TROPHIC RELATIONSHIPS IN AN ARCTIC MARINE FOODWEB AND IMPLICATIONS FOR TRACE ELEMENT DYNAMICS

By

Larissa-A. Dehn

Recommended: Lawrence K Duffy

Ola F.

Todd O'Hara
Advisory Committee Co-Chair

G.H. Allen
Advisory Committee Co-Chair

Edward C Murphy
Assistant Chair, Department of Biology and Wildlife

Approved:

Dean, College of Natural Science and Mathematics

Susan M. Henrichs
Dean of the Graduate School

August 1, 2005
Date

TROPHIC RELATIONSHIPS IN AN ARCTIC MARINE FOODWEB AND

IMPLICATIONS FOR TRACE ELEMENT DYNAMICS

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

Larissa-A. Dehn, B.S., M.S.

Fairbanks, Alaska

August 2005

ABSTRACT

Tissues of subsistence-harvested Arctic marine and terrestrial mammals and potential prey species were analyzed for isotopes of carbon and nitrogen and selected trace elements describing contaminant pathways in the food web. Feeding habits of ice seals were characterized using stable isotopes and gastric contents analysis. Bearded seals (*Erignathus barbatus*) relied on the benthic food chain. Zooplankton and fishes were significant prey for ringed seals (*Phoca hispida*), while fishes were the principal prey in spotted seals (*Phoca largha*). Gastric prey composition and isotope ratios varied with age and sex. Effects of age, trophic level, and prey prevalence on trace element concentrations in seal tissues were investigated. Most trace elements differed significantly in phocid tissues. Bearded seals had the highest cadmium (Cd) concentrations and spotted seals the lowest. This indicates a connection of Cd with invertebrate prey, while mercury (Hg), in particular the proportion of organic to total Hg (THg), accumulated in the piscivorous food web. Silver (Ag) showed possible association to benthic feeding habits. Altered trace element accumulation patterns were observed in compromised seals. Stable isotopes illustrated belugas (*Delphinapterus leucas*) occupied a higher trophic level than bowheads (*Balaena mysticetus*) and gray whales (*Eschrichtius robustus*). Trace element concentrations also differed significantly among these cetaceans. Observed relationships with age or length in species analyzed were complex and nonlinear rather than previously reported continuous bioaccumulation with age. Cd was similar in belugas and bowheads but lowest in gray whales. THg was highest in belugas and near detection limit in mysticetes, supporting the connection of Hg with fish and Cd with invertebrates. The hepatic selenium (Se):THg ratio exceeded the frequently described equimolarity in all species. Se:THg molar ratios and tissue concentrations of zinc (Zn) may show promise as indicators of immune status and animal health. Polar bears (*Ursus maritimus*) feed on the highest trophic level, though Cd concentrations were either similar to, or significantly lower than those in belugas or ice seals. Conversely, THg increased significantly from seal to bear. Generally, trace elements in Alaska-harvested animals were lower than for other Arctic regions, and trace metal magnification in the Arctic food web was not significant.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AAS - Atomic Absorption Spectrometry

Ag – Silver

BMF – Biomagnification Factor

BrCl – Bromine Chloride

Cd – Cadmium

CO_2 – Carbon Dioxide

Cu – Copper

CVAFS – Cold Vapor Atomic Fluorescence Spectrometer

dw – Dry Weight

EPA - Environmental Protection Agency

FO*i* - Frequency of Occurrence

FWMF – Food Web Magnification Factor

GC - Gas Chromatography

HCl – Hydrochloric Acid

He – Helium

Hg – Mercury

HgII – Divalent Mercury

HNO_3 – Nitric Acid

H_2O_2 – Hydrogen Peroxide

H_2SO_4 – Sulfuric Acid

ICP-MS – Inductively Coupled Plasma Mass Spectrometer

IRMS - Isotope Ratio Mass Spectrometer

MDL - Minimum Detection Limit

MeHg – Methyl Mercury

N_2 – Atmospheric Nitrogen

$NaB(C_2H_5)_4$ – Sodium Tetraethyl Borate

NIST – National Institute for Standards and Technology

NRC - National Research Council

PC - Principal Component

PCA – Principal Components Analysis

SD - Standard Deviation

Se – Selenium

$SnCl_2$ – Stannous Chloride

THg – Total Mercury

TL – Trophic Level

ww – Wet Weight

YOY – Young-of-the-Year

Zn – Zinc

%MeHg – Proportion of MeHg to THg

$\delta^{13}C$ – Stable Isotope Ratio of Carbon

$\delta^{15}N$ – Stable Isotope Ratio of Nitrogen

To my father Wolf who gave me his curiosity, my mother Christina who gave me her strength and my husband Jon who always believed in me.

In the sea, once upon a time,
O my best beloved,
There was a whale,
And he ate fishes.
He ate the starfish and the garfish,
And the crab and the dab,
And the plaice and the dace,
And the skate and his mate,
And the mackereel and the pickereel,
And the really truly twirly-whirly eel.

How the whale got his throat
Rudyard Kipling

ACKNOWLEDGEMENTS

Many, many wonderful people were involved in this memorable stage of my life. This study was made possible by the generosity, trust and hospitality of the many subsistence hunters in Alaska, Russia and Canada who invited me to their homes to share hunting success, stories and "pilaking" (butchering) techniques. And of course, they shared a substantial portion of their food to find its way in my sample bags for analysis. In particular, I thank Charlie Brower, Harry Brower, Tommy Olemaun, Benny Akootchook, Taqulik Hepa, Lolly Hopson and Rebecca Brower, who went out of their way to support my sampling effort.

My sincere appreciation and thanks go to my committee. Erich Follmann has been a wonderful advisor and mentor to me. He shared my joy when things were going good and picked me up with much encouragement and his unwavering optimism when I was falling apart. Erich has always been there and I feel honored that he believed in me all those years ago and took me on as a student of life and science. Dana Thomas showed me that statistics can be fun. I knew I was in trouble when he got thrilled about my unruly data set. But he was there for me every step of the way and I admire his unending patience. Larry Duffy introduced me to the secrets of the mercury detector and still trusted me in his lab after the infamous vial with blubber digest garnished the ceiling. Todd O'Hara navigated with me around my first bowhead whale and pointed out that the Cadillac-sized organ in there is actually the liver.

Very special thanks go to Cheryl Rosa. She is one of the few people understanding the excitement of finding a lesion. She is also one of the very few people that can follow my confused ideas and consequently keep me on track. And I should not forget the hours and days (or even months) we spent in the freezer or sub-sampling tissues that would have been dreary without her. Thank you for being such a great friend. Gay Sheffield is the true sampling queen of the North Slope and the Bering Strait. Sample sizes and more importantly sampling fun would have been a lot less impressive without her. I very much appreciate the help of Torsten Bentzen. He was a real trooper helping me wash through seal guts, and I think he almost enjoyed it in the end. Craig George is one of the most amazing storytellers and listening, I learned so much about the Arctic, the people and the whales.

I am indebted to Gerald Bratton, Robert Taylor, Norma Haubenstock and Tim Howe for their collaboration with the many samples in the labs and their patience in explaining the equipment. I especially thank Victoria Woshner for her contagious fascination and joy with necropsies in the freezing cold. I gratefully acknowledge the assistance of the many sampling wizards in the field, Genna Zelensky, Solissa Visalli, Deborah Burnett, Geoff York, Tania Zenteno-Savin, Bob Elsner, Robert Suydam, Paul Hoekstra, Tami Mau, Leslie Pierce, Lois Harwood and many more. I also thank Ray Highsmith and Bodil Bluhm for collecting amphipods in the Bering Strait and Paul Becker and Steven Christopher who organized interlaboratory comparison exercises and provided marine mammal reference material as part of the QA/QC program. Douglas DeMaster, John Bengtson and many others at NMML coached me on processing and aging of seal teeth and stomach contents analysis.

Last, but most certainly not least, I thank my family for their loving support and especially my parents who were close even when 10,000 miles separated us. I am particularly grateful to my father who was always enthusiastic and never got tired of explaining hyperbolas and decay functions.

My love Jon, you have been the unbiased voice of reason, the magician who fixed the computer; you've read through drafts and listened to talks and defenses, put up with bloody stories, pictures and sometimes very bad olfactory inputs. You cooked when I was writing, massaged my shoulders, watched over my sleep and most importantly, you made me laugh. I love you!

Finally, Fenris and Loki. They don't care much for written thank-you's, but they deserve a big bone for not understanding bad days (nor caring) and washing dark clouds away with the wag of a tail.

Financial support for this study was primarily provided by the Cooperative Institute for Arctic Research (CIFAR). Additional funding was provided by the Experimental Program for Stimulation of Competitive Research (EPSCoR); the IdeA Network for Biomedical Research Excellence (INBRE); the North Slope Borough Department of Wildlife Management; the Institute of Arctic Biology and the Department of Biology and Wildlife, UAF; the US Geological Survey and the Barrow Arctic Science Consortium (BASC).

GENERAL INTRODUCTION

Exposure to contaminants is widespread among marine mammals, and continuous bioaccumulation and biomagnification, the increase in concentration of pollutants with age and trophic level, respectively, of various trace metals and organic compounds (e.g., organochlorines (OC's)) have been repeatedly reported in their tissues (Smith and Armstrong, 1978; Honda et al., 1983, Hansen et al., 1990, Dietz et al., 2000, Woshner et al., 2001a; Watanabe et al., 2002; Bustamante et al., 2004). The Arctic has a comparatively low human population density, is relatively unaffected by industrial activity and is generally considered a pristine environment (Barrie et al., 1992; Bard, 1999). However, the Arctic Ocean has been proposed as the sink for many contaminants (Ponce et al., 1997, Bard, 1999), and, recently, mercury (Hg) was shown to accumulate in polar regions due to surface deposition of reactive HgII during polar sunrise (Ebinghaus et al., 2002; Lindberg et al., 2002).

Commercial whalers and sealers exploited and greatly depleted many marine mammal populations during the 18th and 19th century (Fay, 1982; Lowry et al., 1982; Clapham et al., 1999). Thus, Arctic marine mammals are particularly vulnerable to harmful effects of contaminants, e.g., impaired reproduction, neoplasia, and immune suppression (Gauthier et al., 1998; Beckmen et al., 2003; Brousseau et al., 2003; Derocher et al., 2003; Gauthier et al., 2003; Lie et al., 2004). Marine mammals feed generally at the top of food chains, and some of their unique adaptations (e.g., blubber layer) make them a target for fat-soluble contaminants. Some species are remarkably long-lived, for example bowhead whales (*Balaena mysticetus*) can live in excess of 100 years (George et al., 1999). In addition, marine mammals are a significant cultural and nutritional resource for the Native coastal population of Alaska and other Arctic areas. Subsistence users are concerned about exposure to biomagnifying contaminants in the food web. In fact, concentrations of some contaminants (e.g., cadmium (Cd)) are at levels of concern to marine mammal health and subsistence consumers, compared to tissue concentrations established for domestic animals (Puls, 1994; Bratton et al., 1997). However, the highest tissue concentrations of Cd are noted for bowhead whales and ringed seals (*Phoca hispida*) that feed low in the food chain (Lowry et al., 1980; Lowry and Sheffield, 2002) and those levels are an order of magnitude higher than in top-level Arctic predators, e.g., Arctic fox (*Alopex lagopus*) and polar bear (*Ursus maritimus*) (Prestrud et al., 1994; Woshner et al., 2001a; Woshner et al., 2001b). This illustrates that not all trace elements accumulate with trophic level and that the generally accepted concept of biomagnification may be flawed, warranting further study.

Recent investigations reported elevated cadmium (Cd) concentrations in tissues of ringed seals that do not coincide with lesions associated with metal toxicosis (Woshner 2000; Sonne-Hansen et al. 2002). It was suggested that ringed seals may have adapted to these metal concentrations and, perhaps that these levels can be considered normal background for this species (Woshner 2000; Sonne-Hansen et al. 2002). This is supported by trace metal evidence from ancient human and animal hair from archeological sites in Greenland showing unchanged Cd concentrations in 15th century Inuit mummies compared to modern samples (Hansen et al., 1989). Hg, on the other hand, was slightly lower in hair sampled from mummies in Greenland, and Alaska compared to present-day Inuit populations (Toribara and Muhs, 1984; Hansen et al., 1989; Egeland et al., 1999).

Marine mammal studies usually rely on small sample sizes, and, due to legal limitations (Marine Mammal Protection Act), tissues are commonly collected from stranded or otherwise compromised animals (e.g., during epizootics) and thus may not present the norm of a healthy population. This indicates the need to establish normal reference ranges for marine mammals. Many studies have recognized the importance of adequate baseline data to compare and evaluate effects of contaminants, nutrients and disease factors on animal health, immune status and reproduction, but also temporal and geographic trends and variations in marine and terrestrial animals (Warburton and Seagars 1993; Becker et al. 1997; Krahn et al. 1997; Dunbar et al. 1999a; Dunbar et al. 1999b; Aguirre et al. 2000; Ylitalo et al. 2001; Anan et al. 2002; Aguilar et al. 2002; Kucklick et al. 2002; Lander et al. 2003; Jepson et al. 2005). Baseline data enhance the understanding of anthropogenic and climate effects, are invaluable for effective management strategies and restoration of wildlife habitats, and improve knowledge on natural variability within the ecosystem.

This study aims to provide baseline data of selected essential and potentially toxic trace elements (silver (Ag), cadmium (Cd), copper (Cu), mercury (Hg), selenium (Se) and zinc (Zn)) in tissues of apparently healthy marine and some terrestrial mammals in the Alaskan, Canadian and Russian Arctic sampled during Native subsistence harvests. Reference ranges were also established for stable isotope ratios of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) in muscle of marine and terrestrial mammals and total body homogenates of potential prey. Stable isotopes are commonly used as indicators for trophic relationships and feeding sources. Isotope ratios were supplemented and interpreted using analysis of stomach contents in ice-associated pinnipeds. Further, the effects of animal age, sex, trophic ecology (via stable isotopes) and prey preference on trace element pathways were examined. This will aid in the understanding of basic biology, conservation, and management of these important subsistence species.

Chapter 1 compares feeding ecology of three Arctic phocids, ringed, bearded (*Erignathus barbatus*), and spotted seals (*Phoca largha*), harvested in Alaska and Canada using stomach contents and stable isotope analysis. Results are also interpreted with regard to sex and seal age.

Chapter 2 provides baseline concentrations of selected trace elements in apparently healthy ice seals (ringed, bearded and spotted seal) taken during subsistence harvests in Alaska and Canada. Effects of age, sex, trophic level, and prey selection on trace element pathways are evaluated.

Chapter 3 gives baseline concentrations of selected trace elements and stable carbon and nitrogen isotopes in tissues of Arctic cetaceans. Bowhead and beluga whales (*Delphinapterus leucas*) and gray whales (*Eschrichtius robustus*) were sampled during Native subsistence harvests in Alaska and Russia, and tissues were analyzed to compare and evaluate the effects of age, sex, and trophic position on trace metal concentration.

Chapter 4 identifies trophic relationships in the entire Arctic marine food web and presents food web magnification factors in liver and kidney for Hg, Cd and Ag in this ecosystem. Tissue-specific biomagnification factors are established for selected predator-prey scenarios. Concentration ranges of trace elements and stable isotopes for mammals analyzed in this study are compared to mammals from other Arctic regions.

REFERENCES

Aguilar A, Borrell A, Reijnders PJH. Geographical and temporal variation in levels of organochlorine contaminants in marine mammals. Mar Environ Res 2002; 53: 425-452.

Aguirre AA, Angerbjörn A, Tannerfeldt M, Mörner T. Health evaluation of Arctic fox (*Alopex lagopus*) cubs in Sweden. J Zoo Wildl Med 2000; 31: 36-40.

Anan Y, Kunito T, Ikemoto T, Kubota R, Watanabe I, Tanabe S, Miyazaki N, Petrov EA. Elevated concentrations of trace elements in Caspian seals (*Phoca caspica*) found stranded during the mass mortality events in 2000. Arch Environ Contam Toxicol 2002; 42: 354-362.

Bard, HM. Global transport of anthropogenic contaminants and the consequences for the Arctic marine ecosystem. Mar Pollut Bull 1999; 38: 356-379.

Barrie LA, Gregor D, Hargrave B, Lake R, Muir D, Shearer R, Tracey B, Bidleman T. Arctic contaminants: sources, occurrence and pathways. Sci Total Environ 1992; 122: 1-74.

Becker PR, Wise SA, Thorsteinson L, Koster BJ, Rowles T. Specimen banking of marine organisms in the United States: current status and long-term prospective. Chemosphere 1997; 34: 1889-1906.

Beckmen KB, Blake JE, Ylitalo GM, Stott JL, O'Hara TM. Organochlorine contaminant exposure and associations with hematological and humoral immune functional assays with dam age as factor in free-ranging northern fur seal pups (*Callorhinus ursinus*). Mar Pollut Bull 2003; 46: 594-606.

Bratton GR, Flory W, Spainhour CB, Haubold EM. Assessment of selected heavy metals in liver, kidney, muscle, blubber, and visceral fat of Eskimo harvested bowhead whales *Balaena mysticetus* from Alaska's North Coast. 1997. Barrow, Alaska, Department of Wildlife Management, North Slope Borough, 233 pp.

Brousseau P, De Guise S, Voccia I, Ruby S, Fournier M. Immune status of St. Lawrence estuary beluga whales. 2003. *In* Vos, J. G., Bossart, G. D., Fournier, M., O'Shea, T. J. Toxicology of Marine Mammals. Taylor & Francis, London.

Bustamante P, Morales CF, Mikkelsen B, Dam M, Caurant F. Trace element bioaccumulation in grey seals *Halichoerus grypus* from Faroe Islands. Mar Ecol Prog Ser 2004; 267: 291-301.

Clapham PJ, Young SB, Brownell RL. Baleen whales: conservation issues and the status of the most endangered populations. Mamm Rev 1999; 29: 35-60.

Derocher AE, Wolkers H, Colborn T, Schlabach M, Larsen TS, Wiig Ø. Contaminants in Svalbard polar bear samples archived since 1967 and possible population level effects. Sci Total Environ 2003; 301: 163-174.

Dietz R, Riget F, Cleemann M, Aarkrog A, Johansen P, Hansen, JC. Comparison of contaminants from different trophic levels and ecosystems. Sci Total Environ 2000; 245: 221-231.

Dunbar MR, Cunningham MW, Linda SB. Vitamin A concentrations in serum and liver from Florida panthers. J Wildl Dis 1999; 35: 171-177.

Dunbar MR, Velarde R, Gregg MA, Bray M. Health evaluation of a pronghorn antelope population in Oregon. J Wildl Dis 1999; 35: 510.

Ebinghaus R, Kock HH, Temme C, Einax JW, Löwe AG, Richter A, Burrows JP, Schroeder WH. Antarctic springtime depletion of atmospheric mercury. Environ Sci Technol 2002; 36: 1238-1244.

Egeland GM, Ponce R, Knecht R, Bloom NS, Fair J, Middaugh JP. Trace metals in ancient hair from the Karluk archeological site, Kodiak, Alaska. Int J Circumpolar Health 1999; 58: 52-56.

Fay FH. Ecology and biology of the Pacific Walrus, *Odobenus rosmarus divergens* Illiger. 1982. United States Department of the Interior, Fish and Wildlife Service, North American Fauna 74, Washington, D. C., 279 pp.

Gauthier JM, Dubeau H., Rassart E. Mercury-induced micronuclei in skin fibroblasts of beluga whales. Environ Toxicol Chem 1998; 17: 2487-2493.

Gauthier JM, Dubeau H, Rassart E. Evaluation of genotoxic effects of environmental contaminants in cells of marine mammals, with particular emphasis on beluga whales. 2003. *In* Vos, J. G., Bossart, G. D., Fournier, M., O'Shea, T. J. Toxicology of Marine Mammals. Taylor & Francis, London.

George JC, Bada J, Zeh J, Scott L, Brown SE, O'Hara T, Suydam R. Age and growth estimates of bowhead whales (*Balaena mysticetus*) via aspartic acid racemization. Can J Zool 1999; 77: 571-580.

Hansen JC, Toribara TY, Muhs AG. Trace metals in human and animal hair from the 15th century graves at Qilakitsoq compared with recent samples. Meddelelser im Grønland, Man & Society 1989; 12: 161-167.

Hansen CT, Nielsen CO, Dietz R, Hansen MM. Zinc, cadmium, mercury and selenium in minke whales, belugas and narwhals from West Greenland. Polar Biol 1990; 10: 529-539.

Honda K, Tatsukawa R, Itano K, Miyazaki N, Fujiyama T. Heavy metal concentrations in muscle, liver and kidney tissue of striped dolphin, *Stenella coeruleoalba*, and their variations with body length, weight, age and sex. Agricult Biol Chem 1983; 47: 1219-1228.

Jepsen PD, Bennett PM, Deaville R, Allchin CR, Baker JR, Law RJ. Relationships between polychlorinated biphenyls and health status in harbor porpoises (*Phocoena phocoena*) stranded in the United Kingdom. Environ Toxicol Chem 2005; 24: 238-248.

Krahn MM, Becker PR, Tilbury KL, Stein JE. Organochlorine contaminants in blubber of four seal species: integrating biomonitoring and specimen banking. Chemosphere 1997; 34: 2109-2121.

Kucklick JR, Struntz WDJ, Becker PR, York GW, O'Hara TM, Bohonowych JE. Persistent organochlorine pollutants in ringed seals and polar bears collected from northern Alaska. Sci Total Environ 2002; 287: 45-59.

Lander ME, Harvey JT, Gulland FMD. Hematology and serum chemistry comparisons between free-ranging and rehabilitated harbor seal (*Phoca vitulina richardsi*) pups. J Wildl Dis 2003; 39: 600-609.

Lie E, Larsen HJS, Larsen S, Johansen GM, Derocher AE, Lunn NJ, Norstrom RJ, Wiig O, Skaare JU. Does high organochlorine (OC) exposure impair the resistance to infection in polar bears (*Ursus maritimus*)? Part I: Effect of OCs on the humoral immunity. J Toxicol Environ Health A 2004; 67: 555-582.

Lindberg SE, Brooks S, Lin C-J, Scott KJ, Landis MS, Stevens RK, Goodsite M, Richter A. Dynamic oxidation of gaseous mercury in the Arctic troposphere at polar sunrise. Environ Sci Technol 2002; 36: 1245-1256.

Lowry LF, Frost KJ, Burns JJ. Variability in the diet of ringed seals, *Phoca hispida*, in Alaska. Can J Fish Aquat Sci 1980; 37: 2254-2261.

Lowry LF, Frost KJ, Calkins DG, Swartzman GL, Hills S. Feeding habits, food requirements, and status of Bering Sea marine mammals. 1982. Anchorage, North Pacific Fishery Management Council, 233 pp.

Lowry, LF, Sheffield G. Stomach contents of bowhead whales harvested in the Alaskan Beaufort Sea. In Richardson, W. J. and Thompson, D. H. Bowhead whale feeding in the Eastern Alaskan Beaufort Sea: Update of scientific and traditional information. 2002. Vol. 1. OCS Study MMS 2002-012. LDL Report TA2196-7. LGL Ltd. King City, Ontario, Canada.

Ponce RA, Egeland GM, Middaugh JP, Becker PR. Twenty years of trace metal analyses of marine mammals: Evaluation and Summation of data from Alaska and other Arctic regions. State of Alaska Epidemiology Bulletin 1997; 1: 1-15.

Prestrud P, Norheim G, Sivertsen T, Daae HL. Levels of toxic and essential elements in arctic fox in Svalbard. Polar Biol 1994; 14: 155-159.

Puls, R. Mineral levels in animal health. Diagnostic data. 1994. Sherpa International, Clearbrook, British Columbia, Canada. 356 pp.

Smith TG, Armstrong FAJ. Mercury and selenium in ringed and bearded seal tissues from Arctic Canada. Arctic 1978; 31: 75-84.

Sonne-Hansen C, Dietz R, Leifsson PS, Hyldstrup L, Riget FF. Cadmium toxicity to ringed seals (*Phoca hispida*): an epidemiological study of possible cadmium-induced nephropathy and osteodystrophy in ringed seals (*Phoca hispida*) from Qaanaaq in Northwest Greenland. Sci Total Environ 2002; 295: 167-181.

Toribara TY, Muhs AG. Hair: a keeper of history. Arctic Anthropol 1984; 21: 99-108.

Warburton J, Seagars DJ Heavy metal concentrations in liver and kidney tissues of pacific walrus. Continuation of a baseline study. 1993. Anchorage, Alaska, US Fish and Wildlife Service, 27 pp.

Watanabe I, Kunito T, Tanabe S, Amano M, Koyama Y, Miyazaki N, Petrov EA, Tatsukawa R. Accumulation of heavy metals in Caspian seals (*Phoca caspica*). Arch Environ Contam Toxicol 2002; 43: 109-120.

Woshner VM. Concentrations and interactions of selected elements in tissues of four marine mammal species harvested by Inuit hunters in Arctic Alaska, with an intensive histologic assessment, emphasizing the beluga whale. 2000. Ph.D. thesis, College of Veterinary Medicine, University of Illinois, Urbana-Champaign, IL.

Woshner VM, O'Hara TM, Bratton GR, Beasley VR. Concentrations and interactions of selected essential and non-essential elements in ringed seals and polar bears of Arctic Alaska. J Wildl Dis 2001a; 37: 711-721.

Woshner VM, O'Hara TM, Bratton GR, Suydam RS, Beasley VR. Concentrations and interactions of selected essential and non-essential elements in bowhead and beluga whales of Arctic Alaska. J Wildl Dis 2001b; 37: 693-710.

Ylitalo GM, Matkin CO, Buzitis J, Krahn MM, Jones LL, Rowles T, Stein JE. Influence of life-history parameters on organochlorine concentrations in free-ranging killer whales (*Orcinus orca*) from Prince William Sound, AK. Sci Total Environ 2001; 281: 183-203.

CHAPTER 1

FEEDING ECOLOGY OF PHOCID SEALS IN THE ALASKAN AND CANADIAN ARCTIC[1]

1.1 ABSTRACT

Feeding habits of ringed, bearded, and spotted seals were studied using stomach contents and stable isotopes of nitrogen and carbon. Bearded seals relied heavily on the benthic food chain, with crustaceans and mollusks making up the majority of the prey (Frequency of Occurrence (FO_i) = 97.2% and 83.3%, respectively), but fish were common as well (FO_i = 80.6%). Both zooplankton and fish were significant prey for ringed seals (FO_i = 64.1% and 61.5%, respectively), while fish was the principal prey of spotted seals. In ringed seals, age and sex had a large impact on gastric prey composition. Stomachs of male ringed seals caught in Barrow, Alaska were more likely to contain zooplankton while those of females were more likely to contain fish. Stable nitrogen isotope ratios of ringed seal muscle were significantly higher for females than for males (16.7 ± 0.6‰ and 17.1 ± 0.6‰, respectively), indicating that females occupied a higher trophic level. Stomachs of older ringed seals were more likely to contain gadids, while the presence of zooplankton was age-independent. $\delta^{15}N$ was positively correlated to age in spotted seal muscle, suggesting dependence on higher trophic level prey with increasing age. This was not indicated in the analysis of stomach contents possibly due to

[1] Dehn, L.-A., Sheffield, G. G., Follmann, E. H., Duffy, L. K., Thomas, D. L., O'Hara, T. M. Feeding ecology of phocid seals in the Alaskan and Canadian Arctic. Prepared for submission to Polar Biology.

abundance of spawning herring during summer. $\delta^{13}C$ was positively correlated to age in ringed seals, possibly indicating carbon transfer from maternal tissues to pups during gestation and lactation. $\delta^{15}N$ was significantly highest in spotted seals (17.6 ± 0.9‰) and is in agreement with their fish-dominated diet. $\delta^{15}N$ was not different between ringed and bearded seals harvested in Alaska (16.9 ± 0.6‰ and 16.8 ± 1.0‰, respectively) suggesting both species occupy a similar trophic level during summer, while $\delta^{15}N$ was lowest in walrus (13.6 ± 1.0‰). Carbon-13 was most enriched in bearded seals and walrus (-17.1 ± 0.6‰ and –17.3 ± 0.9‰) reflecting greater use of the benthic ecosystem. Ringed seals from the Canadian Arctic were highly depleted in ^{13}C (-20.4 ± 0.4‰) compared to Alaskan pinnipeds, likely because of influence from the Beaufort Sea versus Chukchi and Bering seas. $\delta^{13}C$ was not statistically different between Alaskan ringed and spotted seals, but variability in spotted seals was high, suggesting importance of both coastal and offshore feeding habitats.

Keywords: Ringed seal, bearded seal, spotted seal, walrus, stable isotopes, feeding ecology, stomach contents analysis, Arctic

1.2 INTRODUCTION

Distribution, movement, and feeding ecology of ringed (*Phoca hispida*), bearded (*Erignathus barbatus*) and spotted (*Phoca largha*) seals are strongly associated with Arctic sea ice (Braham et al. 1984). They are commonly referred to as ice or pagophilic seals, but are adapted to different niches within the sea ice environment, and only some overlap occurs among species (Burns 1970; Simpkins et al. 2003). These seals are an important prey to higher trophic level organisms, including Arctic fox (*Alopex lagopus*), polar bear (*Ursus maritimus*), humans and some walrus (*Odobenus rosmarus*) (Smith 1976; Lowry and Fay 1984; Hammill and Smith 1991; Derocher et al. 2002). Ice seals have significant nutritional and cultural importance to the Native coastal populations of Alaska and other Arctic areas.

Ringed seals are the most abundant and smallest seal in circumpolar Arctic waters. They prefer fast ice, dense pack ice or pressure ridges (Braham et al. 1984). Feeding habits of these seals have been described in the Canadian High Arctic, Svalbard, Greenland and the Bering and Chukchi seas (Lowry et al. 1980a; Bradstreet and Finley 1983; Smith 1987; Siegstad et al. 1998). Major prey includes Arctic cod (*Boreogadus saida*), amphipods, and krill (euphausids and mysids). Seasonal shifts in ringed seal feeding show presence of krill in summer and Arctic cod in winter and spring (Lowry et al. 1980a). Age-related prey prevalence and a decline in the importance of crustaceans with age also have been suggested for ringed seals (Lowry et al. 1980a; Bradstreet and Finley 1983; Smith 1987; Siegstad et al. 1998).

Bearded seals have a circumpolar distribution, prefer pack ice, polynyas, and are rarely found in shorefast ice conditions (Burns 1970). Studies conducted in the Canadian High Arctic, Central Bering and Chukchi seas describe a variety of different benthic and epibenthic prey in bearded seal stomachs (Lowry et al. 1980b; Finley and Evans 1983; Antonelis et al. 1994), but importance of prey types vary by location and age groups. Competition for habitat and foods of bearded seals with benthic feeding Pacific walrus has been suggested (Lowry et al. 1980b; Cleator 1996; Simpkins et al. 2003).

Little information on feeding ecology is available for spotted seals, and only in the past 20 years has this species been differentiated from its close relative, the harbor seal (*Phoca vitulina*) (Burns et al. 1984). In the Alaskan Arctic, spotted seals occupy the Bering Sea ice front during winter and spring and travel to coastal habitats in the Bering, Chukchi and Beaufort seas during the open-water season (Braham et al. 1984; Lowry et al. 1998). Schooling fish, such as Pacific herring (*Clupea pallasii*), capelin (*Mallotus villosus*), smelt (*Osmerus* spp.) and Arctic cod dominate their diet in these areas, while invertebrates, such as shrimp, are found less frequently in their stomachs (Bukhtiyarov et al. 1984). However, there is some indication that prey consumed varies with age with younger seals preying predominately on small crustaceans, while the frequency of fish is higher in adults (Kato 1982; Bukhtiyarov et al. 1984).

Analysis of stomach contents has been used extensively in these earlier studies to determine feeding ecology of pagophilic seals. Recently, it has been repeatedly suggested that analysis of stomach contents or scat of marine mammals is strongly biased and overestimates prey with chitinous structures (e.g., crustaceans, cephalopod beaks) and fish otoliths that resist digestion or are retained in the stomach, and underestimates soft prey such as snails and mussels that are digested within hours (Murie and Lavigne 1986; Gales and Cheal 1992; Bowen 2000; Sheffield et al. 2001). Secondary ingestion of prey, such as digestive tract contents of ingested fish, could also lead to biased prey assessments (Santos et al. 2001). Thus, it is difficult to accurately assess the importance of prey species, prey preference or dietary shifts as the stomach contents only represent a "snapshot" in time. The use of other methods has been suggested by Sheffield et al. (2001) to identify diet, and a combination of classic methods with chemical feeding ecology has proven powerful in dietary reconstructions (Hobson et al. 1997; Burns et al. 1998).

Stable isotopes of carbon and nitrogen have become increasingly more important in studies of trophic ecology. Stable nitrogen isotopes become more enriched in consumer tissues as trophic level increases due to selective incorporation of the heavier isotope in tissues (DeNiro and Epstein 1981). Thus, nitrogen isotope analysis is helpful in establishing trophic level and predator-prey relationships (Kelly 2000). However, tissue-specific turnover rates and enrichment factors are poorly understood and make evaluation of nitrogen isotope ratios difficult. Without prior knowledge of typically ingested prey, the results of stable isotope analysis are difficult to interpret (Gannes et al. 1997). Age and body condition can have substantial impact on nitrogen isotope ratios and their interpretation. Hobson et al. (1997) reported enrichment of nitrogen isotopes in muscle of northern fur seal (*Callorhinus ursinus*) pups compared to their mothers and suggested that pups are feeding on a higher trophic level while being nourished by their mothers.

Similarly, body condition can impact nitrogen isotope ratios as water stress and body protein catabolism during starvation will lead to trophic enrichment (Hobson et al. 1993).

Stable carbon isotopes may enrich in consumer tissues to a minor degree and are therefore less useful in the determination of trophic position or predator-prey relationships (Tieszen et al. 1983; France 1995a). However, species differences in $\delta^{13}C$ can provide insights on feeding location or habitat (Burton and Koch 1999). Schell et al. (1998) showed more depleted carbon isotope values of zooplankton from the Beaufort Sea than in the Bering and Chukchi seas, and a similar pattern can be detected in baleen and muscle of bowhead whales (*Balaena mysticetus*) that migrate between these two regions (Schell et al. 1989; Hoekstra et al. 2002). Carbon isotope signatures have also been used to distinguish between fresh- and saltwater feeding habitats of harbor seals (Smith et al. 1996) and have application in identifying animals that rely on either benthic or pelagic food chains (France 1995b).

Feeding ecology of pagophilic seals in the Alaskan Arctic was last described in the 1980's, and changes in prey distribution may have occurred. Feeding ecology information for these seals has been determined almost solely via stomach contents analysis, and little information is available on stable isotopes in ice seals. Published data on stable isotopes in ringed seals vary greatly. Muir et al. (1995) reports stable nitrogen values of 13.9 ± 1.4‰ for ringed seals harvested in the Canadian Arctic, while ringed seals harvested in Barrow and the Barrow Strait-Lancaster Sound seem to occupy higher trophic levels at 16.9 ± 0.2‰ and 17.3 ± 1.1‰, respectively, for $\delta^{15}N$ (Hobson and Welch 1992; Hoekstra et al. 2002).

The purpose of this study is to a) evaluate and compare feeding ecology of arctic phocids harvested in Alaska and Canada using stomach contents and stable isotope analysis, b) provide baseline data for stable isotopes in muscle of apparently healthy seals and typically ingested prey, and c) discuss isotopes in muscle and prey composition in stomachs with regard to age.

1.3 MATERIALS AND METHODS

1.31 Sampling

All marine mammal samples were obtained during Native subsistence harvests. Basic morphometrics, e.g., body length, blubber thickness and sex were recorded. Seals were grossly examined for lesions and parasites. Lumbar muscle samples and stomachs were collected of ringed and bearded seals in Barrow, Alaska mainly during the summer period from 1996-2001. Ringed seal samples also were collected in Holman, NWT, Canada during summer, 2001. Tissues of spotted seals were collected at Little Diomede and Shishmaref, Alaska in summer 2000 and 2001. Walrus muscle was obtained in Barrow and Little Diomede on an opportunistic basis mainly during summer 1998 and 2003 and serves as a comparison to that of bearded seals. Figure 1.1 shows villages and communities where samples were collected, and Table 1.1 summarizes sample sizes. Muscle tissue was sub-sampled under clean conditions with titanium or ceramic blades on a Teflon covered surface, following the sampling protocol for contaminants by Becker et al. (1999) and stored at -20°C in acid-washed vials or whirlpacks™ until analysis. Several potential prey species were collected or donated by subsistence hunters in

Barrow, Alaska and the Alaskan Bering Strait. Marine mammal samples were collected and analyzed under the authority of Permit Nos. 782-1399 and 358-1585 issued to the Alaska Department for Fish and Game (ADFG) and 932-1489-03 issued to T. Rowles of the Marine Mammal Health and Stranding Response Program.

1.32 Stomach Contents Analysis

Stomachs of ringed and bearded seals were collected by tying off cardiac and pyloric sphincters to avoid spillage, placed into a bag and frozen at -20°C until analysis in Fairbanks. All stomachs of ringed seals harvested in Holman were empty. Stomachs obtained from spotted seals from Little Diomede and Shishmaref were archived and analyzed by the ADFG in Fairbanks.

Stomach contents were weighed to the nearest gram for ringed seals and with a chatillon scale (0.1 pound increments) for bearded seals and sequentially washed through three sieves with mesh sizes 3.96 mm, 1.4 mm and 0.5 mm. Spotted seal stomach contents were sequentially washed through sieves with mesh sizes 1.0 mm and 0.5 mm. Standard reference keys (Rathbun 1929; Akimushkin 1965; Keen and Coan 1974; Butler 1980; Frost and Lowry 1980; Frost 1981; Härkönen 1986; Kathman et al. 1986; Foster 1991; Jensen 1995; Harvey et al. 2000) were used for the identification of fish otoliths and invertebrate prey to the lowest possible taxonomic level. Identifiable prey were sorted, counted and weighed to the nearest milligram. Due to digestive biases on diagnostic tissues of varying endurance (e.g., overestimation of chitinous prey versus under-representation of soft prey, such as echiurid worms and polychaetes) a ranking of prey by weight or numerical frequency of prey in the stomach was not determined and only the frequency of occurrence of prey species i (FO_i method) was noted for all animals. FO_i is defined as the percentage of stomachs that contained one or more individuals of the prey species i:

$$FO_i = (p_i / p_t) * 100$$

where p_i is the number of stomachs with the prey species i and p_t is the number of stomachs with digesta (Hjelset et al. 1999). Nematodes in the stomach and cestodes migrating from the duodenum to the stomach after death were found in all seals, in particular bearded seals on a regular basis and were considered normal (Dunbar 1941; Lauckner 1985). They were not analyzed as a food item and hence not included in p_t.

1.33 Stable Isotopes

Lumbar muscle tissue of ringed, bearded, and spotted seals, as well as total body homogenates of prey were freeze-dried and ground into a fine powder with mortar and pestle. For each sample, 0.2 to 0.4 mg of tissue was weighed into a 4.75 x 4 mm tin capsule, which was then folded into a cube. Samples were analyzed for both stable carbon and nitrogen ratios at the University of Alaska Fairbanks (UAF) using a Finnigan MAT Delta^{Plus}XL Isotope Ratio Mass Spectrometer (IRMS) directly coupled to a Costech Elemental Analyzer (ESC 4010). Samples were flash combusted at 1020°C, followed by on-line chromatographic separation of sample N_2 and CO_2 with He as carrier gas.

Samples analyzed for $^{15}N/^{14}N$ and $^{13}C/^{12}C$ were standardized against atmospheric N_2 and PeeDee Belemnite limestone, respectively. Enrichment of a particular isotope was reported using the following notation and equation:

$$\delta R‰ = ((R_{sample}/R_{standard}) - 1) \times 1000$$

where the differential notation (δR) represents the relative difference between isotopic ratios of the sample and standard gases (i.e., $^{13}C/^{12}C$, $^{15}N/^{14}N$). A laboratory-working standard (Peptone No. P-7750) was analyzed every 10 samples during analysis, and tin capsule blanks were run every 20 samples. Calibrations were made with the use of stable isotope reference materials provided by the National Institute of Standards and Technology (NIST). External instrument reproducibility for both carbon and nitrogen isotope analysis was +/- 0.2‰.

Trophic level (TL) was calculated using the equation provided by Hobson and Welch (1992):

$$TL_{cons} = TL_{cop} + (\delta^{15}N_{cons} - \delta^{15}N_{cop}) / 3.8$$

where TL_{cons} and $\delta^{15}N_{cons}$ are trophic level and nitrogen isotope concentration, respectively, of the consumer, and TL_{cop} (by convention copepods are set at TL 2) and $\delta^{15}N_{cop}$ are trophic level and nitrogen isotope composition, respectively, of copepods. A stepwise trophic level enrichment of 3.8‰ was assumed after Hobson and Welch (1992).

Calculated trophic level was compared to trophic level estimates from stomach contents reported by Pauly et al. (1998) for marine mammals.

1.34 Aging

Jaws and claws were collected from seals after 1997 and stored at –20°C until analysis. Ringed seals harvested in 1996 and 1997 were aged using the keratin layers of claws, which represent a minimum age estimate for the animals (Benjaminsen 1973). Two canines or canine and postcanine (if only one canine was available) were extracted from the upper or lower jaw (depending on availability), submerged in a hot water bath for 30 minutes to avoid damage to the structure of the cementum, and stored in paper envelopes (Matson 1981). All teeth were shipped to Matson's Laboratory, Milltown, Montana for slide preparation. Teeth were prepared in 14 μm sections, placed on glass slides and stained with Giemsa histological stain suitable for cementum analysis. Age was estimated by counting annual growth layers in the cementum of teeth by two independent readers at UAF. Preparation and evaluation of teeth were done doubly blind by randomly assigning an identification number to each tooth with two teeth analyzed per animal. The animal identification and matching teeth were revealed only after all ages were estimated in duplicate. One growth layer per year of age was assumed for all seals (Benjaminsen 1973; Stewart et al. 1996). Maximum variation in age estimates was +/- 1 year for seals younger than 15 years and +/- 5 years in animals older than 15.

1.35 Statistical Analysis

The variables in the data set (age, $\delta^{15}N$ and $\delta^{13}C$) were ranked prior to analysis to to reduce the risk of violations of normality and homogeneity of variance assumptions. Variables were analyzed for sex and location differences using a t-test within a seal species. If no significant differences were detected for each variable, sexes and localities were pooled. A residual analysis was conducted to determine any possible violations of assumptions. Spearman rank correlation was calculated within a species to determine correlations between age and stable isotope ratios. LOESS non-parametric smoothing was utilized on non-ranked raw data to estimate suitable functions between two variables and compare regression surfaces between groups. The Kruskal-Wallis test followed by Tukey's multiple comparison test was used to compare variable means between seal species. For statistical analysis of stomach contents, two categories were established for each seal species based on presence and absence of prey items (0 = absent, 1 = present). These categories were analyzed using logistic regression with interaction term (forward selection) with age and sex (indicator variable) as independent variables for each species. All statistical analyses were performed using SAS (Version 8) with $\alpha = 0.05$. Sigma-Plot (Version 7.0) was used for graphic presentation of data. Results are reported as mean +/- standard deviation (SD) unless otherwise noted.

1.4 Results

1.41 Stomach Contents Analyses

Table 1.2 presents frequency of occurrence (FO_i) of prey in stomachs of bearded, ringed, and spotted seals harvested in Alaska. FO_i of prey was calculated for all stomachs containing digesta. For ringed seals, 50 stomachs were analyzed and 11 were empty or contained only bile, blood or parasites. Of 37 bearded seal stomachs, one was empty and contained only parasites. For spotted seals 43 stomachs were analyzed and 5 contained no food. All 25 stomachs of ringed seals harvested in Holman, Canada were empty.

The frequency of fish was 61.5% in stomachs of ringed seals containing prey (Table 1.2). Of all fish identified, gadids like Arctic cod and saffron cod (*Eleginus gracilis*) were identified most often, followed by Pacific sand lance (*Ammodytes hexapterus*). Zooplankton in stomachs of ringed seals occurred at a frequency of 64.1%. Both euphausids and mysids were consumed in similar proportions (53.8% and 46.2%, respectively), as were amphipods and pandalid shrimp (38.5% and 30.8%, respectively). All other prey were present in less than 10% of stomachs with contents.

Prey diversity in bearded seal stomachs was higher than in ringed and spotted seals with more than 20 different species consumed representing more than 10 animal phyla (Table 1.2). Prey was ingested intact in most cases though only feet of ingested clams and snails were present and only abdomens of predominately gravid female spider crabs (*Hyas coarctatus*) was identified from stomach contents. Crustaceans were found in 97.2% of stomachs. Of the prey species consumed, sculptured shrimp (*Sclerocrangon boreas*) was present most often and occurred in 83.3% of the stomachs. The frequency of fish in bearded seal stomachs was 80.6%, with eelpout (*Lycodes* spp.) making up the majority of teleost prey (58.3%). Other prey identified in more than 50% of the stomachs consisted of northern shrimp (*Pandalus* spp.), amphipods, spider crabs, octopus, Greenland cockle (*Serripes groenlandicus*) and echiurid worms.

Fishes were identified in all spotted seal stomachs containing prey (Table 1.2). Most frequently found was Pacific herring (52.6%), followed by gadid fish (47.4%) and rainbow smelt (23.7%). Capelin, sand lance, and flatfish were found in more than 10% of the stomachs, while other teleosts (e.g., sculpin) were present in less than 10%. Invertebrate prey was mainly comprised of crustaceans (44.7%) with amphipods making up the largest proportion (26.3%). Mollusks occurred in less than 10% of the stomachs.

Generally, there was no statistical difference in the frequency of prey types consumed by male and female bearded and spotted seals. However, bearded seal males were 6 times more likely to contain smelt (odds-ratio (OR) = 6.06, $p = 0.04$) and 7.5 times more likely to contain sea cucumber than females (OR = 7.52, $p = 0.01$). In ringed seals, male stomachs were about 16 times more likely to contain zooplankton than females (OR = 15.87, $p = 0.0004$). This relationship was also significant when mysids and euphausids were analyzed separately ($p = 0.007$ and 0.002 for euphausids and mysids, respectively). Similarly, stomachs of female ringed seals were 5.4 times more likely to contain fish than those of males (OR = 5.42, $p = 0.04$). Sex was not significant when teleost species were analyzed separately.

Older bearded seals were less likely to consume welkes (*Buccinum* spp.) (OR = 0.87, $p = 0.049$). No other age effects were noted in bearded seal diets. In spotted seals, presence of capelin and flatfish increased with increasing age (p = 0.003, OR = 1.90 and p = 0.005, OR = 1.41, for capelin and flatfish, respectively). In ringed seals only the consumption of cod was related to age (p = 0.01) and presence of cod is 1.2 times more likely with increasing age (OR = 1.22), while zooplankton ingestion was age independent.

1.42 Stable Isotopes

Age and isotope composition of spotted seals harvested near Shishmaref and Little Diomede were not statistically different (p = 0.65, 0.83 and 0.12 for age, $\delta^{15}N$ and $\delta^{13}C$, respectively). Therefore these data were pooled to increase sample size and power. Ringed seals harvested in Holman were significantly more depleted in ^{13}C than ringed seals from Barrow (p = <0.0001). Stable nitrogen isotope ratios and age were higher in Canadian ringed seals (p = 0.006 for both $\delta^{15}N$ and age) than in animals sampled in Barrow and therefore seals from Alaska and Canada were analyzed as different groups. Generally, there were no sex differences within species in age composition, stable carbon or stable nitrogen isotope ratios. However, female ringed seals harvested in Barrow had significantly higher stable nitrogen isotope ratios than males harvested in Barrow (p = 0.01) and they were analyzed separately for this variable. Male and female ringed seals from Holman as well as bearded and spotted seal sexes were pooled.

Spearman rank test showed no significant correlation between variables in bearded seals and Holman ringed seals. Age was positively correlated to stable nitrogen isotopes ($p = 0.009$) in spotted seals, and positively correlated to $\delta^{13}C$ in Barrow ringed seals ($p = 0.0007$).

Mean ratios of stable carbon and nitrogen isotopes in seals, walrus and some prey species (analyzed in this study and compiled from literature) are given in Table 1.3. $\delta^{15}N$ in prey is quite variable, ranging from 16.7‰ in large squid to 7.9 ± 0.8‰ in amphipods, reflecting different trophic levesl of these pinniped prey items. Stable nitrogen isotope signatures of ringed, bearded and spotted seal muscle ranged widely. Mean $\delta^{15}N$ in ringed seals was 16.9 ± 0.6‰ (range 15.6‰ to 18.0‰) in Barrow, and 17.2 ± 0.7‰ in Holman (range 14.6‰ to 18.0‰). For bearded seal muscle, mean $\delta^{15}N$ was 16.7 ± 0.9‰ and varied between 15.2‰ and 18.8‰. Mean stable nitrogen isotope ratio in muscle of spotted seals was 17.6 ± 0.9‰ (range 15.9‰ to 19.4‰). Values of $\delta^{13}C$ in ringed seals showed a wide range as well, from -21.3‰ to -18.2‰ in Barrow and –20.9‰ to –19.2‰ in Holman. Mean values in bearded seals were –17.1 ± 0.6‰ (range -18.7‰ to -15.8‰) and ranged from -20.0‰ to –15.9‰ in spotted seals.

Kruskal-Wallis test showed significant differences in variables between seal species ($p = <0.0001$ for $\delta^{15}N$ and $\delta^{13}C$). Tukey's post-hoc test established that stable nitrogen isotope ratios were significantly higher in spotted seals and Holman ringed seals than bearded seals and male Alaskan ringed seals. $\delta^{15}N$ was not statistically different in Holman ringed seals, spotted seals and Barrow ringed seal females. $\delta^{15}N$ was lower in walrus than in the seals. Stable carbon isotopes were significantly more enriched in bearded seals and walrus than any other species, while Canadian ringed seals were most depleted in ^{13}C. No difference in $\delta^{13}C$ was detected between ringed seals from Barrow and spotted seals. Figure 1.2 illustrates $\delta^{15}N$ versus $\delta^{13}C$ in all pinnipeds analyzed.

1.5 DISCUSSION

1.51 Stomach Contents Analyses

1.511 Ringed Seals

Analysis of ringed seal stomachs showed Arctic cod prevalence increased with age and could possibly be related to hunting experience or habitat when foraging. This finding agrees with observations by Lowry et al. (1980a) that ringed seal pups consume less cod than adults, and Bradstreet and Finley (1983) noted a decline in the presence of crustaceans in stomachs with age in ringed seals. However, age was not a significant variable for consumption of zooplankton, crustaceans or invertebrate prey in general in this study. This could be related to the use of the FO_i method, as it tends to overestimate the importance of less commonly or unintentionally ingested prey (Hjelset et al. 1999). It is possible that seals preying on schooling fish, such as Arctic cod, will also ingest krill and amphipods as fish schools feed on zooplankton patches (Lowry and Frost 1981; Hop et al. 1997). Hence a decline in the importance of crustaceans with age cannot be ruled out, as the relationship of numerical frequency of krill with age was not determined. It is possible that ingestion of zooplankton is necessary nutritionally for these seals. Very little is known about nutritional quality of most marine forage or nutritional requirements of seals. Geraci (1975) reported high levels of thiaminase, an enzyme that breaks down thiamine (Vitamin B_1), in herring, smelt and capelin. As a result, captive and wild seal populations sustained exclusively on these fish can suffer from thiamine deficiency. Hence, inclusion of krill in the diet, even in adult animals, could serve to fulfill a dietary requirement.

Analysis of stomach contents in this study showed significant differences in prey composition between male and female ringed seals harvested in the Barrow area. This difference in prey composition between sexes was also detected by means of stable isotope analysis. $\delta^{15}N$ was significantly higher in females than males and stomach contents analysis showed that females were more likely to eat fish, while males consumed more zooplankton. Lowry et al. (1980a) reported that female ringed seals from the Bering Sea ate more fish and less shrimp than did males, but differences were minimal and similar differences in prey selection could not be found in other Arctic regions. Possible explanations for the differences in foods ingested by male and female ringed seals could include sexual segregation and associated differential use of resources. Fedoseev (2000) described segregation of ice-associated seals by age and sex outside the breeding period. Differences in foraging strategy and prey selection between sexes have been indicated for northern elephant seals (*Mirounga angustirostris*) (Le Boeuf et al. 2000) and have been suggested for northern fur seals (Hobson et al. 1997).

Several circumpolar studies have noted that ringed seals prey only on a few key taxa, and Arctic cod and a variety of crustaceans were found as important food items (Fedoseev 1965; Lowry et al. 1980a; Bradstreet and Finley 1983; Smith 1987). Results of this study also show that krill and fishes (in particular Gadidae) make up the majority of ringed seal diet and are consumed in similar frequencies. Lowry et al. (1978) noted that amphipods had a high frequency (69%) in ringed seal stomachs taken near Barrow, but comprised only 4.6% of the total combined volume. Similarly, frequency of amphipods in this study was 39%, but biomass consumed was likely negligible. Amphipods have been reported as a major food item for ringed seals from Svalbard and the Canadian Arctic (Dunbar 1941; Bradstreet and Finley 1983; Smith 1987; Weslawski et al. 1994). However, based on stable nitrogen isotope ratios in ringed seals from Barrow and Holman, and assuming an enrichment factor of 2.4‰ for $\delta^{15}N$ in seal muscle (Hobson et al. 1996), it is highly improbable that amphipods ($\delta^{15}N = 7.9 \pm 0.8$‰) make up a large proportion of the diet in either region. However, large variation in prey availability by region may confound a large scale geographical comparison.

1.512 Bearded Seals

Stomachs of bearded seals examined in this study contained a wide variety of benthic and epibenthic prey and these seals can be characterized as opportunistic generalists. Some aspects of bearded seal diet were similar to those previously reported, but others were markedly different. Antonelis et al. (1994) reported high frequencies of fish in bearded seals sampled in the Bering Sea in spring; the most common teleost species consumed was capelin. Finley and Evans (1983) also found large occurrences of fish in bearded seal stomachs from the Canadian Arctic with gadids being most common. In contrast, Lowry et al. (1980b) did not consider fish important prey for bearded seals based on volumetric measurements, even though frequency of fish ranged from 78% - 82%. However, volume of fish would be severely underestimated when only otoliths or bones are present. Frequency of fish found in stomachs of seals from the Barrow area was similar (80.6%) to occurrences reported by Lowry et al. (1980b), but the most common species consumed was eelpout followed by gadids.

Large regional differences in consumption of clams are seen in bearded seal diets. While frequencies of Greenland cockle in this study are high (50%) and are in agreement with prevalence of cockle reported by Lowry et al. (1980b) in the Bering Sea (63%) others only documented infrequent occurrences ranging from 7-8% in the Canadian Arctic and Svalbard (Antonelis et al. 1994; Hjelset et al. 1999) to absence in diet in the Okhotsk Sea (Pikharev 1941).

Frequency of octopus and echiurid worms in stomachs also varied among locations. Finley and Evans (1983) noted a high frequency of occurrence of octopus in bearded seal stomachs from the Canadian Arctic, but amounts consumed were minimal, while echiurid worms were not found. They concluded that neither octopus nor marine worms were important prey. Antonelis et al. (1994) and Hjelset et al. (1999) came to the same conclusion for bearded seals harvested in the Bering Sea and Svalbard, respectively. Lowry et al. (1980b) found no cephalopods, and echiurids were of minor importance. In contrast, this study showed high frequencies of octopus and echiurids in the diet of bearded seals harvested near Barrow and is in accordance with studies in the Sea of Okhotsk (Pikharev 1941).

The high frequency of occurrence of crustacean prey is in agreement with previous studies, though some regional differences are notable in species composition (Pikharev 1941; Lowry et al. 1980b; Finley and Evans 1983; Antonelis et al. 1994;

Hjelset et al. 1999) Sea cucumber was reported as a minor food item in bearded seal stomachs analyzed from the Canadian Arctic and Okhotsk Sea (Pikharev 1941; Finley and Evans 1983), but was fairly common in seals in this study. These variations in bearded seal diet are likely area specific and reflect the local distribution of available prey in the Arctic. However, there were evident variations in prey composition reported from the Bering and Chukchi seas (Lowry et al. 1980b; Antonelis et al. 1994). Differences in frequencies of occurrence in stomachs of seals collected in this study from the Chukchi Sea could reflect changes in prey distribution or abundance over the past 20 years.

Results of this study show that males are more likely than females to consume sea cucumber and smelt. This could be due to sex-related differential use of feeding habitats and associated small changes in benthic fauna. Tarasevich (1976) suggested that females use habitats closer to shore and avoid feeding at greater depth.

Lowry et al. (1980b) found increased importance of mollusk prey with age and suggested acquired behavior as a possible explanation. Segregation by age and associated differences in diet composition were not evident in this study, though some age-related variations in prey consumption was found for whelk (*Buccinum* spp.), and its prevalence decreased with increasing age.

1.513 Spotted Seals

The high frequency of fish in spotted seal diet is in accordance with studies conducted in the Alaskan and Russian Arctic and the Sea of Okhotsk (Bukhtiyarov et al. 1984; Sobolevskii 1996). The most common species present in spotted seal stomachs analyzed in this study was herring followed by gadids. Bukhtiyarov et al. (1984) noted that herring and smelt are minor foods for spotted seals in spring, but increase in prevalence during summer and fall. In late spring and early summer, spotted seals in the Sea of Okhotsk consumed mostly walleye pollock (*Theragra chalcogramma*), followed by herring (Kato 1982). Pacific herring are abundant in coastal waters during spawning in summer and migrate offshore to their wintering grounds after spawning (Lassuy 1989). Hence spotted seals likely are responding to seasonal availability of forage fish. However, salmon has been described as an important component of the diet in summer during spawning (Sobolevskii 1996; Lowry et al. 2000) but was not identified from stomachs of spotted seals in this study.

Gol'tsev (1971), Kato (1982), and Bukhtiyarov et al. (1984) reported high frequencies of crustacean prey in younger spotted seals while fish and cephalopods made up the majority of the adult diet. However, no age-related differences were found in the consumption of invertebrate prey in this study. This could be associated with the abundance of spawning herring that would make this species seasonally accessible and easy prey for spotted seal pups. However, age was a significant factor in the prevalence of flatfish and capelin in older seals. Bukhtiyarov et al. (1984) reported that older seals are more likely to feed on benthic organisms. Benthic prey may only be available to adults due to restrictions in diving performance of juveniles, as has been suggested for harbor seal pups (Jørgensen et al. 2001). This could explain the presence of flatfish or other benthic prey in adult spotted seals and relative absence in pups.

1.52 Stable Isotopes

1.521 Ringed Seals

Stable nitrogen isotopes varied over the range of one trophic level in ringed seals from both Barrow (15.6‰ to 18.0‰) and Holman (14.6‰ to 18.0‰). This possibly reflects the consumption of either krill or Arctic cod. Hobson et al. (1996) suggested an enrichment factor of 2.4‰ for $\delta^{15}N$ in seal muscle. Assuming a seal feeding exclusively on zooplankton, a muscle $\delta^{15}N$ value of approximately 13‰ could be expected while that of a seal preying on cod would approximate 18‰. These extremes reflect the upper, but not lower ranges of $\delta^{15}N$ found in ringed seals, suggesting minor significance of a zooplankton-exclusive diet. Also, average concentrations of $\delta^{15}N$ (16.9‰) indicate a krill and cod mix for these seals, and are in agreement with stomach contents findings.

While the gastric prevalence of Arctic cod was positively correlated to age, $\delta^{15}N$ showed no age dependence. Several young animals (<1 year) and one fetus displayed elevated ^{15}N values. Hobson et al. (1997) and Das et al. (2003) observed similar nitrogen enrichment in northern fur seal pups and suckling harbor porpoises (*Phocoena phocoena*), respectively, and suggested that milk has isotope signatures comparable to other maternal tissues. Kurle (2002) proposed preferential transfer of ^{15}N to milk. Thus, trophic enrichment could occur in nursing pups or in fetuses during gestation compared to their mothers. Roth and Hobson (2000) theorized that high rate of protein synthesis and catabolism in tissues of juveniles causes excretion of predominantly light nitrogen while the heavier isotope is incorporated and magnified in tissues.

$\delta^{15}N$ was significantly different between male and female ringed seals harvested in Barrow, with females occupying a higher trophic level. Das et al. (2003) made a similar observation with female harbor porpoise being enriched in ^{15}N over males and suggested higher consumption or feeding on larger prey by pregnant or lactating animals. Stomach content analysis of ringed seals confirmed that females are more likely to eat cod and less krill than males, thus explaining the higher trophic status observed from stable nitrogen isotope ratios.

A positive correlation of carbon isotope ratios with age was shown for ringed seals from Barrow, but not for ringed seals from the Canadian Arctic (Figure 1.3). In both locations a similar relationship was noticeable after a LOESS non-parametric smoothing. Thus, the positive relationship observed in Barrow seals was most likely driven by some young-of-the-year (YOY) and one fetus (highlighted as open triangles in Figure 1.3) that are highly depleted in ^{13}C compared to the majority of seals. Body fat is usually depleted in ^{13}C due to selective fractionation (DeNiro and Epstein 1977) and the low values for the fetus and YOY suggest the mobilization and transfer of carbon from body fat of the mother to fetal development and milk production. Wilson et al. (1988) found that 54% of the carbon in milk is derived from body fat in Holstein cows during early lactation. Seal pups receive high-fat milk and are weaned after about 2 months, during nursing they more than double their weight (Kelly 1988).

Carbon isotope signatures in Canadian ringed seals were significantly depleted compared to all other seals. Schell et al. (1998) reported more depleted carbon-13 values in the Beaufort Sea versus continental shelf waters of the Bering and Chukchi seas.

Figure 1.3 illustrates that $\delta^{13}C$ in ringed seals from the Canadian Arctic can clearly be distinguished from ringed seals harvested in Barrow. Since many phocid seals migrate (Lowry et al. 1998) and ringed seals of the Canadian Arctic are known to move towards the Chukchi and Bering seas (Smith 1987; Harwood and Smith 2003), it is possible that some of the ringed seals harvested in the Barrow area have migrated or dispersed from the Beaufort Sea and hence show the characteristic low carbon-13 signature. Geographically, Point Barrow is the separation point for Beaufort and Chukchi seas (Figure 1.1) and seals from either area and with either carbon signature are common and taken by subsistence hunters.

1.522 Bearded Seals and Walrus

Stable isotope analyses of bearded seal muscle show a wide range for $\delta^{15}N$. This variation covers one trophic level (TL), likely reflecting the diverse feeding habits of these seals. The low nitrogen isotope ratios in walrus muscle indicate the reliance of this species on lower trophic level prey. The significance of clams to walrus diet has been emphasized by a variety of reports (Lowry et al. 1980b; Fay 1982; Fay et al. 1984) and is further supported by the $\delta^{15}N$ findings of this study. Fish are generally not present in walrus stomachs and frequency of octopus is negligible (Krylov 1971; Fay et al. 1984).

A comparison of TL, calculated from nitrogen isotope ratios and TL estimates from stomach contents by Pauly et al. (1998), shows that results deviate for bearded seals and walrus while they are in good agreement for ringed and spotted seals (Table 1.2). The estimate by Pauly et al. (1998) for bearded seals is much lower than the isotope calculation. This study showed that fish was of great importance to bearded seals and also suggested a possible shift in prey distribution compared to previous reports from the Bering and Chukchi seas (Lowry et al. 1980b; Antonelis et al. 1994). These changes and an under representation of fish as prey for bearded seals could lead to underestimation of TL based on previous accounts of stomach contents. In contrast, isotope calculations were lower for walrus than reported by Pauly et al. (1998). However, the estimate by Pauly et al. (1998) contained animals that had preyed on other pinnipeds and can thus be considered to occupy a higher TL. While seal-eating walrus are known to exist, they do not represent the norm of the population (Fay 1960; Lowry and Fay 1984). Based on nitrogen isotope ratios obtained it is likely that none of the walrus included in this study had consumed other pinnipeds in the recent past.

Values of $\delta^{13}C$ in bearded seals and walrus show a large distribution, while carbon isotope ratios of walrus have a smaller range (-18.7‰ to -15.8‰ and –17.3‰ to –16.8‰ in bearded seals and walrus, respectively). The more enriched carbon isotope signatures found in bearded seals and walrus compared to ringed and spotted seals (Figure 1.2) are likely due to benthic feeding habits, as confirmed by analyses of stomach contents. Aquatic environments with low turbulence and stagnant boundary layers, such as the benthic ecosystem, are more enriched in ^{13}C (France 1995b). The larger $\delta^{13}C$ range in bearded seals could be related to migration patterns between Beaufort and Chukchi Seas as discussed for ringed seals, but also could be associated with opportunistic feeding on both benthos and plankton. In contrast, the walrus is a specialist and relies almost exclusively on benthic prey. However, sample size for walrus muscle was small and could explain the smaller variation in walrus isotope ratios. Based on these results it is unlikely that walrus and bearded seal have a large overlap in prey utilization, but it is possible that competition between these two pinnipeds is the driving force for a dietary change in bearded seals as suggested by Lowry et al. (1980b).

1.523 Spotted Seals

Nitrogen isotope ratios of spotted seals show that they feed on a higher trophic level than other pinnipeds in this study. This is in accordance with stomach contents analysis as fish occurs at a high frequency in the spotted seal diet. $\delta^{15}N$ is positively correlated with age, suggesting that trophic level increases with increasing age of spotted seals (Figure 1.4). However, mostly young animals were sampled and a 24-year old female (Dio-7-01 is highlighted in Figure 1.4) was excluded from regression analysis to avoid the impact of an influential observation and interpolation over the range of 15 years. A change from low to high trophic level prey is in accordance with studies by Kato (1982) and Bukhtiyarov et al. (1984) who suggested that spotted seal pups feed mainly on crustaceans and that the importance of fish increases with age. However, stomach contents analysis in this study did not show any age effects for invertebrate prey. Considering that stable isotopes in muscle tissue show a signature that reflects feeding habits of these animals over at least a month (Tieszen et al. 1983; Hobson 1993) it is possible that younger seals were feeding on a lower trophic level earlier in the year and switched their diet to take advantage of the abundant spawning herring in early summer.

Hence the importance of invertebrates to immature seals was not detectable by means of stomach contents analysis.

Carbon isotope ratios of spotted seal muscle were not statistically different from ringed seal muscle $\delta^{13}C$. This suggests that both species rely on the planktonic food web rather than the benthic ecosystem as seen for bearded seals and walrus. However, stable carbon isotope ratios were not correlated with age in spotted seals as described for Alaskan ringed seals. Lowry et al. (1998) showed long-distance migration of spotted seals equipped with satellite transmitters in the Chukchi Sea during the open-water season. Seals migrating between the Beaufort and Chukchi seas could therefore have different $\delta^{13}C$ patterns. This possible movement and cyclical foraging of spotted seals in offshore areas and coastal haul-out sites as demonstrated by Lowry et al. (1998) could also result in variable carbon signatures. Smith et al. (1996) showed more depleted carbon isotope ratios in harbor seal populations feeding in freshwater versus saltwater habitats. A similar trend was observed by Hobson et al. (1997) when comparing coastal harbor seals with exclusively marine Steller sea lions (*Eumetopias jubatus*).

In conclusion, dietary habits of three phocids analyzed in this study are markedly different. Bearded seals relied heavily on the benthic food chain, and ringed and spotted seals foraged mainly in the water column. However, prevalence of schooling fish in stomachs was much higher in spotted than in ringed seals. Stable nitrogen and carbon isotope analysis fit well with most dietary compositions based on stomach contents. Most notable was the occupation of a higher trophic level by Alaskan ringed seal females compared to males and an overall prevalence of a krill-fish mix diet in ringed seals.

Nitrogen isotope ratios also demonstrated increasing trophic level in spotted seals with age; however, examination of stomach contents did not indicate different feeding patterns. Nevertheless, the importance of herring to spotted seals of all ages during summer would not have been noticeable with stable isotope analyses alone. This study also documented that age is an important factor when reconstructing pinniped diets and accounts for much of the variability found in stable carbon and nitrogen isotope ratios. We recommend the use of traditional methods, e.g., stomach contents or scat analysis, in combination with chemical feeding ecology to assess dietary habits most accurately when direct observation of feeding behavior is not possible.

1.6 ACKNOWLEDGMENTS

This study would not have been possible without the samples provided by Alaskan and Canadian subsistence hunters in the communities of Barrow, Holman, Little Diomede and Shishmaref, and we thank them all for their support. We greatly appreciate the assistance of C. D. N. Brower, H. Brower, Jr., T. Olemaun, B. Akootchook, T. Hepa, L. Hopson, V. Woshner, R. Elsner, T. Zenteno-Savin, S. Visalli, D. Burnett, G. York and many others in the field and T. Bentzen, T. Howe, N. Haubenstock and P. Hoekstra for support with analysis. We also thank L. Harwood for providing tissues and jaws of ringed seals harvested in Holman, Canada, R. Highsmith and B. Bluhm for collection of amphipod samples from the Bering Strait, and J. Bengtson and D. DeMaster for training in cementum aging and stomach contents analysis. The Frozen Tissue Collection of the University of Alaska Museum provided some of the spotted seal muscle samples.

This study was primarily funded by the Cooperative Institute for Arctic Research (CIFAR). Additional support was provided by the Experimental Program for Stimulation of Competitive Research (EPSCoR); the Biomedical Research Infrastructure Network (BRIN); the North Slope Borough Department of Wildlife Management; the Institute of Arctic Biology and the Department of Biology and Wildlife, UAF; the US Geological Survey; the Barrow Arctic Science Consortium (BASC); and the National Science Foundation (NSF) OPP Grant 9910319.

1.7 REFERENCES

Akimushkin II (1965) Cephalopods of the seas of the U.S.S.R. Israel Program for Scientific Translations. Jerusalem.

Antonelis GA, Melin SR, Bukhtiyarov YA (1994) Early spring feeding habits of bearded seals (*Erignathus barbatus*) in the Central Bering Sea, 1981. Arctic 47: 74-79.

Becker PR, Porter BJ, Mackey EA, Schantz MM, Demiralp R, Wise SA (1999) National Marine Mammal Tissue Bank and Quality Assurance Program: protocols, inventory, and analytical results. Gaithersburg, U.S. Department of Commerce.

Benjaminsen T (1973) Age determination and the growth and age distribution from cementum growth layers of bearded seals at Svalbard. Fiskeridirektoratets skrifter. Serie havundersøkelser 16: 159-170.

Bowen WD (2000) Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. Can J Fish Aquat Sci 57: 898-905.

Bradstreet MSW, Finley KJ (1983) Diet of ringed seals (*Phoca hispida*) in the Canadian High Arctic. Toronto, Ontario, LGL Limited.

Braham HW, Burns JJ, Fedoseev GA, Krogman BD (1984) Habitat partitioning by ice-associated pinnipeds: distribution and density of seals and walruses in the Bering Sea, April, 1976 *In* Soviet-American Cooperative. Research on Marine Mammals. Volume 1 – Pinnipeds. U.S. Department of Commerce, NOAA, NMFS.

Bukhtiyarov YA, Frost KJ, Lowry LF (1984) New information on foods of the spotted seal, *Phoca largha*, in the Bering Sea in spring. *In* Soviet-American Cooperative. Research on Marine Mammals. Volume 1 – Pinnipeds. U.S. Department of Commerce, NOAA, NMFS.

Burns JJ (1970) Remarks on the distribution and natural history of pagophilic pinnipeds in the Bering and Chukchi Seas. J Mamm 51: 445-454.

Burns JJ, Francis HF, Fedoseev GA (1984) Craniological analysis of harbor and spotted seals of the North Pacific Region. *In* Soviet-American Cooperative. Research on

Marine Mammals. Volume 1 - Pinnipeds. U. S. Department of Commerce, NOAA, NMFS.

Burns JM, Trumble SJ, Castellini MA, Testa JW (1998) The diet of Weddell seals in McMurdo Sound, Antarctica as determined from scat collections and stable isotope analysis. Polar Biol 19: 272-282.

Burton RK, Koch PL (1999) Isotopic tracking of foraging and long-distance migration in northeastern Pacific pinnipeds. Oecologia 119: 578-585.

Butler TH (1980) Shrimps of the Pacific coast of Canada. Can Bull Fish Aquat Sci 202: 280p.

Cleator HJ (1996) The status of the bearded seal, *Erignathus barbatus*, in Canada. Can Field-Nat 110: 501-510.

Das K, Lepoint G, Leroy Y, Bouquegneau JM (2003) Marine mammals from the southern North Sea: feeding ecology data from $\delta^{13}C$ and $\delta^{15}N$ measurements. Mar Ecol Prog Ser 263: 287-298.

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

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45: 341-351.

Derocher AE, Wiig O, Andersen M (2002) Diet composition of polar bears in Svalbard and the western Barents Sea. Polar Biol 25: 448-452.

Dunbar MJ (1941) On the food of seals in the Canadian Eastern Arctic. Can J Res 19: 150-155.

Fay FH (1960) Carnivorous walrus and some Arctic zoonoses. Arctic 13: 111-122.

Fay FH (1982) Ecology and biology of the Pacific Walrus, *Odobenus rosmarus divergens* Illiger. United States Department of the Interior, Washington, D. C.

Fay FH, Bukhtiyarov YA, Stoker SW, Shults LM (1984) Foods of the Pacific walrus in winter and spring in the Bering Sea. *In* Soviet-American Cooperative. Research on Marine Mammals. Volume 1 - Pinnipeds. U. S. Department of Commerce, NOAA, NMFS.

Fedoseev GA (1965) Food of the ringed seal. Izvestiia TINRO 59: 216-223.

Fedoseev GA (2000) Population biology of ice-associated forms of seals and their role in the northern Pacific ecosystems. Center for Russian Environmental Policy, Moscow.

Finley KJ, Evans CR (1983) Summer diet of the bearded seal (*Erignathus barbatus*) in the Canadian high Arctic. Arctic 36: 82-89.

Foster N R (1991) Intertidal bivalves – a guide to the common marine bivalves of Alaska. University of Alaska Press.

France RL (1995a) Carbon isotopic variability in the composite pelagic foodweb of four oligotrophic lakes: feeding diversity or metabolic fractionations? J Plankton Res 17: 1993-1997.

France RL (1995b) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Mar Ecol Prog Ser 124: 307-312.

Frost KJ (1981) Descriptive key to the otoliths of gadid fishes of the Bering, Chukchi, and Beaufort Seas. Arctic 34: 55-59.

Frost KJ, Lowry LF (1980) Feeding of ribbon seals (*Phoca fasciata*) in the Bering Sea in spring. Can J Zool 58: 1601-1607.

Gales NJ, Cheal AJ (1992) Estimating diet composition of the Australian sea-lion (*Neophoca cinerea*) from scat analysis: an unreliable technique. Wildl Res 19: 447-456.

Gannes LZ, O'Brien DM, Martinez Del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78: 1271-1276.

Geraci JR (1975) Pinniped nutrition. Rapp. P.-v. Reun. Cons. int. Explor. Mer. 169: 312-323.

Gol'tsev VN (1971) Feeding of the largha seal. Ekologiia 2: 62-70.

Härkönen T (1986) Guide to otoliths of the bony fishes of the Northeast Atlantic. Danbiv ApS, Hellerup, Denmark.

Harvey JT, Loughlin TR, Perez MA, Oxman DS (2000) Relationship between fish size and otolith length for 63 species of fishes from the Eastern North Pacific Ocean. NOAA Technical Report NMFS 150. U.S. Department of Commerce. Seattle, Washington.

Hammill MO, Smith TG (1991) The role of predation in the ecology of the ringed seal in Barrow Strait, Northwest Territories, Canada. Mar Mamm Sci 7: 123-135.

Harwood LA, Smith TG (2003) Movements and diving of ringed seals in the Beaufort and Chukchi seas, 1999-2003. 15th Biennial Conference on the Biology of Marine Mammals, Greensboro, North Carolina.

Hjelset AM, Andersen M, Gjertz I, Lydersen C, Gulliksen B. (1999) Feeding habits of bearded seals (*Erignathus barbatus*) from the Svalbard area, Norway. Polar Biol 21: 186-193.

Hobson, K.A. 1993. Trophic relationships among high Arctic seabirds: insights from tissue-dependent stable-isotope models. Mar Ecol Prog Ser 95: 7-18.

Hobson KA, Alisauskas RT, Clark RG (1993) Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. Condor 95: 388-394.

Hobson KA, Schell DM, Renouf D, Noseworthy, E (1996) Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Can J Fish Aquat Sci 53: 528-533.

Hobson KA, Sease JL, Merrick RL, Piatt JF (1997) Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of nitrogen and carbon. Mar Mamm Sci 13: 114-132.

Hobson KA, Welch HE (1992) Determination of trophic relationships within a High Arctic marine food web using d13C and d15N analysis. Mar Ecol Prog Ser 84: 9-18.

Hoekstra PF, Dehn L-A, George JC, Solomon KR, Muir DCG, O'Hara TM (2002) Trophic ecology of bowhead whales (*Balaena mysticetus*) compared with that of other arctic marine biota as interpreted from carbon-, nitrogen-, and sulfur-isotope signatures. Can J Zool 80: 223-231.

Hop H, Welch HE, Crawford RE (1997) Population structure and feeding ecology of Arctic cod schools in the Canadian high Arctic. Am Fish Soc Symp 19: 68-80.

Jensen GC (1995) Pacific coast crabs and shrimps. Sea Challengers.

Jørgensen C, Lydersen C, Brix O, Kovacs KM (2001) Diving development in nursing harbour seal pups. J Exp Biol 204: 3993-4004.

Kathman RD, Austin WC, Saltman JC, Fulton JD (1986) Identification manual to the mysidacea and euphausiacea of the Northeast Pacific. Can Spec Pub Fish Aquat Sci 93: 411p.

Kato H (1982) Food habits of largha seal pups in the pack ice area. Sci. Rep. Whales Res. Inst 43: 123-136.

Keen AM, Coan E (1974) Marine molluscan genera of Western North America. An illustrated key. Stanford University Press, Stanford, California.

Kelly BP (1988) Ringed seal. *In* Selected marine mammals of Alaska: Species accounts with research and management recommendations. Marine Mammal Commission, Washington.

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

Krylov VI (1971) On the food of the pacific walrus (*Odobenus rosmarus divergens* Ill.). Investigations on marine mammals. Trudy AtlantNIRO 39: 110-116.

Kurle CM (2002) Stable-isotope ratios of blood components from captive northern fur seals (*Callorhinus ursinus*) and their diet: applications for studying the foraging ecology of wild otariids. Can J Zool 80: 902-909.

Lassuy DR (1989) Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest). Pacific herring. US Fish and Wildlife Service Biological Report 82: 1-18.

Lauckner G (1985) Diseases of mammalia: pinnipedia. *In* Diseases of marine mammals. Westholsteinische Verlagsdruckerei Boyens & Co., Heide, Germany.

Lawson JW, Hobson KA (2000) Diet of harp seals (*Pagophilus groenlandicus*) in nearshore northeast Newfoundland: inferences from stable-carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses. Mar Mamm Sci 16: 578-591.

Le Boeuf BJ, Crocker DE, Costa DP, Blackwell SB, Webb PM, Houser DS (2000) Foraging ecology of northern elephant seals. Ecol Monographs 70: 353-382.

Lowry LF, Fay FH (1984) Seal eating by walruses in the Bering and Chukchi Seas. Polar Biol 3: 11-18.

Lowry LF, Frost KJ (1981) Distribution, growth, and foods of Arctic cod (*Boreogadus saida*) in the Bering, Chukchi, and Beaufort seas. Can Field-Nat 95: 186-191.

Lowry LF, Frost KJ, Burns JJ (1978) Food of ringed seals and bowhead whales near Point Barrow, Alaska. Can Field-Nat 92: 67-70.

Lowry LF, Frost KJ, Burns JJ (1980a) Variability in the diet of ringed seals, *Phoca hispida*, in Alaska. Can J Fish Aquat Sci 37: 2254-2261.

Lowry LF, Frost KJ, Burns JJ (1980b) Feeding of bearded seals in the Bering and Chukchi Seas and trophic interaction with Pacific walrus. Arctic 33: 330-342.

Lowry LF, Frost KJ, Douglas RD, DeMaster DP, Suydam RS (1998) Movements and behavior of satellite-tagged spotted seals (*Phoca largha*) in the Bering and Chukchi Seas. Polar Biol 19: 221-230.

Lowry LF, Burkanov VN, Frost KJ, Simpkins MA, Davis R, DeMaster DP, Suydam R, Springer A (2000) Habitat use and habitat selection by spotted seals (*Phoca largha*) in the Bering Sea. Can J Zool 78: 1959-1971.

Matson GM (1981) Workbook for cementum analysis. Matson's Laboratory, Milltown, Montana. 30pp.

Muir DCG, Segstro MD, Hobson KA, Ford CA, Stewart REA, Olpinski S (1995) Can seal eating explain elevated levels of PCBs and organochlorine pesticides in walrus blubber from Eastern Hudson Bay (Canada)? Environ Pollut 90: 335-348.

Murie DJ, Lavigne DM (1986) Interpretation of otoliths in stomach content analyses of phocid seals: quantifying fish consumption. Can J Zool 64: 1152-1157.

Ostrom PH, Lien J, Macko SA (1993) Evaluation of the diet of Sowerby's beaked whale, *Mesoplodon bidens*, based on isotopic comparisons among northwestern Atlantic cetaceans. Can J Zool 71: 858-861.

Pauly D, Trites AW, Capuli E, Christensen V (1998) Diet composition and trophic levels of marine mammals. ICES J Mar Sci 55: 467-481.

Pikharev GA (1941) Some data on the feeding of the far eastern bearded seal. Izvestiia TINRO 20: 101-120.

Rathbun MJ (1929) Canadian Atlantic Fauna. Arthropoda – Decapoda. The Biological Board of Canada. The Atlantic Biological Station. St. Andrews, N. B., Canada.

Roth JD, Hobson KA (2000) Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstructions. Can J Zool 78: 848-852.

Santos MB, Clarke MR, Pierce GJ (2001) Assessing the importance of cephalopods in the diets of marine mammals and other top predators: problems and solutions. Fish Res 52: 121-139.

Schell DM, Barnett BA, Vinette KA (1998) Carbon and nitrogen isotope ratios in zooplankton of the Bering, Chukchi and Beaufort seas. Mar Ecol Prog Ser 162: 11-23.

Schell DM, Saupe SM, Haubenstock N (1989) Natural isotope abundance in bowhead whale (*Balaena mysticetus*) baleen: markers of aging and habitat usage. *In* Stable isotopes in ecological research. Springer-Verlag, Berlin.

Sheffield G, Fay FH, Feder H, Kelly BP (2001) Laboratory digestion of prey and interpretation of walrus stomach contents. Mar Mamm Sci 17: 310-330.

Siegstad H, Neve PB, Heide-Jørgensen MP, Härkönen T (1998) Diet of ringed seal (*Phoca hispida*) in Greenland. *In* Ringed seals in the North Atlantic. NAMMCO Scientific Publications, Tromsø. pp. 229-241.

Simpkins MA, Hiruki-Raring LM, Sheffield G, Grebmeier JM, Bengtson JL (2003) Habitat selection by ice-associated pinnipeds near St. Lawrence Island, Alaska in March 2001. Polar Biol 26: 577-586.

Smith RJ, Hobson KA, Koopman HN, Lavigne DM (1996) Distinguishing between populations of fresh-and saltwater harbour seals (*Phoca vitulina*) using stable-isotope ratios and fatty acid profiles. Can J Fish Aquat Sci 53: 272-279.

Smith TG (1976) Predation of ringed seal pups (*Phoca hispida*) by the arctic fox (*Alopex lagopus*). Can J Zool 54: 1610-1616.

Smith, T.G. (1987) The ringed seal, *Phoca hispida*, of the Canadian Western Arctic. Can Bull Fish Aquat Sci 216: 1-81.

Sobolevskii EI (1996) The distribution and seasonal dynamics of feeding of the spotted seal *Phoca largha* in the Bering Sea. Rus J Mar Biol 22: 199-204.

Stewart REA, Stewart BE, Stirling I, Street E 1996 Counts of growth layer groups in cementum and dentine in ringed seals (*Phoca hispida*). Mar Mamm Sci 12: 383-401.

Tarasevich MN (1976) Biology of the bearded seal (*Erignathus barbatus*). Tr. Akad. Nauk SSSR., Institut Okeanologii 71: 223-225. Fisheries and Marine Service Translation Series No. 3774.

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

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

Wilson GF, Mackenzie DDS, Brookes IM, Lyon GL (1988) Importance of body tissue as sources of nutrients for milk synthesis in the cow, using ^{13}C as a marker. Brit J Nutr 60: 605-617.

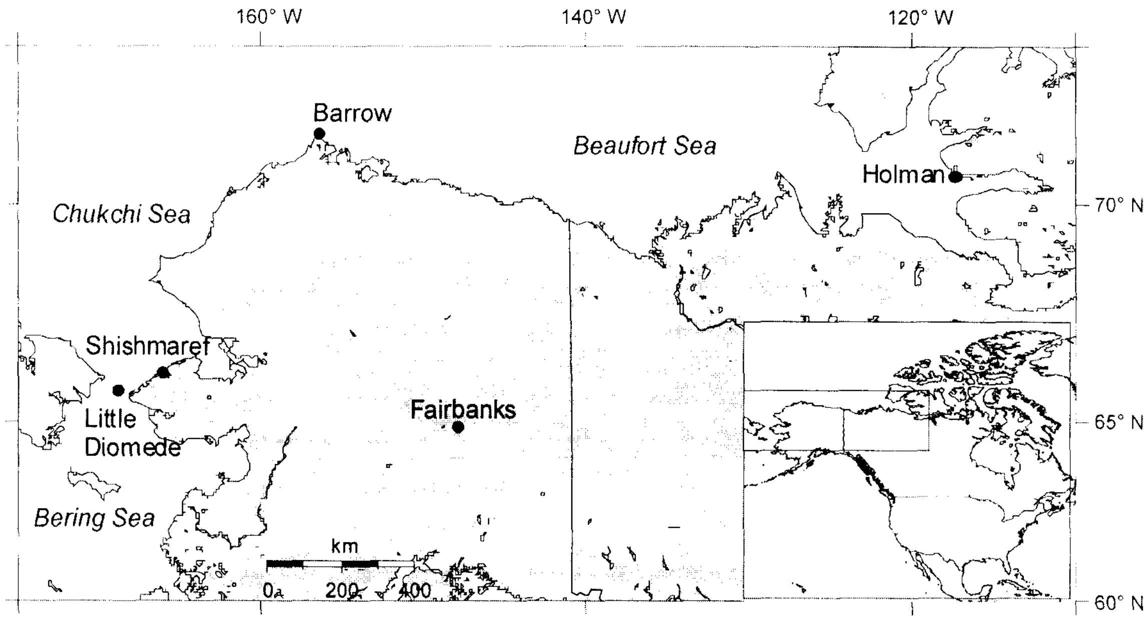


FIGURE 1.1 Alaskan and Canadian communities where samples of subsistence-harvested Arctic pinnipeds were collected. Fairbanks serves as a point of reference.

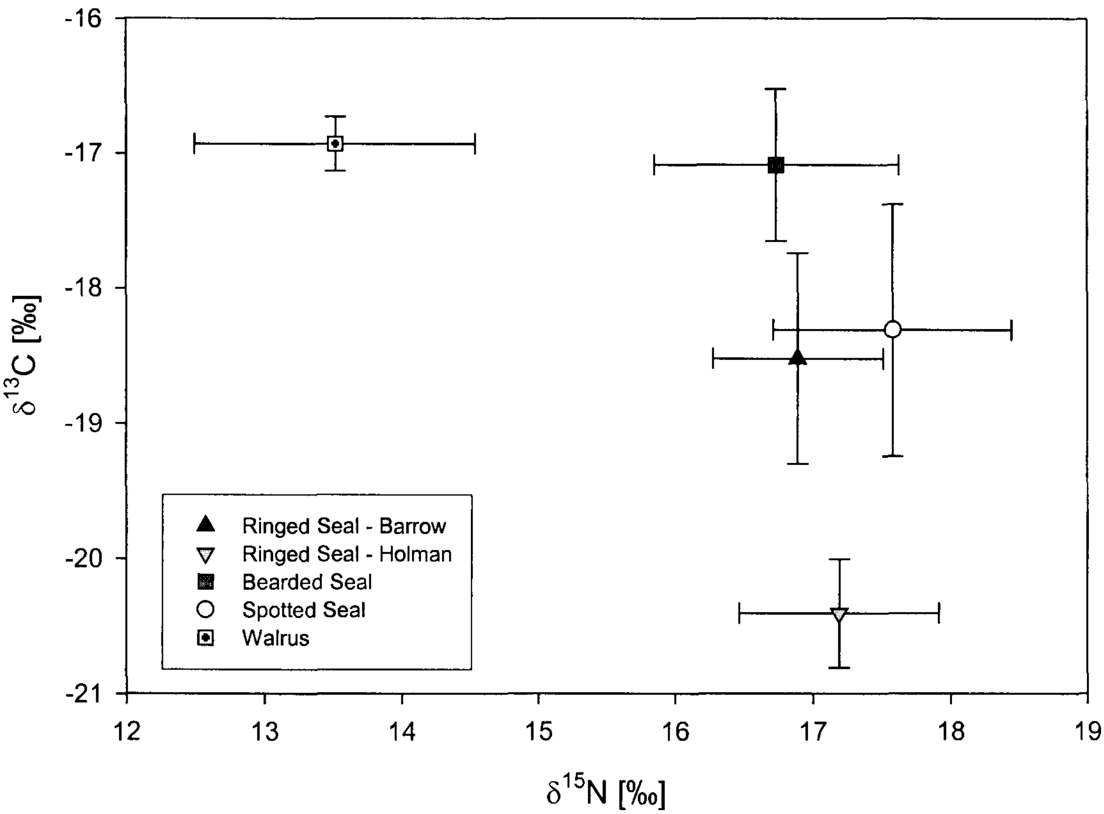


FIGURE 1.2. $\delta^{13}C$ versus $\delta^{15}N$ in ringed seals harvested in Alaska and Canada, and bearded and spotted seals, 1996-2001 and walrus 1998 and 2003 from Alaska. Symbols present the mean values and error bars show the standard deviations.

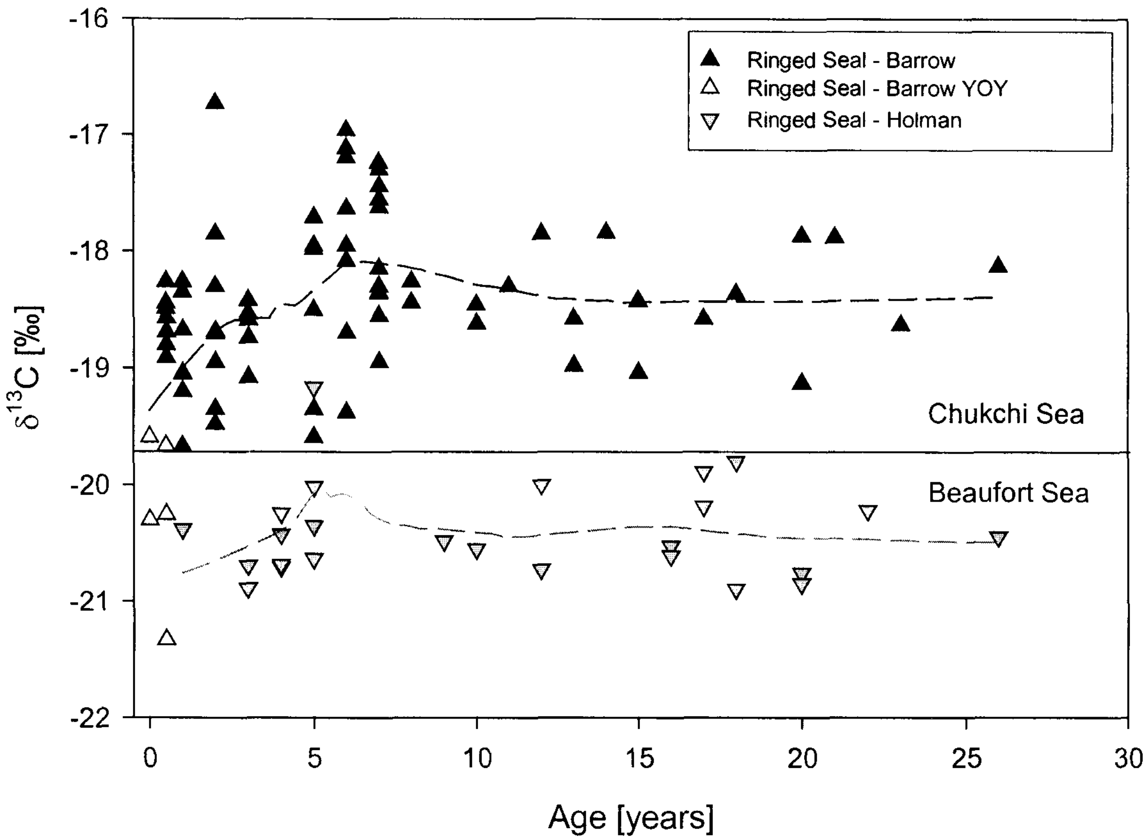


FIGURE 1.3. $\delta^{13}C$ in muscle versus age based on cementum analysis of teeth of ringed seals harvested in Barrow, Alaska, 1996-2001 and Holman, Canada, 2001. Four YOY and 1 fetus harvested in Barrow are highlighted as open triangles. A LOESS nonparametric smoothing (dashed lines) was employed to estimate and compare the regression surface of both groups of seals.

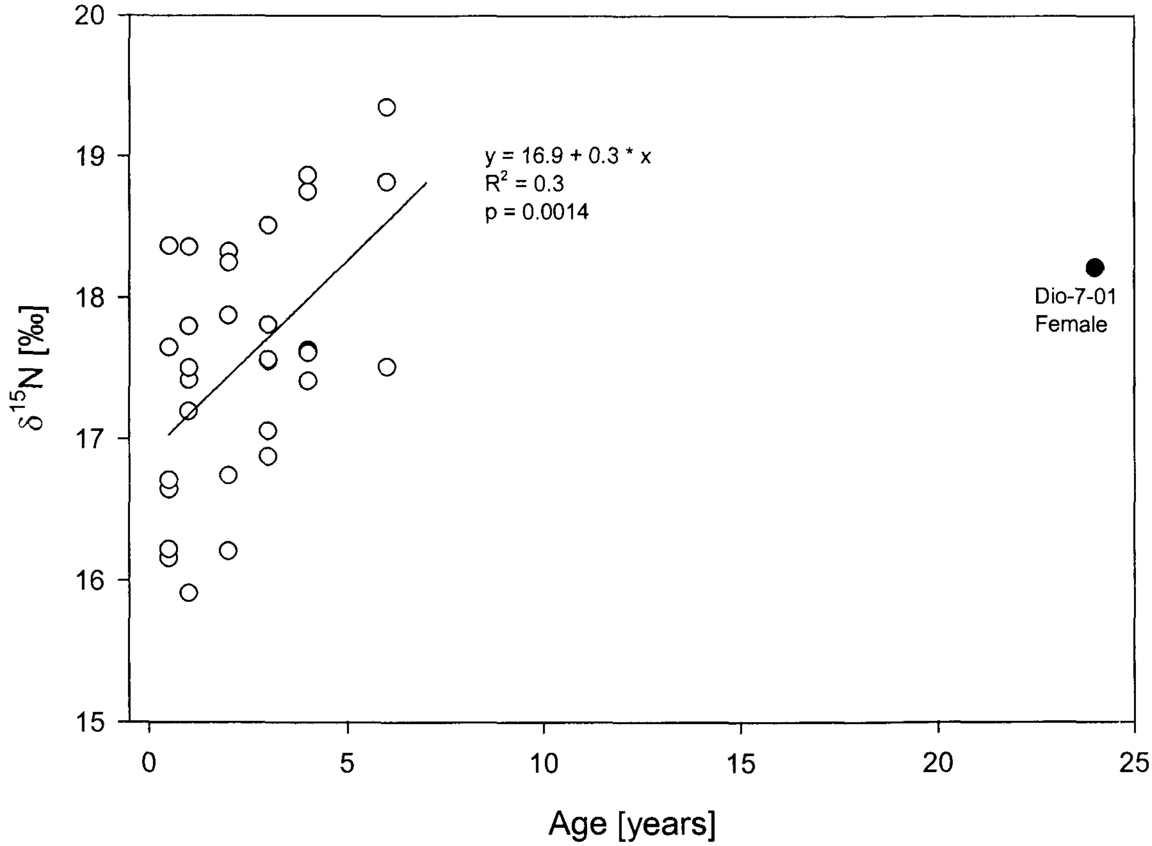


FIGURE 1.4. $\delta^{15}N$ in muscle versus age based on cementum analysis of teeth of spotted seals harvested near Little Diomede and Shishmaref, 2000-2001. A 24-year old spotted seal female (Dio-7-01 highlighted as black circle) was excluded from regression analysis.

TABLE 1.1. Pinniped samples collected in Alaskan and Canadian villages, 1996-2001 and 2003.

Species	Sampling Location	Muscle n	Stomach n
Ringed Seal	Barrow, Alaska	78	50
Ringed Seal	Holman, Canada	25	25
Bearded Seal	Barrow, Alaska	49	37
Spotted Seal	Little Diomede, Shishmaref, Alaska	34	43
Walrus	Barrow, Little Diomede, Alaska	6	-

n : sample size

TABLE 1.2. Frequency of occurrence (FO_i) of prey species identified from bearded, ringed and spotted seal stomachs collected in Alaska, 1996-2001.

Bearded Seals

Species	FO_i[§] [%]
Teleost Fish:	
Eelpout (*Lycodes* spec.)	58.3
Cod (Gadidae)	41.7
Sculpin (Cottidae)	38.9
Rainbow Smelt (*Osmerus mordax*)	22.2
Flatfish (Pleuronectidae)	11.1
All Fish	80.6
Crustaceans:	
Sculptured Shrimp (*Sclerocrangon boreas*)	83.3
Northern Shrimp (*Pandalus* spec.)	63.9
Amphipods	63.9
Spider Crab (*Hyas coarctatus*)	58.3
Hermit Crab (Paguridae)	30.6
Isopoda (*Saduria* spec.)	13.9
Other crustaceans	19.4
All Crustaceans	97.2
Mollusca:	
Octopus (*Octopus* spec.)	69.4
Greenland Cockle (*Serripes groenlandicus*)	50.0
Whelk (*Buccinum* spec.)	38.9
Softshell Mussle (*Mya* spec.)	11.1
Whelk (*Neptunea* spec.)	8.3
All Mollusks	83.3
Marine Worms:	
Echiurida	61.1
Priapulida	16.7
Polychetes	2.8
Echinoderma:	
Sea Cucumber (Holothuroidea)	36.1
Other[¶]	13.9

[§]37 stomachs analyzed, 1 stomach empty
[¶]Includes Sponges, Sea star, Hemichordates, Sipunculids

Ringed Seals

Species	FO_i[§] [%]
Teleost Fish:	
Cod (Gadidae)	46.2
Pacific Sand Lance (*Ammodytes hexapterus*)	20.5
Sculpin (Cottidae)	5.1
Eelpout (*Lycodes* spec.)	5.1
Unidentified fish	7.7
All Fish	61.5
Crustaceans:	
Euphausiacea (*Thysanoessa* spec.)	53.8
Mysidacea (*Mysis* spec. and *Neomysis* spec.)	46.2
Zooplankton (Euphausids and Mysids)	64.1
Amphipoda	38.5
Northern Shrimp (*Pandalus* spec.)	30.8
Sculptured Shrimp (*Sclerocrangon boreas*)	5.1
Isopoda (*Saduria* spec.)	5.1
All Crustaceans	89.7
Marine Worms:	
Echiurida	5.1
Cephalopoda:	
Octopus (*Octopus* spec.)	2.6
Squid	2.6
Other[¶]	10.3

[§]50 stomachs analyzed, 11 stomachs empty
[¶]Includes Feather, *Sclerocrangon* spec., Bryozoa, Hemichordates

Spotted Seals

Species	FO_i[§] [%]
Teleost Fish:	
Pacific Herring (*Clupea pallasii*)	52.6
Arctic Cod (*Boreogadus saida*)	42.1
Saffron Cod (*Eleginus gracilis*)	34.2
All Cod (Gadidae)	47.4
Rainbow Smelt (*Osmerus mordax*)	23.7
Capelin (*Mallotus villosus*)	15.8
Pacific Sand Lance (*Ammodytes hexapterus*)	13.2
Flatfish (Pleuronectidae)	10.5
Prickleback (Stichaeidae)	5.3
Sculpin (Cottidae)	2.6
Snailfish (Liparidae)	2.6
Unidentified Fish	15.8
All Fish	100.0
Crustaceans:	
Amphipods	26.3
Northern Crangon (*Crangon alaskensis*)	13.2
Tanner Crabs (Chionocetes)	2.6
Other Crustaceans	13.2
All Crustaceans	44.7
Mollusca:	
Squid	2.6
Clams	5.3

[§]43 stomachs analyzed, 5 stomachs empty

TABLE 1.3. Stable carbon and nitrogen ratios in Arctic phocids and selected prey species. Values are given as mean +/- standard deviation unless otherwise noted.

TL: trophic level, *n*: sample size

Species	Sampling Location	*n*	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	TL[§]	TL[¶]	Reference
Pinnipeds:							
Ringed Seal (*Phoca hispida*)	Barrow	78	16.9 ± 0.6	-18.5 ± 0.8	4.0	3.8	this study, includes data from Hoekstra et al. 2002
Female	Barrow	30	17.1 ± 0.6	-18.4 ± 0.7	4.1	-	this study, includes data from Hoekstra et al. 2002
Male	Barrow	45	16.7 ± 0.6	-18.5 ± 0.7	4.0	-	this study, includes data from Hoekstra et al. 2002
	Holman	25	17.2 ± 0.7	-20.4 ± 0.4	4.1	-	this study
	Canadian Arctic	9	17.3 ± 1.1	-17.3 ± 0.7	4.1	-	Hobson and Welch 1992
	Canadian Arctic	8	13.9 ± 1.4	-19.7 ± 0.9	3.2	-	Muir et al. 1995
Bearded Seal (*Erignathus barbatus*)	Barrow	49	16.8 ± 1.0	-17.1 ± 0.6	4.0	3.4	this study, includes data from Hoekstra et al. 2002
	Canadian Arctic	4	16.8 ± 0.2	-16.6 ± 0.5	4.0	-	Hobson and Welch 1992
Spotted Seal (*Phoca largha*)	Little Diomede / Shishmaref	34	17.6 ± 0.9	-18.3 ± 0.9	4.2	4.0	this study
Walrus (*Odobenus rosmarus*)	Barrow / Diomede	6	13.5 ± 1.0	-16.9 ± 0.2	3.1	3.4	this study
	Canadian Arctic	6	12.5 ± 0.6	-17.8 ± 0.3	2.9	-	Hobson and Welch 1992
	Canadian Arctic, Akulivik	9	10.9 ± 0.5	-17.3 ± 0.5	2.4	-	Muir et al. 1995
	Canadian Arctic, Inukjuak	12	11.7 ± 0.7	-18.7 ± 0.8	2.7	-	Muir et al. 1995
Teleost Fish:							
Arctic Cod (*Boreogadus saida*)	Barrow	24	15.5 ± 1.0	-20.9 ± 0.4	3.7	-	this study, includes data from Hoekstra et al. 2002
	Canadian Arctic	26	15.2 ± 0.7	-18.9 ± 1.0	3.6	-	Hobson and Welch 1992
	Newfoundland	10	13.7 ± 0.4	-18.8 ± 0.3	3.2	-	Lawson and Hobson 2000
Saffron Cod (*Eleginus gracilis*)	Barrow	1	14.2	-22.0	3.3	-	this study
Capelin (*Mallotus villosus*)	Newfoundland	11	12.2 ± 0.8	-21.4 ± 0.5	2.8	-	Ostrom et al. 1993
	Gulf of Alaska	13	12.4 ± 0.1	-	2.8	-	Hobson et al. 1997
Pacific Herring (*Clupea pallasii*)	Bering Strait	3	13.8 ± 0.9	-20.7 ± 1.7	3.2	-	this study
	Washington	2	14.5 ± 0.3	-	3.4	-	Hobson et al. 1997
Walleye Pollock (*Theragra chalcogramma*)	Bering Strait	6	14.2 ± 2.0	-20.4 ± 2.5	3.3	-	this study
	Gulf of Alaska	24	10.9 ± 0.2	-	2.4	-	Hobson et al. 1997
Pacific Sand Lance (*Ammodytes hexapterus*)	Barrow	1	14.6	-22.5	3.4	-	this study
	Gulf of Alaska	8	11.9 ± 0.1	-	2.7	-	Hobson et al. 1997
Fourhorn Sculpin (*Myoxocephalus quadricornis*)	Canadian Arctic	1	15.2	-18.1	3.6	-	Hobson and Welch 1992
Rainbow Smelt (*Osmerus mordax*)	Barrow	10	14.8 ± 1.0	-21.2 ± 0.8	3.5	-	this study
Flounder (*Hippoglossoides* spp.)	Barrow	3	12.6 ± 0.0	-19.7 ± 0.1	2.9	-	Hoekstra et al. 2002
Snailfish (*Liparis* spp.)	Canadian Arctic	4	15.0 ± 0.4	-17.4 ± 0.5	3.5	-	Hobson and Welch 1992

TABLE 1.3 (continued)

Species	Sampling Location	*n*	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	TL§	TL¶	Reference
Crustaceans:							
Zooplankton (unsorted)	Kaktovik	21	10.4 ± 1.2	-24.9 ± 0.7	2.3	-	this study, includes data from Hoekstra et al. 2002
	Barrow	13	9.9 ± 0.8	-20.3 ± 0.6	2.2	-	this study, includes data from Hoekstra et al. 2002
	Holman	10	10.4 ± 0.5	-24.4 ± 0.6	2.3	-	Hoekstra et al. 2002
Copepods	Canadian Arctic	6	9.2 ± 0.5	-20.4 ± 0.4	2.0	-	Hobson and Welch 1992
	East Chukchi	54/63	10.5 ± 0.2*	-21.8 ± 0.1*	2.3	-	Schell et al. 1998
Euphausids	East Chukchi	33/38	9.7 ± 0.3*	-20.2 ± 0.2*	2.1	-	Schell et al. 1998
	Gulf of Alaska	9	11.2 ± 0.5	-	2.5	-	Hobson et al. 1997
Amphipods (unsorted)	Bering Strait	40	7.9 ± 0.8	-19.9 ± 0.7	1.7	-	this study
Parathemisto libellula	Canadian Arctic	6	11.7 ± 0.7	-20.3 ± 0.4	2.7	-	Hobson and Welch 1992
Onisimus glacialis	Canadian Arctic	4	11.4 ± 0.5	-18.2 ± 1.1	2.6	-	Hobson and Welch 1992
Isopod (*Saduria sabini*)	Barrow	4	13.7 ± 0.5	-17.0 ± 0.5	3.2	-	this study
Isopod (*Saduria entomon*)	Barrow	3	14.3 ± 0.9	-20.7 ± 0.5	3.3	-	this study
Spider crab (*Hyas coarctatus*)	Bering Strait	2	13.4 ± 0.1	-18.4 ± 0.1	3.1	-	this study
Sculptured shrimp (*Sclerocrangon boreas*)	Barrow	1	16.1	-19.8	3.8	-	this study
Northern Shrimp (*Pandalus borealis*)	Newfoundland	10	11.3 ± 0.2	-17.9 ± 0.3	2.6	-	Lawson and Hobson 2000
Mollusca:							
Greenland cockle (*Serripes groenlandicus*)	Bering Strait	1	8.0	-19.2	1.7	-	this study
	Canadian Arctic	7	8.9 ± 0.8	-18.7 ± 0.4	1.9	-	Hobson and Welch 1992
Whelk (*Buccinum* spp.)	Canadian Arctic	5	12.6 ± 0.7	na	2.9	-	Hobson and Welch 1992
Softshell Muscle (*Mya truncata*)	Canadian Arctic	7	9.5 ± 0.7	-19.0 ± 0.4	2.1	-	Hobson and Welch 1992
Octopus (*Octopus* spp.)	Bering Strait	1	9.9	-20.0	2.2	-	this study
Squid	Bering Strait	3	13.6 ± 1.2	-19.9 ± 1.3	3.2	-	this study
Squid - small	Gulf of Alaska	4	9.6 ± 0.5	-	2.1	-	Hobson et al. 1997
Squid - large	Gulf of Alaska	1	16.7	-	4.0	-	Hobson et al. 1997
Arctic squid (*Gonatus fabricii*)	Newfoundland	10	12.3 ± 0.7	-18.5 ± 0.4	2.8	-	Lawson and Hobson 2000
Squid - large (*Illex illecebrosus*)	Newfoundland	2	15.1	-20.0	3.6	-	Ostrom et al. 1993
Squid - small (*Illex illecebrosus*)	Newfoundland	4	9.3 ± 0.1	-19.1 ± 0.4	2.0	-	Ostrom et al. 1993
Echinodermata:							
Sea cucumber	Canadian Arctic	3	9.5 ± 0.5	-19.7 ± 1.2	2.1	-	Hobson and Welch 1992
Urochordata:							
Tunicates	Barrow	3	11.3 ± 1.0	-23.3 ± 0.9	2.6	-	this study
Marine Worms:							
Priapulid	Barrow	1	15.5	-17.2	3.7	-	this study
Echiurid	Barrow	1	9.6	-19.7	2.1	-	this study
Polychaete	Bering Strait	3	10.3 ± 2.6	-19.5 ±1.9	2.3	-	this study

* Standard Error

§ $TL = 2 + (\delta^{15}N_{consumer} - \delta^{15}N_{copepod})/3.8$

¶ after Pauly et al. 1998

CHAPTER 2

TRACE ELEMENTS IN TISSUES OF PHOCID SEALS HARVESTED IN THE ALASKAN AND CANADIAN ARCTIC – INFLUENCE OF AGE AND FEEDING ECOLOGY[2]

2.1 ABSTRACT

Concentrations of selected trace elements (Ag, Cu, Cd, Se, Zn, THg, and MeHg) were measured in tissues of subsistence-harvested ringed (*Phoca hispida* Schreber, 1775), bearded (*Erignathus barbatus* Erxleben, 1777) and spotted seals (*Phoca largha* Pallas, 1811) from Alaska and ringed seals from Canada. Most variables differed significantly in tissues of phocids analyzed. Renal Cd concentrations were highest in ringed seals from Canada and Alaskan bearded seals, while spotted seals had the lowest concentrations. Cd concentrations increased with age to a maximum in ringed and bearded seals, followed by a slow decline with increasing age. Spotted seals had the highest ratio of organic Hg to THg (%MeHg) in liver and bearded seals the lowest ratio. THg in seal tissues followed the opposite trend. %MeHg in ringed and bearded seals followed a hyperbolic decay function with age, but was highly variable in spotted seals. Seals with lesions had a higher relative occurrence of organic mercury in liver. The molar ratio of Se:THg in liver exceeded 1:1 in most seals and was negatively correlated to age

[2] Dehn, L.-A., Sheffield, G. G., Follmann, E. H., Duffy, L. K., Thomas, D. L., Bratton, G. R., Taylor, R. J., O'Hara, T. M. (2005). Trace elements in tissues of phocid seals harvested in the Alaskan and Canadian Arctic – Influence of age and feeding ecology. Canadian Journal of Zoology 83: 726-746.

in ringed and spotted seals. Hepatic Ag was higher in bearded seals compared to ringed and spotted seals. A correlation of Ag with age was not documented.

Keywords: Ringed seal, bearded seal, spotted seal, trace elements, age, feeding ecology, Arctic

2.2 INTRODUCTION

Bioaccumulation of heavy metals has been of growing concern to consumers of subsistence foods in Alaska and other Arctic areas (Ponce et al. 1997; Egeland et al. 1998; Deutch and Hansen 2003). Ice seals such as ringed (*Phoca hispida* Schreber, 1775), bearded (*Erignathus barbatus* Erxleben, 1777) and spotted seals (*Phoca largha* Pallas, 1811) have significant nutritional and cultural importance to the Native coastal population of the Arctic. Marine mammals, including seals, have been reported to accumulate contaminants (e.g., mercury) in their tissues with increasing age and trophic level (Smith and Armstrong 1978; Dietz et al. 2000; Woshner et al. 2001a; Watanabe et al. 2002; Bustamante et al. 2004a). However, accumulation of metals in marine mammal tissues appears to be not solely dependent on trophic position. Bowhead whales (*Balaena mysticetus* Linnaeus, 1758) feed low in the Arctic food chain on pelagic krill (Lowry and Frost 1984). Nevertheless, cadmium (Cd) concentrations in the kidney of bowheads are higher than in top-level consumers of a simple Arctic food chain, e.g., polar bear (*Ursus maritimus* Phipps, 1774) and Arctic fox (*Alopex lagopus* Linnaeus, 1758) (Bratton et al. 1997; Woshner et al. 2001a; Woshner et al. 2001b; Ballard et al. 2003). Hence, it is possible that dietary selection may influence trace element pathways. On the other hand, accumulation of trace elements with age could lead to a higher rate of metal deposition in bowheads since these mysticetes are known to live in excess of 100 years (George et al. 1999), while the life span of Arctic fox usually does not exceed 10 years (Smirnov 1968). Even though the continuous accumulation of metals with age is often assumed, the relationship of trace elements in tissues of marine mammals with age is less well investigated. Egeland et al. (1998) noted the importance of animal age to interpret and identify possible new sources of contamination and to provide recommendations for the consumption of subsistence foods.

Wagemann et al. (1988) suggested that mercury (Hg) is transferred via the placenta from mother to pup in harp seals (*Phoca groenlandica* Erxleben, 1777), while Cd does not cross the placenta. Essential elements like copper (Cu), zinc (Zn) and selenium (Se) are transferred and accumulate in the fetus (Wagemann et al. 1988; Enomoto and Hirunuma 2001; Rombach et al. 2003). Bustamante et al. (2004a) reported a logarithmic relationship of Cd with age in kidney of male grey seals (*Halichoerus grypus* Fabricius, 1791), suggesting equilibrium of Cd intake and elimination with increasing age. Watanabe et al. (2002) found an increase of Cd with age in liver and kidney of Caspian seals (*Phoca caspica* Gmelin, 1788) followed by decline in older seals. This relationship was also described for ringed seals in Greenland (Dietz et al. 1996; Dietz et al. 1998; Sonne-Hansen et al. 2002).

Recent investigations report elevated Cd concentrations in renal and hepatic tissue of ringed seals that do not coincide with lesions associated with metal toxicosis (Woshner 2000; Sonne-Hansen et al. 2002). It was suggested that ringed seals may have adapted to these metal concentrations and perhaps that these levels can in fact be considered normal background for this species (Woshner 2000; Sonne-Hansen et al. 2002). It is likely therefore that ringed seals are exposed to elevated Cd in their diet. Ringed seals experience seasonal dietary shifts, feeding on pelagic zooplankton in summer and Arctic cod (*Boreogadus saida* Lepechin, 1774) in winter and spring (Lowry et al. 1980a). In the

Canadian Arctic, Cd concentrations of typical ringed seal prey such as copepods and amphipods are 5-8 times higher than concentrations in Arctic cod (Bohn and McElroy 1976). Euphausids and mysids have lower concentrations, ranging from 0.25 μg/g dry weight (dw) in mysids to 0.44 μg/g dw in euphausids (Muir et al.1992; Ritterhoff and Zauke 1997).

While metals and trace elements in active metabolic tissues (e.g., liver and kidney) of ringed seals have been well investigated in the circumpolar Arctic there is little information available for trace element concentrations in bearded and spotted seals. Bearded seals rely heavily on benthic food, with crustaceans making up a majority of their prey (Lowry et al. 1980b, Dehn in prep.). Feeding habits of bearded seals are similar to those of Pacific walrus (*Odobenus rosmarus* Linnaeus, 1758). However, Cd concentrations in liver of walrus from Alaska were about 5 times higher (Taylor et al. 1989) than for three bearded seals analyzed by Mackey et al. (1996), while total mercury (THg) in walrus liver was an order of magnitude lower than reported for Canadian bearded seals (Smith and Armstrong 1978).

Recently it has been shown that not only organic compounds may act as endocrine disruptors, but heavy metals like Hg (both organic and inorganic), Cd, cobalt (Co) and lead (Pb) can also have estrogenic effects in humans and rodents (Johnson et al. 2003; Martin et al. 2003). Hence, contaminants have been suggested as a cause for the ongoing decline of harbor seals (*Phoca vitulina* Linnaeus, 1758) in Alaskan waters, a close relative to the spotted seal (Papa and Becker 1998). Both harbor seals and spotted seals are piscivorous, and seasonally abundant schooling fish, e.g., herring (*Clupea* *pallasii* Valenciennes, 1847), capelin (*Mallotus villosus* Müller, 1776) and smelt (*Osmerus* spp. Linnaeus, 1758) dominate their diet (Bukhtiyarov et al. 1984; Hoover 1988; Dehn in prep.). Cd concentrations in kidneys of Alaskan harbor seals range from 0.3-44 μg/g wet weight (ww) and THg in liver ranges from 0.4-72 μg/g ww (Miles et al. 1992). Hepatic tissue concentrations of Cd and THg for one female spotted seal analyzed by Becker et al. (1995a) fell within the lower range reported for harbor seals.

Concentrations of trace elements in most Arctic pinnipeds and accumulation factors and pathways are not well understood, and reference ranges of contaminant levels in healthy seals are needed. The distribution and movement of ringed, bearded and spotted seals are strongly dependent on movements of sea ice, and seals migrate in conjunction with their preferred ice habitat (Burns 1970). Since ice seals occupy a similar habitat but deviate in their feeding ecology, the hypothesis that metal concentrations in tissues could be discriminators for prey in the Arctic marine food web can be tested. These animals also offer the opportunity to study and compare dietary effects of trace elements likely without significant interspecies differences in physiological detoxification mechanisms.

The objectives of this study are to provide baseline concentrations of selected trace elements of apparently healthy seals taken during subsistence harvests in Alaska and Canada, and evaluate the effects of age, trophic level and prey prevalence on trace element pathways and biomagnification in phocid seals.

2.3 MATERIALS AND METHODS

2.31 Sampling

All seal samples were obtained during Native subsistence harvests. Sex and basic morphometrics, e.g., body length and blubber thickness were recorded. Most seals were grossly examined for lesions and parasites. Lumbar muscle, kidney and liver of ringed and bearded seals were collected in Barrow, Alaska mainly during the summer period from 1998-2001. To increase sample size and statistical power, data from ringed seals harvested in Barrow in 1996 and 1997 (Woshner et al. 2001a) were included in the analyses.

Ringed seal samples also were obtained in Holman, NWT, Canada during summer, 2001. Tissues of spotted seals were collected in Little Diomede (n = 18) and Shishmaref (n = 16), Alaska in summer 2000 and 2001. Figure 2.1 shows villages and communities where samples were collected and Table 2.1 summarizes sample sizes. All tissues were sub-sampled under clean conditions with titanium or ceramic blades on a Teflon covered surface, following the sampling protocol for contaminants by Becker et al. (1999) and stored at -20°C in acid-washed scintillation vials or Whirlpacks™ until analysis. Several potential prey species were collected or donated by subsistence hunters in Barrow and the Bering Strait. Marine mammal samples were collected and analyzed under the authority of Permit Nos. 782-1399 and 358-1585 issued to the Alaska Department of Fish and Game and 932-1489-03 issued to Dr. T. Rowles of the Marine Mammal Health and Stranding Response Program.

2.32 Aging

Jaws and claws were collected from seals after 1997 and stored at –20°C until analysis. Ringed seals harvested in 1996 and 1997 were aged using the keratin layers of claws, which provide a minimum age estimate for the animals (Benjaminsen 1973). Two canines or canine and postcanine (if no other canines were available) were extracted from the upper or lower jaw (depending on availability), submerged in a hot water bath for 30 minutes to avoid damage to the structure of the cementum, and stored in paper envelopes (Matson 1981). All teeth were shipped to Matson's Laboratory, Milltown, Montana for slide preparation. Teeth were prepared in 14 μm sections, placed on glass slides and stained with Giemsa histological stain suitable for cementum analysis. Age was estimated by counting annual growth layers in the cementum of teeth by two independent readers at the University of Alaska Fairbanks (UAF). Preparation and evaluation of teeth were done doubly blind by randomly assigning an identification number to each tooth with two teeth analyzed per animal. The animal identification and matching teeth were only revealed after all ages were estimated in duplicate. One growth layer per year of age was assumed for all seals (Benjaminsen 1973; Stewart et al. 1996). Maximum variation in age estimates for seals younger than 15 years was +/- 1 year and in animals older than 15 +/- 5 years.

2.33 Stable Isotope Analyses

Lumbar muscle of ringed, bearded, and spotted seals was freeze-dried and ground into a powder with mortar and pestle. Samples were analyzed for both stable carbon and nitrogen ratios ($\delta^{15}N$ and $\delta^{13}C$) at UAF using a Finnigan MAT Delta^{Plus}XL Isotope Ratio Mass Spectrometer (IRMS) directly coupled to a Costech Elemental Analyzer (ESC 4010). External instrument reproducibility for both carbon and nitrogen isotope analysis was +/- 0.2‰. Detailed methods are described in Dehn (in prep.).

2.34 Trace Element Analyses

Silver (Ag), Cd, Cu, Se and Zn were analyzed at Texas A&M University (TAMU) following US Environmental Protection Agency (EPA) procedures (200.3, 200.7, 200.8 and 200.9) with slight modifications (EPA 1992). Briefly, sub-sampled tissues were freeze-dried to a constant weight and homogenized by ball-milling. Powdered tissue (approximately 0.2 – 0.25 g) was digested in a microwave wet ash procedure using 3:2:1 HNO_3, H_2O_2 and HCl, respectively. A second preparation followed for determination of Se in tissues using excess HCl to completely reduce Se (VI) to Se (IV) in a CPI ModBlock digester. For Alaskan pinnipeds, all metals (except Cu and Zn) were analyzed using Graphite Furnace Atomic Absorption Spectrometry (Perkin-Elmer Model SIMAA 6000 equipped with an AS-72 autosampler and Zeeman background correction). Cu and Zn were determined by Flame Atomic Absorption Spectrometry (Perkin-Elmer Aanalyst 100). Se in all seal tissues was analyzed using Atomic Fluorescence Spectrometry (PSA Millennium Excalibur with CETAC autosampler) and metals (Ag, Cd, Cu and Zn) in seals from the Canadian Arctic were analyzed by ICP-MS (Perkin-Elmer Elan Model 6100 DRC-II). The detection limit was 0.01 μg/g for elements analyzed with Graphite Furnace AAS, Flame AAS and Atomic Fluorescence Spectrometry. The detection limits were 0.01 μg/g for Cd, 0.05 μg/g for Zn and Cu and 0.005 μg/g for Ag using ICP-MS. All element concentrations were expressed as μg/g wet weight (ww) unless otherwise noted.

2.35 Total Mercury Analysis

Total mercury (THg) was analyzed at UAF following the procedure established by Bloom and Crecelius (1983). Briefly, sub-sampled tissues were homogenized and approximately 1 g was digested in 7:3 HNO_3/H_2SO_4 and oxidized with 10% BrCl in 12N HCL. The sample was reduced to Hg^0 with $SnCl_2$ and purged with N_2 onto gold-coated quartz sand traps followed by dual thermal desorption to a Cold Vapor Atomic Fluorescence Spectrometer (Tekran Model-2500 CVAFS Mercury Detector) with argon as the carrier gas. The detection limit was 0.001 μg/g. Concentrations were expressed as μg/g ww unless otherwise noted.

2.36 Methyl Mercury Analysis

Methyl mercury (MeHg) was analyzed at UAF following the procedure established by Bloom (1989). About 1 g of tissue was homogenized and digested in 20% KOH in methanol. Aqueous phase ethylation was initiated with $NaB(C_2H_5)_4$ resulting in volatile methylethylmercury which was purged with N_2 from solution onto a carbotrap™. MeHg was thermally desorbed from the trap and volatile ethyl-analogs separated by isothermal (100°C) gas chromatography (GC) followed by CVAFS (Tekran Model-2500) with argon as the carrier gas. The detection limit was 0.001 μg/g. Concentrations were expressed as μg/g ww unless otherwise noted.

2.37 Quality Control

All trace element analyses have been run with respect to a thorough quality control program (Table 2.2). Reference materials (DOLT-2 and DORM-2) were obtained from the National Research Council, Canada (NRC) and SRM 1946 and BLS 1577b from the National Institute of Standards and Technology (NIST). Marine mammal reference material (liver of pilot and beluga whale) and SRM 1947 were provided by NIST as part of annual interlaboratory comparison exercises for the determination of trace elements in marine mammals (Wise et al. 1993, Christopher 2002, Christopher 2004). Spikes and duplicates of samples as well as method and instrument blanks were run routinely (with each group of 20 samples) during analysis.

2.38 Metal Ratios

The ratio or relative occurrence of organic Hg (MeHg) to total Hg (THg) is referred to as %MeHg in the text and was calculated as:

$$\%\text{MeHg} = (\text{MeHg } \mu g/g \text{ ww} / \text{THg } \mu g/g \text{ ww}) * 100$$

The molar ratio of Se to THg was calculated as:

Se/THg molar ratio = (Se μg/g ww / THg μg/g ww) * (200.59 g/mole / 78.96 g/mole)

where 200.59 g/mole and 78.96 g/mole are the atomic weight of Hg and Se, respectively.

2.39 Statistical Analysis

The variables in the data set (age, $\delta^{15}N$, $\delta^{13}C$, Ag, Cd, Cu, Se, Zn, THg, MeHg, %MeHg and Se/THg molar ratio) were ranked prior to analysis to adjust for violations of normality and homogeneity of variance assumptions. Variables were analyzed for location differences using t-tests within a seal species. If no significant differences were detected for each variable localities were pooled to increase sample size. Two-way ANOVA (with interaction term) followed by Tukey's multiple comparison test was applied to compare variable means among seal species, location and sex. Spearman rank correlation was calculated within a species to determine correlations between the variables. A residual analysis was implemented to determine any possible violations of assumptions. Nonlinear regression analysis and LOESS non-parametric smoothing were utilized on non-ranked raw data to estimate suitable functions between two variables and compare regression surfaces between seal species. Graphing and nonlinear regression analysis were conducted using Sigma-Plot (Version 7.0). All other statistical analyses were performed using SAS (Version 8) with $\alpha = 0.05$. In order to include element concentrations below the minimum detection limit (MDL) into summary statistics and statistical tests, they were expressed as one-half the MDL (Gilbert 1987). Results are reported as mean +/- standard deviation (SD) unless otherwise noted.

2.4 RESULTS

2.41 Trace Element Concentrations and Tissue Distribution

Table 2.3 summarizes mean concentrations of trace elements (Cu, Zn, Cd, Ag, Se, THg and MeHg) and element ratios (%MeHg and Se/THg molar ratio) in tissues of ringed, bearded and spotted seals from Alaska and Canada. For Ag in kidney and muscle, more than 50% of the samples were below the MDL in ringed, bearded, and spotted seals from Alaska.

Generally, concentrations of trace elements were highest in liver, followed by kidney, and lowest in muscle. However, renal concentrations of Cd exceeded levels in liver in all three species of seals in both Alaska and Canada. Zn in bearded seal muscle was higher than in kidney, and renal Se in spotted seals was greater than in liver. Zn in kidney of Holman ringed seals exceeded concentrations in liver and the molar ratio of Se to THg was lower in liver of Alaskan and Canadian ringed, bearded and spotted seals than other tissues.

2.42 Influence of Location

Trace element concentrations of spotted seals harvested near Shishmaref and Little Diomede were not statistically different. Hence, these data were pooled to increase sample size and power. Ringed seals harvested in Holman had significantly higher renal and hepatic concentrations of THg ($p = <0.0001$ for both kidney and liver), Ag ($p = <0.0001$ for both kidney and liver), Cd ($p = <0.0001$ and $p = 0.0004$ for kidney and liver, respectively), Se ($p = 0.003$ and $p = <0.0001$ for kidney and liver, respectively), and MeHg ($p = <0.0001$ and $p = 0.01$ for kidney and liver, respectively) than ringed seals from Barrow. Zn in kidney of Canadian ringed seals was higher than in Alaskan seals (p = 0.0005), while hepatic Zn and Cu in liver and kidney were not different (p = 0.17 for Zn in liver, p = 0.07 and p = 0.18 for Cu in kidney and liver, respectively). The ratio %MeHg in kidney was higher in Canadian seals than in seals from Barrow (p = 0.01), while the latter had higher %MeHg in liver (p = 0.0003). Seals from these two regions were therefore analyzed separately.

2.43 Influence of Sex

There were few sex differences within a species for the variables. Holman ringed seal males had higher renal concentrations of MeHg than females (p = 0.04), while females had higher concentrations of Se and Cd in liver (p = 0.04 and 0.005 for Se and Cd, respectively). Ringed seal females harvested in Barrow had higher hepatic Cd and Ag than did males (p = 0.04 and 0.03 for Cd and Ag, respectively). Similarly, renal Cd and Ag in liver of bearded seals were higher in females than males (p = 0.03 and 0.04 for Cd and Ag, respectively). Using two-way ANOVA, sex had a significant effect on renal Cd (p = 0.01), hepatic Cd (p = 0.006), hepatic Se (p = 0.03) and Ag in liver (p = 0.02) with females having higher concentrations than males in all cases. There were no significant interaction among seal species, location and sex.

2.44 Species Comparison

All variables in all tissues differed significantly among species analyzed and ringed seals from both locations (p = <0.0001 for most comparisons) with the exception of Cd in muscle. Results of the ANOVA and Tukey's multiple comparison test for all variables and tissues are compiled in Table 2.4. The relative proportion of organic Hg to

THg (%MeHg) in muscle was similar in ringed and bearded seals and accounted for about 100% of the THg concentration present in this tissue. Hepatic Zn, Cu, Cd and Ag were significantly higher in bearded seals than Alaskan ringed and spotted seals as well as Cd and Se and in the kidney. Ringed seals from Canada had the highest concentrations of Se in liver; Ag and Cd in liver and renal Cd were similar to concentrations in bearded seals. Average concentrations of Cu and THg in muscle were higher in ringed than in bearded seals. Spotted seals had the lowest concentrations of THg in tissues analyzed, but had the highest molar ratio of Se to THg in kidney and liver and highest renal and hepatic %MeHg. Mean THg and MeHg in liver and kidney were highest in ringed seals from Canada and the molar ratio of Se to THg in these tissues was lowest. Sample size for spotted seal muscle did not allow for an ANOVA comparison with ringed and bearded seals.

2.45 Correlation Between Variables

Spearman rank test showed significant correlations in a variety of variables within and among tissues and are given in Table 2.5 for ringed, bearded and spotted seals from Alaska and Canada. Positive correlations of Cd with age in kidney and Se in liver were noted in ringed, bearded and spotted seals from both Alaska and Canada. Correlations between Se and THg in liver and hepatic Cd to renal Cd were also found in all seals from both locations. Trophic level as determined by $\delta^{15}N$ was negatively correlated to Cd in liver and kidney of Alaskan ringed seals and was positively correlated to renal and/or hepatic MeHg or THg in bearded seals, spotted seals and ringed seals from Canada. Other significant correlations that were consistent among Alaskan species are Ag and Zn with

Cu in liver. Correlations that were noted in all species from both locations are highlighted in Table 2.5 as bold script, and correlations consistent between seal species harvested in Alaska are underlined.

2.46 Trace Element Concentrations in Potential Prey Species

Total body homogenates of potential prey species analyzed for Cu, Zn, Ag, Cd, Se and THg are compiled in Table 2.6. THg in body homogenates was generally low, ranging from 1 ng/g in zooplankton to 70 ng/g in pollock (*Theragra chalcogramma* Pallas, 1814). Cd was highest in invertebrates such as mollusks, sea cucumbers and crustaceans, while Cu was an order of magnitude higher in squid, mussel and benthic crustaceans compared to zooplankton and herring. Concentrations of Ag were higher in benthic and epibenthic species than pelagic zooplankton and the Se to THg molar ratio was highest in isopods and zooplankton (2150:1 and 380:1, respectively) and lowest in salmon (40:1).

2.5 DISCUSSION

2.51 Cadmium

2.511 Species Comparison

There is evidence that invertebrate prey species, in particular cephalopods, have higher levels of Cd than do fish (Bustamante et al. 1998; Bustamante et al. 2003). Canadian ringed seals and bearded seals from Alaska had the highest Cd levels in liver and kidney compared to Barrow ringed seals, and spotted seals had the lowest concentrations. Concentrations of Cd in bearded seal liver were comparable to hepatic Cd in Pacific walrus in Alaska, while concentrations in walrus kidney were higher (9.47 ± 8.26 μg/g and 50.77 ± 21.29 μg/g ww in walrus liver and kidney, respectively) (Taylor et al. 1989). The diet of bearded seals is dominated by benthic and epibenthic species, with fish, crustaceans and mollusks (including cephalopods) making up the majority of ingested prey in the Alaskan Arctic (Lowry et al. 1980b; Dehn in prep.). Walrus dietary habits are similar to those of bearded seals, but clams are their prominent prey, fish are generally not present, and frequency of octopus in walrus stomachs is negligible (Krylov 1971; Fay et al. 1984). However, concentration of Cd was low in the foot of Greenland cockle (*Serripes groenlandicus* Mohr, 1786) and the siphon of soft-shell clam (*Mya truncata* Linnaeus, 1758) from the Canadian Arctic (0.03 ± 0.03 μg/g and 0.21 ± 0.13 μg/g ww, respectively) (Wagemann and Stewart 1994), while Cd in cockle total body homogenates determined for this study was an order of magnitude higher (Table 2.6). Cd content was highest in the digestive gland of clams analyzed from Antarctica (Bargagli et al. 1996). Sheffield et al. (2001) argued that walrus likely excavated the whole clam and did not selectively consume the foot; this is conceivable for bearded seals as well. However, clam organs will digest more rapidly and thus may not be identified from most walrus or seal stomachs (Sheffield et al. 2001). Generally, Cd concentrations were higher in invertebrate prey than fish and higher in benthic than pelagic species (Table 2.6). Marine organisms analyzed from Antarctica showed a similar trend with Cd in benthic invertebrates being as high as 80 μg/g dw (Bargagli et al. 1996). High levels of Cd were reported for hyperiid amphipods (*Parathemisto libellula* Lichtenstein, 1822) and copepods from the Canadian Arctic, averaging 6.31 μg/g and 5.0 μg/g dw, respectively (Bohn and McElroy 1976; Hamanaka and Ogi 1984; Macdonald and Sprague 1988; Ritterhoff and Zauke 1997). Concentrations of Cd in mysids and whole Arctic cod were an order of magnitude lower with 0.17 μg/g and 0.62 μg/g dw, respectively (Bohn and McElroy 1976; Macdonald and Sprague 1988), and Cd in euphausids analyzed from Greenland waters were below detection limit (Dietz et al. 1996; Ritterhoff and Zauke 1997). Cd in Atlantic pilot whales (*Globicephala melas* Traill, 1809) is among the highest reported for marine mammals and can exceed 100 μg/g ww in whole kidneys (Caurant and Amiard-Triquet 1995). Squid has been implicated as being the Cd source for these whales, and concentrations in cephalopods in the Atlantic Ocean can range from 0.1 μg/g to 9.06 μg/g ww (Bustamante et al. 1998). Thus, benthic invertebrate prey consumed by bearded seals and high prevalence of cephalopods in their diet (Dehn in prep.) likely accounted for high concentrations of hepatic and renal Cd.

Ringed seals from the Canadian Arctic have been reported to consume mostly hyperiid amphipods (Bradstreet and Finley 1983; Smith 1987), and this may explain the elevated Cd concentrations found in this study with those of ringed seals from Alaska. However, nitrogen isotope ratios for Canadian ringed seals indicate dependence on higher trophic level prey (Dehn in prep.). Polar bears analyzed by Norstrom et al. (1986) and Braune et al. (1991), as well as ringed seals and beluga whales (*Delphinapterus leucas* Pallas, 1776) examined by Wagemann et al. (1996), followed a similar trend, with Cd being higher in the Eastern than the Western Arctic. Thus, it is likely that ringed seals from Canada are exposed to higher background concentrations of Cd than Alaskan seals.

Renal and hepatic Cd of ringed seals harvested in Holman, Canada fall intermediate to values reported by Wagemann et al. (1996) for ringed seals from Western and Eastern Canada (21.1 ± 14.2 and 47.7 ± 23.3 μg/g ww for seal kidney in western and eastern Canada, respectively). Amphipods are also an important prey species for seals from Greenland (Siegstad et al. 1998), and Cd in the renal cortex is similar to that reported from Eastern Canada (44.5 ± 40.8 μg/g ww) (Sonne-Hansen et al. 2002). However, cortex concentrations of Cd are approximately 25% higher than in whole renicules (Sonne-Hansen et al. 2002). Thus, concentrations of renal Cd in Greenland seals are similar to Canadian ringed seals in this study.

Cd concentrations in liver and kidney are within the range previously reported for ringed seals from Alaska (Becker et al. 1995a; Woshner et al. 2001a). Alaskan ringed seals switch their diet seasonally from zooplankton in summer to Arctic cod in winter and spring (Lowry et al. 1980a). An increased prevalence of Arctic cod with age was noted in ringed seals along with a reduction in the amount of crustaceans consumed (Lowry et al. 1980a, Dehn in prep.). Trophic level ($\delta^{15}N$) in this study was negatively correlated to Cd in Alaskan ringed seals, suggesting association of Cd with low trophic level prey. Hence, the relatively high Cd concentrations in ringed seal tissues are in agreement with the seasonal importance of dietary zooplankton (Lowry et al. 1980a, Dehn in prep.), in contrast to the often assumed insignificance of invertebrate prey to this species.

Spotted seals have been described as piscivorous, but some evidence suggests that taxa of prey consumed vary with age. Younger seals prey mostly on small crustaceans, while the frequency of fish increases in adults (Kato 1982; Bukhtiyarov et al. 1984, Dehn in prep.). The low Cd concentrations found in spotted seal liver and kidney and a diet dominated by fish both support an invertebrate connection to Cd. Similarly, Caspian seals had low Cd concentrations in liver and kidney and seemed to prefer a fish-based diet, while Baikal seals (*Phoca sibirica* Gmelin, 1788) accumulated Cd and fed mostly on invertebrates (Watanabe et al. 2002). Mean concentrations of Cd in kidney of harbor seals are higher than concentrations for closely related spotted seals (6.6 µg/g ww in harbor seal kidney) (Miles et al. 1992). This difference could be due to the age bias toward juvenile spotted seals in this study or could reflect the higher occurrence of cephalopod prey in harbor seals (Lowry et al. 1982; Hoover 1988).

2.512 Cd and Sex

Cd concentrations in liver but not kidney were higher in female ringed seals than in males from both Alaska and Canada. Dehn (in prep.) noted a difference in feeding ecology between sexes of Alaskan ringed seals. However, females occupied a higher trophic level than did males and had higher prevalence of fish in their diet, while males consumed more zooplankton (Dehn in prep.). Thus, the difference in hepatic Cd concentrations between male and female is unlikely related to diet. Bustamante et al. (2004a) suggested the influence of hormonal cycles to Cd metabolism. Adult female seals exhibit higher feeding rates and greater energy content than males as a result of reproductive requirements (Beck et al. 2003). Female rats have been shown to have an increased induction of metallothionein and hence an increased capacity to store essential elements, such as Cu and Zn in preparation for gestation (Suzuki et al. 1990; Chan et al. 1993; Solaiman et al. 2001). However, metallothionein is known to also bind non-essential elements (e.g., Cd) with equal or higher affinity (Das et al. 2000), which could account for the higher concentrations of Cd in liver of ringed seal females. The low amounts of Cd present in prey consumed by spotted seals and a bias in age distribution towards immature seals may explain why a significant difference in Cd between sexes was not documented. Based on element concentrations in typical bearded seal prey (Table 2.6) there is likely a high intake of Cd, Zn and Cu with the diet so that subtle sex differences could not be noted. However, results of the two-way ANOVA, in general, showed that concentrations of Cd in liver and kidney were influenced by sex.

2.513 Cd and Age

Cd in renal tissue ringed and bearded seals from Alaska increase with age to a peak, followed by a slow decline in older animals (Figure 2.2). This relationship was not noted in spotted seals. However, only young animals were available and an age bias could explain why a peak distribution similar to ringed and bearded seals from Alaska was not documented for spotted seals. Ringed seals from Canada showed a continuous, but gradual increase of Cd after a critical age (around age 10). Although Cu and Zn can cross the placental barrier readily, Cd is not transferred from maternal tissues to the fetus during gestation (Wagemann et al. 1988; Itoh et al. 1996). Several studies report induction of metallothionein in the placenta which may serve as a selective filter against toxic effects for the fetus (Itoh et al. 1996; Nordberg and Nordberg 2000; Enomoto and

Hirunuma 2001). Thus, accumulation of Cd begins after birth with dietary uptake. However, some studies report a continuous accumulation of Cd with increasing age or increase of Cd to a maximum, the latter suggesting that dietary input and excretion are balanced (Watanabe et al. 1998; Bustamante et al. 2004a).

A decrease of Cd in kidneys with age as seen in ringed and bearded seals from Alaska was also noted in humans (Marquart and Schaefer 1997), Greenland ringed seals (Dietz et al. 1998; Sonne-Hansen et al. 2002), and Caspian seals (Watanabe et al. 2002). In Caspian seals, this relationship was attributed to a preferential change in diet with age from invertebrates to fish (Watanabe et al. 2002). This explanation is also likely for ringed seals in this study, as they exhibited a similar dietary change with age (Lowry et al. 1980a; Dehn in prep.). A dietary shift from relatively high Cd-containing crustaceans (Bohn and McElroy 1976; MacDonald and Sprague 1988) to lower concentrations in fish (Bustamante et al. 2003) could explain the observed decrease with age. However, in bearded seals, there is no indication of a change in dietary preference with age that would account for a decreased Cd input.

Physiologically, a metallothionein-cadmium complex is filtered through the renal glomerulus and reabsorbed by the proximal tubule cells. Elimination of Cd occurs predominantly with shedding of tubule cells into the urine (Gerhardsson and Skerfving 1996). This process is extremely slow and is in part, responsible for the long biological half-life of about 20-40 years in humans (WHO 2000). The aging process is associated with a variety of changes in renal tissue. The ability to reabsorb Cd (and other essential elements, e.g., Zn) in the proximal tubule cells decreases with age owing to impaired peritubular blood flow and hypoxia caused by glomerulosclerosis (Khan et al. 1999; Sonne-Hansen et al. 2002). The increase in renal connective tissue with age (glomerulosclerosis) is well known (Thomas et al. 1998) and could also account for decreasing Cd concentrations in these seals. In addition, apoptosis (programmed cell death) of renal tubule cells with increasing age, as described by Cardani and Zavanella (2000), may lead to an increased excretion of Cd with shed cells after a critical age. Lastly, involvement of hormones may also explain changes in Cd metabolism. Muraoka (2001) suggested that androgens like testosterone increase renal tubule apoptosis and could thus lead to an increased excretion of Cd with shed tubular cells in males. This is supported by this study, as females had significantly higher renal Cd concentrations than males.

2.52 Mercury

2.521 Species Comparison

Of phocids harvested in Alaska, bearded seals showed the highest concentrations of THg, while spotted seals had the lowest. This finding was unexpected as several studies linked Hg to trophic level and fish consumption (Dietz et al. 1996; Wagemann et al. 1997; Egeland et al. 1998). Fish was identified from all spotted seal stomachs and trophic level ($\delta^{15}N$) was significantly higher in spotted seals than ringed or bearded seals from Alaska. Bearded seals had a high proportion of fish in their diet (Dehn in prep.), which could account for elevated concentrations of THg in their tissues. However, MeHg in bearded seal tissues was low; it is usually associated with fish and readily bioavailable

(Wagemann et al. 1997; Wagemann et al. 2000). If organic Hg (MeHg) is expressed as percentage of THg (%MeHg), piscivorous spotted seals had the highest ratio and bearded seals the lowest ratio in both hepatic and renal tissues. Ringed seals from Alaska consume a mix of fish and crustaceans and showed values intermediate to spotted and bearded seals. This suggests that the ratio of MeHg to THg may be a better indicator for piscivory than THg alone.

Overall, concentrations of THg in liver and kidney were highest for ringed seals from Canada. A geographic increase of Hg from eastern to western Canada has been reported for ringed seal tissues and was attributed to a gradient in natural geological background concentrations (Wagemann et al. 1996). Thus, higher Hg concentrations in ringed seals from Canada than in seals from Alaska are feasible if geologic THg gradients are only localized in the Canadian Arctic. Hepatic THg in bearded seals from Holman was 143 ± 170 μg/g ww (Smith and Armstrong 1978), while concentrations in bearded seals harvested in Barrow were about two orders of magnitude lower. THg and MeHg in liver of ringed seals from Canada in this study were very similar to levels reported by Smith and Armstrong (1978) for Holman seals. In the Greenland Arctic, concentrations of 21.4 ± 52.0 μg/g ww and 13.4 ± 12.2 μg/g ww were found for ringed and bearded seal liver, respectively (Dietz et al. 1990), which are comparable to those from Canada. THg in Alaskan ringed seal tissues are within ranges reported by Becker et al. (1995a) and Woshner et al. (2001a). Hepatic THg of spotted seals in this study was an order of magnitude lower than for 23 harbor seals from Alaska analyzed by Miles et al. (1992).

Several studies reported differences in concentrations of Hg between sexes and suggested that females may be able to excrete Hg, in particular MeHg, through maternal transfer across the placenta, which would leave males with a higher body burden (Wagemann et al. 1988; Becker et al. 1995a, Watanabe et al. 1998; Watanabe et al. 2002). Thomas et al. (1986) showed that female rats fed MeHg cleared Hg faster from body tissues than did males. However, only Holman ringed seal males had higher renal, but not hepatic concentrations of MeHg than females.

2.522 Hg and Age

Several studies describe a positive correlation between THg or MeHg and age in seal liver and kidney (Dietz et al. 1996; Yeats et al. 1999; Wagemann et al. 2000; Woshner et al. 2001a; Watanabe et al. 2002; Bustamante et al. 2004a). This suggests the continuous uptake of Hg / MeHg via diet, slow elimination or storage and thus a relatively long half-life of THg of about 10 years as discussed by Wagemann et al. (2000).

The relative occurrence of MeHg expressed as percentage of THg (%MeHg) in liver versus age in ringed, bearded, and spotted seals from Alaska and Canada is shown in Figure 2.3. A LOESS nonparametric curve fitting (dashed gray line) was used to assess the accuracy of the nonlinear regression. A hyperbolic decay function was fitted to each data set, and is in good agreement with the LOESS fit for bearded seals and ringed seals from both Canada and Alaska. Fetuses are exposed to MeHg, likely through placental transfer from the mother. Transport of Hg across the placental barrier was also described for harp seals, Caspian seals, and humans (Wagemann et al. 1988; Ask et al. 2002;

Watanabe et al. 2002). The percentage of MeHg in liver decreases to a minimum at around 5 years and remains relatively constant with increasing age. This indicates that the dietary uptake of MeHg in these seals remains in equilibrium with physiological detoxification processes. For spotted seals the variability of %MeHg with age is high and the hyperbolic decay function is not a good fit. It is possible that the amount of MeHg in tissues is strongly dependent on the consumption of fish, and the physiological detoxification processes may be outweighed by an increased dietary input of MeHg. In rodent studies, biotransformation of MeHg in the liver was inversely correlated to administered MeHg dose, suggesting saturation of enzyme systems involved in demethylation of Hg (Iverson and Hierlihy 1974; Yamamoto et al. 1986). However, age of spotted seals in this study is biased toward young animals and could also account for the high variability in these animals.

Seals highlighted as open symbols in Figure 2.3 (I and II) were found with hepatic lesions and show a higher relative occurrence of organic mercury in liver (seals were excluded from regression analysis to avoid the impact of influential observations). Lesions were determined during either necropsy and/or histologic assessment by Woshner (2000). Elevated %MeHg could suggest that these seals are compromised in their ability to demethylate MeHg or that the underlying physiological detoxification mechanisms are altered. Thus, caution should be taken when determining element status of stranded or compromised animals, as they may not present the norm of a healthy population. Altered accumulation patterns of some heavy metals in seal tissues during epizootics have also been reported by Frank et al. (1992), Olsson et al. (1994) and Anan et al. (2002).

2.53 Selenium

Se is an essential element and incorporated into antioxidant enzyme systems, most importantly glutathione peroxidase (Bondy 1996). Numerous studies have discussed the protective effect of Se on Hg toxicosis and a molar ratio of 1:1 for Se:THg has been reported in many studies involving marine mammals (Koeman et al. 1973; Smith and Armstrong 1978; Caurant et al. 1994; Dietz et al. 1998; Dietz et al. 2000; Endo et al. 2002; Bustamante et al. 2004a; Decataldo et al. 2004). Other investigators have found ratios deviating from unity to varying degrees (Wagemann and Stewart 1994; Woshner et al. 2001a; Woshner et al. 2001b; Anan et al. 2002). Becker et al. (1995b) found Se:THg molar ratios of 3:1 in belugas harvested in Alaska, while pilot whales from the North Atlantic had a 1:1 ratio. Results of this study showed that the Se:THg molar ratio in liver had a strong negative correlation with age in ringed ($r = -0.48$ and $r = -0.56$ for Alaskan and Canadian ringed seals, respectively) and spotted seals ($r = -0.80$) (Figure 2.4). Wagemann et al. (1988) made a similar observation in harp seal mothers having a 1:1 molar ratio (Se:THg), while their pups had a 6.5:1 Se:THg molar ratio. Se is transported over the placental barrier from mother to fetus (Wagemann et al. 1988; Enomoto and Hirunuma 2001; Yang et al. 2004) and is essential for the maturing antioxidant system, and endocrine, reproductive, and neuronal development (Bedwal and Bahuguna 1994; Watanabe et al. 1999; Bates et al. 2000; Hirunuma et al. 2001).

In general, spotted seals in this study had the highest hepatic Se:THg molar ratio, followed by ringed seals from Alaska (13:1 and 11.7:1 for spotted and ringed seals, respectively). The typical 1:1 molar ratio could only be documented for some older ringed seals from the Canadian Arctic. This is in accordance with Wagemann et al. (2000) and Dietz et al. (2000) who suggested that the classic unity of Se and THg is only found in seals with high concentrations of Hg. However, a molar ratio of 1:1 (Se:THg) would indicate that almost all available Se is bound to Hg. Se is involved in oxy radical scavenging as part of the glutathione peroxidase system and this capability is needed in diving marine mammals to cope with oxidative stress (Zenteno-Savin et al. 2002). Thus, tissue ratios close to 1:1 (Se:THg) could be a possible indicator for compromised health as suggested by Dietz et al. (2000). In spotted seals, renal Se and the Se:THg molar ratio was higher than in liver. The sampling bias was toward young animals and, as described, the correlation between Se:THg molar ratio and age is negative and nonlinear. However, Se in the kidney may be protective against renal damage (Santos et al. 2005). The molar ratio of Se exceeds those of Hg by more than 100 in most prey homogenates and is in agreement with concentrations reported by Dietz et al. (2000).

2.54 Silver

Ag occurs naturally in the earth's crust and as mining deposits (Purcell and Peters 1998). It can erode from natural accumulations or reach the aquatic environment via hydrothermal activity, e.g., black smokers of the Aleutian volcanic arc. Anthropogenic sources of Ag include mining, photographic industry, electronic applications as well as industrial and sewage discharges (Purcell and Peters 1998). None of the anthropogenic sources seem to have significant impact in the Arctic waters of Alaska. Becker et al. (2001) demonstrated lower concentrations of Ag in liver of Cook Inlet beluga whales (close to human activities) than in the more remote Beaufort or Chukchi sea beluga stocks.

Ag has a high affinity to sulfate ions and halogens in water and forms insoluble complexes that precipitate (Bell and Kramer 1999). Thus, Ag has the potential to accumulate in the benthic food chain. Zhang et al. (2001) showed an increase of dissolved Ag with depth, ranging from 4.4 pmol/kg in North Pacific surface waters to 41.5 pmol/kg at over 5 km deep. Sea-floor sediments sampled in the East Siberian Sea and Laptev Sea of the Russian Arctic had concentrations of 0.082 μg/g (Presley 1997) and Ag in the vicinity of hydrothermal vents had concentrations as high as 86 μg/g (Hein et al. 1999). Marine mammals feeding in the benthic ecosystem could therefore have a higher exposure to Ag than animals that feed pelagically. This is supported by the findings of this study as benthic-feeding bearded seals had the highest concentrations of hepatic Ag whereas pelagic ringed and spotted seals had low concentrations. The high levels of Ag in liver of ringed seals from Canada are not likely to be explained by dietary selection. Although benthic or epibenthic prey is consumed by these seals, they are preying mostly in the water column (Bradstreet and Finley 1983; Smith 1987). Thus, it is possible that Ag is higher in the Canadian Arctic becaue of either natural geologic sources or anthropogenic impact as has been discussed for the St. Lawrence estuary (Gobeil 1999).

Benthic invertebrates that seem to accumulate Ag (and Cu) at considerable levels are cephalopods, bivalves, and crustaceans, while flatfish retained less than 20% of an administered Ag dose (Martin and Flegal 1975; Berthet et al. 1992; Rouleau et al. 2000; Bustamante et al. 2004b). The blood of mollusks and crustaceans contains hemocyanin, a Cu-based respiratory pigment. Ag is chemically similar to Cu and has a high affinity to sulfur ligands. Thus, an incidental uptake of Ag instead of Cu or binding-site competition seems possible. Saeki et al. (2001) indicated that Ag interferes with Cu metabolism and Cu transport. Martin and Flegal (1975) described a strong positive correlation of Ag and Cu in cephalopods. These elements are also correlated in liver of Alaskan seals in this study and in polar bears, ringed seals, and bowhead and beluga whales (Woshner et al. 2001a; Woshner et al. 2001b).

The higher concentrations of hepatic Ag in bearded seals and the high occurrence of octopus and large crustaceans in stomachs of these seals (Dehn in prep.) support a linkage of benthic invertebrates to Ag. Similarly, concentrations of Ag in liver of Northern fur seals (*Callorhinus ursinus* Linnaeus, 1758) are comparable to levels found in bearded seals from Alaska and are in agreement with the cephalopod-dominated diet of fur seals (Mori et al. 2001; Saeki et al. 2001). Very high concentrations of Ag have been reported in beluga whales from Alaska and this appears to be unique for this species (Becker et al. 1995b; Mackey et al. 1996; Becker et al. 2001; Woshner et al. 2001b). In contrast to pinnipeds analyzed by Saeki et al. (2001), Ag was not correlated to age in seals of this study. Reasons for higher concentrations of hepatic Ag in females than in males in this study are likely the same as discussed for Cd, e.g., increased induction of metallothionein in reproductive females and increased rate of feeding.

2.55 Copper and Zinc

Cu and Zn are essential elements and thus are regulated within tight biological margins. However, Cu and Zn seem to mirror the concentration patterns described for Ag and Cd, with benthic- and invertebrate-feeding bearded seals showing higher concentrations than piscivorous and pelagic ringed and spotted seals from Alaska and Canada. Wagemann and Stewart (1994) reported concentrations of hepatic Cu and Zn of walrus from Canada that were within the range of ringed seals, but were lower than for bearded seals analyzed in this study (9.7 ± 7.7 and 45.2 ± 10.3 μg/g ww for walrus Cu and Zn, respectively).

Zn, Cu, Cd and Ag are commonly intercorrelated (Das et al. 2000; Woshner et al. 2001a; Bustamante et al. 2004a), suggesting induction of metallothionein and possible competition or paralleling increase in metal-binding sites. Braune et al. (1991) and Woshner et al. (2001a) also described correlations of Hg with Cu and Zn in liver of polar bears. However, Hg does not seem to be correlated to Cu and Zn in pinnipeds and cetaceans, indicating that Hg induces metallothionein in terrestrial, but not in marine animals as has been discussed by Das et al. (2000) and Decataldo et al. (2004).

Several studies report higher concentrations of Cu and Zn in juvenile and subadult animals (Wagemann et al. 1988; Wagemann 1989; Watanabe et al. 1998), and both elements readily cross the placental barrier. Metallothionein in the fetus is higher than in juveniles and adults, likely to accommodate the increased demand for Cu and Zn in developing and growing tissues (Bremner and Beattie 1990; Teigen et al. 1999). In Arctic ruminants, the fetus stores large amounts of Cu for tissue development and growth during late gestation, as dietary Cu and maternal reserves may be marginal (Rombach et al. 2003). On the other hand, Bremner and Beattie (1990) postulated that the accumulation of Cu in fetal tissues may be due to limited efficiency of bilary excretion mechanisms. For seals analyzed in this study, Cu or Zn and age were only inversely correlated in ringed seals from Canada.

2.6 SUMMARY AND CONCLUSION

In conclusion, age and diet can substantially affect trace element concentrations in tissues of phocid seals. Cd concentrations appear to be connected to invertebrates, and bearded seals that are dependent on this type of prey exhibit the highest renal and hepatic concentrations, while spotted seals have the lowest. Renal Cd increases with age to a peak, followed by a gradual decline with increasing age. This suggests physiological changes associated with aging in the kidney, such as increased apoptosis and shedding of proximal tubule cells. THg in liver and kidney was unexpectedly lower in piscivorous spotted seals than bearded seals. Hepatic %MeHg on the other hand was highest in spotted seals and lowest in bearded seals, indicating that this metal ratio may be a good indicator of piscivory. Seals with hepatic lesions had higher %MeHg in liver than healthy seals, implying that these animals may be compromised in their ability to demethylate MeHg. Se is strongly correlated to THg in liver and kidney and the Se:THg molar ratio is inversely correlated to age in ringed and spotted seals. A 1:1 (Se:THg) molar ratio in liver was only documented in older ringed seals from Canada. Ag showed a possible connection to the benthic food chain and was positively correlated to Cu in seals from Alaska. Concentrations of Cu and Zn are more difficult to interpret as they are essential elements and regulated within tight biological margins. However, Cu and Zn mirror tissue distribution patterns described for Ag and Cd and these metals are often intercorrelated, suggesting induction of metallothionein and binding site competition.

2.7 ACKNOWLEDGMENTS

This study would not have been possible without the samples provided by Alaskan and Canadian subsistence hunters in the communities of Barrow, Holman, Little Diomede and Shishmaref, and we thank them all for their support. We greatly appreciate the assistance of C. D. N. Brower, H. Brower, Jr., T. Olemaun, B. Akootchook, T. Hepa, L. Hopson, V. Woshner, B. Elsner, T. Zenteno-Savin, S. Visalli, D. Burnett, G. York, and many others in the field and T. Bentzen for support with analysis. We also thank L. Harwood for providing tissues and jaws of ringed seals harvested in Holman, Canada, and P. Hoekstra for shipment of these samples. J. Bengtson and D. DeMaster arranged for training in cementum aging techniques and P. Becker and S. Christopher provided marine mammal reference material and coordinated interlaboratory comparison exercises for the determination of trace elements in marine mammals. The comments of two anonymous reviewers improved the manuscript. This study was primarily funded by the Cooperative Institute for Arctic Research (CIFAR). Additional support was provided by the

Experimental Program for Stimulation of Competitive Research (EPSCoR); the Biomedical Research Infrastructure Network (BRIN); the North Slope Borough Department of Wildlife Management; the Institute of Arctic Biology and the Department of Biology and Wildlife, UAF; the US Geological Survey; the Barrow Arctic Science Consortium (BASC); and the National Science Foundation (NSF) OPP Grant 9910319.

2.8 REFERENCES

Anan, Y., Kunito, T., Ikemoto, T., Kubota, R., Watanabe, I., Tanabe, S., Miyazaki, N., and Petrov, E.A. 2002. Elevated concentrations of trace elements in Caspian seals (*Phoca caspica*) found stranded during the mass mortality events in 2000. Arch. Environ. Contam. Toxicol. **42:** 354-362.

Ask, K., Akesson, A.B.M., and Vahter, M. 2002. Inorganic mercury and methylmercury in placentas of Swedish women. Environmental Health Perspectives, **110:** 523-526.

Ballard, W.B., Cronin, M.A., Robards, M.D., and Stubblefield, W.A. 2003. Heavy metal concentrations in Arctic foxes, *Alopex lagopus*, in the Prudhoe Bay oil field, Alaska. Can. Field-Nat. **117:** 119-121.

Bargagli, R., Nelli, L., Ancora, S., and Focardi, S. 1996. Elevated cadmium accumulation in marine organisms from Terra Nova Bay (Antarctica). Polar Biol. **16:** 513-520.

Bates, J.M., Spate, V.L., Morris, J.S., Germain, D.L.S., and Galton, V.A. 2000. Effects of selenium deficiency on tissue selenium content, deiodinase activity, and thyroid hormone economy in the rat during development. Endocrinology, **141:** 2490-2500.

Beck, C.A., Bowen, W.D., and Iverson, S.J. 2003. Sex differences in the seasonal patterns of energy storage and expenditure in a phocid seal. J. Anim. Ecol. **72:** 280-291.

Becker, P.R., Mackey, E.A., Schantz, M.M., Demiralp, R., Greenberg, R.R., Koster, B.J., Wise, S.A., and Muir, D.C.G. 1995a. Concentrations of chlorinated hydrocarbons, heavy metals and other elements in tissues banked by the Alaska Marine Mammal Tissue Archival Project. NISTIR 5620, U. S. Department of Commerce, Gaithersburg, MD.

Becker, P.R., Mackey, E.A., Demiralp, R., Suydam, R., Early, G., Koster, B.J., and Wise, S.A. 1995b. Relationship of silver with selenium and mercury in the liver of two species of toothed whales (Odontocetes). Mar. Pollut. Bull. **30:** 262-271.

Becker, P.R., Porter, B.J., Mackey, E.A., Schantz, M.M., Demiralp, R., and Wise, S.A. 1999. National Marine Mammal Tissue Bank and Quality Assurance Program:

protocols, inventory, and analytical results. NISTIR 6279, U. S. Department of Commerce, Gaithersburg, MD.

Becker, P.R., Pugh, R.S., Schantz, M.M., Mackey, E.A., Demiralp, R., Epstein, M.S., Donais, M.K., Porter, B.J., Wise, S.A., and Mahoney, B.A. 2001. Persistent chlorinated compounds and elements in tissues of Cook Inlet beluga whales, *Delphinapterus leucas*, banked by the Alaska Marine Mammal Tissue Archival Project. NISTIR 6702, National Institute of Standards and Technology, Gaithersburg, MD.

Bedwal, R.S., and Bahuguna, A. 1994. Zinc, copper and selenium in reproduction. Experientia (Basel), **50:** 626-640.

Bell, R.A., and Kramer, J.R. 1999. Structural chemistry and geochemistry of silver-sulfur compounds: Critical review. Environ. Toxicol. Chem. **18:** 9-22.

Benjaminsen, T. 1973. Age determination and the growth and age distribution from cementum growth layers of bearded seals at Svalbard. Fiskeridirektoratets skrifter. Serie havundersøkelser, **16:** 159-170.

Berthet, B., Amiard, J.C., Amiard-Triquet, C., Martoja, R., and Jeantet, A.Y. 1992. Bioaccumulation, toxicity and physico-chemical speciation of silver in bivalve molluscs: ecotoxicological and health consequences. Sci. Total Environ. **125:** 97-122.

Bloom, N. 1989. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapor atomic fluorescence detection. Can. J. Fish. Aquat. Sci. **46:** 1131-1140.

Bloom, N.S., and Crecelius, E.A. 1983. Determination of mercury in seawater at sub-nanogram per liter levels. Mar. Chem. **14:** 49-59.

Bohn, A., and McElroy, R.O. 1976. Trace metals (As, Cd, Cu, Fe and Zn) in Arctic cod, *Boreogadus saida*, and selected zooplankton from Strathcona Sound, northern Baffin Island. J. Fish. Res. Board Can. **33:** 2836-2840.

Bondy, S.C. 1996. Oxygen generation as a basis for neurotoxicity by metals. *In* Toxicology of Metals. *Edited by* L. W. Chang. CRC Lewis Publishers, Boca Raton, FL. pp. 699-706.

Bradstreet, M.S.W., and Finley, K.J. 1983. Diet of ringed seals (*Phoca hispida*) in the Canadian High Arctic. LGL Limited. Toronto, Ontario.

Bratton, G.R., Flory, W., Spainhour, C.B., and Haubold, E.M. 1997. Assessment of selected heavy metals in liver, kidney, muscle, blubber, and visceral fat of Eskimo harvested bowhead whales *Balaena mysticetus* from Alaska's North Coast. Department of Wildlife Management, North Slope Borough, Barrow, AK.

Braune, B.M., Norstrom, R.J., Wong, M.P., Collins, B.T., and Lee, J. 1991. Geographical distribution of metals in livers of polar bears from the Northwest Territories, Canada. Sci. Total Environ. **100:** 283-299.

Bremner, I., and Beattie, J.H. 1990. Metallothionein and the trace minerals. Annu. Rev. Nutr. **10:** 63-83.

Bukhtiyarov, Y.A., Frost, K.J., and Lowry, L.F. 1984. New information on foods of the spotted seal, *Phoca largha*, in the Bering Sea in spring. U. S. Department of Commerce, NOAA, NOAA Technical Report, NMFS No.12 : 55- 59.

Burns, J.J. 1970. Remarks on the distribution and natural history of pagophilic pinnipeds in the Bering and Chukchi Seas. J. Mammal. **51:** 445-454.

Bustamante, P., Caurant, F., Fowler, S.W., and Miramand, P. 1998. Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. Sci. Total Environ. **220:** 71-80.

Bustamante, P., Bocher, P., Cherel, Y., Miramand, P., and Caurant, F. 2003. Distribution of trace elements in the tissues of benthic and pelagic fish from the Kerguelen Islands. Sci. Total Environ. **313:** 25-39.

Bustamante, P., Morales, C.F., Mikkelsen, B., Dam, M., and Caurant, F. 2004a. Trace element bioaccumulation in grey seals *Halichoerus grypus* from Faroe Islands. Mar. Ecol. Prog. Ser. **267:** 291-301.

Bustamante, P., Teyssie, J.-L., Danis, B., Fowler, S.W., Miramand, P., Cotret, O., and Warnau, M. 2004b. Uptake, transfer and distribution of silver and cobalt in tissues of common cuttlefish *Sepia officinalis* at different stages of its life cycle. Mar. Ecol. Prog. Ser. **269:** 185-195.

Cardani, R., and Zavanella, T. 2000. Age-related cell proliferation and apoptosis in the kidney of male Fischer 344 rats with observations on a spontaneous tubular cell adenoma. Toxicologic Pathology, **28:** 802-806.

Caurant, F., Amiard, J.C., Amiard-Triquet, C., and Sauriau, P.G. 1994. Ecological and biological factors controlling the concentrations of trace elements (As, Cd, Cu, Hg, Se, Zn) in delphinids *Globicephala melas* from the North Atlantic Ocean. Mar. Ecol. Prog. Ser. **103:** 207-219.

Caurant, F., and Amiard-Triquet, C. 1995. Cadmium contamination in pilot whales *Globicephala melas*: Source and potential hazard to the species. Mar. Pollut. Bull. **30:** 207-210.

Chan, H.M., Tamura, Y., Cherian, M.G., and Goyer, R. 1993. Pregnancy-associated changes in plasma metallothionein concentration and renal cadmium accumulation in rats. Proc. Soc. Exp. Biol. Med. **202:** 420-427.

Christopher, S.J. 2002. NIST / NOAA National marine analytical quality assurance program. Description and results of the 2001 interlaboratory comparison exercise for the determination of trace elements in marine mammals. National Institute of Standards and Technology, Charleston, SC.

Christopher, S.J. 2004. NIST / NOAA National marine analytical quality assurance program. Description and results of the 2003 interlaboratory comparison exercise for the determination of trace elements in marine mammals. National Institute of Standards and Technology, Charleston, SC.

Das, K., Debacker, V., and Bouquegneau, J.M. 2000. Metallothioneins in marine mammals. Cell. Mol. Biol. **46:** 283-294.

Decataldo, A., Di Leo, A., Giandomenico, S., and Cardellicchio, N. 2004. Association of metals (Mercury, cadmium and zinc) with metallothionein-like proteins in storage organs of stranded dolphins from the Mediterranean sea (Southern Italy). J. Environ. Monit. **6:** 361-367.

Dehn, L.-A. In preparation. Trophic relationships in an Arctic marine foodweb and implications for trace element dynamics. Ph.D. thesis, Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, AK.

Deutch, B., and Hansen, J.C. 2003. AMAP Greenland and the Faroe Islands 1997-2001. Dancea. Danish Cooperation for Environment in the Arctic. Danish Environmental Protection Agency. 234 pp.

Dietz, R., Nielsen, C.O., Hansen, M.M., and Hansen, C.T. 1990. Organic mercury in Greenland birds and mammals. Sci. Total Environ. **95:** 41-51.

Dietz, R., Riget, F., and Johansen, P. 1996. Lead, cadmium, mercury and selenium in Greenland marine animals. Sci. Total Environ. **186:** 67-93.

Dietz, R., Paludan-Müller, P., Agger, C.T., and Nielsen, C.O. 1998. Cadmium, mercury, zinc and selenium in ringed seals (*Phoca hispida*) from Greenland and Svalbard. NAMMCO Scientific Publications, Tromsø. pp. 242-273.

Dietz, R., Riget, F., Cleemann, M., Aarkrog, A., Johansen, P., and Hansen, J.C. 2000. Comparison of contaminants from different trophic levels and ecosystems. Sci. Total Environ. **245:** 221-231.

Egeland, G.M., Feyk, L.A., and Middaugh, J.P. 1998. The use of traditional foods in a healthy diet in Alaska: risks in perspective. State of Alaska Epidemiology Bulletin, **2:** 1-140.

Endo, T., Haraguchi, K., and Sakata, M. 2002. Mercury and selenium concentrations in the internal organs of toothed whales and dolphins marketed for human consumption in Japan. Sci. Total Environ. **300:** 15-22.

Enomoto, S., and Hirunuma, R. 2001. Fetoplacental transport of various trace elements in pregnant rat using the multitracer technique. RIKEN Review, **35:** 31-34.

Fay, F.H., Bukhtiyarov, Y.A., Stoker, S.W., and Shults, L.M. 1984. Foods of the Pacific walrus in winter and spring in the Bering Sea. NOAA Technical Report, NMFS No.12 : 81- 88.

Frank, A., Galgan, V., Roos, A., Olsson, M., Petersson, L.R., and Bignert, A. 1992. Metal concentrations in seals from Swedish waters. Ambio, **21:** 529-538.

George, J.C., Bada, J., Zeh, J., Scott, L., Brown, S.E., O'Hara, T., and Suydam, R. 1999. Age and growth estimates of bowhead whales (*Balaena mysticetus*) via aspartic acid racemization. Can. J. Zool. **77:** 571-580.

Gerhardsson, L., and Skerfving, S. 1996. Concepts on biological markers and biomonitoring for metal toxicity. *In* Toxicology of Metals. *Edited by* L. W. Chang. CRC Lewis Publishers, Boca Raton, FL. pp 81-107.

Gilbert, R. O. 1987. Statistical methods for environmental pollution monitoring. John Wiley and Sons, Inc., New York.

Gobeil, C. 1999. Silver in sediments from St. Lawrence River and estuary and the Saguenay Fjord. Environ. Sci. Technol. **33:** 2953-2957.

Hamanaka, T., and Ogi, H. 1984. Cadmium and zinc concentrations in the hyperiid amphipod, *Parathemisto libellula* from the Bering Sea. Bull. Fac. Fish. Hokkaido Univ. **35:** 171-178.

Hein, J.R., Koski, R.A., Embley, R.W., Reid, J., and Chang, S.-W. 1999. Diffuse-flow hydrothermal field in an oceanic fracture zone setting, Northeast Pacific: Deposit composition. Exploration and Mining Geology, **8:** 299-322.

Hirunuma, R., Ozaki. T., Endo, K., Yasui, H., Sakurai, H., and Enomoto, S. 2001. Uptake of various elements in selenium-deficient rats: Application of the multitracer technique. RIKEN Review, **35:** 40-44.

Hoover, A.A. 1988. Harbor seal. *In* Selected marine mammals of Alaska: Species accounts with research and management recommendations. *Edited by* J. W. Lentfer. Marine Mammal Commission, Washington, D.C. pp. 125-157.

Itoh, N., Fujita, Y., Nakanishi, H., Kawai, Y., Mayumi, T., Hawang, G.S., Min, K., Onosake, S., Muto, N., and Tanaka, K. 1996. Binding of Cd to metallothionein in the placenta of Cd-treated mouse. J. Toxicol. Sci. **21:** 19-27.

Iverson, F., and Hierlihy, S.L. 1974. Biotransformation of methyl mercury in the guinea pig. Bull. Environ. Contam. Toxicol. **11:** 85-91.

Johnson, M.D., Kenny, N., Stoica, A., Hilakivi-Clarke, L., Singh, B., Chepko, G., Clarke, R., Sholler, P.F., Lirio, A.A., Foss, C., Reiter, R., Trock, B., Paik, S., and Martin, M.B. 2003. Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. Nature Medicine, **9:** 1081-1084.

Kato, H. 1982. Food habits of largha seal pups in the pack ice area. Sci. Rep. Whales Res. Inst. (Tokyo), **43:** 123-136.

Khan, S., Cleveland, R.P., Koch, C.J., and Schelling, J.R. 1999. Hypoxia induces renal tubular epithelial cell apoptosis in chronic renal disease. Lab. Investig. **79:** 1089-1099.

Koeman, J.H., Peeters, W.H.M., Koudstaal-Hol, C.H.M., Tijoe, P.S., and de Goeij, J.J.M. 1973. Mercury-selenium correlations in marine mammals. Nature (Lond.), **245**: 385-386.

Krylov, V.I. 1971. On the food of the pacific walrus (*Odobenus rosmarus divergens* Ill.). Investigations on marine mammals. Trudy AtlantNIRO, **39:** 110-116.

Lowry, L.F., Frost, K.J., and Burns, J.J. 1980a. Variability in the diet of ringed seals, *Phoca hispida*, in Alaska. Can. J. Fish. Aquat. Sci. **37:** 2254-2261.

Lowry, L.F., Frost, K.J., and Burns, J.J. 1980b. Feeding of bearded seals in the Bering and Chukchi Seas and trophic interaction with Pacific walrus. Arctic, **33:** 330-342.

Lowry, L.F., Frost, K.J., Calkins, D.G., Swartzman, G.L., and Hills, S. 1982. Feeding habits, food requirements, and status of Bering Sea marine mammals. North Pacific Fishery Management Council, Anchorage, AK.

Lowry, L.L., and Frost, K.J. 1984. Foods and feeding of bowhead whales in western and northern Alaska. Sci. Rep. Whales Res. Inst. (Tokyo), **35:** 1-16.

Macdonald, C.R., and Sprague, J.B. 1988. Cadmium in the marine invertebrate and arctic cod in the Canadian Arctic. Distribution and ecological implications. Mar. Ecol. Prog. Ser. **47:** 17-30.

Mackey, E.A., Becker, P.R., Demiralp, R., Greenberg, R.R., Koster, B.J., and Wise , S.A. 1996. Bioaccumulation of vanadium and other trace metals in livers of Alaskan cetaceans and pinnipeds. Arch. Environ. Contam. Toxicol. **30:** 503-512.

Marquart, H., and Schaefer, S.G. 1997. Lehrbuch der Toxikolgie. Spektrum Akademischer Verlag, Heidelberg.

Martin, J.H., and Flegal, A.R. 1975. High copper concentrations in squid livers in association with elevated levels of silver, cadmium, and zinc. Mar. Biol. **30:** 51-55.

Martin, M.B., Reiter, R., Pham, T., Avellanet, Y.R., Camara, J., Lahm, M., Pentecost, E., Pratap, K., Gilmore, B.A., Divekar, S., Dagata, R.S., Bull, J.L., and Stoica, A. 2003. Estrogen-like activity of metals in Mcf-7 breast cancer cells. Endocrinology, **144:** 2425-2436.

Matson, G.M. 1981. Workbook for cementum analysis. Matson's Laboratory, Milltown, MT.

Miles, A.K., Calkins, D.G., and Coon, N.C. 1992. Toxic elements and organochlorines in harbor seals (*Phoca vitulina richardsi*), Kodiak, Alaska, USA. Bull. Environ. Contam. Toxicol. **48:** 727-732.

Mori, J., Kubodera, T., and Baba, N. 2001. Squid in the diet of northern fur seals, *Callorhinus ursinus*, caught in the western and central North Pacific Ocean. Fish. Res. **52:** 91-97.

Muir, D.C.G., Wagemann, R., Hargrave, B.T., Thomas, D.J., Peakall, D.B., and Norstrom, R.J. 1992. Arctic ecosystem contamination. Sci. Total Environ. **122:** 75-134.

Muraoka, K. 2001. Effects of testosterone replacement on renal function and apoptosis on mesangial renal tubule cells in rats. Yonago Acta medica, **41:** 37-44.

Nordberg, M., and Nordberg, G.F. 2000. Toxicological aspects of metallothionein. Cell. Mol. Biol. **46:** 451-463.

Norstrom, R.J., Schweinsburg, R.E., and Collins, B.T. 1986. Heavy metals and essential elements in liver of the polar bear (*Ursus maritimus*) in the Canadian Arctic. Sci. Total Environ. **48:** 195-212.

Olsson, M., Karlsson, B., and Ahnland, E. 1994. Diseases and environmental contaminants in seals from the Baltic and Swedish west coast. Sci. Total Environ. **154:** 217-227.

Papa, R.S., and Becker, P.R. 1998. Alaska harbor seal (*Phoca vitulina*) contaminants. NISTIR 6211, U. S. Department of Commerce, Gaithersburg, MD.

Ponce, R.A., Egeland, G.M., Middaugh, J.P., and Becker, P.R. 1997. Twenty years of trace metal analyses of marine mammals: Evaluation and Summation of data from Alaska and other Arctic regions. State of Alaska Epidemiology Bulletin, **1:** 1-15.

Presley, B.J. 1997. A review of Arctic trace metal data with implications for biological effects. Mar. Pollut. Bull. **35:** 226-234.

Purcell, T.W., and Peters, J.J. 1998. Historical impacts of environmental regulation of silver. Environ. Toxicol. Chem. **18:** 3-8.

Ritterhoff, J., and Zauke, G.-P. 1997. Trace metals in field samples of zooplankton from Farm Strait and the Greenland Sea. Sci. Total Environ. **199:** 255-270.

Rombach, E.P., Barboza, P.S., and Blake, J.E. 2003. Cost of gestation in an Arctic ruminant: copper reserves in muskoxen. Comp. Biochem. Physiol. C **134:** 157-168.

Rouleau, C., Gobeil, C., and Tjälve, H. 2000. Accumulation of silver from the diet in two marine benthic predators: the snow crab (*Chionoecetes opilio*) and American plaice (*Hippoglossoides platessoides*). Environ. Toxicol. Chem. **9:** 631-637.

Saeki, K., Nakajima, M., Loughlin, T.R., Calkins, D.C., Baba, N., Kiyota, M., and Tatsukawa, R. 2001. Accumulation of silver in the liver of three species of pinnipeds. Environ. Pollut. **112:** 19-25.

Santos, F.W., Zeni, G., Rocha, J.B., Weis, S.N., Fachinetto, J.M., Favero, A.M., and Nogueira, C.W. 2005. Diphenyl diselenide reverses cadmium-induced oxidative damage on mice tissues. Chem. Biol. Interact. **151:** 159-165.

Sheffield, G., Fay, F.H., Feder, H., and Kelly, B.P. 2001. Laboratory digestion of prey and interpretation of walrus stomach contents. Mar. Mamm. Sci. **17:** 310-330.

Siegstad, H., Neve, P.B., Heide-Jørgensen, M.P., and Härkönen, T. 1998. Diet of ringed seal (*Phoca hispida*) in Greenland. *In* Ringed seals in the North Atlantic. NAMMCO Scientific Publications, Tromsø. pp. 229-241.

Smirnov, V.S. 1968. Analysis of Arctic fox population dynamics and methods of increasing the Arctic fox harvest. Problems of the North, **11:** 81-101.

Smith, T.G., and Armstrong, F.A.J. 1978. Mercury and selenium in ringed and bearded seal tissues from Arctic Canada. Arctic, **31:** 75-84.

Smith, T.G. 1987. The ringed seal, *Phoca hispida*, of the Canadian Western Arctic. Can. Bull. Fish. Aquat. Sci. **216:** 1-81.

Solaiman, D., Jonah, M.M., Miyazaki, W., Ho, G., and Bhattacharyya, M.H. 2001. Increased metallothionein in mouse liver, kidneys, and duodenum during lactation. Toxicological Sciences, **60:** 184-192.

Sonne-Hansen, C., Dietz, R., Leifsson, P.S., Hyldstrup, L., and Riget, F.F. 2002. Cadmium toxicity to ringed seals (*Phoca hispida*): an epidemiological study of possible cadmium-induced nephropathy and osteodystrophy in ringed seals (*Phoca hispida*) from Qaanaaq in Northwest Greenland. Sci. Total Environ. **295:** 167-181.

Stewart, R.E.A., Stewart, B.E., Stirling, I., and Street, E. 1996. Counts of growth layer groups in cementum and dentine in ringed seals (*Phoca hispida*). Mar. Mamm. Sci. **12:** 383-401.

Suzuki, K.T., Tamagawa, H., Takahashi, K., and Shimojo. 1990. Pregnancy-induced mobilization of copper and zinc bound to renal metallothionein in cadmium-loaded rats. Toxicology, **60:** 199-210.

Taylor, D.L., Schliebe, S., and Metske, H. 1989. Contaminants in Blubber, Liver and Kidney Tissue of Pacific Walruses. Mar. Pollut. Bull. **20:** 465-468.

Teigen, S.W., Andersen, R.A., Daae, H.L., and Skaare, J.U. 1999. Heavy metal content in liver and kidneys of grey seals (*Halichoerus grypus*) in various life stages correlated with metallothionein levels: some metal-binding characteristics of this protein. Environ. Toxicol. Chem. **18:** 2364-2369.

Thomas, D.J., Fisher, H.L., Sumler, M.R., Marcus, A.H., Mushak, P., and Hall, L.L. 1986. Sexual differences in the distribution and retention of organic and inorganic mercury in methyl mercury-treated rats. Environ. Res. **41:** 219-234.

Thomas, S.E., Anderson, S., Gordon, K.L., Oyama, T.T., Shankland, S.J., and Johnson, R.J. 1998. Tubulointerstitial disease in aging: Evidence for underlying peritubular capillary damage, a potential role for renal ischemia. J. Am. Soc. Nephrol. **9:** 231-242.

US Environmental Protection Agency. 1992. Methods for the determination of metals in environmental samples. CRC Press, Boca Raton, FL.

Wagemann, R., Stewart, R.E.A., Lockhart, W.L., Stewart, B.E., and Povoledo, M. 1988. Trace metals and methyl mercury: associations and transfer in harp seal (*Phoca groenlandica*) mothers and their pups. Mar. Mamm. Sci. **4:** 339-355.

Wagemann, R. 1989. Comparison of heavy metals in two groups of ringed seals (*Phoca hispida*) from the Canadian Arctic. Can. J. Fish. Aquat. Sci. **46:** 1558-1563.

Wagemann, R., and Stewart, R.E.A. 1994. Concentrations of heavy metals and selenium in tissues and some foods of walrus (*Odobenus rosmarus rosmarus*) from eastern Canadian Arctic and sub-Arctic, and associations between metals, age, and gender. Can. J. Fish. Aquat. Sci. **51:** 426-436.

Wagemann, R., Innes, S., and Richard, P. 1996. Overview and regional and temporal differences of heavy metals in Arctic and ringed seals in the Canadian Arctic. Sci. Total Environ. **186:** 41-66.

Wagemann, R., Trebacz, E., Hunt, R., and Boila, G. 1997. Percent methylmercury and organic mercury in tissues of marine mammals and fish using different experimental and calculation methods. Environ. Toxicol. Chem. **16:** 1859-1866.

Wagemann, R., Trebacz, E., Boila, G., and Lockhart, W.L. 2000. Mercury species in the liver of ringed seals. Sci. Total Environ. **261:** 21-32.

Watanabe, C., Yoshida, K, Kasanuma, Y., Kun, Y., and Satoh, H. 1999. In utero methylmercury exposure differentially affects the activities of selenoenzymes in the fetal mouse brain. Environ. Res. **80:** 208-214.

Watanabe, I., Tanabe, S., Amano, M., Miyazaki, N., Petrov, E.A., and Tatsukawa, R. 1998. Age-dependent accumulation of heavy metals in Baikal seal (*Phoca sibirica*) from the Lake Baikal. Arch. Environ. Contam. Toxicol. **35:** 518-526.

Watanabe, I., Kunito, T., Tanabe, S., Amano, M., Koyama, Y., Miyazaki, N., Petrov, E.A., and Tatsukawa, R. 2002. Accumulation of heavy metals in Caspian seals (*Phoca caspica*). Arch. Environ. Contam. Toxicol. **43:** 109-120.

WHO 2000. Cadmium. World Health Organization Regional Publications, Copenhagen, Denmark.

Wise, S.A., Schantz, M.M., Koster, B.J., Demiralp, R., Mackey, E.A., Greenberg, R.R., Burow, M., Ostapczuk, P., and Lillestolen, T.I. 1993. Development of frozen whale blubber and liver reference materials for the measurement of organic and inorganic contaminants. Fresenius J. Anal. Chem. **345:** 270-277.

Woshner, V.M. 2000. Concentrations and interactions of selected elements in tissues of four marine mammal species harvested by Inuit hunters in Arctic Alaska, with an intensive histologic assessment, emphasizing the beluga whale. Ph.D. thesis, College of Veterinary Medicine, University of Illinois, Urbana-Champaign, IL.

Woshner, V.M., O'Hara, T.M., Bratton, G.R., and Beasley, V.R. 2001a. Concentrations and interactions of selected essential and non-essential elements in ringed seals and polar bears of Arctic Alaska. J. Wildl. Dis. **37:** 711-721.

Woshner, V.M., O'Hara, T.M., Bratton, G.R., Suydam, R.S., and Beasley, V.R. 2001b. Concentrations and interactions of selected essential and non-essential elements in bowhead and beluga whales of Arctic Alaska. J. Wildl. Dis. **37:** 693-710.

Yamamoto, R., Suzuki, T., Satoh, H., and Kawai, K. 1986. Generation and dose as modifying factors of inorganic mercury accumulation in brain, liver, and kidneys of rats fed methylmercury. Environ. Res. **41:** 309-318.

Yang, J., Kunito, T., Anan, Y., Tanabe, S., and Miyazaki, N. 2004. Total and subcellular distribution of trace elements in the liver of a mother-fetus pair of Dall's porpoises (*Phocoenoides dalli*). Mar. Pollut. Bull. **48:**1122-1129.

Yeats, P., Stenson, G., and Hellou, J. 1999. Essential elements and priority contaminants in liver, kidney, muscle and blubber of harp seal beaters. Sci. Total Environ. **243/244:** 157-167.

Zenteno-Savin, T., Clayton-Hernandez, E., and Elsner, R. 2002. Diving seal: are they a model for coping with oxidative stress? Comp. Biochem. Physiol. C **133:** 527-536.

Zhang, Y., Amakawa, H., and Nozaki, Y. 2001. Oceanic profiles of dissolved silver: precise measurements in the basins of western North Pacific, Sea of Okhotsk, and the Japan Sea. Mar. Chem. **75:** 151-163.

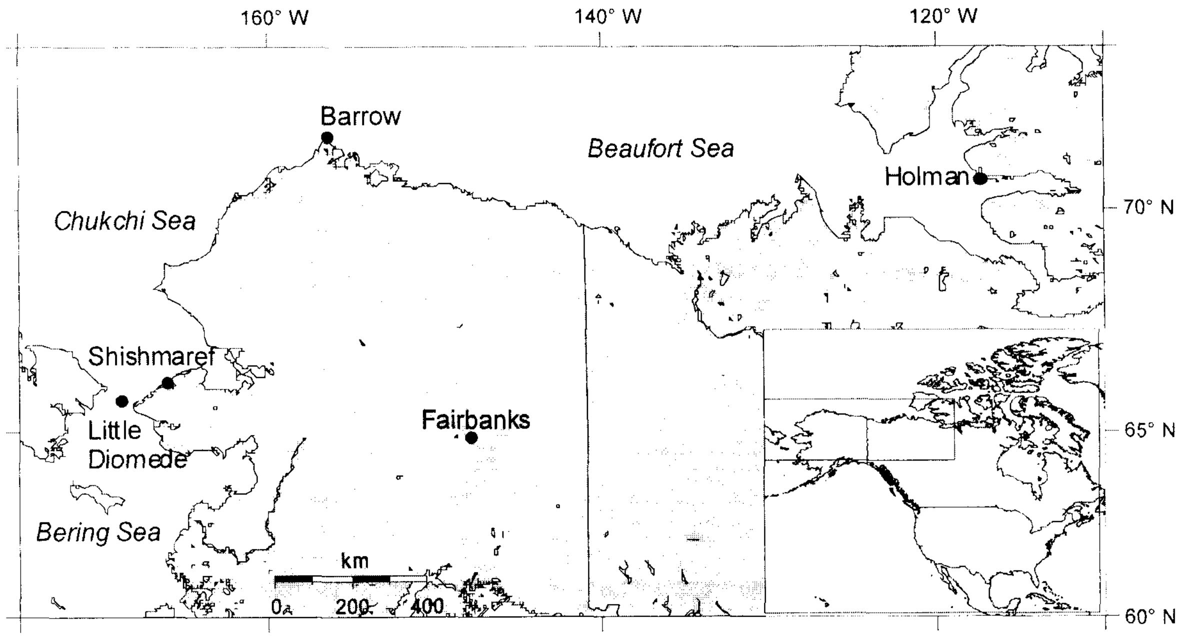


FIGURE 2.1. Alaskan and Canadian villages and communities where samples of subsistence-harvested Arctic pinnipeds were collected. Fairbanks serves as a point of reference.

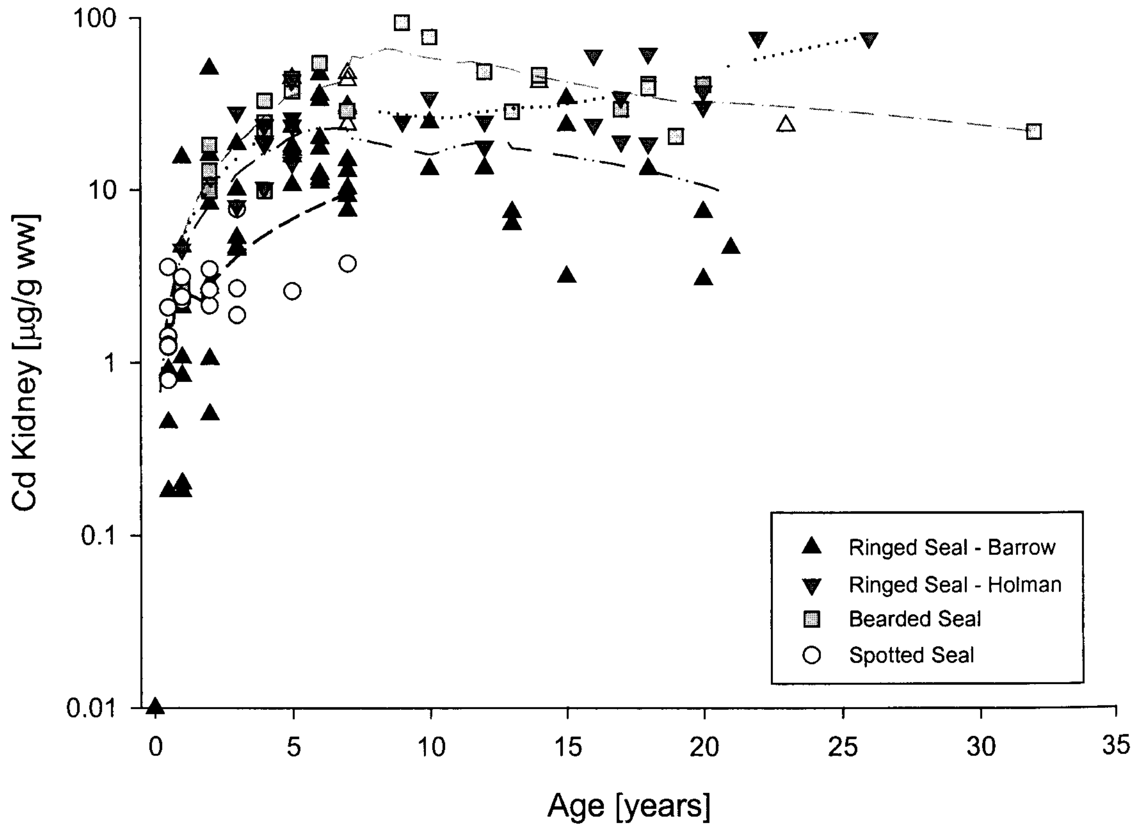


FIGURE 2.2. Age based on cementum analysis of teeth versus renal Cd [µg/g ww] in ringed (*Phoca hispida*), bearded (*Erignathus barbatus*) and spotted seals (*Phoca largha*) harvested in Alaska, 1996-2001 and ringed seals from Holman, Canada, 2001. The Y-axis is given as logarithmic scale. A LOESS nonparametric smoothing (dashed lines) was employed to estimate and compare the regression surface. Five ringed seals (highlighted as open triangles) were found with lesions and excluded from regression analysis.

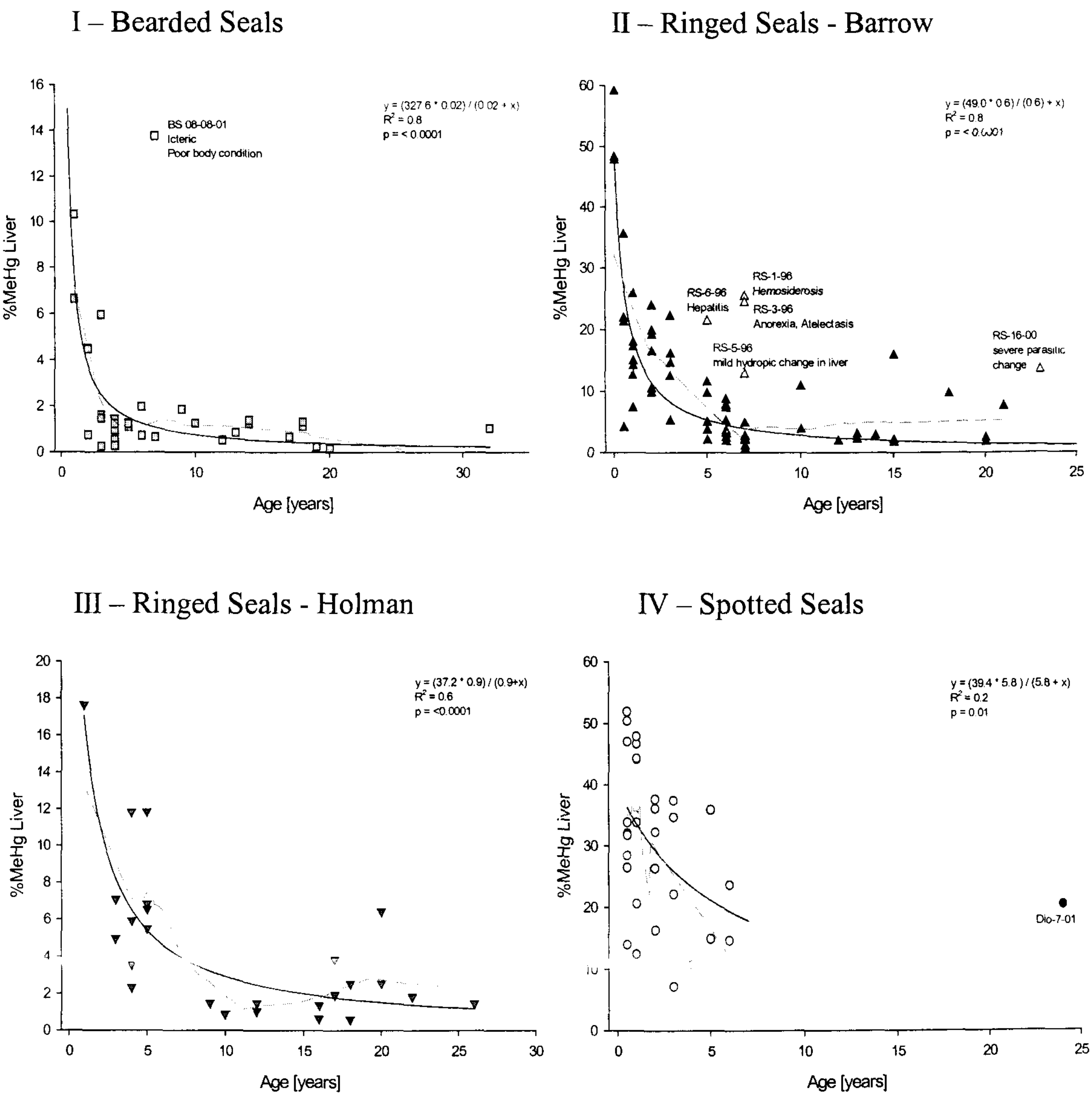


FIGURE 2.3. Age based on cementum analysis of teeth versus %MeHg in liver of bearded (I), ringed (II) and spotted seals (IV) harvested in Alaska, 1996-2001 and ringed seals (III) from Holman, Canada, 2001. A hyperbolic decay function was fitted to each data set and a LOESS nonparametric smoothing (dashed lines) was employed to estimate and compare the regression surface. Five Alaska ringed seals (highlighted as open triangles) and one bearded seal (highlighted as open square) were found with lesions and excluded from regression analysis. A 24-year old spotted seal female (Dio-7-01 highlighted as black circle) was excluded to avoid the impact of an influential observation.

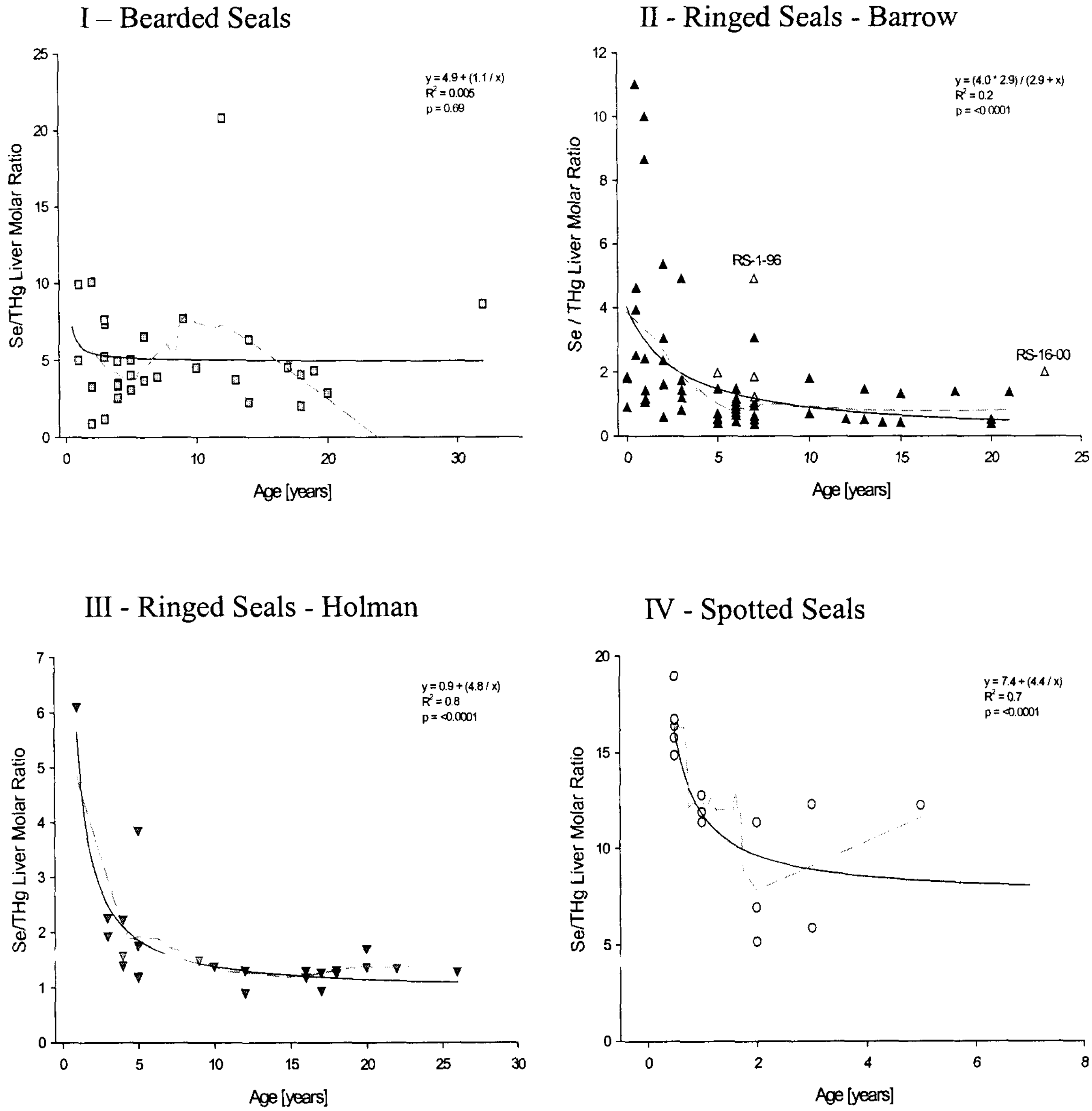


FIGURE 2.4. Age based on cementum analysis of teeth versus the hepatic Se/THg molar ratio of bearded (I), ringed (II) and spotted seals (IV) harvested in Alaska, 1996-2001 and ringed seals (III) from Holman, Canada, 2001. A hyperbolic decay function was fitted to the data of Alaska harvested ringed seals and an inverse polynomial was fitted to the other data sets. A LOESS nonparametric smoothing (dashed lines) was employed to estimate the regression surface. Five ringed seals (highlighted as open triangles) were found with lesions and excluded from regression analysis.

TABLE 2.1. Seal samples collected in Alaskan and Canadian villages, 1996-2001.

			Number of samples collected					
Species	**Sampling Date**	**Sampling Location**	**Males**	**Females**	**Unknown Sex**	**Kidney**	**Liver**	**Muscle**
Ringed Seal	1996-2001	Barrow	39	26	1	66	66	63
Ringed Seal	2001	Holman	12	13	-	25	25	-
Bearded Seal	1998-2001	Barrow	14	21	3	27	38	34
Spotted Seal	2000-2001	Little Diomede/Shishmaref	24	10	-	34	34	3

TABLE 2.2. Results for trace element analysis of reference materials for quality assurance / quality control. Concentrations are given in µg/g ww.
n.e. = not established

		Ag	Cd	Cu	Se	Zn	THg	MeHg
Dogfish muscle	Dorm - 2							
	Certified value	0.041 ± 0.09	0.043 ± 0.008	2.34 ± 0.16	1.4 ± 0.09	25.6 ± 2.3	4.64 ± 0.26	4.47 ± 0.32
	Measured Mean	0.042	0.044	2.075	1.314	23.294	4.522	3.943
	Standard Deviation	0.003	0.005	0.197	0.097	1.446	0.248	0.125
	% Recovery	101.7	101.6	88.7	93.9	91.0	97.5	88.2
	n	11	11	11	36	11	21	8
Dogfish Liver	Dolt - 2							
	Certified value	0.608 ± 0.032	20.8 ± 0.5	25.8 ± 1.1	6.06 ± 0.49	85.8 ± 2.5	2.14	0.693 ± 0.06
	Measured Mean	0.612	19.824	25.730	5.363	89.514	-	-
	Standard Deviation	0.031	0.803	1.426	0.375	6.928	-	-
	% Recovery	100.6	95.3	99.7	88.5	104.3	-	-
	n	53	45	53	27	53	-	-
Dogfish Liver	Dolt - 3							
	Certified value	1.20 ± 0.07	19.4 ± 0.6	31.2 ± 1.0	7.06 ± 0.48	86.6 ± 2.4	3.37 ± 0.14	n.e.
	Measured Mean	-	-	-	6.712	-	-	-
	Standard Deviation	-	-	-	0.434	-	-	-
	% Recovery	-	-	-	95.1	-	-	-
	n	-	-	-	12	-	-	-
Bovine liver	SRM 1577b							
	Certified value	0.039 ± 0.007	0.5 ± 0.03	160 ± 8.0	0.73 ± 0.06	127 ± 16.0	n.e.	n.e.
	Measured Mean	0.039	0.490	165.680	-	125.708	-	-
	Standard Deviation	0.002	0.027	5.327	-	8.328	-	-
	% Recovery	100.5	98.1	103.5	-	99.0	-	-
	n	21	13	13	-	19	-	-
Lake Superior Fish	SRM 1946							
	Certified value	n.e.	0.002 ± 0.0003	0.476 ± 0.06	0.491 ± 0.043	3.10 ± 0.18	0.433 ± 0.009	0.394 ± 0.015
	Measured Mean	-	-	-	-	-	0.425	0.344
	Standard Deviation	-	-	-	-	-	0.021	0.027
	% Recovery	-	-	-	-	-	98.2	87.4
	n	-	-	-	-	-	10	10
Pilot whale liver	QC91LH1							
	Certified value	0.181 ± 0.005	8.51 ± 0.22	2.96 ± 0.20	11.0 ± 0.3	32.2 ± 0.7	28.2 ± 1.1	1.36
	Measured Mean	-	-	-	-	-	27.931	1.397
	Standard Deviation	-	-	-	-	-	1.465	0.079
	% Recovery	-	-	-	-	-	99.0	102.7
	n	-	-	-	-	-	25	30
Beluga whale liver	QC97LH2							
	Consensus mean*	13.24 ± 2.41	2.433 ± 0.040	13.10 ± 0.188	24.35 ± 0.484	26.92 ± 0.359	40.31 ± 1.28	n.e.
	Analyzed Mean	-	-	-	-	-	42.031	1.505
	Standard Deviation	-	-	-	-	-	2.603	0.091
	% Recovery	-	-	-	-	-	104.3	-
	n	-	-	-	-	-	11	11
Lake Superior Fish	SRM 1947							
	Consensus mean*	0.018 ± 0.006	0.0012 ± 0.0002	0.421 ± 0.015	0.441 ± 0.023	2.63 ± 0.08	0.251 ± 0.013	n.e.
	Analyzed Mean	-	-	-	-	-	0.251	0.242
	Standard Deviation	-	-	-	-	-	0.014	0.035
	% Recovery	-	-	-	-	-	99.9	-
	n	-	-	-	-	-	5	5

*established by 19 laboratories (Christopher 2002, Christopher 2004)

TABLE 2.3 Mean trace element concentration ± standard deviation (SD) in μg/g ww, concentration range and sample size (*n*) in tissues of ringed seals (*Phoca hispida*) harvested in Alaska and Canada, and bearded (*Erignathus barbatus*) and spotted seals (*Phoca largha*), 1996-2001.

Ringed Seal - Barrow

	$\delta^{15}N$	$\delta^{13}C$	Zn	Cu	Cd	Se	Ag	THg	MeHg	%MeHg	Se/THg Molar Ratio
Liver											
Mean ± SD	-	-	47.71 ± 9.29	10.82 ± 5.17	3.64 ± 3.01	4.49 ± 3.94	0.11 ± 0.13	2.47 ± 3.15	0.11 ± 0.08	12.02 ± 11.99	11.72 ± 13.91
Range	-	-	28.50 - 77.40	4.50 - 30.04	0.01 - 11.84	0.34 - 25.77	0.01 - 0.69	0.06 - 16.55	0.01 - 0.50	0.56 - 59.03	2.04 - 70.86
n	-	-	66	66	66	66	66	64	64	64	64
Kidney											
Mean ± SD	-	-	39.04 ± 11.78	7.54 ± 3.87	14.70 ± 13.98	2.72 ± 0.89	0.01 ± 0.01*	0.45 ± 0.27	0.05 ± 0.03	14.93 ± 14.00	25.01 ± 23.44
Range	-	-	16.38 - 80.90	2.06 -25.57	0.01 - 50.70	0.91 - 5.81	0.01 - 0.04	0.05 - 1.06	0.01 - 0.16	2.51 - 77.54	6.82 - 139.58
n	-	-	66	66	66	66	66	63	47	47	63
Muscle											
Mean ± SD	16.89 ± 0.62	-18.52 ± 0.78	25.34 ± 8.99	1.52 ± 0.94	0.16 ± 0.34	0.46 ± 0.17	0.01 ± 0.00*	0.10 ± 0.16	0.07 ± 0.07	81.79 ± 25.21	21.76 ± 17.73
Range	15.62 - 18.49	-21.33 - -16.73	13.25 - 53.10	0.93 - 7.97	0.01 - 1.87	0.19 - 0.99	0.01 - 0.01	0.01 - 1.06	0.01 - 0.38	21.48 - 135.82	0.67 - 104.34
n	78	78	63	63	58	62	59	59	47	47	56

* more than 50% of samples below MDL

Ringed Seal - Holman

	$\delta^{15}N$	$\delta^{13}C$	Zn	Cu	Cd	Se	Ag	THg	MeHg	%MeHg	Se/THg Molar Ratio
Liver											
Mean ± SD	-	-	43.99 ± 6.93	9.25 ± 4.87	6.65 ± 4.20	12.13 ± 8.42	0.55 ± 0.56	22.65 ± 17.54	0.52 ± 0.19	4.48 ± 4.20	1.74 ± 1.08
Range	-	-	27.30 - 55.70	2.28 - 19.40	1.20 - 18.10	3.56 - 39.60	0.05 - 2.74	1.48 - 71.96	0.26 - 0.88	0.60 - 17.65	0.91 - 6.11
n	-	-	25	25	25	25	25	25	25	25	25
Kidney											
Mean ± SD	-	-	50.63 ± 14.64	8.38 ± 3.18	30.44 ± 19.56	3.16 ± 0.06	0.02 ± 0.01	1.94 ± 0.74	0.32 ± 0.14	17.98 ± 8.05	4.79 ± 2.53
Range	-	-	28.50 - 76.60	3.62 - 20.20	4.54 - 77.1	2.31 - 4.59	0.004 - 0.05	0.79 - 3.71	0.11 - 0.70	7.64 - 40.73	2.19 - 17.83
n	-	-	25	25	25	25	25	25	25	25	25
Muscle											
Mean ± SD	17.19 ± 0.72	-20.41 ± 0.40	-	-	-	-	-	-	-	-	-
Range	14.64 - 18.00	-20.90 - -19.17	-	-	-	-	-	-	-	-	-
n	25	25	-	-	-	-	-	-	-	-	-

TABLE 2.3 (continued)

Bearded Seal

	$\delta^{15}N$	$\delta^{13}C$	Zn	Cu	Cd	Se	Ag	THg	MeHg	%MeHg	Se/THg Molar Ratio
Liver											
Mean ± SD	-	-	57.48 ± 10.37	22.69 ± 7.99	8.66 ± 7.03	5.27 ± 3.51	0.34 ± 0.24	3.84 ± 3.55	0.06 ± 0.08	2.29 ± 2.99	5.48 ± 3.95
Range	-	-	36.80 - 81.00	9.64 - 39.60	0.57 - 33.62	0.75 - 23.20	0.01 - 1.13	0.64 - 20.44	0.004 - 0.49	0.19 - 13.77	0.89 - 20.81
n	-	-	38	38	38	38	38	34	34	34	33
Kidney											
Mean ± SD	-	-	39.00 ± 10.75	5.81 ± 1.17	31.47 ± 20.90	4.90 ± 1.49	0.01 ± 0.00*	0.58 ± 0.28	0.01 ± 0.02	2.35 ± 2.00	28.12 ± 21.88
Range	-	-	25.11 - 69.48	3.38 - 7.84	1.30 - 94.47	2.83 - 9.35	0.01 - 0.01	0.21 - 1.50	0.001 - 0.07	0.08 - 8.26	6.90 - 115.85
n	-	-	27	27	30	27	27	26	26	26	25
Muscle											
Mean ± SD	16.74 ± 0.89	-17.09 ± 0.57	41.48 ± 21.13	0.86 ± 0.31	0.08 ± 0.10	0.81 ± 0.24	0.01 ± 0.00*	0.03 ± 0.02	0.03 ± 0.02	74.52 ± 26.58	78.80 ± 47.16
Range	15.23 - 18.84	-18.72 - -15.81	16.70 - 87.89	0.54 - 1.94	0.01 - 0.42	0.45 - 1.37	0.01 - 0.01	0.01 - 0.09	0.001 - 0.08	2.41 - 110.23	13.38 - 219.71
n	47	47	34	34	34	34	34	33	33	33	32

* more than 50% of samples below MDL

Spotted Seal

	$\delta^{15}N$	$\delta^{13}C$	Zn	Cu	Cd	Se	Ag	THg	MeHg	%MeHg	Se/THg Molar Ratio
Liver											
Mean ± SD	-	-	39.74 ± 6.79	10.20 ± 3.46	0.39 ± 0.48	1.45 ± 0.69	0.08 ± 0.03	0.68 ± 0.68	0.15 ± 0.11	29.08 ± 12.66	12.92 ± 3.97
Range	-	-	25.48 - 53.19	5.88 - 17.63	0.09 - 2.18	0.71 - 2.97	0.01 - 0.13	0.10 - 2.62	0.02 - 0.53	7.12 - 51.87	5.14 - 18.96
n	-	-	17	17	17	17	17	34	34	34	17
Kidney											
Mean ± SD	-	-	26.09 ± 4.04	3.78 ± 0.80	2.58 ± 1.56	3.58 ± 1.25	0.01 ± 0.00*	0.31 ± 0.21	0.07 ± 0.06	20.11 ± 11.76	50.86 ± 18.31
Range	-	-	20.69 - 33.06	2.61 - 5.48	0.79 - 7.76	2.22 - 6.31	0.01 - 0.01	0.08 - 0.90	0.004 - 0.23	1.75 - 46.09	18.15 - 76.66
n	-	-	18	18	18	18	18	34	34	34	18
Muscle											
Mean ± SD	17.58 ± 0.86	-18.31 ± 0.93	23.95 ± 9.73	1.22 ± 0.10	0.02 ± 0.03	0.67 ± 0.16	0.01 ± 0.00*	0.13 ± 0.03	0.10 ± 0.02	77.48 ± 3.14	15.82 ± 3.07
Range	15.91 - 19.35	-20.01 - -15.93	17.44 - 35.13	1.12 - 1.32	0.01 - 0.06	0.49 - 0.81	0.01 - 0.01	0.10 - 0.15	0.08 - 0.11	75.26 - 79.70	13.65 - 17.99
n	34	34	3	3	3	3	3	2	2	2	2

* more than 50% of samples below MDL

TABLE 2.4. Tukey grouping and p-values of all variables in tissues of ringed (RS), bearded (BS) and spotted seals (SS) from Alaska, 1996-2001 and ringed seals (HRS) from Holman, Canada, 2001.

Variable	Tissue	Tukey grouping	p-value
Age	-	[§] HRS > BS = RS > SS	<0.0001
$\delta^{15}N$	Muscle	SS > RS = BS; SS = HRS; RS = HRS; HRS > BS	0.0006
$\delta^{13}C$	Muscle	BS > SS = RS > HRS	<0.0001
Zn	Kidney	HRS > BS = RS > SS	<0.0001
	Liver	BS > RS > SS; HRS = RS; HRS = SS	<0.0001
	Muscle	BS > RS	0.0004
Cu	Kidney	HRS = RS > BS > SS	<0.0001
	Liver	BS > HRS = RS = SS	<0.0001
	Muscle	RS > BS	<0.0001
Cd	Kidney	BS = HRS > RS > SS	<0.0001
	Liver	BS = HRS > RS > SS	<0.0001
	Muscle	BS = RS	0.048
Ag	Kidney *	-	-
	Liver	HRS = BS > RS = SS	<0.0001
	Muscle *	-	-
Se	Kidney	BS > SS = HRS > RS	<0.0001
	Liver	HRS > BS > RS > SS	<0.0001
	Muscle	BS > RS	<0.0001
THg	Kidney	HRS > BS = RS > SS	<0.0001
	Liver	HRS > BS > RS > SS	<0.0001
	Muscle	RS > BS	<0.0001
MeHg	Kidney	HRS > RS = SS > BS	<0.0001
	Liver	HRS > RS = SS > BS	<0.0001
	Muscle	RS > BS	<0.0001
%MeHg	Kidney	HRS = SS > RS > BS	<0.0001
	Liver	SS > RS > HRS > BS	<0.0001
	Muscle	BS = RS	0.98
Se/THg molar ratio	Kidney	SS > BS = RS > HRS	<0.0001
	Liver	SS > RS > BS > HRS	<0.0001
	Muscle	BS > RS	<0.0001

[§] HRS - Ringed seal, Holman

RS - Ringed seal, Barrow

BS - Bearded seal

SS - Spotted seal

* more than 50% of samples below MDL

TABLE 2.5. Correlation matrix of all variables in tissues of ringed seals harvested in Alaska and Canada, and bearded and spotted seals from Alaska, 1996-2001. Only significant relationships were noted and the slope of correlated variables is indicated by either + (positive) or – (negative). Correlated variables that are highlighted in bold are consistent for all species from both locations. Correlations consistent between seal species harvested in Alaska are underlined.

Ringed Seal - Barrow

		Liver	Kidney	Muscle
	Age	+Cd, **+Se**, +THg, +MeHg, -%MeHg	+Zn, **+Cd**, +Se	+δ^{13}C, -Zn, -Cu, +Cd, +MeHg
	δ^{13}C	+Cd, +Se, +THg, +MeHg	+Zn, +Cd, -%MeHg	-δ^{15}N, -Cu, -Se
	δ^{15}N	-Cd	-Zn, -Cd	-
Liver	Zn	+Cu, +Cd, +Ag	+Zn, +Cd	-Cu, -Se
	Cu	+Ag	-	-
	Cd	+Ag, +Se, +THg, +MeHg, -%MeHg	+Zn, **+Cd**, +THg	-Cu, +Cd, -Se, +MeHg
	Ag	+Se, +THg, +MeHg, +%MeHg	+Cd, +THg	-
	Se	**+THg**, +MeHg, +%MeHg	+Zn, +Cd, +Se, +THg, +MeHg	-Cu, +Cd, +MeHg
	THg	+MeHg, **-%MeHg**	+Zn, +Cd, +Se, +THg, +MeHg	-Cu, +Cd, +THg, +MeHg
	MeHg	-	+Zn, +Cd, +THg, +MeHg	-Cu, +THg, +MeHg
	%MeHg	-	-Cd, -THg, +%MeHg	+Cu, -MeHg
Kidney	Zn	-	+Cu, +Cd, +THg, -%MeHg	-Cu, +Cd, -Se
	Cu	-	+THg	-%MeHg
	Cd	-	+THg	-Cu, +Cd, -Se
	Ag *	-	-	-
	Se	-	+THg	-Zn, -Cu, +Se
	THg	-	**+MeHg**, -%MeHg	-Cu, +Cd, +MeHg
	MeHg	-	**+%MeHg**	-Cu, +Cd, +Se, +THg, +MeHg
	%MeHg	-	-	-
Muscle	Zn	-	-	-Se
	Cu	-	-	-
	Cd	-	-	+MeHg
	Ag *	-	-	-
	Se	-	-	+THg, +MeHg
	THg	-	-	+MeHg
	MeHg	-	-	+%MeHg

* more than 50% of samples below MDL

TABLE 2.5 (continued)

Ringed Seal - Holman

		Liver	Kidney
	Age	-Zn, -Cu, **+Se**, +THg, -%MeHg	+Zn, **+Cd**, +Ag
	$\delta^{13}C$	-	-
	$\delta^{15}N$	+MeHg	+THg, +MeHg
Liver	Zn	-	-
	Cu	-	-Ag
	Cd	+Se, +Ag, +THg, -%MeHg	+Zn, **+Cd**
	Ag	+Se, +THg, -%MeHg	-
	Se	**+THg**, -%MeHg	+Cd, +Ag
	THg	**-%MeHg**	-
	MeHg	-	-
	%MeHg	-	-Cd, -Ag
Kidney	Zn	-	+Cd
	Cu	-	-
	Cd	-	+Ag, -MeHg, -%MeHg
	Ag	-	-MeHg
	Se	-	-
	THg	-	**+MeHg**, -%MeHg
	MeHg	-	**+%MeHg**
	%MeHg	-	-

TABLE 2.5 (continued)

Bearded Seal

		Liver	Kidney	Muscle
	Age	+Cd, **+Se**, -%MeHg	+Zn, **+Cd**	+Cd
	$\delta^{13}C$	+THg	-	+THg
	$\delta^{15}N$	-Ag	+MeHg, +%MeHg	+THg, +MeHg
Liver	Zn	+Cu, +Cd, +Se	+Zn	+Zn, -MeHg, -%MeHg
	Cu	+Ag	-	-MeHg
	Cd	+Se, -%MeHg	+Zn, **+Cd**, -%MeHg	+Cd, -MeHg, -%MeHg
	Ag	+THg	-	-
	Se	**+THg**, -%MeHg	+Zn, +Cd	+Se, -%MeHg
	THg	**-%MeHg**	+THg	-
	MeHg	+%MeHg	+THg, +MeHg, +%MeHg	+THg
	%MeHg	-	-	+Cu
Kidney	Zn	-	+Cu, +Cd	+Zn, +Cd, -%MeHg
	Cu	-	+Cd	-
	Cd	-	-%MeHg	+Cd
	Ag *	-	-	-
	Se	-	-	-
	THg	-	**+MeHg**	+THg, +MeHg
	MeHg	-	**+%MeHg**	-Se, +THg, +MeHg
	%MeHg	-	-	-Se, +THg, MeHg
Muscle	Zn	-	-	+Cu, +Cd
	Cu	-	-	-
	Cd	-	-	-
	Ag *	-	-	-
	Se	-	-	-
	THg	-	-	+MeHg
	MeHg	-	-	+%MeHg

* more than 50% of samples below MDL

TABLE 2.5 (continued)

Spotted Seal

		Liver	Kidney	Muscle
	Age	+Cd, **+Se**, +THg, +MeHg	**+Cd**, +Se, +THg, +MeHg	+δ^{15}N, +Zn
	δ^{13}C	+THg, +MeHg	+THg	-Se
	δ^{15}N	+THg	-	+Zn
Liver	Zn	-	-	-
	Cu	+Cd, +Ag	+MeHg	-
	Cd	+MeHg	**+Cd**, +Se, +THg, +MeHg	-
	Ag	-	-	-
	Se	**+THg**, +MeHg	-Cu, +Se, +THg	-
	THg	+MeHg, **-%MeHg**	+Se, +THg, +MeHg	-
	MeHg	-	+Cd, **+Se**, +THg, +MeHg	-
	%MeHg	-	-THg, -MeHg	-
Kidney	Zn	-	+Cu	-
	Cu	-	-Se	-
	Cd	-	+Se, +THg, +MeHg	-
	Ag *	-	-	-
	Se	-	+THg, +MeHg	-
	THg	-	**+MeHg**	-
	MeHg	-	**+%MeHg**	-
	%MeHg	-	-	-
Muscle	Zn	-	-	-
	Cu	-	-	+Cd
	Cd	-	-	-
	Ag *	-	-	-
	Se	-	-	-
	THg	-	-	-
	MeHg	-	-	-

* more than 50% of samples below MDL

TABLE 2.6 Mean trace element concentration ± standard deviation (SD) in μg/g ww, concentration range and sample size (*n*) in total body homogenates of selected prey species. n. d. = not determined

	Species	Cd	Cu	Zn	Ag	Se	THg	Se/THg Molar Ratio
Teleost Fish								
Mean ± SD	Arctic cod	0.19 ± 0.07	5.49 ± 1.03	80.82 ± 10.35	0.06 ± 0.03	2.99 ± 0.23	n.d.	n.d.
Range	(*Boreogadus saida*)	0.10 - 0.26	4.11 - 6.71	63.00 - 92.00	0.04 - 0.11	2.74 - 3.31	-	-
n		6	6	6	6	6	-	-
Mean ± SD	Walleye Pollock	0.10 ± 0.05	1.24 ± 0.49	25.21 ± 6.09	0.25 ± 0.13	0.40 ± 0.11	0.02 ± 0.03	121.16 ± 86.15
Range	(*Theragra chalcogramma*)	0.03 - 0.15	0.75 - 1.98	17.92 - 32.76	0.16 - 0.47	0.24 - 0.54	0.004 - 0.07	15.56 - 245.81
n		5	5	5	5	5	5	5
Mean ± SD	Pacific Herring	0.17 ± 0.07	0.94 ± 0.39	22.50 ± 3.20	0.18 ± 0.12	0.50 ± 0.25	0.02 ± 0.02	108.73 ± 60.92
Range	(*Clupea pallasii*)	0.11 - 0.24	0.49 - 1.21	19.23 - 25.62	0.07 - 0.31	0.22 - 0.66	0.004 - 0.04	38.87 - 150.81
n		3	3	3	3	3	3	3
Mean ± SD	King Salmon	0.13	1.22	52.37	0.17	0.31	0.02	41.47
Range	(*Oncorhynchus tshawytscha*)	-	-	-	-	-	-	-
n		1	1	1	1	1	1	1
Mean ± SD	Chum Salmon	0.14	1.72	25.66	0.15	0.42	0.02	55.49
Range	(*Oncorhynchus keta*)	-	-	-	-	-	-	-
n		1	1	1	1	1	1	1
Mollusks								
Mean ± SD	Squid	0.25 ± 0.24	8.53 ± 3.06	22.55 ± 5.27	0.16 ± 0.07	0.27 ± 0.05	0.01 ± 0.004	93.47 ± 49.94
Range		0.09 - 0.52	6.11 - 11.97	17.49 - 28.00	0.12 - 0.25	0.21 - 0.32	0.01 - 0.01	42.03 - 141.76
n		3	3	3	3	3	3	3
Mean ± SD	Octopus	0.14	2.23	64.17	0.05	0.24	0.01	100.23
Range	(*Octopus* spp.)	-	-	-	-	-	-	-
n		1	1	1	1	1	1	1
Mean ± SD	Greenland cockle	1.76	11.40	45.40	0.16	4.14	n.d	n.d
Range	(*Serripes groenlandicus*)	-	-	-	-	-	-	-
n		1	1	1	1	1	-	-
Crustaceans								
Mean ± SD	Zooplankton	0.22 ± 0.10	0.76 ± 0.64	28.67 ± 8.22	0.04 ± 0.03	0.38 ± 0.14	0.01 ± 0.002	382.98 ± 300.76
Range	(unsorted)	0.04 - 0.44	0.11 - 2.93	13.36 - 47.51	0.01 - 0.09	0.15 - 0.67	0.001 - 0.01	170.92 - 976.52
n		21	21	21	21	21	6	6
Mean ± SD	Amphipods	0.87	39.50	89.50	1.66	1.96	n.d.	n.d.
Range	Gammaridae	-	-	-	-	-	-	-
n		1	1	1	1	1	-	-
Mean ± SD	Isopods	0.89 ± 0.48	73.58 ± 24.58	65.72 ± 10.29	1.44 ± 0.68	2.64 ± 1.01	0.004 ± 0.00	2156.01 ± 1170.95
Range	(*Saduria* spp.)	0.13 - 1.60	41.10 - 107.00	51.20 - 87.90	0.64 - 2.93	1.94 - 5.44	0.004 - 0.01	1328.03 ± 2984.00
n		10	10	10	10	10	2	2
Mean ± SD	Sculptured Shrimp	9.15	36.20	50.60	0.79	1.24	n.d.	n.d.
Range	(*Sclerocrangon boreas*)	-	-	-	-	-	-	-
n		1	1	1	1	1	-	-
Mean ± SD	Hermit crab	1.23	19.80	52.30	0.14	0.80	0.01	292.75
Range	Paguridae	-	-	-	-	-	-	-
n		1	1	1	1	1	1	1
Echinodermata								
Mean ± SD	Sea Cucumber	1.65 ± 0.89	3.21 ± 3.38	52.34 ± 13.23	0.04 ± 0.04	3.56	n.d.	n.d.
Range		1.03 - 2.28	0.82 - 5.60	42.99 - 61.70	0.01 - 0.07	-	-	-
n		2	2	2	2	1	-	-
Urochordata								
Mean ± SD	Tunicate	0.28	7.50	53.8	0.12	1.55	n.d.	n.d.
Range		-	-	-	-	-	-	-
n		1	1	1	1	1	-	-

CHAPTER 3

STABLE ISOTOPE AND TRACE ELEMENT STATUS OF SUBSISTENCE HUNTED BOWHEAD (*BALAENA MYSTICETUS*) AND BELUGA WHALES (*DELPHINAPTERUS LEUCAS*) IN ALASKA AND GRAY WHALES (*ESCHRICHTIUS ROBUSTUS*) IN CHUKOTKA[3]

3.1 ABSTRACT

Tissues of bowhead, beluga, and gray whales were analyzed for Ag, Cd, Cu, Se, Zn, THg and MeHg (belugas only). $\delta^{15}N$ and $\delta^{13}C$ in muscle were used to estimate trophic position and feeding habitat, respectively. Trace element concentrations in tissues were significantly different among whale species. Hepatic Ag was higher in belugas than bowheads and gray whales. Gray whales had lower Cd concentrations in liver and kidney than bowhead and belugas and a sigmoid correlation of Cd with length was noted for all whales. Renal and hepatic Se and THg were higher in belugas than in baleen whales. The hepatic molar ratio of Se:THg exceeded 1:1 in all species and was negatively correlated to body length. Hepatic and renal Zn in subsistence-harvested gray whales was lower than concentrations for stranded whales. Se:THg molar ratios and tissue concentrations of Zn may show promise as potential indicators of immune status and animal health.

[3] Dehn, L.-A., Follmann, E. H., Rosa, C., Duffy, L. K., Thomas, D. L., Bratton, G. R., Taylor, R. J., O'Hara, T. M. Stable isotope and trace element status of subsistence hunted bowhead (*Balaena mysticetus)* and beluga whales (*Delphinapterus leucas*) in Alaska and gray whales (*Eschrichtius robustus*) in Chukotka. Submitted to Marine Pollution Bulletin.

Keywords: Bowhead whale, beluga whale, gray whale, trace elements, stable isotopes, feeding ecology, Arctic

3.2 INTRODUCTION

Bowhead (*Balaena mysticetus*), beluga (*Delphinapterus leucas*) and gray whales (*Eschrichtius robustus*) have been of subsistence and cultural importance to the Inuit of Alaska and other Arctic areas for centuries. Accumulation of toxic elements is of growing concern to the consumers of subsistence foods in Alaska and Russia, and the cold water of the Arctic has been proposed as a sink for many contaminants (Ponce et al., 1997, Egeland et al., 1998, Bard, 1999). Continuous bioaccumulation and biomagnification of trace elements have been repeatedly reported in marine mammal tissues (Honda et al., 1983, Hansen et al., 1990, Dietz et al., 2000, Woshner et al., 2001a). In addition, the effect of longevity in cetaceans, in particular the bowhead whale (George et al., 1999), may lead to high levels of trace element accumulation.

The bowhead whale is the largest mysticete in Arctic waters. The Bering-Chukchi-Beaufort seas stock (BCBS or western Arctic stock) of bowheads migrates annually from the Bering Sea in winter to the Beaufort Sea in summer (Moore and Reeves, 1993). Commercial whalers decimated the bowhead population in the 19th century, but the BCBS stock is recovering at an estimated rate of 3.4% a year and sustains a controlled subsistence harvest (George et al., 2004). However, effects of offshore and coastal industrial development and thus health status and contaminant burden are of great importance for conservation and management of this culturally important species. Generally, trace elements in tissues of bowhead whales are low compared to other cetaceans, e.g., beluga, narwhal (*Monodon monoceros*), minke whale (*Balaenoptera acutorostrata*) and harbor porpoise (*Phocoena phocoena*) (Honda et al., 1987, Hansen et al., 1990, Mackey et al., 1995, Wagemann et al., 1996, Woshner et al., 2001a). Several studies have linked the lower trace element concentrations found in mysticetes to their low position in the food chain (Honda et al., 1987, Hansen et al., 1990, Bratton et al. 1997, Woshner et al., 2001a). However, compared to domestic animals, cadmium (Cd) concentrations in kidneys of bowheads are at levels of concern to whale health and subsistence consumers (Puls, 1994, Bratton et al., 1997). The levels are an order of magnitude higher than in top-level Arctic predators, e.g., Arctic fox (*Alopex lagopus*) and polar bear (*Ursus maritimus*) (Woshner et al., 2001a, 2001b, Ballard et al., 2003). Thus, it is evident that some trace elements do not accumulate with trophic level, but rather are prey-specific (Watanabe et al., 2002, Dehn et al., 2005). Bowheads feed low in the Arctic food chain on pelagic krill, primarily euphausids and copepods (Lowry, 1993, Lowry and Sheffield, 2002). Other prey is comprised of amphipods, Arctic cod (*Boreogadus saida*), and epibenthic species, but the presence of these species is likely due to incidental ingestion or age-related feeding differences (Lowry, 1993, Hazard and Lowry, 1994).

Gray whales are primitive mysticetes and unique in their reliance on benthic invertebrates (Rice and Wolman, 1971). Benthic gammaridean amphipods (e.g., *Ampelisca* spp.) are found most commonly in their stomachs (Rice and Wolman, 1971, Bogoslovskaya et al., 1981). The eastern Pacific stock of gray whales migrates annually from their feeding grounds in the Bering and Chukchi seas in summer to its calving grounds in Baja California and the Gulf of California in winter (Rice and Wolman, 1971). In 1999 the number of gray whales involved in fatal strandings increased from an average of about 50 animals per year to 274 animals (Le Boeuf et al., 2000). Contaminants, in particular sediment-associated compounds, have been proposed as possible causes for the die-offs (Varanasi et al., 1994, Le Boeuf et al., 2000, De Luna and Rosales-Hoz, 2004). However, very little baseline information is available on trace elements in healthy gray whales, making inference on the cause of strandings difficult. Tilbury et al. (2002) have reported trace element concentrations in juvenile gray whales harvested in Russia for subsistence use. Other information on trace elements consists of data of stranded animals (mostly juveniles) of varying specimen condition (Varanasi et al., 1994, Méndez et al., 2002, Ruelas-Inzunza and Paez-Osuna, 2002, De Luna and Rosales-Hoz, 2004).

The beluga is a medium sized odontocete and is widely distributed throughout the Arctic. Five stocks of beluga whales are currently recognized in Alaskan waters, including the small isolated Cook Inlet stock (O'Corry-Crowe et al., 1997). Little information is available on beluga feeding ecology. Various species of fishes have been identified from stomach contents (Seaman et al., 1982). However, belugas routinely carry out deep benthic dives for foraging (Martin et al., 1998, Martin and Smith, 1999), and benthic and epibenthic prey (e.g., octopus, shrimp, polychaetes) seem to be of importance (Seaman et al., 1982). A close relative of belugas, the narwhal, shows a high prevalence of deep benthic prey, e.g., halibut (*Reinhardtius hippoglossoides*), cephalopods, and crustaceans (Finley and Gibb, 1982). Beluga and narwhal habitats often overlap in the Canadian High Arctic, and it has been suggested that both utilize similar prey (Finley and Gibb, 1982, Richard et al., 1994). Some beluga stocks are declining in Alaska and Canada, and contaminants have been proposed as significant factors in this decline

(Wagemann et al., 1990, Gauthier et al., 1998, Becker et al., 2000). Belugas in the isolated St. Lawrence estuary are exposed to elevated contaminants and show signs of disease (e.g., neoplasia) and immune suppression associated with contaminant burden (Gauthier et al., 1998, Martineau et al., 2003, Brousseau et al., 2003, Gauthier et al., 2003).

Bowheads, beluga, and gray whales utilize very different trophic niches in the Arctic marine food web, and a comparison between tissues of these cetaceans may help to discriminate trace element pathways. Thus, it is of importance to identify trophic level and predator-prey relationships for these species. Studies of feeding ecology that rely on fecal or stomach contents analysis are strongly biased toward prey with identifiable hard parts and often underestimate soft prey (Sheffield et al., 2001). In addition, the frequency of empty stomachs is high in marine mammals, especially during migration, and empty stomachs will not yield any prey-based information (Rice and Wolman, 1971, Oliver et al., 1983, Kasuya, 1995).

Stable isotopes have been increasingly important in feeding ecology studies. They occur naturally, and nitrogen isotope ratios of prey are reflected in tissues of the consumer with slight enrichment occurring at each trophic step (Kelly, 2000). Carbon isotope ratios are not reliable indicators of trophic position, but are powerful in distinguishing between benthic and pelagic foodwebs, inshore vs. offshore environments and fresh- and saltwater habitats (Tieszen et al., 1983, France, 1995, Smith et al., 1996, Burton and Koch, 1999). Schell et al. (1998) showed distinct regional differences in carbon isotope signatures of zooplankton from the Beaufort Sea versus the Bering and

Chukchi seas. These differences have also been detected in muscle and baleen of migrating bowhead whales (Schell et al., 1989, Hoekstra et al., 2002) and are apparent in muscle of ringed seals (*Phoca hispida*) originating from either the Canadian or Alaskan Arctic (Dehn, in prep.). However, little or no comparative information is available on stable carbon and nitrogen isotope ratios in tissues of bowheads, belugas, and gray whales.

The objectives of this study are to provide reference concentrations of selected trace elements and stable isotopes of apparently healthy whales taken during subsistence harvests in Alaska and Russia to evaluate the effects of age (length) and trophic level ($\delta^{15}N$) on trace element pathways and biomagnification in Arctic cetaceans. This may aid in the conservation and management of these important subsistence species.

3.3 MATERIALS AND METHODS

3.31 Sample Collection

All whale samples were obtained during Native subsistence harvests. Basic morphometrics, e.g., body length, blubber thickness, and sex were recorded. Standard body length (rostrum to fluke notch) was used as a proxy for age (Sergeant and Brodie, 1969, Rice and Wolman, 1971, George et al., 1999). Most whales were grossly examined for lesions. Bowhead epidermis, lumbar muscle, kidney, and liver were predominantly collected in Barrow, Alaska during either spring or fall harvest 1998-2001. Data from bowheads harvested during 1995-1997 (Woshner et al., 2001a) and harvested from 1983 to 1990 (Bratton et al., 1997) were included in the data set. Samples of belugas harvested in Point Lay and Wainwright, Alaska in 1998-1999 were combined with data obtained during 1996-1997 (Woshner et al., 2001a) and 1992-1995 (Tarpley et al., 1995) that displayed the appropriate biological variables to increase sample size and statistical power. Tissues of gray whales were sampled in Lorino and Lavrentiya, Russia in 2001. Amphipods and zooplankton were obtained in the Bering Strait and near Kaktovik, Alaska, respectively.

Figure 3.1 shows villages and communities where samples were collected and Table 3.1 summarizes sample sizes. All tissues were sub-sampled under clean conditions with titanium or ceramic blades on a Teflon covered surface, following the sampling protocol for contaminants by Becker et al. (1999) and stored at -20°C in acid-washed scintillation vials or Whirlpacks™ until analysis. Marine mammal samples were collected and analyzed under the authority of Permit No. 932-1489-03 issued to Dr. T. Rowles of the Marine Mammal Health and Stranding Response Program.

3.32 Stable Isotope Analyses

Muscle of bowhead, beluga, and gray whales and total body homogenates of prey (unsorted zooplankton and amphipods) were freeze-dried and ground into a fine powder with mortar and pestle. For each sample, 0.2 to 0.4 mg of tissue was weighed into a 4.75 x 4 mm tin capsule, which were folded into a cube. Samples were analyzed for both stable isotope ratios of carbon and nitrogen at the University of Alaska Fairbanks (UAF) using a Finnigan MAT Delta^{Plus}XL Isotope Ratio Mass Spectrometer (IRMS) directly coupled to a Costech Elemental Analyzer (ESC 4010). Samples were flash-combusted at

1020°C, followed by on-line chromatographic separation of sample N_2 and CO_2 with He as carrier gas. Samples analyzed for $^{15}N/^{14}N$ and $^{13}C/^{12}C$ were standardized against atmospheric N_2 and PeeDee Belemnite limestone, respectively. Enrichment of a particular isotope was reported using the following notation and equation:

$$\delta R‰ = ((R_{sample}/R_{standard}) - 1) \times 1000$$

where the differential notation (δR) represents the relative difference between isotopic ratios of the sample and standard gases (i.e., $^{13}C/^{12}C$, $^{15}N/^{14}N$). A laboratory-working standard (Peptone No. P-7750) was analyzed every 10 samples during analysis and tin capsule blanks were run every 20 samples. Calibrations were made with the use of stable isotope reference materials provided by the National Institute of Standards and Technology (NIST). External instrument reproducibility for both carbon and nitrogen isotope analysis was +/- 0.2‰.

3.33 Trace Element Analyses

Silver (Ag), cadmium (Cd), copper (Cu), selenium (Se), and zinc (Zn) were analyzed at Texas A&M University (TAMU) following US Environmental Protection Agency (EPA) procedures (200.3, 200.7, 200.8 and 200.9) with slight modifications (EPA, 1992). Briefly, sub-sampled tissues were homogenized, and approximately 0.8 – 1.0 g of sample was digested in a microwave wet ash procedure using HNO_3, H_2O_2 and HCl. A second preparation followed for determination of Se in tissues using excess HCl to completely reduce Se (VI) to Se (IV) in a CPI ModBlock digester. For bowhead and beluga whales, Cd and Ag were analyzed using Graphite Furnace Atomic Absorption Spectrometry (Perkin-Elmer Model SIMAA 6000 equipped with an AS-72 autosampler and Zeeman background correction). Cu and Zn were determined by Flame Atomic Absorption Spectrometry (Perkin-Elmer Aanalyst 100). Se in all whale tissues was analyzed using Atomic Fluorescence Spectrometry (PSA Millennium Excalibur with CETAC autosampler) and metals (Ag, Cd, Cu and Zn) in gray whales were analyzed by ICP-MS (Perkin-Elmer Elan Model 6100 DRC-II). The detection limit was 0.01 μg/g for elements analyzed with Graphite Furnace AAS, Flame AAS and Atomic Fluorescence Spectrometry. The detection limits were 0.01 μg/g for Cd, 0.05 μg/g for Zn and Cu and 0.005 μg/g for Ag using ICP-MS. All element concentrations are expressed as μg/g wet weight (ww) unless otherwise noted.

3.34 Total Mercury

Total mercury (THg) was analyzed at UAF following the procedure established by Bloom and Crecelius (1983). Briefly, sub-sampled tissues were homogenized, and approximately 1 g of tissue was digested in 7:3 HNO_3/H_2SO_4 and oxidized with 10% BrCl in 12N HCL. The sample was reduced to Hg^0 with $SnCl_2$ and purged with N_2 onto gold-coated quartz sand traps followed by dual thermal desorption to a Cold Vapor Atomic Fluorescence Spectrometer (Tekran Model-2500 CVAFS Mercury Detector) with argon as carrier gas. The detection limit was 0.001 μg/g. Concentrations are expressed as μg/g wet weight (ww) unless otherwise noted.

3.35 Methyl Mercury

Methyl mercury (MeHg) was analyzed in beluga tissues at UAF following the procedure established by Bloom (1989). About 1 g of tissue was homogenized and digested in 20% KOH in methanol. Aqueous phase ethylation was initiated with $NaB(C_2H_5)_4$ resulting in volatile methylethylmercury which was purged with N_2 from solution onto a carbotrap™. MeHg was thermally desorbed from the trap and volatile ethyl-analogs were separated by isothermal (100°C) gas chromatography (GC) followed by CVAFS (Tekran Model-2500) with argon as carrier gas. The detection limit was 0.001 μg/g. Concentrations are expressed as μg/g wet weight (ww) unless otherwise noted.

3.36 Quality Control

All trace element analyses were performed under a thorough quality control program (Table 3.2). Reference materials (DOLT-2, DOLT-3 and DORM-2) were obtained from the National Research Council, Canada (NRC) and BLS 1577b from National Institute for Standards and Technology (NIST). Marine mammal reference material (liver of pilot and beluga whale) was provided by NIST as part of annual interlaboratory comparison exercises for the determination of trace elements in marine mammals (Wise et al., 1993, Christopher, 2002, Christopher, 2004). Spikes and duplicate samples as well as method and instrument blanks were run routinely (with each group of 20 samples) during analysis.

3.37 Trace Element Ratios

The ratio or relative occurrence of organic Hg (MeHg) to total Hg (THg) is referred to as %MeHg in the text and was calculated (for belugas only) as:

$$\%MeHg = (MeHg\ \mu g/g\ ww\ /\ THg\ \mu g/g\ ww) * 100$$

The molar ratio of Se to THg was calculated for all three species as:

$$Se{:}THg\ molar\ ratio = (Se\ \mu g/g\ ww\ /\ THg\ \mu g/g\ ww) * (200.59\ g/mole\ /\ 78.96\ g/mole)$$

where 200.59 g/mole and 78.96 g/mole are the atomic weights of Hg and Se, respectively.

3.38 Statistical Analysis

The variables in the data set (body length, $\delta^{15}N$, $\delta^{13}C$, Ag, Cd, Cu, Se, Zn, THg, MeHg, %MeHg, and Se:THg molar ratio) were ranked prior to analysis to reduce the risk of violations of normality and homogeneity of variance assumptions. Two-way ANOVA (with interaction term – species * sex) followed by Tukey's multiple comparison test was applied to compare variable means between cetacean species and sex. Sample sizes did not allow for a comparison between localities. A residual analysis was implemented to determine possible violations of assumptions. Spearman rank correlation was calculated within a species to determine correlations between the variables. LOESS smoothing followed by nonlinear regression analysis was utilized on non-ranked raw data to estimate suitable functions between two variables and compare regression surfaces between whale species. Graphing and nonlinear regression analyses were conducted using Sigma-Plot (Version 7.0). All other statistical analyses were performed using SAS (Version 8) with $\alpha = 0.05$. In order to include element concentrations below the minimum detection limit (MDL) in summary statistics and statistical tests, they were expressed as one-half the MDL (Gilbert, 1987). If more than 50% of samples had element concentrations below the MDL they were highlighted in summary statistics and excluded from further statistical tests. Results are reported as mean +/- standard deviation (SD) unless otherwise noted. In addition, the sample median is reported as it is robust to outliers and is appropriate for censored data sets.

3.4 RESULTS

3.41 Stable Isotopes

Stable carbon and nitrogen isotope ratios were significantly different for the three cetacean species analyzed ($p = <0.0001$ for both $\delta^{13}C$ and $\delta^{15}N$). $\delta^{15}N$ was highest in belugas (16.8 ± 0.6‰), followed by bowheads (13.3 ± 0.6‰) then gray whales (12.0 ± 0.9‰). Carbon isotope values were more enriched in gray whales (-17.3 ± 1.0‰) than in bowheads (-20.7 ± 0.8‰) and belugas were intermediate (-18.4 ± 0.6‰). Averages, standard deviations, medians and ranges of stable carbon and nitrogen isotope ratios in whales are given in Table 3.3. No sex difference and no significant interaction term (species * sex) were noted for $\delta^{13}C$ and $\delta^{15}N$ in the three whale species.

Nitrogen and carbon isotope ratios in the mysticete prey examined were significantly different (p = <0.0001 for both $\delta^{13}C$ and $\delta^{15}N$). Total body homogenates of unsorted zooplankton (including copepods and euphausids) had higher $\delta^{15}N$ than homogenates of benthic gammaridean amphipods (10.4 ± 1.2‰ and 7.9 ± 0.8‰ for zooplankton and amphipods, respectively). In contrast, amphipods showed more enriched ^{13}C values than zooplankton (-24.9 ± 0.7‰ and –19.9 ± 0.7‰ for zooplankton and amphipods, respectively). Figure 3.2 illustrates $\delta^{13}C$ versus $\delta^{15}N$ in all cetacean groups analyzed and in bowhead and gray whale prey (pelagic zooplankton and benthic amphipods, respectively).

3.42 Trace Element Concentrations and Tissue Distribution

Table 3.3 summarizes mean, standard deviation, median, and concentration range of trace elements (Cu, Zn, Cd, Ag, Se, THg and MeHg) and element ratios (%MeHg and Se:THg molar ratio) in epidermis, muscle, liver and kidney of bowheads, belugas and gray whales. For Ag in epidermis and muscle and Cd in epidermis, more than 50% of the samples were below the MDL in the three whale species analyzed. Ag in kidney was below MDL in more than 50% of bowheads and gray whales only.

Generally, concentrations of trace elements were highest in liver, followed by kidney and lowest in muscle and epidermis. However, renal concentrations of Cd exceeded levels in liver in all three species. Epidermal Se was higher in gray whales and belugas than concentrations in muscle and kidney, while Se in bowhead kidney showed the highest concentrations and muscle the lowest. The Se:THg molar ratio was highest in epidermis for all species analyzed. Zn was higher in bowhead muscle than in any other tissue, while belugas had the highest Zn concentration in the epidermis. MeHg was only analyzed in beluga tissues and accounted for approximately 100% of the THg measured in muscle and epidermis, while kidney and liver had less than 15% MeHg.

3.43 Species Comparison

All variables in all tissues differed significantly among species analyzed, except for Zn in liver (Table 3.4). Zn in kidney and epidermis and hepatic Ag were higher in belugas than in bowheads and gray whales. Se and THg were also highest in beluga tissues compared to bowhead and gray whale tissues, while the Se:THg ratio was lowest in belugas. Cd concentrations were higher in bowheads and belugas than in gray whales, and Cu was highest in gray whale and beluga tissues and was lowest in bowheads. Results of the ANOVA and Tukey's multiple comparison tests for all variables and tissues are compiled in Table 3.4. No sex differences were detected for variables analyzed in this study, and no significant interaction between whale species and sex was noted.

3.44 Correlation Between Variables

Significant Spearman rank correlations were found between many variables within and among tissues (Table 3.5) for all whale species. Positive correlations of renal and hepatic Cd with length, hepatic THg with length, and Se and THg in kidney were noted in all species. Hepatic Cd was positively correlated to Se and THg in liver in bowheads, belugas, and gray whales. THg in liver and renal Se were negatively correlated with trophic level (based on $\delta^{15}N$) in mysticetes only. Other significant correlations consistent for bowheads and gray whales included Cu with Ag in liver and hepatic Ag with THg. Hepatic Se was correlated to hepatic THg in belugas and bowheads, but not in gray whales. Similarly, Cu in liver was negatively correlated with length in bowheads and belugas, but was not correlated in gray whales. Hepatic %MeHg was negatively correlated to length in belugas, while THg and MeHg were positively correlated to length in all beluga tissues. Significant correlations that were noted in all species are underlined in Table 3.5, and correlations consistent among mysticetes were highlighted in bold script.

3.5 DISCUSSION

3.51 Stable Isotopes

Beluga whales occupy a higher trophic level (based on $\delta^{15}N$) than both mysticete species analyzed. Seaman et al. (1982) suggested competition for prey between belugas and piscivorous spotted seals (*Phoca largha*). However, nitrogen isotope ratios were lower in belugas than reported for spotted seals, indicating that this odontocete does not eat fish exclusively (Dehn, in prep.). Though a variety of fish species are clearly important to the beluga diet, cephalopods and shrimp are commonly eaten and 90-100% of stomachs analyzed by Seaman et al. (1982) contained invertebrates. $\delta^{15}N$ in beluga muscle analyzed in this study was similar to values reported for narwhal in Greenland

(16.3 ± 1.0‰) and belugas from the St. Lawrence estuary (15.1‰ to 16.3‰ range), suggesting similar prey utilization by these whales (Lesage et al., 2001, Dietz et al., 2004). Narwhals rely on deep benthic prey, with squid, octopus, and fish making up the majority of the diet, while crustaceans were present in about 60% of stomachs (Finley and Gibb, 1982).

Stable nitrogen isotope ratios suggest that bowheads are foraging on a higher trophic level than gray whales, thus pointing to differences in prey consumed. Typical bowhead prey (e.g., euphausids and copepods) had higher $\delta^{15}N$ than benthic gammaridean amphipods that make up the majority of the gray whale diet (Figure 3.2). This indicates a stepwise trophic enrichment of 3.0‰ for $\delta^{15}N$ in whale muscle, corresponding to enrichment factors reported by Hobson et al. (1996). Stable nitrogen isotope ratios of unsorted amphipods and zooplankton in this study are in agreement with values reported for gray whale prey (7.0 ± 0.4‰ and 8.3 ± 0.3‰ for *Ampelisca eschrichti* and *Ampelisca macrocephala*, respectively) and bowhead dietary items (9.6 ± 1.6‰ and 10.1 ± 0.7‰ for euphausids and copepods, respectively) (Highsmith and Coyle, 1991). Minke whale nitrogen isotope ratios in baleen are generally similar to those found in bowhead muscle (12‰ - 14‰ and 12‰ – 15‰ for minke and bowhead whales, respectively) with baleen and muscle tissue showing comparable isotope ratios (Hobson and Schell, 1998, Born et al., 2003, Hobson et al., 2004). However, in contrast to bowheads, minke whales are known to consume capelin (*Mallotus villosus*) and herring (*Clupea harengus*) and may switch to krill only if fish is not available (Sigurjónsson et al., 2000, Haug et al., 2002).

Carbon-13 is significantly enriched in gray whales as compared to bowhead and beluga whales (Figure 3.2). Stagnant boundary layers and low turbulence, as found in benthic ecosystems, will lead to enrichment of ^{13}C (France, 1995), thus explaining the enriched carbon isotope values in benthic-feeding gray whales. In contrast, bowheads feed mostly in the water column on euphausids and copepods (Lowry, 1993), displaying the more depleted carbon isotope signatures of the pelagic foodweb. Two bowhead whale fetuses exhibited highly depleted carbon isotope signatures (highlighted in Figure 3.2). Selective fractionation of carbon isotopes leads to depleted ^{13}C in body fat compared to other tissues (DeNiro and Epstein, 1977), and the low concentrations in fetuses suggest mobilization and transfer of maternal carbon to fetal development.

Gray whales are enriched in ^{13}C relative to their prey by 2.6 ‰, while bowheads show a shift of 4.2‰. However, carbon isotope signatures are significantly different between Arctic regions, and bowhead whales migrating from the Beaufort Sea in fall are more depleted in ^{13}C than whales migrating from the Bering Sea in spring (Schell et al., 1989, Hoekstra et al., 2002). Bowheads analyzed in this study were taken during both spring and fall harvests; therefore, the sample includes animals with both carbon signatures. Zooplankton was obtained from the Beaufort Sea and can be expected to have more depleted carbon isotope values compared to invertebrates from continental shelf waters of the Bering and Chukchi seas (Schell et al., 1998). Thus, a shift of $\delta^{13}C$ from zooplankton to bowheads of about 3‰ is more plausible and coincides with enrichment factors noticed for gray whales.

Belugas have intermediate $\delta^{13}C$ values between bowhead and gray whales, suggesting that both pelagic and benthic foods are important components of their diet. As discussed, narwhals rely heavily on epibenthic prey, but Arctic cod is also of importance (Finley and Gibb, 1982). Similarly, saffron cod (*Eleginus gracilis*), shrimp, and octopus dominate the beluga diet (Seaman et al., 1982). The majority of beluga stomachs analyzed by Seaman et al. (1982) contained sediment and pebbles, and about 27% of narwhal stomachs contained sand (Finley and Gibb, 1982). Carbon isotope signatures of belugas in this study and narwhal (Dietz et al., 2004) were almost indistinguishable (–18.5 ± 0.4‰ and –18.4 ± 0.6‰ for narwhal and beluga, respectively), further supporting similar prey utilization by these two odontocetes.

3.52 Trace Elements

3.521 Mercury and Selenium

The beluga occupies a higher trophic level compared to the mysticetes analyzed in this study, and, as expected, concentrations of Hg in all beluga tissues are up to two orders of magnitude higher. Hepatic Hg in this work falls within the range reported for Alaska belugas by Becker et al. (1995) and Woshner et al. (2001a), but is higher than for belugas analyzed from the Cook Inlet stock (Becker et al., 2000). Hepatic Hg concentrations of belugas harvested in Alaska are intermediate to values reported by Wagemann et al. (1996) for whales from Western and Eastern Canada (11.3 ± 7.1 μg/g ww and 19.2 ± 32.7 μg/g ww, respectively, in liver), while belugas from the St. Lawrence Estuary were an order of magnitude higher (Wagemann et al., 1990). Thus, concentrations of Hg in tissues ranges widely in these whales and may be interpreted as differences in feeding habits and feeding grounds due to geographically separate stocks or populations (Becker et al., 1995, Kunito et al., 2002, Born et al., 2003). THg and MeHg in this study were positively correlated to length (as a proxy for age), and heterogeneity in age structure of the sampled beluga stocks may also explain the large variations in Hg concentrations reported for this species. However, concentrations of THg in belugas in this study are low compared to other odontocetes, e.g., rough-toothed dolphins (*Steno bredanensis*), pilot whales (*Globicephala melas*), and striped dolphins (*Stenella coeruleoalba*) (Honda et al., 1983, Becker et al., 1995, Caurant et al., 1996, Mackey et al., 2003).

THg in tissues of bowhead and gray whales is very low in comparison to other mysticetes, e.g., minke whales (Hansen et al., 1990, Born et al., 2003). The overall higher concentrations of Hg in minke whale tissues in contrast to bowhead and gray whales are likely due to the higher prevalence of fish in the minke whale diet (Haug et al., 2002). Fish consumption has been correlated to elevated Hg levels in a variety of studies (Dietz et al., 1996, Wagemann et al., 1997, Egeland et al., 1998), and the low concentrations of THg in bowhead and gray whales are in agreement with the invertebrate-dominated diet of these mysticetes. THg in tissues of bowheads is comparable to levels reported by Bratton et al. (1997) and Woshner et al. (2001a). Hg concentrations in gray whales analyzed in this study are among the lowest reported for marine mammals and MeHg analyzed in four stranded gray whales accounted for 22%, 18% and 75% of the THg burden in liver, kidney and muscle, respectively (Ruelas-Inzunza et al., 2003). Tissue levels of THg in whales stranded along the Pacific West coast of North America were lower (0.06 ± 0.01 μg/g ww for gray whale liver) (Varanasi et al., 1994) than for juvenile gray whales sampled during subsistence harvests in Russia (0.16 ± 0.06 μg/g ww for liver) (Tilbury et al., 2002) and whales examined in this study.

THg is positively correlated to length (as a proxy for age) in liver of all three species analyzed in this study ($r = 0.43$, 0.57 and 0.55 for bowhead, beluga and gray whale, respectively). In addition, MeHg was positively correlated to length in all beluga tissues ($r = 0.45$, 0.38, 0.60 and 0.53 for liver, kidney, muscle and epidermis, respectively). This likely results from the continuous uptake of Hg / MeHg via diet, slow elimination, or storage (e.g., tiemannite), and thus a relatively long half-life of THg of about 10 years as discussed by Wagemann et al. (2000). However, studies on captive bottlenose dolphins (*Tursiops truncatus*) inferred that only about 50% of ingested Hg (administered as dietary fish) is retained and the remainder is eliminated via bilary excretion in the feces, while pulmonary elimination is negligible (Nigro et al., 2002). A half-life of less than 1000 days for Hg in whale tissues was postulated (Nigro et al., 2002). Wagemann et al. (1996) suggested that the epidermis of cetaceans might be a significant route of elimination of Hg compounds. For belugas in this study, about 100% of THg in epidermis was present as organic Hg. The highest Hg concentration is found in the outer epidermal layer, and during skin molt approximately 14% of epidermal MeHg can be eliminated (Wagemann et al., 1996). The fraction of MeHg (%MeHg) in beluga liver was inversely correlated to length ($r = -0.46$). A similar decay function of hepatic

%MeHg with age was also described for other marine mammals (Becker et al., 1995, 2000, Dehn et al., 2005).

Storage of Hg as biologically inert tiemannite (Hg-Se granules) requires Se and thus leads to the often discussed protective effect of Se on Hg toxicosis. This is supported by a strong correlation between THg and Se in liver of beluga and bowhead whales ($r = 0.72$ and 0.58 for beluga and bowhead, respectively). This relationship is commonly observed in marine mammal tissues (Koeman et al, 1973, Mackey et al., 1996, 2003, Das et al., 2004a), although it was not noted for gray whales in this study ($r = 0.31$, $p = 0.11$). Several authors report that both elements occur in a 1:1 ratio when expressed as molar concentrations (Koeman et al., 1973, Caurant et al., 1994, Becker et al., 1995, Dietz et al., 2000, Endo et al., 2002). Unity between Se and Hg on a molar basis was only observed in one adult beluga whale, and other cetaceans in this study had ratios in liver of approximately 10:1 in belugas or 100:1 (Se:THg) for mysticetes (Figure 3.3). Se is an essential element and is incorporated into selenoproteins involved in hormone homeostasis, reproduction, and anti-oxidant enzyme systems, e.g., glutathione peroxidase (Bedwal and Bahuguna, 1994, Bates et al., 2000, Whanger, 2001). A molar ratio of 1:1 would indicate that all available Se is bound to Hg, leaving animals, in particular diving marine mammals, vulnerable to oxidative stress. Becker et al. (1995) and Ikemoto et al. (2004) argued that not only Hg binds Se, but also Ag may compete for binding sites on Se in belugas and other marine mammals, thus making the assumed unity of Hg:Se even more improbable. In addition, Figure 3.4 shows that the Se:THg molar ratio is negatively correlated to body length in all three species, and a similar decay function of Se:THg with age was also noted for ice seals (Dehn et al., 2005). This illustrates the importance of Se for maturation of the antioxidant system and endocrine, reproductive, and neuronal development (Bedwal and Bahuguna, 1994, Hagmar et al., 1998, Whanger, 2001). Thus, tissue ratios of Se:THg close to 1:1 could suggest compromised health of the animals (Dietz et al., 2000, Dehn et al., 2005). Wagemann et al. (2000) postulated that only marine mammals with exceedingly high concentrations of Hg show a Se:THg ratio approaching unity. Se in liver of stranded gray whales was higher than for subsistence-harvested gray whales examined here (Varanasi et al., 1994). Hepatic Se concentrations have been reported to be elevated in stranded and emaciated animals (Bennet et al., 2001, Das et al., 2004a). Ketone body metabolism requires Se, and it is possible that during starvation Se is elevated to increase turnover of lipids and ketone bodies (Olsson, 1985).

Epidermal Se in this study was highest in belugas and lowest in bowhead whales. Elevated concentrations of Se were also reported in epidermis of harbor porpoise from Greenland and belugas and narwhals from the Canadian Arctic (Dietz et al., 1990, Paludan-Müller et al., 1993, Wagemann et al., 1996). Se in epidermis does not seem to be associated with Hg as tiemannite, as most of the Hg in epidermis is present in the organic form. Paludan-Müller et al. (1993) considered a possible storage mechanism or excretion of Se via skin molt as described for Hg. Leccia et al. (1993) and Burke et al. (2003) suggested that Se in the form of glutathione peroxidase protects against ultraviolet (UV) induced skin damage and carcinogenesis caused by generation of reactive oxygen species. As belugas lose their skin pigmentation with adulthood at approximately 6 years of age (Sergeant, 1973), it is feasible that this species will need more UV protection than the dark pigmented bowhead whale. Se in epidermis of belugas analyzed in this study was higher than reported by Wagemann et al. (1996) for belugas and narwhals from the Canadian Arctic. However, epidermal Se concentrations in harbor porpoise exceeded levels established for belugas by an order of magnitude (Dietz et al., 1990, Paludan-Müller et al., 1993).

3.522 Cadmium

Although bowheads feed low in the food chain, renal and hepatic Cd concentrations of bowheads were similar to or even higher than those of belugas that occupy a higher trophic level. Renal and hepatic Cd in gray whales was considerably lower than in both bowheads and belugas. Most invertebrates have higher Cd levels than fish (Bohn and McElroy, 1976, Bustamante et al., 2003), and cephalopods, in particular, display elevated Cd concentrations (Martin and Flegal, 1975, Bustamante et al., 1998a, 1998b). High variability in Cd levels has been described for different species of crustaceans that make up typical mysticete prey. Elevated levels of Cd were reported for pelagic amphipods (*Parathemisto libellula*) and copepods, ranging from 3 μg/g dry weight (dw) in copepods to 11 μg/g dw in amphipods (Bohn and McElroy, 1976, Hamanaka and Ogi, 1984, Macdonald and Sprague, 1988). However, benthic amphipods have very low concentrations ranging from 0.2 to 1.3 μg/g ww in the Alaskan Beaufort Sea (Presley, 1997). Similarly, concentrations of Cd in mysids and euphausids are up to an order of magnitude lower than in *Parathemisto* and copepods (Hamanaka and Ogi, 1984, Macdonald and Sprague, 1988), and Cd concentrations in euphausids from

Greenland are below the limit of detection (Dietz et al, 1996). However, the overall lower concentrations of Cd in gray whale prey would not account for the 1 to 2 orders of magnitude difference in renal Cd levels of the two mysticetes. According to daily consumption estimates based on body mass and metabolic rate, adult bowheads consume between 1083 and 1453 kg of prey, while adult gray whales take in approximately 268 to 538 kg of amphipods (Tamura and Ohsumi, 2000, Thompson, 2002). In addition, gray whales feed mainly for about 5 months in their summering grounds, while there is still disagreement about seasonality of bowhead feeding (Rice and Wolman, 1971, Schell et al., 1989, Lowry, 1993, Hoekstra et al., 2002). Thus, differences in prey Cd concentrations and daily and seasonal intake of prey may account for the overall higher exposure to Cd in bowhead whales compared to gray whales. Renal Cd concentration in minke whales is intermediate to bowheads and gray whales, and the seasonal importance of fish to this species may explain much of this difference (Hansen et al., 1990, Haug et al., 2002).

The relatively high concentrations of Cd in kidneys of belugas indicate the importance of invertebrates and/or cephalopods to this species. Bustamante et al. (1998a) suggested that cephalopods, in particular benthic rather than neritic species, are a main vector for Cd in the food chain. Cd levels in narwhal tissues are higher than established for belugas in this study, ranging from 0.76 to 168 μg/g ww (Wagemann et al., 1996). This may indicate higher prevalence of invertebrates and cephalopods in narwhals or regional differences in Cd concentrations as described by Wagemann et al. (1996). Belugas from the St. Lawrence estuary are considered highly polluted, though Cd levels in these whales are among the lowest compared to belugas from other Arctic regions (Wagemann et al., 1990). Similarly, renal Cd of the declining Cook Inlet beluga stock is markedly lower than for other Alaska belugas (Becker et al., 2000). Feeding habits of Cook Inlet belugas are strongly correlated with salmon runs and other seasonally abundant fish, while invertebrates seem uncommon in their diet (Huntington, 2000). Thus, limited abundance of invertebrate prey or dietary preference for fish may explain the low Cd concentrations in these isolated beluga stocks.

Renal Cd was positively correlated to length in all three species ($r = 0.83$, 0.40 and 0.84 for bowhead, beluga and gray whale, respectively). The correlation is sigmoid rather than linear, showing plateaus of Cd levels during the fetal and neonate stage and later in adult life (Figure 3.5). Cd concentrations are below detection limit in two bowhead fetuses (Figure 3.5), suggesting that Cd is not transferred from mother to fetus in this species. Some studies report that the placenta may serve as a selective Cd filter (Itoh et al., 1996, Enomoto and Hirunuma, 2001). Almost linear accumulation of Cd begins at birth via oral exposure (e.g., milk, prey). With increasing length (as a proxy for age) Cd increases to a maximum in midlife and plateaus such that Cd intake and excretion are balanced. Renal Cd concentrations at the point of curve saturation are 29.4, 10.6 and 4.7 μg/g ww in bowhead, beluga and gray whales, respectively (Figure 3.5).

Metallothionein is involved in intracellular binding of divalent elements (e.g., Cd, Cu, Zn) at the renal glomerulus, though Cd is reabsorbed at the proximal tubules along with the biologically similar essential elements. The biological half-life for Cd in humans is estimated at 20-40 years as Cd is continuously accumulated until tubule cells and associated Cd are shed in the urine (Gerhardsson and Skerfving, 1996). Changes in renal physiology associated with the aging process (e.g., apoptosis, glomerulosclerosis) may lead to an increased excretion of Cd with shed cells or decreased absorption efficiency due to impaired peritubular blood flow after a critical age (Khan et al., 1999, Cardani and Zavanella, 2000). Thus, increase of Cd levels to a maximum or even decreases in renal Cd concentrations after a critical age are plausible and have been described for other marine mammals (Dietz et al., 1998, Watanabe et al., 2002, Dehn et al., 2005).

3.523 Silver

The high concentrations of Ag in beluga liver have long been a mystery and several authors have discussed possible causes for Ag accumulation (Becker et al. 1995, 2000, Woshner et al. 2001a). Cephalopods have been associated with elevated concentrations of Ag (Martin and Flegal, 1975). Benthic snow crabs (*Chionoecetes opilio*) retained 90% of an administered Ag dose and the biological half-life was above 1000 days, while flatfish retained only 16% and rate of Ag elimination was 10 to 100 times faster (Rouleau et al., 2000). Thus, much higher concentrations of Ag can be reached in benthic invertebrates. As discussed above, belugas rely heavily on cephalopods; however, hepatic Ag concentrations were an order of magnitude higher in belugas than in some pinnipeds (e.g., northern fur seal (*Callorhinus ursinus*), bearded seal (*Erignathus barbatus*)) and cetaceans (e.g., pilot whale) that also rely heavily on octopus and squid (Mackey et al., 1996, Becker et al., 2000, Saeki et al., 2001, Dehn et al., 2005). Thus, the concentrations of Ag found in belugas could reflect a unique dietary source, as has been suggested by Becker et al. (1995), or feeding location, e.g., vicinity to volcanic or hydrothermal activity (Hein et al., 1999). It is also conceivable that belugas have a predilection for Ag accumulation or this trace metal serves an unknown nutritional need. Currently, there is no data available on Ag concentrations in tissues of narwhals to determine if Ag accumulation is a peculiarity in the Family Monodontidae.

Hepatic Ag concentrations of pelagic bowheads and benthic-feeding gray whales were 2-3 orders of magnitude lower than concentrations in beluga whales, suggesting that mysticete prey, e.g., zooplankton and benthic amphipods, are not retaining Ag to the extent that large crustaceans and cephalopods do. Copepods have low accumulation potential (~17% of Ag is retained), and zooplankton molt their chitinous carapace so that Ag adsorbed to the exoskeleton is shed, hence not bioaccumulated (Ratte, 1999).

Ag in liver was not correlated to body length in gray whales or belugas ($r = -0.08$ and -0.29 for gray whales and beluga, respectively), but was negatively correlated in bowheads ($r = -0.37$). Juvenile and subadult bowhead whales have higher hepatic Ag levels than adults (Figure 3.6). Hazard and Lowry (1984) reported benthic prey in a juvenile bowhead and considered that the baleen plates of young bowhead whales are not long enough to filter plankton efficiently from the water column. Hence, differences in feeding ecology could explain higher concentrations of hepatic Ag in juvenile bowheads. However, Ag is also elevated in juvenile gray whales and belugas (Figure 3.6). Ag and Cu are commonly intercorrelated, and Saeki et al. (2001) indicated that Ag interferes with Cu metabolism and transport. Bremner and Beattie (1990) postulated that Cu accumulates in fetal tissues due to limited efficiency of bilary excretion mechanisms. Thus, co-accumulation of Cu and Ag in liver of juvenile whales or limited bilary excretion may account for elevated concentrations of Ag.

3.524 Copper and Zinc

Homeostasis of the essential trace metals Cu and Zn is tightly regulated. Both metals are excreted from the liver with bile or pancreatic secretions. Excretion via kidneys is limited, though Zn may be removed in urine following muscle catabolism mediated by interleukin-1 (Cousins, 1985). Cu and Zn are required for bone formation and are part of the antioxidant enzyme system superoxide dismutase. In addition, Zn contributes to tissue growth, wound-healing, and immune function and protects against UV-radiation in the epidermis (Rostan et al., 2002). Thus, Zn is found in its highest concentration in the epidermis of beluga whales, and is lower in bowhead and gray whales.

Concentrations of renal and hepatic Zn of stranded gray whales (Varanasi et al., 1994) were higher than levels of subsistence-harvested animals analyzed in this study. Zn is an inhibitor of gluconeogenesis, its absorption increases during malnutrition, and it competes with Cu for receptor binding sites (Cousins, 1985, Das et al., 2004b). In addition, high dietary Zn decreases Cu absorption (Cousins, 1985). Cu secretion from the liver is increased and its absorption is negatively influenced by stress (e. g., glucocorticoids), while elevated Zn concentrations and altered Zn kinetics are a response to stressors, poor body condition, and infection (Cousins, 1985, 1986, Frank et al., 1992, Bennet et al., 2001, Das et al., 2004b, Ilbäck et al., 2004). Thus, tissue concentrations of Zn could provide a possible indication of immune status and health in cetaceans.

Cu in liver is inversely correlated to length in bowheads and beluga, but not gray whales, such that fetal and juvenile tissues have the highest hepatic concentrations (Figure 3.7). The negative correlation of Cu with age has been noticed by a variety of studies (Honda et al., 1983, Wagemann et al. 1988, 1990, Caurant et al., 1994, Woshner et al., 2001a). Liver metallothionein is increased during fetal growth, and concentrations of Cu in a Dall's porpoise (*Phocoenoides dalli*) fetus exceeded levels found in maternal tissues by 6 times (Yang et al., 2004). Thus, tissue growth, development, and DNA synthesis may require increased concentrations of Cu and Zn. However, limited efficiency of bilary excretion mechanisms in fetus and subadult animals, as discussed above, may lead to accumulation of Cu in juveniles (Bremner and Beattie, 1990).

3.6 SUMMARY AND CONCLUSION

Stable nitrogen isotope ratios indicate that belugas occupied the highest trophic level. $\delta^{15}N$ values also established that bowheads forage on a higher trophic level than gray whales, thus pointing to differences in prey species consumed. Typical bowhead prey had higher nitrogen isotope ratios than benthic amphipods that make up the majority of the gray whale diet. Carbon isotope values were enriched in gray whales and are likely due to their benthic feeding habits. Bowheads feed mostly in the water column, displaying depleted ^{13}C values of the pelagic foodweb. Belugas take an intermediate position, suggesting that both pelagic and benthic prey are important components. Cd concentrations in whale tissues seem to be associated with invertebrate prey and are indicative of Cd levels in prey species consumed and ingested biomass. The relationship between Cd and length was sigmoid in all three species. The molar ratio of Se to THg was inversely correlated to length. The observed ratios exceeded the classic 1:1 ratio by one or two orders of magnitude, and Se:THg ratios close to unity may indicate compromised health. High concentrations of Ag in liver of belugas were noticed in this study; mysticetes had much lower Ag concentrations. Juvenile and subadult whales of all species possessed higher hepatic Ag than adults, mirroring accumulation patterns observed for hepatic Cu. Se and Zn occurred in high concentrations in cetacean epidermis. Both elements are likely involved in protection against reactive oxygen species and UV radiation. Subsistence-harvested gray whales had strikingly lower concentrations of Zn in liver and kidney than stranded gray whales. Thus, Zn status may be useful in the evaluation of body condition, immune status, and animal health.

3.7 ACKNOWLEDGMENTS

This study would not have been possible without the samples provided by Alaskan and Russian subsistence hunters and whaling captains in the communities of Barrow, Kaktovik, Wainwright, Point Lay, Point Hope, Savoonga, Lorino and Lavrentiya, and we thank them all for their support. We greatly appreciate the assistance of H. Brower, Jr., C. D. N. Brower, B. Akootchook, T. Hepa, C. George, R. Suydam, G. Zelensky, G. Sheffield, V. Woshner, G. York and many others in the field and N. Haubenstock, T. Howe, T. Bentzen and P. Hoekstra for support with analysis. We also thank P. Krahn and G. Ylitalo for making additional gray whale samples available and R. Highsmith and B. Bluhm for collection of amphipod samples from the Bering Strait. P. Becker and S. Christopher provided marine mammal reference material and coordinated interlaboratory comparison exercises for the determination of trace elements in marine mammals. This study was primarily funded by the Cooperative Institute for Arctic Research (CIFAR). Additional support was provided by the Experimental Program for Stimulation of Competitive Research (EPSCoR); the Biomedical Research Infrastructure Network (BRIN); the North Slope Borough Department of Wildlife Management; the Institute of Arctic Biology and the Department of Biology and Wildlife, UAF.

3.8 REFERENCES

Ballard, W. B., Cronin, M. A., Robards, M. D. & Stubblefield, W. A. 2003. Heavy metal concentrations in Arctic foxes, *Alopex lagopus*, in the Prudhoe Bay oil field, Alaska. Canadian Field-Naturalist 117, 119-121.

Bard, H. M. 1999. Global transport of anthropogenic contaminants and the consequences for the Arctic marine ecosystem. Marine Pollution Bulletin 38, 356-379.

Bates, J. M., Spate, V. L., Morris, J. S., Germain, D. L. S. & Galton, V. A. 2000. Effects of selenium deficiency on tissue selenium content, deiodinase activity, and thyroid hormone economy in the rat during development. Endocrinology 141, 2490-2500.

Becker, P. R., Mackey, E. A., Demiralp, R., Suydam, R., Early, G., Koster, B. J. & Wise, S. A. 1995. Relationship of silver with selenium and mercury in the liver of two species of toothed whales (Odontocetes). Marine Pollution Bulletin 30, 262-271.

Becker, P. R., Porter, B. J., Mackey, E. A., Schantz, M. M., Demiralp, R., & Wise, S. A. 1999. National Marine Mammal Tissue Bank and Quality Assurance Program: protocols, inventory, and analytical results. U. S. Department of Commerce, Gaithersburg.

Becker, P. R., Krahn, M. M., Mackey, E. A., Demiralp, R., Schantz, M. M., Epstein, M. S., Donais, M. K., Porter, B. J., Muir, D. C. G. & Wise, S. A. 2000. Concentrations and polychlorinated biphenyls (PCB's), chlorinated pesticides, and heavy metals and other elements in tissues of belugas, *Delphinapterus leucas*, from Cook Inlet, Alaska. Marine Fisheries Review 62, 81-98.

Bedwal, R. S. and Bahuguna, A. 1994. Zinc, copper and selenium in reproduction. Experientia 50, 626-640.

Bennet, P. M., Jepson, P. D., Law, R. J., Jones, B. R., Kuiken, Y., Baker, J. R., Rogan, E. & Kirkwood, J. K. 2001. Exposure to heavy metals and infectious disease mortality in harbour porpoises from England and Wales. Environmental Pollution 112, 33-40.

Bloom, N. S. and Crecelius, E. A. 1983. Determination of mercury in seawater at sub-nanogram per liter levels. Marine Chemistry 14, 49-59.

Bloom, N. 1989. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapor atomic fluorescence detection. Canadian Journal of Fisheries and Aquatic Science 46, 1131-1140.

Bogoslovskaya, L. S., Vorogov, L. M. & Semenova, T. N. 1981. Feeding habits of the gray whale off Chukotka. Report to the Scientific Committee of the International Whaling Commission 31, 507-510.

Bohn, A. and McElroy, R. O. 1976. Trace metals (As, Cd, Cu, Fe and Zn) in Arctic cod, *Boreogadus saida*, and selected zooplankton from Strathcona Sound, northern Baffin Island. Journal of the Fisheries Research Board of Canada 33, 2836-2840.

Born, E. W., Outridge, P., Riget, F. F., Hobson, K. A., Dietz, R., Øien, N. & Haug, T. 2003. Population substructure of North Atlantic minke whales (*Balaenoptera acutorostrata*) inferred from regional variation of elemental and stable isotopic signatures in tissues. Journal of Marine Systems 43, 1-17.

Bratton, G. R., Flory, W., Spainhour, C. B. & Haubold, E. M. 1997. Assessment of selected heavy metals in liver, kidney, muscle, blubber, and visceral fat of Eskimo harvested bowhead whales *Balaena mysticetus* from Alaska's North Coast. Department of Wildlife Management, North Slope Borough, Barrow, Alaska.

Bremner, I. and Beattie, J. H. 1990. Metallothionein and the trace minerals. Annual Review of Nutrition 10, 63-83.

Brousseau, P., De Guise, S., Voccia, I., Ruby, S. & Fournier, M. 2003. Immune status of St. Lawrence estuary beluga whales. *In* Vos, J. G., Bossart, G. D., Fournier, M., O'Shea, T. J. Toxicology of Marine Mammals. Taylor & Francis, London.

Burke, K. E., Clive, J., Combs, G. F. & Nakamura, R. M. 2003. Effects of topical L-selenomethionine with topical and oral vitamin E on pigmentation and skin cancer induced by ultraviolet irradiation in Skh:2 hairless mice. Journal of the American Academy of Dermatology 49, 458-472.

Burton, R. K. and Koch, P. L. 1999. Isotopic tracking of foraging and long-distance migration in northeastern Pacific pinnipeds. Oecologia 119, 578-585.

Bustamante, P., Caurant, F., Fowler, S. W. & Miramand, P. 1998a. Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. The Science of the Total Environment 220, 71-80.

Bustamante, P., Cherel, Y., Caurant, F. & Miramand, P. 1998b. Cadmium, copper and zinc in octopuses from Kerguelen Islands, Southern Indian Ocean. Polar Biology 19, 264-271.

Bustamante, P., Bocher, P., Cherel, Y., Miramand, P. & Caurant, F. 2003. Distribution of trace elements in the tissues of benthic and pelagic fish from the Kerguelen Islands. The Science of the Total Environment 313, 25-39.

Cardani, R. and Zavanella, T. 2000. Age-related cell proliferation and apoptosis in the kidney of male Fischer 344 rats with observations on a spontaneous tubular cell adenoma. Toxicologic Pathology 28, 802-806.

Caurant, F., Amiard, J. C., Amiard-Triquet, C. & Sauriau, P. G. 1994. Ecological and biological factors controlling the concentrations of trace elements (As, Cd, Cu, Hg, Se, Zn) in delphinids *Globicephala melas* from the North Atlantic Ocean. Marine Ecology Progress Series 103, 207-219.

Caurant, F., Navarro, M. & Amiard, J.-C. 1996. Mercury in pilot whales: possible limits to the detoxification process. The Science of the Total Environment 186, 95-104.

Cousins, R. J. 1985. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiological Reviews 65, 238-309.

Cousins, R. J. 1986. Toward a molecular understanding of zinc metabolism. Clinical Physiology and Biochemistry 4, 20-30.

Christopher, S. J. 2002. NIST / NOAA National marine analytical quality assurance program. Description and results of the 2001 interlaboratory comparison exercise for the determination of trace elements in marine mammals. NIST.

Christopher, S. J. 2004. NIST / NOAA National marine analytical quality assurance program. Description and results of the 2003 interlaboratory comparison exercise for the determination of trace elements in marine mammals. NIST.

Das, K., Holsbeek, L., Browning, J., Siebert, U., Birkun, A. & Bouquegneau, J.-M. 2004a. Trace metal and stable isotope measurements ($\delta^{13}C$ and $\delta^{15}N$) in the harbour porpoise *Phocoena phocoena relicta* from the Black Sea. Environmental Pollution 131, 197-204.

Das, K., Siebert, U., Fontaine, M., Jauniaux, T., Holsbeek, L. & Bouquegneau, J.-M. 2004b. Ecological and pathological factors related to trace metal concentrations in harbour porpoises *Phocoena phocoena* from the North Sea and adjacent areas. Marine Ecology Progress Series 281, 283-295.

Dehn, L.-A., Sheffield, G. G., Follmann, E. H., Duffy, L. K., Thomas, D. L., Bratton, G. R., Taylor, R. J. & O'Hara, T. M. (2005). Trace elements in tissues of phocid seals harvested in the Alaskan and Canadian Arctic – Influence of age and feeding ecology. Canadian Journal of Zoology 83, 726-746.

Dehn, L.-A. In preparation. Trophic relationships in an Arctic marine foodweb and implications for trace element dynamics. Ph.D. thesis, Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, AK.

De Luna, C. J. and Rosales-Hoz, L. 2004. Heavy metals in tissues of gray whales *Eschrichtius robustus*, and in sediments of Ojo de Liebre Lagoon in Mexico. Bulletin of Environmental Contamination and Toxicology 72, 460-466.

DeNiro, M. J. and Epstein, S. 1977. Mechanisms of carbon isotope fractionation associated with lipid synthesis. Science 197, 261-263.

Dietz, R., Nielsen, C. O., Hansen, M. M. & Hansen, C. T. 1990. Organic mercury in Greenland birds and mammals. The Science of the Total Environment 95, 41-51.

Dietz, R., Nørgaard, J. & Hansen, J. C. 1998. Have arctic marine mammals adapted to high cadmium levels? Marine Pollution Bulletin 36, 490-492.

Dietz, R., Riget, F., Cleemann, M., Aarkrog, A., Johansen, P. & Hansen, J. C. 2000. Comparison of contaminants from different trophic levels and ecosystems. The Science of the Total Environment 245, 221-231.

Dietz, R., Riget, F., Hobson, K. A., Heide-Jørgensen, M. P., Møller, P., Cleemann, M., de Boer, J. & Glasius, M. 2004. Regional and inter annual patterns of heavy metals, organochlorines and stable isotopes in narwhals (*Monodon monoceros*) from West Greenland. The Science of the Total Environment 331, 83-105.

Egeland, G. M., Feyk, L. A. & Middaugh, J. P. 1998. The use of traditional foods in a healthy diet in Alaska: risks in perspective. State of Alaska Epidemiology Bulletin 2, 1-140.

Endo, T., Haraguchi, K. & Sakata, M. 2002. Mercury and selenium concentrations in the internal organs of toothed whales and dolphins marketed for human consumption in Japan. The Science of the Total Environment 300, 15-22.

Enomoto, S. and Hirunuma, R. 2001. Fetoplacental transport of various trace elements in pregnant rat using the multitracer technique. RIKEN Review 35, 31-34.

Finley, K. J. and Gibb, E. J. 1982. Summer diet of the narwhal (*Monodon monoceros*) in Pond Inlet, northern Baffin Island. Canadian Journal of Zoology 60, 3353-3363.

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

Frank, A., Galgan, V., Roos, A., Olsson, M., Petersson, L. R. & Bignert, A. 1992. Metal concentrations in seals from Swedish waters. Ambio 21, 529-538.

Gauthier, J. M., Dubeau, H. & Rassart, E. 1998. Mercury-induced micronuclei in skin fibroblasts of beluga whales. Environmental Toxicology and Chemistry 17, 2487-2493.

Gauthier, J. M., Dubeau, H. & Rassart, E. 2003. Evaluation of genotoxic effects of environmental contaminants in cells of marine mammals, with particular emphasis on beluga whales. *In* Vos, J. G., Bossart, G. D., Fournier, M., O'Shea, T. J. Toxicology of Marine Mammals. Taylor & Francis, London.

George, J. C., Bada, J., Zeh, J., Scott, L., Brown, S. E., O'Hara, T. & Suydam, R. 1999. Age and growth estimates of bowhead whales (*Balaena mysticetus*) via aspartic acid racemization. Canadian Journal of Zoology 77, 571-580.

George, J. C., Zeh, J., Suydam, R. & Clark, C. 2004. Abundance and population trend (1978-2001) of western Arctic bowhead whales surveyed near Barrow, Alaska. Marine Mammal Science 20, 755-773.

Gerhardsson, L. and Skerfving, S. 1996. Concepts on biological markers and biomonitoring for metal toxicity. *In* Chang, L. W. Toxicology of Metals. CRC Lewis Publishers, Boca Raton.

Gilbert, R. O. 1987. Statistical methods for environmental pollution monitoring. John Wiley and Sons, Inc., New York.

Hagmar, L., Persson-Moschos, M., Akesson, B. & Schütz, A. 1998. Plasma levels of selenium, selenoprotein P and glutathione peroxidase and their correlations to fish intake and serum levels of thyrotropin and thyroid hormones: A study on Latvian fish consumers. European Journal of Clinical Nutrition 52, 796-800.

Hansen, C. T., Nielsen, C. O., Dietz, R. & Hansen, M. M. 1990. Zinc, cadmium, mercury and selenium in minke whales, belugas and narwhals from West Greenland. Polar Biology 10, 529-539.

Haug, T., Lindstrøm, U. & Nilssen, K. T. 2002. Variations in minke whale (*Balaenoptera acutorostrata*) diet and body condition in response to ecosystem changes in the Barents Sea. Sarsia 87, 409-422.

Hazard, K. W. and Lowry, L. F. 1984. Benthic prey in a bowhead whale from the Northern Bering Sea. Arctic 37, 166-168.

Hein, J. R., Koski, R. A., Embley, R. W., Reid, J. & Chang, S.-W. 1999. Diffuse-flow hydrothermal field in an oceanic fracture zone setting, Northeast Pacific: Deposit composition. Exploration and Mining Geology 8, 299-322.

Highsmith, R. C. and Coyle, K. O. 1991. Amphipod life histories: community structure, impact of temperature on decoupled growth and maturation rates, productivity, and P:B ratios. American Zoologist 31, 861-873.

Hobson, K. A., Schell, D. M., Renouf, D. & Noseworthy, E. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals:

implications for dietary reconstructions involving marine mammals. Canadian Journal of Fisheries and Aquatic Science 53, 528-533.

Hobson, K. A. and Schell, D. M. 1998. Stable carbon and nitrogen isotope patterns in baleen from eastern Arctic bowhead whales (*Balaena mysticetus*). Canadian Journal of Fisheries and Aquatic Science 55, 2601-2607.

Hobson, K. A., Riget, F. F., Outridge, P. M., Dietz, R. & Born, E. 2004. Baleen as biomonitor of mercury content and dietary history of North Atlantic minke whales (*Balaenoptera acutorostrata*): combining elemental and stable isotope approaches. The Science of the Total Environment 331, 69-82.

Hoekstra, P. F., Dehn, L.-A., George, J. C., Solomon, K. R., Muir, D. C. G. & O'Hara, T. M. 2002. Trophic ecology of bowhead whales (*Balaena mysticetus*) compared with that of other arctic marine biota as interpreted from carbon-, nitrogen-, and sulfur-isotope signatures. Canadian Journal of Zoology 80, 223-231.

Honda, K., Tatsukawa, R., Itano, K., Miyazaki, N. & Fujiyama, T. 1983. Heavy metal concentrations in muscle, liver and kidney tissue of striped dolphin, *Stenella coeruleoalba*, and their variations with body length, weight, age and sex. Agricultural and Biological Chemistry 47, 1219-1228.

Honda, K., Yamamoto, Y., Kata, H. & Tatsukawa, R. 1987. Heavy metal accumulations and their recent changes in Southern minke whales *Balaenoptera acutorostrata*. Archives of Environmental Contamination and Toxicology 16, 209-216.

Huntington, H. P. 2000. Traditional knowledge of the ecology of belugas, *Delphinapterus leucas*, in Cook Inlet, Alaska. Marine Fisheries Review 62, 134-140.

Ikemoto, T., Kunito, T., Tanaka, H., Baba, N., Miyazaki, N. & Tanabe, S. 2004. Detoxification mechanism of heavy metals in marine mammals and seabirds: interaction of selenium with mercury, silver, copper, zinc, and cadmium in liver. Archives of Environmental Contamination and Toxicology 47, 402-413.

Ilbäck, N.-G., Glynn, A. W., Wikberg, L., Netzel, E. & Lindh, U. 2004. Metallothionein is induced and trace element balance changed in target organs of a common viral infection. Toxicology 199, 241-250.

Itoh, N., Fujita, Y., Nakanishi, H., Kawai, Y., Mayumi, T., Hawang, G.S., Min, K., Onosake, S., Muto, N. & Tanaka, K. 1996. Binding of Cd to metallothionein in the placenta of Cd-treated mouse. Journal of Toxicological Sciences 21, 19-27.

Kasuya, T. 1995. Overview of cetacean life histories: an essay in their evolution. *In* Whales, seals, fish and man. Elsevier Science B. V., New York.

Kelly, J. F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78, 1-27.

Khan, S., Cleveland, R. P., Koch, C. J. & Schelling, J. R. 1999. Hypoxia induces renal tubular epithelial cell apoptosis in chronic renal disease. Laboratory Investigation 79, 1089-1099.

Koeman, J. H., Peeters, W. H. M. & Koudstaal-Hol, C. H. M. 1973. Mercury-selenium correlations in marine mammals. Nature 245, 385-386.

Kunito T., Watanabe I., Yasunaga G., Fujise Y. & Tanabe S. 2002. Using trace elements in skin to discriminate the populations of minke whales in southern hemisphere. Marine Environmental Research 53, 175-197.

Le Boeuf, B. J., Pérez-Cortés, M. H., Urbán-Ramirez, J., Mate, B. & Ollervides, F. 2000. High gray whale mortality and low recruitment in 1999: potential causes and implications. Journal of Cetacean Research and Management 2, 85-99.

Leccia, M. T., Richard, M. J., Beani, J. C., Faure, H., Monjo, A. M., Cadet, J., Amblard & P., Favier, A. 1993. Protective effect of selenium and zinc on UV-A damage in human skin fibroblasts. Photochemistry and Photobiology 58, 548-553.

Lesage, V., Hammill, M. O. & Kovacs, K. M. 2001. Marine mammals and the community structure of the Estuary and Gulf of St. Lawrence, Canada: evidence from stable isotope analysis. Marine Ecology Progress Series 210, 203-221.

Lowry, L. F. 1993. Foods and Feeding Ecology. *In* Burns, J. J., Montague, J. J., Cowles, C. J. The bowhead whale. The Society for Marine Mammalogy. Special Publication Number 2.

Lowry, L. F. and Sheffield, G. 2002. Stomach contents of bowhead whales harvested in the Alaskan Beaufort Sea. In Richardson, W. J. and Thompson, D. H. Bowhead whale feeding in the Eastern Alaskan Beaufort Sea: Update of scientific and traditional information. Vol. 1. OCS Study MMS 2002-012. LDL Report TA2196-7. LGL Ltd. King City, Ontario, Canada.

Macdonald, C. R. and Sprague, J. B. 1988. Cadmium in the marine invertebrate and arctic cod in the Canadian Arctic. Distribution and ecological implications. Marine Ecology Progress Series 47, 17-30.

Mackey, E. A., Becker, P. R., Demiralp, R., Greenberg, R. R., Koster, B. J. & Wise , S. A. 1996. Bioaccumulation of vanadium and other trace metals in livers of Alaskan cetaceans and pinnipeds. The Archives of Environmental Contamination and Toxicology 30, 503-512.

Mackey, E. A., Demiralp, R., Becker, P. R., Greenberg, R. R., Koster, B. J. & Wise, S. A. 1995. Trace element concentrations in cetacean liver tissues archived in the National Marine Mammal Tissue Bank. The Science of the Total Environment 175, 25-41.

Mackey, E. A., Oflaz, R. D., Epstein, M. S., Buehler, B., Porter, B. J., Rowles, T., Wise, S. A. & Becker, P. R. 2003. Elemental composition of liver and kidney tissues of rough-toothed dolphins (*Steno bredanensis*). Archives of Environmental Contamination and Toxicology 44, 523-532.

Martin, J. H. and Flegal, A. R. 1975. High copper concentrations in squid livers in association with elevated levels of silver, cadmium, and zinc. Marine Biology 30, 51-55.

Martin, A. R., Smith, T. G. & Cox, O. P. 1998. Dive form and function in belugas (*Delphinapterus leucas*) of the Canadian high arctic. Polar Biology 20, 218-228.

Martin, A. R. and Smith, T. G. 1999. Strategy and capability of wild belugas, *Delphinapterus leucas*, during deep, benthic diving. Canadian Journal of Zoology 77, 1783-1793.

Martineau, D., Mikaelian, I., Lapointe, J.-M., Labelle, P. & Higgins, R. 2003. Pathology of cetaceans. A case study: Beluga from the St. Lawrence estuary. *In* Vos, J. G., Bossart, G. D., Fournier, M., O'Shea, T. J. Toxicology of Marine Mammals. Taylor & Francis, London.

Méndez, L., Alvarez-Castaneda, S. T., Acosta, B. & Sierra-Beltran, A. P. 2002. Trace metals in tissues of gray whale (*Eschrichtius robustus*) carcasses from the Northern Pacific Mexican Coast. Marine Pollution Bulletin 44, 217-221.

Moore, S. E. and Reeves, R. R. 1993. Distribution and Movement. *In* Burns, J. J., Montague, J. J., Cowles, C. J. The bowhead whale. The Society for Marine Mammalogy. Special Publication Number 2.

Nigro, M., Campana, A., Lanzillotta, E. & Ferrara, R. 2002. Mercury exposure and elimination rates in captive bottlenose dolphins. Marine Pollution Bulletin 44, 1071-1075.

O'Corry-Crowe, G. M., Suydam, R. S., Rosenberg, A., Frost, K. J. & Dizon, A. E. 1997. Phylogeography, population structure and dispersal patterns of the beluga whale *Delphinapterus leucas* in the western Nearctic revealed by mitochondrial DNA. Molecular Ecology 6, 955-970.

Oliver, J. S., Slattery, P. N., Silberstein, M. A. & O'Connor, E. F. 1983. A comparison of gray whale, *Eschrichtius robustus*, feeding in the Bering Sea and Baja California. Fishery Bulletin 81, 513-522.

Olsson, U. 1985. Impaired ketone body metabolism in the selenium deficient rat. Possible implications. Metabolism 34, 933-938.

Paludan-Müller, P., Agger, C. T., Dietz, R. & Kinze, C. C. 1993. Mercury, cadmium, zinc, copper and selenium in harbour porpoise (*Phocoena phocoena*) from West Greenland. Polar Biology 13, 311-320.

Ponce, R. A., Egeland, G. M., Middaugh, J. P. & Becker, P. R. 1997. Twenty years of trace metal analyses of marine mammals: Evaluation and Summation of data from Alaska and other Arctic regions. State of Alaska Epidemiology Bulletin 1, 1-15.

Presley, B. J. 1997. A review of Arctic trace metal data with implications for biological effects. Marine Pollution Bulletin 35, 226-234.

Puls, R. 1994. Mineral levels in animal health. Diagnostic data. Sherpa International, Clearbrook, British Columbia, Canada. 356 pp.

Ratte, H. T. 1999. Bioaccumulation and toxicity of silver compounds: A review. Environmental Toxicology and Chemistry 18, 89-108.

Rice, D. W. and Wolman, A. A. 1971. The life history and ecology of the gray whale (*Eschrichtius robustus*). The American Society of Mammalogists Special Publication No. 3.

Richard, P., Weaver, P., Dueck, L. & Barber, D. 1994. Distribution and numbers of Canadian High Arctic narwhals (*Monodon monoceros*) in August 1984. Meddelelser om Gronland, Bioscience 39, 41-50.

Rostan, E. F., DeBuys, H. V., Madey, D. L. & Pinnell, S. R. 2002. Evidence supporting zinc as an important antioxidant for skin. International Journal of Dermatology 41, 606-611.

Rouleau, C., Gobeil, C. & Tjälve, H. 2000. Accumulation of silver from the diet in two marine benthic predators: the snow crab (*Chionoecetes opilio*) and American plaice (*Hippoglossoides platessoides*). Environmental Toxicology and Chemistry 9, 631-637.

Ruelas-Inzunza, J. and Paez-Osuna, F. 2002. Distribution of Cd, Cu, Fe, Mn, Pb and Zn in selected tissues of juvenile whales stranded in the SE Gulf of California (Mexico). Environment International 28, 325-329.

Ruelas-Inzunza, J. R., Horvat, M., Perez-Cortes, H. & Paez-Osuna, F. 2003. Methylmercury and total mercury in tissues of gray whales (*Eschrichtius robustus*) and spinner dolphins (*Stenella longirostris*) stranded along the lower Gulf of California, Mexico. Ciencias Marinas 29, 1-8.

Saeki, K., Nakajima, M., Loughlin, T. R., Calkins, D. C., Baba, N., Kiyota, M. & Tatsukawa, R. 2001. Accumulation of silver in the liver of three species of pinnipeds. Environmental Pollution 112, 19-25.

Schell, D. M., Saupe, S. M. & Haubenstock, N. 1989. Bowhead whale (*Balaena mysticetus*) growth and feeding as estimated by d13C techniques. Marine Biology 103, 433-443.

Schell, D. M., Barnett, B. A. & Vinette, K. A. 1998. Carbon and nitrogen isotope ratios in zooplankton of the Bering, Chukchi and Beaufort seas. Marine Ecology Progress Series 162, 11-23.

Seaman, G. A., Lowry, L. F. & Frost, K. J. 1982. Foods of belukha whales (*Delphinapterus leucas*) in Western Alaska. Cetology 44, 1-19.

Sergeant, D. E. and Brodie, P. F. 1969. Body size in white whales, *Delphinapterus leucas*. Journal of the Fisheries Research Board of Canada 26, 2561-2580.

Sergeant, D. E. 1973. Biology of white whales (*Delphinapterus leucas*) in Western Hudson Bay. Journal of the Fisheries Research Board of Canada 30, 1065-1090.

Sheffield, G., Fay, F. H., Feder, H. & Kelly, B. P. 2001. Laboratory digestion of prey and interpretation of walrus stomach contents. Marine Mammal Science 17, 310-330.

Sigurjónsson, J., Galan, A. & Vikingsson, G. A. 2000. A note on stomach contents of minke whales (*Balaenoptera acutorostrata*) in Icelandic waters. *In* Vikingsson, G. A., Kapel, F. O. Minke whales, harp and hooded seals: major predators in the North Atlantic ecosystem. NAMMCO Scientific Publications 2, Tromsø.

Smith, R. J., Hobson, K. A., Koopman, H. N. & Lavigne, D. M. 1996. Distinguishing between populations of fresh-and saltwater harbour seals (*Phoca vitulina*) using stable-isotope ratios and fatty acid profiles. Canadian Journal of Fisheries and Aquatic Science 53, 272-279.

Tamura, T. and Ohsumi, S. 2000. Regional assessments of prey consumption by marine cetaceans in the world. Report to the Scientific Committee of the International Whaling Commission. SC/52/E6. Adelaide, Australia.

Tarpley, R. J., Wade, T. L. & Haubold, E. M. 1995. Toxicological studies in tissues of the beluga whale *Delphinapterus leucas* along northern Alaska with an emphasis on public health implications of subsistence utilization. Final report to the Alaska Beluga Whale Committee. North Slope Borough, Department of Wildlife Management. Barrow, Alaska.

Thompson, D. H. 2002. Energetics of bowhead whales. In Richardson, W. J. and Thompson, D. H. Bowhead whale feeding in the Eastern Alaskan Beaufort Sea: Update of scientific and traditional information. Vol. 1. OCS Study MMS 2002-012. LDL Report TA2196-7. LGL Ltd. King City, Ontario, Canada.

Tieszen, L. L., Boutton, T. W., Tesdahl, K. G. & Slade, N. A. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecologia 57, 32-37.

Tilbury, K. L., Stein, J. E., Krone, C. A., Brownell, R. L., Blokhin, S. A., Bolton, J. L. & Ernest, D. W. 2002. Chemical contaminants in juvenile gray whales

(*Eschrichtius robustus*) from subsistence harvest in Arctic feeding grounds. Chemosphere 47, 555-564.

US Environmental Protection Agency. 1992. Methods for the determination of metals in environmental samples. CRC Press, Boca Raton.

Varanasi, U., Stein, J. E., Tilbury, K. L., Meador, J. P., Sloan, C. A., Clark, R. C. & Chan, S.-L. 1994. Chemical contaminants in gray whales (*Eschrichtius robustus*) stranded along the west coast of North America. The Science of the Total Environment 145, 29-53.

Wagemann, R., Stewart, R. E. A., Lockhart, W. L., Stewart, B. E. & Povoledo, M. 1988. Trace metals and methyl mercury: associations and transfer in harp seal (*Phoca groenlandica*) mothers and their pups. Marine Mammal Science 4, 339-355.

Wagemann, R., Stewart, R. E. A., Beland, P. & Desjardins, C. 1990. Heavy metals and selenium in tissues of beluga whales, *Delphinapterus leucas*, from the Canadian Arctic and the St. Lawrence estuary. Canadian Bulletin of Fisheries and Aquatic Science 224, 191-206.

Wagemann, R., Innes, S. & Richard, P. 1996. Overview and regional and temporal differences of heavy metals in Arctic whales and ringed seals in the Canadian Arctic. The Science of the Total Environment 186, 41-66.

Wagemann, R., Trebacz, E., Hunt, R. & Boila, G. 1997. Percent methylmercury and organic mercury in tissues of marine mammals and fish using different experimental and calculation methods. Environmental Toxicology and Chemistry 16, 1859-1866.

Wagemann, R, Trebacz, E, Boila, G & Lockhart, WL. 2000. Mercury species in the liver of ringed seals. Sci Total Environ; 261: 21-32.

Watanabe, I., Kunito, T., Tanabe, S., Amano, M., Koyama, Y., Miyazaki, N., Petrov, E. A. & Tatsukawa, R. 2002. Accumulation of heavy metals in Caspian seals (*Phoca caspica*). Archives of Environmental Contamination and Toxicology 43, 109-120.

Whanger, P.D. 2001. Selenium and the brain. Nutritional Neuroscience 4, 81-97.

Wise, S. A., Schantz, M. M., Koster, B. J., Demiralp, R., Mackey, E. A., Greenberg, R. R., Burow, M., Ostapczuk, P. & Lillestolen, T. I. 1993. Development of frozen whale blubber and liver reference materials for the measurement of organic and inorganic contaminants. Fresenius Journal of Analytical Chemistry 345, 270-277.

Woshner, V. M., O'Hara, T. M., Bratton, G. R., Suydam, R. S. & Beasley, V. R. 2001a. Concentrations and interactions of selected essential and non-essential elements in bowhead and beluga whales of Arctic Alaska. Journal of Wildlife Diseases 37, 693-710.

Woshner, V. M., O'Hara, T. M., Bratton, G. R. & Beasley, V. R. 2001b. Concentrations and interactions of selected essential and non-essential elements in ringed seals and polar bears of Arctic Alaska. Journal of Wildlife Diseases 37, 711-721.

Yang, J., Hunito, T., Anan, Y., Tanabe, S. & Miyazaki, N. 2004. Total and subcellular distribution of trace elements in the liver of a mother-fetus pair of Dall's porpoises (*Phocoenoides dalli*). Marine Pollution Bulletin 48, 1122-1129.

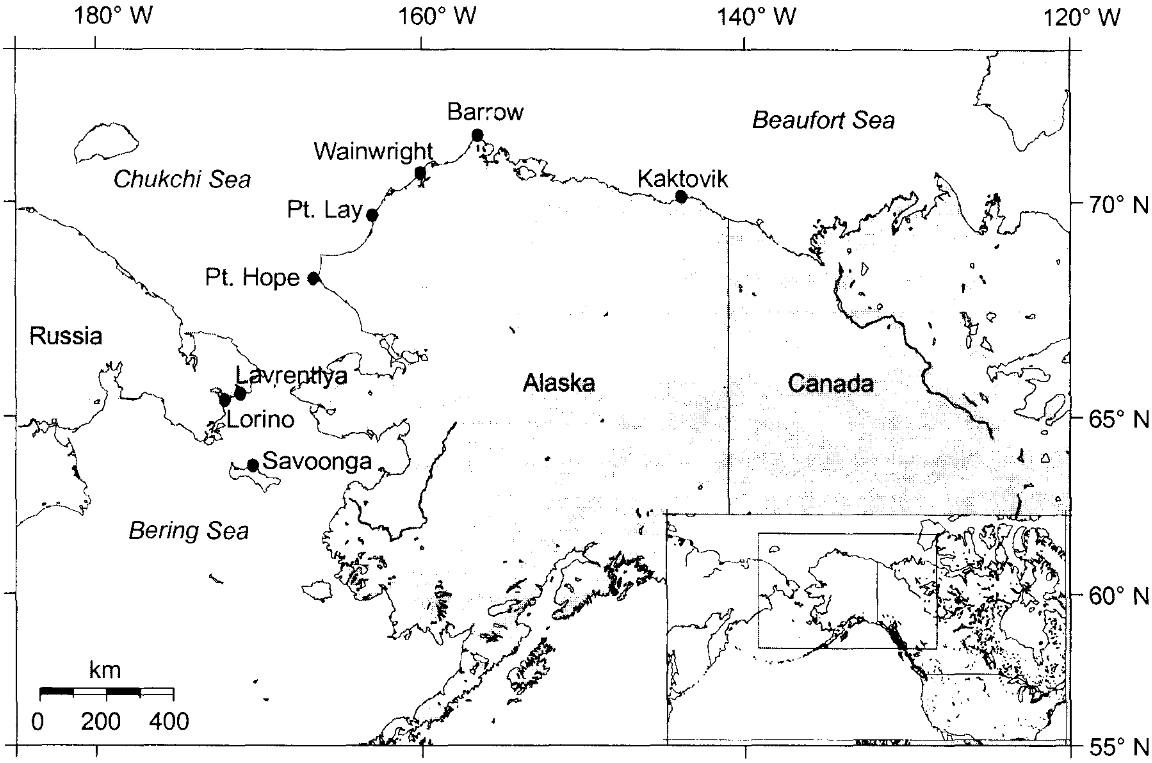


FIGURE 3.1. Alaskan and Russian villages and communities where samples of subsistence harvested Arctic cetaceans were collected.

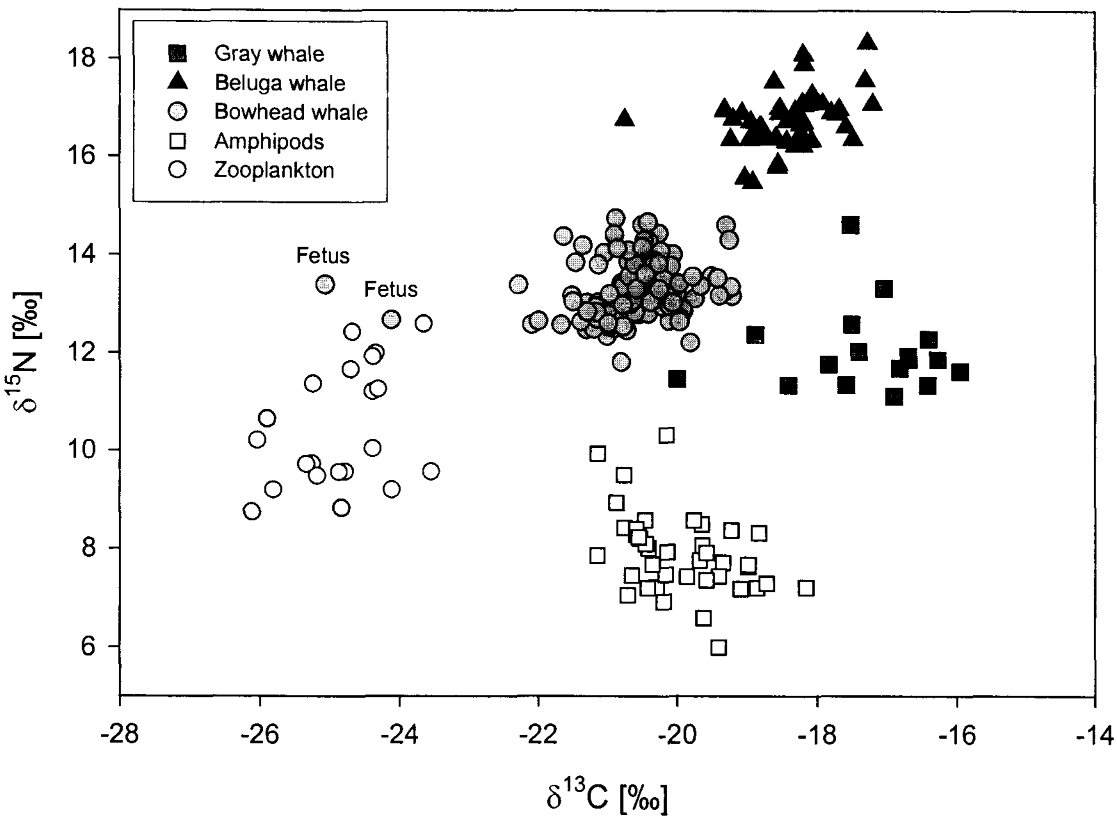


FIGURE 3.2. $\delta^{15}N$ versus $\delta^{13}C$ in bowhead, beluga and gray whales harvested in Alaska and Chukotka. Zooplankton and amphipods were collected in Kaktovik and the Bering Strait, respectively.

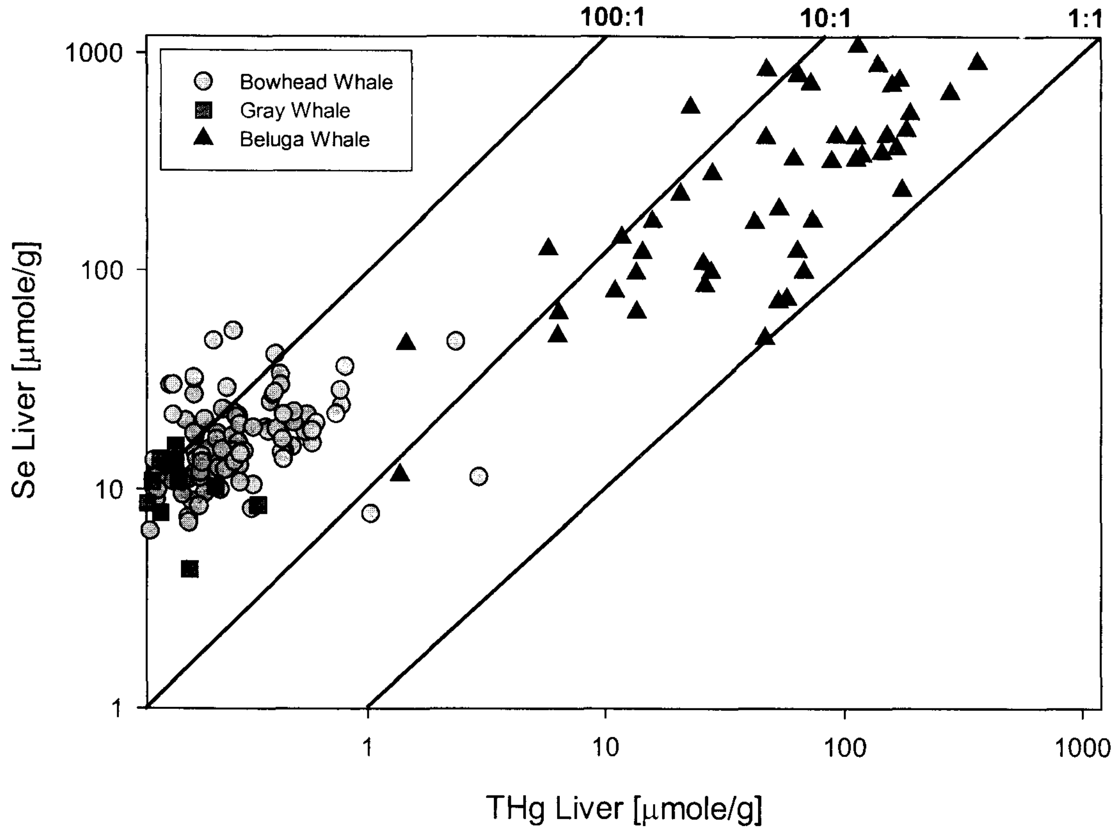


FIGURE 3.3. Hepatic THg [µmole/g] versus hepatic Se [µmole/g] of bowhead, beluga and gray whales. The lines indicate the 1:1, 10:1 and 100:1 molar ratio of Se:THg.

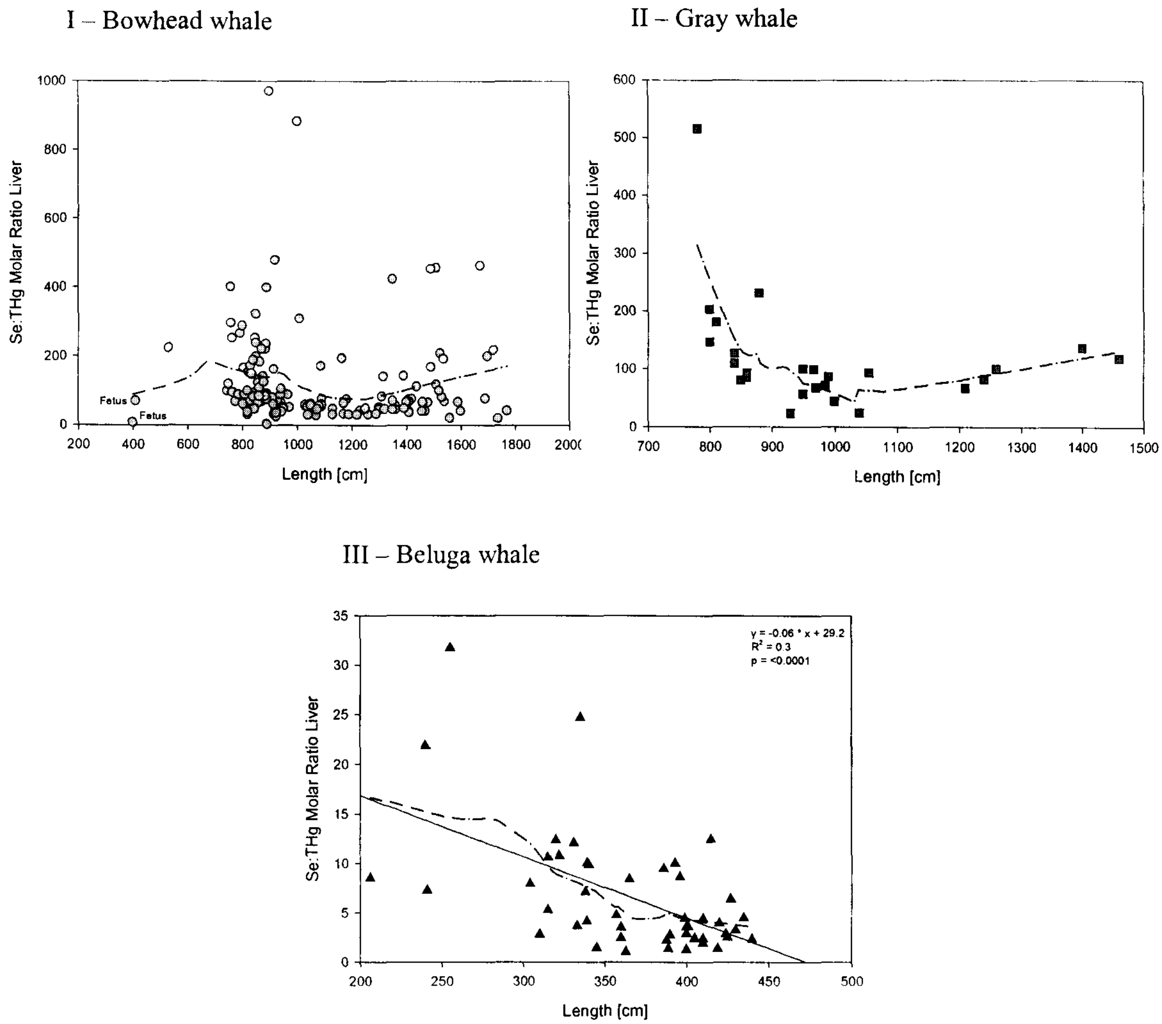


FIGURE 3.4. Hepatic Se:THg molar ratio versus length [cm] in bowhead (I), beluga (III) and gray whales (II). LOESS nonparametric smoothing (dashed lines) was employed to estimate the regression surface. Linear regression parameters are given for beluga whales.

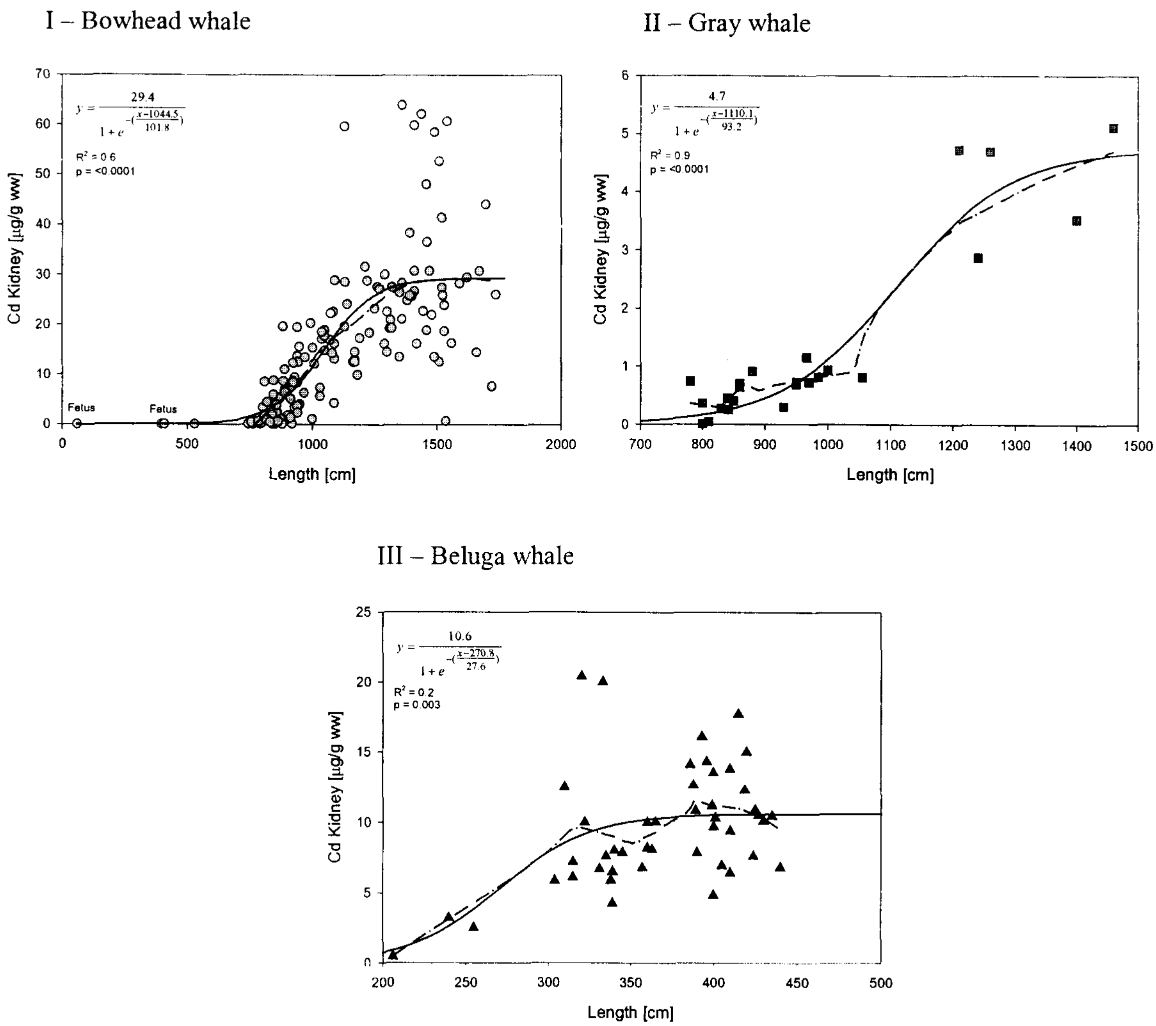


FIGURE 3.5. Renal Cd [µg/g ww] versus length [cm] in bowhead (I), beluga (III) and gray whales (II). A sigmoid function was fitted to the data sets and LOESS nonparametric smoothing (dashed lines) was employed to estimate and compare the regression surface.

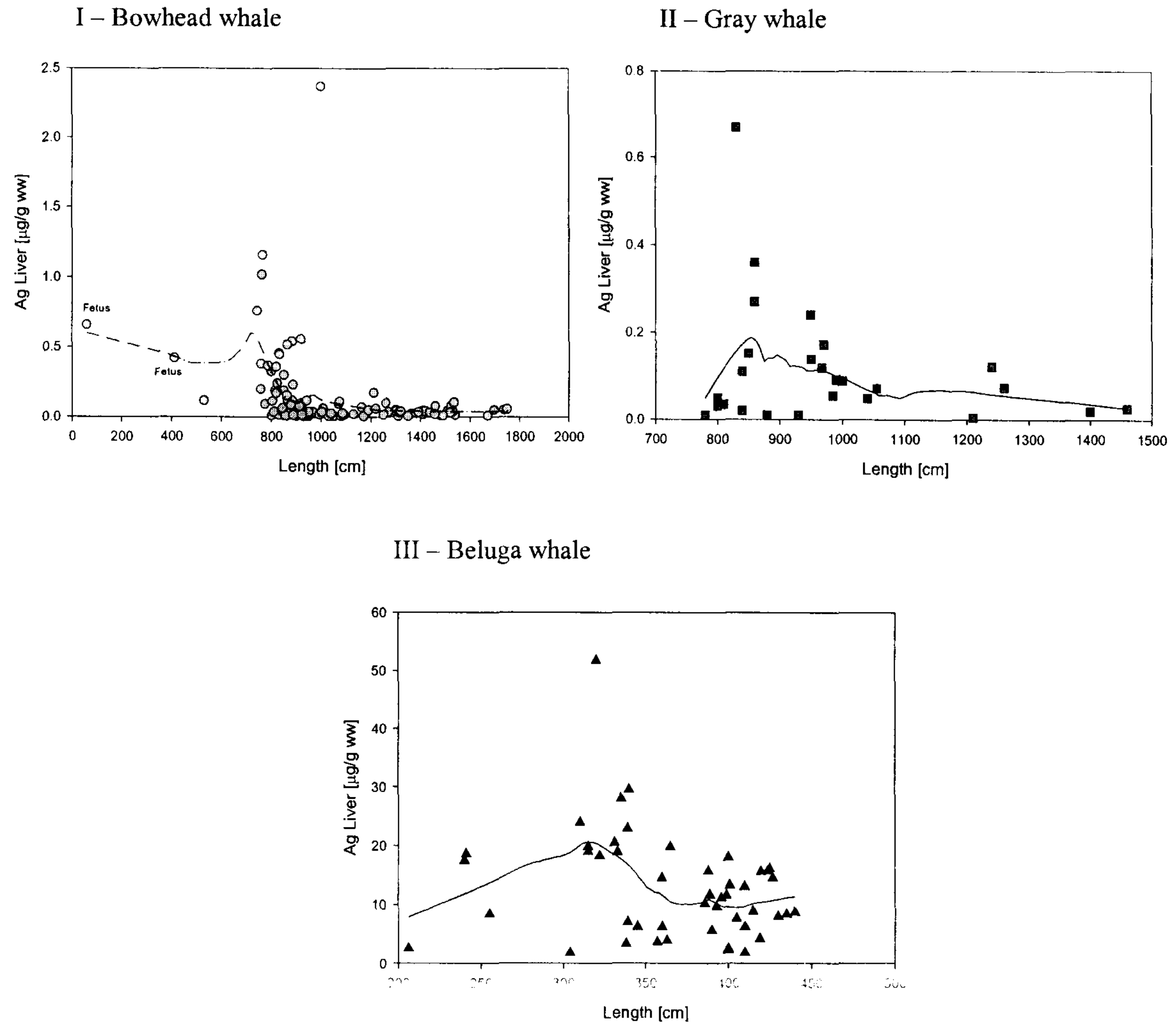


FIGURE 3.6. Hepatic Ag [µg/g ww] versus length [cm] in bowhead (I), beluga (III) and gray whales (II). LOESS nonparametric smoothing (dashed lines) was employed to estimate and compare regression surfaces.

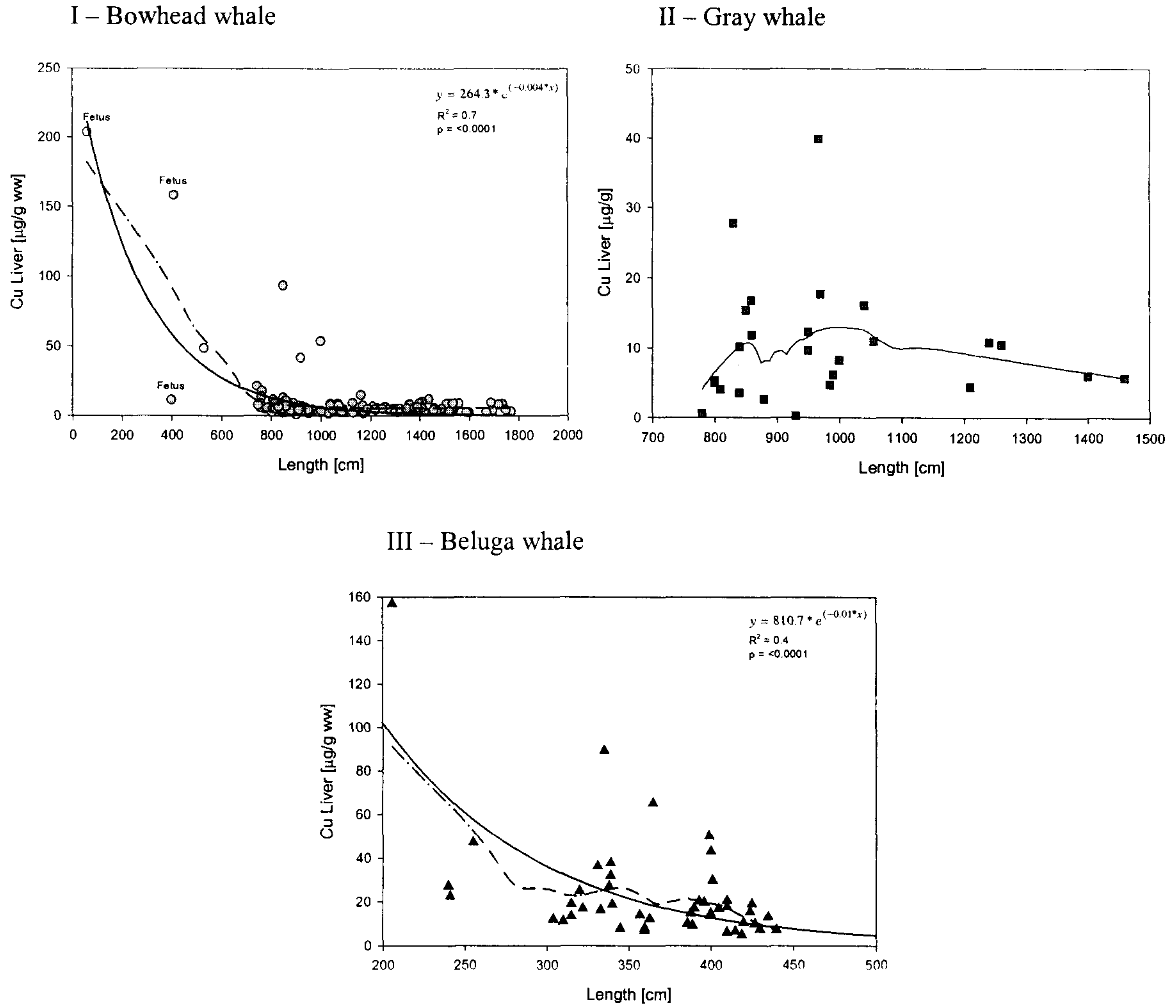


FIGURE 3.7. Hepatic Cu [µg/g ww] versus length [cm] in bowhead (I), beluga (III) and gray whales (II). An exponential decay function was fitted to the data for bowheads and beluga whales. LOESS nonparametric smoothing (dashed lines) was employed to estimate and compare the regression surface of all data sets.

TABLE 3.1. Whale samples collected in Alaskan and Russian villages.

Species	Sampling Location	Epidermis	Muscle	Kidney	Liver
			n		
Bowhead whale	Barrow	96	77	140	143
	Kaktovik	4	9	12	14
	Wainwright	-	-	4	3
	Savoonga	-	-	-	1
Beluga whale	Pt. Lay	32	31	49	51
	Wainwright	2	-	2	2
	Pt. Hope	2	9	9	9
	Barrow	4	4	3	4
	Kaktovik	1	1	1	1
Gray whale	Lorino / Lavrentiya	27	17	28	29

n: sample size

TABLE 3.2. Results for trace element analysis of reference materials for quality assurance / quality control. Concentrations are given in µg/g ww.
n.e. = not established

		Ag	Cd	Cu	Se	Zn	THg	MeHg
Dogfish muscle	Dorm - 2							
	Certified value	0.041 ± 0.09	0.043 ± 0.008	2.34 ± 0.16	1.4 ± 0.09	25.6 ± 2.3	4.64 ± 0.26	4.47 ± 0.32
	Measured Mean	-	-	-	1.349	-	4.545	3.879
	Standard Deviation	-	-	-	0.080	-	0.246	0.112
	% Recovery	-	-	-	96.4	-	98.0	86.8
	n	-	-	-	25	-	17	5
Dogfish Liver	Dolt - 2							
	Certified value	0.608 ± 0.032	20.8 ± 0.5	25.8 ± 1.1	6.06 ± 0.49	85.8 ± 2.5	2.14	0.693 ± 0.06
	Measured Mean	0.606	21.567	25.684	5.529	92.651	2.053	-
	Standard Deviation	0.068	1.113	1.562	0.310	10.304	0.094	-
	% Recovery	99.7	103.7	99.5	91.2	108.0	95.9	-
	n	23	15	25	30	25	8	-
Dogfish Liver	Dolt-3							
	Certified value	1.20 ± 0.07	19.4 ± 0.6	31.2 ± 1.0	7.06 ± 0.48	86.6 ± 2.4	3.37 ± 0.14	n.e.
	Measured Mean	1.044	18.1	31.406	6.541	83.286	3.389	-
	Standard Deviation	0.194	1.3	0.930	0.548	1.810	0.355	-
	% Recovery	87.0	93.2	100.7	92.6	96.2	100.6	-
	n	12	12	10	10	10	10	-
Bovine liver	SRM 1577b							
	Certified value	0.039 ± 0.007	0.50 ± 0.03	160 ± 8.0	0.73 ± 0.06	127 ± 16.0	n.e.	n.e.
	Measured Mean	0.042	0.515	167.971	0.707	134.845	-	-
	Standard Deviation	0.001	0.045	2.117	0.049	8.366	-	-
	% Recovery	108.2	103.1	105.0	96.9	106.2	-	-
	n	6	18	11	6	18	-	-
Pilot whale liver	QC91LH1							
	Certified value	0.181 ± 0.005	8.51 ± 0.22	2.96 ± 0.20	11.0 ± 0.3	32.2 ± 0.7	28.2 ± 1.1	1.36
	Measured Mean	0.194	8.90	3.21	11.0	32.3	27.460	1.378
	Standard Deviation	0.002	0.08	0.01	0.2	0.0	2.441	0.062
	% Recovery	107.0	104.6	108.4	100.4	100.5	97.4	101.3
	n	3	3	3	3	3	17	10
Beluga whale liver	QC97LH2							
	Consensus mean*	13.24 ± 2.41	2.433 ± 0.040	13.10 ± 0.188	24.35 ± 0.484	26.92 ± 0.359	40.31 ± 1.28	n.e.
	Analyzed Mean	23.48	2.497	12.91	23.93	26.63	39.982	1.468
	Standard Deviation	0.434	0.0162	0.039	0.344	0.081	2.822	0.090
	% Recovery	177.3	102.6	98.5	98.3	98.9	99.2	-
	n	5	5	5	5	5	7	10

*established by 19 laboratories (Christopher 2002, Christopher 2004)

TABLE 3.3. Mean trace element concentration ± standard deviation (SD) in µg/g ww, concentration range, median and sample size (n) in tissues of bowhead, beluga and gray whales harvested in Alaska and Russia.

Bowhead whale

	$\delta^{15}N$	$\delta^{13}C$	Zn	Cu	Cd	Se	Ag	THg	Se:THg Molar Ratio
Liver									
Median	-	-	31.60	4.89	3.93	1.06	0.04	0.04	74.41
Mean ± SD	-	-	35.99 ± 17.08	9.13 ± 21.67	7.27 ± 8.97	1.23 ± 0.69	0.13 ± 0.28	0.05 ± 0.07	121.62 ± 136.20
Range	-	-	6.99 - 135.11	1.09 - 203.81	0.03 - 50.91	0.06 - 4.19	0.002 - 2.37	0.001 - 0.59	3.88 - 971.83
n	-	-	161	161	161	161	127	154	151
Kidney									
Median	-	-	24.80	1.85	12.66	1.45	0.01*	0.03	156.68
Mean ± SD	-	-	25.90 ± 9.20	2.27 ± 1.17	15.08 ± 14.94	1.45 ± 0.43	0.01 ± 0.01*	0.03 ± 0.03	252.96 ± 251.18
Range	-	-	9.07 - 56.31	0.76 - 7.94	0.01 - 64.00	023 - 3.21	0.002 - 0.06	0.001 - 0.18	20.25 - 1386.00
n	-	-	156	156	156	157	128	145	144
Muscle									
Median	13.16	-20.62	33.85	0.65	0.04	0.20	0.002*	0.02	31.52
Mean ± SD	13.28 ± 0.62	-20.65 ± 0.82	35.38 ± 9.64	0.65 ± 0.10	0.07 ± 0.10	0.21 ± 0.07	0.003 ± 0.001*	0.02 ± 0.01	134.22 ± 596.58
Range	11.81 - 14.74	-25.06 - -19.20	9.47 - 74.10	0.47 - 1.07	0.01 - 0.61	0.08 - 0.77	0.001 - 0.01	0.00 - 0.05	11.06 - 5255.00
n	110	110	86	86	86	86	84	123	79
Epidermis									
Median	-	-	13.82	0.37	0.01*	0.71	0.002*	0.01	198.97
Mean ± SD	-	-	14.20 ± 2.63	0.38 ± 0.07	0.01 ± 0.01*	0.70 ± 0.23	0.003 ± 0.003*	0.01 ± 0.01	480.87 ± 897.66
Range	-	-	10.50 - 28.80	0.25 - 0.70	0.01 - 0.07	0.24 - 1.42	0.002 - 0.03	0.00 - 0.04	44.72 - 5855.00
n	-	-	100	100	100	100	100	98	98

*more than 50% of samples below MDL

TABLE 3.3 (continued)

Gray whale

	$\delta^{15}N$	$\delta^{13}C$	Zn	Cu	Cd	Se	Ag	THg	Se:THg Molar Ratio
Liver									
Median	-	-	29.70	9.66	0.24	0.83	0.06	0.02	99.58
Mean ± SD	-	-	41.07 ± 51.78	18.90 ± 34.67	0.47 ± 0.63	0.83 ± 0.26	0.11 ± 0.14	0.02 ± 0.01	127.81 ± 101.98
Range	-	-	9.57 - 300.48	0.24 - 154.45	0.01 - 2.20	0.34 - 1.32	0.004 - 0.67	0.004 - 0.07	23.80 - 515.81
n	-	-	29	29	29	29	29	28	28
Kidney									
Median	-	-	19.27	2.55	0.71	1.56	0.004*	0.02	293.67
Mean ± SD	-	-	20.09 ± 5.12	2.51 ± 0.71	1.19 ± 1.50	1.50 ± 0.40	0.01 ± 0.001*	0.01 ± 0.01	401.50 ± 345.38
Range	-	-	14.30 - 33.30	1.34 - 4.64	0.01 - 5.11	0.50 - 2.24	0.003 - 0.01	0.001 - 0.03	128.02 - 1805.00
n	-	-	28	28	28	28	28	28	28
Muscle									
Median	11.87	-17.05	33.50	2.80	0.01	0.19	0.004*	0.02	28.65
Mean ± SD	12.04 ± 0.86	-17.32 ± 1.03	39.47 ± 18.68	3.17 ± 2.54	0.02 ± 0.01	0.19 ± 0.04	0.004 ± 0.0004*	0.02 ± 0.01	27.09 ± 9.05
Range	11.12 - 14.62	-20.00 - -15.96	19.10 - 74.80	0.46 - 8.01	0.01 - 0.05	0.13 - 0.29	0.003 - 0.004	0.01 - 0.04	9.93 - 45.71
n	17	17	17	17	17	17	17	17	17
Epidermis									
Median	-	-	18.10	1.00	0.01*	3.36	0.003*	0.01	1251.47
Mean ± SD	-	-	16.71 ± 6.77	1.58 ± 2.17	0.01 ± 0.001*	3.75 ± 2.08	0.01 ± 0.003*	0.01 ± 0.01	1717.00 ± 1560.00
Range	-	-	0.03 - 26.04	0.01 - 8.29	0.01 - 0.01	0.85 - 10.60	0.003 - 0.01	0.001 - 0.03	180.04 - 7582.00
n	-	-	27	27	27	27	27	24	24

*more than 50% of samples below MDL

TABLE 3.3 (continued)

Beluga whale

	$\delta^{15}N$	$\delta^{13}C$	Zn	Cu	Cd	Se	Ag	THg	MeHg	%MeHg	Se:THg Molar Ratio
Liver											
Median	-	-	36.80	17.00	2.84	25.70	11.33	11.99	1.41	13.20	4.46
Mean ± SD	-	-	36.21 ± 8.29	24.98 ± 27.23	3.05 ± 1.52	31.39 ± 25.95	12.84 ± 9.09	15.95 ± 15.17	1.43 ± 0.78	17.60 ± 14.57	6.91 ± 6.37
Range	-	-	18.50 - 53.20	4.90 - 156.84	0.05 - 7.05	0.93 - 113.20	1.77 - 51.70	0.28 - 72.48	0.19 - 3.89	2.51 - 63.10	1.05 - 31.73
n	-	-	67	67	67	67	48	48	46	46	48
Kidney											
Median	-	-	33.89	1.99	10.20	4.86	0.05	3.53	0.48	11.50	2.86
Mean ± SD	-	-	34.49 ± 5.71	1.98 ± 0.29	10.16 ± 4.25	5.04 ± 1.96	0.05 ± 0.03	4.41 ± 3.00	0.50 ± 0.32	12.80 ± 5.13	4.99 ± 6.69
Range	-	-	24.04 - 49.30	1.29 - 2.92	0.46 - 20.40	1.65 - 10.82	0.01 - 0.15	0.10 - 12.26	0.07 - 1.67	4.98 - 28.98	1.31 - 42.14
n	-	-	64	64	64	64	32	46	44	44	46
Muscle											
Median	16.72	-18.32	28.40	1.01	0.03	0.32	0.01*	1.10	1.05	96.05	0.84
Mean ± SD	16.74 ± 0.56	-18.41 ± 0.62	31.72 ± 12.16	0.96 ± 0.33	0.06 ± 0.06	0.38 ± 0.19	0.01 ± 0.00*	1.13 ± 0.63	1.04 ± 0.52	94.63 ± 10.90	1.26 ± 1.05
Range	15.48 - 18.34	-20.75 - -17.21	16.30 - 66.66	0.41 - 1.51	0.01 - 0.21	0.20 - 1.26	0.01 - 0.01	0.13 - 3.27	0.13 - 2.40	56.83 - 138.20	0.30 - 4.62
n	49	49	45	45	32	45	47	46	46	46	45
Epidermis											
Median	-	-	86.84	0.55	0.01*	7.05	0.01*	0.51	0.54	97.14	35.12
Mean ± SD	-	-	82.52 ± 36.00	0.53 ± 0.13	0.01 ± 0.003*	7.96 ± 5.06	0.01 ± 0.002*	0.63 ± 0.39	0.63 ± 0.39	95.99 ± 6.83	60.08 ± 81.30
Range	-	-	12.50 - 160.12	0.23 - 0.83	0.01 - 0.02	2.66 - 32.91	0.01 - 0.01	0.03 - 1.52	0.06 - 1.48	75.38 - 111.15	5.56 - 447.15
n	-	-	41	41	35	41	41	39	37	37	39

*more than 50% of samples below MDL

TABLE 3.4. Tukey grouping and p-values of variables in tissues of bowhead (BM), beluga (DL) and gray whales (ER) from Alaska and Russia.

Variable	Tissue	Tukey grouping	p-value
$\delta^{15}N$	Muscle	[§]DL > BM > ER	<0.0001
$\delta^{13}C$	Muscle	ER > DL > BM	<0.0001
Zn	Kidney	DL > BM > ER	<0.0001
	Liver	DL = BM = ER	0.07
	Muscle	BM = ER, BM > DL, ER = DL	0.005
	Epidermis	DL > ER > BM	<0.0001
Cu	Kidney	ER = DL, ER > BM, DL = BM	0.01
	Liver	DL > ER > BM	<0.0001
	Muscle	ER = DL > BM	<0.0001
	Epidermis	DL = ER > BM	<0.0001
Cd	Kidney	BM = DL > ER	<0.0001
	Liver	BM = DL > ER	<0.0001
	Muscle	BM = DL > ER	0.04
	Epidermis*	-	-
Ag	Kidney*	-	-
	Liver	DL > ER = BM	<0.0001
	Muscle*	-	-
	Epidermis*	-	-
Se	Kidney	DL > ER = BM	<0.0001
	Liver	DL > BM > ER	<0.0001
	Muscle	DL > BM > ER	<0.0001
	Epidermis	DL > ER > BM	<0.0001
THg	Kidney	DL > BM > ER	<0.0001
	Liver	DL > BM > ER	<0.0001
	Muscle	DL > ER = BM	<0.0001
	Epidermis	DL > ER = BM	<0.0001
Se:THg molar ratio	Kidney	ER > BM > DL	<0.0001
	Liver	ER = BM > DL	<0.0001
	Muscle	ER = BM > DL	<0.0001
	Epidermis	ER > BM > DL	<0.0001

[§]DL - Beluga whale

BM - Bowhead whale

ER - Gray whale

*more than 50% of samples below MDL

TABLE 3.5. Correlation matrix of all variables in tissues of bowhead, beluga and gray whales. Only significant relationships were noted and slope of correlated variables is indicated by either + (positive) or – (negative). Correlated variables that are underlined are consistent for all whale species. Correlations consistent between mysticetes are highlighted in bold script.

Bowhead whale

		Liver	Kidney	Muscle	Epidermis
	Length	-Cu, <u>+Cd</u>, -Ag, +Se, <u>+THg</u>	+Zn, <u>+Cd</u>, +Se, +THg	$-\delta^{15}N$, +Cd, +Se, +THg	+Zn, +Se, +THg
	$\delta^{13}C$	+Zn, +Cu, +Se	-Cu	$+\delta^{15}N$	-
	$\delta^{15}N$	+Cu, -Cd, +Ag, -Se, **-THg**	-Zn, -Cd, **-Se**, -THg	+Cu, -Cd, -Se, -THg	-Se, -THg
Liver	Zn	+Cu, +Cd, +Ag, +Se, +THg	<u>+Zn</u>, +Cd, +THg	-	-
	Cu	**+Ag**	+Cu, -Cd, +Ag	-THg	-Se, -THg
	Cd	-Ag, <u>+Se</u>, <u>+THg</u>	+Zn, <u>+Cd</u>, +Se, +THg	+Cd, +Se, +THg	+Zn, +Se, +THg
	Ag	**-THg**	-Cd, -Se, -THg	-THg	-Zn, -THg
	Se	+THg	+Zn, +Cu, <u>+Cd</u>, +Se, <u>+THg</u>	+Cd, +Se, +THg	+Zn, +Cu, **+Se**, <u>+THg</u>
	THg	-	+Zn, +Cd, +Se, +THg	+Cd, +Se, +THg	+Se, <u>+THg</u>
Kidney	Zn	-	+Cd, +Se, +THg	+Cd, +Se, +THg	**+Zn**, +Se, +THg
	Cu	-	+Se	-	-
	Cd	-	<u>+Se</u>, +THg	+Cd, +Se, +THg	+Zn, +Se, +THg
	Ag*	-	-	-	-
	Se	-	<u>+THg</u>	+Cd, +Se, +THg	**+Se**, +THg
	THg	-	-	+Cd, +Se, +THg	+Zn, **+Se**, <u>+THg</u>
Muscle	Zn	-	-	+Se	-
	Cu	-	-	-	-Se
	Cd	-	-	+THg	+THg
	Ag*	-	-	-	-
	Se	-	-	+THg	-Se, +THg
	THg	-	-	-	+Se, <u>+THg</u>
Epidermis	Zn	-	-	-	+Cu, +Se
	Cu	-	-	-	+Se
	Cd*	-	-	-	-
	Ag*	-	-	-	-
	Se	-	-	-	**+THg**

*more than 50% of samples below MDL

TABLE 3.5 (continued)

Gray whale

		Liver	Kidney	Muscle	Epidermis
	Length	+Cd, +THg	+Cu, +Cd	-	-Zn
	$\delta^{13}C$	-	-	-	-
	$\delta^{15}N$	**-THg**	**-Se**	-	-
Liver	Zn	-	+Zn	-	+Zn
	Cu	**+Ag**	-	-	+Zn
	Cd	+Se, +THg	+Cd, -Zn	-	+Cu, -Zn
	Ag	**-THg**	-	-Se	+THg
	Se	-	+Cd, +THg	-Cu	**+Se**, +THg
	THg	-	-	-Zn	+THg
Kidney	Zn	-Cd	-	-	+Cu, +Zn
	Cu	-	-Se, -THg	-	-Se
	Cd	-	+Se	-	-Zn
	Ag*	-	-	-	-
	Se	-	+THg	-	**+Se**
	THg	-	-	-	**+Se**, +THg
Muscle	Zn	-	-	-	-Zn
	Cu	-	-	-	
	Cd	-	-	-	-
	Ag*	-	-	-	-
	Se	-	-	-	-
	THg	-	-	-	-
Epidermis	Zn	-	-	-	-
	Cu	-	-	-	-
	Cd*	-	-	-	-
	Ag*	-	-	-	-
	Se	-	-	-	**+THg**

*more than 50% of samples below MDL

Table 3.5 (continued)

Beluga whale

		Liver	Kidney	Muscle	Epidermis
	Length	-Cu, +Cd, +Se, +THg, +MeHg, -%MeHg	+Cd, +Se, +THg, +MeHg	-Cu, +THg, +MeHg	+THg, +MeHg
	$\delta^{13}C$	-Cu, +Cd, +Se, +MeHg	-Cu, +Cd, +MeHg	+$\delta^{15}N$, -Cu, -Se, +THg, +MeHg	-Zn, -Cu, +THg
	$\delta^{15}N$	-Cu, +Se, +MeHg	-Cu, +Cd, +Se, +THg, +MeHg	+THg, +MeHg	-Zn, -Cu
Liver	Zn	+Cd	+Zn, +Cd, +Se	-Cu	+Se
	Cu	-THg, -MeHg	+Cu, -Cd, -MeHg	-THg, -MeHg	+Zn, +Cu
	Cd	+Ag, +Se, +THg, +MeHg	+Zn, -Cu, +Cd, +Ag, +Se, +THg, +MeHg	-Cu, +THg, +MeHg	-Cu, +THg, +MeHg
	Ag	+Se	+Zn, +Ag, +Se	+%MeHg	+Se
	Se	+THg, +MeHg, -%MeHg	+Zn, +Cd, +Ag, +Se, +THg, +MeHg, -%MeHg	-Cu, +THg, +MeHg	-Cu, +THg, +MeHg, +%MeHg
	THg	+MeHg, -%MeHg	+Zn, +Cd, +Ag, +Se, +THg, +MeHg, -%MeHg	+Se, +THg, +MeHg	+THg, +MeHg
	MeHg	-	-Cu, +Cd, +Ag, +Se, +THg, +MeHg	-Cu, +THg, +MeHg	-Cu, +THg, +MeHg
	%MeHg	-	-Zn, -Cd, -Ag, -THg, +%MeHg	-Cd, -Se, -THg, -MeHg, +%MeHg	-Zn, -THg, -MeHg
Kidney	Zn	-	+Cd, +Ag, +Se, +THg, +MeHg, -%MeHg	-	+THg, +MeHg
	Cu	-	-%MeHg	+Cd, +Se	+Zn
	Cd	-	+Ag, +Se, +THg, +MeHg	-Cu, +THg, +MeHg	-Cu, +THg, +MeHg
	Ag	-	+Se, +THg, +MeHg	+THg	+THg
	Se	-	+THg, +MeHg, -%MeHg	-Cu, +THg, +MeHg	-Cu, +THg, +MeHg
	THg	-	+MeHg, -%MeHg	+THg, +MeHg	+THg, +MeHg
	MeHg	-	-	-Cu, +THg, +MeHg	-Cu, +THg, +MeHg, +%MeHg
	%MeHg	-	-	-Se	-Zn
Muscle	Zn	-	-	-	-%MeHg
	Cu	-	-	-THg, -MeHg	+Cu, -THg, -MeHg
	Cd	-	-	-	+Zn
	Ag*	-	-	-	-
	Se	-	-	-	+Zn
	THg	-	-	+MeHg	-Cu, +THg, +MeHg
	MeHg	-	-	-	-Cu, +THg, +MeHg
	%MeHg	-	-	-	-
Epidermis	Zn	-	-	-	-
	Cu	-	-	-	-THg, -%MeHg
	Cd*	-	-	-	-
	Ag*	-	-	-	-
	Se	-	-	-	-
	THg	-	-	-	+MeHg
	MeHg	-	-	-	+%MeHg

*more than 50% of samples below MDL

CHAPTER 4

TROPHIC ECOLOGY OF ARCTIC MARINE BIOTA AND IMPLICATIONS FOR TRACE METAL DYNAMICS[4]

4.1 ABSTRACT

Tissues of subsistence-harvested Arctic mammals were analyzed for silver (Ag), cadmium (Cd), and total mercury (THg). Muscle (or total body homogenates of potential fish and invertebrate prey) was analyzed for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes to establish trophic interactions within the Arctic food chain. Food web magnification factors (FWMFs) and biomagnification factors for selected predator-prey scenarios (BMFs) were calculated to describe pathways of heavy metals in the Alaskan Arctic. FWMFs in this study indicate that magnification of selected heavy metals in the Arctic food web is not significant. Biomagnification of Cd occurs mainly in kidneys; calculated BMFs are higher for hepatic THg than renal THg for all predator-prey scenarios with the exception of polar bears (*Ursus maritimus*). In bears, the accumulation of renal THg is approximately 6 times higher than in liver. Magnification of hepatic Ag is minimal for all selected predator-prey scenarios. Though polar bears occupy a higher trophic level than belugas (*Delphinapterus leucas*), based on $\delta^{15}N$, the metal concentrations are either not statistically different between the two species or lower for

---

[4] Dehn, L.-A. Follmann, E. H., Thomas, D. L., Sheffield, G. G., Rosa, C., Duffy, L. K., O'Hara, T. M. Trophic ecology of Arctic marine biota and implications for trace metal dynamics. Prepared for submission to The Science of the Total Environment.

bears. Similarly, concentrations of renal and hepatic Cd are significantly lower or not statistically different in polar bears compared to ringed (*Phoca hispida*) and bearded seals (*Erignathus barbatus*), their primary prey. THg, on the other hand, increased significantly from seal to polar bear tissues. Mean $\delta^{15}N$ was lowest in muscle of Arctic fox (*Alopex lagopus*) and foxes also show the lowest levels of Hg, Cd and Ag in liver and kidney compared to the other species analyzed. These values are in good agreement with a diet dominated by terrestrial prey. Metal deposition in animal tissues is strongly dependent on biological factors such as diet, age, sex, body condition and health, and caution should be taken when interpreting biomagnification of dynamic and actively regulated trace metals.

Keywords: Arctic, food web, biomagnification, trace metals, stable isotopes, arctic fox, polar bear, bowhead whale, beluga whale, gray whale, ringed seal, bearded seal, spotted seal, ribbon seal

4.2 INTRODUCTION

Trace metals are widely distributed and have multiple natural (e.g., degassing of the earth's crust) and anthropogenic inputs (e.g., fossil fuel burning, mining) into the environment (Presley, 1997; Pyle and Mather, 2003; Trefry, et al. 2003; AMAP, 2004). The Arctic is generally considered a pristine environment as it is sparsely populated by humans and relatively unaffected by industrial activity (Barrie et al., 1992; Bard, 1999). However, concentrations of some contaminants in Arctic marine mammals are at levels of concern to subsistence users and animal health (Gauthier et al., 1998; Deutch and Hansen, 2000; Belles-Isles et al., 2002; Lalancette et al., 2003). Of particular importance in marine mammals are bioaccumulation and biomagnification, the increase in concentration of contaminants in tissues with age and trophic level, respectively. Several Arctic marine mammal populations were decimated by commercial whalers and sealers in the18th and 19th centuries and are therefore especially vulnerable to harmful effects of pollutants, e.g., effects on reproduction, body condition, and immune status (Fay, 1982; Lowry et al., 1982; Clapham et al., 1999). Additionally, marine mammals are long-lived, e.g., bowhead whales (*Balaena mysticetus*) are reported to live in excess of 100 years (George et al., 1999); many species are top-level predators, and the Inuit coastal population of the Arctic has been nutritionally and culturally dependent on marine resources for centuries.

Mercury (Hg) may accumulate in polar regions due to surface deposition of reactive, divalent Hg (HgII) during polar sunrise (Ebinghaus et al., 2002; Lindberg et al., 2002). It is released during snow melt and thus made available to the marine food chain where it is subjected to microbial methylation (Boening, 2000; Ebinghaus et al., 2002; Lindberg et al., 2002). Transfer efficiency from phytoplankton to copepods is four times greater for organic Hg (MeHg) than HgII (Morel et al., 1998). Furthermore, fish muscle contains approximately 100% bioavailable MeHg, therefore leading to high potential of Hg magnification in the piscivorous food chain (Bryan and Langston, 1992; Wagemann et al., 1997; Baeyens et al., 2003). On the other hand, cadmium (Cd) is found in higher concentrations in kidney and liver of marine mammals that feed low in the Arctic food chain, e.g., bowhead whales and walrus (*Odobenus rosmarus*), than in top-level Arctic predators such as polar bear (*Ursus maritimus*) and Arctic fox (*Alopex lagopus*) (Warburton and Seagars, 1993; Prestrude et al., 1994; Wagemann and Stewart, 1994; Bratton et al., 1997; Woshner et al., 2001a; Woshner et al., 2001b). Marine invertebrates and cephalopods in particular have been suggested to be main vectors of Cd in the food chain, while fish generally have lower concentrations (Bustamante et al., 1998; Bustamante et al., 2003).

Silver (Ag) is associated with sewage wastes, but also occurs naturally in the earth's crust and in mining deposits (Purcell and Peters, 1998). Elevated concentrations (>50 ppm) of Ag also have been reported in the vicinity of hydrothermal vents (Hein et al., 1999). Ag has a high affinity to sulfur ligands and halogens in water and sediments (Bell and Kramer, 1999) and may thus have the potential to accumulate in the benthic food web. Cephalopods, bivalves, and crustaceans can accumulate Ag at high concentrations, usually in conjunction with copper (Cu), required by some of these taxa as the central atom in their blood pigment, hemocyanin (Martin and Flegal, 1975; Berthet et al., 1992; Rouleau et al., 2000; Bustamante et al., 2004a).

In order to address trophic relationships and biomagnification of contaminants in the Arctic, stable nitrogen isotopes have been commonly used (Fisk et al., 2001; Hobson et al., 2002; Hop et al., 2002; Hoekstra et al., 2003). Nitrogen isotope ratios of prey are reflected in tissues of the consumer, with slight enrichment occurring at each trophic step (Kelly, 2000). Stable isotopes of carbon are generally used to provide information on spatial habitat use and carbon sources rather than trophic relationships as they enrich in consumer tissues only to a minor degree (Schell et al., 1989; France, 1995; Burton and Koch, 1999; Dehn, in prep.). However, there are difficulties and limitations with the interpretation of stable isotopes. Nitrogen isotope ratios can be influenced by age, body condition, water stress, body protein catabolism associated with starvation and urea recycling during hibernation (Hobson et al., 1993; Barboza et al., 1997; Hobson et al., 1997; Fernandez-Mosquera et al., 2001; Dehn, in prep.). In addition, turnover rates and tissue and species-specific isotopic fractionation factors are poorly understood. Substantial variations in trophic shift have been reported for animals consuming either high versus low protein food or invertebrate versus plant diets (McCutchan et al., 2003; Sponheimer et al., 2003). Furthermore, differences in metabolic rate could result in different species-specific fractionation rates (Bearhop et al., 2002). For the marine ecosystem, trophic enrichment of ^{15}N can range from 2.4 ‰ for pinniped and bird muscle (Hobson and Clark, 1992; Hobson et al., 1996) to 3.0 ‰ for whale muscle (Dehn et al., submitted), and a general fractionation of 3.8 ‰ has been reported for muscle or total body homogenates of other food web components (Hobson and Welch, 1992; Hobson et al., 2002). Thus, caution should be taken when nitrogen isotopes are used to calculate trophic level based on general enrichment factors. Nevertheless, stable isotope signatures are powerful tools in establishing basic trophic interactions.

The purpose of this study is to identify trophic relationships (via $\delta^{15}N$) of selected heavy metals in the Arctic marine food chain, and compare trace element concentrations within this ecosystem. Foodweb magnification factors (FWMF) and biomagnification factors (BMF) are calculated to describe and quantify biomagnification of heavy metals in the Alaskan Arctic.

4.3 MATERIAL AND METHODS

4.31 Field Sampling and Tissue Processing

All marine mammal samples were obtained during native subsistence harvests. Ringed (*Phoca hispida*) and bearded seal (*Erignathus barbatus*) lumbar muscle, kidney and liver were collected in Barrow, Alaska, mainly during summer from 1998-2001. To increase sample size and statistical power, data from ringed seals harvested in Barrow during summer 1996 and 1997 (Woshner et al., 2001a) were included in the analyses. Ringed seal samples were also obtained in Holman, Canada, during summer, 2001. Tissues of spotted seals (*Phoca largha*) were collected in Little Diomede and Shishmaref, Alaska, in summer 2000 and 2001. Ribbon seal (*Phoca fasciata*) samples were acquired in Little Diomede and Hooper Bay, Alaska, during summer 2003. Walrus muscle was obtained in Barrow and Little Diomede on an opportunistic basis mainly during summer 1998 and 2003.

Epidermis, lumbar muscle, kidney and liver of bowheads were predominantly collected in Barrow either during spring or fall harvest 1998-2001. Data from bowheads harvested during 1995-1997 (Woshner et al., 2001b) and 1983 to 1990 (Bratton et al., 1997) were included in the data set. Samples of beluga whales (*Delphinapterus leucas*) harvested in Point Lay and Wainwright, Alaska, in 1998-1999 were combined with data obtained during 1996-1997 (Woshner et al., 2001b) and 1992-1995 (Tarpley et al., 1995) that displayed the appropriate biological variables to increase sample size and statistical power. Tissues of gray whales (*Eschrichtius robustus*) were sampled in Lorino and Lavrentiya, Russia, in 2001.

Polar bear samples were obtained opportunistically from subsistence hunters during winter 1999 and 2000 near Barrow. Bears sampled during 1996 and 1997 were analyzed for renal mercury (THg) and stable isotopes. Additional data for these bears (Woshner et al., 2001a) were included in the analyses. Personnel of the North Slope Borough Public Health Office Veterinary Clinic collected Arctic fox tissues in winter 1999 and spring 2000 in Barrow as part of the Animal Damage Control Program. Inuit hunters in Holman collected additional samples of Arctic fox in November 1999. Several potential prey species were collected or donated by subsistence hunters in Barrow and the Alaskan Bering Strait. Figure 4.1 shows Arctic villages and communities where samples were collected.

All tissues were sub-sampled under clean conditions with titanium or ceramic blades on a Teflon covered surface, following the sampling protocol for contaminants by Becker et al. (1999), and stored at -20°C in acid-washed scintillation vials or Whirlpacks™ until analysis. Marine mammal samples were collected and analyzed under the authority of Permit Nos. 782-1399 and 358-1585 issued to the Alaska Department of Fish and Game and 932-1489-03 issued to T. Rowles of the Marine Mammal Health and Stranding Response Program.

4.32 Stable Isotope Analyses

Muscle of marine and terrestrial mammals and total body homogenates of potential prey were freeze-dried and ground into a fine powder with mortar and pestle. For each sample, 0.2 to 0.4 mg of tissue was weighed into a 4.75 x 4 mm tin capsule, which was folded into a cube. Samples were analyzed for both stable carbon and nitrogen ratios at the University of Alaska Fairbanks (UAF) using a Finnigan MAT Delta^{Plus}XL Isotope Ratio Mass Spectrometer (IRMS) directly coupled to a Costech Elemental Analyzer (ESC 4010). Samples were flash combusted at 1020°C, followed by on-line chromatographic separation of sample N_2 and CO_2 with He as carrier gas. Samples analyzed for $^{15}N/^{14}N$ and $^{13}C/^{12}C$ were standardized against atmospheric N_2 and PeeDee Belemnite limestone, respectively. Enrichment of a particular isotope was reported using the equation:

$$\delta R‰ = ((R_{sample}/R_{standard}) - 1) \times 1000$$

where the differential notation (δR) represents the relative difference between isotopic ratios of the sample and standard gases (i.e., $^{13}C/^{12}C$, $^{15}N/^{14}N$). A laboratory-working standard (Peptone No. P-7750) was analyzed every 10 samples during analysis, and tin capsule blanks were run every 20 samples. Calibrations were made with the use of stable isotope reference materials provided by the National Institute of Standards and Technology (NIST). External instrument reproducibility for both carbon and nitrogen isotope analysis was ± 0.2 ‰.

4.33 Trace Metal Analyses

Silver (Ag) and cadmium (Cd) were analyzed at Texas A&M University (TAMU) following US Environmental Protection Agency (EPA) procedures (200.3, 200.7, 200.8 and 200.9) with slight modifications (EPA, 1992). Briefly, sub-sampled tissues were freeze-dried to a constant weight and homogenized by ball-milling. Powdered tissue (approximately 0.2 – 0.25 g) was digested in a microwave wet ash procedure using HNO_3, H_2O_2 and HCl. Whale tissues were homogenized and about 0.8-1.0 g of sample was subjected to a wet tissue digestion in the microwave wet ash procedure. Cd and Ag were analyzed using either Graphite Furnace Atomic Absorption Spectrometry (Perkin-Elmer Model SIMAA 6000 equipped with an AS-72 autosampler and Zeeman background correction) or by ICP-MS (Perkin-Elmer Elan Model 6100 DRC-II). The detection limit was 0.01 μg/g for elements analyzed with Graphite Furnace AAS and 0.01 μg/g for Cd and 0.005 μg/g for Ag using ICP-MS.

Total mercury (THg) was analyzed at UAF following the procedure established by Bloom and Crecelius (1983). Briefly, sub-sampled tissues were homogenized and approximately 1 g of tissue was digested in 7:3 HNO_3/H_2SO_4 and oxidized with 10 % BrCl in 12N HCL. The sample was reduced to Hg^0 with $SnCl_2$ and purged with N_2 onto gold-coated quartz sand traps followed by dual thermal desorption to a Cold Vapor Atomic Fluorescence Spectrometer (Tekran Model-2500 CVAFS Mercury Detector) with argon as carrier gas. The detection limit was 0.001 μg/g. All trace element concentrations are expressed as μg/g wet weight (ww) unless otherwise noted.

4.34 Statistical Analysis and Trophic Transfer Calculations

MANOVA was used to simultaneously compare means of $\delta^{15}N$, $\delta^{13}C$, Ag in liver, Cd in liver and kidney, and THg in liver and kidney between 9 marine and terrestrial mammal groups sampled (polar bear, Arctic fox from Barrow, ringed seal from both Holman and Barrow, bearded seal, spotted seal, bowhead whale, beluga whale and gray whale). The variables in the data set were ranked prior to analysis to reduce the risk of violations of normality and homogeneity of variance assumptions (Conover, 1999). Multiple one-way ANOVAs were implemented (controlled for Type I error using the Bonferroni approach with $\alpha = 0.001$) as follow-up tests to determine pairs of groups that are different in specific dependent variables.

Principal components analysis (PCA) was used on standardized data (Z scores / correlation matrix) to identify patterns and highlight similarities and differences between marine and terrestrial mammals. PCA on the correlation matrix is appropriate when adjustment for large variance differences between variables is necessary or when variables are not measured in comparable units (Johnson, 1998).

Simple linear regression analysis was used on all animals in the data set to determine food web magnification factors (FWMF) based on the relationship between $\delta^{15}N$ (as indicator for trophic position) and each metal concentration. The slope of the linear regression gives the mean rate of increase (FWMF) for selected trace metals (Cd and THg in liver and kidney and hepatic Ag) in this Arctic food web (Fisk et al., 2001).

Biomagnification factors (BMF) were calculated for selected predator-prey scenarios as:

$$BMF = (Metal_{Predator} / Metal_{Prey}) / (\delta^{15}N_{Predator} / \delta^{15}N_{Prey})$$

where $Metal_{Predator}$ and $Metal_{Prey}$ are the concentrations of selected metals in µg/g ww of the predator and prey, respectively (adapted from Hoekstra et al., 2003). BMF was corrected for differences in trophic position (based on $\delta^{15}N$) between predator and prey. BMF was calculated for liver and kidney in marine mammals. For invertebrates and fish, trace element concentrations of total body homogenates were used for calculations.

Statistical analyses were performed using SAS (Version 8) with 5 % significance level unless otherwise noted. Sigma-Plot (Version 7.0) was used for graphic presentation of data and linear regression. In order to include element concentrations below the minimum detection limit (MDL) into summary statistics and statistical tests, they were expressed as one-half the MDL (Gilbert, 1987). However, none of the tissues analyzed for trace metals showed more than 50 % of samples below MDL. Results are reported as mean ± standard deviation (SD) unless otherwise noted.

4.4 RESULTS

4.41 Stable Isotopes and Trace Metals

Significant differences (p = <0.0001) in the multivariate mean vectors of ranked values of $\delta^{15}N$, $\delta^{13}C$, Ag in liver, Cd and THg in liver and kidney among the different Arctic mammal groups were found using MANOVA. Table 4.1 summarizes the results of the follow-up pair-wise comparisons using multiple one-way ANOVAs. Though polar bears occupy a higher trophic level than belugas (based on $\delta^{15}N$), the metal concentrations between the two species were either not statistically different or were significantly lower for polar bears. Similarly, concentrations of renal and hepatic Cd were either significantly lower or not statistically different in polar bears compared to ringed and bearded seals, their primary prey. Mean $\delta^{15}N$ was lowest in muscle of Arctic fox, and foxes also show the lowest levels of Hg, Cd and Ag in liver and kidney compared to the other groups.

Mean values of stable carbon and nitrogen isotopes in Arctic marine and terrestrial mammals (analyzed in this study and compiled from literature) are given in Table 4.2. Stable carbon and nitrogen isotope signatures in muscle range widely. Mean $\delta^{15}N$ in muscle of belugas and ice seals (ringed, bearded and spotted seals) are not statistically different, though values are higher than for mysticetes. Stable nitrogen isotope ratios are significantly higher in bowheads than gray whales and $\delta^{15}N$ in typical prey of baleen whale (zooplankton and amphipods) is also isotopically distinct (Figure 4.2). Carbon-13 is enriched in bearded seal, walrus, and gray whale compared to other species and most depleted in Arctic fox. Figure 4.2 illustrates $\delta^{15}N$ versus $\delta^{13}C$ in marine and terrestrial vertebrates and some potential prey species.

The first two principal components (PC1 and PC2) explained 60 % of the variability in marine and terrestrial animals harvested in Alaska and Canada (Figure 4.3). Variables with the most weight in PC1 were $\delta^{15}N$ and THg in kidney and liver. The separation in PC2 was largely driven by a positive loading of renal and hepatic Cd (Figure 4.3). The nine groups are distinct, though ringed and bearded seal are similar and some overlap occurs between polar bear and beluga whale and also between bowhead and gray whale. Arctic fox can clearly be differentiated from the other species. The spread in PC1 closely resembles the trophic structure of the groups with the exception of spotted seals (see Figure 4.2) and is in agreement with the high loading of $\delta^{15}N$ on PC1. Spotted seals had comparatively low concentrations of THg in tissues based on their trophic standing. Table 4.3 gives means, standard deviations and ranges of concentrations for selected metals of mammals analyzed in this study and data compiled from literature for mammals from other Arctic regions.

4.42 Food Web Magnification and Biomagnification Factors

Magnification of renal Cd and THg and Ag in liver versus $\delta^{15}N$ in Arctic marine biota is illustrated in Figure 4.4. FWMFs ranged from 0.22 for Ag in liver to 1.86 for renal Cd (Table 4.4). The linear regression relationship was only significant for hepatic and renal THg ($p = 0.02$ and $p = 0.01$ for liver and kidney, respectively). A slope greater than 1 indicates accumulation in the food web (for FWMFs) or accumulation from predator to prey (for BMFs), while values less than 1 suggest active elimination of the element or interrupted trophic transfer. A two-tailed t-test was used to determine if the slope of the linear regression is statistically different from unity. Hepatic Ag and Cd had slopes significantly smaller than 1, while the slope of all other variables was not statistically different from unity (Table 4.4).

In this study, biomagnification of Cd occurred mainly in the kidney, while calculated BMFs were higher for hepatic THg than renal THg with the exception of polar bears. In bears the accumulation of renal THg was approximately 6 times higher than in liver (Table 4.4). Similarly, concentrations of THg in Arctic fox were higher in kidneys than in liver (Table 4.3). Accumulation of hepatic Ag was minimal for all selected predator-prey scenarios. The highest BMFs (above 350) were calculated for THg from zooplankton and shrimp to liver of ringed and bearded seals, respectively. Cd magnification from zooplankton to Arctic cod (*Boreogadus saida*) was not observed, but concentrations of THg (corrected for $\delta^{15}N$) increased 3-fold. Similarly, Cd was not transferred from ringed seal to polar bear in either kidney or liver, while THg increased significantly.

4.5 DISCUSSION

4.51 Stable Isotopes

The low trophic standing (based on $\delta^{15}N$) determined for Arctic fox in this study indicates that these animals are not feeding or scavenging on remains of pinnipeds or other large vertebrates as suggested by Chesemore (1968) and Smith (1976). Arvicoline rodents are the primary terrestrial food source of Arctic fox (Fay and Stephenson, 1989; Anthony et al., 2000). Mean nitrogen isotope ratios established for tundra voles (*Microtus oeconomus*) are approximately 7.0 ‰ (Schmutz and Hobson, 1998) and range from means of 9.3 ‰ to 11.3 ‰ in muscle of Arctic foxes from Holman and Barrow, respectively. These values are in good agreement with a diet dominated by microtine rodents, assuming a trophic enrichment of 3.5 ‰ for fox muscle (Roth and Hobson, 2000). However, based on mean nitrogen isotope ratios, caribou or reindeer (*Rangifer tarandus*) cannot be excluded as a food source for Arctic fox and have been described as fox prey in Svalbard (Prestrud, 1992; Szepanski et al., 1999; Frafjord, 2002). The depleted ^{13}C values for Arctic fox in this study from both Alaska and Canada also indicate that the animals rely on a terrestrial rather than marine diet (Angerbjörn et al., 1994; Kelly, 2000). Roth (2003) suggested that foxes feed on marine resources only when lemming populations are depleted. The capture years of foxes in this study (1999 and 2000) fell within high lemming (*Lemmus* spp. and *Dicrostonyx* spp.) and vole (*Microtus* spp. and *Clethrionomys* spp.) population cycles in the Alaskan and Canadian Arctic though geographical variation can be substantial (Krebs et al., 2002).

Polar bears occupy the highest trophic level (based on $\delta^{15}N$). In the Alaskan Arctic they prey mostly on ringed and bearded seals (Smith, 1980; Hammill and Smith, 1991). A number of reports show that bears in the circumpolar Arctic opportunistically hunt belugas and narwhals (*Monodon monoceros*), feed on carrion (e.g., bowhead and gray whale carcasses) and may prey on terrestrial ungulates (e.g., caribou) (Lowry et al., 1987; Smith and Sjare, 1990; Derocher et al., 2000; Miller et al., 2004). Mean nitrogen isotope ratios in muscle of ringed seals and polar bears suggest a ^{15}N trophic enrichment of 3.7 ‰, assuming a bear feeding exclusively on ringed seal. However, the close range of mean nitrogen isotope ratios in ringed, bearded and spotted seals and belugas and narwhals (Table 4.2) made it difficult to discern the proportions of these potential prey species to the polar bear diet. Based on mean $\delta^{15}N$ ratios in bear muscle in this study it is improbable that bowhead or gray whale carrion made up a considerable fraction of their annual diet. Enriched ^{13}C values in polar bears suggest reliance on the marine rather than terrestrial food chain. This is in accordance with Ramsay and Hobson (1991), who concluded that terrestrial food sources are negligible in the polar bear diet.

The diet of pagophilic seals is highly variable, and this is reflected by the large, but overlapping ranges of $\delta^{15}N$ (Figure 4.2). However, spotted seal $\delta^{15}N$ is slightly higher than that of other seals and is in agreement with a higher frequency of fish in spotted seal diet (Bukhtiyarov et al., 1984; Dehn, in prep.). Invertebrates make up the majority of the bearded seal prey; they are seasonally important to ringed seals, and invertebrate consumption is dependent on age in ringed seals (Lowry et al., 1980a; Lowry et al., 1980b; Dehn, in prep.). Little is known about ribbon seal feeding due to their remote distribution in the pack ice. Available reports suggest that nektobenthos, e.g., walleye pollock (*Theragra chalcogramma*) and cephalopods are of importance to adult ribbon seals, while pups and juveniles utilize small crustaceans (Shustov, 1965; Frost and Lowry, 1980; Fedoseev, 2000). Stable nitrogen isotopes in assumed prey of adult ribbon seals range from 13.6 ± 1.2 ‰ in cephalopods to 14.2 ± 2.0 ‰ in pollock (Figure 4.2). Mean $\delta^{15}N$ values of ribbon seals (16.0 ± 1.2 ‰) are in good agreement with the proposed prey, assuming a nitrogen fractionation rate of 2.4 ‰ from prey to seal muscle (Hobson et al., 1996). Nitrogen isotope ratios are low in walrus muscle compared to that of other pinnipeds (Table 4.2), and the significance of clams in their diet has been emphasized (Lowry et al., 1980b; Fay, 1982; Fay et al., 1984). Mean $\delta^{15}N$ values in Greenland cockle (*Serripes groenlandicus*) homogenates (commonly found in walrus stomachs) range from 8.0 ‰ to 8.9 ‰ (Hobson and Welch, 1992; Dehn, in prep.). Bearded seals and walrus are significantly enriched in carbon-13 compared to the other seals. Both feed benthically, and the enriched carbon-13 values reflect the use of this habitat (France, 1995). In contrast, ringed, spotted and ribbon seals are pelagic feeders most of the year (Burns, 1970; Fedoseev, 2000).

Beluga whales feed on a higher trophic level (based on $\delta^{15}N$) than either bowhead and gray whales. This finding is in accordance with a diet dominated by fish described for belugas, though cephalopods and other invertebrates are commonly eaten (Seaman et al., 1982; Huntington, 2000). Nitrogen isotope ratios are not different for ringed, bearded and spotted seals (Table 4.1), and, as described above, ice seals also feed opportunistically on a wide variety of prey so that competition for prey between ice seals and belugas is probable (Seaman et al., 1982). In contrast, bowhead and gray whales are fairly specialized filter-feeding predators. Bowheads consume primarily pelagic euphausids and copepods (Lowry, 1993; Lowry and Sheffield, 2002), while gray whales are unique in their reliance on benthic gammaridean amphipods (Rice and Wolman, 1971; Bogoslovskaya et al., 1981). Both prey types (zooplankton and amphipods) are isotopically distinct, thus leading to discernable isotope ratios in muscle of bowhead and gray whale (Figure 4.2). Bowheads feed mostly in the water column, and their depleted carbon-13 value in muscle is indicative for the pelagic foodweb. Gray whales display the enriched ^{13}C signature of the benthic food chain and their ratios are not different from other benthic feeders, e.g., bearded seal (Figure 4.2). Belugas had intermediate $\delta^{13}C$ values between bowhead and gray whales, suggesting that both pelagic and benthic foods are important components of their diet.

4.52 Trace Metals

A comparison with existing data from other Arctic regions (Table 4.3) shows generally lower concentrations of Cd and Hg in liver and kidney of subsistence-harvested marine and terrestrial mammals in Alaska. Hepatic Ag is rarely reported for Arctic mammals. Ag was highest in liver of belugas compared to other species by 1 to 2 orders of magnitude. These high concentrations have long been a mystery and possible causes for Ag accumulation have been discussed (Becker et al.; 1995, Becker et al.; 2000; Woshner et al., 2001a; Dehn et al., submitted). Ag shows a possible connection to the benthic food chain or cephalopod and crustacean prey (Martin and Flegal, 1975; Rouleau et al., 2000), as bearded seals and walrus (aside from belugas) have higher hepatic Ag concentrations than pelagic feeding bowhead whales, spotted seals, and harp seals (*Phoca groenlandica*).

Table 4.3 shows that the highest Cd concentrations in the circumpolar Arctic have been measured in tissues of hooded seals (*Cystophora cristata*) from Greenland (Julshamn and Grahl-Nielsen, 2000). Hepatic THg levels were also highest for hooded seals, which are deep divers and feed on a variety of fish and squid (Hauksson and Bogason, 1997; Folkow and Blix, 1999). Bustamante et al. (1998) indicated that cephalopods are a main vector in the trophic flux of Cd, while fish accumulate and transfer Hg (Baeyens et al., 2003). Hg accumulation from sediments has also been suggested as a dominant pathway for Hg uptake (Bryan and Langston, 1992) and could explain elevated concentrations of Hg in deep-diving hooded seals. Interestingly, piscivorous spotted seals had the lowest THg concentrations in their tissues compared to other Arctic pinnipeds (Table 4.3). However, Dehn et al. (2005) reported that the ratio of organic to inorganic Hg was highest in tissues of spotted seals and they suggested that this ratio might be a better indicator for piscivory than THg alone.

The lowest concentrations of metals selected in this study were found in Arctic fox. This is in agreement with the low trophic position established for this species. Concentrations of THg and Cd measured in liver of red-backed vole (*Clethrionomys rutilus*) sampled from Inuvik, Northwest Territories, Canada were 0.08 μg/g and 0.12 μg/g ww for THg and Cd, respectively (after Lodenius et al., 2002, a conversion factor of 0.27 was used for liver to transform Cd concentrations from dry weight to wet weight)

(Poole et al., 1998). On the other hand, concentrations of THg and Cd in liver of caribou and reindeer from Svalbard and Greenland exceed levels established for rodents by orders of magnitude (Borch-Iohnsen et al., 1996; Aastrup et al., 2000). Cd concentrations were higher in Arctic foxes from Svalbard than in foxes from Alaska and Canada analyzed in this study, while THg levels were comparable at a similar mean age (Prestud et al., 1994). Arvicoline rodents are absent from Svalbard with the exception of some small and isolated vole populations (Fuglei and Øritsland, 1999). Thus, Arctic fox in Svalbard rely either on the marine ecosystem for food or utilize reindeer carrion, explaining the higher levels of Cd found in their tissues (Prestrud, 1992; Pond et al., 1995, Frafjord, 2002).

In comparison, THg concentrations in tissues of polar bears from Alaska and Greenland were higher than in bears from Svalbard. Cd concentrations were similar in bears sampled in Alaska and Svalbard, while Cd in tissues of Greenland bears was higher (Table 4.3). This is in agreement with a geographic increase of Hg from eastern to western Arctic Canada reported for a variety of marine mammal tissues, which was attributed to a gradient in natural geological concentrations (Wagemann et al., 1996). In contrast, Cd concentrations were higher in the Eastern than the Western Arctic in polar bears, ringed seals and beluga whales (Norstrom et al., 1986; Braune et al., 1991; Wagemann et al., 1996).

Belugas, narwhals and harbor porpoise (*Phocoena phocoena*) have a higher trophic standing (Table 4.2) than mysticetes, and concentrations of THg in odontocete tissues are up to two orders of magnitude higher (Table 4.3). Renal and hepatic concentrations of Cd, on the other hand, are similar or even higher in filter-feeding bowheads than in belugas. Renal and hepatic Cd in gray whales was considerably lower than in other cetaceans from polar waters (Table 4.3).

4.53 Food Web Magnification

The calculation of FWMFs in this study indicates that magnification of selected heavy metals in the Arctic foodweb is not significant or FWMFs are significantly smaller than unity, suggesting active elimination of some metals rather than magnification (Table 4.4). In addition, low R^2-values of the regressions illustrate that the underlying relationships lack linearity (Figure 4.4). The calculation of these transfer factors makes a variety of assumptions that may not be representative for the biomagnification of heavy metals. It is assumed that the contaminant burden originates from the prey and that the tissue under evaluation is a good proxy for total body burden (Fisk et al., 2001; McGeer et al., 2003). However, metal uptake and adsorption over the gills and other body surfaces may be an important route of exposure for invertebrates and fish (Rainbow, 1996; Laporte et al., 2002; Rainbow and Black, 2004; Wood et al., 2004) and uptake via ingested seawater or lungs cannot be excluded in marine mammals (Law, 1996).

In contrast to organochlorines (OC's) that are mainly concentrated in lipid-rich tissues (e.g., blubber), trace metal storage and detoxification may be more organ specific and metal dependent, e.g., Cd accumulation in kidney, lead (Pb) in bone and arsenic (As) in blubber and liver (Hu et al., 1998; Gallien et al., 2001; Kubota et al., 2001; Ebisuda et al., 2003). It should be considered that most trace metals are dynamic, actively regulated, dependent on transport molecules, and subjected to binding site competition (McGeer et al., 2003). Tracing pathways of Hg can be particularly challenging, depending on its chemical form and presence of demethylation processes. Hg can accumulate in the brain via exposure to MeHg and Hg vapor that can cross the blood-brain-barrier, and is there converted to inert HgII (Sichak et al., 1986; Aschner and Aschner, 1990). MeHg is also known to accumulate in muscle, epidermis, hair, and other keratinized structures and is transferred to the fetus via the placenta (Wagemann et al., 1988; Shi et al., 1990; Wagemann et al., 1990; Dehn et al., 2005; Dehn et al., submitted). Hg is commonly associated with selenium (Se) in liver as biologically inert Se-Hg granules (tiemannite), and this has led to the often-discussed protective effect of Se on Hg toxicosis (Koeman et al., 1973; Danscher and Møller-Madsen, 1985; Caurant et al., 1996; Nigro et al., 2002; Arai et al., 2004; Decataldo et al., 2004). Renal metallothionein may be involved in the detoxification and accumulation of Hg in terrestrial, but not marine mammals (Caurant et al., 1996; Satoh et al., 1997; Das et al., 2000; Decataldo et al., 2004). Active elimination and demethylation of Hg can also occur with bilary excretion and the reticuloendothelial system, respectively (Suda and Takahashi, 1990; Nigro et al., 2002; Berntssen et al., 2004). Wagemann et al. (1996) suggested that approximately 14 % of epidermal MeHg could be actively eliminated via skin molt in cetaceans. Similarly, Cd can be bound to metallothionein or deposited in storage granules (Gallien et al., 2001; Das et al., 2002; Decataldo et al., 2004) and is thus not bioavailable to predators and disconnected from trophic transfer (Groten et al., 1990; Groten et al., 1991; Lind et al., 2001). Non-essential Cd is chemically similar to zinc (Zn), an essential element regulated within tight homeostatic margins. Among others, this leads to interactions of Cd and Zn, binding site competition on metallothionein, re-absorption of both essential and toxic elements in renal tubule cells and increased uptake of Cd when dietary Zn is marginal (Cousins, 1986; Reeves and Chaney, 2004; Barbier et al., 2005). Correspondingly, Ag is chemically comparable to essential copper (Cu) and can interfere with Cu metabolism and transport (Saeki et al., 2001). Trophic transfer of Ag can be interrupted by adsorption of Ag to the carapace of crustaceans and shedding of this exoskeleton and associated Ag (Ratte, 1999). It is also possible that trophic transfer of Ag only occurs in the benthic food chain as described above. Molting is considered a primary pathway of Ag excretion in pinnipeds (Saeki et al., 2001).

Furthermore, metal deposition in animal tissues is strongly dependent on numerous physical and biological factors, e.g., geography, sex, age, health status and body condition (Honda et al., 1983; Wagemann et al., 1996; Watanabe et al., 2002; Dehn et al., 2005; Dehn et al., submitted). The induction of metallothionein and thus binding potential of some trace metals is itself influenced by a variety of factors. Liver metallothionein is increased in fetus and in pregnant or lactating females to store essential elements, thus giving the opportunity for other non-essential elements (e.g., Cd) to compete for binding sites (Bremner and Beattie, 1990; Teigen et al., 1999; Solaiman et al., 2001). This may lead to differences in metal accumulation patterns between males and females and juvenile and adult animals. Altered accumulation mechanisms of heavy metals have been described for compromised, stranded or starving animals and during epizootics. For example, elevated Zn concentrations and altered Zn kinetics are a response to stressors, poor body condition, and infection (Frank et al., 1992; Olsson et al., 1994; Bennet et al., 2001; Anan et al., 2002; Das et al., 2004a; Ilbäck et al., 2004; Dehn et al., submitted).

4.54 Biomagnification

BMF calculations are based on trace metal comparisons of predator and assumed prey (corrected for $\delta^{15}N$ ratio differences) and are summarized in Table 4.4. It is assumed that the selected comparisons are representative of simple predator-prey relationships; however, that may not be true. For example, bearded seals in particular have a diverse diet (Lowry et al., 1980b; Dehn, in prep), ringed seals are known to shift their diet with age and season, have prey selection differences based on sex (Lowry et al., 1980a; Dehn, in prep.), and diets of some species, such as ribbon seals and belugas, are largely unknown (Frost and Lowry, 1980; Seaman et al., 1982). The extent to which polar bears may be scavenging on remains of subsistence-harvested or stranded marine mammal carcasses or terrestrial food sources, as discussed above, is not well understood (Derocher et al., 2000; Miller et al., 2004). The calculation of BMF's also assumes that predators completely consume the prey. However, studies have suggested that some polar bears will selectively feed on seal blubber (Stirling and McEwan, 1975, Smith, 1980), and thus are exposed to very low concentrations of THg, Cd and Ag (Woshner et al, 2001a). On the other hand, THg and other trace elements like Se and Zn are higher in muscle and also in the epidermis of cetaceans, and Hg occurs mainly as bioavailable MeHg in these tissues (Paludan-Müller et al., 1993; Wagemann et al., 1996; Dehn et al., submitted). This could explain why Cd does not biomagnify from prey to polar bear, whereas THg does.

Overall, calculation of BMFs between predator and potential prey warrants similar concerns as discussed above, e.g., internal metal dynamics, essentiality and binding site competition. This becomes evident when comparing BMFs of THg in kidney and liver between marine and terrestrial mammals. Accumulation of THg is higher in kidneys of terrestrial mammals than marine species (Table 4.3 and Table 4.4). This is most likely due to differences in accumulation mechanisms and, as discussed above, renal metallothionein is involved in binding and bioconcentration of Hg in terrestrial but not marine mammals (Caurant et al., 1996; Satoh et al., 1997; Das et al., 2000; Decataldo et al., 2004).

The very high BMFs calculated for THg from zooplankton and shrimp to ringed and bearded seals, respectively, indicate that the seals do not exclusively depend on these dietary items. In addition, fish, not invertebrates, may be the main route by which seals are exposed to Hg as discussed above. The opposite may be true for Cd, as several studies have observed higher concentrations of Cd in marine mammals that rely on invertebrates rather than fish (Watanabe et al., 2002; Bustamante et al., 2004b; Dehn et al., 2005; Dehn et al., submitted). Exceptionally high concentrations of Cd have been reported for invertebrates from polar waters (Bohn and McElroy, 1976; Hamanaka and Ogi, 1984; Macdonald and Sprague, 1988; Ritterhoff and Zauke, 1997), while fish generally have lower concentrations (Bohn and McElroy, 1976; Bustamante et al., 2003). It is notable that Ag in this study has a very low accumulation potential in marine mammal tissues with the exception of beluga tissues. This indicates that Ag is either actively eliminated in the species analyzed except for belugas or that belugas have a predilection for Ag for reasons yet unknown. Overall, more data from polar regions is needed, particularly at the base of the food chain, to accurately assess biomagnification of heavy metals in the Arctic.

4.6 SUMMARY AND CONCLUSION

In conclusion, concentrations of trace elements in tissues of marine and some terrestrial mammals harvested in Alaska are generally lower than reported for other Arctic regions. Magnification of the selected heavy metals (THg, Cd and Ag) was not significant for the components of the Arctic food web analyzed in this study. Calculation of BMFs suggested organ-specific accumulation of Cd in the kidney. Concentrations of THg and BMFs were higher in liver than kidney for all selected predator-prey scenarios with the exception of polar bear and Arctic fox, reflecting the involvement of renal metallothionein in binding and accumulation of THg in terrestrial but not marine mammals. Hepatic Ag has low magnification potential in the food web and in mammalian tissues with the exception of beluga tissues. Polar bears occupied the highest trophic level, though concentrations of trace metals were not statistically different or significantly lower in bears than in belugas. Correspondingly, Cd did not biomagnify from ice seal to polar bear tissues, while THg increased significantly. The calculations of BMFs from predator to prey usually assume the complete ingestion of prey though polar bears may selectively feed on seal or whale blubber, muscle and epidermis. Mean $\delta^{15}N$ in muscle of belugas and ice seals were not statistically different and the close range of mean nitrogen isotope ratios made it difficult to discern the proportions of these potential prey species to the polar bear diet. The low trophic standing (based on $\delta^{15}N$) and equally low concentrations of trace metals determined for Arctic fox sampled in this study is in agreement with a diet dominated by arvicoline rodents or other low trophic level terrestrial prey. A variety of assumptions are made when calculating trophic transfer factors that may not be suitable for trace metals. Some of these elements are actively regulated or deposited as inert storage molecules, and thus made unavailable for trophic transfer. In addition, trace metals are affected by basic, biological factors such as sex, reproductive status, age and body condition. Therefore careful deliberation should be given to the interpretation of biomagnification and transfer of trace elements.

4.7 ACKNOWLEDGEMENTS

We thank the subsistence hunters and whaling captains in the communities of Barrow, Holman, Little Diomede, Shishmaref, Kaktovik, Wainwright, Point Lay, Point Hope, Savoonga, Hooper Bay, Lorino and Lavrentiya for their cooperation and support in providing samples for this study. We greatly appreciate the assistance of the North Slope Borough (NSB) Public Health Office Veterinary Clinic and the Department of Wildlife Management for arranging Arctic fox samples and R. Highsmith and B. Bluhm for collection of amphipod samples from the Bering Strait. This study would not have been possible without the guidance and helping hands of C. D. N. Brower, H. Brower, Jr., B. Akootchook, T. Olemaun, T. Hepa, L. Hopson, C. George, R. Suydam, G. Zelensky, V. Woshner, R. Elsner, T. Zenteno-Savin, S. Visalli, D. Burnett, G. York and many others in the field and G. Bratton, R. Taylor, N. Haubenstock, T. Howe, T. Bentzen and P.

Hoekstra for support with analysis. We also thank L. Harwood for providing tissues and jaws of ringed seals harvested in Holman, Canada. P. Becker and S. Christopher provided marine mammal reference material and coordinated interlaboratory comparison exercises for the determination of trace elements in marine mammals. This study was primarily funded by the Cooperative Institute for Arctic Research (CIFAR). Additional support was provided by the Experimental Program for Stimulation of Competitive Research (EPSCoR); the IdeA Network for Biomedical Research Excellence (INBRE); the North Slope Borough Department of Wildlife Management; the Institute of Arctic Biology and the Department of Biology and Wildlife, UAF.

4.8 REFERENCES

Aastrup P, Riget F, Dietz R, Asmund G. Lead, zinc, cadmium, mercury, selenium and copper in Greenland caribou and reindeer (*Rangifer tarandus*). Sci Total Environ 2000; 245: 149-159.

AMAP. Persistent toxic substances, food security and indigenous peoples of the Russian North. Final Report. Oslo, Norway, 2004, 192 pp.

Anan Y, Kunito T, Ikemoto T, Kubota R, Watanabe I, Tanabe S, Miyazaki N, Petrov EA. Elevated concentrations of trace elements in Caspian seals (*Phoca caspica*) found stranded during the mass mortality events in 2000. Arch Environ Contam Toxicol 2002; 42: 354-362.

Angerbjörn A, Hersteinsson P, Liden K, Nelson E. Dietary variation in arctic foxes (*Alopex lagopus*) - an analysis of stable carbon isotopes. Oecologia 1994; 99: 226-232.

Anthony RM, Barten NL, Seiser PE. Foods of Arctic foxes (*Alopex lagopus*) during winter and spring in western Alaska. J Mamm 2000; 81: 820-828.

Arai T, Ikemoto T, Hokura A, Terada Y, Kunito T, Tanabe S, Nakai I. Chemical forms of mercury and cadmium accumulated in marine mammals and seabirds as determined by XAFS analysis. Environ Sci Technol 2004; 38: 6468-6474.

Aschner M, Aschner JL. Mercury neurotoxicity: mechanism of blood-brain barrier transport. Neurosci Biobehav Rev 1990; 14: 169-176.

Baeyens W, Leermakers M, Papina T, Saprykin A, Brion N, Noyen J, De Gieter M, Elskens M, Goeyens L. Bioconcentration and biomagnification of mercury and methylmercury in North Sea and Schelt Estuary fish. Arch Environ Contam Toxicol 2003; 45: 498-508.

Barbier O, Dauby A, Jacquillet G, Tauc M, Poujeol P, Cougnon M. Zinc and cadmium interactions in a renal cell line derived from rabbit proximal tubule. Nephron Physiol 2005; 99: 74-84.

Barboza PS, Farley SD, Robbins CT. Whole-body urea cycling and protein turnover during hyperphagia and dormancy in growing bears (*Ursus americanus* and *U. arctos*). Can J Zool 1997; 75: 2129-2136.

Bard HM. Global transport of anthropogenic contaminants and the consequences for the Arctic marine ecosystem. Mar Pollut Bull 1999; 38: 356-379.

Barrie LA, Gregor D, Hargrave B, Lake R, Muir D, Shearer R, Tracey B, Bidleman T. Arctic contaminants: sources, occurrence and pathways. Sci Total Environ 1992; 122: 1-74.

Bearhop S, Waldron S, Votier SC, Furness RW. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. Physiol Biochem Zool 2002; 75: 451-458.

Becker PR, Mackey EA, Demiralp R, Suydam R, Early G, Koster BJ, Wise SA. Relationship of silver with selenium and mercury in the liver of two species of toothed whales (Odontocetes). Mar Pollut Bull 1995; 30: 262-271.

Becker PR, Porter BJ, Macke EA, Schantz MM, Demiralp R, Wise SA. National Marine Mammal Tissue Bank and Quality Assurance Program: protocols, inventory, and analytical results. 1999. Gaithersburg, U. S. Department of Commerce. NISTIR 6279, 183 pp.

Becker PR, Krahn MM, Mackey EA, Demiralp R, Schantz MM, Spstein MS, Donais MK, Porter BJ, Muir DCG, Wise SA. Concentrations and polychlorinated biphenyls (PCB's), chlorinated pesticides, and heavy metals and other elements in tissues of belugas, *Delphinapterus leucas*, from Cook Inlet, Alaska. Mar Fish Rev 2000; 62: 81-98.

Bell RA, Kramer JR. Structural chemistry and geochemistry of silver-sulfur compounds: Critical review. Environ Toxicol Chem 1999; 18: 9-22.

Belles-Isles M, Ayotte P, Dewailly E, Weber J-P, Roy R. Cord blood lymphocyte functions in newborns from a remote maritime population exposed to organochlorines and methylmercury. J Toxicol Environ Health A 2002; 65: 165-182.

Bennett PM, Jepson PD, Law RJ, Jones BR, Kuiken T, Baker JR, Rogan E, Kirkwood JK. Exposure to heavy metals in infectious disease mortality in harbour porpoises from England and Wales. Environ Pollut 2001; 112: 33-40.

Berntssen MH, Hylland K, Lundebye AK, Julshamn K. Higher faecal excretion and lower tissue accumulation of mercury in Wistar rats from contaminated fish than from methylmercury chloride added to fish. Food Chem Toxicol 2004; 40: 1359-1366.

Berthet B, Amiard JC, Amiard-Triquet C, Martoja M, Jeantet AY. Bioaccumulation, toxicity and physico-chemical speciation of silver in bivalve molluscs: ecotoxicological and health consequences. Sci Total Environ 1992; 125: 97-122.

Bloom NS, Crecelius EA. Determination of mercury in seawater at sub-nanogram per liter levels. Mar Chem 1983; 14: 49-59.

Boening DW. Ecological effects, transport, and fate of mercury: a general review. Chemosphere 2000; 40: 1335-1351.

Bogoslovskaya LS, Vorogov LM, Semenova TN. Feeding habits of the gray whale off Chukotka. Rep Int Whal Commn 1981; 31: 507-510.

Bohn A, McElroy RO. Trace metals (As, Cd, Cu, Fe and Zn) in Arctic cod, *Boreogadus saida*, and selected zooplankton from Strathcona Sound, northern Baffin Island. J Fish Res Bd Can 1976; 33: 2836-2840.

Borch-Iohnsen B, Nilssen KJ, Norheim G. Influence of season and diet on liver and kidney content of essential elements and heavy metals in Svalbard reindeer. Biol Trace Elem Res 1996; 56: 235-247.

Born EW, Outridge P, Riget FF, Hobson KA, Dietz R, Øien N, Haug T. Population substructure of North Atlantic minke whales (*Balaenoptera acutorostrata*) inferred from regional variation of elemental and stable isotopic signatures in tissues. J Mar Sys 2003; 43: 1-17.

Bratton GR, Flory W, Spainhour CB, Haubold EM. Assessment of selected heavy metals in liver, kidney, muscle, blubber, and visceral fat of Eskimo harvested bowhead whales *Balaena mysticetus* from Alaska's North Coast. 1997. Barrow, Alaska, Department of Wildlife Management, North Slope Borough, 233 pp.

Braune BM, Norstrom RJ, Wong MP, Collins BT, Lee J. Geographical distribution of metals in livers of polar bears from the Northwest Territories, Canada. Sci Total Environ 1991; 100: 283-299.

Bremner I, Beattie JH. Metallothionein and the trace minerals. Annu Rev Nutr 1990; 10: 63-83.

Bryan GW, Langston WJ. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. Environ Pollut 1992; 76: 89-131.

Bukhtiyarov YA, Frost KJ, Lowry LF. New information on foods of the spotted seal, *Phoca largha*, in the Bering Sea in spring. U.S. Department of Commerce, NOAA Technical Report 1984; 12: 55- 59.

Burns JJ. Remarks on the distribution and natural history of pagophilic pinnipeds in the Bering and Chukchi Seas. J Mamm 1970; 51: 445-454.

Burton RK, Koch PL. Isotopic tracking of foraging and long-distance migration in northeastern Pacific pinnipeds. Oecologia 1999; 119: 578-585.

Bustamante P, Caurant F, Fowler SW, Miramand P. Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. Sci Total Environ 1998; 220: 71-80.

Bustamante P, Bocher P, Cherel Y, Miramand P, Caurant F. Distribution of trace elements in the tissues of benthic and pelagic fish from the Kerguelen Islands. Sci Total Environ 2003; 313: 25-39.

Bustamante P, Teyssie J-L, Danis B, Fowler SW, Miramand P, Cotret O, Warnau M. Uptake, transfer and distribution of silver and cobalt in tissues of common cuttlefish *Sepia officinalis* at different stages of its life cycle. Mar Ecol Prog Ser 2004a; 269: 185-195.

Bustamante P, Morales CF, Mikkelsen B, Dam M, Caurant F. Trace element bioaccumulation in grey seals *Halichoerus grypus* from Faroe Islands. Mar Ecol Prog Ser 2004b; 267: 291-301.

Caurant F, Navarro M, Amiard J-C. Mercury in pilot whales: possible limits to the detoxification process. Sci Total Environ 1996; 186: 95-104.

Chesemore DL. Notes on the food habits of Arctic foxes in northern Alaska. Can J Zool 1968; 46: 1127-1130.

Clapham PJ, Young SB, Brownell RL. Baleen whales: conservation issues and the status of the most endangered populations. Mamm Rev 1999; 29: 35-60.

Conover WJ. Practical nonparametric statistics. John Wiley and Sons, Inc., New York, 1999, 584 pp.

Cousins RJ. Toward a molecular understanding of zinc metabolism. Clin Physiol Biochem 1986; 4: 20-30.

Danscher G, Møller-Madsen B. Silver amplification of mercury sulfide and selenide: a histochemical method for light and electron microscopic localization of mercury in tissue. J Histochem Cytochem 1985; 33: 219-228.

Das K, Debacker V, Bouquegneau JM. Metallothioneins in marine mammals. Cell Mol Biol 2000; 46: 283-294.

Das K, Jacob V, Bouquegneau JM. White-sided dolphin metallothioneins: purification, characterization and potential role. Comp Biochem Physiol C 2002; 131: 245-251.

Das K, Lepoint G, Leroy Y, Bouquegneau JM. Marine mammals from the southern North Sea: feeding ecology data from $\delta^{13}C$ and $\delta^{15}N$ measurements. Mar Ecol Prog Ser 2003; 263: 287-298.

Das K, Siebert U, Fontaine M, Jauniaux T, Holsbeek L, Bouquegneau J-M. Ecological and pathological factors related to trace metal concentrations in harbour porpoises *Phocoena phocoena* from the North Sea and adjacent areas. Mar Ecol Prog Ser 2004a; 281: 283-295.

Das K, Holsbeek L, Browning J, Siebert U, Birkun A , Bouquegneau J-M. Trace metal and stable isotope measurements ($\delta^{13}C$ and $\delta^{15}N$) in the harbour porpoise *Phocoena phocoena relicta* from the Black Sea. Environ Pollut 2004b; 131: 197-204.

Decataldo A, Di Leo A, Giandomenico S, Cardellicchio N. Association of metals (Mercury, cadmium and zinc) with metallothionein-like proteins in storage organs of stranded dolphins from the Mediterranean sea (Southern Italy). J Environ Monit 2004; 6: 361-367.

Dehn, L-A. Trophic relationships in an Arctic marine foodweb and implications for trace element dynamics. Ph.D. thesis, Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, Alaska, in preparation.

Dehn L-A, Follmann EH, Rosa C, Duffy LK, Thomas DL, Bratton GR, Taylor RJ, O'Hara TM. Stable isotope and trace element status of subsistence hunted bowhead (*Balaena mysticetus*) and beluga whales (*Delphinapterus leucas*) in Alaska and gray whales (*Eschrichtius robustus*) in Chukotka. Mar Pollut Bull submitted.

Dehn L-A, Sheffield GG, Follmann EH, Duffy LK, Thomas DL, Bratton GR, Taylor RJ, O'Hara TM. Trace elements in tissues of phocid seals harvested in the Alaskan and Canadian Arctic – Influence of age and feeding ecology. Can J Zool; 2005; 83: 726-746.

Derocher AE, Wiig Ø, Bangjord G. Predation of Svalbard reindeer by polar bears. Polar Biol 2000; 23: 675-678.

Deutch B, Hansen JC. High human plasma levels of organochlorine compounds in Greenland. Dan Med Bull 2000; 47: 132-137.

Dietz R, Riget F, Johansen P. Lead, cadmium, mercury and selenium in Greenland marine animals. Sci Total Environ 1996; 186: 67-93.

Dietz R, Paludan-Müller P, Agger CT, Nielsen CO. Cadmium, mercury, zinc and selenium in ringed seals (*Phoca hispida*) from Greenland and Svalbard. NAMMCO Scientific Publications, Tromsø, 1998, pp. 242-273.

Ebinghaus R, Kock HH, Temme C, Einax JW, Löwe AG, Richter A, Burrows JP, Schroeder WH. Antarctic springtime depletion of atmospheric mercury. Environ Sci Technol 2002; 36: 1238-1244.

Ebisuda K, Kunito T, Fujihara J, Kubota R, Shibata Y, Tanabe S. Lipid-soluble and water-soluble arsenic compounds in blubber of ringed seal (*Pusa hispida*). Talanta 2003; 61: 779-787.

Fay FH. Ecology and biology of the Pacific Walrus, *Odobenus rosmarus divergens* Illiger. United States Department of the Interior, Fish and Wildlife Service, North American Fauna 74, Washington, D. C., 1982, 279 pp.

Fay FH, Bukhtiyarov YA, Stoker SW, Shults LM. Foods of the Pacific walrus in winter and spring in the Bering Sea. NOAA Technical Report, NMFS 1984; 12: 81- 88.

Fay FH, Stephenson RO. Annual, seasonal and habitat-related variation in feeding habits of the arctic fox (*Alopex lagopus*) on St. Lawrence Island, Bering Sea. Can J Zool 1989; 67: 1986-1994.

Fedoseev GA. Population biology of ice-associated forms of seals and their role in the northern Pacific ecosystems. Center for Russian Environmental Policy, Moscow, 2000, 271 pp.

Fernandez-Mosquera D, Vila-Taboada M, Grandal-d'Anglade A. Stable isotopes data ($\delta^{13}C$, $\delta^{15}N$) from the cave bear (*Ursus spelaeus*): a new approach to its palaeoenvironment and dormancy. Proc R Soc London, Ser B 2001; 268: 1159-1164.

Fisk AT, Hobson KA, Norstrom RJ. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the northwater polynya marine food web. Environ Sci Technol 2001; 35: 732-738.

Folkow LP, Blix AS. Diving behaviour of hooded seals (*Cystophora cristata*) in the Greenland and Norwegian seas. Polar Biol 1999; 22: 61-74.

Frafjord K. Predation on an introduced vole *Microtus rossiaemeridionalis* by Arctic fox *Alopex lagopus* on Svalbard. Wildlife Biol 2002; 8: 41-47.

France RL. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Mar Ecol Prog Ser 1995; 124: 307-312.

Frank A, Galgan V, Roos A, Olsson M, Petersson LR, Bignert A. Metal concentrations in seals from Swedish waters. Ambio 1992; 21: 529-538.

Frost KJ, Lowry LF. Feeding of ribbon seals (*Phoca fasciata*) in the Bering Sea in spring. Can J Zool 1980; 58: 1601-1607.

Fuglei E, Øritsland NA. Seasonal trends in body mass, food intake and resting metabolic rate, and induction of metabolic depression in arctic foxes (*Alopex lagopus*) at Svalbard. J Comp Physiol B 1999; 169: 361-369.

Gallien I, Caurant F, Bordes M, Bustamante P, Miramand P, Fernandez B, Quellard N, Babin P. Cadmium-containing granules in kidney tissue of the Atlantic white-sided dolphin (*Lagenorhyncus acutus*) off the Faroe Islands. Comp Biochem Physiol C 2001; 130: 389-395.

Gauthier JM, Dubeau H, Rassart E. Mercury-induced micronuclei in skin fibroblasts of beluga whales. Environ Toxicol Chem 1998; 17: 2487-2493.

George JC, Bada J, Zeh J, Scott L, Brown SE, O'Hara T, Suydam R. Age and growth estimates of bowhead whales (*Balaena mysticetus*) via aspartic acid racemization. Can J Zool 1999; 77: 571-580.

Gilbert RO. Statistical methods for environmental pollution monitoring. John Wiley and Sons, Inc., New York, 1987, 320 pp.

Groten JP, Sinkeldam EJ, Luten JB, van Bladeren PJ. Comparison of the toxicity of inorganic and liver-incorporated cadmium: a 4-wk feeding study in rats. Food Chem Toxicol 1990; 28: 435-441.

Groten JP, Sinkeldam EJ, Luten JB, van Bladeren PJ. Cadmium accumulation and metallothionein concentrations after 4-week dietary exposure to cadmium chloride or cadmium-metallothionein in rats. Toxicol Appl Pharmacol 1991; 111: 504-513.

Hamanaka T, Ogi H. Cadmium and zinc concentrations in the hyperiid amphipod, *Parathemisto libellula* from the Bering Sea. Bull Fac Fish Hokkaido Univ 1984; 35: 171-178.

Hammill MO, Smith TG. The role of predation in the ecology of the ringed seal in Barrow Strait, Northwest Territories, Canada. Mar Mamm Sci 1991; 7: 123-135.

Hansen CT, Nielsen CO, Dietz R, Hansen MM. Zinc, cadmium, mercury and selenium in minke whales, belugas and narwhals from West Greenland. Polar Biol 1990; 10: 529-539.

Hauksson E, Bogason V. Comparative feeding of grey (*Halichoerus grypus*) and common seals (*Phoca vitulina*) in coastal waters of Iceland, with a note on the diet of hooded (*Cystophora cristata*) and harp seals (*Phoca groenlandica*). J Northw Atl Fish Sci 1997; 22:125-135.

Hein JR, Koski RA, Embley RW, Reid J, Chang S-W. Diffuse-flow hydrothermal field in an oceanic fracture zone setting, Northeast Pacific: Deposit composition. Explor Min Geol 1999; 8: 299-322.

Hobson KA, Clark RG. Assessing avian diets using stable isotopes II: Factors influencing diet-tissue fractionation. Condor 1992; 94: 189-197.

Hobson KA, Welch HE. Determination of trophic relationships within a high Arctic marine food web using d13C and d15N analysis. Mar Ecol Prog Ser 1992; 84: 9-18.

Hobson KA, Alisauskas RT, Clark RG. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. Condor 1993; 95: 388-394.

Hobson KA, Schell DM, Renouf D, Noseworthy E. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Can J Fish Aquat Sci 1996; 53: 528-533.

Hobson KA, Sease JL, Merrick RL, Piatt JF. Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of nitrogen and carbon. Mar Mamm Sci 1997; 13: 114-132.

Hobson KA, Fisk AT, Karnovsky N, Holst M, Gagnon J-M, Fortier M. A stable isotope ($\delta^{13}C$, $\delta^{15}N$) model for the North Water foodweb: implications for evaluating trophodynamics and the flow of energy and contaminants. Deep-Sea Res II 2002; 49: 5131-5150.

Hoekstra PF, Dehn L-A, George JC, Solomon KR, Muir DCG, O'Hara TM. Trophic ecology of bowhead whales (*Balaena mysticetus*) compared with that of other arctic marine biota as interpreted from carbon-, nitrogen-, and sulfur-isotope signatures. Can J Zool 2002; 80: 223-231.

Hoekstra PF, O'Hara TM, Fisk AT, Borga K, Solomon KR , Muir DCG. Trophic transfer of persistent organochlorine contaminants (OCs) within an Arctic marine food web from the southern Beaufort-Chukchi Seas. Environ Pollut 2003; 124: 509-522.

Honda K, Tatsukawa R, Itano K, Miyazaki N, Fujiyama T. Heavy metal concentrations in muscle, liver and kidney tissue of striped dolphin, *Stenella coeruleoalba*, and their variations with body length, weight, age and sex. Agric Biol Chem 1983; 47: 1219-1228.

Hop H, Borga K, Gabrielsen GW, Kleivane L, Skaare JU. Food web magnification of persistent organic pollutants in poikilotherms and homeotherms from the Barents Sea. Environ Sci Technol 2002; 36: 2589-2597.

Hu H, Rabinowitz M, Smith D. Bone lead as a biological marker in epidemiologic studies of chronic toxicity: conceptual paradigms. Environ Health Perspect 1998; 106: 1-8.

Huntington HP. Traditional knowledge of the ecology of belugas, *Delphinapterus leucas*, in Cook Inlet, Alaska. Mar Fish Rev 2000; 62: 134-140.

Ilbäck N-G, Glynn AW, Wikberg L, Netzel E, Lindh U. Metallothionein is induced and trace element balance changed in target organs of a common viral infection. Toxicology 2004; 199: 241-250.

Johnson DE. Applied multivariate methods for data analysis. Duxbury Press, Pacific Grove, California. 1998, 567 pp.

Julshamn K, Grahl-Nielsen O. Trace element levels in harp seal (*Pagophilus groenlandicus*) and hooded seal (*Cystophora cristata*) from the Greenland Sea. A multivariate approach. Sci Total Environ 200; 250: 123-133.

Kelly JF. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can J Zool 2000; 78: 1-27.

Koeman JH, Peeters WHM, Koudstaal-Hol CHM. Mercury-selenium correlations in marine mammals. Nature 1973; 245: 385-386.

Koeman JH, van de Ven WS, de Goeij JJ, Tjioe PS, van Haaften JL. Mercury and selenium in marine mammals and birds. Sci Total Environ 1975; 3: 279-287.

Krebs CJ, Kenney AJ, Gilbert S, Danel K, Angerbjörn A, Erlinge S, Bromley RG, Shank C, Carriere S. Synchrony in lemming and vole populations in the Canadian Arctic. Can J Zool 2002; 80: 1323-1333.

Kubota R, Kunito T, Tanabe S. Arsenic accumulation in the liver tissue of marine mammals. Environ Pollut 2001; 115: 303-312.

Lalancette A, Morin Y, Measures LFM. Contrasting changes of sensitivity by lymphocytes and neutrophils to mercury in developing grey seals. Develop Comp Immunol 2003; 27: 735-747.

Laporte JM, Andres S, Mason RP. Effect of ligands and other metals on the uptake of mercury and methylmercury across the gills and the intestine of the blue crab (*Callinectes sapidus*). Comp Biochem Physiol C 2002; 131: 185-196.

Law RJ. Metals in marine mammals. In: Beyer WN, Heinz GH, Redmon-Norwood AW, editors. Environmental contaminants in wildlife: interpreting tissue concentrations. CRC Press, Inc., Boca Raton, Florida, 1996, pp. 357-376.

Lesage V, Hammill MO, Kovacs KM. Marine mammals and the community structure of the Estuary and Gulf of St. Lawrence, Canada: evidence from stable isotope analysis . Mar Ecol Prog Ser 2001; 210: 203-221.

Lind Y, Engman J, Jorhem L, Glynn AW. Cadmium absorption in mice: effects of broiling on bioavailability of cadmium in foods of animal origin. J Toxicol Environ Health A 2001; 62: 269-280.

Lindberg SE, Brooks S, Lin C-J, Scott KJ, Landis MS, Stevens RK, Goodsite M, Richter A. Dynamic oxidation of gaseous mercury in the Arctic troposphere at polar sunrise. Environ Sci Technol 2002; 36: 1245-1256.

Lodenius M, Soltanpour-Gargari A, Tulisalo E, Henttonen H. Effects of ash application on cadmium concentration in small mammals. J Environ Qual 2002; 31: 188-192.

Lowry LF, Frost KJ, Burns JJ. Variability in the diet of ringed seals, *Phoca hispida*, in Alaska. Can J Fish Aquat Sci 1980a; 37: 2254-2261.

Lowry LF, Frost KJ, Burns JJ. Feeding of bearded seals in the Bering and Chukchi Seas and trophic interaction with Pacific walrus. Arctic 1980b; 33: 330-342.

Lowry LF, Frost KJ, Calkins DG, Swartzman GL, Hills S. Feeding habits, food requirements, and status of Bering Sea marine mammals. 1982. Anchorage, North Pacific Fishery Management Council, 233 pp.

Lowry LF, Burns JJ, Nelson RR. Polar bear, *Ursus maritimus*, predation on belugas, *Delphinapterus leucas*, in the Bering and Chukchi Seas. Can Field-Nat 1987; 101: 141-146.

Lowry LF. Foods and feeding ecology. In: Burns JJ, Montague JJ, Cowles CJ, editors. The bowhead whale. The Society for Marine Mammalogy, Special Publication 2, 1993, pp. 201-238.

Lowry LF, Sheffield G. Stomach contents of bowhead whales harvested in the Alaskan Beaufort Sea. In: Richardson WJ, Thompson DH, editors. Bowhead whale feeding in the Eastern Alaskan Beaufort Sea: Update of scientific and traditional information. Vol. 1. 2002. OCS Study MMS 2002-012. LDL Report TA2196-7. LGL Ltd. King City, Ontario, Canada, pp. 1810-1828.

Macdonald CR, Sprague JB. Cadmium in the marine invertebrate and arctic cod in the Canadian Arctic. Distribution and ecological implications. Mar Ecol Prog Ser 1988; 47: 17-30.

Martin JH, Flegal AR. High copper concentrations in squid livers in association with elevated levels of silver, cadmium, and zinc. Mar Biol 1975; 30: 51-55.

McCutchan JH, Lewis WM, Kendall C, McGrath CC. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. OIKOS 2003; 102: 378-390.

McGeer JC, Brix KV, Skeaff JM, DeForest DK, Brigham SI, Adams WJ, Green A. Inverse relationship between bioconcentration factor and exposure concentration for metals: implications for hazard assessment of metals in the aquatic environment. Environ Toxicol Chem 2003; 22: 1017-1037.

Medvedev N, Panichev N, Hyvärinen. Levels of heavy metals in seals of Lake Ladoga and the White Sea. Sci Total Environ 1997; 206: 95-105.

Miles AK, Calkins DG, Coon NC. Toxic elements and organochlorines in harbor seals (*Phoca vitulina richardsi*), Kodiak, Alaska, USA. Bull Environ Contam Toxicol 1992; 48: 727-732.

Miller S, Proffitt K, Schliebe S. Demography and behavior of polar bears feeding on marine mammal carcasses. Second Interim Report. 2004. U.S. Department of the Interior. Mineral Management Service. Alaska Outer Continental Shelf Region, 22 pp.

Morel FMM, Kraepiel AML, Amyot M. The chemical cycle and bioaccumulation of mercury. Annu Rev Ecol Sys 1998; 29: 543-566.

Muir DCG, Segstro MD, Hobson KA, Ford CA, Stewart REA, Olpinski S. Can Seal Eating Explain Elevated Levels of PCBs and Organochlorine Pesticides in Walrus Blubber from Eastern Hudson Bay (Canada)? Environ Pollut 1995; 90: 335-348.

Nigro M, Campana A, Lanzillotta E, Ferrara R. Mercury exposure and elimination rates in captive bottlenose dolphins. Mar Pollut Bull2002; 44: 1071-1075.

Norheim G, Skaare JU, Wiig Ø. Some heavy metals, essential elements, and chlorinated hydrocarbons in polar bear (*Ursus maritimus*) at Svalbard. Environ Pollut 1992; 77: 51-57.

Norstrom RJ, Schweinsburg RE, Collins BT. Heavy metals and essential elements in liver of the polar bear (*Ursus maritimus*) in the Canadian Arctic. Sci Total Environ 1986; 48: 195-212.

Olsson M, Karlsson B, Ahnland E. Diseases and environmental contaminants in seals from the Baltic and Swedish west coast. Sci Total Environ 1994; 154: 217-227.

Paludan-Müller P, Agger CT, Dietz R, Kinze CC. Mercury, cadmium, zinc, copper and selenium in harbour porpoise (*Phocoena phocoena*) from West Greenland. Polar Biol 1993; 13: 311-320.

Polischuk SC, Hobson KA, Ramsay MA. Use of stable-carbon and -nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. Can J Zool 2001; 79: 499-511.

Pond CM, Mattacks CA, Gilmour I, Johnston MA, Pillinger CT, Prestrud P. Chemical and carbon isotopic composition of fatty acids in adipose tissue as indicators of dietary history in wild arctic foxes (*Alopex lagopus*) on Svalbard. J Zool, Lond 1995; 236: 611-623.

Poole KG, Elkin BT, Bethke RW. Organochlorine and heavy metal contaminants in wild mink in western Northwest Territories, Canada. Arch Environ Contam Toxicol 1998; 34: 406-413.

Presley BJ. A review of arctic trace metal data with implications for biological effects. Mar Pollut Bull 1997; 35: 226-234.

Prestrud P. Food habits and observations of the hunting behaviour of Arctic foxes, *Alopex lagopus*, in Svalbard. Can Field-Nat 1992; 106: 225-236.

Prestrud P, Norheim G, Sivertsen T, Daae HL. Levels of toxic and essential elements in arctic fox in Svalbard. Polar Biol 1994; 14: 155-159.

Purcell TW, Peters JJ. Sources of silver in the environment. Environ Toxicol Chem 1998; 17: 539-546.

Pyle DM, Mather TA. The importance of volcanic emissions for the global atmospheric mercury cycle. Atmos Environ 2003; 37: 5115-5124.

Rainbow PS. Heavy metals in Aquatic Invertebrates. In: Beyer WN, Heinz GH, Redmon-Norwood AW, editors. Environmental contaminants in wildlife: interpreting tissue concentrations. CRC Press, Inc., Boca Raton, Florida, 1996, pp. 405-425.

Rainbow PS, Black WH. Cadmium, zinc and the uptake of calcium by two crabs, *Carcinus maenas* and *Eriocheir sinensis*. Aquat Toxicol 2005; 72: 45-65.

Ramsay MA, Hobson KA. Polar bears make little use of terrestrial food webs: evidence from stable-carbon isotope analysis. Oecologia 1991; 86: 598-600.

Ratte HT. Bioaccumulation and toxicity of silver compounds: A review. Environ Toxicol Chem 1999; 18: 89-108.

Reeves PG, Chaney RL. Marginal nutritional status of zinc, iron, and calcium increases cadmium retention in the duodenum and other organs of rats fed rice-based diets. Environ Res 2004; 96: 311-322.

Rice DW, Wolman AA. The life history and ecology of the gray whale (*Eschrichtius robustus*). The American Society of Mammalogists 1971; Special publication 3.

Ritterhoff J, Zauke G-P. Trace metals in field samples of zooplankton from the Farm Strait and the Greenland Sea. Sci Total Environ 1997; 199: 255-270.

Roth JD, Hobson KA. Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstructions. Can J Zool 2000; 78: 848-852.

Roth JD. Variability in marine resources affects arctic fox population dynamics. J Anim Ecol 2003; 72: 668-676.

Rouleau C, Gobeil C, Tjälve H. Accumulation of silver from the diet in two marine benthic predators: the snow crab (*Chionoecetes opilio*) and American plaice (*Hippoglossoides platessoides*). Environ Toxicol Chem 2000; 9: 631-637.

Saeki K, Nakajima M, Loughlin TR, Calkins DC, Baba N, Kiyota M, Tatsukawa R. Accumulation of silver in the liver of three species of pinnipeds. Environ Pollut 2001; 112: 19-25.

Satoh M, Nishimura N, Kanayama Y, Naganuma A, Suzuki T, Tohyama C. Enhanced renal toxicity by inorganic mercury in metallothionein-null mice. J Pharmacol Exp Ther 1997; 283: 1529-1533.

Schell DM, Saupe SM, Haubenstock N. Natural isotope abundance in bowhead whale (*Balaena mysticetus*) baleen: markers of aging and habitat usage. In: Rundel PW, Ehleringer JR, Nagy KA, editors. Stable isotopes in ecological research. Springer-Verlag, Berlin, 1989, pp. 261-269.

Schmutz JA, Hobson KA. Geographic, temporal, and age-specific variation in diets of glaucous gulls in Western Alaska. Condor 1998; 100: 119-130.

Seaman GA, Lowry LF, Frost KJ. Foods of belukha whales (*Delphinapterus leucas*) in Western Alaska. Cetology 1982; 44: 1-19.

Shi CY, Lane AT, Clarkson TW. Uptake of mercury by the hair of methylmercury-treated newborn mice. Environ Res 1990; 51: 170-181.

Shustov AP. The food of ribbon seals in the Bering Sea. Izvestiia TINRO 1965; 59: 178-183.

Sichak SP, Mavis RD, Finkelstein JN, Clarkson TW. An examination of the oxidation of mercury vapor by rat brain homogenate. J Biochem Toxicol 1986; 1: 53-68.

Smith TG, Armstrong FAJ. Mercury in seals, terrestrial carnivores, and principal food items of the Inuit, from Holman, N. W. T. J Fish Res Bd Can 1975; 32: 795-801.

Smith TG. Predation of ringed seal pups (*Phoca hispida*) by the arctic fox (*Alopex lagopus*). Can J Zool 1976; 54: 1610-1616.

Smith TG, Armstrong FAJ. Mercury and selenium in ringed and bearded seal tissues from Arctic Canada. Arctic 1978; 31: 75-84.

Smith TG. Polar bear predation of ringed and bearded seals in the land-fast sea ice habitat. Can J Zool 1980; 58: 2201-2209.

Smith TG, Sjare B. Predation of belugas and narwhals by polar bears in nearshore areas of the Canadian High Arctic. Arctic 1990; 43: 99-102.

Solaiman D, Jonah MM, Miyazaki W, Ho G, Bhattacharyya MH. Increased metallothionein in mouse liver, kidneys, and duodenum during lactation. Toxicol Sci 2001; 60: 184-192.

Sponheimer M, Robinson T, Ayliffe L, Roeder B, Hammer J, Passey B, West A, Cerling T, Dearing D, Ehleringer J. Nitrogen isotopes in mammalian herbivores: hair $\delta^{15}N$ values from a controlled feeding study. Int J Osteoarch 2003; 13: 80-87.

Stirling I, McEwan E. The caloric value of whole ringed seals (*Phoca hispida*) in relation to polar bear (*Ursus maritimus*) ecology and hunting behavior. Can J Zool 1975; 53: 1021-1027.

Suda I, Takahashi H. Effect of reticuloendothelial system blockade on the biotransformation of methyl mercury in the rat. Bull Environ Contam Toxicol 1990; 44: 609-615.

Szepanski MM, Ben-David M, Van Ballenberghe V. Assessment of anadromous salmon resources in the diet of Alexander Archipelago wolf using stable isotope analysis. Oecologia 1999; 120: 327-335.

Tarpley RJ, Wade TL, Haubold EM. Toxicological studies in tissues of the beluga whale *Delphinapterus leucas* along northern Alaska with an emphasis on public health implications of subsistence utilization. Final report to the Alaska Beluga Whale Committee. 1995. North Slope Borough, Department of Wildlife Management. Barrow, Alaska.

Taylor DL, Schliebe S, Metske H. Contaminants in Blubber, Liver and Kidney Tissue of Pacific Walruses. Mar Pollut Bull 1989; 20: 465-468.

Teigen SW, Andersen RA, Daae HL, Skaare JU. Heavy metal content in liver and kidneys of grey seals (*Halichoerus grypus*) in various life stages correlated with metallothionein levels: some metal-binding characteristics of this protein. Environ Toxicol Chem 1999; 18: 2364-2369.

Tilbury KL, Stein JE, Krone CA, Brownell RL, Blokhin SA, Bolton JL, Ernest DW. Chemical contaminants in juvenile gray whales (*Eschrichtius robustus*) from subsistence harvest in Arctic feeding grounds. Chemosphere 2002; 47: 555-564.

Trefry JH, Rember RD, Trocine RP, Brown JS. Trace metals in sediments near offshore oil exploration and production sites in the Alaskan Arctic. Environ Geol 2003; 6: 1-12.

US Environmental Protection Agency. Methods for the determination of metals in environmental samples. CRC Press, Boca Raton, 1992, 352 pp.

Wagemann R, Stewart REA, Lockhart WL, Stewart BE, Povoledo M. Trace metals and methyl mercury: associations and transfer in harp seal (*Phoca groenlandica*) mothers and their pups. Mar Mamm Sci 1988; 4: 339-355.

Wagemann R. Comparison of heavy metals in two groups of ringed seals (*Phoca hispida*) from the Canadian Arctic. Can J Fish Aquat Sci 1989; 46: 1558-1563.

Wagemann R, Stewart REA, Beland P, Desjardins C. Heavy metals and selenium in tissues of beluga whales, *Delphinapterus leucas*, from the Canadian Arctic and the St. Lawrence estuary. Can Bull Fish Aquat Sci 1990; 224: 191-206.

Wagemann R, Stewart REA. Concentrations of heavy metals and selenium in tissues and some foods of walrus (*Odobenus rosmarus rosmarus*) from eastern Canadian Arctic and sub-Arctic, and associations between metals, age, and gender. Can J Fish Aquat Sci 1994; 51: 426-436.

Wagemann R, Innes S, Richard P. Overview and regional and temporal differences of heavy metals in Arctic whales and ringed seals in the Canadian Arctic. Sci Total Environ 1996; 186: 41-66.

Wagemann R, Trebacz E, Hunt R, Boila G. Percent methylmercury and organic mercury in tissues of marine mammals and fish using different experimental and calculation methods. Environ Toxicol Chem 1997; 16: 1859-1866.

Warburton J, Seagars DJ Heavy metal concentrations in liver and kidney tissues of pacific walrus. Continuation of a baseline study. 1993. Anchorage, Alaska, US Fish and Wildlife Service, 27 pp.

Watanabe I, Kunito T, Tanabe S, Amano M, Koyama Y, Miyazaki N, Petrov EA, Tatsukawa R. Accumulation of heavy metals in Caspian seals (*Phoca caspica*). Arch Environ Contam Toxicol 2002; 43: 109-120.

Wood CM, McDonald MD, Walker P, Grosell M, Barimo JF, Playle RC, Walsh PJ. Bioavailability of silver and its relationship to ionregulation and silver speciation across a range of salinities in the gulf toadfish (*Opsanus beta*). Aquat Toxicol 2004; 70: 137-157.

Woshner VM, O'Hara TM, Bratton GR, Beasley VR. Concentrations and interactions of selected essential and non-essential elements in ringed seals and polar bears of Arctic Alaska. J Wildl Dis 2001a; 37: 711-721.

Woshner VM, O'Hara TM, Bratton GR, Suydam RS, Beasley VR. Concentrations and interactions of selected essential and non-essential elements in bowhead and beluga whales of Arctic Alaska. J Wildl Dis 2001b; 37: 693-710.

Yeats P, Stenson G, Hellou J. Essential elements and priority contaminants in liver, kidney, muscle and blubber of harp seal beaters. Sci Total Environ 1999; 243/244: 157-167.

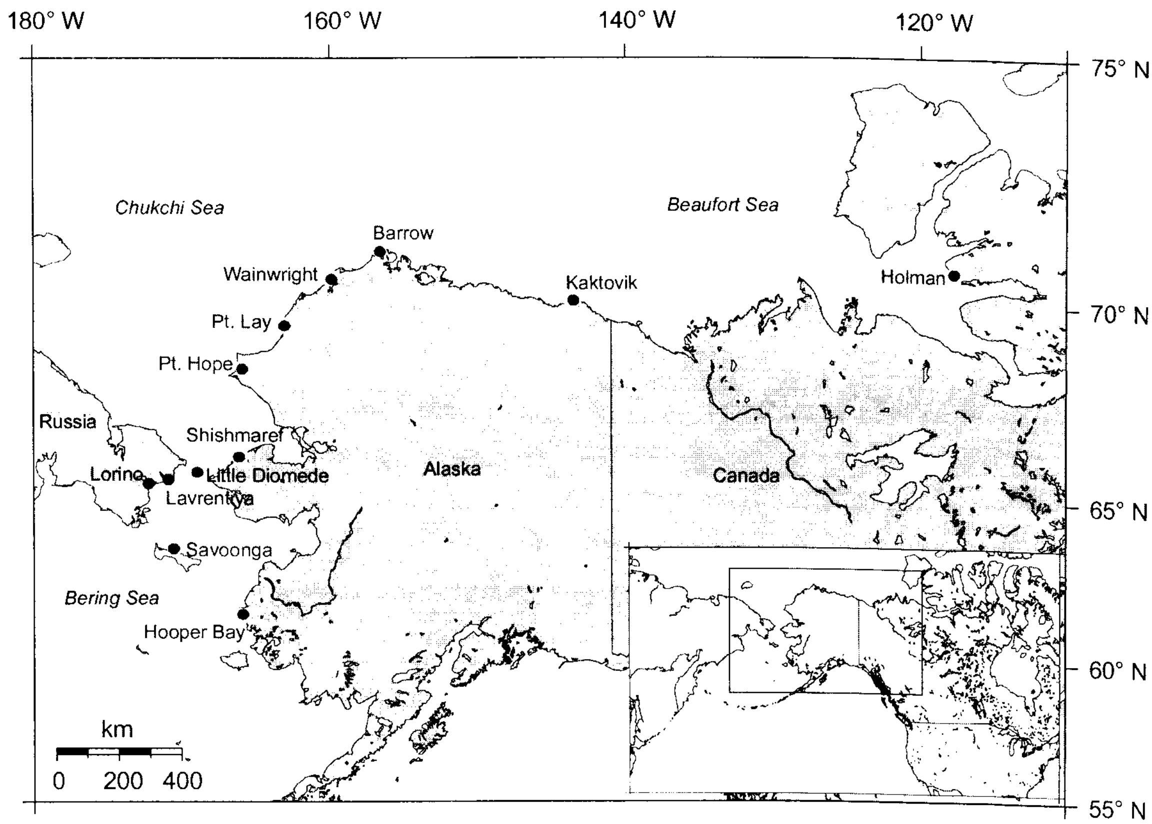


FIGURE 4.1. Alaskan, Russian and Canadian villages and communities where samples of subsistence-harvested Arctic marine and terrestrial mammals were collected.

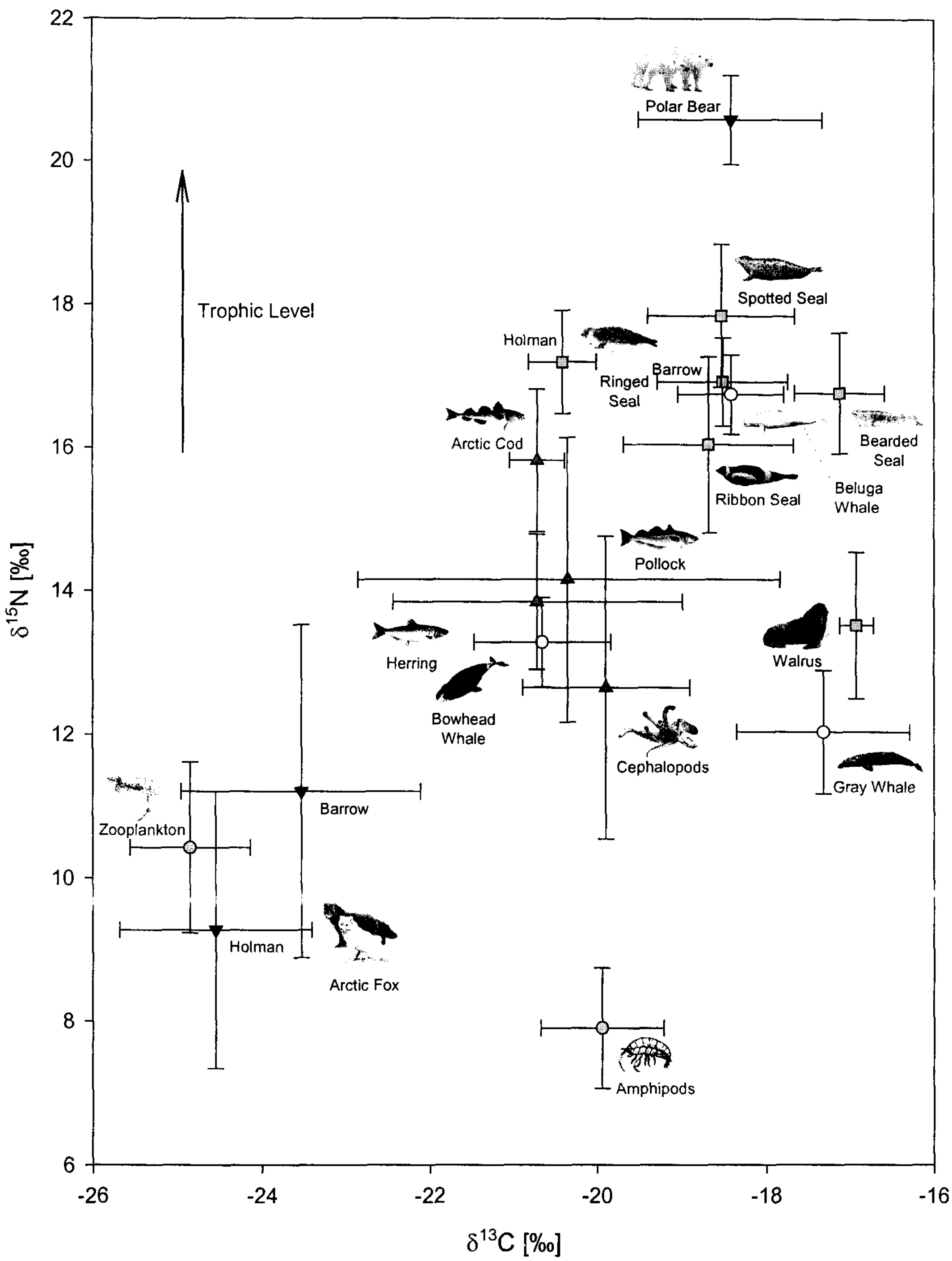


FIGURE 4.2. Trophic structure of an Arctic marine food web as determined from stable carbon and nitrogen isotope ratios. Symbols present the mean values and error bars show the standard deviations for both $\delta^{13}C$ and $\delta^{15}N$.

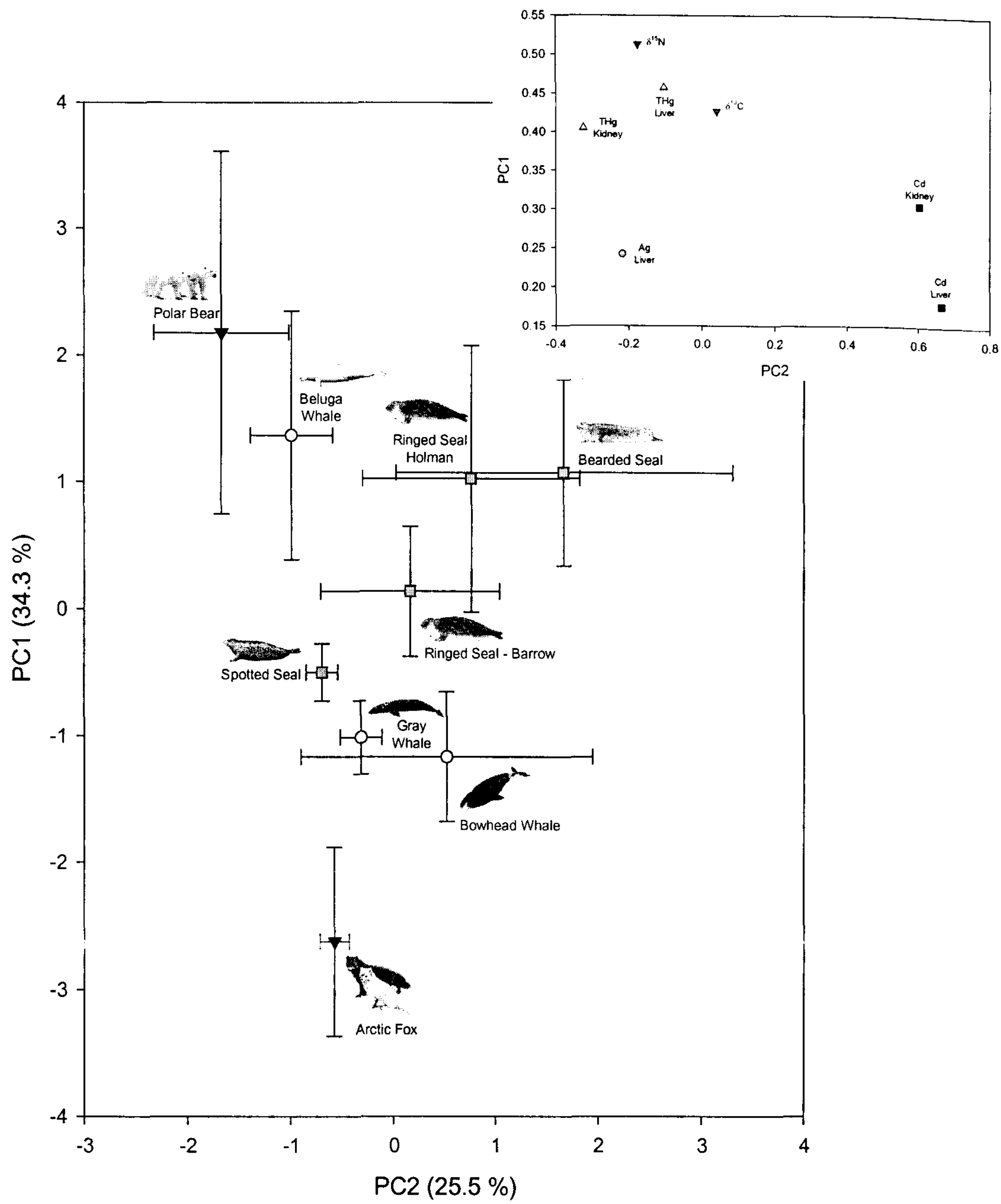


FIGURE 4.3. Principal component (PC) 2 versus 1 from standardized data for Arctic marine and terrestrial mammals. The variables used were Ag in liver, Cd and THg in kidney and liver and stable carbon and nitrogen isotopes. Symbols present the mean values and error bars show the standard deviations. The graph in the upper right shows the contribution of variables associated with the principle components in a loading plot.

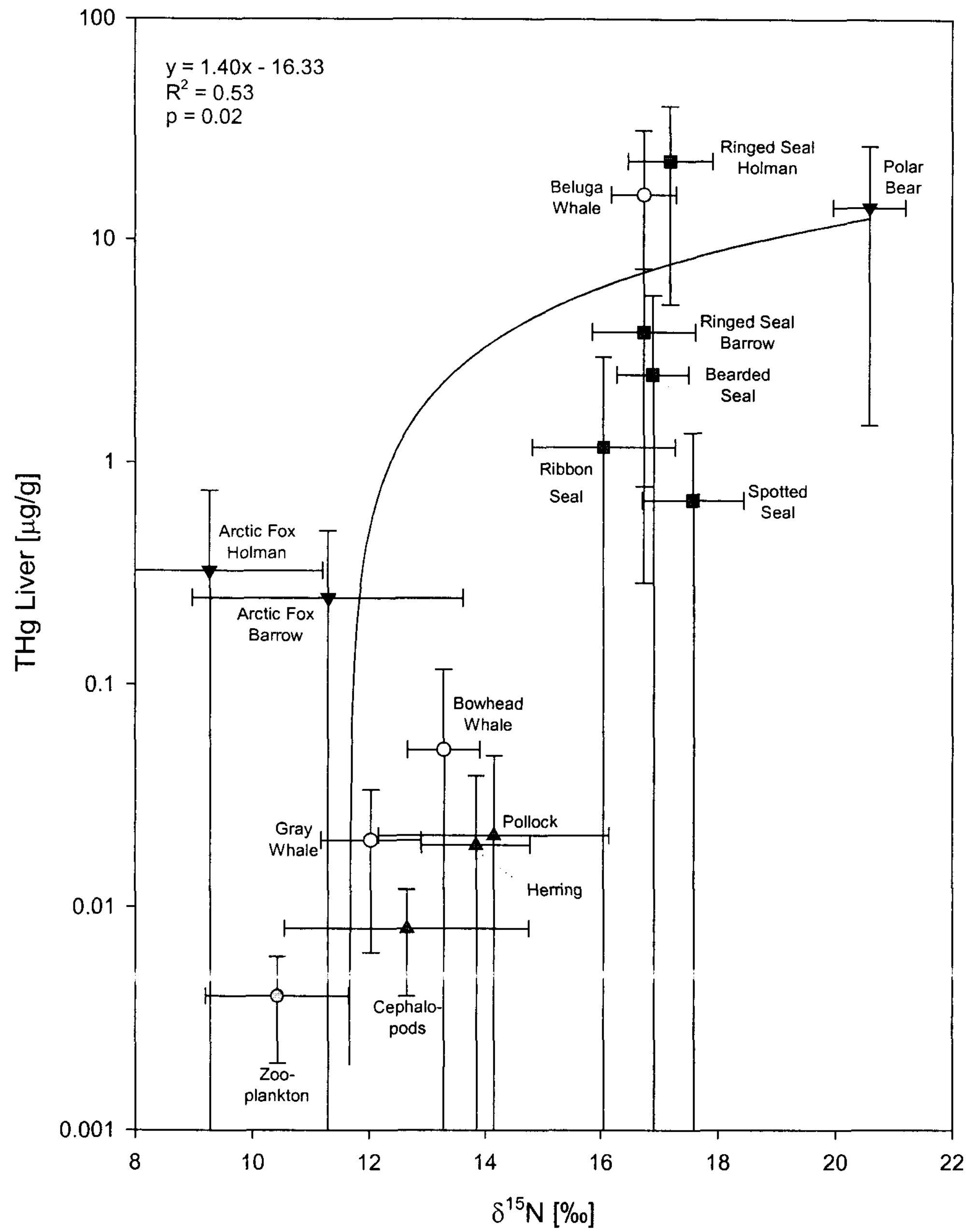


FIGURE 4.4a Trophic structure (based on $\delta^{15}N$) versus THg (a) and Ag (b) in liver and renal Cd (c) (or total body homogenates in case of invertebrates and fish) in μg/g ww in an Arctic marine food web. The Y-axis is given as logarithmic scale. Symbols present the mean values and error bars show the standard deviations. The slope of the linear regression gives the Food Web Magnification Factor (FWMF).

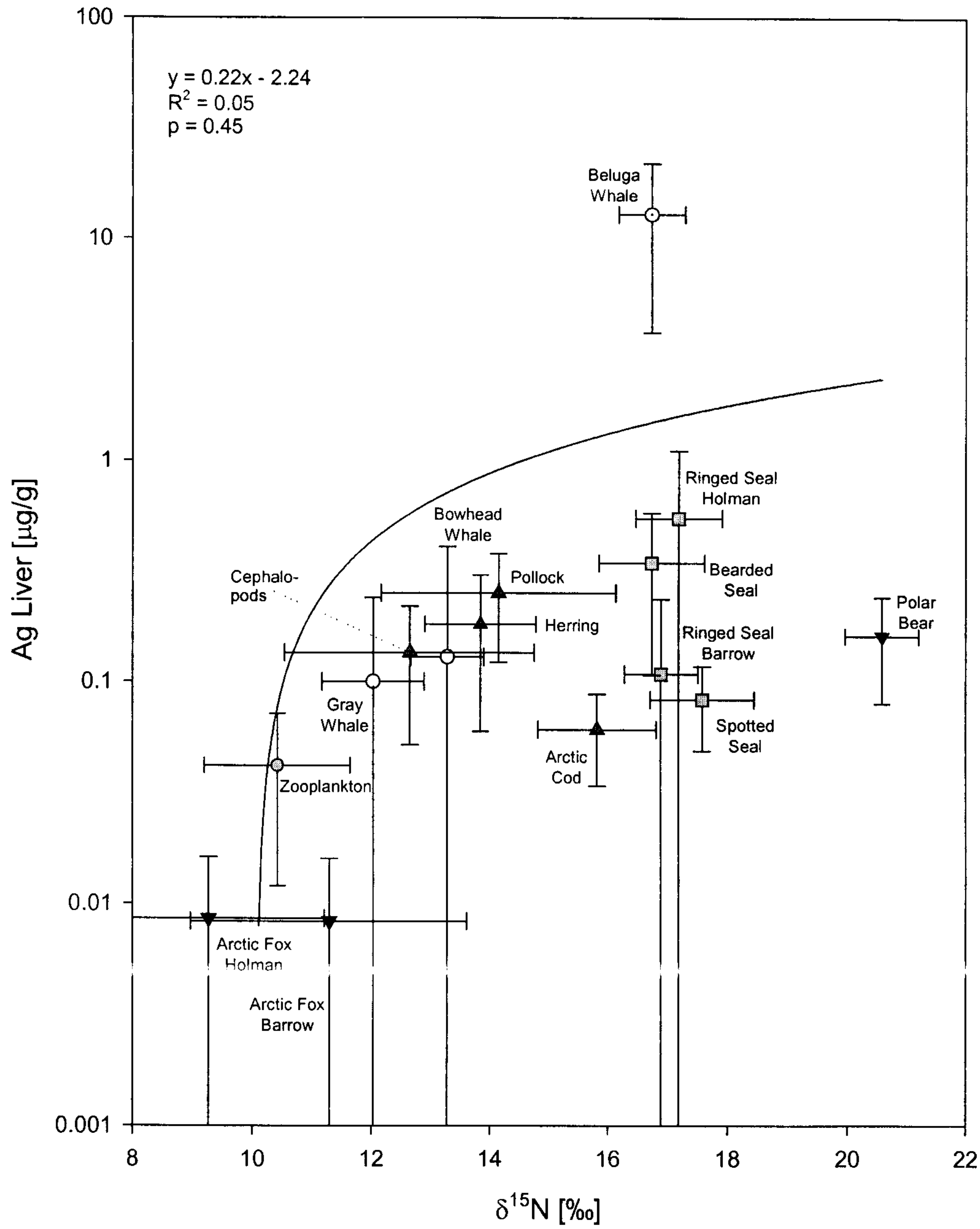
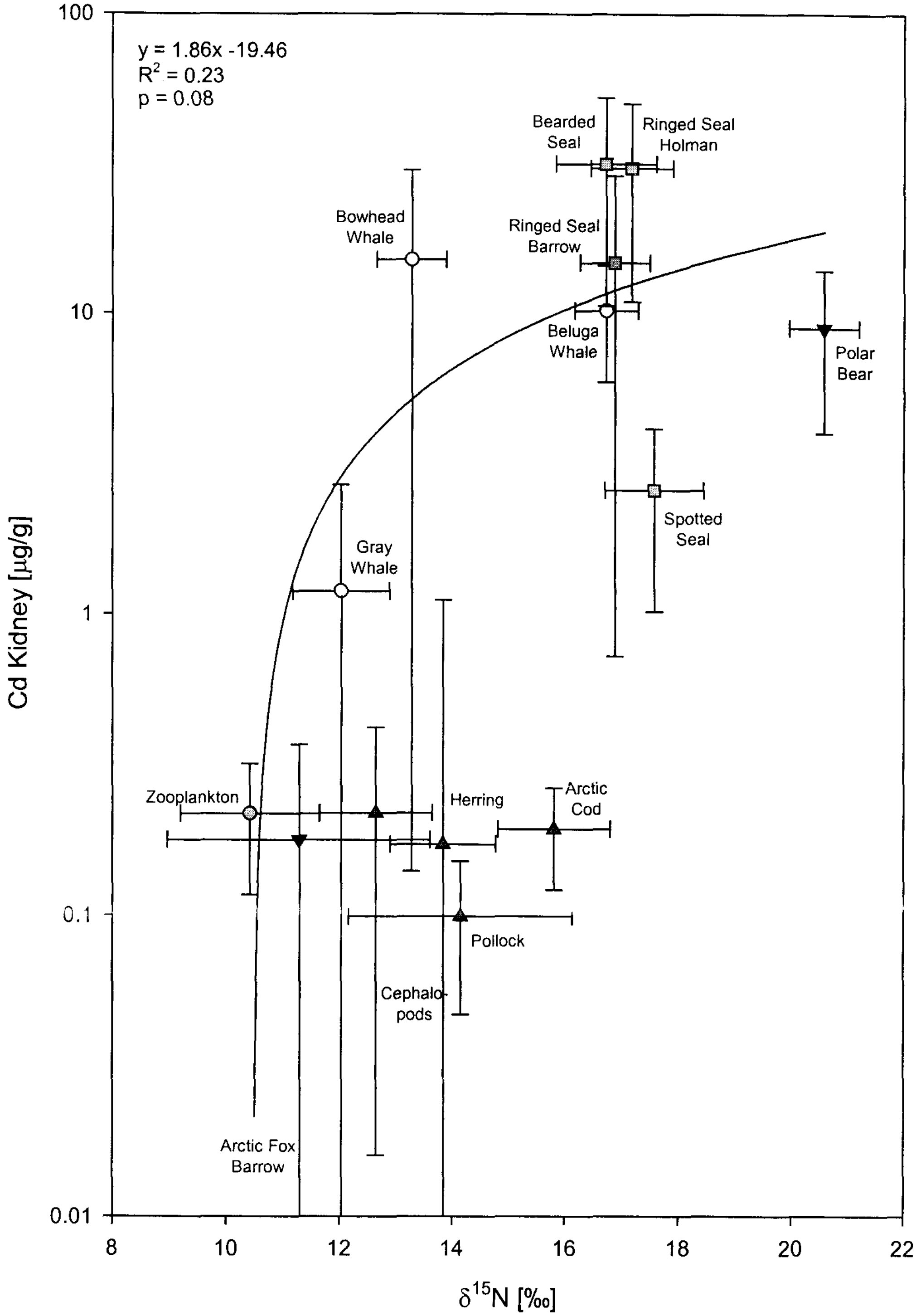

y = 0.22x - 2.24
R² = 0.05
p = 0.45
Beluga Whale
Ringed Seal Holman
Bowhead Whale
Bearded Seal
Cephalo-pods
Pollock
Herring
Polar Bear
Ringed Seal Barrow
Gray Whale
Spotted Seal
Arctic Cod
Zooplankton
Arctic Fox Holman
Arctic Fox Barrow
Ag Liver [µg/g]
δ15N [‰]
100
10
1
0.1
0.01
0.001
8
10
12
14
16
18
20
22

FIGURE 4.4b



y = 1.86x -19.46
R^2 = 0.23
p = 0.08
Bearded Seal
Ringed Seal Holman
Bowhead Whale
Ringed Seal Barrow
Beluga Whale
Polar Bear
Spotted Seal
Gray Whale
Zooplankton
Herring
Arctic Cod
Pollock
Cephalo-pods
Arctic Fox Barrow
Cd Kidney [µg/g]
δ15N [‰]
100
10
1
0.1
0.01
8
10
12
14
16
18
20
22

FIGURE 4.4c

TABLE 4.1. Results of multiple ANOVAs as follow-up test to MANOVA. Pair-wise comparisons that showed no significant differences (↔) between groups are highlighted in gray. Significant differences in variables are labeled with up or down arrows, meaning the mean for group A is either significantly larger (↑) or significantly smaller (↓) than that of group B.

Group Comparison				Cadmium		Total Mercury		Silver
Group A	**Group B**	**$\delta^{13}C$**	**$\delta^{15}N$**	**Kidney**	**Liver**	**Kidney**	**Liver**	**Liver**
Polar bear	Beluga whale	↔	↑	↔	↓	↔	↔	↓
	Gray whale	↓	↑	↑	↔	↑	↑	↑
	Bowhead whale	↑	↑	↔	↓	↑	↑	↑
	Bearded seal	↓	↑	↓	↓	↑	↑	↔
	Ringed seal, Barrow	↔	↑	↔	↓	↑	↑	↑
	Ringed seal, Holman	↑	↑	↓	↓	↑	↔	↔
	Spotted seal	↑	↑	↔	↔	↑	↑	↔
	Arctic fox, Barrow	↑	↑	↑	↑	↑	↑	↑
Beluga whale	Gray whale	↓	↑	↑	↑	↑	↑	↑
	Bowhead whale	↑	↑	↔	↔	↑	↑	↑
	Bearded seal	↓	↔	↓	↓	↑	↑	↑
	Ringed seal, Barrow	↔	↔	↔	↔	↑	↑	↑
	Ringed seal, Holman	↑	↓	↓	↓	↔	↔	↑
	Spotted seal	↑	↔	↔	↑	↑	↑	↑
	Arctic fox, Barrow	↑	↑	↑	↑	↑	↑	↑
Gray whale	Bowhead whale	↑	↔	↓	↓	↔	↔	↔
	Bearded seal	↔	↓	↓	↓	↓	↓	↓
	Ringed seal, Barrow	↑	↓	↓	↓	↓	↓	↔
	Ringed seal, Holman	↑	↓	↓	↓	↓	↓	↓
	Spotted seal	↑	↓	↔	↔	↓	↓	↔
	Arctic fox, Barrow	↑	↔	↔	↔	↓	↓	↑
Bowhead whale	Bearded seal	↓	↓	↓	↓	↓	↓	↓
	Ringed seal, Barrow	↓	↓	↔	↔	↓	↓	↓
	Ringed seal, Holman	↔	↓	↓	↓	↓	↓	↓
	Spotted seal	↓	↓	↔	↑	↓	↓	↔
	Arctic fox, Barrow	↑	↑	↑	↑	↓	↓	↑
Bearded seal	Ringed seal, Barrow	↑	↔	↑	↑	↔	↔	↑
	Ringed seal, Holman	↑	↓	↔	↔	↓	↓	↔
	Spotted seal	↑	↔	↑	↑	↑	↑	↔
	Arctic fox, Barrow	↑	↑	↑	↑	↔	↑	↑
Ringed seal, Barrow	Ringed seal, Holman	↑	↔	↓	↓	↓	↓	↓
	Spotted seal	↔	↔	↑	↑	↔	↑	↔
	Arctic fox, Barrow	↑	↑	↑	↑	↔	↑	↑
Ringed seal, Holman	Spotted seal	↓	↔	↑	↑	↑	↑	↑
	Arctic fox, Barrow	↑	↑	↑	↑	↑	↑	↑
Spotted seal	Arctic fox, Barrow	↑	↑	↔	↔	↔	↔	↑

↔ no significant difference
↑ significantly larger
↓ significantly smaller

TABLE 4.2. Stable carbon and nitrogen ratios in selected Arctic marine and terrestrial mammals. Values are given as mean ± standard deviation for muscle tissue unless otherwise noted (n = sample size).

Species	Location	$\delta^{15}N$	$\delta^{13}C$	Source
Terrestrial Mammals				
Polar bear *Ursus maritimus*	Barrow, Alaska	20.6 ± 0.6 n = 10	-18.4 ± 1.1 n = 10	this study
Polar bear *Ursus maritimus*	Canada	21.1 ± 0.3 n = 3	-18.2 ± 0.3 n = 3	Hobson and Welch (1992)
Polar bear (pregnant female) *Ursus maritimus*	Churchill, Canada	18.5 ± 1.2* n = 5	-19.8 ± 0.2* n = 5	Polischuk et al. (2001)
Polar bear (cub of year) *Ursus maritimus*	Churchill, Canada	21.4 ± 0.6* n = 9	-19.7 ± 0.2* n = 9	Polischuk et al. (2001)
Arctic fox *Alopex lagopus*	Barrow, Alaska	11.3 ± 2.3 n = 27	-23.5 ± 1.4 n = 27	this study
Arctic fox *Alopex lagopus*	Holman, Canada	9.3 ± 1.9 n = 20	-24.5 ± 1.1 n = 20	this study
Cetaceans / Odontocetes				
Beluga whale *Delphinapterus leucas*	Point Hope, Point Lay, Alaska	16.5 ± 0.6 n = 49	-18.4 ± 0.6 n = 49	this study
Beluga whale (male) *Delphinapterus leucas*	St. Lawrence Estuary, Canada	15.8 ± 0.6 n = 11	-16.7 ± 0.2 n = 11	Lesage et al. (2001)
Beluga whale (female) *Delphinapterus leucas*	St. Lawrence Estuary, Canada	15.1 ± 0.4 n = 16	-17.3 ± 0.2 n = 16	Lesage et al. (2001)
Beluga whale *Delphinapterus leucas*	Baffin, Canada	16.0 ± 0.2 n = 30	-17.7 ± 0.2 n = 30	Hobson et al. (2002)
Beluga whale *Delphinapterus leucas*	Greenland	16.9 ± 0.2 n = 40	-17.6 ± 0.1 n = 40	Hobson et al. (2002)
Narwhal *Monodon monoceros*	Greenland	16.0 ± 0.1 n = 89	18.2 ± 0.03 n = 89	Hobson et al. (2002)
Harbor porpoise *Phocoena phocoena*	Black Sea	12.0 ± 0.6 n = 46	-20.5 ± 0.4 n = 46	Das et al. (2004b)
Harbor porpoise *Phocoena phocoena*	Irish Coast	14.1 ± 1.6 n = 7	-16.5 ± 0.7 n = 7	Das et al. (2003)
Cetaceans / Mysticetes				
Gray whale *Eschrichtius robustus*	Lorino, Lavrentia, Russia	12.0 ± 0.9 n = 17	-17.3 ± 1.0 n = 17	this study
Bowhead whale *Balaena mysticetus*	Barrow, Kaktovik, Alaska	13.4 ± 0.7 n = 122	-20.6 ± 0.9 n = 122	this study includes data from Hoekstra et al. (2002)
Bowhead whale *Balaena mysticetus*	Eastern Arctic	13.2 ± 0.7 n = 3	-18.2 ± 0.3 n = 3	Hobson et al. (2002)
Minke whale *Balaenoptera acutorostrata*	West Greenland	12.2 ± 1.0 n = 43	-18.2 ± 0.4 n = 43	Born et al. (2003)
Minke whale *Balaenoptera acutorostrata*	Svalbard, Norway	11.9 ± 0.9 n = 16	-19.7 ± 0.2 n = 16	Born et al. (2003)

TABLE 4.2 (continued)

Species	Location	$\delta^{15}N$	$\delta^{13}C$	Source
Pinnipeds				
Bearded seal *Erignathus barbatus*	Barrow	16.7 ± 0.9 n = 47	-17.1 ± 0.6 n = 47	this study includes data from Hoekstra et al. (2002)
Bearded seal *Erignathus barbatus*	Canada	16.8 ± 0.1 n = 5	-16.6 ± 0.3 n = 5	Hobson et al. (2002)
Ringed seal *Phoca hispida*	Barrow, Alaska	16.9 ± 0.6 n = 78	-18.5 ± 0.8 n = 78	this study includes data from Hoekstra et al. (2002)
Ringed seal *Phoca hispida*	Holman, Canada	17.2 ± 0.7 n = 25	-20.4 ± 0.4 n = 25	this study
Ringed seal *Phoca hispida*	Canada	13.9 ± 1.4 n = 8	-19.7 ± 0.9 n = 8	Muir et al. 1995
Ringed seal *Phoca hispida*	Thule, Greenland	17.0 ± 0.1 n = 100	-19.4 ± 0.1 n = 100	Hobson et al. (2002)
Spotted seal *Phoca largha*	Little Diomede, Shishmaref, Alaska	17.6 ± 0.9 n = 34	-18.3 ± 0.9 n = 34	this study
Ribbon seal *Phoca fasciata*	Little Diomede, Hooper Bay, Alaska	16.0 ± 1.2 n = 40	-18.7 ± 1.0 n = 40	this study
Harbor seal *Phoca vitullina*	Copper River Delta, Alaska	18.6 ± 0.3 n = 9	-17.6 ± 0.2 n = 9	Hobson et al. (1997)
Harp seal (female) *Phoca groenlandica*	St. Lawrence Estuary, Canada	13.7 ± 0.7 [&] n = 7	-17.6 ± 0.3 [&] n = 7	Lesage et al. (2001)
Hooded seal (male) *Cystophora cristata*	Gulf of St. Lawrence, Canada	16.5 ± 0.7 [&] n = 7	-16.9 ± 0.4 [&] n = 7	Lesage et al. (2001)
Grey seal (male) *Halichoerus grypus*	Gulf of St. Lawrence, Canada	15.6 ± 0.7 [&] n = 8	-17.4 ± 0.5 [&] n = 8	Lesage et al. (2001)
Walrus *Odobenus rosmarus*	Barrow, Little Diomede, Alaska	13.52 ± 1.02 n = 6	-16.93 ± 0.20 n = 6	this study
Walrus *Odobenus rosmarus*	Akulivik, Canada	10.9 ± 0.5 n = 9	-17.3 ± 0.5 n = 9	Muir et al. (1995)
Walrus *Odobenus rosmarus*	Inukjuak, Canada	11.7 ± 0.7 n = 12	-18.7 ± 0.8 n = 12	Muir et al. (1995)
Walrus *Odobenus rosmarus*	Canada	12.5 ± 0.6 n = 6	-17.8 ± 0.3 n = 6	Hobson and Welch (1992)

[*] plasma
[&] blood cells

TABLE 4.3. Concentrations of Cd and THg in liver and kidney and hepatic Ag of selected Arctic marine and terrestrial mammals. Values are given as mean ± standard deviation in μg/g ww unless otherwise noted. Concentration ranges are included whenever available (n = sample size).

			Cadmium		Total Mercury		Silver	
Species	**Location**	**Age / Length [cm]***	**Kidney**	**Liver**	**Kidney**	**Liver**	**Liver**	**Source**
Terrestrial Mammals								
Polar bear *Ursus maritimus*	Barrow, Alaska	10.6 ± 8.7 range: 2 - 29 n = 11	8.96 ± 4.94 range: 1.40 - 19.60 n = 26	0.47 ± 0.21 range: 0.10 - 1.22 n = 26	16.62 ± 13.53 range: 1.56 - 45.90 n = 25	14.01 ± 12.53 range: 1.50 - 54.26 n = 26	0.16 ± 0.08 range: 0.05 - 0.35 n = 23	this study includes data from Woshner et al. (2001a)
Polar bear *Ursus maritimus*	Svalbard, Norway	>2 - n = 16	8.10 ± 7.20 range: 0.30 - 19.00 n = 7	0.60 ± 0.30 range: <0.1 - 1.2 n = 16	4.90 ± 6.6 range: 0.50 - 21.00 n = 7	2.6 ± 2.0 range: 0.10 - 6.00 n = 16	n.a. - -	Norheim et al. (1992)
Polar bear *Ursus maritimus*	Greenand	>6 - n = 8	19.70 ± 1.51 * - n = 8	1.67 ± 1.39 * - n = 8	20.90 ± 2.22 * - n = 8	21.60 ± 1.26 * - n = 8	n.a. - -	Dietz et al. (1996)
Arctic fox *Alopex lagopus*	Barrow, Alaska	0.5 ± 0 range: 0.5 - 0.5 n = 27	0.178 ± 0.19 range: 0.03 - 1.00 n = 27	0.04 ± 0.03 range: <0.01 - 0.15 n = 27	0.43 ± 0.46 range: 0.03 - 2.29 n = 26	0.25 ± 0.25 range: 0.02 - 1.26 n = 26	0.01 ± 0.01 range: <0.01 - 0.03 n = 27	this study
Arctic fox *Alopex lagopus*	Holman, Canada	1.3 ± 1.9 range: 0.5 - 8 n = 20	n.a. - -	0.10 ± 0.11 range: <0.01 - 0.40 n = 19	n.a. - -	0.32 ± 0.42 range: <0.01 - 1.85 n = 19	0.01 ± 0.01 range: <0.01 - 0.03 n = 19	this study
Arctic fox *Alopex lagopus*	Holman, Canada	- - -	n.a.	n.a.	n.a.	0.76 ± 1.12 - n = 16	n.a.	Smith and Armstrong (1975)
Arctic fox *Alopex lagopus*	Svalbard, Norway	1.4 ± 2.4 range: 0 - 9 n = 91	2.20 ± 2.30 range: 0.20 - 13.00 n = 95	0.53 ± 0.50 range: 0.10 - 2.40 n = 94	n.a. - -	0.37 ± 0.43 range: 0.01 - 2.20 n = 93	n.a. - -	Prestrud et al. (1994)
Cetaceans / Odontocetes								
Beluga whale *Delphinapterus leucas*	Point Hope, Point Lay, Alaska	364.8 ± 55.6* range: 206 - 440 n = 47	10.16 ± 4.25 range: 0.46 - 20.40 n = 64	3.05 ± 1.52 range: 0.05 - 7.05 n = 67	4.41 ± 3.00 range: 0.10 - 12.26 n = 46	15.95 ± 15.17 range: 0.28 - 72.48 n = 48	12.84 ± 9.09 range: 1.77 - 50.70 n = 48	this study, includes data from Tarpley et al. (1995) and Woshner et al. (2001b)
Beluga whale *Delphinapterus leucas*	Point Hope, Point Lay, Alaska	374.4 ± 38.4 range: 310 - 434 n = 14	n.a. - -	n.a. - -	n.a. - -	30.51 ± 27.68 range: 1.40 - 72.90 n = 10	27.66 ± 24.67 range: 10.1 - 107.4 n = 14	Becker et al. (1995)

TABLE 4.3 (continued)

Species	Location	Age / Length [cm]*	Cadmium Kidney	Cadmium Liver	Total Mercury Kidney	Total Mercury Liver	Silver Liver	Source
Beluga whale (males) *Delphinapterus leucas*	Cook Inlet, Alaska	397.7 ± 19.9* range: 374 - 422 n = 6	*n.a.* - -	<1 range: <0.44 - <1 n = 6	*n.a.* - -	5.45 ± 3.47 range: 2.98 - 11.42 n = 6	6.78 ± 4.17 range: 1.51 - 11.61 n = 6	Becker et al. (2000)
Beluga whale *Delphinapterus leucas*	Mackenzie Delta, Canada	13.9 ± 5.5 range: 2 - 42 n = 43	9.55 ± 4.47 range: 0.63 - 22.90 n = 43	2.32 ± 1.47 range: 0.22 - 6.42 n = 43	2.23 ± 1.71 range: 0.19 - 9.42 n = 43	11.97 ± 12.38 range: 0.13 - 49.50 n = 42	n.a. - -	Wagemann et al. (1990)
Beluga whale *Delphinapterus leucas*	Grise Fiord, Canada	5.6 ± 4.8 range: 1 - 21 n = 17	9.16 ± 4.86 range: 1.18 - 19.88 n = 1[illegible]	3.07 ± 3.55 range: 0.16 - 15.95 n = 17	1.49 ± 1.07 range: 0.34 - 4.14 n = 17	2.08 ± 1.94 range: 0.36 - 7.41 n = 17	n.a. - -	Wagemann et al. (1990)
Beluga whale *Delphinapterus leucas*	St. Lawrence Estuary, Canada	17.5 ± 8.8 range: 0 - 30 n = 35	1.52 ± 0.89 range: <0.0[illegible] - 3.88 n = 30	0.15 ± 0.11 range: <0.01 = 0.40 n = 30	6.40 ± 9.20 range: 0.32 - 49.48 n = 30	33.52 ± 42.83 range: 0.38 - 498.96 n = 30	n.a. - -	Wagemann et al. (1990)
Beluga whale *Delphinapterus leucas*	Greenland	- - -	10.30 [a] range: <0.02 - 28.70 n = 36	2.21 [a] range: <0.02 - 8.54 n = 40	1.29 [a] range: <0.01 - 8.88 n = 37	1.77 [a] range: 0.07 - 30.80 n = 40	n.a. - -	Hansen et al. (1990)
Narwhal *Monodon monoceros*	Greenland	- - -	39.1 [a] range: <0.02 - 125.00 n = 93	10.8 [a] range: <0.02 - 73.70 n = 90	1.22 [a] range: 0.014 - 4.61 n = 94	5.26 [a] range: <0.01 - 42.80 n = 86	n.a. - -	Hansen et al. (1990)
Narwhal *Monodon monoceros*	Eastern Arctic	420 ± 57* range: 280 - 470 n = 26	54.1 ± 24.1 range: 15.80 - 113.00 n = 55	29.70 ± 25.40 range: 2.44 - 137.00 n = 55	1.93 ± 1.12 range: 0.29 - 6.92 n = 55	10.80 ± 8.05 range: 0.32 - 37.20 n = 55	n.a. - -	Wagemann et al. (1996)
Harbor porpoise *Phocoena phocoena*	Greenland	- - -	13.20 [a] range: 0.11 - 72.50 n = 26	3.25 [a] range: 0.06 - 11.70 n = 44	0.92 [a] range: 0.19 - 2.51 n = 26	4.17 [a] range: 0.48 - 20.70 n = 44	n.a. - -	Paludan-Müller et al. (1993)
Cetaceans / Mysticetes								
Gray whale *Eschrichtius robustus*	Lorino, Lavrentia, Russia	977.7 ± 185.0* range: 780 - 1460 n = 27	1.19 ± 1.50 range: 0.01 - 5.11 n = 28	0.46 ± 0.63 range: 0.01 - 2.20 n = 29	0.01 ± 0.01 range: <0.01 - 0.03 n = 28	0.02 ± 0.01 range: <0.01 - 0.07 n = 28	0.10 ± 0.14 range: <0.01 - 0.67 n = 29	this study
Gray whale *Eschrichtius robustus*	Mechigmenskiy Zaliv, Russia	840 ± 49.3* range: 780 - 950 n = 15	0.59 ± 0.11 - n = 6	0.21 ± 0.04 - n = 5	0.03 ± 0.001 - n = 6	0.16 ± 0.06 - n = 5	0.31 ± 0.06 - n = 5	Tilbury et al. (2002)
Bowhead whale *Balaena mysticetus*	Barrow, Kaktovik, Alaska	1093.0 ± 305.7* range: 60 - 1770 n = 178	15.04 ± 14.94 range: <0.01 - 64.00 n = 156	7.27 ± 8.97 range: 0.03 - 50.91 n = 161	0.03 ± 0.03 range: <0.01 - 0.18 n = 145	0.05 ± 0.07 range: <0.01 - 0.59 n = 154	0.13 ± 0.28 range: <0.01 - 2.37 n = 127	this study, includes data from Bratton et al. (1997) and Woshner et al. (2001b)
Minke whale *Balaenoptera acutorostrata*	Greenland	- - -	3.72 [a] range: 1.71 - 5.62 n = 13	0.90 [a] range: 0.50 - 1.45 n = 17	0.28 [a] range: 0.17 - 1.20 n = 13	0.39 [a] range: 0.14 - 2.68 n = 17	n.a. - -	Hansen et al. (1990)
Minke whale *Balaenoptera acutorostrata*	West Greenland	- - -	16.90 ± 11.30 - n = 4[illegible]	3.89 ± 3.12 - n 36	0.86 ± 0.84 - n = 39	1.00 ± 1.12 - n = 36	n.a. - -	Born et al. (2003)

TABLE 4.3 (continued)

Species	Location	Age / Length [cm]*	Cadmium Kidney	Cadmium Liver	Total Mercury Kidney	Total Mercury Liver	Silver Liver	Source
Minke whale	Svalbard,	-	15.40 ± 8.70	3.76 ± 1.49	0.69 ± 0.34	0.73 ± 0.35	n.a.	Born et al. (2003)
Balaenoptera acutorostrata	Norway	-	-	-	-	-	-	
		-	n = 16	n = 16	n = 16	n = 16	-	
Pinnipeds								
Bearded seal	Barrow, Alaska	9.6 ± 8.4	31.47 ± 20.90	8.66 ± 7.03	0.58 ± 0.28	3.84 ± 3.55	0.34 ± 0.24	this study
Erignathus barbatus		range: 0.5 - 32	range: 1.30 - 94.47	range: 0.57 - 33.62	range: 0.21 - 1.50	range: 0.64 - 20.44	range: <0.01 - 1.13	
		n = 43	n = 30	n = 38	n = 26	n = 34	n = 38	
Bearded seal	Holman, Canada	8.5	n.a.	n.a.	n.a.	143 ± 170	n.a.	Smith and Armstrong (1978)
Erignathus barbatus		-	-	-	-	-	-	
		-	-	-	-	n = 6	-	
Bearded seal	Hudson Bay, Canada	4.9	n.a.	n.a.	n.a.	26.18 ± 26.13	n.a.	Smith and Armstrong (1978)
Erignathus barbatus		-	-	-	-	-	-	
		-	-	-	-	n = 56	-	
Ringed seal	Barrow, Alaska	6.6 ± 6.5	14.70 ± 13.98	3.64 ± 3.01	0.45 ± 0.27	2.47 ± 3.15	0.11 ± 0.13	this study
Phoca hispida		range: 0 - 27	range: <0.01 - 50.70	range: <0.01 - 11.84	range: 0.05 - 1.06	range: 0.06 - 16.55	range: <0.01 - 0.69	includes data from Woshner et al. (2001)
		n = 79	n = 66	n = 66	n = 63	n = 64	n = 66	
Ringed seal	Holman, Canada	11.0 ± 7.4	30.44 ± 19.56	6.65 ± 4.20	1.94 ± 0.74	22.65 ± 17.54	0.55 ± 0.56	this study
Phoca hispida		range: 1 - 26	range: 4.54 - 77.10	range: 1.20 - 18.10	range: 0.79 - 3.71	range: 1.48 - 71.96	range: 0.05 - 2.74	
		n = 25	n = 25	n = 25	n = 25	n = 25	n = 25	
Ringed seal	Holman, Canada	8.1	n.a.	n.a.	n.a.	25.54 ± 15.00	n.a.	Smith and Armstrong (1978)
Phoca hispida		-	-	-	-	-	-	
		-	-	-	-	n = 112	-	
Ringed seal	Western Arctic	7.4 ± 5.1	21.10 ± 14.20	5.60 ± 3.14	2.05 ± 1.34	32.90 ± 35.20	n.a.	Wagemann et al. (1996)
Phoca hispida		range: 0 - 38	range: 0.12 - 87.10	range: <0.01 - 15.90	range: 0.25 - 7.15	range: 0.23 - 219.00	-	
		n = 145	n = 144	n = 142	n = 144	n - 145	-	
Ringed seal	Eastern Arctic	6.1 ± 4.6	47.70 ± 23.30	11.90 ± 9.20	1.49 ± 0.58	8.34 ± 7.03	n.a.	Wagemann et al. (1996)
Phoca hispida		range: 0 - 22	range: 8.98 - 111.00	range: 0.22 - 44.60	range: 0.64 - 2.88	range: 0.36 - 38.70	-	
		n = 114	n = 35	n = 115	n = 35	n = 115	-	
Ringed seal	Greenland	5.4	33.5 ▪	7.79 ▪	0.96 ▪	2.59 ▪	n.a.	Dietz et al. (1998)
Phoca hispida		-	-	-	-	-	-	
		n = 455	n = 456	n = 454	n = 246	n = 248	-	
Ringed seal	Lake Ladoga,	-	0.50 ± 0.48	0.31 ± 0.17	6.15 ± 4.20	35.40 ± 49.15	n.a.	Medvedev et al. (1997)
Phoca hispida ladogensis	Russia	-	range: 0.03 - 1.91	range: 0.07 - 0.57	range: 1.04 - 13.2	range: 0.41 - 170.60	-	
		-	n = 16	n = 19	n = 11	n = 21	-	
Ringed seal	White Sea	-	0.10 ± 0.04	0.18 ± 0.07	0.42 ± 0.15	4.20 ± 11.90	n.a.	Medvedev et al. (1997)
Phoca hispida		-	range: 0.06 - 0.18	range: 0.07 - 0.30	range: 0.19 - 0.77	range: 0.27 - 45.51	-	
		-	n = 14	n = 13	n = 14	n = 14	-	
Ringed seal	Canada	-	49.09 ± 39.41	10.23 ± 6.41	1.31 ± 0.68	8.75 ± 6.65	n.a.	Wagemann (1989)
Phoca hispida		-	0.47 - 143.49	0.10 - 20.51	0.36 - 2.93	0.71 - 21.84	-	
		-	n = 15	n = 15	n = 15	n = 15	-	

TABLE 4.3 (continued)

Species	Location	Age / Length [cm]*	Cadmium Kidney	Cadmium Liver	Total Mercury Kidney	Total Mercury Liver	Silver Liver	Source
Caspian seal *Phoca caspica*	Caspian Sea	11.5 ± 11.2 range: 0 - 41.5 n = 40	9.50 ± 11.00 range: 0.01 - 55.00 n = 42	1.10 ± 1.70 range: 0.10 - 11.00 n = 42	1.60 ± 1.30 range: 0.30 - 8.40 n = 42	15.00 ± 26.00 range: 0.30 - 150.00 n = 42	n.a. - -	Watanabe et al. (2002)
Baikal Seal *Phoca sibirica*	Lake Baikal, Russia	- - -	2.00 ± 1.10 range: <0.02 - 4.30 n = 59	0.28 ± 0.45 range: <0.01 - 3.50 n = 58	1.80 ± 0.80 0.60 - 3.60 n = 40	2.30 ± 2.60 0.20 - 9.10 n = 41	n. a. - -	Watanabe et al. (1998)
Spotted seal *Phoca largha*	Little Diomede, Shishmaref, Alaska	2.8 ± 3.8 range: 0.5 - 24 n = 42	2.58 ± 1.56 range: 0.79 - 7.76 n = 18	0.39 ± 0.48 range: 0.09 - 2.18 n = 17	0.31 ± 0.21 range: 0.08 - 0.90 n = 34	0.68 ± 0.68 range: 0.10 - 2.62 n = 34	0.08 ± 0.03 range: 0.01 - 0.13 n = 17	this study
Ribbon seal *Phoca fasciata*	Little Diomede, Hooper Bay, Alaska	5.9 ± 6.4 range: 0.5 - 25 n = 42	n.a. - -	n.a. - -	0.50 ± 0.35 range: 0.13 - 1.61 n = 40	1.17 ± 1.79 range: 0.18 - 8.52 n = 39	n.a. - -	this study
Harp seal *Phoca groenlandica*	Labrador	<1 - n = 10	7.93 ± 2.65 - n = 10	2.27 ± 0.95 - n = 10	n. a. - -	n. a. - -	0.07 ± 0.03 - n = 10	Yeats et al. (1999)
Harp seal *Phoca groenlandica*	Magdalen Islands, Canada	11.6 ± 5.0 range: 5 - 24 n = 20	23.90 ± 10.39 range: 9.49 - 10.39 n = 20	6.64 ± 2.99 range: 2.57 - 16.09 n = 20	0.83 ± 0.44 range: 0.45 - 2.44 n = 20	10.38 ± 8.10 range: 1.26 - 30.20 n = 20	n.a. - -	Wagemann et al. (1988)
Harp seal (male) *Phoca groenlandica*	Greenland	Adult - n = 8	50.00 ± 20.00 range: 27.20 - 94.10 n = 8	16.00 ± 5.00 range: 9.40 - 22.40 n = 8	0.70 ± 0.40 range: 0.33 - 1.30 n = 8	2.00 ± 3.00 range: 0.35 - 7.70 n = 8	n.a. - -	Julshamn and Grahl-Nielsen (2000)
Harp seal (female) *Phoca groenlandica*	Greenland	Adult - n = 15	50.00 ± 20.00 range: 24.20 - 101.00 n = 15	14.00 ± 6.00 range: 3.60 - 25.10 n = 15	0.70 ± 0.60 range: 0.10 - 2.30 n = 15	2.00 ± 2.00 range: 0.45 - 6.80 n = 15	n.a. - -	Julshamn and Grahl-Nielsen (2000)
Harbor seal *Phoca vitullina*	Kodiak, Alaska	9.3 ± 6.9 range: 1 - 22 n = 23	6.60 range: 0.30 - 44.00 n = 23	n.a. - -	n.a. - -	5.00 range: 0.40 - 72.00 n = 23	0.21 ± 0.15 ^ - n = 58	Miles et al. (1992) ^ Ag by Saeki et al. (2001)
Hooded seal *Cystophora cristata*	Greenland	1 ± 0 range: 1 - 1 n = 8	18.70 ± 1.46 * - n = 8	3.47 ± 1.49 * - n = 8	1.40 ± 1.59 * - n = 8	7.85 ± 1.33 - n = 8	n.a. - -	Dietz et al. (1996)
Hooded seal (males) *Cystophora cristata*	Greenland	Adult - n = 5	100.00 ± 40.00 range: 46.00 - 156.00 n = 5	30.00 ± 30.00 range: 5.50 - 55.50 n = 5	11.00 ± 7.00 range: 5.20 - 23.20 n = 5	70.00 ± 70.00 range: 15.50 - 179.00 n = 5	n.a. - -	Julshamn and Grahl-Nielsen (2000)
Hooded seal (females) *Cystophora cristata*	Greenland	Adult - n = 15	140.00 ± 40.00 range: 86.30 - 207.00 n = 15	40.00 ± 20.00 range: 12.90 - 86.10 n = 15	3.00 ± 1.00 range: 0.95 - 4.80 n = 15	20.00 ± 20.00 range: 2.70 - 74.10 n = 15	n.a. - -	Julshamn and Grahl-Nielsen (2000)
Grey seal *Halichoerus grypus*	Faroe Islands	7.1 ± 6.9 - n = 68	15.80 ± 24.60 range: 0.39 - 155.00 n = 68	5.06 ± 8.63 range: 0.06 - 51.90 n = 68	2.93 ± 2.47 range: 0.41 - 15.90 n = 68	59.70 ± 70.40 range: 1.13 - 238.00 n = 68	n.a. - -	Bustamante et al. (2004b)

TABLE 4.3 (continued)

Species	Location	Age / Length [cm]*	Cadmium Kidney	Cadmium Liver	Total Mercury Kidney	Total Mercury Liver	Silver Liver	Source
Walrus	Bering Strait	-	38.63 ± 19.02 %	8.25 ± 5.06 %	0.26 ± 0.25 %	1.25 ± 1.82 %	0.45 ± 0.33 %	Warburton and Seagars (1993)
Odobenus rosmarus		-	range: 0.83 - 106.17	range: 0.29 - 25.94	range: 0.07 - 1.75	range: 0.08 - 11.07	range: 0.21 - 1.53	
		-	n=50	n=53	n=50	n=53	n=53	
Walrus	*Bering Strait*	-	*46.52 ± 20.19*	*9.47 ± 8.26*	*n.a.*	*1.50 ± 3.18*	*n.a.*	*Taylor et al. (1989)*
Odobenus rosmarus		-	-	-	-	-	-	
		-	n = 42	n = 65	-	n = 62	-	
Walrus	Canada	-	56.61 ± 28.54	11.24 ± 6.58	0.32 ± 0.12	1.35 ± 1.08	n.a.	Wagemann and Stewart (1994)
Odobenus rosmarus		-	range: 0.03 - 130.85	range: 0.03 - 40.96	range: 0.07 - 0.74	range: 0.01 - 5.68	-	
		-	n = 112	n = 116	n = 112	n = 117	-	

n.a. = not analyzed
* geometric mean
♣ sample median
% a factor of 0.299 was used for liver and 0.232 for kidney to convert walrus tissue concentrations from dry weight to wet weight (after Wagemann and Stewart (1994))

TABLE 4.4. Food Web Magnification Factors (FWMF) and Biomagnification Factors (BMF) of selected heavy metals in an Arctic marine food web.

					Selected Biomagnification Factors (BMF)[&]						
	FWMF[#]	R^2	p-value	p-value H_0: a=1[$]	Zooplankton* ↓ Bowhead Whale	Zooplankton* ↓ Arctic Cod*	Zooplankton* ↓ Ringed Seal[%]	Arctic Cod* ↓ Ringed Seal[%]	Sculptured Shrimp* ↓ Bearded Seal	Herring* ↓ Spotted Seal	Ringed Seal[%] ↓ Polar Bear
Cd Kidney	1.86	0.23	0.08	0.40	54.30	0.58	41.60	71.32	3.32	11.79	0.50
Cd Liver	0.31	0.11	0.24	0.02	26.17	-	10.30	17.65	0.91	1.79	0.11
Ag Liver	0.22	0.05	0.45	0.006	2.41	0.96	1.59	1.66	0.42	0.36	1.22
THg Kidney	1.00	0.42	0.01	1.00	6.47	3.29	68.79	20.88	55.54	12.81	30.59
THg Liver	1.40	0.53	0.02	0.45	10.01	-	381.12	115.68	370.16	27.97	4.65

* Total Body Homogenate

[%] Ringed seal harvested in Barrow was used for calculations

[&] BMF = ($Metal_{Predator}$ / $Metal_{Prey}$) / ($\delta^{15}N_{Predator}$ / $\delta^{15}N_{Prey}$)

[#] Foodweb Magnification Factor (slope of linear regression)

R^2 = Coefficient of determination of linear regression

p-value of linear regression

[$] p-value associated with testing the null hypothesis (H_0) of the slope (a) equal to 1

GENERAL CONCLUSIONS

This study showed complex interactions among different Arctic species, their trophic position, specific feeding habits and biological factors, such as sex, season, reproductive status and age. Stable isotopes of carbon and nitrogen have been established as powerful tools in animal ecology. However, it is often underestimated that stable isotopes are highly dynamic and are influenced by age, body condition, gestation or lactation, water stress, body protein catabolism, and urea recycling, among others (Hobson et al., 1993; Barboza et al., 1997; Hobson et al., 1997; Fernandez-Mosquera et al., 2001). Their interpretation is further complicated by unpredictable or poorly understood turnover rates and tissue- and species-specific isotopic fractionation factors (Gannes et al., 1997; Bearhop et al., 2002). On the other hand, analysis of stomach contents or scat may be biased and overestimate prey that resist digestion and have identifiable hard parts (e.g., fish otoliths, cephalopod beaks), and underestimate soft prey (Murie and Lavigne, 1986; Gales and Cheal, 1992; Bowen, 2000; Sheffield et al., 2001). It is often difficult to assess seasonal importance of prey as stomach contents only present a single point in time. In addition, occurrences of individuals with empty stomachs are common in marine mammals, and thus no prey-based information can be obtained. The use of traditional methods, such as fecal or stomach contents analysis in combination with chemical feeding ecology, e.g., stable isotope analysis, describe dietary habits most accurately if direct observation of feeding behavior is not possible.

This research also demonstrated that concentrations of trace elements in marine mammals were substantially influenced by a variety of factors, including age and diet.

Age was identified as one of the most important factors in explaining variability of trace elements, stable isotopes, and prey prevalence in biological systems. Animal age is of significance in the interpretation and identification of possible new sources of contaminants and in providing recommendations for the consumption of subsistence foods (Egeland et al., 1998). However, estimates of animal age can be challenging. Aging based on growth layers in keratin of claws and dentine or cementum of teeth and other calcified structures is widely used (Benjaminsen, 1973; Matson, 1981; Stewart et al., 1996; Childerhouse et al., 2004). This method assumes that the deposition of growth layers is predictable, although studies on known-age beluga whales (*Delphinapterus leucas*) have shown that between one and two growth layers can be deposited annually (Goren et al., 1987). Teeth of female black bears (*Ursus americanus*), and potentially other species, have the capability to re-absorb or reduce production of calcified material during cub rearing, thus making age assessments difficult (Harshyne et al., 1998). In the absence of easily accessible age-defining structures, as is the case for baleen whales, other methods have been developed, such as ear plug counts, aspartic acid racemization, and stable carbon isotope oscillations in baleen (Rice and Wolman, 1971, Schell et al., 1989, George et al., 1999). Linear accumulation of metals with age is often assumed although this study demonstrated more complex nonlinear relationships affected by physiological processes, for example, changes in renal physiology associated with advanced age. Juveniles, fetuses and gestating or lactating females have a higher demand for essential elements like zinc (Zn) and copper (Cu). However, chemical similarity of these elements with cadmium (Cd), silver (Ag) and other heavy metals leads to interactions and binding site competition on transport and storage molecules. Similarly, selenium (Se) is involved in oxy radical scavenging, needed to cope with oxidative stress, particularily in diving species. In addition, Se is required in juveniles for maturation of antioxidant defenses and neuronal development. Many studies have observed a protective effect of Se on mercury (Hg) toxicosis due to an inert and non-toxic Hg-Se storage complex. It was argued that both elements occur in a 1:1 molar ratio (Koeman et al., 1973; Smith and Armstrong, 1978; Caurant et al., 1994; Dietz et al., 2000; Bustamante et al., 2004). However, results of this study showed few animals approaching Se:Hg unity and it is likely that the often reported 1:1 molar ratio may in fact be an indicator for compromised health. Other evidence also suggests that metal accumulation patterns are altered in emaciated or diseased animals. Seals with hepatic lesions had a higher proportion of methyl Hg (MeHg) to total Hg (THg) in liver than healthy seals, suggesting they may have been challenged in their ability to demethylate MeHg. Similarly, tissue concentrations of Zn and Zn status may be useful in the evaluation of body condition, immune status, and animal health. This further illustrates the importance of high quality baseline data to evaluate and interpret effects of contaminants.

This work established that trace element concentrations can be discriminators for characteristic prey species and ecological relationships in the Arctic. Cd concentrations appear to be associated with a diet comprised mostly of invertebrate prey, while Hg is linked to the fish-based food chain. However, the ratio of MeHg to THg may be a better indicator for piscivory than THg alone. Ag has potential to accumulate in the benthic food web and seems particularly linked to cephalopods and large crustaceans. However, biomagnification of Hg, Cd and Ag could not be documented when calculating food web magnification factors using data on the entire Arctic food chain. Further, accumulation of trace metals is organ-specific, and this study established that Cd accumulates predominantly in the kidney, whereas Hg accumulates mainly in the liver of marine mammals, but in the kidney of terrestrial mammals. When calculating trophic transfer factors, assumptions are made that may not be suitable for trace metals. It is often assumed that the tissue under investigation is a good proxy for total body burden of the contaminant and it is further assumed that metals are static. It is important to recognize that metals are tissue-specific, accumulate differently in marine and terrestrial species, and thus are affected by adaptive capabilities and detoxification mechanisms of the animal. Metal accumulation is dependent on age, gender, season, and diet, and accumulation patterns may be altered with disease or other stressors. Additionally, metals are dynamic, actively regulated and eliminated, dependent on transport molecules and subject to binding site competition, and this can lead to unpredictable or disrupted trophic transfer. In general, concentrations of trace metals in tissues of marine and terrestrial mammals harvested in Alaska are low compared to other Arctic regions. Future efforts should include determination of Cd bound to metallothionein and continued monitoring of MeHg in the Arctic food chain to assess bioavailability of these high priority contaminants and possible risks to the subsistence consumer.

REFERENCES

Barboza PS, Farley SD, Robbins CT. Whole-body urea cycling and protein turnover during hyperphagia and dormancy in growing bears (*Ursus americanus* and *U. arctos*). Can J Zool 1997; 75: 2129-2136.

Bearhop S, Waldron S, Votier SC, Furness RW. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. Physiol Biochem Zool 2002; 75: 451-458.

Benjaminsen T. Age determination and the growth and age distribution from cementum growth layers of bearded seals at Svalbard. Fiskeridirektoratets skrifter. Serie havundersøkelser 1973; 16: 159-170.

Bowen WD. Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. Can J Fish Aquatic Sci 2000; 57: 898-905.

Bustamante P, Morales CF, Mikkelsen B, Dam M, Caurant F. Trace element bioaccumulation in grey seals *Halichoerus grypus* from Faroe Islands. Mar Ecol Prog Ser 2004; 267: 291-301.

Caurant F, Amiard JC, Amiard-Triquet C, Sauriau PG. Ecological and biological factors controlling the concentrations of trace elements (As, Cd, Cu, Hg, Se, Zn) in delphinids *Globicephala melas* from the North Atlantic Ocean. Mar Ecol Prog Ser 1994; 103: 207-219.

Childerhouse S, Dickie G, Hessel G. Ageing live New Zealand sea lions (*Phocarctos hookeri*) using the first post-canine tooth. Wildl Res 2004; 31: 177-181.

Dietz R, Ridget F, Born EW. An assessment of selenium to mercury in Greenland marine animals. Sci Total Environ 2000; 245: 15-24.

Egeland GM, Feyk LA, Middaugh JP. The use of traditional foods in a healthy diet in Alaska: risks in perspective. State of Alaska Epidemiology Bulletin 1998; 2: 1-140.

Fernandez-Mosquera D, Vila-Taboada M, Grandal-d'Anglade A. Stable isotopes data ($\delta^{13}C$, $\delta^{15}N$) from the cave bear (*Ursus spelaeus*): a new approach to its palaeoenvironment and dormancy. Proc R Soc London, Ser B 2001; 268: 1159-1164.

Gales NJ, Cheal AJ. Estimating diet composition of the Australian sea-lion (*Neophoca cinerea*) from scat analysis: an unreliable technique. Wildl Res 1992; 19: 447-456.

Gannes LZ, O'Brien DM, Rio CMD. Stable isotopes in animal ecology: Assumptions, caveats, and call for more laboratory experiments. Ecology 1997; 78: 1271-1276.

George JC, Bada J, Zeh J, Scott L, Brown SE, O'Hara T, Suydam R. Age and growth estimates of bowhead whales (*Balaena mysticetus*) via aspartic acid racemization. Can J Zool 1999; 77: 571-580.

Goren AD, Brodie PF, Spotte S, Ray GC, Kaufman HW, Gwinnett AJ, Sciubba JJ, Buck JD. Growth layer groups (GLGs) in the teeth of an adult belukha whale (*Delphinapterus leucas*) of known age: evidence for two annual layers. Mar Mam Sci 1987; 3: 14-21.

Harshyne WA, Deifenach DR, Alt GA, Matson GM. Analysis of error from cementum-annuli age estimates of known-age Pennsylvania black bears. J Wildl Manage 1998; 62:1281-1291.

Hobson KA, Alisauskas RT, Clark RG. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. Condor 1993; 95: 388-394.

Hobson KA, Sease JL, Merrick RL, Piatt JF. Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of nitrogen and carbon. Mar Mamm Sci 1997; 13: 114-132.

Koeman JH, Peeters WHM, Koudstaal-Hol CHM. Mercury-selenium correlations in marine mammals. Nature 1973; 245: 385-386.

Matson GM. Workbook for cementum analysis. Milltown, Montana, Matson's Laboratory, 1981, 31 pp.

Murie DJ, Lavigne DM. Interpretation of otoliths in stomach content analyses of phocid seals: quantifying fish consumption. Can J Zool 1986; 64: 1152-1157.

Rice DW, Wolman AA. The life history and ecology of the gray whale (*Eschrichtius robustus*). The American Society of Mammalogists 1971; Special publication 3.

Schell DM, Saupe SM, Haubenstock N. Natural isotope abundance in bowhead whale (*Balaena mysticetus*) baleen: markers of aging and habitat usage. In: Rundel PW, Ehleringer JR, Nagy KA, editors. Stable isotopes in ecological research. Springer-Verlag, Berlin, 1989, pp. 261-269.

Sheffield G, Fay FH, Feder H, Kelly BP. Laboratory digestion of prey and interpretation of walrus stomach contents. Mar Mam Sci 2001; 17: 310-330.

Smith TG, Armstrong FAJ. Mercury and selenium in ringed and bearded seal tissues from Arctic Canada. Arctic 1978; 31: 75-84.

Stewart REA, Stewart BE, Stirling I, Street E. Counts of growth layer groups in cementum and dentine in ringed seals (*Phoca hispida*). Marine Mammal Science 1996; 12: 383-401.