starry Flounder	SFL
Arctic Flounder	AFL
Lake white fish	LWF
Themisto libellula	TLIB
Mysids	MYS
Shrimp	SHP
Anonyx	ANX
A. malmgremi	AMI

outflow, representing Mackenzie shelf Arctic cod. Only adult Arctic cod longer than 110 mm were selected for analysis.

Fish in the brackish water of the Mackenzie Delta were collected from the shoreline out to the 20m isobath via community-based sampling programs and on board the CCGS *Nahidik* using gill nets. Species collected near the shoreline from community based sampling included rainbow smelt (*Osmerus mordax)*, arctic cisco (*Coregonus autumnalis),* least cisco (*Coregonus sardinella)*, and species collected from the CCGS *Nahidik* included pacific herring *(Clupea palasii)*, and saffron cod (*Eleginus gracilis*). Fish characteristic of the epibenthic habitat, such as the starry flounder *(Platichthys stellatus),* arctic flounder (*Pleuronectes glacialis)* and fourhorn sculpin (*Myoxocephalus quadricornis*) were also collected in this region using gill nets from shore and the CCGS *Nahidik.* For our study, all fish species will be referred to by their common names. Only adult fish were used (> 200 mm).

Epibenthic animals were collected in the fall of 2003 and spring/summer of 2004 with a modified MACER-GIROQ sled that collected organisms living directly on and within 60 cm of the seafloor (Choe and Deibel 2000). The sled was equipped with a 500-μm net, a partially closed cod end, and a door that opened when the sled was in contact with the sea floor. As soon as the sled came on board, the contents of the cod end were gently rinsed and placed into coolers. Cooler contents were then rinsed with surface water to remove mud from the samples. The decapods *Eualus* spp. and *Bythocaris* spp. (which will be referred to as 'shrimp'), the amphipods *Anonyx* spp. and *Acanthostephia malmgreni,* as well as four mysid genera which were pooled (*Psuedamma* spp., *Erythrops* spp., *Mysis* spp., and *Michthyops* spp.) were picked from

the samples and identified onboard ship. *Themisto libellula* were collected during the 2004 fall cruise of the CCGS *Amundsen* using integrated vertical tows taken with a double Tucker Trawl (200 μm mesh and 500 μm mesh) and from depth-stratified samples taken with a Hydrobios multi-net (200 μm mesh).

5.2.2 Fatty acid extraction

Lipids were extracted from the inner blubber layer of beluga and from whole fish. Beluga blubber extraction was previously described in Chapter 4. Whole homogenized fish and invertebrate samples were used to give the best representation of the prey fatty acid signatures in beluga diet (Budge et al. 2002). Fish and larger invertebrates were homogenized in a blender and subsampled in duplicate. Lipids were extracted from 1.5g of the prey homogenate using a 2:1 chloroform-methanol (30ml) mixture containing 0.01% BHT (v/v/w) to minimize oxidation during sample processing (Folch et al. 1957). The lipid phase was collected, washed, and filtered through anhydrous sodium sulphate and evaporated under nitrogen to obtain the total lipid weight. The lipid was used to prepare the fatty acid methyl esters by transesterfication with Hilditch reagent (0.5 N H_2SO_4 in methanol). The samples were heated for 1h at 100°C. Fatty acid methyl ester samples were analyzed using gas chromatography (GC; Hewlett Packer HP Series 6890) with a mass spectrometer detector (Hewlett Packard 5973). Inlet temperature was 250°C, and the temperature program started at 153°C for 2 min, then ramped up at 2.3°C min^{-1}, was held at 174°C for 0.2 min and then ramped up at 2.5°C min^{-1} and hold at 220°C for 3 min, as described previously (Budge et al. 2002). A silica column (30m x 0.25mm ID) coated with 50% cyanopropyl polysiloxane (0.25 μm film thickness; J&W DB-23) was used.

Helium was the carrier gas and was equipped with an oxygen scrubber. Up to 66 fatty acid methyl esters were identified according with to verification by of ion mass spectroscopy and known standard mixtures (Nu Check Prep.). To analyze the smaller invertebrates including *A. malmgreni,* mysid genera and *T. libelulla* the method for small aquatic invertebrates was used (Parrish 1999). *A. malmgreni,* mysids and several shrimp and *Anonyx* were analyzed at Memorial University, Newfoundland (C. Parrish). Fatty acid methyl esters were derivatized using BF3-methanol and extracts were analyzed on Varian 3400 GC on an Omegawax column with a flame ionization detector (FID) following Budge and Parrish (1998).

Fatty acid peaks were integrated and expressed as a mass percent of total fatty acids. Fatty acid identifications were checked on all chromatograms and reintegrated if necessary. Each fatty acid was described using the shorthand nomenclature of A:Bn-X, where A represents the number of carbon atoms, B the number of double bonds, and X the position of the double bond closest to the terminal methyl group.

5.2.3 Data Analysis

Percent fatty acid values were log transformed prior to analyses to normalize the data. Although 65 fatty acids were identified, only the fatty acids known to biotransfer (Iverson et al., 2004) were used in the analyses below. Multivariate analyses were carried out using SYN-TAX© Ordination 2000 (Budapest, Hungary) while Systat 11® (Systat Software Inc., 2004, San Jose, CA) was used for univariate statistics.

5.2.3.1 Food web and Prey Discrimination

Mean percent fatty acid content of prey items was explored using a principle component analysis (PCA) of the covariance matrix. All prey listed in the Methods section above were included in the analysis. The results from the PCA were used to place prey items into ecological prey groups that may represent potential prey types to beluga. A discriminant function analysis (DFA) (with cross-validation Jackknife tests) was used to discriminate among apparent prey groups using first and second PCA axis scores of individual prey items, rather than mean values. Prey groups were created to assist in differentiating among prey items that were similar as well as to evaluate their relative importance to beluga diet. The three lower trophic level organisms *T. libellula*, mysids and *A. malmgreni* were not included in the ecological prey groups in the DFA because they are not likely part of the beluga diet (Chapter 3).

5.2.3.2 Beluga Dietary Preference

The fatty acid signature of the inner beluga blubber layer reflects its diet; however, modification of some fatty acids occurs during uptake and metabolism. Fatty acid metabolism has been measured and modelled in pinnipeds to quantify the proportion of prey species in the predator diet (c.a., QFASA, Iverson et al. 2004). Metabolic modification limits the opportunity of quantifying beluga diet in this study, however, multivariate ordination techniques such as PCA and DFA have succeeded for qualitative diet analyses (Iverson et al. 1997, Dahl et al. 2000, Budge et al. 2002, Bradshaw et al. 2003). Thus, in this paper we used a PCA combining prey and beluga fatty acids to show how beluga compares in relation to possible prey items. Due to metabolic processes, we expect beluga to plot separately from prey. Therefore, to interpret this PCA we examined results from separate prey and beluga analyses. The

fatty acids that best described beluga variation were selected based on highest and lowest PCA axis factor loadings (> or < ± 0.015) (Chapter 4) for two evaluations. First we examined whether the beluga length relationship (previously found to relate to beluga fatty acid profiles) was maintained in the combined prey and beluga PCA using linear regressions. Second, we examined the abundance of these key beluga fatty acids among the ecological prey groups using an analysis of variance. To support the methods above as well as to demonstrate how to interpret the PCA plot we show the relationships between beluga and prey PCA scores and levels of a key fatty acid (20:1(n-9)).

5.3 Results

5.3.1 Food web and Prey Differentiation

In the food web PCA, the first axis separated pelagic, brackish water and bottom-feeding fish from each other (57% variance explained) (Figure 5.2a). The two Arctic cod groups (ACD and SACD) had positive scores on the first axis, but were further apart from one another than were two of the flounder species with negative scores on the first axis (SFL and AFL; Figure 5.2a). The second PCA axis separated fish from invertebrates, accounting for 13% of the variance.

The DFA supported the segregation observed in the PCA of the different prey groups by habitat and feeding behaviour. The following prey groups were defined: 1) Invertebrates, including shrimp and *Anonyx*. 2) Bottom-feeding fish, including lake white fish and two flounder species. 3) Pelagic fish, including Arctic cod from two sites

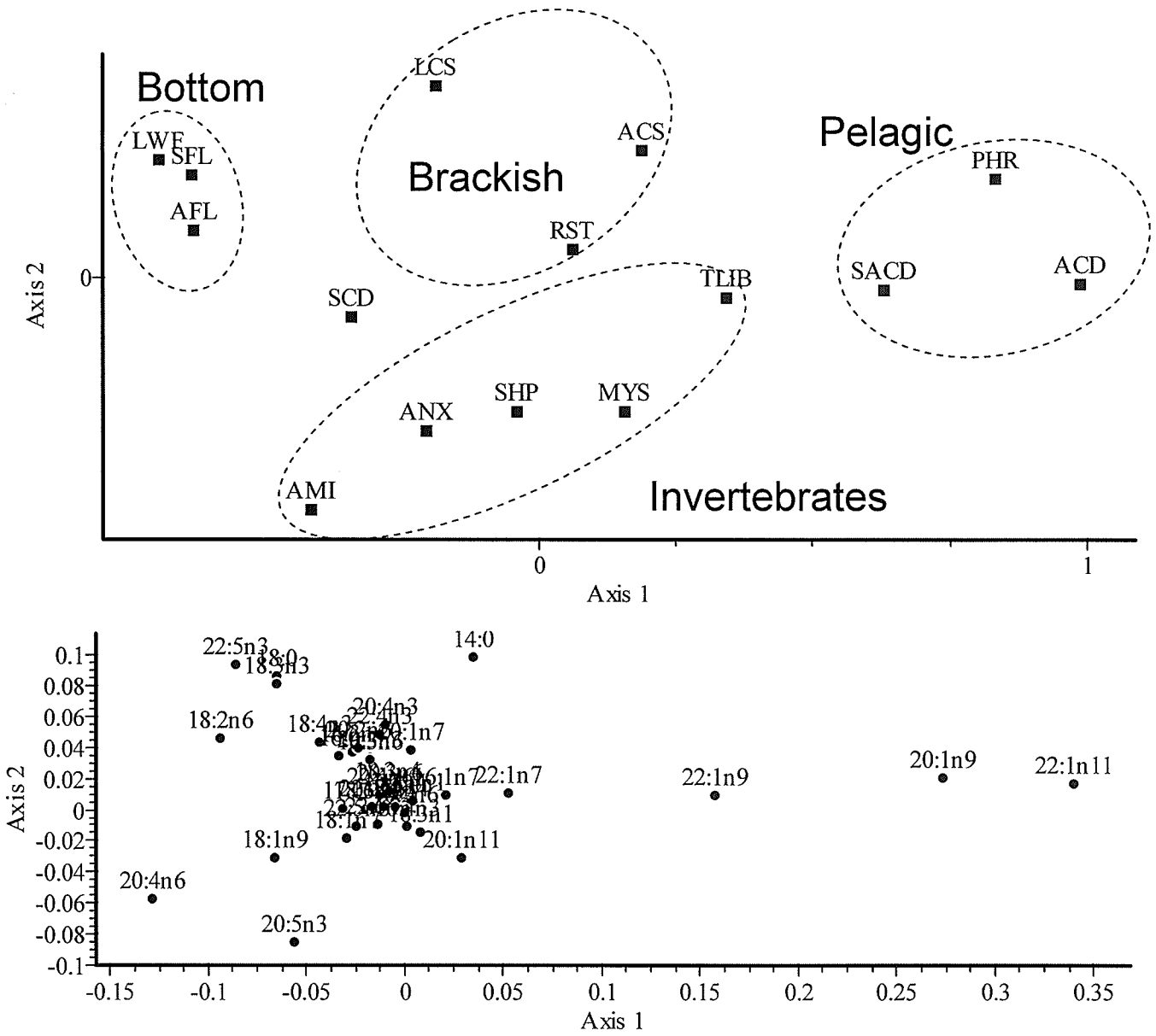

Figure 5.2. Principle component analysis of prey fatty acids (axis 1: 57%; axis2: 13% of the total variance) **A.** Prey plot, dashed lines representing groups discriminated using a discriminant function analysis. **B.** Variable plot of fatty acids known to biotransfer. Fatty acid positions describe the position of prey on the species plot, and the location indicates the importance, those in the centre have the lowest importance relative to those furthest from the origin.

and Pacific herring. 4) Brackish water fish, including least cisco, Arctic cisco, and rainbow smelt. 5) Saffron cod comprised a distinct group since it did not appear to fit into any of the other prey groups. Although there was some overlap, the above five prey groups were statistically distinguishable ($P < 0.001$, Table 5.2), indicating prey community differentiation along 2 gradients in the large study region; an offshore-nearshore (shelf) gradient and a depth gradient. Pelagic fish were most dissimilar from the other prey groups (Figure 5.2a). Although bottom-feeding fish likely have a different food source than do brackish water fish, their proximity to the brackish fish on the PCA may arise because they were all collected from the Mackenzie Shelf region. The factor loading plot revealed that the long chain monounsaturated C_{20} and C_{22} were important fatty acids driving the prey distribution along the first axis, whereas 22:5(n-3), 18:3(n-3) and 20:5(n-3) were important in driving the second axis (Figure 5.2b)

5.3.2 Beluga Dietary Preference

Eighty percent of the variance in the prey and beluga fatty acid composition was explained by the first two PCA axes (axis 1: 67.5%, axis 2: 12.4%)(Figure 5.3). Individual beluga scores plotted similarly to scores in the PCA of beluga only (Chapter 4) and prey ecological groups observed in the food web PCA were conserved in the combined food web and beluga PCA (Figure 5.2). As expected, prey and beluga scores plotted separately on the combined PCA due to metabolic alterations of fatty acids by beluga. However, the diagonal orientation of points in Figure 5.3 contains important information requiring interpretation. The first PCA axis shows that the beluga diet was most similar to pelagic prey and least similar to near-bottom prey (Figure 5.3a). The second axis highlights important trends for beluga and prey, where beluga is distributed

Table 5.2 Summary of the fatty acids known to biotransfer as % fatty acids of the total, in ecological prey groups.
Marine fish: pacific herring and arctic cod from shelf and offshore; Brackish fish: Rainbow smelt, least cisco, arctic cisco,
Bottom-feeding fish: lake white fish, starry and arctic flounder; Invertebrates: shrimp and anonyx.
Results from Discriminant function Jacknife reclassification listed as percent

	Ecological Prey Groups				
	Marine Fish (n = 68)	Brackish (n = 49)	Saffron Cod (n = 12)	Bottom Fish (n = 27)	Invertebrates (n = 40)
Saturated FA (%)					
14.00	3.36 ± 0.13	4.15 ± 0.10	2.38 ± 0.18	3.25 ± 0.11	1.80 ± 0.15
16.00	12.44 ± 0.30	15.73 ± 0.28	15.96 ± 0.63	15.04 ± 0.31	12.82 ± 0.42
17.00	0.08 ± 0.01	0.16 ± 0.02	0.18 ± 0.01	0.30 ± 0.01	0.17 ± 0.02
18.00	1.84 ± 0.08	2.37 ± 0.10	3.18 ± 0.16	3.16 ± 0.16	1.73 ± 0.16
Subtotal	17.72	22.41	21.70	21.74	16.52
Monounsaturated fatty acids (%)					
16:1(n-7)	14.61 ± 0.48	15.51 ± 0.53	13.44 ± 1.13	15.65 ± 0.63	14.51 ± 0.75
18:1(n-9)	6.71 ± 0.19	10.02 ± 0.43	11.24 ± 0.67	10.71 ± 0.39	13.02 ± 1.10
18:1(n-7)	4.65 ± 0.15	4.84 ± 0.12	6.44 ± 0.35	5.78 ± 0.34	8.80 ± 0.76
20:1(n-11)	1.04 ± 0.07	0.39 ± 0.04	0.18 ± 0.02	1.03 ± 0.17	0.79 ± 0.11
20:1(n-9)	10.85 ± 0.42	4.44 ± 0.33	2.33 ± 0.24	0.91 ± 0.05	3.60 ± 0.71
20:1(n-7)	1.75 ± 0.08	1.42 ± 0.12	0.76 ± 0.08	2.48 ± 0.32	1.51 ± 0.14
22:1(n-11)	10.79 ± 0.47	3.27 ± 0.41	0.70 ± 0.27	0.17 ± 0.03	2.57 ± 0.68
22:1(n-9)	2.31 ± 0.12	0.80 ± 0.08	0.21 ± 0.07	0.12 ± 0.01	0.66 ± 0.16
22:1(n-7)	0.63 ± 0.04	0.33 ± 0.04	0.06 ± 0.02	0.13 ± 0.01	0.36 ± 0.05
subtotal	53.34	41.02	35.35	36.98	45.82
Polyunsaturated fatty acids (%)					
16:2(n-6)	0.03 ± 0.00	0.05 ± 0.00	0.03 ± 0.01	0.06 ± 0.00	0.01 ± 0.00
16:2(n-4)	0.28 ± 0.01	0.18 ± 0.01	0.19 ± 0.02	0.41 ± 0.04	0.20 ± 0.03
16:3(n-6)	0.41 ± 0.02	0.52 ± 0.02	0.38 ± 0.04	0.63 ± 0.03	0.25 ± 0.03
16:3(n-4)	0.18 ± 0.01	0.44 ± 0.03	0.30 ± 0.05	0.51 ± 0.04	0.14 ± 0.02
16:4(n-3)	0.07 ± 0.01	0.07 ± 0.01	0.03 ± 0.01	0.06 ± 0.01	0.17 ± 0.03
16:4(n-1)	0.18 ± 0.02	0.40 ± 0.03	0.17 ± 0.03	0.32 ± 0.11	0.15 ± 0.03
18:2(n-6)	0.54 ± 0.02	1.17 ± 0.11	0.89 ± 0.07	2.48 ± 0.22	1.03 ± 0.11
18:2(n-4)	0.14 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	0.16 ± 0.01	0.19 ± 0.02
18:3(n-6)	0.08 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	0.30 ± 0.02	0.18 ± 0.02
18:3(n-4)	0.05 ± 0.01	0.18 ± 0.01	0.08 ± 0.02	0.10 ± 0.01	0.11 ± 0.04
18:3(n-3)	0.29 ± 0.02	0.86 ± 0.19	0.41 ± 0.06	1.23 ± 0.08	0.32 ± 0.06
18:3(n-1)	0.06 ± 0.00	0.06 ± 0.02	0.04 ± 0.01	0.06 ± 0.01	0.01 ± 0.00
18:4(n-3)	0.50 ± 0.04	1.41 ± 0.13	0.93 ± 0.08	1.28 ± 0.06	0.79 ± 0.10
18:4(n-1)	0.06 ± 0.00	0.17 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	0.08 ± 0.02
20:2(n-6)	0.18 ± 0.01	0.33 ± 0.02	0.37 ± 0.04	0.42 ± 0.03	0.18 ± 0.02
20:3(n-6)	0.06 ± 0.01	0.11 ± 0.01	0.08 ± 0.02	0.14 ± 0.01	0.07 ± 0.01
20:4(n-6)	0.45 ± 0.03	0.73 ± 0.05	1.47 ± 0.14	1.83 ± 0.08	1.97 ± 0.25
20:3(n-3)	0.09 ± 0.01	0.20 ± 0.02	0.15 ± 0.02	0.24 ± 0.02	0.07 ± 0.05
20:4(n-3)	0.36 ± 0.02	0.81 ± 0.05	0.59 ± 0.04	0.49 ± 0.03	0.28 ± 0.03
20:5(n-3)	8.85 ± 0.27	9.92 ± 0.23	16.06 ± 0.95	11.58 ± 0.30	15.72 ± 0.73
22:2(n-6)	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.25 ± 0.07	0.06 ± 0.02
21:5(n-3)	0.15 ± 0.01	0.25 ± 0.01	0.16 ± 0.05	0.27 ± 0.01	0.24 ± 0.02
22:4(n-6)	0.09 ± 0.01	0.12 ± 0.01	0.07 ± 0.03	0.18 ± 0.02	0.07 ± 0.03
22:5(n-6)	0.12 ± 0.01	0.21 ± 0.04	0.19 ± 0.04	0.24 ± 0.02	0.22 ± 0.03
22:4(n-3)	0.16 ± 0.02	0.37 ± 0.05	0.23 ± 0.07	0.28 ± 0.04	0.18 ± 0.06
22:5(n-3)	0.93 ± 0.04	2.18 ± 0.10	1.64 ± 0.20	2.67 ± 0.11	1.04 ± 0.12
22:6(n-3)	9.95 ± 0.51	11.14 ± 0.41	14.75 ± 1.35	9.26 ± 0.67	9.00 ± 0.70
subtotal	24.30	32.22	39.54	35.50	32.74
Total	95.36	95.65	96.60	94.22	95.08
Discriminant classification (% correct)					
	88	55	75	66	72

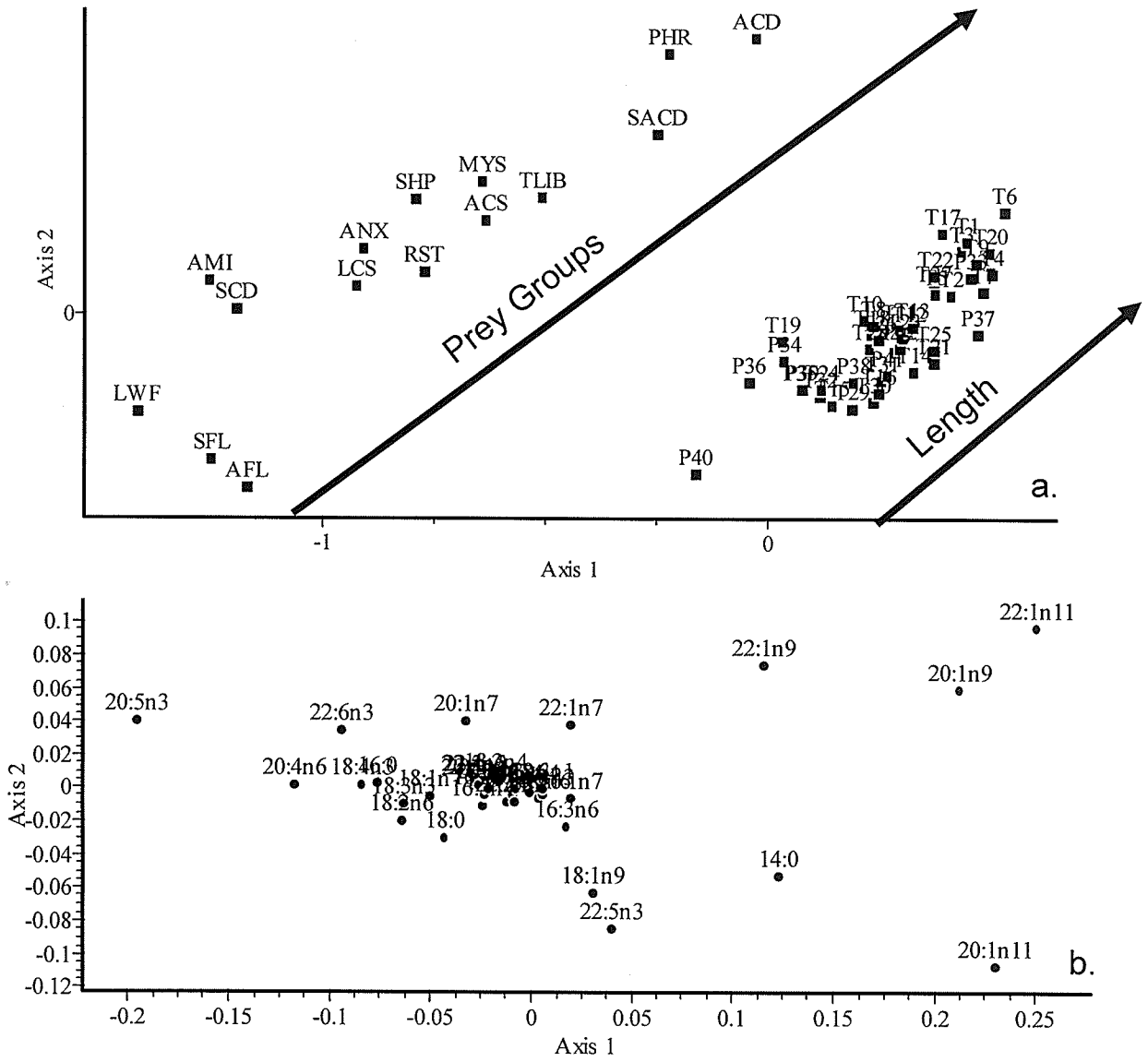

Figure 5.3. Principle component analysis of the 40 dietary fatty acids in prey items and belugas (axis 1: 68%; axis 2: 12%). **A.** Beluga and prey plot show the first and second axis describe the beluga length as well as the marine gradient in prey. Prey names found on table 5.1, beluga labelled T and P correspond to Tuktoyaktuk and Paulatuk. **B.** Variable displaying the fatty acids driving the beluga and prey plot. The location of the fatty acids indicates the importance, those in the centre have the lowest importance relative to those furthest from the origin. Figure 5.4 helps with the interpretation

according to length, and the prey groups are described by their offshore-shelf and depth gradients. The position of prey and beluga scores on PCA axis 1 is strongly correlated with the relative content of the herbivorous copepod signature fatty acid, 20:1(n-9) (Figure 5.4). Accordingly, we found that those fatty acids that were positively related to beluga length were highest in pelagic prey, and those fatty acids that were negatively related to beluga length were lowest in pelagic prey (Table 5.3).

5.4 Discussion

5.4.1 Food web and Prey Differentiation

Fatty acids effectively partitioned prey items into groups associated with their habitat and ecology. Not much is known about the prey species examined here; thus, the fatty acid analysis provided some new information about their feeding ecology. For example, saffron cod was collected in the Mackenzie Shelf region, yet was located near the invertebrates and bottom-feeders in the PCA, revealing clear differences from brackish water and pelagic fish. Invertebrates were separated from fish on the second PCA axis, yet they maintained a consistent pattern with the ecological groups, whereby invertebrates feeding on marine plankton derived material (e.g. mysids, *T. libellula* (Pakhomov and Perissinotto 1996, Richoux et al. 2004)) were farther toward the pelagic end of the axis than those feeding in the near-bottom environment (e.g. flounders) were located toward the bottom-feeding fish on the left end of the first axis.

The fatty acid variable plot (factor loadings) provided important information about those fatty acids most responsible for the separation of prey into ecological groups. Fatty acids separating the pelagic fish Arctic cod and Pacific herring from

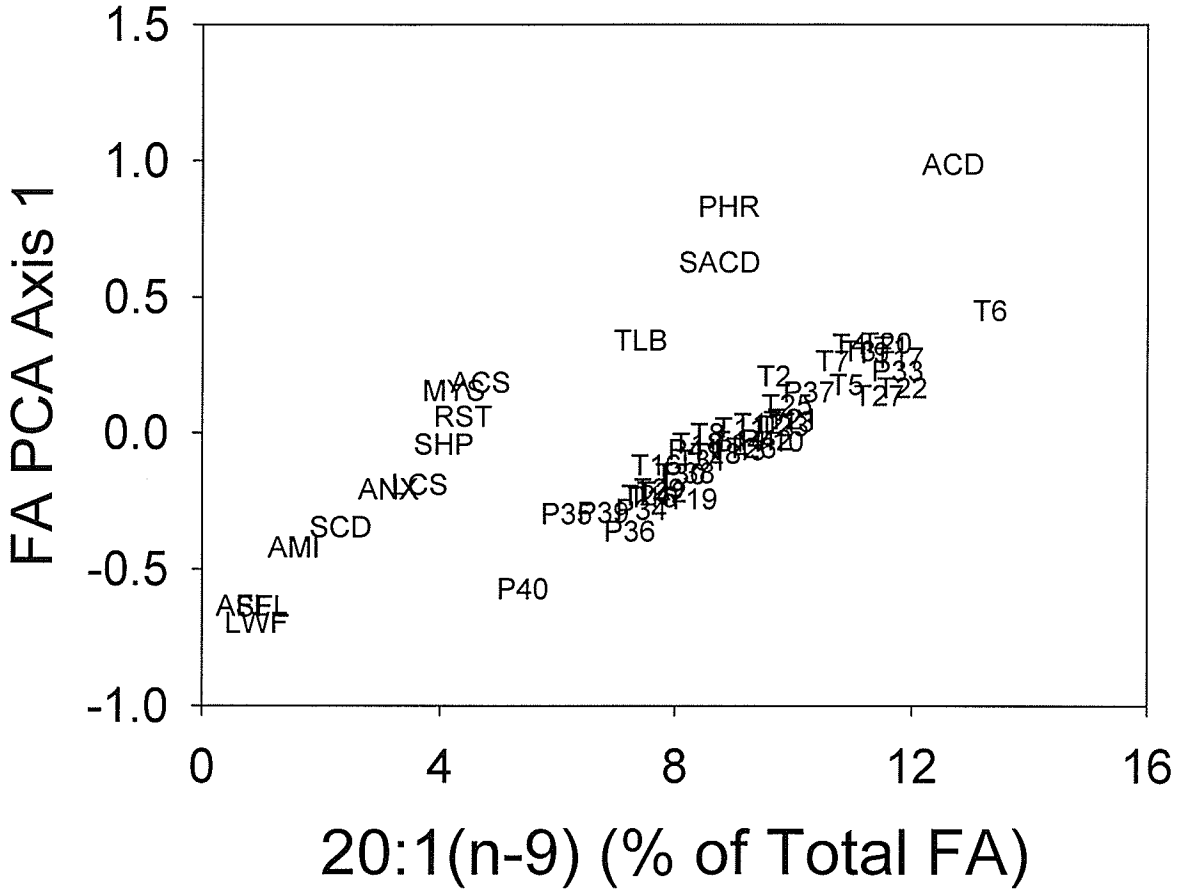

Figure 5.4. The percent of total of the marine FA 20:1(n-9) in all beluga and prey plotted against principle component analysis (PCA) axis 1 scores from separate PCA analyses. This relationship demonstrates a similar trend among prey and beluga for the same fatty acid. Beluga PCA 1 scores relate to beluga length and prey marine diet. Prey abbreviations are found on on Table 5.1 and 'T' and 'P' refer to beluga harvested at Tuktoyaktuk and Paulatuk, Northwest Territories respectively.

Table 5.3. Key fatty acids selected from beluga PCA that had the highest scores (< or > 0.015). Linear regression results of beluga length and individual fatty acids, described as positive or negative (+/-). The mean abundance in ecological prey groups (% of total fatty acids). An ANOVA was used to determine if concentrations differed among the prey groups.

	Beluga				Prey Eco Groups					
	FA Scores		Length Regression		Average Percent of FA of the Total					
FA	PCA1	PCA 2	R^2	P_{value} (+/-)	Brackish	Marine	SCD	Bottom	Inverts	P_{value}
14:00	0.002	-0.028	0.007	0.584(-)	0.723	0.642	0.535	0.643	0.443	< 0.001
16:00	-0.039	0.031	0.232	0.001(-)	1.239	1.141	1.241	1.226	1.155	< 0.001
16:1(n-7)	0.003	-0.074	0.001	0.807(-)						0.3
16:2(n-4)	0.009	-0.013	0.103	0.035(+)	0.075	0.110	0.076	0.151	0.078	< 0.001
16:3(n-6)	-0.004	-0.022	0.000	0.19(+)	0.187	0.154	0.142	0.220	0.097	< 0.001
16:4(n-3)	-0.003	-0.022	0.085	0.057(+)	0.030	0.031	0.012	0.027	0.066	< 0.001
16:4(n-1)	-0.017	0.002	0.152	0.01(-)	0.145	0.071	0.068	0.101	0.060	< 0.001
18:00	-0.034	0.066	0.129	0.018(-)	0.533	0.455	0.629	0.620	0.428	< 0.001
18:1(n-9)	-0.037	-0.018	0.106	0.033(-)	1.043	0.892	1.097	1.082	1.133	< 0.001
18:1(n-7)	-0.034	0.013	0.197	0.003(-)	0.779	0.759	0.881	0.826	0.946	< 0.001
18:4(n-3)	-0.031	0.008	0.131	0.017(-)	0.377	0.170	0.289	0.367	0.243	< 0.001
20:1(n-9)	0.073	0.002	0.260	< 0.001(+)	0.709	1.071	0.523	0.289	0.552	< 0.001
20:1(n-7)	0.063	0.016	0.145	0.012(+)	0.371	0.438	0.247	0.485	0.391	< 0.001
20:5(n-3)	-0.077	0.016	0.359	< 0.001(+)	1.051	1.000	1.241	1.119	1.231	< 0.001
22:1(n-11)	0.108	0.033	0.199	0.003(+)	0.552	1.058	0.202	0.067	0.401	< 0.001
22:1(n-9)	0.106	0.028	0.158	0.008(+)	0.246	0.516	0.079	0.053	0.183	< 0.001
22:1(n-7)	0.041	0.021	0.116	0.025(+)	0.120	0.212	0.025	0.056	0.132	< 0.001
21:5(n-3)	-0.015	0.013	0.091	0.05(-)	0.099	0.064	0.066	0.107	0.094	< 0.001
22:5(n-3)	-0.047	0.054	0.265	< 0.001(-)	0.505	0.290	0.420	0.576	0.302	< 0.001
22:6(n-3)	-0.027	0.028	0.100	0.039(-)	1.091	1.032	1.198	1.003	0.993	< 0.001

brackish water and bottom-feeding fish were the long chain C_{20} and C_{22} monounsaturates. The fatty acids 20:1(n-9) and 22:1(n-11) are well known indicators of calanoid copepod diets, because they accumulate to high levels in copepods (Lee et al. 1971, Sargent and Henderson 1986, Kattner et al. 1989). Calanoid copepods are known to synthesise these fatty acids de novo and at high levels from a phytoplankton diet (Sargent 1976). The C_{20} and C_{22} monounsaturates increase in relative concentration at each trophic level, and thus can be used as a biomarker of food webs based upon the pelagic herbivorous copepods. Alternatively, high levels may result from feeding heavily on copepods. Therefore, we conclude that Arctic cod and Pacific herring either feed directly on calanoid copepods, or on predators of copepods, similar to observations made in the Barents Sea (Dahl et al. 2000) and in Prince William Sound (Iverson et al. 2002).

The separation between offshore and shelf Arctic cod on axis 1 of the PCA plot suggests that the offshore fish were feeding at a higher trophic level or more heavily on calanoid copepods than were Arctic cod collected on the shelf. Stable isotope analysis supports the former possibility, because offshore Arctic cod had higher $\delta^{15}N$ values (Chapter 3). Therefore, it is likely that offshore Arctic cod had a diet comprised of both copepods and the copepod predator *T. libellula*, which has also been observed in the guts of Barents Sea Arctic cod (Lonne and Gulliksen 1989). On the other hand, the C_{20} and C_{22} levels may also describe habitat, whereby the offshore Arctic cod were exposed to higher levels associated with regional food web sources; however, without a measurement of fatty acids and $\delta^{15}N$ levels at the bottom of the food web it is difficult

to discern if diet or habitat use differences resulted in observed trends among the Arctic cod.

Although lake white fish and flounders have very different morphologies. their similar bottom feeding ecology was revealed by their similar fatty acid signatures. The essential fatty acid 20:5(n-3) (eicosapentaenoic acid; EPA) had a high, negative factor loadings on the first PCA axis, indicating they were high in bottom-feeding fish (Kakela et al. 2005), as has been reported in other Arctic benthic organisms (Graeve et al. 1997). Fish are unable to synthesize essential fatty acids; therefore, nutritional requirements must be met by dietary sources. The EPA originate primarily from diatoms (Viso and Marty 1993) and suggesting that bottom-feeding fish depend on a food web based upon sinking phytodetritus from water column blooms.

Other fatty acids that scored high on the negative axis included 20:4(n-6) (arachidonic acid; AA), 18:2(n-6) and 18:3(n-3) that were found to reflect a non-marine, freshwater diet source in harbour seals (*Phoca vitulina*) (Smith et al. 1996). These fatty acids may reflect terrigeneous carbon sources to both the bottom feeding fish and the brackish water fish. The brackish water fish were not grouped tightly together, suggesting relatively high inter-specific variability in their feeding ecology. Their placement in the center region of the first PCA axis suggests that their diet may include fatty acids from both a pelagic food web where carbon sources originate from phytoplankton, as well as from a benthic food web where carbon can have terrigeneous and phytodetritus carbon sources. Thus, the first PCA axis describes the 'depth and shore' food web sources to evaluate trends in the beluga diet, whereby offshore Arctic

cod have the highest C_{20} and C_{22} monounsaturate levels and lowest terrigenous fatty acids (e.g. EPA, 18:2(n-6)) and the reverse is true for the bottom-feeding fish.

5.4.2 Beluga Dietary Preference

Results from the combined beluga and prey PCA indicate that pelagic prey such as Arctic cod and Pacific herring may be the most important prey items for adult Beaufort Sea beluga, whereas bottom-feeding fish did not appear to be as significant. Similar results have been found for beluga diets estimated from fatty acid near Svalbard (Dahl et al. 2000, Birkeland et al. 2005). Here, we found a shore gradient in beluga fatty acids, whereby the largest beluga had the highest offshore diet signal (e.g. high 20:1(n-9) and 22:1(n-11), low 18:2(n-6)) relative to smaller whales. This suggests that larger belugas fed predominantly on Arctic cod collected from the offshore region because they had the strongest pelagic signal among prey.

The yearling beluga (animal P40, 1.9 m long) had the lowest level of offshore, pelagic fatty acids, which is different than observations for calves in the same size range in Svalbard that were found to have very similar fatty acid profiles as adults (Birkeland et al. 2005). Birkeland et al., (2005) suggested calve blubber fatty acid are better described by the physiological structure and function of blubber rather than their diet, which we were unable to investigate here without milk samples. The second smallest and youngest whale (animal P36), also had a low pelagic fatty acid content, similar to other small whales, suggesting that small whales have different diets from larger adult beluga. More specifically, the smaller whales appeared to have a more diverse diet, similar to fatty acid results in juvenile grey seals (*Halichoerus grypus*) (Beck et al. 2007). The diverse diet in juvenile grey seals was thought to be caused by

inexperience as well as by diving limitations relative to adult males (Beck et al. 2007). These factors are likely to be common among young seals and belugas. Tracking studies showed smaller males were found in open-water regions near the mainland coast (Chapter 2) likely accounting for their lower pelagic fatty acid content that reflects a pelagic and nearshore diet where the copepod signal was less strong. Similarly, hooded seals (*Cystophora cristata*) that remained near the coastline had a more diverse diet that incorporated arctic cod, whereas those near the sea ice were found to predominantly feed on arctic cod and squid (Haug et al. 2007).

Fatty acid results suggesting that larger beluga fed predominantly on offshore Arctic cod refutes our hypothesis that males selecting ice-covered regions feed on epibenthic prey such as flounder (Chapter 2, 3). Our hypothesis was based upon the assumption that the largest belugas might feed on epibenthic prey because of observed deep-diving behaviour in ice-covered regions (Richard et al. 1997). Diving and feeding in offshore, heavily ice covered habitats would be energetically expensive relative to feeding in shallow, open-water habitats. Belugas are size dimorphic, thus larger males need to maintain mass by adjusting foraging behaviour to either feed on energy rich prey or feed more often (Boyd et al. 1997). Therefore, greater prey abundance, mass or energy must be available to the large males in the offshore habitats to compensate for the energetic costs of deep diving. The Arctic cod collected offshore were shown to be semi-pelagic, spending time near the ocean bottom (Benoit et al. Accepted, Deibel et al. In Press), however they are also known to occur at high concentrations underneath the sea ice (Gradinger and Bluhm 2004). Thus it is unclear if beluga selecting this habitat

are expending energy diving, however predation and mortality risks of this habitat is greater than nearshore open water regions (Chapter 2).

Largest beluga feeding in offshore regions on Arctic cod is supported by measurements of mercury concentrations and $\delta^{15}N$ that show larger beluga and offshore Arctic cod had the highest mercury and $\delta^{15}N$ values, whereas smaller beluga and shelf Arctic cod and brackish water fish had lower mercury and $\delta^{15}N$ levels (Chapter 3, 4). Our findings support the differences between shelf and offshore beluga habitat use and mercury uptake. Thus, larger beluga using and feeding in the offshore habitat are exposed to higher dietary mercury levels relative to smaller beluga selecting the open-water, shelf habitat, supporting our findings that mercury biomagnification processes relate to both beluga length and habitat and that both of these factors are needed to predict beluga muscle mercury levels (Chapter 4)

5.5 Conclusion

Here, we report that fatty acids can be used to partition likely beluga prey items into groups that are related to their feeding and ecology. In general, beluga appeared to feed predominantly on pelagic fish. However, larger beluga appeared to prefer offshore Arctic cod, whereas medium and smaller beluga incorporated shelf Arctic cod and brackish water prey species. The proposed variation in beluga diet based upon signature fatty acids was supported by the differences in mercury concentrations and $\delta^{15}N$ values in both prey and beluga. In addition, beluga diet analysis provided new information about Arctic cod ecology, as well as predator-prey interactions that is linked with

habitat. Given the currently observed and predicted sea ice loss in the western Canadian Arctic, we predict changes in the distribution and abundance of Arctic cod that will disrupt beluga feeding patterns, habitat use and food web dynamics.

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6.0 Summary and Conclusions

6.1 What are Beaufort Sea belugas eating? And what are the levels of Hg in their diets?

Incorporating beluga behaviour and food web dynamics into the analysis of dietary Hg uptake provided new perspectives on Hg processes in beluga. Habitat use findings from Chapter 2 provided the basis for the inclusion of predator behaviour in Hg analysis, as well as provided direction for food web delineation (Figure 1.2). Beaufort Sea beluga whales segregated into groups described by size and gender that were then used to evaluate the presence of variation in dietary composition. Habitats described by sea ice concentration, bathymetry and distance to coastlines were used as a guide when assigning food webs to beluga habitats. The following three food webs were paired with beluga segregation groups:

1) Small male and female belugas using shallow, open-water habitats were paired with the estuarine-shelf food web;

2) Medium male and large female belugas selecting the ice edge were paired with pelagic food webs, of the Amundsen Gulf offshore food web;

3) Large male belugas selecting high levels of sea ice concentration do so in deep waters where they dive up to 800 m and were paired with the epibenthic food web.

Assessing beluga - food web pairs revealed that the belugas hypothesized to feed in the epibenthic and Amundsen Gulf food webs had the highest Hg levels and matched well with high Hg levels in associated food webs. The estuarine-shelf belugas had the lowest Hg levels, which also corresponded with the low Hg food web of the near shore delta region. It was surprising to find that prey items from Mackenzie shelf had the lowest Hg levels, given the significantly large local source of Hg and MeHg from the Mackenzie River (Leitch et al., 2007). The higher levels of Hg in the offshore food webs suggested that the Hg from the Mackenzie River discharge only became available for biological uptake after leaving the shelf region perhaps in response to different physio-chemical properties in the more saline environment. This observation was supported by large scale regional Hg trends in copepods and arctic cod (Stern and Macdonald, 2005).

The importance of food web structure and Hg sources at the bottom of the food web were revealed in the pelagic (Amundsen Gulf) and epibenthic food webs. The length of the pelagic food web may have caused Hg levels to be higher in arctic cod and beluga relative to the shelf arctic cod and beluga. High Hg level environments such as sediment and detritus likely resulted in high Hg levels in the epibenthic food web organisms. Apart from the different Hg levels among the three food webs, Hg biomagnified at a consistent rate that was comparable to food web biomagnification factors found elsewhere. This enabled the novel approach of using biomagnification factors as a tool to validate the beluga pairing with food webs. The results from this approach suggested the first two pairings were likely, but pairing of the larger male

beluga with epibenthic prey was less likely and/or requires further investigation into the energy and Hg metabolism of the unique organisms in this food web.

Results from Chapter 3 also suggested the importance of beluga length over age as a diet discriminator of Hg levels in beluga muscle. Although this appeared to be a valid trend, it was not proved until the incorporation of beluga fatty acid signature analysis in Chapter 4. Under the premise that dietary composition related to length because of the habitat use analysis findings (Chapter 2), multivariate statistics were used to isolate the biological variables describing habitat use, and to define diet preference using fatty acid data. The importance of beluga harvest site and length in predicting diet supported the hypothesis that feeding variation among beluga is driven by differences in habitat use. One important consequence of this finding was its application to the analysis of Hg in beluga as it related to length. For the first time we demonstrated Hg levels in beluga muscle Hg resulted from Hg biomagnification processes up food webs; more so than bioaccumulation with time. Therefore, the habitat resource selection prescribed to energy needs and predation avoidance did describe differences in diet that ultimately impacted beluga Hg levels. However, the final question still remained; what are Beaufort Sea beluga whales eating? Thus, food web pairings described above were analyzed in more detail with the use of fatty acids in beluga and prey items in Chapter 5.

Fatty acids were found to effectively partition possible prey into groups associated with their habitat and ecology. With the use of well known marine fatty acids (e.i. 20:1(n-9) and 22:1(n-11)) a gradient of marine food web species described the prey items. Overall results from the fatty acid analysis revealed that beluga fed

predominantly on the marine fish: arctic cod and pacific herring. In support of Chapter 2, 3 and 4, beluga diet varied with size. The larger beluga appeared to prefer the offshore arctic cod, and medium and smaller sized beluga preferred arctic cod collected from the Mackenzie shelf and other near shore fish. The matching of beluga length and to the gradient of marine prey was supported by the more diverse fatty acid signature found in the juvenile belugas. The fatty acid classification and interpretation of beluga diet were supported by the Hg and $\delta^{15}N$ trends in beluga and prey.

Results of the fatty acid analysis of beluga diet were slightly different than the original pairings proposed in Chapter 3. The pairing of the largest beluga with epibenthic prey such as flounders and shrimp no longer seemed likely according to the fatty acid analysis; however, the premise of the hypothesized pairing was based on beluga habitat use and diving behaviour which matches with feeding on offshore arctic cod found at deep ocean depths. Therefore, results show that the habitat use of Beaufort Sea belugas that range from smaller whales using open-water habitats regions to the largest belugas using heavy ice covered habitats, matched well with preferential feeding of different the arctic cod types. The dietary composition of beluga also provided new information about arctic cod habitat use and behaviour in the eastern Beaufort Sea. Perhaps at an earlier arctic cod life stage prefers shallow open water regions, whereas the older arctic cod may choose deep ocean depths. Beluga dive data from satellite telemetry may provide a better means of addressing this variation in arctic cod occurrence. Finally, this thesis concludes that beluga in general feed on marine prey, and larger beluga are exposed to higher dietary Hg levels associated with offshore

feeding on arctic cod, whereas smaller beluga are exposed to lower Hg levels in diet in the near shore and shelf regions.

This conclusion revealed that dietary Hg levels varied with habitat, whereby the shelf was a lower source than the offshore food web. Therefore, beluga habitat use and associated food web complexity were found to be important factors driving beluga Hg levels. In addition, the variation of Hg in regional habitats may in part be related to arctic cod in deep ocean regions being exposed to high Hg levels through detritus food web links (seen in epibenthic food web). However, given the similar $\delta^{13}C$ among the two arctic cod groups this scenario appears unlikely because if offshore arctic cod were receiving more detrial sources the $\delta^{13}C$ should be higher. Thus, it is more likely that Hg leaving the Mackenzie plume only becomes bioavailable for uptake in offshore habitats. Further analyses on arctic cod feeding behaviour and Hg bioavailability are required to validate this claim.

6.1 Retrospective and Prospective Trends

The high levels of Hg observed in the late 1990's were reported for beluga liver tissue. Liver Hg is representative of bioaccumulation over time because it is the site of MeHg demethylation to mercuric-selenide species that are not bioavailable and no longer toxic (detoxification process). As such, it is suggested that Beaufort Sea beluga Hg levels in 1990's be re-evaluated with the muscle tissue Hg analysis. If the muscle Hg levels are also found to be high, then the reported high Hg levels can be attributed to dietary sources of Hg. This would call for an analysis of food web trends as well as habitat alterations that may have occurred and resulted in altered feeding behaviour and

Hg uptake. If however, muscle Hg levels are not higher, it would suggest physiological processes, specifically demethylation in the liver may have been responsible and should be further investigated. A better understanding of the causes or these historic Hg fluctuations may help to link historical events such as shifts in sea ice, or the Arctic Oscillation with Hg processes.

Under future scenarios of climate warming and sea ice depletion, beluga habitat and habitat use will be altered. It appears that belugas select habitats based on the resource availability related to diet and mortality risks. It appears that the sea ice features may be selected due to the presence of arctic cod, either at the edge or coincidentally at the ocean bottom; the use of open-water habitats may relate more to predation avoidance or survival techniques. Given that sea ice plays an important role in the arctic cod life cycle (Gradinger and Bluhm, 2004), the removal or alteration of it will equate to the removal or alteration of beluga food resources. Although beluga can feed on other shelf species (e.g. pacific herring, arctic cisco), the selection of arctic cod likely relates to energy/ nutrition or density/availability given larger beluga prefer this prey even though the associated physical habitat presents greater risks. Thus, a reduction in sea ice will likely result in changes in food availability. This may lead to nutritional or energetic stress in beluga, but also perhaps to reduced Hg intake. Beaufort Sea beluga may choose to travel further in search of abundant prey; however, the risk/reward of this habitat modification may not ultimately work in favour of the continued success of this behaviour.

6.2 References

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