NUTRIENT AND CONTAMINANT DYNAMICS IN THE MARINE FOOD WEB OF KOTZEBUE SOUND (ALASKA)

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A

THESIS

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ABSTRACT

The objectives of these studies were to document nutrient and contaminant concentrations in upper trophic level organisms of the Kotzeue, Alaska marine food web; address associated risks and benefits to human consumers of these species; understand the drivers of nutrient and contaminant patterns and concentrations; and test the limitations of chemical feeding ecology tools used to trace nutrient and contaminant pathways within this food web. Tissues of subsistence harvested animals were analyzed for nutrients, contaminants and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$). Foods derived from sheefish (*Stenodus leucicthys*) and spotted seal (*Phoca largha*) provide numerous essential nutrients, with limited risk from contaminant exposure. Food processing altered nutrient and contaminant concentrations and stable isotope ratios, warranting the evaluation of foods as they are ultimately consumed when determining the risks and benefits of traditional diets. $\delta^{13}C$ and $\delta^{15}N$, common chemical tracers of feeding ecology and contaminant pathways in food webs, varied widely by tissue type. $\delta^{15}N$ and mercury did not differ consistently among seal tissues. Consequently, when utilizing stable isotopes as tracers of feeding ecology and mercury exposure, the specific tissue consumed and the processed state of the tissue should be considered. Bioaccumulation patterns differed between sheefish and spotted seals in relation to their respiratory physiology and persistent organic pollutant (POP) partitioning behavior between lipids and the respiratory medium (i.e., air versus water). Certain POPs that do not bioaccumulate in fish due to rapid excretion across the gills into surrounding waters (low

K_{OW}) do bioaccumulate in seals if not efficiently eliminated via the lungs to the air (high K_{OA}). Thus, K_{OW} alone cannot predict bioaccumulation in mammals. Regulatory guidelines must incorporate K_{OA} into chemical risk-assessments for air-breathing species, including humans and marine mammals. Ringed (*Phoca hispida*), spotted and bearded (*Erignathus barbatus*) seals had distinct blubber fatty acid (FA) signatures. Blubber of ringed and spotted seals exhibited significant stratification relative to both FA degree of unsaturation and carbon chain length. FA stratification appears largely driven by the steep temperature gradient of blubber, except in the case of polyunsaturated FA (PUFA) which may be maintained in the inner blubber for rapid mobilization to meet physiological requirements.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

LIST OF ABBREVIATIONS

AAS	atomic absorption spectrometry
AF	atomic fluorescence
Ag	silver
AMAP	Arctic Monitoring and Assessment Programme
AMDR	Acceptable Macronutrient Distribution Range
AMMTAP	Alaska Marine Mammal Tissue Archival Project
ANOVA	analysis of variance
AOAC	Association of Analytical Communities
As	arsenic
ASET	Applied Science, Engineering and Technology Laboratory
BAF	bioaccumulation factor
BCF	bioconcentration factor
BDL	below detection limit
BHT	butylated hydroxyl-toluene
BW	body weight
C	carbon
Ca	calcium
CBZ	chlorobenzenes
Cd	cadmium
CHL	chlordanes

Cr	chromium
Cu	copper
CVAFS	cold vapor atomic fluorescence spectrometry
CVD	cardiovascular disease
DBI	double bond index
DCB	dichlorobenzene
DCM	dichloromethane
DDD	dichlorodiphenyldichloroethane
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DRI	Daily Recommended Intake
DV	Daily Value
dw	dry weight
EA-IRMS	element analyzer isotope ratio mass spectrpmetry
FA	fatty acid
FAME	fatty acid methyl ester
FASA	fatty acid signature analysis
Fe	iron
FID	flame ionization detection
GC	gas chromatography
GIT	gastrointestinal tract
GSH-Px	glutathione peroxidase

HCB	hexachlorobenzene
HCH	hexachlorocyclohexane
HCl	hydrochloric acid
Hg	mercury
H_2O_2	hydrogen peroxide
HNO_3	nitric acid
ICP-MS	inductively coupled mass plasma mass spectrometry
ICP-OES	inductively coupled plasma optical emission spectrometry
INBRE	Alaska IDeA Networks for Biomedical Research Excellence
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K	potassium
K_{OA}	octanol-air partition coefficient
K_{OW}	octanol-water partition coefficient
KOH	potassium hydroxide
LCFA	long-chain fatty acid
LCMUFA	long-chain monounsaturated fatty acid
LOQ	level of quantification
lw	lipid weight
MDI	Minimum Daily Intake
MDL	minimum detection limit
MeHg	methyl mercury

Mg	magnesium
MMHSRP	Marine Mammal Health and Stranding Response Program
Mn	manganese
m.p.	melting point
Mo	molybdenum
MUFA	monounsaturated fatty acid
N	nitrogen
n-3 FA	omega-3 fatty acid
Na	sodium
$NaB(C_2H_5)_4$	sodium tetraethylborate
N_{atm}	atmospheric nitrogen
NCRR	National Center for Research Resources
NIH	National Institutes of Health
NIST	National Institutes of Standards and Technology
NLET	National Laboratory for Environmental Testing
NMI FA	non-methylene interrupted fatty acid
NOAA	National Oceanic and Atmospheric Administration
NWRI	National Water Research Institute
OC	organochlorine
OH	organohalogen
Pb	lead
PBDE	polybriminated diphenyl ether

PBT	persistent, bioaccumulative and toxic
PC	principal component
PCA	principal components analysis
PCB	polychlorinated biohenyl
PECB	pentachlorobenzene
POP	persistent organic pollutant
ppb	parts per million
ppm	parts per billion
PTDI	provisional tolerable daily intake
PUFA	polyunsaturated fatty acid
QA/QC	quality assurance and quality control
QSAR	quantitative structure activity relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RfD	reference dose
RSD	relative standard deviation
SCMUFA	short-chain monounsaturated fatty acid
SD	standard deviation
SFA	saturated fatty acid
Se	selenium
SI	stable isotope
SRM	standard reference material

TAMU	Texas A&M University
TCB	trichorobenzene
TDIL	tolerable daily intake limit
THg	total mercury
trans-FA	trans-fatty acid
TTCB	tetrachlorobenzene
WTL	Wildlife Toxicology Laboratory
ww	wet weight
UAA	University of Alaska Anchorage
UAF	University of Alaska Fairbanks
UFA	unsaturated fatty acid
UL	upper limit
USEPA	United States Environmental Protection Agency
VPDB	Vienna Peedee Belemnite
Zn	zinc
%Δ	percent change
$\delta^{13}C$	stable isotope ratio of carbon
$\delta^{15}N$	stable isotope ratio of nitrogen

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INTRODUCTION

The Arctic is undergoing rapid change. Interannual warming in this region is nearly twice that of the global average (Johannessen *et al.* 2004, Graversen *et al.* 2008). Global climate change has led to rising air and ocean temperatures, reduced sea-ice extent and shifts in species distribution and abundances (Comiso 2002, Gaston *et al.* 2003, Rigor and Wallace 2004, Serreze *et al.* 2000). Further, in many regions, the traditional subsistence lifestyle and diet is being replaced by a more Westernized way of life (Murphy *et al.* 1995, Nobmann *et al.* 2005). As a result, wildlife and human nutrition, contaminant exposure, and health status are being impacted (Bersamin *et al.* 2007, Burek *et al.* 2008, Carrie *et al.* 2010, Kraemer *et al.* 2005, Lamon *et al.* 2009, Macdonald 2005, Macdonald *et al.* 2005, McKinney *et al.* 2009, Noyes *et al.* 2009, Prowes *et al.* 2009). It is critical that we characterize current nutrient and contaminant status so that we may monitor and understand these shifts over time and begin to assess their implications for wildlife species and the humans who rely upon them for nutrition, livelihood and the of preservation cultural traditions. Further, we must characterize the factors that control how these chemical components are transferred through food webs in order to better predict concentrations and effects on species, including humans, within this shifting landscape.

Implications of Cultural and Climactic Change on Human Diets in Rural Alaska

Marine mammals and fish are well known resources for subsistence users of northwest Alaska. Residents of these communities depend on these and other wildlife for nutritional, economic, cultural, and spiritual reasons. However, several factors threaten food security, including contaminants, climate change, access to animals, industrial development, and integration of western culture into traditional lifestyles (Caufield 2002, Duhaime 2002).

A number of factors have led to a decrease in the contribution of subsistence foods to the diets of Alaska Natives. Rising air and ocean temperatures have resulted in reduced sea-ice extent, thinner ice and early timing of spring break-up (Vinnikov *et al.* 1999, Stabeno *et al.* 2001, Clement *et al.* 2004, Stroeve *et al.* 2005, Moore and Laidre 2006), thus reducing access to marine resources. Subsequent shifts in species abundances and distribution have been reported. These changes affect not only humans, but resonate throughout the Arctic marine food web. Gaston *et al.* (2003) linked changes in forage fish populations, inferred from changes in the diet composition of thick-billed murres (*Uria lomvia*), with warming trends in Northern Hudson Bay. Changes in prey distribution in the Canadian Arctic have caused shifts in the diets of polar bears (*Ursus maritimus*: Iverson *et al.* 2006). In Alaska, a general shift from arctic to subarctic conditions has coincided with a northward progression of the pelagic-dominated ecosystem of the southern Bering Sea into more northern regions (Overland and Stabeno 2004, Grebmeier *et al.* 2006, Mueter and Litzow 2008).

Many inhabitants of the rural Alaskan coast rely on the sea-ice as a platform to hunt and fish, with the marine species representing the majority of their diet. The changing sea-ice conditions and wildlife availability have impacted the ability of Alaska Natives to harvest certain subsistence species, such as fish and marine mammals (Krupnik 2002, Ray and McCormick-Ray 2004). This had led to dietary shifts that often include the replacement of traditional foods with store-bought alternatives that are far less nutritious.

A cultural shift away from the traditional subsistence way of life is also taking place across rural Alaska. Numerous studies have documented a negative correlation between age and the contribution of subsistence foods to the diet of Alaska Natives (Murphy *et al.* 1995, Nobmann *et al.* 2005, Wilkinson *et al.* 2007). In addition, dietary choices are significantly altered by the availability of store-bought foods, which has increased in recent years. Further, many traditional foods do not have equivalent store-bought replacements (e.g., seal blubber, a lipid rich food high in nutritious omega-3 fatty acids).

Communities are often encouraged by public health officials to continue eating traditional foods (Arnold and Middaugh 2004, Verbrugge and Middaugh 2004). At the same time local residents fear that the presence of environmental contaminants in wildlife species may be making their subsistence foods unsafe. This so called "Arctic Dilemma" (AMAP 1998) may be steering residents away from traditionally healthy subsistence diets to store-bought, processed foods (Blanchet *et al.* 2000, Dewailly *et al.* 2002, Booth and Zeller 2005). Changes in diet as a result of such fears may be more harmful than the

contaminants themselves (Egeland *et al.* 1998). In addition, many store-bought foods contain levels of certain contaminants similar to those found in many subsistence foods (Hites *et al.* 2004, O'Hara *et al.* 2005, Rawn *et al.* 2006).

The combination of all these factors has likely contributed to a decrease in subsistence foods in the diet of some Alaska Natives. The proportion of traditional foods in the diet is directly related to both macro- and micro-nutrient intake (Bersamin *et al.* 2007). These foods are rich in a variety of essential nutrients and are generally considered healthy food choices (Ballew *et al.* 2006, Moses et al. 2009a, b). Decreased intake of subsistence foods has been associated with negative health outcomes in Alaska Native populations. Until recently, obesity, cardiovascular disease, and diabetes were rarely reported among Alaska Natives. Today, chronic disease is emerging as a major concern in this population. Over the past decade the prevalence of obesity has increased dramatically (Rith-Najarian *et al.* 2002, McLaughlin *et al.* 2004, Ebbesson *et al.* 2005a, b, c, d). Diet and physical activity, both linked to subsistence activities, are key factors in the prevention or development of obesity and other chronic disease (Adler *et al.* 1996).

Missing from the current discussion is an understanding of the risks and benefits of traditional foods. Residents of rural Alaska are in need of balanced information to evaluate the nutritional value of subsistence foods relative to the potential contaminant exposure from these same items. Until such information is collected, the overall health implications of the ongoing dietary shift to a more Westernized diet cannot be fully

understood. By studying nutrient and contaminant concentrations in wildlife species one can thus gain insight into human health outcomes, highlighting the intricate linkage between wildlife and human health in this region.

The Effects of Climate Change on Contaminant Exposure in the Arctic

Climate change and variability are having profound effects on contaminant pathways to the Arctic. Environmental contaminants such as heavy metals and persistent organic pollutants (POPs) are primarily released at lower latitudes and reach the Arctic via long-range transport in air and water currents and via biotransport. Climate variability within source regions may cause increases in arable lands and the abundance of agricultural pests or certain disease vectors (e.g., mosquito-borne diseases) leading to increased application of certain pesticides (Kraemer *et al.* 2005). Increased industrialization may also increase the global emissions of some contaminants, such as heavy metals or acid rain producing chemicals, and subsequently the mass reaching the Arctic.

A growing body of literature indicates that warming events may also increase the global transport of certain contaminants to polar regions, independent of changes in their use (Lamon *et al.* 2009). Climate change will greatly affect the environmental fate and behavior of toxicants by altering chemical, physical and biological drivers of partitioning among biotic and abiotic components of the environment (Dalla Valle *et al.* 2007, Macdonald *et al.* 2005, Noyes *et al.* 2009). For example, the volatilization of hexachlorocyclohexane (HCH), an insecticide, from soils is positively correlated with

temperature, which may ultimately lead to increased concentrations in northern regions (Ma *et al.* 2003). Increased microbial activity with increasing temperatures may lead to greater rates of conversion of inorganic mercury into methyl mercury (Loseto *et al.* 2004). Further, the cryosphere acts as a sink for several POPs in the circumpolar north, including HCH, chlordane, DDT, HCB and PCBs (Macdonald *et. al* 2000, Melnikov *et al.* 2003). Melting snow, ice and permafrost as a result of rising temperatures may lead to increased remobilization of these components into the environment (Kraemer *et al.* 2005).

Within the Arctic, increased microbial activity with increasing temperatures may lead to greater rates of conversion of inorganic mercury into methylmercury (Loseto *et al.* 2004). Greater access to shipping routes within the Arctic as a result of longer ice-free periods and increased oil and gas exploration may increase the levels of PAHs in the Arctic environment (Kraemer *et al.* 2005).

These changes in contaminant concentrations in the abiotic environment will presumably coincide with similar shifts in exposure and concentrations among arctic biota. For example rising mercury levels over time in burbot (*Lota lota*), beluga whales (*Delphinapterus leucas*), and ringed seals (*Phoca hispida*) have been linked to climate warming and reduced sea ice cover in the Arctic (Carrie *et al.* 2010, Loseto *et al.* 2008, Gaden *et al.* 2009). In addition, dietary shifts as a result of the effects of climate change on species distribution, as discussed above, can alter contaminant pathways within food

webs. Changes in the timing of sea ice breakup in western Hudson Bay have led to a greater proportion of open water-associated seals relative to ice-associated species in the diet of polar bears (*Ursus maritimus*) resulting in increased levels of chlorinated and brominated POPs in this species (McKinney *et al.* 2009).

Changes in trophic structure, food sources, and feeding behavior may also impact the processes of bioaccumulation and biomagnification within food webs (AMAP 2004, Furnell and Schweinsburg 1984, Macdonald *et al.* 2005, Olafsdottir *et al.* 1998, Stirling *et al.* 1999, Noyes *et al.* 2009). Impacts of climate change on processes such as altered primary production may lead to the addition or removal of trophic levels, in turn shifting predator positions within food webs leading to variability in bioaccumulation and biomagnification (Macdonald *et al.* 2003).

In addition to effects on toxicant concentrations and bioaccumulation patterns in humans and wildlife, contaminant toxicity may also be enhanced by increasing temperatures, in part due to shifts in temperature-induced metabolism (Monserrat and Bianchini 1995, Boone and Bridges 1999, Lydy *et al.* 1999, Gaunt and Baker 2000, Capkin *et al.* 2006, Buckman *et al.* 2007). Conversely, species ability to cope with elevated temperatures while maintaining homeostasis may be impaired by the presence of toxicants, as the two may act together as co-stressors on an individual (Broomhall 2004). Further, by reducing their ability to mount an effective immune response, increased contaminant burdens may render humans and wildlife more susceptible to diseases that may spread to new regions

with changing climate (Brevik *et al.* 2004, Burek *et al.* 2008, de Swart *et al.* 1996, Gilbertson *et. al* 2003, Kajiwara *et al.* 2002, Sagerup *et al.* 2000).

Predictive Tools for Nutrient and Contaminant Exposure and Food Web Dynamics

Considering the changes taking place in nutrient and contaminant dynamics within the Arctic, it is important to document current concentrations of these components and anticipated effects on humans and wildlife so that future shifts can be monitored. Further, we must understand the environmental, biological, and chemical drivers controlling the movement of these chemicals through the food web so that we may predict the impact of these changes. Two chemical tools commonly used for studying the movement of nutrients and contaminants through food webs are stable isotopes of carbon and nitrogen and fatty acid signature analysis. To properly apply these tools, we must fully characterize their variability within and among tissues, individuals and species and understand the drivers of the variability that is present. Further, we must test the assumptions associated with the use of the tools to determine whether they apply across all situations. If achieved, these chemical tools have the potential to reveal a great deal about not only feeding ecology, but also animal physiology and the health of individuals, populations, species and the Arctic ecosystem.

General Objectives of the Thesis

When viewed within the context of the rapid cultural and environmental change that is occurring, and anticipated to continue, within the Arctic, the importance of understanding

nutrient and contaminant dynamics within Alaska becomes obvious. We can gain insight into human and wildlife health and physiology and develop better predictive models for the movement of these components through food webs and the potential impacts of global climate change. This thesis specifically investigates nutrient and contaminant dynamics in the marine subsistence food web of Kotzebue Sound in northwest Alaska.

The species included in the following studies, sheefish and ice seals, were specifically chosen for a number of reasons. They represent nutritionally and culturally important subsistence resources to the human inhabitants of this region. Because they are upper trophic level species in the marine food web, they are useful models for humans with a similar dependence on marine-based prey items. Their high trophic position also positions them as sentinels of contamination within the marine environment due to their ability to bioaccumulate environmental contaminants. In addition, because many arctic marine predators are long-lived with broad geographic distributions, they can be utilized to gain insight into ecosystem processes and structure over large temporal and spatial scales. Knowledge of the health, contaminant exposure and nutritional status of these species can provide integrated information on ecosystem health and functioning at lower trophic levels. Finally, because of their dependence on and close-association with the sea ice, the ice seals may serve as sensitive indicators of the effects of climate change on the arctic ecosystem.

Chapters 1 and 2 examine nutrients and contaminants in two species commonly consumed by subsistence users in Kotzebue, AK. We expand previous studies by evaluating additional tissues (foods) and including the effects of food processing. Both animal health and human intake perspectives are employed by intensively examining animals taken and consumed by subsistence hunters. We emphasize that animal health and human health are intimately linked in this scenario. Spotted seal (*Phoca largha*) and sheefish (*Stenodus leucicthys*) were selected with input from local project participants, because they represent two major food groups (fish and marine mammals) and because their top trophic positions allows them to be good indicators of contaminants present in the marine food web. The unique focus of this study is measuring contaminants and nutrients in an integrated fashion, utilizing both raw product and the actual food items consumed. We evaluate changes in food composition as a result of various preparation methods. Such research is necessary to provide the balanced information regarding nutrients and contaminants that is needed to develop integrated, quantitative models that public health officials can utilize for effective interventions based on actual food items consumed. The information presented here is intended to serve as a basic risk-benefit analysis. We do not intend to provide consumption advice, as that is the responsibility of public health agencies.

The specific objectives of Chapters 1 and 2 were: 1) determine the concentrations of select nutrients and contaminants in spotted seal and sheefish tissues commonly utilized as subsistence foods, 2) determine the effects of traditional food processing methods on

the concentrations of these nutrients and contaminants, and 3) relate the levels of nutrients and contaminants in these subsistence foods to established nutrient and contaminant intake criteria. These studies provide a comprehensive evaluation of the nutritional benefits and subsequent toxicological risks associated with the consumption of two important subsistence species in northwest AK.

Chapter 3 evaluates the variation in Hg concentration, $\delta^{13}C$ and $\delta^{15}N$ in two important subsistence species of northwest Alaska, focusing on tissues commonly utilized as food. Spotted seal (*Phoca largha*) and sheefish (*Stenodus leucicthys*) were selected for this study with input from local project participants, because they represent two major food groups (fish and marine mammals) in this region. In addition, seals represent the major prey item of polar bears, a tissue-selective apex predator of this region. $\delta^{13}C$ and $\delta^{15}N$ are important chemical tools used to assess feeding ecology and trace the movement of caontaminants within food webs. We assessed Hg concentration in parallel with $\delta^{13}C$ and $\delta^{15}N$ in common food tissues from these species both prior to and after food processing imitating traditional cooking methods. In addition, we describe the variation in Hg and stable isotopes within a single species among tissues commonly consumed by humans and certain selectively feeding wildlife species. The subsequent impact on the use of stable isotopes as dietary biomarkers and chemical tracers of Hg exposure in humans and selectively feeding wildlife, such as polar bears, is discussed. This study tests certain assumptions and limitations associated with the use of stable isotopes to gain insight into nutrient and contaminant dynamics in the arctic marine food web of Kotzebue Sound.

Chapter 4 tests the validity of applying partitioning-based contaminant bioaccumulation models developed in aquatic species to air-breathing organisms based on the hypothesis that respiratory elimination of organochlorines (OCs) will differ with respiratory physiology. We compare the relative roles of the octanol-water (K_{OW}) and octanol-air (K_{OA}) partition coefficients, respective measure of lipid-water and lipid-air partitioning, in determining OC concentrations in gilled versus an air-breathing arctic vertebrates in the same relative time, space, and trophic level. Concentrations of various OCs in analogous spotted seal and sheefish tissues were compared in the context of their physical-chemical properties (i.e., K_{OW} and K_{OA}) to determine whether these chemical properties differentially affect bioaccumulation potential in sheefish and spotted seals. This study tests the applicability of current regulatory criteria for classifying bioaccumulative substances developed for aquatic organisms to air-brathing species. The health and exposure implications for mammals, including humans and polar bears, are discussed.

Chapter 5 quantifies fatty acids (FA) at three blubber depths in ringed, bearded and spotted seals at two locations along the ventral midline. It adds to the existing literature by examining these species in a different location (Kotzebue, Alaska) and includes measures of FA concentration on blubber strata. The main objectives of Chapter 5 were to (1) expand the limited baseline data set of FA concentrations and profiles in the blubber of three Alaskan ice seals, (2) characterize the variation in FA with blubber depth (strata) and body location in these species, and (3) investigate the role of certain chemical

properties of FA (i.e., carbon chain length and number of double bonds) in determining the location of FA within stratified blubber. These data provide a baseline on which future shifts in the marine food webs of Alaska and this arctic ecosystem could be based and aids in defining the need for standardized sampling in these species. Such information is essential for effectively monitoring ecosystem and population health.

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CHAPTER 1

Inorganic nutrients and contaminants in subsistence species of Alaska: linking wildlife and human health[1]

1.1 ABSTRACT

Objectives. Determine inorganic nutrient and contaminant concentrations in subsistence foods consumed by Alaska Natives, concentration changes related to common preparation methods and provide a basic risk-benefit analysis for these foods.

Study Design. Eleven essential and six non-essential elements were measured in foods derived from spotted seals and sheefish.

Methods. Essential nutrients in foodstuffs were compared to Daily Recommended Intake (DRI) criteria. Non-essential elements were compared to Tolerable Daily Intake Limits (TDIL). These comparisons serve as a risk-benefit analysis, not as consumption advice.

Results. Cooking altered nutrient and contaminant concentrations. Spotted seal muscle and kidney are rich in Fe and Se; liver in Cu, Fe, Mo and Se; and sheefish muscle in Se. TDIL was exceeded in a 100g serving of seal for THg in raw and fried liver and boiled kidney; MeHg in dried muscle and raw and fried liver; Cd in raw and boiled kidney; and As in raw and rendered blubber. Arsenic exceeded TDIL in sheefish muscle. However, toxicity potential is likely reduced by the element form (i.e., organic As, inorganic Hg) and the presence of protective nutrients, such as Se.

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Conclusions. Preparation methods alter wildlife tissues from their raw state, significantly affecting element concentrations. Direct evaluation of actual food items is warranted to determine risk-benefits ratios of traditional diets. Traditional foods provide many essential nutrients with a very limited risk from contaminants. We encourage continued consumption of traditional foods and urge public health agencies to develop applicable models for providing consumption advice incorporating food processing considerations.

KEY WORDS

Spotted seal, sheefish, essential elements, heavy metals, subsistence

1.2 INTRODUCTION

Marine mammals and fish are well known resources for subsistence users of northwest Alaska (AK). Residents of these communities depend on these and other wildlife for nutritional, economic, cultural, and spiritual reasons. However, several issues threaten food security, including contaminants, climate change, access to animals, industrial development and integration of western culture into traditional lifestyles (1,2).

Until recently, obesity, cardiovascular disease (CVD) and diabetes were rarely reported among AK Natives. Today, chronic disease is emerging as a major concern in this population. Over the past decade the prevalence of obesity has increased among AK Natives (3,4). Diet and physical activity, both linked to subsistence activities, are key factors in the prevention or development of obesity and other chronic disease. Potential consequences of a shift from traditional subsistence-based diets to Western store-bought foods include decreased nutritive value and increased risk of obesity, diabetes and CVD. Biomedical professionals have documented that negative impacts have, or will likely, result (5-9), but these relationships are largely unknown.

Changes to traditional diets in AK result from both local and global factors. Local choices are significantly altered by the availability of store-bought foods, which can often be less nutritious than subsistence alternatives. At the same time, global sources are "contaminating" the arctic food chain with various chemicals (e.g., chlorinated pesticides, heavy metals, radioisotopes). Although known health benefits are associated with

consumption of traditional foods, there is concern about the presence of environmental contaminants.

Northwest AK receives contaminants from both local and global sources. These contaminants have been detected in fish, wildlife and local store bought foods (10-12). Although the impact on local health has not been fully determined, fear of contaminants may be steering residents away from traditionally healthy subsistence diets to store-bought, processed foods (13-15). It has been suggested that changes in diet as a result of such fears may be more harmful than the contaminants themselves (16). Further study must be completed before these relationships can be soundly determined.

Although reports document the presence of contaminants in wildlife tissues, they are incomplete in many ways. Few account for the nutritive value of the tissues in which the contaminants are measured or how food processing affects chemical composition. Most wildlife contaminants studies have focused on tissues that are convenient to sample or biomagnify contaminants and are not conducted from the perspective of a consumer (i.e., do not included tissues specifically utilized as food). Nutrients have rarely been addressed, or addressed only in raw tissues for basic nutrients. Although some have examined nutrients and contaminants in subsistence use (17-22), these studies dealt primarily with the North Slope of AK and did not focus on the consumer. Studies on actual marine food items, as they are consumed by AK Natives, are limited (12,23).

Without these data, intake of contaminants and nutrients cannot be adequately estimated for subsistence communities consuming these foods.

Here, we examine nutrients and contaminants in two species commonly consumed by subsistence users in Kotzebue, AK. We expand previous studies by evaluating additional tissues (foods) and including the effects of food processing. Both animal health and human intake perspectives are employed by intensively examining animals taken and consumed by subsistence hunters. We emphasize that animal health and human health are intimately linked in this scenario. The unique focus of this study is measuring contaminants and nutrients in an integrated fashion, utilizing both raw product and the actual food items consumed. We evaluate changes in food composition as a result of various preparation methods. Such research is necessary to provide the balanced information regarding nutrients and contaminants that is needed to develop integrated, quantitative models that public health officials can utilize for effective interventions based on actual food items consumed. The information presented here is intended to serve as a basic risk-benefit analysis. We do not intend to provide consumption advice, as that is the responsibility of public health agencies.

Spotted seal (*Phoca largha*) and sheefish (*Stenodus leucicthys*) were selected based on availability of subsistence animals with input from local project participants, the community and hunters regarding the most frequently consumed species. Fish and marine mammals comprise the majority of subsistence harvested foods in Kotzebue. The

Native Village of Kotzebue 2002-2004 Harvest Survey (24) reports that fish represent 27% of the subsistence harvest by weight with sheefish making up 45% of the total fish harvest. Marine mammals account for another 26% of the total harvest by weight, with ice seals (spotted, ringed, bearded) accounting for 98% of this harvest.

1.3 MATERIALS AND METHODS

1.3.1 Sample Collection

Samples were collected in October 2004 and March 2005 at Kotzebue, AK (66.90°N, 162.59°W) under Marine Mammal Health and Stranding Response Program (MMHSRP) permit #932-1489-05. Blubber, muscle, liver and kidney samples from spotted seals (*Phoca largha*; n=5) and muscle from sheefish (*Stenodus leucicthys*; n=8) were collected from legally subsistence harvested animals for chemical analyses using Whirl-Pak or Scienceware polyethylene bags. Blubber and liver sub-samples were provided to the Alaska Marine Mammal Tissue Archival Project (AMMTAP) according to the methods of Becker et al. (25). Skin samples (1 cm^2) were provided to the Alaska Department of Fish and Game Arctic Marine Mammal Program for genetic analyses including confirmation of species identification.

All animals were assessed for gross general health prior to sampling to allow for data interpretation in the context of animal condition. Collection of nutrient and contaminant samples was performed as previously described (19). Samples were immediately frozen

at -20°C, shipped to the University of Alaska Fairbanks (UAF) and stored at -80°C until analysis.

1.3.2 Morphometrics and Age Estimation

Spotted seal and sheefish morphometrics appear in Table 1.1. Seal length was measured as the straight line distance from the tip of the nose to both the base and the tip of the tail. Girth was measured at the axillary and umbilical positions. Blubber thickness was measured as the distance from below the epidermis to the blubber-muscle interface at the axillary and umbilical positions via an incision along the ventral midline from the thoracic inlet to anus. Sheefish length was measured as the straight line distance from tip of mandible to fork of tail. Sex and body mass were determined for both species.

Seal age was estimated by counting annual growth layers in the cementum of teeth as described by Dehn et al. (17). Sheefish were aged by counting otolith annual growth increments as described in Brown et al. (26). Each slide was read in triplicate by each of three independent readers.

1.3.3 Food Processing

A portion of each tissue was "food processed" to mimic traditional cooking methods. Spotted seal blubber (ventral midline) was rendered to produce oil. 125g of full thickness blubber from each individual was wrapped in cheese cloth and suspended within a 1000mL glass beaker at room temperature. Oil was allowed to drip from the blubber

until no further appreciable oil was produced (approximately 30 days). Rendered oil was transferred to 40mL clear borosilicate trace clean I-Chem vials (Chase Scientific Glass) and stored at -20°C. Muscle and kidney were boiled by placing approximately 125g into 600mL of ultrapure water in a 1000mL glass beaker and boiling for 20 minutes on a VWR model 355 hotplate. Muscle was also dried by placing 125g of muscle strips (1x3x15cm) in a Precision model 45EG gravity convection oven for 12 hours at 65°C. Liver was fried by placing 14g of butter into a stainless steel frying pan and heating on a VWR model 355 hot plate on the highest setting. When the butter was melted, 125g of liver was placed in the pan for ten minutes, flipping every two minutes with a stainless steel spoon. Processed muscle, liver and kidney were sub-sampled and stored at -80°C until analysis.

Sheefish muscle was baked, dried and smoked both with and without skin on the filets. Baking was carried out at 425°F (218°C) for 20 minutes in a conventional oven (Kenmore Model 911.9349180). Muscle was dried as described above for seals. Finally, muscle was smoked with 50/50 mesquite/hickory wood chips in an electric smoker (Brinkman Model 810-7080-K) for two hours. Processed sheefish muscle was sub-sampled and stored at -80°C until analysis.

1.3.4 Elements Analyses

Raw and food processed tissues were analyzed for essential [calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo),

potassium (K), selenium (Se), sodium (Na), zinc (Zn)] and non-essential elements [arsenic (As), cadmium (Cd), lead (Pb), total mercury (THg), methyl mercury (MeHg), silver (Ag)]. Elements in tissues are reported on a ppm (μg/g) or ppb (ng/g) ww basis.

Elements were analyzed at Texas A&M University (TAMU) and/or the UAF according to US Environmental Protection Agency (EPA) procedures (27) as previously described (18) with minor modifications. Briefly, 0.8g (ww) of homogenized, sub-sampled tissue was digested by a microwave procedure using nitric acid (HNO_3), hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl). In preparation for Se analysis, an aliquot of each digest was heated (95°C, 1 hour) with excess HCl to reduce all Se(VI) to Se(IV).

Ag, As, Cd, Cr, Cu, Mn, Mo and Pb in seals and Ag, As, Cd, Cr, Cu, Fe, Mn, Mo, Pb, Se and Zn in sheefish were analyzed at TAMU using a Perkin Elmer Sciex Elan Model 6100 DRC-II inductively coupled plasma mass spectrometer (ICP-MS). Ca, Fe, K, Mg, Na and Zn in seal samples were analyzed at TAMU using a Spectro Ciros Vision ICP optical emissions spectrometer (ICP-OES). Ca, K, Mg and Na in sheefish were analyzed at the UAF on a Perkin Elmer AAnalyst 800 atomic absorption spectrometer (AAS) using flame ionization. Finally, Se in seals was determined at TAMU by hydride generation with a PSA Millennium System atomic fluorescence (AF) detector.

An aliquot of each digest was diluted 1:4 with 7% HCl for THg analysis. THg in seal tissues was measured at TAMU with a CETAC Quick Trace Mercury Analyzer. THg in

sheefish was analyzed at the UAF using a purge-and-burn technique with cold vapor atomic fluorescence spectrometric (CVAFS) detection on an Amalgamation Control Module equipped with a Model III detector (Brooks Rand) as previously described (10,11,17,18).

MeHg analyses of seals and sheefish were carried out at the UAF using a purge-and-burn technique with CVAFS detection, as established previously by Bloom (28). In short, approximately 0.25g dry weight (dw) of tissue was digested in 25% KOH in methanol. Aqueous phase ethylation was activated using 1% sodium tetraethylborate ($NaB(C_2H_5)_4$) to produce methylethyl mercury. Methylethyl mercury was purged from solution with N_2 gas and collected on Tenax® traps (Brooks Rand). MeHg was thermally desorbed from the traps, analogs separated using isothermal (100°C) gas chromatography, and detected via CVAFS on a Model III detector.

The proportion of THg present as MeHg (%MeHg) in each tissue was determined according to the equation:

$$\%MeHg = MeHg\ (ng/g)/THg\ (ng/g) \times 100$$

1.3.5 Detection limits

The minimum detection limit (MDL) for each element was determined in terms of tissue ww concentrations. In spotted seal tissues, the MDL (ng/g) were 500 (Ca, Mg, K, Na),

125 (Fe), 50 (Cu, Zn), 25 (Mo), 12.5 (Cr, As), 10 (Mn, Se), 8 (THg), 7 (MeHg), 5 (Cd), 2.5 (Ag), and 1.25 (Pb). In sheefish tissues, the MDL (ng/g) were 200,000 (Na), 4000 (Ca), 3000 (Mg), 750 (K), 200 (Fe), 80 (Cu, Mn, Zn), 40 (Mo), 20 (Cr, Se, As, Pb), 8 (Cd), 7 (MeHg), and 4 (THg, Ag).

1.3.6 QA/QC

Element analyses were held to strict QA/QC standards to assure the accuracy and precision of the results. For every 12 samples, at least five QA/QC samples were run, including a method blank, method blank spike, sample spike, sample duplicate and standard reference materials (SRM). SRM were DOLT-2 dogfish liver tissue (Institute for Environmental Chemistry, National Research Council), 1577b Bovine Liver and 1946 Lake Superior Fish Tissue (National Institute of Standards and Technology). Method blanks were held <10% of MDL for the element analyzed. Method blank spikes, sample spikes and SRM were kept within 80-120% recovery. Sample duplicate validation criterion was percent difference <20%.

1.3.7 Calculations and Statistics

1.3.7.1 Concentration Changes Due to Food Processing

Element concentrations were determined in tissues before and after food processing, as described above. Concentration changes due to food processing were calculated as:

$$\% \text{ Change} = \frac{(C_p - C_r)}{(C_r)} \times 100$$

where C_r is the concentration (ppm) of the raw tissue and C_p is the concentration (ppm) of the processed tissue. Thus, (C_p-C_r) represents the absolute concentration change due to food processing, where positive values indicate an increase and negative values indicate a decrease. To account for changes in water content during food processing, absolute and percent change were calculated on both a ww and dw basis. Significance was determined using a paired t-test ($p<0.05$). Water content was determined by freeze drying a 1g sample to a constant mass on a Labconco FreeZone 4.5L Benchtop Freeze Dryer.

1.3.7.2 Daily Recommended Intakes (DRI) and Upper Limits (UL) for Essential Elements

Essential element concentrations (ww) in raw and food processed tissues were compared to Daily Recommended Intakes (DRI) and Upper Limits (UL) (29). The contribution (%) of one serving (100g) of each food product to element DRI/UL was determined. Serving size was chosen as an intermediate to the serving sizes for meats and fish established by the United States Department of Agriculture (57-85g) and the Food and Drug Administration (140g). K and Na were not included in this analysis since these macronutrients do not have DRIs. The reference group for DRI/UL calculations was adult men, ages 31-50 years. It should be noted that recommended intakes vary by cohort (i.e. age, sex, pregnancy/lactation status).

1.3.7.3 Tolerable Daily Intake Limits (TDIL) for Non-Essential Elements

Non-essential element concentrations (ww) in raw and processed tissues were compared to the provisional tolerable daily intake (PTDI) for As, Cd, Pb, THg and MeHg and to the reference dose (RfD) established by the US EPA for Ag (30-34).

Assuming a reference consumer body weight (BW) of 70kg, tolerable daily intake limits (TDIL) of food products for each non-essential element were calculated according to:

$$\text{TDIL (g)} = \frac{\text{PTDI or RfD (µg/kg/day) x BW (kg)}}{C_t \text{ (µg/g, ww)}}$$

where C_t is the mean concentration of the contaminant in the food tissue. The TDIL represents the amount of a particular food a 70kg consumer could safely eat daily throughout their entire lifespan without risk of adverse effect from a given contaminant.

1.4 RESULTS

1.4.1 Essential and Non-Essential Element Concentrations and Changes Due to Food Processing

Concentrations of essential (Ca, Cr, Cu, Fe, Mg, Mn, Mo, K, Se, Na, Zn) and non-essential (As, Cd, Pb, THg, MeHg, Ag) elements in raw and food processed spotted seal and sheefish tissues are summarized in Tables 1.2 through 1.5. Changes in element concentrations as a result of food processing on a ww and dw basis are shown in Tables

1.6 through 1.9. Statistically significant changes ranged from -96.4% (K with rendering of seal blubber) to +217% (As with drying of seal muscle) on a ww basis and from -96.4% (K with rendering of seal blubber) to -13.9% (Mg with frying of seal liver) on a dw basis.

1.4.2 Contribution to Daily Reference Intakes (DRI) and Upper Limits (UL)

The contribution of a 100g serving of each food product to the DRI for essential elements is shown in Table 1.10. Elements present at >100% of DRI in spotted seal tissues included Cu and Mo in raw and fried liver and Fe and Se in all raw and processed muscle, liver and kidney. In sheefish Se exceeded 100% in muscle dried with skin.

In two cases tissues exceeded the UL for an element for a single serving. Dried seal muscle contributes 122% of the UL for Fe. Raw kidney provides 132% of the UL for Se. No element exceeded the UL in a serving of sheefish prepared by any method investigated.

1.4.3 Contribution to Tolerable Daily Intake Limit (TDIL)

Tolerable daily intake levels (TDIL) for non-essential elements are shown in Table 1.11. In spotted seal tissues, elements present above the TDIL in a 100g serving were THg in raw and fried liver, MeHg in dried muscle and raw and fried liver, Cd in raw and boiled kidney and As in raw and rendered blubber. In sheefish muscle, As was above the TDIL in all raw and processed samples.

1.4.4 Percent Methylmercury (% MeHg)

%MeHg in raw and food processed tissues is shown in Tables 1.4 and 1.5. Muscle had the highest %MeHg, followed by liver and kidney. Both THg and MeHg were below the MDL in all blubber samples, thus %MeHg was not determined. %MeHg in muscle (71.2-104% for seals and sheefish) was significantly higher than both seal liver (22.9% raw and 26.4% fried) and kidney (19.9% raw and 20.4% boiled), but the %MeHg in liver and kidney were not significantly different. %MeHg did not change significantly in any spotted seal tissue when food processed by any method. Three processing methods (baking with skin, drying without skin and drying with skin) resulted in significant increases in %MeHg in sheefish muscle. No concurrent significant change in THg concentration was observed. There were no significant differences in %MeHg in sheefish muscle processed by the same method whether skin was present during processing or not.

1.5 DISCUSSION

Alaska is unique within the United States in that a significant proportion of its population depends on fish and wildlife as major food sources. Shifts from traditional subsistence diets to "westernized", store-bought diets have coincided with increases in adverse health issues among AK Natives, including obesity, cardiovascular disease and diabetes (3,4,6-9). Although contaminants are generally lower in arctic species than their counterparts from lower latitudes, they remain a concern due to the importance of these species as food sources. The relative benefits of nutritional contributions must be weighed against

potential risks posed by the presence of contaminants. Store-bought alternatives to subsistence foods are often limited, may not provide the same level of nutrition and may contain appreciable levels of contaminants themselves (12). Numerous studies have concluded that the risks of consuming nutritionally inferior commercial foods outweigh the risks posed by the contaminant intake associated with a subsistence diet (5,16,20,35-37). However, the recent release of an AK based fish consumption advisory (38) for Hg has blurred this issue.

Contaminant and nutrient studies in AK have focused primarily on establishing baseline concentration data for use in species monitoring over space and time. Thus, the tissues studied have not necessarily been those utilized most frequently by human consumers for food. Nor have they investigated how contaminants or nutrients may change when a tissue is processed for food. Therefore, true intake of nutrients and contaminants by subsistence users, critical components of a risk-benefit analysis of traditional foods, is unknown. This study focuses on known food tissues in important subsistence species of northwest AK and documents that these foods contain numerous important nutrients with very limited risk from contaminants and that element concentrations can be significantly altered through food processing.

1.5.1 Essential and Non-Essential Element Concentrations

All elements, except As and Pb, were lower in seal blubber than in muscle, liver or kidney. Typically either liver or kidney contained the highest concentration of a given

element. In most cases element concentrations in raw seal tissues were similar to those previously reported for northern ice seals (11,17,39-45). Concentrations of Ca, Cr, K, Na and MeHg in blubber and Ca and K in kidney were not sufficiently represented in the literature to make an adequate comparison. In this study, As was approximately two-fold higher in blubber and muscle of spotted seals than previously reported for ringed and harp seals (11,44). This result could be a marker for piscivory or locally elevated As levels from geologic sources and/or activities such as mining. Mg was one-third to one-half that reported in the same studies. Very little data on elements in sheefish exists in published literature. Therefore, the data in this study fills an important gap and was not compared with existing information for this species and region.

1.5.2 Changes due to Food Processing

Significant changes in nutrient and contaminant concentrations of tissues resulted from food processing in several cases. Changes were determined not only on a ww basis, but also a dw basis to account for concentration changes resulting from changes in water content. This is a critical consideration for determining risks and benefits to human consumers as the contribution to DRI or TDIL is subsequently affected and potential mechanisms for compositional changes can be proposed.

As an example, raw sheefish muscle provides 53.3% of the DRI for Se per serving. This value does not change significantly for baked fish, but if the fish consumed is smoked or dried, the contribution to DRI increases to 70.0/66.9% (cooked without/with skin) and

93.3/106%, respectively. Similarly, the contribution to TDIL for Cd in spotted seal kidney decreases significantly from 449% to 368% when kidney is boiled as compared to raw. Therefore the contribution to TDIL is overestimated if only raw kidney is considered.

Thus, preparation method must be considered when assessing nutrients and contaminants in traditional foods. By basing DRI and TDIL determinations only on concentrations in raw tissues, the contribution to DRI or TDIL may be grossly under- or overestimated for the actual food items consumed.

1.5.3 Contributions to Daily Reference Intakes (DRI) and Upper Limits (UL)

As expected, traditionally prepared foods provide a number of nutrients at >100% of the DRI per 100g serving. In addition, these foods provide many nutrients in moderate amounts (i.e., 10-100% per 100g serving) while others, such as Ca and Cr, were not represented in any tissue at ≥10% of their respective DRI's. These results support the assertion that traditional foods represent an important, nutritious part of a balanced diet.

In addition to the danger posed by a lack of nutrition, some essential elements can become harmful at excessive doses. Therefore, UL have been developed in addition to minimal requirements. Fe in dried seal muscle and Se in raw kidney exceeded the UL for these elements. But, it should be noted though that UL are *daily* limits. The seasonal nature of subsistence foods makes it extremely unlikely that any given food item would

be eaten every day of the year. In addition, 100g may be an overestimate of a typical serving size for dried seal meat. Finally, raw kidney is not an abundant food item as compared to the mass of other tissues and is included in this study primarily for comparison to processed kidney. Based on these considerations, there does not appear to be a significant threat of essential element toxicosis from consuming these traditional foods. Element interactions are another important consideration, but are beyond the scope of this work.

1.5.4 Contribution to Tolerable Daily Intake Limits

Although Alaskan wildlife is generally less contaminated than wildlife at lower latitudes, several contaminants are still detectable in all tissues of these animals. The non-essential elements investigated may have either natural and/or anthropogenic sources. Contaminant levels approached or exceeded TDIL in some cases.

When interpreting TDIL, one must remember that these values are very conservative and represent the amount that can be consumed *every day over an entire lifetime* without risk of adverse health effects. Due to the seasonality of subsistence foods, it is extremely unlikely that any food item studied would be eaten every day of the year for an entire lifetime. On the other hand, these risks are only those originating from individual food items. Humans consume a range of foods and must also consider contaminant intake from multiple sources. Further, the above assessments do not take into account the chemical form of some elements, a critical factor for assessing potential toxicity.

It is not our intent that comparisons of element concentrations to DRI and TDIL be interpreted as consumption advice. This analysis was utilized to put concentration values into a useful context in terms of human consumption guidelines and to facilitate comparisons between tissues and between the current study and the available published literature. We recognize that it is the responsibility of public health agencies to consider the information presented here and to provide consumption advice accordingly. The data has been provided to public health agencies in Alaska including the Alaska Division of Public Health, Department of Health and Social Services and the Alaska Native Tribal Health Consortium.

1.5.5 Arsenic Speciation Considerations

Only total As was measured in this study. The chemical form of the As present was not determined. Like Hg, As can exist in organic or inorganic forms, yet the PTDI does not take into consideration the relative amounts of each form present. Inorganic As is of greater concern in terms of toxic effects to a consumer. It is well known that As in marine mammals is primarily in the organic form (46). Studies have shown that >90% of the As present is organic with >70% being organic arsenobetaine (47-50). Similarly, fish muscle contains 75-100% organic As (51-53). Therefore, the fact that As was above TDIL in seal blubber and sheefish muscle should be interpreted carefully. It is likely that the levels present in this study do not pose a toxicological risk, but a complete investigation of the As speciation in these tissues is needed to make this conclusion with greater certainty.

1.5.6 Percent Methylmercury (% MeHg)

When considering implications of Hg in foods, it is critical to take into account the chemical and physical forms represented. MeHg is the main form of concern since it can be present in appreciable amounts, is relatively bioavailable and is a known neurotoxicant (54). This is particularly true for the developing central nervous systems of fetuses and children (55), making them the cohort of greatest concern. Inorganic mercury is considered less toxic because it has lower bioavailability and may be bound to selenium in insoluble Hg-Se complexes. MeHg is present in fish and marine mammals, but whether it occurs at levels that may cause subtle neurotoxic effects in human consumers of these species has been widely debated. Long term studies indicate that the benefits obtained from the nutrients (e.g., Se, ω-3 fatty acids) in these foods outweigh any danger posed by the presence of MeHg (37).

%MeHg must be considered together with the THg concentration. A tissue with low %MeHg can still contribute significant levels of MeHg if the THg concentration is substantial. For example, the %MeHg in spotted seal liver (22.9% and 26.4% for raw and fried, respectively) is much lower than that in muscle (86.4%, 71.2% and 103% for raw, boiled and dried). However, because the THg level in liver (1991 and 2510ng/g for raw and fried) is greater than in muscle (182, 261 and 406ng/g for raw, boiled and dried), a serving of liver contributes more MeHg to the diet than an equivalent serving of muscle.

1.5.7 Mercury-Selenium Interactions

Another important consideration for determining potential toxicity of Hg in foods is the relative ratio of Hg to Se in the tissue. Studies suggest Se may be highly effective in reducing Hg toxicity, though human data is lacking (56). The exact mechanism of the protective role of Se against Hg toxicity is unknown. The leading hypothesis is that Se protects against Hg by forming insoluble complexes with Hg, rendering it non-bioavailable (57). Others suggest the mechanism may be related to the antioxidant properties of Se, for example as glutathione peroxidase (GSH-Px), which may protect against Hg toxicity and be involved in demethylation of MeHg (58). For human consumers, intake of tissues with Se in excess of Hg could potentially aid in reducing the bioavailability of Hg and/or mitigating the systemic toxic effects of Hg.

In all tissues studied, Se:THg molar ratio was significantly >1 (student t-test; $p<0.05$). In spotted seals, Se:Hg was lowest in liver (4.84-5.22), followed by muscle (7.05-10.7) and highest in kidney (17.8-31.5). Se:Hg in sheefish muscle was similar (8.35-10.3) to that found in seals. These results indicate that although these traditional foods contain Hg, they are also rich in Se, which may help to counteract any toxic effects of Hg.

1.6 CONCLUSIONS

Cooking can have significant effects on the concentration of elements in a tissue, illustrating the importance of looking at the actual food items consumed when considering the risks and benefits of a traditional diet.

Spotted seal and sheefish tissues were abundant sources of several nutrients. The consumption of these traditional foods does not appear to pose a significant threat due to essential elements exceeding their established UL. Although certain non-essential elements exceeded their respective TDIL in certain food items, considerations of element interactions (Se/Hg), bioavailabilty (Hg-Se complexes) and chemical form (organic vs. inorganic Hg or As) as well as the seasonal nature of subsistence food use, lead to the conclusion that the risk posed by contaminant intake via these items is relatively low.

Overall, the results suggest that the traditional foods investigated provide an array of nutrients accompanied by a very limited risk of contaminant toxicosis. Therefore, we encourage the continuation of traditional food consumption as a nutritious part of a balanced diet. Because the current work was interpreted in terms of a 70kg male human consumer, the data would need to be reevaluated for other consumer cohorts, particularly children and women of childbearing age who may have different nutrient requirements or capacity to tolerate contaminants without ill effect. The data presented here could be used by public health agencies in the future to support the development of cohort-specific consumption advice. It is our intent that the data presented here be treated as a risk-benefit analysis, not consumption advice, which should be provided only by appropriate public health agencies. Finally, we encourage public health agencies to develop models or algorithms to assess overall food safety and quality for underrepresented diets, such as the subsistence diet of many Alaska residents.

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Table 1.1. Spotted seal and sheefish harvest, age, sex and morphometric information including animal identification (ID), AMMTAP[a] ID, harvest date, sex, age, mass, length, girth and blubber thickness

Animal ID	Species	AMMTAP ID	Harvest Date	Sex	Age (yrs) [median (range)]	Mass (kg)	Length (cm)[b]	Girth (cm)[c]	Blubber Thickness (cm)[d]
KOTZ-01-04	Spotted Seal	692-SPSL-015	25-Oct-2004	Male	6 (3-8)	95.2	122/129	92/90	5.5 / 5.2
KOTZ-02-04	Spotted Seal	692-SPSL-016	25-Oct-2004	Female	5 (4-6)	87.1	119/125	93/80	3.5 / 3.6
KOTZ-03-04	Spotted Seal	692-SPSL-017	25-Oct-2004	Male	5 (4-7)	105.2	131/137	98/89	5.2 / 6.0
KOTZ-04-04	Spotted Seal	692-SPSL-018	25-Oct-2004	Male	6 (5-8)	57.0	NA[e]	NA[e]	4.0 / 4.0
KOTZ-05-04	Spotted Seal	692-SPSL-019	25-Oct-2004	Male	3 (2-4)	60.3	106/115	70/68	4.4 / 4.8
KOTZ-01-05	Sheefish	NA	22-Mar-2005	Male	14 (14-19)	5.2	83.0	NA	NA
KOTZ-02-05	Sheefish	NA	22-Mar-2005	Male	15 (15-18)	5.0	79.9	NA	NA
KOTZ-03-05	Sheefish	NA	22-Mar-2005	Female	20 (19-21)	5.2	81.0	NA	NA
KOTZ-04-05	Sheefish	NA	22-Mar-2005	Male	22 (22-25)	5.5	83.1	NA	NA
KOTZ-05-05	Sheefish	NA	22-Mar-2005	Female	20 (19-23)	6.5	87.7	NA	NA
KOTZ-06-05	Sheefish	NA	22-Mar-2005	Female	22 (20-25)	6.7	90.1	NA	NA
KOTZ-07-05	Sheefish	NA	22-Mar-2005	Female	23 (23-23)	4.8	86.6	NA	NA
KOTZ-08-05	Sheefish	NA	22-Mar-2005	Female	17 (17-18)	5.6	79.8	NA	NA

[a] Alaska Marine Mammal Tissue Archival Project (AMMTAP)

[b] Spotted seal length is the straight line distance from tip of nose to base of tail/tip of the tail. Sheefish length was measured from tip of mandible to fork of tail.

[c] Girth is reported here as axillary/umbilical (cm).

[d] Blubber thickness is reported here as axillary/umbilical (cm), measured along the ventral midline.

[e] No length or girth measurements available because body was distorted during transport and storage (could not be appropriately positioned for measurement).

Table 1.2. Essential element concentrations in raw and processed tissues of spotted seals (n=5) harvested in Kotzebue, Alaska (2004). Mean (± 1 SD), median and range (ppm[a] or ppb[b] ww) are reported.[c]

Element	Blubber Raw	Blubber Rendered	Muscle Raw	Muscle Boiled	Muscle Dried	Liver Raw	Liver Fried	Kidney Raw	Kidney Boiled
Ca[a]	7.34 (±0.60)	1.09 (±0.16)	32.0 (±4.8)	35.1 (±8.2)	85.0 (±15.8)	36.8 (±4.2)	67.6 (±25.2)	65.1 (±2.4)	73.3 (±22.9)
	7.41	1.02	31.9	36.9	77.1	39.5	51.7	65.1	83.9
	7.02-8.11	0.97–1.37	25.5–38.7	21.8–44.3	74.4–112	32.1–40.4	46.5–95.9	62.8–68.8	35.1–92.3
Cu[a]	0.12 (±0.01)		1.30 (±0.20)	1.48 (±0.22)	3.13 (±0.75)	16.4 (±8.6)	17.4 (±7.2)	4.09 (±0.42)	3.52 (±1.83)
	0.11	BDL[c]	1.23	1.38	3.18	12.6	16.6	4.15	3.55
	0.10–0.13		1.08-1.55	1.31-1.84	1.90-3.93	7.07-26.4	10.3-26.1	3.43-4.46	0.92-6.02
Fe[a]	4.83 (±2.80)		204 (±53)	271 (±74)	549 (±146)	392 (±151)	431 (±149)	136 (±30)	182 (±39)
	3.58	BDL[c]	217	297	579	389	468	119	186
	3.01-9.68		115-257	163-357	323-705	170-543	198-591	107-181	139-238
Mg[a]	8.10 (±0.91)	1.13 (±0.39)	233 (±12)	214 (±25)	602 (±115)	200 (±9)	225 (±15)	154 (±10)	141 (±47)
	7.76	1.04	228	204	618	201	225	154	167
	7.43-9.67	0.75-1.78	221-252	186-248	411-724	188-212	210-248	140-167	62.7-177
Mn[b]	38.4 (±2.3)	16.7 (±2.1)	169 (±48)	173 (±31)	381 (±12)	5306 (±469)	6408 (±407)	1066 (±92)	1027 (±359)
	38.6	17.1	165	178	421	5340	6490	1080	1070
	34.9-41.4	13.5-19.2	109-240	126-211	166-481	4700-5780	5750-6800	919-1170	425-1320
Mo[b]						552 (±63)	801 (±89)	122 (±12)	152 (±78)
	BDL[c]	BDL[c]	BDL[c]	BDL[c]	BDL[c]	560	822	118	179
						472-633	664-900	109-135	147-232
K[a]	140 (±35)	4.66 (±1.62)	3234 (±253)	2120 (±377)	8922 (±1628)	3084 (±246)	3172 (±325)	2568 (±211)	1344 (±401)
	147	3.81	3100	2190	9140	2940	3290	2560	1550
	102-191	3.34-7.12	2970-3520	1620-2610	6360-10900	2900-3470	2740-3560	2280-2800	860-1690
Se[b]	138 (±25)	14.4 (±10.0)	649 (±191)	700 (±202)	1655 (±606)	2992 (±1010)	3806 (±1053)	5274 (±652)	3776 (±1407)
	142	10.7	605	643	1810	2820	3850	5240	4040
	107-173	7.94-32.0	414-801	517-1030	906-2240	2000-4300	2540-5030	4360-5960	1480-5250
Na[a]	150 (±13)	48.1 (±5.2)	569 (±129)	357 (±78)	1592 (±326)	883 (±69)	1044 (±186)	1858 (±133)	1036 (±337)
	152	46.6	565	341	1500	905	971	1920	1170
	129-165	42.3-56.4	375-708	271-463	1170-2030	790-948	841-1320	1700-1980	538-1350
Zn[a]	3.30 (±0.68)	1.82 (0.54)	19.6 (±3.6)	27.6 (±5.8)	54.4 (±10.0)	42.1 (±4.8)	54.3 (±3.1)	26.2 (±1.4)	35.8 (±11.5)
	3.25	1.67	19.2	28.0	51.4	40.2	54.9	26.5	37.0
	2.43-4.22	1.34-2.74	15.5-25.3	21.1-34.9	41.8-68.5	37.6-47.6	49.7-58.2	23.8-27.6	16.9-46.3

[a] Ca, Cu, Fe, Mg, K, Na and Zn are reported in ppm ww.
[b] Mn, Mo and Se are reported in ppb ww.
[c] BDL = Below Detection Limit. All samples were BDL for Cr.

Table 1.3. Essential element concentrations in raw and processed sheefish muscle (n=8) harvested in Kotzebue, Alaska (2005). Mean (± 1 SD), median and range (ppm[a] or ppb[b] ww) are reported.[c]

Element	Raw	Baked No Skin	Baked With Skin	Dried No Skin	Dried With Skin	Smoked No Skin	Smoked With Skin
Ca[a]	32.6 (±40.0)	15.4 (±5.8)	12.3 (±4.5)	43.6 (±64.6)	100 (±39)	13.0 (±4.8)	21.2 (±24.3)
	21.2	15.2	11.4	20.3	103	12.0	13.2
	5.9-129	6.8-23.4	7.9-22.1	16.3-203	20.2-303	7.8-23.1	8.7-81.1
Cu[a]	323 (±96)	480 (±199)	394 (±130)	675 (±296)	836 (±904)	482 (±141)	430 (±165)
	304	430	352	614	468	460	415
	246-544	245-731	262-623	385-1151	365-3007	309-716	239-770
Fe[a]	2.85 (±1.11)	3.75 (±1.33)	4.39 (±3.75)	6.51 (±3.13)	4.35 (±2.15)	3.30 (±2.19)	3.39 (±1.40)
	2.45	3.87	3.13	5.99	3.68	2.43	3.19
	1.92-5.20	1.44-5.67	2.22-13.5	2.18-11.7	3.11-9.58	1.95-8.48	1.72-6.46
Mg[a]	414 (±58)	431 (±74)	389 (±51)	704 (±162)	815 (±151)	511 (±136)	374 (±33)
	385	435	386	751	843	529	373
	368-538	269-509	318-462	431-875	601-1067	353-736	337-432
Mn[b]	188 (±56)	219 (±43)	208 (±40)	307 (±82)	386 (±227)	252 (±66)	253 (±20)
	165	232	211	298	278	237	253
	123-300	144-264	160-278	179-416	213-883	186-378	211-274
K[a]	5486 (±379)	5760 (±550)	5394 (±587)	9474 (±1343)	10968 (±1860)	6884 (±1139)	6499 (±1407)
	5536	5630	5320	9541	11011	6549	5953
	4481-5957	5167-6676	4802-6394	7495-11301	8708-14595	5510-9163	5100-9182
Se[b]	293 (±30)	320 (±36)	301 (±32)	513 (±134)	584 (±50)	385 (±47)	368 (±42)
	286	317	295	474	594	372	362
	265-344	277-379	251-354	347-730	505-655	333-476	310-452
Na[a]	565 (±118)	658 (±180)	623 (±168)	1063 (±328)	1026 (±319)	640 (±158)	610 (±143)
	605	607	565	989	981	603	609
	414-704	449-920	439-856	765-1825	642-1717	493-980	360-825
Zn[a]	3.81 (±0.34)	4.83 (±0.79)	4.40 (±0.55)	7.87 (±1.61)	8.07 (±1.35)	5.07 (±0.54)	5.27 (±0.94)
	3.81	4.63	4.20	8.34	7.62	5.29	4.93
	3.47-4.47	4.01-6.15	3.78-5.42	4.95-9.52	6.73-10.4	4.24-5.64	4.31-7.26

[a] Ca, Fe, Mg, K, Na and Zn are reported in ppm ww.
[b] Cu, Mn and Se are reported in ppb ww.
[c] BDL = Below Detection Limit. All samples were BDL for Cr and Mo.

Table 1.4. Non-essential element concentrations in raw and processed tissues of spotted seals (n=5) harvested in Kotzebue, Alaska (2004-2005). Mean (± 1 SD), median and range (ppb ww) are reported.

	THg	MeHg	%MeHg	Cd	As	Ag	Pb
Spotted Seal					2704 (±254)		41.5 (±13.4)
Blubber	BDL[a]	BDL[a]	-----	BDL[a]	2630	BDL[a]	36.1
Raw					2410-3060		26.1-60.2
Spotted Seal					2580 (±348)		33.1 (±9.3)
Blubber	BDL[a]	BDL[a]	-----	BDL[a]	2570	BDL[a]	30.6
Rendered					2110-3070		25.3-49.3
Spotted Seal	182 (±46)	149 (±17)	86.4 (±23.9)		197 (±70)		5.73 (±0.95)
Muscle	196	146	72.0	BDL[a]	172	BDL[a]	5.13
Raw	133-242	128-172	64.9-119		141-319		4.96-6.89
Spotted Seal	261 (±74)	179 (±42)	71.2 (±21.0)		200 (±21)		4.34 (±0.89)
Muscle	294	182	61.4	BDL[a]	209	BDL[a]	4.41
Boiled	161-343	112-226	57.8-108		175-219		3.32-5.65
Spotted Seal	406 (±111)	415 (±125)	103 (±17)		583 (±249)		7.82 (±6.65)
Muscle	413	430	110	BDL[a]	532	BDL[a]	5.13
Dried	248-559	289-596	73.0-116		370-991		4.34-19.7
Spotted Seal	1991 (±1170)	314 (±35)	22.9 (±15.2)	478 (±155)	415 (±154)	43.0 (± 21.1)	10.5 (±4.4)
Liver	2500	326	13.6	374	362	44.1	8.94
Raw	615-3140	253-340	10.4-41.1	349-671	304-686	20.9-69.3	6.93-17.9
Spotted Seal	2510 (±1524)	415 (±60)	26.4 (±21.3)	456 (±111)	557 (±159)	61.8 (±32.1)	16.1 (±9.8)
Liver	3440	442	12.9	440	493	60.6	15.2
Fried	835-3730	336-482	9.91-57.8	351-626	432-828	24.9-112	4.91-30.8
Spotted Seal	444 (±132)	83.9 (±13.2)	19.9 (±5.8)	3488 (±538)	281 (±58)		16.5 (±4.4)
Kidney	437	84.4	19.0	3740	296	BDL[a]	15.9
Raw	336-661	63.8-99.8	13.6-29.4	2830-4040	182-333		11.1-21.3
Spotted Seal	576 (±200)	113 (±26)	20.4 (±4.4)	2417 (±1273)	541 (±559)		7.79 (±1.45)
Kidney	526	100	21.1	2270	279	BDL[a]	7.47
Boiled	399-908	90.9-151	15.2-25.1	616-4090	245-1540		6.66-10.2

[a] BDL = Below Detection Limit

Table 1.5. Non-essential element concentrations in raw and processed muscle of sheefish (n=8) harvested in Kotzebue, Alaska (2004-2005). Mean (± 1 SD), median and range (ppb ww) are reported.[a]

	THg	MeHg	%MeHg	As
Sheefish	87.7 (±34.5)	69.0 (±23.9)	80.0 (±13.9)	6236 (±3030)
Muscle	76.7	64.5	76.2	6466
Raw	62.5-169	45.5-117	64.1-99.8	2059-10547
Sheefish	103 (±39)	93.8 (±28.2)	95.8 (±23.9)	5563 (±2437)
Muscle	94.8	87.7	85.5	6147
Baked (No Skin)	58.2-185	66.3-160	76.2-148	1979-8309
Sheefish	102 (±40)	91.9 (±35.4)	90.0 (±7.3)	5810 (±2949)
Muscle	96.9	81.7	89.6	6086
Baked (With Skin)	62.5-191	56.4-169	79.7-98.5	1695-10455
Sheefish	158 (±63)	161 (±66)	104 (±17)	10175 (±4863)
Muscle	133	159	108	10978
Dried (No Skin)	97.8-268	102-306	75.7-125	3326-16697
Sheefish	161 (±66)	156 (±46)	99.8 (±15.2)	10945 (±5720)
Muscle	140	153	97.5	11543
Dried (With Skin)	106-311	94.5-256	82.3-123	2816-18962
Sheefish	108 (±39)	85.6 (±28.5)	80.6 (±14.6)	6772 (±3658)
Muscle	101	83.5	76.5	7101
Smoked (No Skin)	68.4-195	49.1-148	65.9-109	2120-11827
Sheefish	105 (±43)	84.6 (±27.5)	82.4 (±7.8)	5735 (±2646)
Muscle	95.8	80.0	84.8	5819
Smoked (With Skin)	70.0-198	57.4-135	68.4-92.2	2486-10272

[a] Cd, Ag and Pb were below detection in all raw and processed tissues.

Table 1.6. Absolute (ppm[a] or ppb[b]) and percent change (%Δ) [mean (±1 SD)][c] in essential and non-essential elements on a ***wet weight*** basis as a result of food processing for tissues of spotted seals (n=5) harvested in Kotzebue, Alaska (2004).[d]

Element	Blubber Δ with Rendering	Muscle Δ with Boiling	Muscle Δ with Drying	Liver Δ with Frying	Kidney Δ with Boiling
Ca	**-6.25 (±0.69)**	+3.18 (±6.73)	**+53.0 (±16.5)**	**+30.8 (±23.1)**	+8.20 (±21.4)
	-85.0 (±3.1)%	+9.9 (±22.7)%	**+170 (±59)%**	**+81.8 (±55.7)%**	+12.0 (±33.4)%
Cu	NA[d]	+0.18 (±0.34)	**+1.82 (±0.69)**	+0.95 (±8.19)	**-0.57 (±1.75)**
		+16.2 (±26.8)%	**+140.5 (±56.3)%**	+20.8 (±57.6)%	**-14.2 (±41.4)%**
Fe	**-2.88 (±0.55)**	**+67.0 (±33.1)**	+345 (±106)	+38.8 (±273)	+45.8 (±35.2)
	-89.7 (±5.0)%	**+33.5 (±14.1)%**	+170.8 (±36.4)%	+43.4 (±118.9)%	+36.7 (±31.8)%
Mg	**-6.97 (±1.10)**	-19.2 (±31.6)	**+369 (±112)**	+24.8 (±20.2)	-12.3 (±40.2)
	-85.8 (±5.1)%	-7.9 (±13.2)%	**+159 (±49)%**	+12.7 (±11.0)%	-9.0 (±28.1)%
Mn	**-21.7 (±2.7)**	+4.80 (±25.5)	**+212 (±106)**	**+1102 (±464)**	-38.8 (±299)
	-56.4 (±5.5)%	+5.7 (±14.9)%	**+128 (±71)%**	**+21.3 (±9.8)%**	-4.9 (±30.9)%
Mo	NA[d]	NA[d]	NA[d]	**+249 (±72)**	+60.3 (±35.6)
				+45.7 (±15.0)%	+49.4 (±28.3)%
K	**-135 (±36)**	**-1114 (±523)**	**+5688 (±1785)**	+88.0 (±464)	**-1224 (±420)**
	-96.4 (±2.0)%	**-33.9 (±14.1)%**	**+179 (±64)%**	+3.5 (±15.0)%	**-47.5 (±16.4)%**
Se	**-124 (±26)**	+51.6 (±135)	**+1007 (±487)**	**+814 (±387)**	-1498 (±1849)
	-89.5 (±6.5)%	+9.9 (±18.0)%	**+155 (±66)%**	**+29.6 (±13.5)%**	-26.1 (±31.4)%
Na	**-103 (±11)**	**-212 (±133)**	**+1023 (±269)**	+161 (±196)	**-822 (±411)**
	-68.0 (±3.2)%	**-35.1 (±17.0)%**	**+185 (±52)%**	+18.8 (±22.3)%	**-43.6 (±19.8)%**
Zn	**-1.48 (±0.99)**	+7.98 (±6.57)	**+34.8 (±12.0)**	**+12.2 (±7.3)**	+9.62 (±12.1)
	-42.2 (±23.2)%	+44.3 (±40.4)%	**+188 (±95)%**	**+30.8 (±20.1)%**	+37.7 (±45.6)%
THg	NA[d]	**+79.4 (±62.1)**	**+225 (±103)**	**+519 (±415)**	+132 (±204)
		+47.2 (±45.2)%	**+130 (±68)%**	**+24.6 (±17.5)%**	+33.9 (±45.1)%
MeHg	NA[d]	+29.6 (±38.5)	**+266 (±130)**	**+101 (±42)**	+28.9 (±26.3)
		+19.8 (±27.3)%	**+184 (±101)%**	**+32.2 (±12.8)%**	+36.5 (±33.8)%
Cd	NA[d]	NA[d]	NA[d]	+21.8 (±207)	+1071 (±1624)
				+4.2 (±43.0)%	-27.0 (±43.4)%
As	-124 (±473)	+2.60 (±64.6)	**+386 (±254)**	+142 (±279)	+261 (±551)
	-3.8 (±15.7)%	+8.2 (±25.7)%	**+217 (±163)%**	+51.9 (±76.8)%	+91.8 (±180.1)%
Ag	NA[d]	NA[d]	NA[d]	+18.9 (±29.7)	NA[d]
				+54.9 (±69.6)%	
Pb	-8.40 (±18.3)	**-1.39 (±1.58)**	-2.09 (±7.12)	+5.64 (±12.2)	**-5.35 (±7.50)**
	-12.3 (±38.3)%	**-36.1 (±22.2)%**	-46.0 (±53.7)%	+118.0 (±191)%	**-40.1 (±26.3)%**

[a] Ca, Cu, Fe, Mg, K, Na and Zn are reported in ppm ww.

[b] Mn, Mo, Se, THg, MeHg, As, Ag, Cd and Pb are reported in ppb ww.

[c] **Bold** text represents statistically significant changes ($p < 0.05$).

[d] NA = Not available because both raw and processed samples were below the analytical detection limit. All samples NA for Cr.

Table 1.7. Absolute (ppm[a] or ppb[b]) and percent change (%Δ) [mean (±1 SD)][c] in essential and non-essential elements on a ***wet weight*** basis as a result of food processing for sheefish muscle (n=8) harvested in Kotzebue, Alaska (2005).[d]

Element	Δ with Baking (No Skin)	Δ with Baking (With Skin)	Δ with Drying (No Skin)	Δ with Drying (With Skin)	Δ with Smoking (No Skin)	Δ with Smoking (With Skin)
Ca	-17.1 (±37.4)	-20.3 (±36.2)	11.0 (±27.1)	67.4 (±101)	-19.6 (±40.5)	-11.4 (±51.4)
	-7.14 (±88.4)%	-34.2 (±40.8)%	+48.8 (±107.2)%	+436 (±702)%	-21.8 (±60.6)%	+48.2 (±205.8)%
Cu	+157 (±229)	+70.5 (±185)	**+352 (±297)**	+513 (±906)	+159 (±213)	+106 (±193)
	+58.5 (±83.7)%	+31.2 (±58.9)%	**+116 (±92)%**	+164 (±267)%	+62.7 (±72.6)%	+39.6 (±60.0)%
Fe	+899 (±1778)	+1543 (±4352)	**+3658 (±3187)**	+1502 (±2818)	+457 (±2091)	+542 (±1782)
	+48.4 (±82.2)%	+91.0 (±210)%	**+144 (±136)%**	+80.7 (±136)%	+19.8 (±62.9)%	+30.9 (±62.9)%
Mg	+16.7 (±75.3)	-25.2 (±74.5)	**+290 (±154)**	**+401 (±151)**	+97.5 (±134)	**-39.9 (±40.5)**
	+4.90 (±19.6)%	-4.73 (±17.2)%	**+71.4 (±41.7)%**	**+99.0 (±41.9)%**	+24.5 (±35.0)%	**-8.92 (±8.38)%**
Mn	+31.1 (±47.8)	+20.0 (±35.7)	**+119 (±46)**	**+198 (±219)**	**+63.6 (±72.2)**	**+65.3 (±52.0)**
	+21.6 (±29.7)%	+14.4 (±21.0)%	**+65.9 (±28.5)%**	**+112 (±139)%**	**+39.9 (±43.7)%**	**+43.3 (±36.1)%**
K	+274 (±751)	-92.8 (±538)	**+3988 (±1325)**	**+5482 (±1770)**	**+1397 (±1181)**	**+1012 (±1450)**
	+5.63 (±14.5)%	-1.58 (±9.39)%	**+73.2 (±26.2)%**	**+100 (±32)%**	**+25.9 (±22.2)%**	**+18.9 (±27.1)%**
Se	+26.3 (±35.2)	+7.50 (±27.5)	**+220 (±118)**	**+291 (±66)**	**+91.3 (±39.7)**	**+75.0 (±31.0)**
	+9.4 (±12.4)%	+2.9 (±9.4)%	**+74.1 (±36.9)%**	**+101 (±29)%**	**+31.5 (±13.1)%**	**+25.9 (±10.8)%**
Na	+93.3 (±124)	+58.9 (±130)	**+499 (±296)**	**+461 (±284)**	+75.0 (±138)	+45.6 (±145)
	+16.9 (±20.3)%	+11.0 (±20.3)%	**+91.5 (±51.0)%**	**+84.8 (±47.4)%**	+15.3 (±24.2)%	+11.0 (±33.1)%
Zn	**+1.02 (±0.87)**	**+0.59 (±0.67)**	**+4.06 (±1.47)**	**+4.26 (±1.56)**	**+1.25 (±0.74)**	**+1.46 (±0.89)**
	+27.7 (±24.6)%	**+16.3 (±17.7)%**	**+106 (±38)%**	**+114 (±49)%**	**+34.3 (±22.1)%**	**+38.6 (±23.3)%**
THg	**+15.0 (±14.3)**	**+14.8 (±9.8)**	**+69.8 (±38.4)**	**+73.8 (±32.8)**	**+20.3 (±10.2)**	**+17.3 (±13.8)**
	+17.9 (±19.7)%	**+16.9 (±11.9)%**	**+80.4 (±37.9)%**	**+83.9 (±19.2)%**	**+24.3 (±14.0)%**	**+19.2 (±15.1)%**
MeHg	**+24.8 (±12.5)**	**+22.9 (±17.1)**	**+91.8 (±47.7)**	**+87.0 (±33.9)**	**+16.6 (±14.0)**	**+15.6 (±15.2)**
	+39.9 (±24.8)%	**+33.8 (±21.5)%**	**+134 (±52)%**	**+137 (±64)%**	**+26.6 (±24.2)%**	**+25.2 (±25.4)%**
As	-0.67 (±1.17)	-0.43 (±0.77)	**+3.94 (±1.97)**	**+4.71 (±3.27)**	+0.54 (±1.57)	-0.50 (±0.78)
	-8.28 (±14.3)%	-7.70 (±10.4)%	**+64.0 (±15.5)%**	**+72.5 (±32.5)%**	+6.50 (±22.2)%	-4.99 (±12.7)%

[a] Ca, Fe, Mg, K, Na, Zn and As are reported in ppm ww.

[b] Cu, Mn, Se, THg and MeHg are reported in ppb ww.

[c] **Bold** text represents statistically significant changes ($p < 0.05$).

[d] NA = Not available because both raw and processed samples were below the analytical detection limit. All samples were NA for Cr, Mo, Cd, Ag and Pb.

Table 1.8. Absolute (ppm[a] or ppb[b]) and percent change (%Δ) [mean (±1 SD)][c] in essential ad non-essential elements on a ***dry weight*** basis as a result of food processing for tissues of spotted seals (n=5) harvested in Kotzebue, Alaska (2004).[e]

Element	Spotted Seal Blubber Δ with Rendering[d]	Spotted Seal Muscle Δ with Boiling	Spotted Seal Muscle Δ with Drying	Spotted Seal Liver Δ with Frying	Spotted Seal Kidney Δ with Boiling
Ca	**-6.25 (±0.69)**	-12.1 (±21.5)	-6.53 (±11.9)	+45.9 (±52.6)	-66.2 (±79.9)
	-85.0 (±3.1)%	-11.3 (±21.1)%	-5.4 (±11.9)%	+38.6 (±40.9)%	-23.6 (±27.9)%
Cu	NA[e]	-0.42 (±0.94)	-0.72 (±0.74)	-9.90 (±24.4)	**-8.12 (±5.67)**
		-6.9 (±19.7) %	-15.5 (±14.8) %	-8.04 (±44.3) %	**-43.9 (±29.7) %**
Fe	**-2.88 (±0.55)**	+39.1 (±114)	-58.4 (±116)	-204 (±794)	-58.9 (±171)
	-89.7 (±5.0)%	+8.0 (±17.6)%	-2.6 (±24.2)%	+11.9 (±99.6)%	-7.10 (±26.4) %
Mg	**-6.97 (±1.10)**	**-211 (±88)**	-74.6 (±91.1)	**-89.9 (±65.6)**	**-245 (±145)**
	-85.8 (±5.1)%	**-26.1 (±8.9)%**	-9.2 (±10.8)%	**-13.9 (±10.2)%**	**-37.8 (±23.1) %**
Mn	**-21.7 (±2.7)**	-104 (±105)	**-132 (±104)**	-1294 (±1568)	**-1503 (±1061)**
	-56.4 (±5.5)%	-14.8 (±14.1)%	**-21.6 (±10.0)%**	-7.36 (±8.70)%	**-34.2 (±26.1) %**
Mo	NA[e]	NA[e]	NA[e]	+190 (±231)	-89.5 (±212)
				+11.4 (±14.0)%	-17.4 (±40.8) %
K	**-135 (±36)**	**-5149 (±852)**	-287 (±1356)	-2090 (±1245)	**-7485 (±1090)**
	-96.4 (±2.0)%	**-47.3 (±8.5)%**	-2.7 (±12.5)%	-21.0 (±11.8)%	**-66.5 (±9.9)%**
Se	**-124 (±26)**	-314 (±404)	-296 (±392)	-194 (±1284)	**-12559 (±6779)**
	-89.5 (±6.5)%	-11.4 (±16.6)%	-10.6 (±15.4)%	-1.06 (±11.2)%	**-51.6 (±21.8)%**
Na	**-103 (±11)**	-948 (±355)	26.1 (±269)	-272 (±509)	**-5110 (±1602)**
	-68.0 (±3.2)	-48.3 (±11.4)%	+0.7 (±13.9)%	-9.16 (±18.5)%	**-62.6 (±13.9)%**
Zn	**-1.48 (±0.99)**	+7.31 (±20.3)	-1.21 (±34.5)	-2.11 (±20.5)	-13.9 (±44.5)
	-42.2 (±23.2)%	+16.7 (±35.8)%	+10.0 (±63.6)%	-0.19 (±15.4)%	-10.3 (±36.2)%
THg	NA[e]	+85.2 (±209)	-137 (±245)	-155 (±361)	-628 (±966)
		+20.8 (±46.5)%	-13.9 (±43.4)%	-5.10 (±11.7)%	-21.6 (±35.8)%
MeHg	NA[e]	-25.2 (±107)	-17.6 (±133)	+9.95 (±111)	-47.8 (±108)
		-4.51 (±21.1)%	-2.37 (±26.7)%	+1.00 (±11.0)%	-10.5 (±27.3)%
Cd	NA[e]	NA[e]	NA[e]	-0.41 (±0.64)	**-9.25 (±5.86)**
				-19.7 (±36.3)%	**-53.5 (±30.1)%**
As	-124 (±473)	-112 (±158)	+34.4 (±218)	+40.3 (±813)	+127 (±1070)
	-3.8 (±15.7)%	-13.7 (±18.1)%	+7.1 (±34.8)%	+17.4 (±65.2)%	+9.54 (±78.1)%
Ag	NA[e]	NA[e]	NA[e]	+14.9 (±83.3)	NA[e]
				+19.1 (±57.6)%	
Pb	-8.40 (±18.3)	**-7.75 (±6.23)**	-9.85 (±11.4)	+5.51 (±30.3)	**-54.5 (±25.7)**
	-12.3 (±38.3)%	**-36.1 (±22.2)%**	-46.0 (±53.7)%	+37.3 (±114)%	**-68.0 (±14.7)%**

[a] Ca, Cu, Fe, Mg, K, Na, Zn and Cd are reported in ppm dw.

[b] Mn, Mo, Se, THg, MeHg, As, Ag and Pb are reported in ppb dw.

[c] **Bold** text represents statistically significant changes ($p < 0.05$).

[d] Changes in blubber are based on ww values which are assumed to be essentially equivalent to dw values (i.e., 0% water content).

[e] NA = Not available because either raw or processed samples were below the analytical detection limit. All samples were NA for Cr.

Table 1.9. Absolute (ppm[a] or ppb[b]) and percent change (%Δ) [mean (±1 SD)][c] in essential and non-essential on a ***dry weight*** basis as a result of food processing for sheefish muscle (n=8) harvested in Kotzebue, Alaska (2005).[d]

Element	Δ with Baking No Skin	Δ with Baking With Skin	Δ with Drying No Skin	Δ with Drying With Skin	Δ with Smoking No Skin	Δ with Smoking With Skin
Ca	-82.0 (±165)	-94.6 (±159)	**-51.2 (±60.1)**	+76.5 (±220)	-96.2 (±175)	-70.4 (±205)
	-22.7 (±70.6)%	-50.4 (±27.4)%	**-31.0 (±46.1)%**	+168 (±311)%	-44.8 (±39.9)%	+10.6 (±159.9)%
Cu	+251 (±732)	-62.2 (±703)	-83.7 (±578)	+276 (±1373)	+121 (±776)	-3.60 (±715)
	+30.1 (±68.0)%	+4.88 (±51.2)%	-1.24 (±39.2)%	+27.0 (±101)%	+21.9 (±61.1)%	+5.26 (±46.7)%
Fe	+829 (±6051)	+2462 (±14822)	+190 (±6582)	-2414 (±6900)	-1601 (±7044)	-1133 (±6536)
	+21.3 (±66.7)%	+54.9 (±179)%	+11.3 (±58.4)%	-8.29 (±60.0)%	-12.1 (±47.8)%	-2.20 (±47.8)%
Mg	-208 (±344)	**-420 (±238)**	**-353 (±274)**	+79.0 (±366)	-150 (±423)	**-518 (±125)**
	-11.8 (±20.7)%	**-23.4 (±13.5)%**	**-21.4 (±16.4)%**	+4.51 (±23.0)%	-8.63 (±26.8)%	**-31.9 (±8.3)%**
Mn	-31.8 (±154)	-92.4 (±114)	**-178 (±178)**	+56.7 (±400)	-11.3 (±217)	+4.85 (±230)
	-0.17 (±18.5)%	-10.8 (±12.8)%	**-22.4 (±18.2)%**	+8.66 (±57.7)%	+1.21 (±26.5)%	+7.14 (±27.8)%
K	-2695 (±3377)	**-4755 (±2272)**	**-4276 (±3363)**	+1363 (±4521)	-1641 (±3733)	-2196 (±5363)
	-11.9 (±15.0)%	**-22.5 (±11.2)%**	**-19.5 (±14.8)%**	+5.40 (±21.1)%	-7.72 (±17.6)%	-10.6 (±24.6)%
Se	-109 (±203)	**-227 (±129)**	**-231 (±168)**	+59.7 (±271)	-45.2 (±186)	-76.3 (±129)
	-8.3 (±16.5)%	**-19.0 (±9.2)%**	**-19.8 (±15.2)%**	+6.6 (±25.5)%	-3.3 (±16.6)%	-5.9 (±10.4)%
Na	-100 (±445)	-269 (±599)	-269 (±703)	-108 (±693)	-391 (±487)	**-450 (±378)**
	-2.95 (±17.58)%	-11.7 (±23.0)%	-9.56 (±30.7)%	-1.01 (±31.38)%	-15.2 (±21.9)%	**-18.5 (±16.9)%**
Zn	+0.71 (±3.19)	-1.35 (±3.06)	-0.78 (±3.21)	+1.73 (±4.50)	-0.41 (±3.12)	+0.59 (±3.46)
	+6.27 (±22.9)%	-8.01 (±18.5)%	-4.10 (±22.2)%	+13.3 (±32.8)%	-1.35 (±21.7)%	+4.07 (±21.3)%
THg	-1.54 (±63.6)	-23.2 (±50.2)	-56.1 (±103)	-0.26 (±56.5)	-28.6 (±44.3)	-29.9(±40.6)
	-1.3 (±21.7)%	-7.7 (±15.7)%	-15.5 (±26.3)%	-3.3 (±17.6)%	-9.2 (±13.9)%	-11.2 (±12.8)%
MeHg	+39.2 (±53.9)	+18.6 (±71.1)	+20.2 (±73.2)	+59.1 (±86.7)	-23.1 (±49.2)	-17.9 (±58.6)
	+15.9 (±18.4)%	+6.29 (±23.3)%	+9.18 (±29.4)%	+24.2 (±36.6)%	-7.8 (±16.4)%	-6.1 (±21.2)%
As	**-6.28 (±6.51)**	**-6.14 (±3.48)**	**-5.33 (±3.23)**	-1.36 (±5.13)	**-4.81 (±4.44)**	**-7.07 (±4.61)**
	-23.0 (±17.0)%	**-27.1 (±12.8)%**	**-23.8 (±10.8)%**	-9.44 (±18.9)%	**-22.1 (±16.9)%**	**-28.9 (±11.0)%**

[a] Ca, Fe, Mg, K, Na, Zn and As are reported in ppm dw.

[b] Cu, Mn, Se, THg and MeHg are reported in ppb dw.

[c] **Bold** text represents statistically significant changes ($p < 0.05$).

[d] NA = Not available because both raw and processed samples were below the analytical detection limit. All samples were NA for Cr, Mo, Cd, Ag and Pb.

Table 1.10. Mean percent (%) contribution[a,b] of one serving (100g ww) of spotted seal (n=5) and sheefish (n=8) tissue to the Daily Reference Intake (DRI)[c] for select essential elements

		Element:	Ca	Cu	Fe	Mg	Mn	Mo	Se	Zn
		DRI:	1000 mg/day	900 µg/day	8 mg/day	420 mg/day	1.8 mg/day	45 µg/day	55 µg/day	11 mg/day
Species	Tissue	Processing								
Spotted Seal	Blubber	Raw	0.07	1.28	6.04	0.19	0.21	BDL[d]	25.1	3.00
Spotted Seal	Blubber	Rendered	0.01	BDL[d]	BDL[d]	0.03	0.09	BDL[d]	2.62	1.65
Spotted Seal	Muscle	Raw	0.32	14.5	**256**	5.54	0.94	BDL[d]	**118**	17.8
Spotted Seal	Muscle	Boiled	0.35	16.4	**339**	5.09	0.96	BDL[d]	**127**	25.1
Spotted Seal	Muscle	Dried	0.85	34.7	**686**	14.3	2.12	BDL[d]	**301**	49.5
Spotted Seal	Liver	Raw	0.37	**183**	**490**	4.77	29.5	**123**	**544**	38.3
Spotted Seal	Liver	Fried	0.68	**193**	**539**	5.36	35.6	**178**	**692**	49.4
Spotted Seal	Kidney	Raw	0.65	45.4	**170**	3.66	5.92	26.2	**959**	23.8
Spotted Seal	Kidney	Boiled	0.73	39.1	**227**	3.37	5.71	41.0	**687**	32.6
Sheefish	Muscle	Raw	0.33	3.59	3.56	9.86	1.05	BDL[d]	53.3	3.47
Sheefish	Muscle	Baked (No Skin)	0.15	5.33	4.68	10.3	1.22	BDL[d]	58.2	4.39
Sheefish	Muscle	Baked (With Skin)	0.12	4.38	5.49	9.26	1.16	BDL[d]	54.7	4.00
Sheefish	Muscle	Dried (No Skin)	0.44	7.49	8.13	16.8	1.70	BDL[d]	93.3	7.15
Sheefish	Muscle	Dried (With Skin)	1.00	9.29	5.44	19.4	2.15	BDL[d]	**106**	7.34
Sheefish	Muscle	Smoked (No Skin)	0.13	5.36	4.13	12.2	1.40	BDL[d]	70.0	4.61
Sheefish	Muscle	Smoked (With Skin)	0.21	4.77	4.24	8.90	1.41	BDL[d]	66.9	4.79

[a] **Bold** text highlights contributions of >100% of DRI of a given element by a 100g meal of the specified tissue.

[b] Contributions to the DRI of K and Na are not included because no DRI exists for these elements.

[c] Reference group used for DRI analysis is men ages 31-50.

[d] BDL = No contribution to DRI calculated because element was below detection limit in tissue. All samples were BDL for Cr.

Table 1.11. Mean percent (%) contribution of one meal (100g ww) of spotted seal (n=5) and sheefish (n=8) tissue to the toxicological reference dose for select non-essential elements[a]

		Element:	THg	MeHg	Cd	As	Ag	Pb
		PTDI/RfD[b,c] (µg/kg/day):	0.71	0.27	1.0	2.1[d]	5.0	3.57
Species	Tissue	Processing						
Spotted Seal	Blubber	Raw	BDL[e]	BDL[e]	BDL[e]	**(184)**	BDL[e]	1.7
Spotted Seal	Blubber	Rendered	BDL[e]	BDL[e]	BDL[e]	**(176)**	BDL[e]	1.3
Spotted Seal	Muscle	Raw	36.6	78.8	BDL[e]	(13.4)	BDL[e]	0.2
Spotted Seal	Muscle	Boiled	52.5	94.7	BDL[e]	(13.6)	BDL[e]	0.2
Spotted Seal	Muscle	Dried	81.7	**220**	BDL[e]	(39.7)	BDL[e]	0.3
Spotted Seal	Liver	Raw	**401**	**166**	68.3	(28.2)	1.2	0.4
Spotted Seal	Liver	Fried	**505**	**220**	65.1	(37.9)	1.8	0.6
Spotted Seal	Kidney	Raw	89.3	44.4	**499**	(19.1)	BDL[e]	0.7
Spotted Seal	Kidney	Boiled	**116**	59.8	**346**	(36.8)	BDL[e]	0.3
Sheefish	Muscle	Raw	17.7	36.5	BDL[e]	**(424)**	BDL[e]	BDL[e]
Sheefish	Muscle	Baked (No Skin)	20.7	49.6	BDL[e]	**(378)**	BDL[e]	BDL[e]
Sheefish	Muscle	Baked (With Skin)	20.5	48.6	BDL[e]	**(395)**	BDL[e]	BDL[e]
Sheefish	Muscle	Dried (No Skin)	31.8	85.2	BDL[e]	**(694)**	BDL[e]	BDL[e]
Sheefish	Muscle	Dried (With Skin)	32.4	82.5	BDL[e]	**(748)**	BDL[e]	BDL[e]
Sheefish	Muscle	Smoked (No Skin)	21.7	45.3	BDL[e]	**(461)**	BDL[e]	BDL[e]
Sheefish	Muscle	Smoked (With Skin)	21.1	44.8	BDL[e]	**(390)**	BDL[e]	BDL[e]

[a] **Bold** text highlights contributions of >100% of PTDI/RfD of a given element by a 100g meal of the specified tissue.

[b] THg, MeHg, Cd, As, Pb: Provisional Tolerable Daily Intake (PTDI): Joint FAO/WHO Expert Committee on Food Additives (JECFA)

[c] Ag: Reference Dose (RfD): United States Environmental Protection Agency (US EPA)

[d] ( ) indicate that the PTDI for As refers to inorganic As, while total As was measured for this study and used for RfD contribution calculations. Because As is expected to be primarily organic in these tissues, contribution is likely overestimated here (see Discussion).

[e] BDL = No contribution to toxicological reference dose calculated because element was below detection limit in tissue.

CHAPTER 2

Organic nutrients and contaminants in subsistence species of Alaska: concentrations and relationship to food processing method[2]

2.1 ABSTRACT

Objectives. Determine nutrient and contaminant concentrations, concentration changes related to common preparation methods, and provide a basic risk-benefit analysis for select subsistence foods consumed by residents of Kotzebue, Alaska.

Study Design. Eleven organic nutrients and 156 persistent organic pollutants (POPs) were measured in foods derived from spotted seals and sheefish.

Methods. Nutrients in foodstuffs were compared to Daily Recommended Intake criteria. POPs were compared to Tolerable Daily Intake Limits (TDIL).

Results. Cooking, as well as absence/presence of skin during sheefish processing, altered nutrient and contaminant concentrations in seals and fish. Sheefish muscle and seal blubber were particularly rich in omega-3 fatty acids and seal liver in vitamin A. Seal liver exceeded the recommended upper limit for vitamin A. POP contribution to TDIL was <25% in all tissues except blubber, in which four POPs were present at >25% TDIL. No POPs exceeded TDIL in a serving of any tissue studied. The most prominent concerns identified were levels of vitamin A in spotted seal liver and certain POPs in

[2] Published as: Moses, S.K., A.V. Whiting, D.C.G. Muir, X. Wang, T.M. O'Hara. 2009. Inorganic nutrients and contaminants in subsistence species of Alaska: linking wildlife and human health. Int J Circumpolar Health 68(4):354-71.

blubber, warranting consideration when determining how much and how often these foods should be consumed.

Conclusions. Preparation methods altering tissues from their raw state significantly affect nutrient and contaminant concentrations, thus direct evaluation of actual food items is highly recommended to determine risk-benefits ratios of traditional diets. Traditional foods provide essential nutrients with very limited risk from contaminants. We encourage the consumption of traditional foods and urge public health agencies to develop applicable models to assess overall food safety and quality.

KEY WORDS

Spotted seal, sheefish, organic nutrients, persistent organic pollutants, subsistence

2.2 INTRODUCTION

Fish and wildlife are important resources to the residents of northwest Alaska (AK). In Kotzebue, AK (USA), fish and marine mammals comprise the majority (70%) of subsistence harvested foods with sheefish representing 45% of the total fish harvest and ice seals (spotted, ringed, bearded) accounting for 98% of the marine mammal harvest (1).

Obesity, cardiovascular disease and diabetes were rarely reported among AK Natives historically. Today chronic disease is emerging as a major concern in this population as obesity has increased among AK Natives (2,3). A shift from traditional subsistence-based diets to Western store bought foods may include decreased nutritive value and physical activity and increased risk of obesity, diabetes and cardiovascular disease. Biomedical professionals have documented that negative impacts have, or will likely, result from decreased subsistence food use (2,4-10).

Although health benefits from consumption of traditional foods exist, there is concern about the presence of environmental contaminants. Contaminants enter the arctic food chain from both local (11,12) and global sources (13). Anxiety about contaminants may be steering residents away from traditionally healthy subsistence diets to store-bought, processed foods, which are as a whole, less nutritious and are also known to contain contaminants in northern AK (14-16). Decreased nutritional quality as a result of such

changes in diet may actually be more harmful than the contaminants in subsistence foods themselves (17), given the levels of contaminants present and the quantities consumed.

Previous studies have documented the presence of contaminants in wildlife tissue, but many are incomplete in that they do not account for the nutritional value of the tissues or how food processing may impact chemical composition. Investigators that have previously examined contaminants and nutrients in subsistence use species (18-23) did not primarily conduct their studies from the perspective of the consumer. Studies on actual marine food items, as they are consumed by AK Natives, are limited (14,24). Without these data, intake of contaminants and nutrients cannot be adequately estimated for subsistence communities consuming these foods.

We add to existing wildlife studies in Alaska by examining the nutrient and contaminant content of tissues from two species commonly consumed by subsistence users in Kotzebue, AK. The selected species can also be used as models for related species and tissues that are consumed. We expand the previous studies by evaluating additional tissues (foods) and including the effects of food processing on these tissues. Both animal health and human intake perspectives are employed by intensively examining animals taken and consumed by subsistence hunters. Spotted seal (*Phoca largha*) and sheefish (*Stenodus leucicthys*) were selected with input from local project participants, because they represent two major food groups (fish and marine mammals) and because their top

trophic positions allows them to be good indicators of contaminants present in the marine food web.

The unique focus of this study is measuring contaminants and nutrients in an integrated fashion, utilizing both the raw product and the actual food items consumed. This research is necessary to provide balanced information regarding contaminants and nutrients and to provide the information needed to develop integrated, quantitative models that public health officials can utilize for effective recommendations and interventions based on actual food items consumed. We emphasize that animal health and human health are intimately linked in this scenario.

The specific aims of the current study were: 1) determine the concentrations of select nutrients and contaminants in spotted seal and sheefish tissues commonly utilized as subsistence foods, 2) determine the effects of traditional food processing methods on the concentrations of these nutrients and contaminants, and 3) relate the levels of nutrients and contaminants in these subsistence foods to established nutrient and contaminant intake criteria. The study presented here focuses on organic nutrients and contaminants. Inorganic nutrients and contaminants in these species were reported by Moses et al. (25). Together, these studies provide a comprehensive evaluation of the nutritional benefits and subsequent toxicological risks associated with the consumption of two important subsistence species in northwest AK.

2.3 MATERIALS AND METHODS

2.3.1 Sample Collection, Morphometrics and Aging

Sample collection and permission (MMHSRP permit #932-1489-05), storage, morphometric measurements and age estimation have been previously described in detail and reported (25). Samples were collected in October 2004 and March 2005 at Kotzebue, AK. Blubber, muscle, liver and kidney samples from spotted seals (*Phoca largha*; n=5) and muscle from sheefish (*Stenodus leucicthys*; n=8) were collected from legally subsistence harvested animals for chemical analyses.

2.3.2 Food Processing

A portion of each tissue was "food processed" to mimic traditional cooking methods as described in Moses et al. (25). Processing methods for seal tissues included rendering of blubber; boiling and drying of muscle; frying of liver and boiling of kidney. Sheefish muscle was baked, dried and smoked both with and without skin on the filets.

2.3.3 Nutrients

Nutrient analyses were conducted by Maxxam Analytics (Mississauga, Ontario, Canada). All samples of the same species/tissue/processing method were pooled (spotted seals, n=5; sheefish, n=8), homogenized and a single nutrient concentration obtained in order to meet the large sample size requirement for these analytes. Similar sample masses from each individual were included in the pooled sample, thus analytic results represent a proximate average concentration. Nutrients investigated include total fat, saturated fat,

cis-monounsaturated fatty acids (MUFA), cis-polyunsaturated FA (PUFA), trans-FA, omega-3 PUFA, omega-6 PUFA, cholesterol, beta carotene, and vitamins A and C.

FA profiles were generated according to Association of Analytical Communities (AOAC) method 996.06. Cholesterol was analyzed according to AOAC method 976.26, vitamin A and beta carotene according to AOAC methods 922.04 and 922.06, and vitamin C according to AOAC methods 967.22 and 984.26 (26).

2.3.4 Persistent Organic Pollutants (POPs)

Persistent organic pollutants (POPs) including organochlorine pesticides, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) were determined in spotted seal and sheefish tissues according to previously described quantitative methods (27-29). Briefly, samples were extracted with dichloromethane (DCM), followed by drying and concentration of extracts. Extracts were analyzed using single column capillary GC. Analyses were carried out at the National Laboratory for Environmental Testing (NLET) at the National Water Research Institute (NWRI, Burlington, Ontario, Canada).

Quantification of POPs was performed using internally (NLET) prepared spiking standards containing known concentrations of POPs. Standard reference materials (SRM 1588a: organics in cod liver oil) from the National Institutes of Standards and Technology (NIST, Gaithersburg, MD, USA) were used to confirm the accuracy and

reproducibility of the analytical methods. A calibration check standard was run every six samples, followed by a spike or SRM for further QA/QC. Method blanks were run in order to blank correct analyzed contaminant concentrations. The minimum detection limit (MDL) for POPs in tissues varied depending upon analyte and sample size. Individual MDL are reported in Tables 2.2 and 2.3.

POPs determined in spotted seal tissues were 1,3,5-, 1,2,4- and 1,2,3-trichlorobenzene (TCB); hexchlorobutadiene; 1,2,4,5- and 1,2,3,4-tetrachlorobenzene (TTCB); pentachlorobenzene (PECB); hexachlorobenzene (HCB); 3,4,5,6-tetrachloroveratrole; pentachloroanisole; α-, β- and γ-hexachlorocyclohexane (HCH); heptachlor; octachlorostyrene; heptachlor epoxide; oxychlordane; α- and γ-chlordane; cis- and trans-nonachlor; dieldrin; o,p- and p,p-dichlorodiphenyldichloroethylene (DDE); o,p- and p,p-dichlorodiphenyldichloroethane (DDD); o,p- and p,p-dichlorodiphenyltrichloroethane (DDT); methoxychlor; mirex; PCB congeners (IUPAC designations in order of elution: 1, 3, 4/10, 7/9, 6, 8/5, 19, 12/13, 18, 15/17, 24/27, 16/32, 54/29, 26, 25, 31/28, 50, 33/20, 53, 51, 22, 45, 46, 52, 43, 49, 47/48, 44, 59, 42, 71/41/64, 40, 100, 63, 74, 70/76/98, 66, 95, 91, 55, 56/60, 92, 84, 101, 99, 119, 83, 97, 81/87, 85, 136, 110, 82, 151, 135/144, 147, 107, 149, 118, 133, 114, 134/131, 146, 153, 132, 105, 141, 179, 137, 176, 130, 163/138, 158, 129, 178, 175, 182/187, 183, 128, 167, 185, 174, 177, 202/171, 156, 173, 157/200, 172, 197, 180, 193, 191, 199, 170/190, 198, 201, 203/196, 189, 208/195, 207, 194, 205, 206, 209) ("/" indicates co-eluters); and PBDE congeners (in order of elution: 17, 28/33, 49, 47, 66, 100, 99, 85, 154, 153, 138, 183, 190). POPs determined in sheefish

were the same as above, except that 1,3-, 1,2- and 1,4-dichlorobenzene (DCB) were additionally analyzed but PBDE congeners were not analyzed in fish.

Some concentrations are reported as sums of contaminant groups. These include ΣPCB (sum of all PCB congeners detected), ΣPCB_{10} (sum of PCB congeners 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180), ΣDDT (sum of o,p- and p,p-substituted DDT, DDE and DDD), ΣCHL (sum of oxychlordane; methoxychlor; heptachlor; heptachlor epoxide; α- and γ-chlordane; and cis- and trans-nonachlor), ΣHCH (sum of α-, β- and γ-HCH isomers), ΣCBZ [sum of 1,2-, 1,3-, and 1,4-DCB; 1,3,5-, 1,2,4- and 1,2,3-trichlorobenzene (TCB); 1,2,4,5- and 1,2,3,4-tetrachlorobenzene (TTCB); PECB and HCB], and ΣPBDE (sum of all PBDE congeners detected).

2.3.5 Calculations and Statistics

2.3.5.1 Concentrations and Summary Statistics

POP concentration summary statistics are reported for each tissue type/processing method combination for which the analyte was above the MDL in >50% of samples of that group. When >50% but <100% of samples were above the MDL, a value of ½ MDL was used for all samples that were below the minimum detection limit (BDL) to calculate summary statistics.

2.3.5.2 Concentration Changes Due to Food Processing

Nutrient and POP concentrations (ww) were determined in tissues prior to and after food processing, as described above. Concentration changes due to food processing were calculated as:

$$\% \text{ Change} = \frac{(C_p - C_r)}{(C_r)} \times 100$$

where C_r is the concentration of the raw tissue and C_p is the concentration of the processed tissue. Thus, $(C_p - C_r)$ represents the absolute concentration change due to food processing, where positive values indicate an increase and negative values indicate a decrease. Percent change was not calculated if either the raw or processed samples were BDL. To account for changes in water or lipid content during processing, absolute and percent change were calculated on a ww, dry weight (dw) and lipid weight (lw) basis. Significance was determined using a paired t-test ($p<0.05$). In addition, t-tests were repeated with the inclusion of a Bonferroni correction for multiple comparisons to account for potential Type I error arising from the use of multiple t-tests within a family (i.e. tissue/processing combination).

2.3.5.3 Reference Intake Values for Organic Nutrients

Nutrient concentrations (ww) in raw and processed tissues were compared to reference intake values (Daily Reference Intake (DRI)/Upper Limit (UL), Acceptable

Macronutrient Distribution Range (AMDR) or Daily Value (DV), as appropriate) (30). The contribution (%) of one serving (100g) of each food product to the reference values was determined. The recommendation for trans-FA intake is "as low as possible while consuming a nutritionally adequate diet", thus % contributions to recommended intakes need not be calculated. Reference intake values for cis-MUFA and cis-PUFA were not available, therefore these nutrients were not included in this analysis. The reference group used for calculations was adult men, ages 31-50 years. It should be noted that recommended intakes vary by cohort (i.e. age, sex, pregnancy/lactation status).

2.3.5.4 Tolerable Daily Intake Limits (TDIL) for Persistent Organic Pollutants

POP concentrations (ww) in raw and processed tissues were compared to established tolerable daily intakes (TDI). Assuming a reference consumer body weight (BW) of 70kg, tolerable daily intake limits (TDIL) of food products for each POP were calculated according to:

$$\text{TDIL (g)} = \frac{\text{TDI (ng/kg/day) x BW (kg)}}{C_t \text{ (ng/g, ww)}}$$

where, C_t is the mean concentration of the contaminant in the food tissue. The TDIL represents the amount of a particular food a 70kg consumer could safely eat daily throughout their entire lifespan without risk of adverse effect from a given contaminant.

The oral reference dose (RfD) established by the United States Environmental Protection Agency (31) was utilized as the TDI for hexachlorobutadiene, dieldrin, mirex, ΣCHL and ΣHCH. TDI guidelines established by Health Canada (32) were utilized for ΣPCB, ΣDDT and ΣCBZ. The TDI value for DDT was used to calculate the TDIL for ΣDDT, a group which contains both DDT and its DDE and DDD metabolites. The TDI used to calculate the TDIL for ΣCBZ was the mean of all contaminants included in this group. TDIL calculations for tetrachloroveratrole, octachlorostyrene, pentachloroanisole and ΣPBDE are not presented here because no official intake criteria exist for these compounds.

2.4 RESULTS

2.4.1 Organic Nutrient and Contaminant Concentrations and Changes Due to Food Processing

Concentrations of nutrients and POPs in raw and food processed spotted seal and sheefish tissues are summarized in Tables 2.1, 2.2 and 2.3. In spotted seals, all fat and fatty acid classes were highest in raw and rendered blubber. Cholesterol was highest in organ meats (liver and kidney) and lowest in muscle and blubber products. Vitamin A was highest in liver followed by blubber, kidney and finally muscle. Vitamin C and beta carotene were very low or below MDL in all tissues. Generally, spotted seal and sheefish muscle had similar organic nutrient concentrations.

Changes in concentrations as a result of food processing on a ww, dw and lw basis are shown in Tables 2.4-2.7. Seven statistically significant changes were noted, ranging from -95.6% (vitamin A in boiled seal kidney) to +583% (octachlorostyrene in sheefish muscle dried without skin) on a ww basis, from -97.2% (vitamin A in boiled seal kidney) to +468 (tetrachloroveratrole in rendered blubber) on a dw basis, and from -96.7% (vitamin A in boiled seal kidney) to +413% (tetrachloroveratrole in rendered blubber) on a lw basis. When a Bonferroni correction was applied to account for multiple pair-wise comparisons, only the change in ΣPBDE in seals remained statistically significant, and only on a dw basis for muscle when boiled (-38.1%) or dried (-69.4%) and kidney when boiled (-56.2%).

2.4.2 Contributions to Daily Intake Reference Values and Tolerable Daily Intake Limits (TDIL)

Nutrient concentrations (ww) in a meal-sized portion (100g) of each tissue were compared to recommended daily intake criteria for those nutrients for which guidelines were available (Table 2.8). Nutrients present at >100% of the minimum daily intake (MDI) reference values in spotted seal tissues were total fat and omega-3 PUFA in raw and rendered blubber, vitamin A in raw and fried liver and raw blubber. No nutrients were present at >100% of MDI value in sheefish muscle processed by any method investigated.

In eight cases seal tissues exceeded nutrient ULs for a single serving: total fat in rendered blubber (104%), omega-3 PUFA in raw and rendered blubber (511% and 393%, respectively), vitamin A in raw and fried liver (913% and 1050%), and cholesterol in fried liver (162%) and raw and boiled kidney (149% and 240%). No raw or processed sheefish muscle exceeded the UL for any nutrient in a single serving.

Tolerable daily intake limits (TDIL) for POPs are shown in Table 2.9. No POP exceeded TDIL in a 100g serving of any of the seal or sheefish tissues studied. Only in blubber did any POP exceed 25% of the respective TDIL.

2.5 DISCUSSION

Results of the current study indicate that foods derived from spotted seal and sheefish are nutritious, containing particularly substantial amounts of omega-3 PUFA and vitamin A. Contaminants in these food items were present at relatively low levels, posing a very minimal threat to the consumer. The levels of nutrients and contaminants in the wildlife tissues were significantly affected by tissue type and food preparation method.

2.5.1 Organic Nutrient and Contaminant Concentrations

Increasing POP concentrations were observed with increasing fat content in tissues, as would be expected for the lipophilic contaminants. Thus, blubber, both raw and rendered consistently had the highest concentration of POPs. In general the next highest concentrations were found for liver, followed by kidney and lowest in muscle, with some

exceptions. POP concentrations have been reported for numerous arctic marine mammal and fish species (21-23,33-37), although rarely in food processed tissues. The concentrations determined in spotted seals and sheefish were well within reported ranges for similar species and tended to be relatively low by comparison.

2.5.2 Changes Due to Food Processing

In several cases, significant changes in either nutrient or contaminant concentrations of tissues resulted from food processing. These changes were determined not only on a wet weight basis, but also on a dry weight and lipid weight basis to account for concentration changes resulting from changes in basic composition such as water or lipid content. This is a critical consideration for determining risks and benefits to human consumers as the contribution to recommended daily intake criteria or TDIL is subsequently affected and potential mechanisms for compositional changes can be proposed as well.

Some analytes, while showing a significant change with processing on a ww basis, did not change significantly on a dw basis indicating a likely concentration change due to variations in water content, as dehydration results in increased concentration. For example, cis-PUFA, omega-3 FA, omega-6 FA and cholesterol increased in seal muscle on a ww basis when dried, but showed no significant change on a dw basis in the same tissue, suggesting that the increase was a result of decreased water content. Similarly, some analytes showed significant changes with processing on a ww basis, but not on a lw basis. For example, sheefish muscle (without skin) increased significantly in dieldrin,

octachlorostyrene, ΣCHL, ΣHCH and ΣCBZ when dried on a ww basis, but did not change significantly on a lw basis, indicating the ww changes observed resulted from variations in total lipid content during processing. Finally, other analytes showed significant changes on a ww, dw and lw basis, thus simple water loss or change in % lipid cannot explain the results. For example, vitamin A significantly decreased in spotted seal tissues on a ww, dw and lw basis when boiled, suggesting that the change was independent of variations in water or lipid content of the tissue and that vitamin A is actually lost during processing and may explain why vitamin A toxicosis is not a common health problem in this population or others that consume internal organs from certain marine mammals.

It is worth noting that the presence or absence of skin on the sheefish filets during processing affected the nutrient and contaminant concentrations in the final muscle product. With the exception of cholesterol in dried and smoked muscle and trans-FA in dried muscle, all nutrients were higher in filets processed with skin on versus those prepared without skin. With the exception of hexachlorobutadiene and pentachloroanisole in baked muscle, all POPs were higher in filets baked or smoked with skin on the filets versus their counterparts processed without skin. In contrast, muscle dried with skin on had higher POP concentrations in all cases except ΣHCH. Thus, the presence of skin during food processing may be an important consideration when providing consumption advice for sheefish.

Concentration changes on a ww basis, along with meal size, are the most important factors when determining human intake of nutrients and contaminants, as they represent the tissue as it is actually consumed. Thus, preparation method must be considered when assessing the nutritional benefits provided by traditional foods. By basing calculations of contributions to recommended daily intake, upper intake limits (nutrients) and TDIL (contaminants) only on concentrations in raw tissues, contributions may be grossly under- or overestimated for the actual food items consumed. These very basic findings support an "end-of-the fork" approach to assessing nutrient and contaminant risks and benefits.

2.5.3 Contributions to Daily Intake Reference Values

As expected, traditionally prepared foods provide a number of nutrients at >100% of recommended daily intake per 100g serving. In addition, these foods provide many nutrients in moderate amounts (i.e., 10-100% per serving) while others, such as vitamin C and beta carotene, are not present in any tissue at ≥10% of the recommended intake value. These results support the assertion that traditional foods represent an important, nutritious part of a balanced diet.

Certain nutrient ratios, such as omega-6 to omega-3 PUFA, are also important considerations. An optimal omega-6 to omega-3 ratio has not yet been established, but it is known that shifts from traditional diets based on fish and marine mammals to Western diets rich in saturated fats from dairy, meats and vegetable oils greatly increases omega-6 PUFA to omega-3 PUFA ratio (38). The higher omega-6 to omega-3 PUFA ratios found

in Western diets can be as high as 10-25:1 and may increase inflammatory processes, promoting chronic diseases (39). In contrast, traditional diets based on fish and game meats provide an omega-6 to omega-3 ratio closer to 1:1 or less (38,40). In this study, the ratio in spotted seal tissues ranged from 0.15:1 (raw blubber) to 1.96:1 (boiled kidney) and from 0.07:1 to 0.15:1 in sheefish muscle. The high intake of omega-3 PUFA relative to omega-6 PUFA likely provides protection to consumers against chronic diseases such as diabetes and metabolic syndrome (41).

In addition to the health consequences posed by a lack of proper nutrition, some nutrients can become detrimental at excessive doses. Therefore, upper limits (UL) have been developed in addition to the minimal requirements. In eight cases spotted seal tissues exceeded the UL for a nutrient for a single serving, including total fat in rendered blubber, omega-3 PUFA in raw and rendered blubber, vitamin A in raw and fried liver and cholesterol in fried liver, raw and boiled kidney.

It should be noted that the UL are *daily* limits. Because of the seasonal nature of subsistence foods, it would be extremely unlikely that any given food item would be eaten every day of the year. In addition, 100g may be an overestimate of a typical serving size for items such as blubber, which is commonly consumed as a dipping oil, and an underestimate for other tissues such as muscle. The major concern in terms of nutrient ULs based on the current data would be excessive vitamin A intake resulting from the consumption of spotted seal liver products. Serving size and intake frequency

should be kept in mind when consuming these items. For some processing methods, vitamin A was greatly reduced providing a potential mechanism for protection against excessive vitamin A intake. Interactions of nutrients with each other as well as with contaminants are another important consideration, but are not within the scope of this work.

2.5.4 Contribution to Tolerable Daily Intake Limits (TDIL)

Although Alaskan wildlife is generally less contaminated than wildlife at lower latitudes, several contaminants are still detectable in all tissues of these animals. Contaminant levels approached TDIL in some cases, but never exceeded TDIL.

In spotted seals, all POPs were at <10% of their respective TDIL per serving of any type of muscle, liver or kidney. Only raw and rendered blubber, the tissues with the highest lipid content, contained POPs approaching TDIL in a single serving. Raw and rendered blubber contained >25% of the TDIL per serving for dieldrin, ΣPCB, ΣCHL and ΣHCH. Sheefish did not exceed 3% TDIL for any POP. The rank order of apparent risks posed by contaminants in the subsistence foods studied here are dieldrin, followed by ΣPCB, ΣCHL and ΣHCH in blubber products.

When interpreting TDIL, one must remember that these values are very conservative, including a large safety factor, and represent the amount that can be consumed *every day over an entire lifetime* without risk of adverse health effects. Because of the seasonality

of subsistence foods, it is extremely unlikely that any of the food items studied here would be eaten every day of the year for an entire lifetime. On the other hand, the risks here outline only those originating from the specified individual food items. Human consumers consume many foods and thus must also consider intake of contaminants from multiple sources. To assess overall exposure to a human consumer would require detailed diet survey data and proper biosampling of human tissues such as serum, whole blood or subcutaneous fat.

2.5.5 POPs in Store-Bought Foods

To place these findings on spotted seal and sheefish into context, we compare concentrations to what may be found in alternative store-bought foods. It is well known that store-bought foods contain a wide range of nutrient levels. But, in addition, these foods are also known to contain organic contaminants at levels that may be similar to or exceed those in some subsistence foods. For example, Hites et al. (42) found that several organic contaminants, including ΣPCB, dioxins, toxaphene and dieldrin, were consistently higher in farmed than wild salmon. Farm raised salmon on the Canadian market were found to have ΣPCB concentrations as high as 45.1 ng/g ww and 29.1 ng/g ww in farm raised trout (43). These levels exceed ΣPCB found in all sheefish samples, with the exception of two samples of muscle dried without skin which had ΣPCB levels of 34 ng/g ww. O'Hara et al. (14) also quantified POPs in store-bought foods from Barrow, AK and found concentrations to be comparable to many marine based subsistence foods such as fish and marine mammals. The single exception is any blubber

based subsistence food. Because lipid content significantly affects POP concentrations, blubber consistently has the highest concentration of POPs of any subsistence or store-bought food item, as there is no blubber-like alternative available commercially in AK.

2.6 CONCLUSIONS

Cooking method and tissue type can have significant effects on the concentration of nutrients and contaminants, illustrating the importance of considering the actual food items consumed when assessing the risks and benefits of a traditional diet. In addition to tissue type and processing method, it was found that the presence or absence of skin on sheefish filets during food processing had consistent affects on nutrient and contaminant concentrations, justifying this as another important consideration when providing fish consumption advice.

Spotted seal and sheefish tissues were abundant sources of several nutrients, particularly vitamin A in seal liver and omega-3 PUFA in seal muscle and liver and sheefish muscle. The consumption of these traditional foods does not appear to pose any significant threat of nutrients exceeding their established UL, except for the cases of vitamin A in spotted seal liver and omega-3 PUFA in blubber, which may be greatly reduced during cooking. Some preparation methods greatly reduce vitamin A content. Although certain POPs approached their respective TDIL in blubber-based food items, none exceeded TDIL. The seasonal nature of subsistence food use and the fact that blubber is frequently consumed in servings less than 100g lead to the conclusion that the risk posed by

contaminant intake resulting from these items is relatively low in most cases. One should be cognizant of the levels of vitamin A in seal liver and omega-3 PUFA and certain POPs in spotted seal blubber and take the associated risks in to consideration when making decisions about portion size and intake frequency of these foods for themselves and their families. But, we stress that outright avoidance is likely not a good choice due to the obvious nutritional benefits provided by these foods.

Overall, these results suggest that the traditional foods investigated provide an array of nutrients accompanied by a limited risk of contaminant exposure. Therefore, we encourage the continuation of traditional food consumption as a nutritious part of a balanced diet. The lack of certain nutrients, such as vitamin C and beta carotene, in all tissues studied underline the importance of eating a balanced and varied diet in order to meet the minimum daily intake criteria for all important nutrients. Because the current work was interpreted in terms of a 70 kg male human consumer, the data would need to be reevaluated for other consumer cohorts, particularly children and women of childbearing age who may have different nutrient requirements or vulnerabilities to contaminants. The data presented here have been provided to and could be used by public health agencies in the future to support the development of cohort-specific consumption advice in a format that enhances communication for local residents. Finally, we encourage public health agencies to develop models or algorithms to assess overall food safety and quality for underrepresented diets, such as the marine-based subsistence diet of many AK residents.

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Table 2.1. Average[a] concentration of select organic nutrients (ww) in raw and processed tissues of spotted seals (pooled, n=5) and sheefish (pooled, n=8) harvested in Kotzebue, Alaska (2004-2005)[b]

	Total Fat g/100g	Saturated Fat g/100g	cis-MUFA[c] g/100g	cis-PUFA[c] g/100g	trans-FA[c] g/100g	Omega-3 PUFA[c] g/100g	Omega-6 PUFA[c] g/100g	Omega-6 PUFA: Omega-3 PUFA[c] -----	Cholesterol mg/100g	Vitamin A µg/100g	Vitamin C µg/100g
Spotted Seal											
Raw Blubber	75.5	12.8	40.9	15.8	2.59	13.8	2.05	0.15:1	48.0	1030	<0.05
Rendered Blubber	81.2	14.8	47.6	12.5	2.79	10.6	1.82	0.17:1	46.0	377	<0.05
Raw Muscle	3.48	0.915	2.15	0.177	0.083	0.115	0.062	0.54:1	60.0	2	<0.05
Boiled Muscle	1.84	0.496	1.03	0.189	0.046	0.137	0.053	0.39:1	86.0	<1	<0.05
Dried Muscle	4.47	1.16	2.60	0.410	0.102	0.279	0.130	0.47:1	185	<1	0.5
Raw Liver	3.45	0.982	1.19	1.06	0.071	0.698	0.366	0.52:1	291	27400	0.7
Fried Liver	4.36	1.46	1.47	1.16	0.089	0.724	0.438	0.60:1	487	31500	0.9
Raw Kidney	3.16	0.846	1.63	0.492	0.058	0.224	0.268	1.20:1	447	406	0.8
Boiled Kidney	4.31	1.25	2.26	0.540	0.075	0.188	0.352	1.96:1	720	18.0	0.5
Sheefish Muscle											
Raw	2.91	0.584	1.36	0.769	0.073	0.712	0.057	0.08:1	53	7	<0.05
Baked without Skin	2.30	0.474	0.997	0.674	0.052	0.629	0.045	0.07:1	51	8	<0.05
Baked with Skin	8.08	1.58	4.58	1.49	0.088	1.34	0.146	0.11:1	60	12	<0.05
Dried without Skin	6.02	1.24	2.84	1.48	0.187	1.38	0.112	0.08:1	98	15	<0.05
Dried with Skin	9.06	1.85	4.82	1.90	0.105	1.72	0.177	0.10:1	96	19	0.05
Smoked without Skin	3.40	0.687	1.52	0.977	0.066	0.905	0.072	0.08:1	59	17	<0.05
Smoked with Skin	5.85	1.09	3.05	1.41	0.061	1.29	0.119	0.15:1	57	18	<0.05

[a] Nutrients were analyzed in pooled homogenates that contained approximately equal masses of individual samples from each animal.
[b] Beta carotene was below the analytical detection limit (BDL) in all tissues.
[c] MUFA = monounsaturated fatty acid. FA = fatty acid. PUFA = polyunsaturated fatty acid.

Table 2.2. Organic contaminants (ng/g ww) in various raw and processed tissues of spotted seals (n=5) harvested in Kotzebue, Alaska (2004). Mean (± 1 SD), median and range are reported.

Contaminant	MDL[a]	*Blubber* Raw	*Blubber* Rendered	*Muscle* Raw	*Muscle* Boiled	*Muscle* Dried	*Liver* Raw	*Liver* Fried	*Kidney* Raw	*Kidney* Boiled
Hexachlorobutadiene	0.001/0.012	0.092 (±0.074)[c]	0.117 (±0.070)	BDL[b]	BDL[b]	BDL[b]	BDL[b]	BDL[b]	BDL[b]	BDL[b]
		0.066	0.133							
		0.012-0.184	0.012-0.185							
Tetrachloroveratrole	0.003/0.029	0.329 (±0.323)[c]	1.45 (±1.08)	BDL[b]	BDL[b]	BDL[b]	BDL[b]	BDL[b]	BDL[b]	BDL[b]
		0.234	1.47							
		0.038-0.875	0.374-2.77							
Dieldrin	0.024/0.236	32.6 (±19.1)	33.5 (±18.8)	0.984 (±1.343)	0.443 (±0.304)	1.98 (±2.23)	1.44 (±0.28)	1.66 (±0.37)	0.508 (±0.226)	1.56 (±1.39)
		25.0	21.8	0.363	0.396	1.29	1.51	1.69	0.365	1.30
		14.5-59.1	18.9-59.1	0.170-3.35	0.084-0.829	0.331-5.83	0.992-1.71	1.12-2.02	0.315-0.765	0.344-8.35
Octachlorostyrene	<0.001	1.09 (±0.38)	1.24 (±0.29)	0.027 (±0.023)	0.019 (±0.011)	0.054 (±0.037)	0.125 (±0.032)	BDL[b]	0.069 (±0.062)[c]	0.034 (±0.024)[c]
		0.871	1.18	0.015	0.020	0.033	0.120		0.063	0.041
		0.793-1.55	0.966-1.73	0.009-0.060	0.007-0.032	0.023-0.106	0.089-0.170		0.001-0.168	0.001-0.056
Pentachloroanisole	<0.001	0.040 (±0.037)[c]	BDL[b]	BDL[b]	BDL[b]	0.036 (±0.008)	BDL[b]	BDL[b]	BDL[b]	BDL[b]
		0.057				0.037				
		0.001-0.075				0.028-0.049				
Mirex	<0.001	5.79 (±3.61)	4.87 (±2.31)	0.096 (±0.057)	0.038 (±0.031)[c]	0.161 (±0.076)	0.201 (±0.069)	0.195 (±0.085)	0.037 (±0.011)	0.066 (±0.100)[c]
		4.92	3.74	0.070	0.046	0.178	0.214	0.252	0.034	0.025
		1.43-11.0	3.26-8.86	0.045-0.169	0.001-0.067	0.059-0.255	0.104-0.293	0.102-0.266	0.021-0.048	0.001-0.243
∑PCB	3.3/32	298 (±110)	290 (±90)	11.4 (±5.9)	10.5 (±5.7)	16.1 (±9.1)	33.0 (±6.3)	38.9 (±11.1)	18.0 (±9.2)	16.2 (±11.7)
		256	293	10.6	11.1	12.5	32.2	36.7	14.7	11.7
		201-446	183-395	5.88-21.3	5.26-19.1	6.49-30.1	26.8-42.6	26.3-51.3	11.6-34.1	7.38-36.5
$\sum PCB_{10}$	1.2/12	127 (±49)	132 (±47)	3.98 (±2.62)	2.78 (±1.11)	6.47 (±3.56)	8.75 (±1.95)	8.63 (±2.35)	4.04 (±1.86)	5.13 (±4.39)
		107	141	2.78	3.19	4.97	8.68	7.65	3.74	3.36
		83.3-185	71.1-178	2.09-8.30	1.60-4.15	2.58-11.8	6.50-10.8	6.23-11.2	2.29-7.04	2.72-13.0
∑DDT	0.23/2.25	119 (±53)	106 (±66)	2.27 (±2.88)	1.19 (±0.78)	4.56 (±3.73)	5.20 (±1.67)	5.65 (±2.06)	0.783 (±0.281)	3.20 (±4.20)
		124	114	0.654	1.18	2.75	4.13	4.76	0.780	1.52
		67.2-192	8.11-169	0.646-7.30	0.367-1.99	1.21-10.5	3.90-7.53	3.16-7.95	0.440-1.07	0.498-10.7
∑CHL	0.041/0.40	115 (±65)	126 (±54)	2.56 (±2.74)	1.29 (±0.99)	5.56 (±4.39)	3.76 (±0.57)	3.84 (±1.37)	1.17 (±0.21)	3.57 (±5.39)
		94.9	103	1.17	1.71	3.67	3.67	4.23	1.11	1.44
		69.9-229	85.1-214	0.809-7.29	0.123-2.43	1.67-12.5	2.99-4.56	1.95-5.11	0.913-1.44	0.352-13.2
∑HCH	0.012/0.119	89.7 (±34.6)	92.0 (±39.2)	2.96 (±2.63)	0.754 (±0.643)	5.09 (±5.11)	1.62 (±0.54)	1.57 (±0.66)	0.672 (±0.164)	1.91 (±2.57)
		83.8	95.2	3.19	0.979	2.49	1.36	1.48	0.732	0.856
		54.0-146	51.2-140	0.612-7.08	0.020-1.42	1.74-14.0	1.22-2.52	0.69-2.34	0.446-0.859	0.320-6.45
∑CBZ	0.003/0.30	12.6 (±2.3)	15.3 (±2.8)	0.691 (±0.417)	0.370 (±0.214)	0.912 (±0.509)	1.15 (±0.29)	1.28 (±0.23)	0.659 (±0.161)	1.14 (±0.77)
		12.1	14.5	0.978	0.381	0.660	1.06	1.26	0.727	0.831
		10.8-16.5	12.3-18.9	0.225-1.03	0.155-0.624	0.462-1.68	0.810-1.59	0.986-1.57	0.425-0.823	0.697-2.52
∑PBDE	0.22/1.73	4.75 (±1.56)	3.95 (±1.29)	0.415 (±0.062)	0.300 (±0.110)	0.346 (±0.62)	0.806 (±0.311)	0.601 (±0.142)	0.529 (±0.101)	0.364 (±0.136)
		3.82	4.04	0.401	0.343	0.336	0.649	0.650	0.518	0.320
		3.52-6.82	2.70-5.98	0.351-0.518	0.22-0.373	0.271-0.442	0.517-1.17	0.350-0.696	0.398-0.682	0.265-0.603

[a] MDL = Minimum detection limit (ng/g ww). The first MDL reported applies to muscle, liver and kidney samples and the second to blubber samples. If only one MDL is reported, that MDL applies to all tissues for that analyte.
[b] BDL = Below Detection Limit. Summary statistics were not calculated for compounds with ≥50% of samples BDL.
[c] One or two out of five samples were BDL. A value of ½ MDL (MDL=0.001 ng/g) was used for these samples to calculate summary statistics.

Table 2.3. Organic contaminants (ng/g ww) in raw and processed muscle of sheefish (n=8) harvested in Kotzebue, Alaska (2005). Mean (± 1 SD), median and range are reported.

Contaminant	MDL[a]	Raw	Baked Without Skin	Baked With Skin	Dried Without Skin	Dried With Skin	Smoked Without Skin	Smoked With Skin
Hexachlorobutadiene	0.001	BDL[b]	0.008 (±0.006)[c]	BDL[b]	0.015 (±0.007)[c]	0.010 (±0.005)[c]	0.011 (±0.008)[c]	0.012 (±0.009)[c]
			0.008		0.015	0.010	0.009	0.015
			0.001-0.016		0.001-0.023	0.001-0.017	0.001-0.026	0.001-0.024
Tetrachloroveratrole	0.001	0.047 (±0.046)	0.033 (±0.035)[c]	0.061 (±0.027)	0.073 (±0.029)	0.060 (±0.033)	0.052 (±0.052)	0.053 (±0.020)
		0.035	0.026	0.075	0.080	0.042	0.041	0.051
		0.006-0.149	0.001-0.109	0.019-0.086	0.015-0.114	0.022-0.112	0.011-0.177	0.025-0.083
Dieldrin	0.011	0.270 (±192)	0.120 (±0.104)[c]	0.257 (±0.212)[c]	0.431 (±0.179)	0.401 (±0.205)	0.254 (±0.188)	0.415 (±0.170)
		0.242	0.113	0.288	0.434	0.369	0.208	0.445
		0.043-0.595	0.011-0.284	0.011-0.505	0.080-0.649	0.163-0.768	0.062-0.629	0.108-0.632
Octachlorostyrene	<0.001	0.063 (±0.056)	0.056 (±0.028)	0.081 (±0.035)	0.177 (±0.128)	0.113 (±0.89)[c]	0.072 (±0.035)	0.129 (±0.051)
		0.044	0.048	0.078	0.142	0.092	0.063	0.128
		0.011-0.174	0.022-0.097	0.033-0.135	0.025-0.410	0.001-0.282	0.025-0.134	0.045-0.205
Pentachloroanisole	<0.001	BDL[b]	0.014 (±0.010)[c]	0.010 (±0.010)[c]	BDL[b]	BDL[b]	BDL[b]	BDL[b]
			0.010	0.007				
			0.001-0.032	0.001-0.025				
Mirex	<0.001	0.061 (±0.056)	0.042 (±0.019)	0.070 (±0.038)	0.134 (±0.096)	0.103 (±0.061)	0.057 (±0.031)	0.103 (±0.044)
		0.044	0.038	0.062	0.109	0.082	0.046	0.092
		0.020-0.186	0.019-0.070	0.024-0.133	0.023-0.303	0.043-0.211	0.016-0.104	0.037-0.164
∑PCB	1.44	9.54 (±6.74)	6.01 (±3.98)	9.09 (±4.01)	16.7 (±11.9)	12.4 (±6.0)	8.95 (±4.56)	11.0 (±4.7)
		7.45	5.19	9.68	14.4	11.6	8.49	10.4
		3.22-23.6	1.86-14.6	3.29-14.9	1.72-34.5	5.73-22.0	3.58-14.3	4.05-16.4
$\sum PCB_{10}$	0.53	2.39 (±1.80)	1.46 (±0.88)	2.61 (±1.20)	4.83 (±3.47)	3.94 (±2.00)	2.56 (±1.19)	3.40 (±1.44)
		1.58	1.33	2.80	4.12	3.49	2.59	3.16
		0.859-6.15	0.53-2.95	0.914-4.45	0.53-10.1	1.78-7.34	0.927-4.09	1.33-5.10
∑DDT	0.10	1.56 (±1.45)	0.778 (±0.417)	1.46 (±0.93)	3.25 (±2.26)	2.56 (±1.42)	1.29 (±0.64)	2.56 (±1.14)
		0.971	0.833	1.39	2.57	2.19	1.16	2.35
		0.406-4.51	0.113-1.35	0.10-3.05	0.510-6.97	0.940-4.90	0.455-2.23	0.874-4.12
∑CHL	0.02	1.99 (±1.62)	1.44 (±1.30)	2.03 (±1.12)	4.06 (±2.19)	3.33 (±1.59)	1.76 (±0.93)	3.31 (±1.35)
		1.52	1.08	1.92	3.61	3.14	1.46	3.34
		0.445-4.66	0.327-4.48	0.266-3.76	0.740-7.27	1.62-5.94	0.749-3.30	1.31-5.13
∑HCH	0.01	0.397 (±0.232)	0.317 (±0.204)	0.627 (±0.389)	0.797 (±0.399)	0.907 (±0.614)	0.454 (±0.362)	0.694 (±0.278)
		0.438	0.238	0.735	0.700	0.844	0.409	0.752
		0.058-0.752	0.071-0.591	0.092-1.07	0.111-1.25	0.183-1.88	0.078-1.19	0.239-1.06
∑CBZ	0.001	2.15 (±1.15)	1.64 (±0.90)	1.81 (±1.09)	3.87 (±1.58)	2.03 (±1.05)	2.15 (±1.40)	2.20 (±0.79)
		2.33	1.47	1.53	4.02	1.77	1.95	2.39
		0.381-3.95	0.504-2.86	0.315-3.31	0.692-5.54	0.945-4.32	0.790-5.00	0.519-3.01

[a] MDL = Minimum detection limit (ng/g ww).

[b] BDL = Below Detection Limit. Summary statistics were not calculated for compounds with ≥50% of samples BDL.

[c] One to three out of eight samples were BDL. A value of ½ MDL (MDL=0.001 ng/g) was used for these samples to calculate summary statistics.

Table 2.4. Absolute[a] and percent change (%Δ) in organic nutrients on a ***wet weight*** basis as a result of food processing for tissues of spotted seal (pooled, n=5) and sheefish (pooled, n=8) harvested in Kotzebue, Alaska (2004-2005).[b]

	Total Fat	Saturated Fat	cis-MUFA[c]	cis-PUFA[c]	trans-FA[c]	Omega-3 PUFA[c]	Omega-6 PUFA[c]	Cholesterol	Vitamin A	Vitamin C
Spotted Seal										
Blubber Δ with Rendering	+5.7	+2.0	+6.7	+3.3	+0.20	-3.2	-0.23	-2.0	**-653**	NA[d]
	+7.55%	+15.6%	+16.4%	-20.9%	+7.72%	-23.2%	-11.2%	-4.17%	**-63.4%**	
Muscle Δ with Boiling	-1.64	-0.419	**-1.12**	+0.012	-0.037	+0.022	-0.009	+26.0	NA[d]	NA[d]
	-47.1%	-45.8%	**-52.1%**	+6.78%	-44.6%	+19.1%	-14.5%	+43.3%		
Muscle Δ with Drying	+0.99	+0.245	+0.45	**+0.233**	+0.019	**+0.164**	**+0.068**	**+125**	NA[d]	NA[d]
	+28.4%	+26.8%	+20.9%	**+132%**	+22.9%	**+143%**	**+110%**	**+208%**		
Liver Δ with Frying	+0.91	+0.478	+0.28	+0.10	+0.018	+0.026	+0.072	**+196**	+4100	+0.2
	+26.4%	+48.7%	+23.5%	+9.43%	+25.4%	+3.72%	+19.7%	**+67.4%**	+15.0%	+28.6%
Kidney Δ with Boiling	+1.15	+0.404	+0.63	+0.048	+0.017	-0.036	+0.084	+273	**-388**	-0.3
	+36.4%	+47.8%	+38.7%	+9.76%	+29.3%	-16.1%	+31.3%	+61.1%	**-95.6%**	-37.5%
Sheefish Muscle										
Δ with Baking without Skin	-0.61	-0.11	-0.363	-0.095	-0.021	-0.083	-0.012	-2	-1	NA[d]
	-21.0%	-18.8%	-26.7%	-12.4%	-28.8%	-11.7%	-21.1%	-3.8%	-14.3%	
Δ with Baking with Skin	**+5.17**	**+0.996**	**+3.22**	**+0.721**	+0.007	**+0.628**	**+0.089**	+7	**+5**	NA[d]
	+178%	**+171%**	**+237%**	**+93.8%**	+9.59%	**+88.2%**	**+156%**	+13.2%	**+71.4%**	
Δ with Drying without Skin	**+2.07**	**+0.656**	**+1.48**	**+0.711**	**+0.114**	**+0.668**	**+0.055**	**+45**	**+8**	NA[d]
	+71.1%	**+112%**	**+109%**	**+92.5%**	**+156%**	**+93.8%**	**+96.5%**	**+84.9%**	**+114%**	
Δ with Drying with Skin	**+6.15**	**+1.266**	**+3.46**	**+1.131**	+0.032	**+1.008**	**+0.120**	**+43**	**+12**	NA[d]
	+211%	**+217%**	**+254%**	**+147%**	+43.8%	**+142%**	**+211%**	**+81.1%**	**+171%**	
Δ with Smoking without	+0.49	+0.103	+0.16	+0.201	-0.013	+0.193	+0.015	+6	**+10**	NA[d]
Skin	+16.8%	+17.6%	+11.8%	+26.1%	-17.8%	+27.1%	+25.3%	+11.3%	**+143%**	
Δ with Smoking with Skin	**+2.94**	**+0.506**	**+1.69**	**+0.641**	-0.012	**+0.578**	**+0.062**	+4	**+11**	NA[d]
	+101%	**+86.6%**	**+124%**	**+83.4%**	-16.4%	**+81.2%**	**+109%**	+7.55%	**+157%**	

[a] Fat (total and saturated) and fatty acid classes are reported in g/100g wet weight (ww), cholesterol in mg/100g (ww) and vitamin A and vitamin C in μg/100g (ww).
[b] Bold text indicates changes of >50% and bold underline changes >100% as a result of food processing.
[c] MUFA = monounsaturated fatty acid. FA = fatty acid. PUFA = polyunsaturated fatty acid.
[d] Absolute and percent change not available (NA) because either raw, food processed or both tissues were below the minimum detection limit (BDL). Beta carotene was NA in all tissues.

Table 2.5. Absolute[a] and percent change (%Δ) in organic nutrients on a ***dry weight*** basis as a result of food processing for tissues of spotted seal (pooled, n=5) and sheefish (pooled, n=8) harvested in Kotzebue, Alaska (2004-2005).[b,c]

	Total Fat	Saturated Fat	cis-MUFA[c]	cis-PUFA[c]	trans-FA[c]	Omega-3 PUFA[c]	Omega-6 PUFA[c]	Cholesterol	Vitamin A	Vitamin C
Spotted Seal										
Blubber Δ with Rendering[d]	+5.7	+2.0	+6.7	+3.3	+0.20	-3.2	-0.23	-2.0	**-653**	NA[e]
	+7.55%	+15.6%	+16.4%	-20.9%	+7.72%	-23.2%	-11.2%	-4.17%	**-63.4%**	
Muscle Δ with Boiling	**-6.67**	**-1.72**	**-4.41**	-0.080	**-0.15**	-0.014	-0.064	+32.2	NA[e]	NA[e]
	-57.2%	**-56.1%**	**-61.2%**	-13.6%	**-55.1%**	-3.57%	-30.8%	+16.0%		
Muscle Δ with Drying	**-6.33**	**-1.68**	**-4.11**	-0.10	**-0.16**	-0.058	-0.053	+19.5	NA[e]	NA[e]
	-54.3%	**-54.9%**	**-57.0%**	-17.6%	**-56.3%**	-13.7%	-25.4%	+9.70%		
Liver Δ with Frying	-0.39	+0.42	-0.22	-0.55	-0.010	-0.46	-0.10	+254	-10641	**-1702**
	-3.60%	+13.4%	-5.77%	-16.5%	-4.38%	-20.9%	-8.71%	+26.7%	-12.3%	**-97.2%**
Kidney Δ with Boiling	-11.7	-0.19	-0.78	-0.63	-0.043	-0.45	-0.18	+64.5	-0.043	**-2.07**
	-12.5%	-5.20%	-11.0%	-29.6%	-17.0%	-46.2%	-15.7%	+3.35%	-1.93%	**-59.9%**
Sheefish Muscle										
Δ with Baking without Skin	-3.96	-0.75	-2.11	-0.83	-0.12	-0.75	-0.078	-42.2	-1.42	NA[e]
	-34.4%	-32.6%	-39.1%	-27.2%	-40.9%	-26.7%	-34.5%	-20.1%	-5.12%	
Δ with Baking with Skin	**+13.6**	**+2.60**	**+8.85**	**+1.59**	-0.015	+1.35	**+0.23**	-23.3	+9.58	NA[e]
	+118%	**+112%**	**+164%**	**+52.1%**	-5.35%	+47.8%	**+101%**	-11.1%	+34.6%	
Δ with Drying without Skin	-0.46	-0.033	-0.17	-0.32	+0.055	-0.28	-0.020	-29.7	-0.15	NA[e]
	-3.97%	-1.44%	-3.07%	-10.7%	+18.9%	-10.0%	-8.79%	-14.2%	-0.53%	
Δ with Drying with Skin	**+726**	**+1.52**	**+4.61**	+0.89	-0.071	+0.75	**+0.14**	-10.8	+11.7	NA[e]
	+63.0%	**+65.9%**	**+85.6%**	+29.4%	-24.7%	+26.5	**+62.6%**	-5.15%	+42.1%	
Δ with Smoking without	-1.80	-0.35	-1.04	-0.25	-0.10	-0.23	-0.020	-41.2	**+20.9**	NA[e]
Skin	-15.7%	-15.1%	-19.3%	-8.28%	-34.7%	-8.23%	-8.80%	-19.6%	**+75.3%**	
Δ with Smoking with Skin	+5.87	+0.93	**+3.68**	+1.15	-0.11	+1.02	**+0.13**	-40.3	**+25.8**	NA[e]
	+51.0%	+40.2%	**+68.4%**	+37.7%	-37.3%	+36.1%	**+56.8%**	-19.2%	**+93.1%**	

[a] Fat (total and saturated) and fatty acid classes are reported in g/100g wet weight (dw), cholesterol in mg/100g (dw) and vitamin A and vitamin C in µg/100g (dw).
[b] Bold text indicates changes of >50% as a result of food processing.
[c] MUFA = monounsaturated fatty acid. FA = fatty acid. PUFA = polyunsaturated fatty acid.
[d] Changes in blubber are based on wet weight values which are assumed to be essentially equivalent to dry weight values (i.e., 0% water content).
[e] Absolute and percent change not available (NA) because either raw, food processed or both tissues were below the minimum detection limit (BDL). Beta carotene was NA in all tissues.

Table 2.6. Absolute[a] and percent change (%Δ) in organic nutrients on a ***lipid adjusted weight*** basis as a result of food processing for tissues of spotted seal (pooled, n=5) and sheefish (pooled, n=8) harvested in Kotzebue, Alaska (2004-2005).[b,c]

	Saturated Fat	cis-MUFA[c]	cis-PUFA[c]	trans-FA[c]	Omega-3 PUFA[c]	Omega-6 PUFA[c]	Cholesterol	Vitamin A	Vitamin C
Spotted Seal									
Blubber Δ with Rendering[d]	+0.013	+0.045	-0.055	+0.0001	-0.052	-0.005	-0.069	**-9.00**	NA[d]
	+7.51%	+8.21%	-26.4%	+0.16%	-28.6%	-17.5%	-10.9%	**-65.7%**	
Muscle Δ with Boiling	+0.007	-0.058	**+0.052**	+0.001	**+0.041**	**+0.011**	**+29.5**	NA[d]	NA[d]
	+2.52%	-9.39%	**+102%**	+4.82%	**+125%**	**+61.7%**	**+171%**		
Muscle Δ with Drying	-0.003	-0.036	**+0.041**	-0.001	**+0.029**	**+0.011**	**+24.1**	NA[d]	NA[d]
	-1.30%	-5.85%	**+80.3%**	-4.33%	**+88.9%**	**+63.2%**	**+140%**		
Liver Δ with Frying	+0.050	-0.008	-0.041	-0.0002	-0.036	-0.006	+27.3	-717	+0.004
	+17.6%	-2.25%	-13.4%	-0.81%	-17.9%	-5.31%	+32.3%	-9.03%	+1.74%
Kidney Δ with Boiling	+0.022	+0.009	-0.030	-0.001	-0.027	-0.003	+25.6	**-124**	-0.14
	+8.33%	+1.66%	-19.5%	-5.19%	-38.5%	-3.70%	+18.1%	**-96.7%**	-54.2%
Sheefish Muscle									
Δ with Baking without Skin	+0.005	-0.034	+0.029	-0.003	+0.029	-0.00002	+3.96	+1.07	NA[d]
	+2.69%	-7.25%	+10.9%	-9.88%	+11.8%	-0.11%	+21.7%	+44.6%	
Δ with Baking with Skin	-0.005	+0.10	-0.080	**-0.014**	-0.079	-0.002	**-10.8**	-0.92	NA[d]
	-2.56%	+21.3%	-30.2%	**-56.6%**	-32.2%	-7.75%	**-59.2%**	-38.3%	
Δ with Drying without Skin	+0.005	+0.004	-0.018	+0.006	-0.015	-0.001	-1.93	+0.086	NA[d]
	+2.64%	+0.94%	-6.97%	+23.8%	-6.31%	-5.02%	-10.6%	+3.58%	
Δ with Drying with Skin	+0.004	+0.065	-0.055	**-0.014**	-0.055	-0.0001	-7.62	-0.31	NA[d]
	+1.75%	+13.8%	-20.6%	**-53.8%**	-22.4%	-0.26%	-41.8%	-12.8%	
Δ with Smoking without	+0.001	-0.020	+0.023	-0.006	+0.022	+0.002	-0.86	**+2.59**	NA[d]
Skin	+0.68%	-4.34%	+8.74%	-22.6%	+8.79%	+8.11%	-4.72%	**+108%**	
Δ with Smoking with Skin	-0.014	+0.054	-0.023	**-0.015**	-0.024	+0.001	-8.47	+0.67	NA[d]
	-7.16%	+11.6%	-8.79%	**-58.4%**	-9.88%	+3.85%	-46.5%	+27.9%	

[a] Saturated fat and fatty acid classes are reported in g/g lipid weight (lw), cholesterol in mg/g (lw) and vitamin A and vitamin C in μg/g (lw).

[b] Bold text indicates changes of >50% as a result of food processing.

[c] MUFA = monounsaturated fatty acid. FA = fatty acid. PUFA = polyunsaturated fatty acid.

[d] Absolute and percent change not available (NA) because either raw, food processed or both tissues were below the minimum detection limit (BDL). Beta carotene was NA in all tissues.

Table 2.7. Absolute (ng/g) and percent change (%Δ) [mean (±1 SD)] in organic contaminants on a wet weight (ww), dry weight (dw) and lipid adjusted weight (lw) basis as a result of food processing for various tissues of spotted seals (n=5) and sheefish (n=8) harvested on Kotzebue, Alaska (2004)[a].

Wet Weight			**Dry Weight**			**Lipid Adjusted Weight**		
Tissue/Processing	Contaminant	Δ with Processing	Tissue/Processing	Contaminant	Δ with Processing	Tissue/Processing	Contaminant	Δ with Processing
Spotted Seal			Spotted Seal			Spotted Seal		
Blubber/Rendered	Tetrachloroveratrole	+1.12 (±0.81) +468 (±305)%	Blubber/Rendered	Tetrachloroveratrole	+1.12 (±0.81) +468 (±305)%	Blubber/Rendered	Tetrachloroveratrole	+1.08 (±0.79) +413 (±283)%
Blubber/Rendered	ΣCBZ	+2.75 (±2.11) +22.9 (±18.2)%	Blubber/Rendered	ΣCBZ	+2.75 (±2.11) +22.9 (±18.2)%	Muscle/Dried	Mirex	-1.43 (±0.58) -44.0 (±20.0)%
Muscle/Dried	ΣPBDE	-0.07 (±0.05) -16.6 (±11.8)%	Muscle/Boiled	ΣPBDE	-0.54 (±0.21) -38.1 (±14.5)%	Muscle/Dried	ΣPCB_{10}	-68.6 (±38.1) -48.3 (±16.0)%
Liver/Fried	Dieldrin	+0.22 (±0.17) +15.4 (±10.6)%	Muscle/Dried	ΣPCB	-17.0 (±11.5) -44.2 (±27.2)%	Muscle/Dried	ΣPBDE	-16.2 (±11.5) -72.9 (±12.0)%
Sheefish Muscle			Muscle/Dried	ΣPBDE	-0.97 (±0.16) -69.4 (±8.8)%	Liver/Fried	ΣDDT	-13.3 (±6.2) -15.9 (±11.3)%
Dried (No Skin)	Dieldrin	+0.16 (±0.19) +154 (±261)%	Liver/Fried	ΣHCH	-1.36 (±1.06) -26.9 (±21.3)%	Liver/Fried	ΣHCH	-7.05 (±4.01) -25.7 (±18.6)%
Dried (No Skin)	Octachlorostyrene	+0.11 (±0.13) +583 (±1262)%	Kidney/Boiled	ΣPBDE	-1.30 (±0.39) -56.2 (±10.4)%	Kidney/Boiled	Mirex	-0.71 (±0.34) -54.5 (±24.8)%
Dried (No Skin)	ΣCHL	+2.07 (±2.28) +270 (±503)%	Sheefish			Kidney/Boiled	ΣPBDE	-15.3 (±8.5) -74.0 (±9.1)
Dried (No Skin)	ΣHCH	+0.40 (±0.29) +213 (±354)%	None			Sheefish		
Dried (No Skin)	ΣCBZ	+1.71 (±1.77) +173 (±289)%				None		
Smoked (With Skin)	Octachlorostyrene	+0.07 (±0.06) +278 (±354)%						
Smoked (With Skin)	ΣHCH	+0.30 (±0.33) +175 (±239)%						

[a] Only statistically significant changes ($p < 0.05$) are shown. For all changes displayed, none remain significant if a Bonferroni corrections for multiple comparisons is included.

Table 2.8. Mean percent (%) contribution[a] (% of minimum reference value/% of maximum reference value) of one serving (100g ww) of spotted seal (pooled, n=5) and sheefish (pooled, n=8) tissue to the Daily Reference Intake (DRI)/Upper Limit (UL), Acceptable Macronutrient Distribution Range (AMDR) or Daily Value (DV) for select organic nutrients[b]

DRI/UL, AMDR, or DV[c]	Total Fat 44-78 g/day	Saturated Fat ND[e]/20 g/day	Omega-3 PUFA 1.6-2.7 g/day	Omega-6 PUFA 11-22 g/day	Cholesterol ND[e]/300 mg/day	Vitamin A 900-3000 µg/day	Vitamin C 90-2000 mg/day
Spotted Seal							
Raw Blubber	**172**/96.8	ND/64.0	**863/511**	18.6/9.32	ND/16.0	**114**/34.3	BDL[d]
Rendered Blubber	**185/104**	ND/74.0	**663/393**	16.5/8.27	ND/15.3	41.9/12.6	BDL[d]
Raw Muscle	7.91/4.46	ND/4.58	7.19/4.26	0.56/.28	ND/20.0	0.22/0.07	BDL[d]
Boiled Muscle	4.18/2.36	ND/2.48	8.56/5.07	0.48/0.24	ND/28.7	BDL[d]	BDL[d]
Dried Muscle	10.2/5.73	ND/5.80	17.4/10.3	1.18/0.59	ND/61.7	BDL[d]	<0.01/<0.01
Raw Liver	7.84/4.42	ND/4.91	43.6/25.9	3.33/1.66	ND/97.0	**3044/913**	<0.01/<0.01
Fried Liver	9.91/5.59	ND/7.30	45.3/26.8	3.98/1.99	ND/**162**	**3500/1050**	0.01/<0.01
Raw Kidney	7.18/4.05	ND/4.23	14.0/8.30	2.44/1.22	ND/**149**	45.1/13.5	<0.01/<0.01
Boiled Kidney	9.80/5.53	ND/6.25	11.8/6.96	3.20/1.60	ND/**240**	2.00/0.60	<0.01/<0.01
Sheefish Muscle							
Raw	6.61/3.73	ND/2.92	44.5/26.4	0.52/0.26	ND/17.7	0.78/0.23	BDL[d]
Baked without Skin	5.23/2.95	ND/2.37	39.3/23.3	0.41/0.20	ND/17.0	0.89/0.27	BDL[d]
Baked with Skin	18.4/10.4	ND/7.90	83.8/49.6	1.33/0.66	ND/20.0	1.33/0.40	BDL[d]
Dried without Skin	13.7/7.72	ND/6.20	86.3/51.1	1.02/0.51	ND/32.7	1.67/0.50	BDL[d]
Dried with Skin	20.6/11.6	ND/9.25	**108**/63.7	0.80/0.40	ND/32.0	2.11/0.63	<0.01/<0.01
Smoked without Skin	7.73/4.36	ND/3.44	56.6/33.5	0.65/0.33	ND/19.7	1.89/0.57	BDL[d]
Smoked with Skin	13.3/7.50	ND/5.45	80.6/47.8	1.08/0.54	ND/19.0	2.00/0.60	BDL[d]

[a] **Bold** text highlights a contribution of >100% of the minimum or maximum reference value of a given nutrient by a 100g meal of the specified tissue.

[b] Recommendation for trans-fatty acids is "as low as possible while consuming a nutritionally adequate diet". No criteria for cis-monounsaturated or cis-polyunsaturated fatty acids exist. Thus % contributions to recommended intakes could not be calculated for these nutrients.

[c] DRI/UL criteria used for vitamin A and vitamin C. AMDR criteria used for total fat, omega-3 and omega-6 polyunsaturated fatty acids. DV criteria used for saturated fat and cholesterol (MND = Minimum required intake has not been determined).

[d] BDL = No contribution to DRI calculated because element below detection limit. Beta carotene was BDL in all tissues.

[e] ND = Not determined because no minimum intake criteria exists.

Table 2.9. Mean percent (%) contribution of one meal (100g ww) of spotted seal (n=5) and sheefish (n=8) tissue to the tolerable daily intake limit (TDIL) for select persistent organic pollutants (POPs)[a]

	Hexachlorobutadiene	Dieldrin	Mirex	ΣPCB	ΣDDT	ΣCHL	ΣHCH	ΣCBZ
TDI (mg/kg/day)[b,c]	0.0002	0.00005	0.0002	0.001	0.01	0.0005	0.0003	0.061
Spotted Seal								
Raw Blubber	0.07	**93.1**	4.14	**42.6**	1.70	**32.9**	**42.7**	2.06
Rendered Blubber	0.08	**95.7**	3.48	**41.4**	1.51	**36.0**	**43.8**	2.50
Raw Muscle	BDL[d]	2.81	0.07	1.63	0.03	0.73	1.41	0.11
Boiled Muscle	BDL[d]	1.27	0.03	1.50	0.02	0.37	0.36	0.06
Dried Muscle	BDL[d]	5.66	0.12	2.30	0.07	1.59	2.42	0.15
Raw Liver	BDL[d]	4.11	0.14	0.43	0.07	1.07	0.77	0.19
Fried Liver	BDL[d]	4.74	0.14	5.56	0.08	1.10	0.75	0.21
Raw Kidney	BDL[d]	1.45	0.03	2.57	0.01	0.33	0.32	0.11
Boiled Kidney	BDL[d]	4.46	0.05	2.31	0.05	1.02	0.91	0.19
Sheefish Muscle								
Raw	BDL[d]	0.77	0.04	1.36	0.02	0.57	0.19	0.35
Baked without Skin	0.01	0.34	0.03	0.86	0.01	0.69	0.15	0.27
Baked with Skin	BDL[d]	0.73	0.05	1.30	0.02	0.58	0.30	0.30
Dried without Skin	0.01	1.23	0.10	2.39	0.05	1.16	0.38	0.63
Dried with Skin	0.01	1.15	0.07	1.77	0.04	0.94	0.43	0.33
Smoked without Skin	0.01	0.73	0.04	1.28	0.02	0.50	0.22	0.35
Smoked with Skin	0.01	1.19	0.07	1.57	0.04	0.95	0.33	0.36

[a] **Bold** text highlights contributions of >25% of the TDIL for a given POP by a 100g meal of the specified tissue.
[b] Hexachlorobutadiene, dieldrin, mirex, ΣCHL, ΣHCH and ΣCBZ: Reference Dose (RfD): United States Environmental Protection Agency (U.S. EPA). ΣPCB and ΣDDT: Tolerable Daily Intake (TDI): Health Canada.
[c] TDIL calculations for tetrachloroveratrole, octachlorostyrene, pentachloroanisole and ΣPBDE not included here because no intake criteria exist for these compounds.
[d] BDL = No contribution to toxicological reference dose calculated because element was below detection limit in tissue.

CHAPTER 3

Tissue type and food processing effects on stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) as chemical tracers of feeding ecology and mercury exposure[3]

3.1 ABSTRACT

Stable isotopes of carbon (C) and nitrogen (N) are useful markers of feeding ecology and tools for assessing contaminants (e.g., mercury exposure). Documenting differences in carbon and nitrogen stable isotope signatures between species, tissue types and foods processed via different cooking techniques is warranted if these tools are to be used as dietary biomarkers in human and wildlife populations. Further, understanding the variation in $\delta^{13}C$ and $\delta^{15}N$ relative to mercury concentrations among tissue types and the effects of food processing on these relationships will allow for improved applications of stable isotope information to research on contaminant pathways in food webs. The objectives of the current study were to 1) document changes in mercury (Hg), $\delta^{13}C$ and $\delta^{15}N$ with food processing imitating traditional cooking methods in the tissues of two important subsistence species of northwest Alaska, spotted seals and sheefish, and 2) describe the variation in Hg, $\delta^{13}C$ and $\delta^{15}N$ within a single species among tissues commonly consumed by humans and certain selectively feeding wildlife species. We found that Hg concentration and $\delta^{13}C$ and $\delta^{15}N$ vary widely by tissue type within a species and that food preparation methods can produce small, but statistically significant,

[3] Moses, S.K., A.V. Whiting, T.M. O'Hara. Tissue type and food processing effects on stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) as chemical tracers of feeding ecology and mercury exposure. Prepared for submission to Science of the Total Environment.

changes in the stable C and N isotope signatures of fish and wildlife tissues. $\delta^{15}N$ and Hg did not differ in a consistent (i.e., parallel) fashion among seal tissues, thus complicating the use for scenarios where preferential consumption of specific tissues may occur. Consequently, when stable isotopes are used as chemical tracers of feeding ecology and mercury exposure, the specific tissue consumed, and to a lesser degree the processed state of the actual food items, should be considered.

KEY WORDS

stable isotope, mercury, selective feeding, cooking, spotted seal, sheefish

3.2 INTRODUCTION

In recent decades, stable isotopes of carbon (C) and nitrogen (N) have been applied widely as feeding ecology tools in the study of wildlife populations. It is well established that the stable isotope ratios in an organism reflect those of its food source, with certain predictable modifications (i.e, fractionation, trophic enrichment) (Kelly, 2000; Hobson and Welch, 1992; Peterson and Fry, 1987). Due to the trophic enrichment of N (i.e., the systematic increase in the proportion of ^{15}N relative to ^{14}N with increasing trophic level), information about the trophic position of an organism within a food web can be gained through measurements of $\delta^{15}N$ of the organism's tissues. Determination of $\delta^{13}C$ can provide additional information regarding trophic status as well as an indication of carbon sources (e.g., benthic versus pelagic prey or C_3 versus C_4 plants).

Recently, stable isotopes of C and N have been used to study human populations, including Greenland Inuit where a strong indication of a marine-based diet was noted (Buchardt et al., 2007). In Alaska Natives, $\delta^{15}N$ and $\delta^{13}C$ are being utilized as dietary biomarkers to elucidate the relative contribution of marine versus terrestrial foods to the diet and the subsequent linkages between nutrition and chronic disease in this population (Nash et al., 2009; O'Brien et al., 2009; Wilkinson et al., 2007).

In an extension of feeding ecology studies, it is becoming increasingly common to incorporate stable isotope data into investigations of environmental contaminant exposure in humans and wildlife. The synthesis of $\delta^{15}N$ and $\delta^{13}C$ measurements with the

quantification of organismal contaminant concentrations can aid in the determination of possible contaminant exposure pathways within food webs. C and N isotopic signatures have been used to study mercury (Hg) dynamics within arctic species including invertebrates, fish, seals, walrus, seabirds and polar bears (Atwell et al., 1998; Cardona-Marek et al., 2009; Dehn et al., 2006; Horton et al., 2009). A positive correlation between trophic level, as indicated by $\delta^{15}N$, and Hg concentrations both within species and across species within a food web has typically been observed.

Isotopes partition non-randomly in the environment and within organisms. Isotopic ratios of C and N vary by tissue depending on processes such as isotopic routing and tissue turnover rate (Gannes et al., 1997). Thus, the tissue in which $\delta^{15}N$ and $\delta^{13}C$ are measured will influence the magnitude of the values obtained and hence the interpretation of the data with respect to dietary history. Further, the isotopic ratio of C and N assimilated from prey items will be affected if the predator selectively feeds on certain tissue types relative to others or as compared to consuming whole prey. This is largely due to the fact that C and N signatures vary by tissue type. This also results in part as a result of isotopic routing, which causes some tissues of an organism to more closely reflect certain dietary fractions (e.g., lipid, carbohydrate, or protein) due to differential routing of nutrients to different tissues or body compartments (Schwartz, 1991). Yet, the specific tissue types consumed (i.e., muscle vs. organ vs. lipid) are rarely discussed, despite the fact that isotope patterns are known to vary widely by tissue type. Further, the

effects of food processing on the isotopic signatures of food products and the potential impacts on assessing contaminants and feeding ecology are relatively unknown.

When stable isotopes of C and N are utilized in feeding ecology and studies addressing contaminant dynamics within food webs, a number of assumptions, some untested, are often made (Gannes et al., 1997). Two common assumptions are that prey items are consumed whole, as opposed to preferential targeting of specific tissues for consumption, and that $\delta^{13}C$ and $\delta^{15}N$ are not significantly affected by food processing. Generation of data documenting differences in stable isotope signatures between species, tissues and foods processed via different cooking techniques is warranted if stable isotopes continue to be used as dietary biomarkers and tracers of contaminant exposure in human and wildlife populations. Understanding the variation in $\delta^{13}C$ and $\delta^{15}N$ relative to mercury concentrations among tissue types and the effects of cooking on these relationships can improve applications of stable isotope information to research on contaminant pathways in food webs.

Here, we evaluate the variation in Hg concentration, $\delta^{13}C$ and $\delta^{15}N$ in two important subsistence species of northwest Alaska, focusing on tissues commonly utilized as food. Spotted seal (*Phoca largha*) and sheefish (*Stenodus leucicthys*) were selected for this study with input from local project participants, because they represent two major food groups (fish and marine mammals) in this region. In Kotzebue, Alaska (USA), fish and marine mammals comprise the majority (70%) of subsistence harvested foods with

sheefish representing 45% of the total fish harvest and ice seals (spotted, ringed, bearded) accounting for 98% of the marine mammal harvest (Whiting, 2006). In addition, seals represent the major prey item of polar bears, a tissue-selective apex predator of this region. We assessed Hg concentration in parallel with $\delta^{13}C$ and $\delta^{15}N$ in common food tissues from these species both prior to and after food processing imitating traditional cooking methods. In addition, we describe the variation in Hg and stable isotopes within a single species among tissues commonly consumed by humans and certain selectively feeding wildlife species. The subsequent impact on the use of stable isotopes as dietary biomarkers and chemical tracers of Hg exposure in humans and selectively feeding wildlife, such as polar bears, is discussed.

3.3 MATERIALS AND METHODS

3.3.1 Sample Collection

Samples were collected during the winter of 2004-5 at Kotzebue, AK (66.90°N, 162.59°W) under Marine Mammal Health and Stranding Response Program (MMHSRP) permit #932-1489-05. Blubber, muscle, liver and kidney samples from spotted seals (*Phoca largha*; n=5) and muscle from sheefish (*Stenodus leucicthys*; n=8) were collected from legally subsistence harvested animals for chemical analyses using Whirl-Pak or Scienceware polyethylene bags. Seal blubber and liver sub-samples were provided to the Alaska Marine Mammal Tissue Archival Project (AMMTAP) according to the methods of Becker et al. (1991). Skin samples (1 cm^2) were provided to the Alaska Department of

Fish and Game Arctic Marine Mammal Program for genetic analyses including confirmation of species identification. All animals were assessed for gross general health prior to sampling and found to be in excellent condition. Samples were immediately frozen at -20°C, shipped frozen to the University of Alaska Fairbanks (UAF) and stored at -80°C until analysis.

3.3.2 Morphometrics, Age Estimation and Food Processing

Determination of spotted seal and sheefish age, sex, length and body mass as well as spotted seal girth and blubber thickness was performed as described in Moses et al. (2009). Results are presented in Table 3.1. A portion of each tissue was "food processed" imitating traditional cooking methods. Detailed processing methods have been reported previously (Moses et al., 2009). In short, spotted seal blubber was rendered to produce oil, muscle was both boiled and dried, liver was fried and kidney was boiled. Sheefish muscle was baked, smoked and dried.

3.3.3 Stable Isotope Analysis

Tissue samples (1g) were freeze dried to a constant mass on a Labconco FreeZone 4.5L benchtop freeze dryer. Stable isotopes of C and N were analyzed at the Alaska Stable Isotope Facility as described by Wilkenson et al. (2007). Each sample was analyzed in duplicate and the mean value used for all subsequent data analysis. Data are presented in delta notation:

$$\delta X = (R_{sample}-R_{standard})/(R_{standard})*1000‰$$

where, X = ^{13}C or ^{15}N and R is the ratio of the heavier to the lighter isotope of C or N. International standards used were Vienna Peedee Belemnite (VPDB) for C and atmospheric nitrogen (N_{atm}) for N. Absolute concentrations of N and ^{15}N in each tissue were calculated from these results. Concentrations are reported on a dry weight (dw) basis in order to account for variation in water content among tissue types and due to processing methods.

3.3.4 Calculation of Changes due to Food Processing

Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and the concentrations of N, ^{15}N and THg were determined in tissues before and after food processing, as described above. Analyte changes due to food processing were calculated as:

$$\% \text{ Change } = \frac{(X_p-X_r)}{(X_r)} \times 100$$

where X_r is the analyte concentration of the raw tissue, X_p is the analyte concentration of the processed tissue and X = N, ^{15}N or THg. Thus, (X_p-X_r) represents the absolute change in analyte concentration due to food processing, where positive values indicate an increase and negative values a decrease. Because isotopic signatures are reported in per

mil (‰) units, absolute change in $\delta^{13}C$ and $\delta^{15}N$, but not % change, was calculated for these values.

3.3.5 Mercury (Hg) Analysis

Total Hg was analyzed at Texas A&M University (TAMU) and UAF according to United States Environmental Protection Agency procedures (U.S. EPA, 1992) with minor modifications as previously described (Moses et al., 2009). Briefly, 0.8g (wet weight, ww) of homogenized tissue was digested by a microwave procedure using nitric acid (HNO_3), hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl). An aliquot of each digest was diluted 1:4 with 7% HCl for Hg analysis. Hg in seal tissues was measured at TAMU with a CETAC Quick Trace Mercury Analyzer. Hg in sheefish was analyzed at the UAF using a purge-and-burn technique with cold vapor atomic fluorescence spectrometric (CVAFS) detection on an Amalgamation Control Module equipped with a Model III detector (Brooks Rand). Hg concentrations are reported on a dw basis to account for variation in water content among tissue types and due to food processing.

3.3.6 Statistics

The statistical significance of the changes in $\delta^{13}C$, $\delta^{15}N$, N, ^{15}N, and Hg as a result of food processing was determined using a paired t-test ($p<0.05$). Statistically significant differences in $\delta^{13}C$, $\delta^{15}N$, N and THg among raw spotted seal tissues were detected via single-factor analysis of variance (ANOVA) using SAS statistical software (version 9.1; SAS Institute Inc.).

3.4 RESULTS

3.4.1 Effect of Tissue Type on $\delta^{13}C$ and $\delta^{15}N$

The isotopic ratios of C and N were determined in raw and food processed tissues of spotted seal and sheefish (Table 3.2, Figure 3.1). Mean (±1SD) $\delta^{13}C$ was -19.47 (±0.94)‰ in spotted seal muscle, -20.39 (±0.27)‰ in seal liver, -20.74 (±0.58)‰ in seal kidney, -22.50 (±0.68)‰ in sheefish muscle, and -25.75 (±0.46)‰ in seal blubber. ANOVA of $\delta^{13}C$ in raw spotted seal tissues revealed that $\delta^{13}C$ values in muscle and liver as well as in liver and kidney were not statistically different (i.e., $p>0.05$). Blubber $\delta^{13}C$ was significantly depleted relative to the other three seal tissues. $\delta^{13}C$ in muscle was significantly greater than in kidney.

Mean $\delta^{15}N$ was 16.28 (±0.77)‰ in sheefish muscle, 17.09 (±0.66)‰ in spotted seal muscle, 18.33 (±0.54)‰ in seal liver, and 18.40 (±0.63)‰ in seal kidney. $\delta^{15}N$ was not available for blubber due to the negligible protein content of this tissue. ANOVA of $\delta^{15}N$ in raw spotted seal tissues showed that $\delta^{15}N$ in muscle was significantly ($p<0.05$) lower than in liver or kidney. Mean $\delta^{15}N$ values were not significantly different between liver and kidney.

3.4.2 Effect of Food Processing Technique on $\delta^{13}C$ and $\delta^{15}N$

Changes in $\delta^{13}C$ and $\delta^{15}N$ in spotted seal and sheefish tissues as a result of processing are shown in Table 3.3. A statistically significant change ($p<0.05$) in $\delta^{13}C$, a 0.27‰ depletion, was observed in spotted seal liver when fried with butter. No seal tissues

exhibited a significant change in $\delta^{15}N$ as a result of any of the processing methods investigated, but sheefish muscle showed a significant increase in $\delta^{15}N$ as a result of all three processing methods investigated (i.e., baking, drying, smoking).

3.4.3 Effect of Tissue Type on Mercury and Nitrogen Concentration

The concentration of Hg, total N, and ^{15}N in tissues of spotted seal and sheefish are shown in Table 3.4. In spotted seals, the mean Hg concentration was highest in liver (6296 ±3698 ng/g dw), followed by kidney (1934 ±629 ng/g dw), and lowest in muscle (623 ±189 ng/g dw), although the concentrations in kidney and muscle were not statistically different ($p>0.05$). Hg was below the level of quantification in seal blubber. The mean Hg concentration in sheefish muscle (349 ±139 ng/g dw) was significantly lower than in any seal tissue, with the exception of blubber.

N content was significantly greater in spotted seal muscle (13.6 g/100g dw) and sheefish muscle (13.4 g/100g dw) than spotted seal kidney (11.8 g/100g dw) and liver (11.8 g/100g dw). The nitrogen concentration in seal kidney and liver were not significantly different. As expected, the vast majority of the nitrogen in these tissues was ^{14}N, with ^{15}N representing 0.40% of the total nitrogen present in all raw tissues.

3.4.4 Effect of Food Processing Technique on Mercury and Nitrogen Concentration

Changes in the concentrations of Hg, total N and ^{15}N in tissues of spotted seal and sheefish as a result of food processing are shown in Table 3.5. None of these changes were statistically significant ($p \geq 0.05$).

3.5 DISCUSSION

3.5.1 $\delta^{13}C$ and $\delta^{15}N$ as Chemical Tracers of Feeding Ecology

We found that $\delta^{13}C$ and $\delta^{15}N$ vary widely by tissue type within a species and that food preparation methods can produce small (0.3‰), but statistically significant, changes in the stable C and N isotope signatures of fish and wildlife tissues. This finding has implications for the use of $\delta^{13}C$ and $\delta^{15}N$ measurements in feeding ecology studies of humans and wildlife species that feed selectively on certain tissues of their prey.

Humans are perhaps the most familiar example of a tissue-selective feeder, but certain wildlife species are know to target specific prey tissues as well. When resources are abundant, bear species are known to exhibit size and sex selective feeding strategies (Quinn and Kinnison, 1999). In years of high salmon densities, brown (*Ursus arctos*) and black bears (*Ursus americanus*) preferentially consume the most lipid rich tissues of salmon, such as eggs and brain (Gende et al., 2001; Ruggerone et al., 2000). In addition, brown bears select feeding habitats that allow for predation of salmon with higher energy content (i.e., lipid and protein reserves), such as those fish at earlier stages of the migration to spawning grounds (Gende et al., 2004). Polar bears (*Ursus maritimus*) also

exhibit selective feeding strategies, such as targeting pups as prey during times of high seal productivity (Stirling and Archibald, 1977). Regardless of seal abundance, polar bears will often consume only the lipid-rich blubber layer of seals, their main prey species, leaving behind significant quantities of meat and organ tissue to be scavenged by other species such as the arctic fox (*Alopex lagopus*) (Stirling and McEwan, 1975).

Significant differences in both C and N isotope signatures were observed among spotted seal tissues. Within the four tissues analyzed (i.e., blubber, muscle, liver and kidney), $\delta^{13}C$ varied by greater than 6‰ between the most enriched and depleted tissue types, muscle and blubber, respectively. Since $\delta^{13}C$ increases by approximately 1‰ per trophic level in pelagic food webs (Rau et al., 1983), a failure to consider which tissue types are being consumed could lead to gross errors when estimating trophic relationships within food webs. However, $\delta^{13}C$ is not commonly used for trophic level determination. More commonly, $\delta^{13}C$ is utilized as an indicator of carbon source to an organism. Therefore, the contribution of benthic versus pelagic prey items to the total diet of seal consumers could be significantly over- or under-estimated if the consumer is incorrectly assumed to ingest whole prey or tissues other than those that are actually selectively consumed. Thus, the proportion of specific tissues consumed could alter isotopic signature of ingested and assimilated C without changing trophic interactions.

$\delta^{15}N$ values were more consistent across seal tissues than $\delta^{13}C$, with muscle being depleted by approximately 1.3‰ relative to liver and kidney. But, $\delta^{15}N$ was not

measurable in blubber due to the negligible protein content. Thus, species feeding selectively on seal blubber, such as polar bears and humans, would not reflect the $\delta^{15}N$ signature of this major food source due to the extremely low amount of N available for assimilation. As a result, if based on $\delta^{15}N$ alone, the trophic structure of the prey items would be poorly reconstructed for individuals primarily consuming lipids as compared to those utilizing more protein rich tissues.

Assuming a N enrichment of 3.8‰ per trophic level in this marine food web (Hobson and Welch, 1992), the 1.3‰ variation between muscle and liver/kidney in seals represents a difference of one-third of a trophic level in the resulting calculated trophic position. If a tissue other than the one that is primarily consumed is selected for chemical analysis, this variability could lead to errors in estimations of seal diet composition or the contribution of this species to the diet of a predator.

In addition to being tissue-selective feeders, humans prepare their foods using a wide variety of cooking techniques. Certain wildlife scavengers will consume "processed" (e.g., desiccated, oxidized, heated, microbially digested) foods as well. The effects of food processing on the isotopic signatures of food products warrant consideration. This may be particularly important when considering the traditional diets of Alaska Natives, which are comprised of foods and cooking techniques that are uncommon in the rest of the United States (i.e, rendering of blubber into oil, fermentation, smoking and drying of meats) and are therefore less well characterized.

The few studies that have investigated alteration of isotopic signatures by cooking have focused on applications in reconstructing prehistoric human diets. Two studies focusing plant materials recorded $\delta^{13}C$ enrichment of up to 1.5‰ as a result of charring (Marino and DeNiro, 1987; Poole et al., 2002). In contrast, another study found no effect of cooking on $\delta^{13}C$ in a maize-deer meat mixture (Hart et al., 2007). No studies have investigated the effects of cooking on $\delta^{15}N$. Data on whether variations in C and N isotopic signatures occur as a result of modern cooking methods is lacking, yet represents an important consideration if $\delta^{15}N$ and $\delta^{13}C$ are to be used to reconstruct diets or explain contaminant exposure in human populations.

$\delta^{13}C$ and $\delta^{15}N$ were significantly altered in seal and fish tissues as a result of certain common food preparation methods utilized by Alaska Natives, although to a much lesser degree than the variation characterized among tissue types. There was a statistically significant decrease in $\delta^{13}C$ in spotted seal liver when fried and an increase in $\delta^{15}N$ in sheefish muscle when baked, dried and smoked. Frying seal liver was the only process that significantly affected $\delta^{13}C$ and was also the only process that introduced an additional component (i.e., butter). Butter tends to be more depleted in carbon than was observed for raw spotted seal liver, with $\delta^{13}C$ values generally ranging from -20 to -33‰ (Rossman et al., 2000). Therefore, it would be reasonable to expect that the addition of butter when processing seal liver could result in a significant decrease in $\delta^{13}C$ as compared to raw liver alone. Thus, the observed decrease in $\delta^{13}C$ may have resulted

from this addition, although this hypothesis was not directly tested. Multi-component food items are important to assess, but are beyond the scope of the current study.

Although the isotopic changes resulting from food processing were small, this potential source of error could lead to a skewed assignment of the trophic position of prey items. One cannot assume that cooking has no effect. If the $\delta^{15}N$, or trophic, resolution is poor among prey items being distinguished, this could affect determinations of the relative contributions of individual foods to overall diet. In addition, sample preparation methods that desiccate samples using heat-based techniques prior to isotopic analysis may induce shifts in isotopic signatures. Therefore, other methods, such as freeze drying, may be more appropriate.

The $\delta^{15}N$ enrichment in sheefish muscle as a result of cooking was not accompanied by any significant alteration of total N or ^{15}N concentration (dw) in this tissue. A possible mechanistic explanation for this observation is that during cooking some degree of protein hydrolysis is occurring and a small proportion of the amino acids, or other nitrogenous products, are volatilized. If hydrolysis and volatilization occur preferentially for those compounds containing the lighter isotope (^{14}N), no change would be observed in the overall concentration of ^{15}N. The decrease in ^{14}N could be sufficient to alter $\delta^{15}N$, but the simultaneous decreases in ^{14}N and total N could be undetected due to the large amount of N present and the fact that this N pool consists primarily (>99.5%) of ^{14}N.

The current work demonstrates that cooking can alter the isotopic composition of a tissue from its raw state and that $\delta^{13}C$ and $\delta^{15}N$ among tissue types will vary widely within the same individuals (spotted seals). It should be noted, however, that even when statistically significant changes in $\delta^{13}C$ and $\delta^{15}N$ were induced by food processing, the changes were relatively small, ranging from 0.27 to 0.41‰. Differences in $\delta^{13}C$ and $\delta^{15}N$ by tissue type within a species were much greater. Failing to acknowledge this variation could potentially lead to errors in dietary reconstructions if only the species consumed is recorded and not the specific tissue type.

3.5.2 $\delta^{13}C$ and $\delta^{15}N$ as Chemical Tracers of Mercury Exposure

Methyl Hg is a potent neurotoxicant that biomagnifies to considerable concentrations in marine and freshwater food webs. This contaminant is of particular concern in the Arctic due to the heavy reliance of subsistence users on high trophic level aquatic prey items, such as fish and marine mammals (Arnold and Middaugh, 2004). The presence of Hg has been documented in numerous fish and wildlife species within Alaska. Moses et al. (2009) investigated Hg concentrations in two important subsistence marine species of northwest Alaska in the context of both wildlife and human health. Hg exposure to human consumers of these species was estimated, noting the potential for Hg to be present near or above established intake criteria in certain cases depending on species, tissue and food preparation method.

Hg concentration differed significantly among spotted seal tissues. As discussed above, both $\delta^{13}C$ and $\delta^{15}N$ also showed significant variation among tissue types. Correlations between $\delta^{15}N$, or trophic level, and Hg concentrations are often utilized in an attempt to understand the dynamics of Hg within food webs and the trophic relationships between the organisms within that food web. Because Hg is bioaccumulative, an increase in Hg concentration is generally observed with increasing trophic level, as measured by $\delta^{15}N$. But, $\delta^{15}N$ and Hg did not differ in a consistent fashion among seal tissues. Hg was greatest in liver, followed by kidney/muscle (not statistically distinct) and below the level of quantification (<LOQ) in blubber. In contrast, muscle was depleted in N relative to liver and kidney and $\delta^{15}N$ was not measurable in blubber due to the low protein content of this tissue. Thus, Hg concentration and $\delta^{15}N$ do rank in the same order among the four seal tissues investigated. As a result, the utility of $\delta^{15}N$ to explain Hg concentrations and exposure pathways is likely affected by tissue selection of the consumer.

In addition to the $\delta^{15}N$ variation among tissue types, N enrichment was also observed as a result of the three food processing techniques applied to sheefish muscle (i.e., baking, smoking and drying). Although $\delta^{15}N$ increased significantly, Hg concentrations were not significantly altered in this tissue when cooked. Hg and $\delta^{15}N$ are not necessarily affected in the same manner (i.e., increase, decrease or no change) by traditional food processing. This could potentially affect the utility of $\delta^{15}N$ as an indicator of Hg exposure from cooked, wildlife-based foods if such issues are not accounted for.

3.6 CONCLUSIONS

Stable isotopes of C and N are useful markers of feeding ecology and mercury exposure. Studies utilizing isotopic signatures for these purposes sometimes rest on assumptions that are untested or may not hold true. Two common assumptions are that prey items are consumed whole rather than specific tissues targeted preferentially and that $\delta^{13}C$ and $\delta^{15}N$ are not significantly affected by cooking. We found that $\delta^{13}C$ and $\delta^{15}N$ vary widely by tissue type within a seal species and that food preparation methods can produce small changes in the stable C and N isotope signatures of fish and wildlife tissues.

Changes in $\delta^{13}C$ and $\delta^{15}N$, both among tissue types and as a result of food processing, do not occur in tandem with the observed changes in Hg concentration. Consequently, when stable isotopes are used as chemical tracers of feeding ecology and mercury exposure, the specific tissue consumed as well as the processed state of the actual food items should be considered.

These potential sources of error for dietary reconstructions and contaminant pathway determinations could be minimized or eliminated by basing measurements instead on the isotopic signatures of the actual food tissues. We should analyze tissues prepared as they are ultimately consumed, rather than basing interpretations solely on the isotopic signatures of raw food tissues or simply on a species only, rather than tissue specific, basis. We emphasize that stable isotopes of C and N remain a useful tool for studies of feeding ecology and contaminant pathways within food webs.

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Table 3.1 Animal identification (ID), AMMTAP[a] ID, harvest date, sex, age, mass, length, girth and blubber thickness of spotted seals and sheefish sampled in Kotzebue, Alaska (2004-2005)

Animal ID	Species	AMMTAP ID	Harvest Date	Sex	Age in years [median (range)]	Mass (kg)	Length (cm)[b]	Girth (cm)[c]	Blubber Thickness (cm)[d]
KOTZ-01-04	Spotted Seal	692-SPSL-015	25-Oct-2004	Male	6 (3-8)	95.2	122/129	92/90	5.5 / 5.2
KOTZ-02-04	Spotted Seal	692-SPSL-016	25-Oct-2004	Female	5 (4-6)	87.1	119/125	93/80	3.5 / 3.6
KOTZ-03-04	Spotted Seal	692-SPSL-017	25-Oct-2004	Male	5 (4-7)	105.2	131/137	98/89	5.2 / 6.0
KOTZ-04-04	Spotted Seal	692-SPSL-018	25-Oct-2004	Male	6 (5-8)	57.0	NA[e]	NA[e]	4.0 / 4.0
KOTZ-05-04	Spotted Seal	692-SPSL-019	25-Oct-2004	Male	3 (2-4)	60.3	106/115	70/68	4.4 / 4.8
KOTZ-01-05	Sheefish	NA	22-Mar-2005	Male	14 (14-19)	5.2	83.0	NA	NA
KOTZ-02-05	Sheefish	NA	22-Mar-2005	Male	15 (15-18)	5.0	79.9	NA	NA
KOTZ-03-05	Sheefish	NA	22-Mar-2005	Female	20 (19-21)	5.2	81.0	NA	NA
KOTZ-04-05	Sheefish	NA	22-Mar-2005	Male	22 (22-25)	5.5	83.1	NA	NA
KOTZ-05-05	Sheefish	NA	22-Mar-2005	Female	20 (19-23)	6.5	87.7	NA	NA
KOTZ-06-05	Sheefish	NA	22-Mar-2005	Female	22 (20-25)	6.7	90.1	NA	NA
KOTZ-07-05	Sheefish	NA	22-Mar-2005	Female	23 (23-23)	4.8	86.6	NA	NA
KOTZ-08-05	Sheefish	NA	22-Mar-2005	Female	17 (17-18)	5.6	79.8	NA	NA

[a] Alaska Marine Mammal Tissue Archival Project (AMMTAP)

[b] Spotted seal length is the straight line distance from tip of nose to base of tail/tip of the tail. Sheefish length was measured from tip of mandible to fork of tail.

[c] Girth is reported as axillary/umbilical.

[d] Blubber thickness is reported as axillary/umbilical (cm), measured along the ventral midline.

[e] No length or girth measurements available because body was distorted during transport and storage (could not be appropriately positioned for measurement).

Table 3.2 Mean (±1 SD), median and range (minimum, maximum) $\delta^{13}C$ and $\delta^{15}N$ of raw and processed tissues of spotted seals (*Phoca largha*, n=5) and sheefish (*Stenodus leucicthys*, n=8) from Kotzebue, AK (USA)

Species	Tissue	Processing Type	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Spotted Seal	Blubber	Raw	-25.75 (±0.46)	NA[a]
			-25.76	
			-26.34, -25.26	
		Rendered	-26.10 (±0.48)	NA[a]
			-26.38	
			-26.42, -25.24	
	Muscle	Raw	-19.47 (±0.94)	17.09 (±0.66)
			-19.11	16.88
			-21.17, -18.60	16.45 – 18.20
		Boiled	-19.15 (±0.40)	17.59 (±0.54)
			-19.19	17.88
			-19.73, -18.61	16.82 – 18.10
		Dried	-19.44 (±1.14)	17.52 (±0.48)
			-19.16	17.76
			-21.44, -18.43	16.88 - 17.97
	Liver	Raw	-20.39 (±0.27)	18.33 (±0.54)
			-20.36	18.32
			-20.82, -20.13	17.70 – 18.96
		Fried	-20.66 (±0.17)	18.50 (±0.38)
			-20.68	18.69
			-20.89, -20.43	17.99 – 18.85
	Kidney	Raw	-20.74 (±0.58)	18.40 (±0.63)
			-20.72	18.28
			-21.38, -19.90	17.68 – 18.96
		Boiled	-20.97 (±0.99)	18.59 (±0.35)
			-20.56	18.64
			-22.68, -20.27	17.86 – 18.96
Sheefish	Muscle	Raw	-22.50 (±0.68)	16.28 (±0.77)
			-22.37	16.29
			-23.74, -21.84	14.94 – 17.31
		Baked	-22.72 (±0.93)	16.65 (±0.84)
			-22.39	16.61
			-24.60, -21.55	15.00 – 17.75
		Dried	-22.81 (±1.03)	16.69 (±0.87)
			-22.64	16.64
			-24.45, -21.32	14.88 – 17.87
		Smoked	-22.68 (±0.91)	16.62 (±0.79)
			-22.48	16.67
			-24.20, -21.48	15.00 – 17.72

[a] NA = not available. $\delta^{15}N$ values are not available in blubber due to the negligible protein, and thus nitrogen, content in blubber.

Table 3.3 Absolute (‰) and percent (%) change [mean (±1 SD)] in $\delta^{13}C$ and $\delta^{15}N$ as a result of food processing for various food tissues of spotted seals (n=5) and sheefish (n=8) harvested in Kotzebue, Alaska (2004-2005)[a]

Species	Tissue	Process	$\delta^{13}C$: Absolute (‰) and % Change	$\delta^{15}N$: Absolute (‰) and % Change
Spotted Seal	Blubber	Rendering	-0.35 (±0.46)	NA[b]
			-1.38 (±1.82)%	
	Muscle	Boiling	+0.32 (±0.64)	+0.50 (±0.59)
			+1.54 (±3.01)%	+2.98 (±3.44)%
		Drying	+0.03 (±0.31)	+0.43 (±0.47)
			+0.19 (±1.55)%	+2.55 (±2.75)%
	Liver	Frying	**-0.27 (±0.19)**	+0.17 (±0.60)
			-1.32 (±0.93)%	+0.98 (±3.35)%
	Kidney	Boiling	-0.17 (±0.88)	+0.11 (±0.71)
			-0.84 (±4.14)%	+0.68 (±3.96)%
Sheefish	Muscle	Baking	-0.22 (±0.36)	**+0.37 (±0.18)**
			-0.96 (±1.56)%	**+2.22 (±1.06)%**
		Drying	-0.32 (±0.48)	**+0.41 (±0.25)**
			-1.39 (±2.10)%	**+2.50 (±1.51)%**
		Smoking	-0.19 (±0.28)	**+0.33 (±0.17)**
			-0.81 (±1.24)%	**+2.05 (±1.05)%**

[a]**Bold** entries represent statistically significant changes ($p<0.05$).
[b]$\delta^{15}N$ values are not available in blubber due to negligible nitrogen content in this tissue.

Table 3.4 Mean (±1 SD), median and range (minimum-maximum) dry weight concentrations of mercury (Hg), total nitrogen (N) and ^{15}N in raw and processed tissues of spotted seals (*Phoca largha*, n=5) and sheefish (*Stenodus leucicthys*, n=8) from Kotzebue, AK (USA)[a]

Species	Tissue	Processing Type	Mercury (ng/g)	Nitrogen (g/100g)	^{15}N (mg/100g)
Spotted Seal	Muscle	Raw	623 (±189)	13.6 (±1.0)	54.3 (±3.9)
			664	13.7	54.7
			379-846	12.1-14.9	48.3-59.4
		Boiled	708 (±199)	13.8 (±1.6)	55.0 (±6.3)
			742	14.0	56.1
			459-929	11.4-15.6	45.6-62.4
		Dried	487 (±130)	14.5 (±1.7)	57.7 (±6.7)
			452	14.5	58.0
			326-641	12.4-17.0	49.6-67.9
	Liver	Raw	6296 (±3698)	11.8 (±0.7)	47.3 (±2.9)
			8154	11.7	46.7
			1959-9754	11.1-12.9	44.4-51.4
		Fried	6142 (±3833)	11.6 (±0.5)	46.2 (±2.0)
			8173	11.5	45.7
			1969-9391	11.2-12.4	44.8-49.6
	Kidney	Raw	1934 (±629)	11.8 (±1.1)	47.1 (±4.2)
			1837	12.3	49.0
			1935-2997	10.1-12.7	40.2-40.8
		Boiled	1598 (±520)	11.6 (±1.5)	46.5 (±5.9)
			1463	12.1	48.5
			1074-2432	9.03-12.6	36.1-50.3
Sheefish	Muscle	Raw	349 (±139)	13.4 (±1.0)	53.5 (±4.0)
			312	13.5	53.7
			241-673	11.7-15.0	46.5-59.7
		Baked	337 (±152)	13.0 (±0.8)	51.8 (±3.0)
			284	12.9	51.5
			206-659	11.8-14.2	47.1-56.4
		Dried	321 (±148)	13.0 (±1.2)	51.7 (±4.8)
			284	13.6	54.1
			169-602	11.2-14.1	44.6-56.2
		Smoked	336 (±158)	13.3 (±1.1)	53.1 (±4.4)
			286	13.7	54.5
			199-654	11.5-14.5	45.9-57.6

[a]Hg, N and ^{15}N were below the level of quantification (<LOQ) in seal blubber.

Table 3.5 Absolute and percent (%) change [mean (±1 SD)] in mercury (Hg), total nitrogen (N) and ^{15}N concentrations (dw) as a result of food processing for various food tissues of spotted seals (n=5) and sheefish (n=8) harvested in Kotzebue, Alaska (2004-2005)[a]

Species	Tissue	Process	Hg: Absolute (ng/g) and % Change	N: Absolute (ng/g) and % Change	^{15}N: Absolute (mg/100g) and % Change
Spotted Seal	Muscle	Boiling	+85.3 (±209.7)	+0.19 (±1.57)	+0.79 (±6.29)
			+20.8 (±46.5)%	+1.63 (±11.71)%	+1.68 (±11.74)%
		Drying	-136 (±244)	+0.86 (±2.49)	+3.45 (±9.95)
			-13.9 (±43.4)%	+7.35 (±20.01)%	+7.39 (±20.01)%
	Liver	Frying	-155 (±361)	-0.28 (±1.03)	-1.13 (±4.14)
			-5.10 (±11.56)%	-2.03 (±8.58)%	-2.01 (±8.64)%
	Kidney	Boiling	-336 (±833)	-0.15 (±1.64)	-0.60 (±6.56)
			-21.6 (±35.8)%	-0.74 (±15.07)%	-0.72 (±15.13)%
Sheefish	Muscle	Baking	-12.4 (±48.9)	-0.44 (±0.97)	-1.74 (±3.85)
			-4.48 (±15.01)%	-2.93 (±7.53)%	-2.90 (±7.52)%
		Drying	-28.2 (±63.1)	-0.47 (±1.58)	-1.85 (±6.28)
			-11.0 (±19.7)%	-2.97 (±12.31)%	-2.93 (±12.31)%
		Smoking	-24.2 (±37.1)	-0.10 (±1.34)	-0.37 (±5.35)
			-10.8 (±14.2)%	-0.26 (±10.72)%	-0.23 (±10.72)%

[a]No changes were statistically significant for these analytes ($p<0.05$).
[b]Hg, N, and ^{15}N were below the level of quantification (<LOQ) in seal blubber, therefore changes in these analytes due to food processing could not be determined.

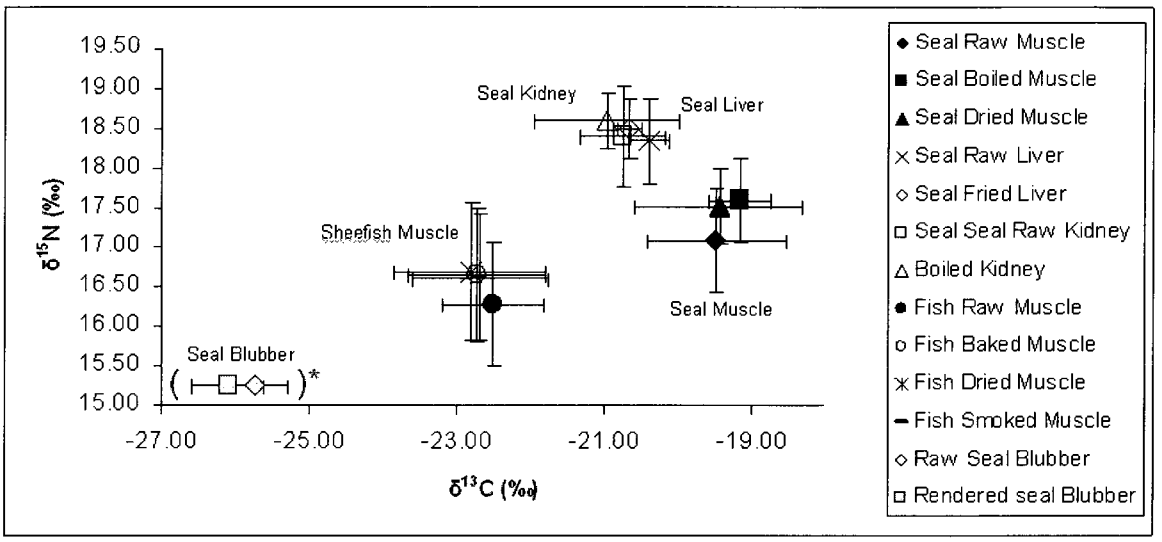

Figure 3.1 Carbon and nitrogen isotope ratios [mean ± 1SD] of raw and food processed tissues of spotted seals (*Phoca largha*, n=5) and sheefish (*Stenodus leucicthys*, n=8) from Kotzebue, AK (USA). *δ^{15}N is not available for spotted seal blubber due to the low protein, and thus N, content of this tissue. The plotted values are intended to represent δ^{13}C values only.

CHAPTER 4

Revised regulatory guidelines are needed to predict bioaccumulation in air-breathing species[4]

4.1 ABSTRACT

Bioaccumulation factors (BAF) are the key parameter for evaluating the likelihood of human and wildlife exposure to a chemical through food consumption. Certain regulatory guidelines allow the octanol-water partition coefficient (K_{OW}), a measure of the relative affinity of a chemical for water versus lipid, to be used as a proxy for BAF. Low K_{OW} chemicals are classified as non-bioaccumulative since they are assumed not to bioaccumulate in aquatic species. We show here that low K_{OW} contaminants can bioaccumulate in an air-breathing species relative to an aquatic species of similar trophic status. Here, respiratory elimination and bioaccumulation are controlled by lipid-air partitioning (i.e., the octanol-air partition coefficient, K_{OA}), rather than lipid-water partitioning (K_{OW}). Thus, K_{OW} alone is inadequate in predicting bioaccumulation in mammals. New regulatory guidelines must utilize bioaccumulation models incorporating K_{OA} into chemical risk-assessments for air-breathing species, including marine mammals and humans.

[4] Moses, S.K., C.L. Lieske, D.C.G. Muir, A.V. Whiting, T.M. O'Hara. Revised regulatory guidelines are needed to predict bioaccumulation in air-breathing species. Prepared for submission to Environmental Science and Technology.

4.2 INTRODUCTION

Recent lawsuits (*1*) related to contaminants in polar bears have enhanced scrutiny of regulatory methods to protect arctic wildlife from the adverse effects of contamination. Persistent organic pollutants (POPs), including organochlorines (OCs), have been detected in biota, even in remote regions of the Arctic (*2, 3*). Risks associated with the release of these chemicals depend on their potential for long-range transport, environmental persistence, toxicity, and capacity to biomagnify and bioaccumulate (*4, 5*).

Biomagnification of lipophilic contaminants is of particular interest in the Arctic where lipid rich prey species and tissues are vitally important energy sources. Additionally, humans in the Arctic occupy a high trophic level due to subsistence consumption of top level predators, like marine fish and mammals.

Biomagnification potential is determined by an organism's ability to absorb, biotransform and eliminate a given compound, which is largely dependent upon its physical-chemical properties. These properties include partition coefficients such as the octanol-water (K_{OW}) and octanol-air (K_{OA}) partition coefficients, which describe the compound's relative solubility in water versus lipid (i.e., octanol) or air, respectively.

Predicting chemical behavior within food webs from physical-chemical properties is an important aspect of the registration and management of POPs, and is crucial for identifying contaminants of concern. The bioaccumulation models most frequently used

to help define criteria for acceptable levels of POPs in food, water and sediments have been derived from data collected from aquatic organisms. Generally, bioaccumulation is considered qualitatively in risk assessments based on a "cut-off" value.

For example, in their persistent, bioaccumulative and toxic (PBT) substance policy statement, the U.S. Environmental Protection Agency (EPA) defines bioaccumulative substances as those with a bioconcentration factor (BCF) >1000 (*6*). In Tier 1 testing of a new, potentially PBT compound, K_{OW} can be measured in lieu of BCF. Any compound with a log K_{OW}<4.2, assumed to be equivalent to a BCF <1000, is classified as non-bioaccumulative. If the same compound is determined to be not persistent in the environment, no further PBT testing is required. The European REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) guidelines defines a bioaccumulative substance as one with BCF>2000 (*7*). If the production volume of a chemical is <100 tons/year, a log K_{OW}<4.5 (measured or QSAR calculated) can be used in place of BCF data to establish that a compound is not bioaccumulative.

The relationship between BCF/BAF and K_{OW} for neutral, lipophilic, organic chemicals has been demonstrated in numerous aquatic and marine food webs in both the laboratory and field (*8-10*). Within these systems, biomagnification at upper trophic levels is significant only for poorly metabolizable, non-polar substances with log K_{OW}>5 (*11, 12*). Below this K_{OW}, compounds are sufficiently hydrophilic to allow their elimination across the gills, even if they have been efficiently absorbed from food.

Recently, the applicability of aquatic bioaccumulation models to air-breathing species has been questioned. K_{OA} is defined as the ratio of the equilibrium concentrations of a chemical in the octanol versus air phase. Volatility, described by K_{OA}, drives respiratory elimination of hydrophobic organic compounds in lunged organisms. As K_{OA} increases, lipid-to-air elimination decreases. If biotransformation is low, high K_{OA} compounds may not undergo adequate depuration to counteract dietary absorption, allowing bioaccumulation relative to gilled species.

Studies highlighting the role of partitioning behavior in bioaccumulation by terrestrial species or marine mammals are rare (*13, 14*). Those that exist suggest K_{OA} may be a better indicator of bioaccumulation potential in air-breathing species (*15, 16*). Thus, lunged organisms may biomagnify low K_{OW} compounds if K_{OA} is sufficiently high. Compounds with log K_{OW}<5 and log K_{OA}>5, may reach levels of concern in upper trophic level birds and mammals, including arctic seals, polar bears and humans.

The objectives of our study were to compare the relative roles of K_{OW} and K_{OA} in determining OC concentrations in gilled versus an air-breathing arctic vertebrates in the same relative time, space, and trophic level.

4.3 EXPERIMENTAL

4.3.1 Sample Collection and Storage

Spotted seals were sampled in October of 2004-2007 and sheefish in March 2005 at Kotzebue, AK (66.90°N, 162.59°W) under Marine Mammal Health and Stranding Response Program (MMHSRP) permit #932-1489-07. Blubber, muscle and liver samples from spotted seals (n=18) and muscle and liver from sheefish (n=8) were collected for chemical analyses from legally subsistence harvested animals. All animals were assessed for gross general health prior to sampling to allow for data interpretation in the context of animal condition and were deemed to be in excellent physical condition. Collection of samples was performed as previously described (*17*). Samples were immediately frozen at -20°C, shipped to the University of Alaska Fairbanks (UAF) and stored at -80°C until analysis.

4.3.2 Morphometrics and Age Estimation

Spotted seal and sheefish harvest date, sex, age and morphometric information appear in Table 4.1. Seal length was measured as the straight line distance from the tip of the nose to both the base and the tip of the tail. Sheefish length was measured as the straight line distance from tip of mandible to fork of tail. Seal age was estimated by counting annual growth layers in the cementum of teeth as described by Dehn et al. (*18*). Sheefish were aged by counting otolith annual growth increments as described in Brown et al. (*19*). Ages were read in triplicate by each of three independent readers.

4.3.3 Organochlorine (OC) Analysis

Organochlorines (OCs) were determined in spotted seal and sheefish tissues according to previously described methods (*20*). Briefly, samples were extracted with dichloromethane (DCM) and quantified using high resolution, single-column capillary gas chromatography with electron capture detection.

Standard reference materials (SRM 1588a: organics in cod liver oil) from the National Institutes of Standards and Technology (Gaithersburg, MD, USA) were used to confirm the accuracy and reproducibility of the analytical methods. A calibration check standard was run every six samples, followed by a spike or SRM for quality assurance and control (QA/QC). Method blanks were included to blank correct analyzed contaminant concentrations and calculate minimum detection limits (MDL). The MDL for OCs in tissues varied depending upon analyte and sample size, ranging from 0.001-14 ng/g wet weight (ww).

OCs (n=108) included in the present study are 1,3,5-, 1,2,4- and 1,2,3-trichlorobenzene (TCB); 1,2,4,5- and 1,2,3,4-tetrachlorobenzene (TTCB); pentachlorobenzene (PECB); hexachlorobenzene (HCB); α-, β- and γ-hexachlorocyclohexane (HCH); heptachlor; heptachlor epoxide; α- and γ-chlordane; dieldrin; o,p- and p,p-dichlorodiphenyldichloroethylene (DDE); o,p- and p,p-dichlorodiphenyldichloroethane (DDD); o,p- and p,p-dichlorodiphenyltrichloroethane (DDT); and the following PCB congeners (IUPAC designations in order of elution): 4/10, 7/9, 6, 8/5, 19, 12/13, 18,

15/17, 24/27, 16/32, 26, 25, 50, 33/20, 53, 22, 45, 46, 52, 49, 47/48, 44, 42, 71/41/64, 100, 63, 74, 70/76/98, 66, 95, 91, 55, 56/60, 92, 84, 101, 99, 119, 83, 97, 81/87, 85, 136, 110, 82, 151, 135/144, 107, 149, 118, 114, 134/131, 146, 153, 132, 105, 141, 179, 137, 163/138, 158, 129, 182/187, 183, 128, 167, 174, 177, 202/171, 156, 157/200, 172, 197, 180, 193, 191, 199, 170/190, 201, 203/196, 189, 208/195, 207, 194, 205, 206, 209 ("/" indicates co-eluting congeners).

OC concentrations are reported in ng/g and were normalized to PCB 153 prior to comparisons between species.

4.3.4 Assignment of K_{OW} and K_{OA} values to Organochlorines (OCs)

K_{OW} and K_{OA} values were derived from measured values reported by Mackay *et al.* (*21*) by determining the median of all reported values at 25°C. To assign a single K_{OW}/K_{OA} value for the cases in which multiple (2-3) PCB congeners co-elute from the GC column, in the case of homologous pairs (i.e., co-eluting pairs with the same degree of chlorination), the reported K_{OW}/K_{OA} values of both compounds were combined and an overall median taken. When co-eluting pairs were non-homologous, any congener present that was more highly chlorinated with 2,3,4,5-, 2,4,5- or 2,3,4- substitution was assumed to be the predominate congener and the median K_{OW}/K_{OA} value for that congener was used to represent the pair. For the remaining co-eluting pairs, the congener least likely to undergo cytochrome P450 biotransformation, as classified by Boon *et al.*

(*22*), was assumed to be the predominate congener and the median K_{OW}/K_{OA} value for this compound was used to represent the pair.

4.3.5 Stable Isotopes and Trophic Level Calculations

Nitrogen (N) stable isotope signatures were determined in sheefish and spotted seal muscle and liver. No value was determined in seal blubber due to the low N content in this tissue. Isotopes were analyzed by the Alaska Stable Isotope Facility using an Elemental Analyzer (Costech Scientific) and a Delta Plus XL Isotope Ratio Mass Spectrometer with a Conflo III interface (Thermo-Finnegan) (EA-IRMS). Each sample was analyzed in duplicate and the mean value was used for all subsequent data analysis. Data are presented in delta notation, where $\delta^{15}N = (R_{sample}-R_{standard})/(R_{standard})*1000‰$ and R is the ratio of the heavier (^{15}N) to the lighter (^{14}N) isotope and the international standard for N is atmospheric nitrogen (N_{atm}). QA/QC was evaluated via the standard deviations of the reference material, peptone.

Trophic level was estimated for each species using $\delta^{15}N$ based on the following equation:

$$TL = 2 + [(\delta^{15}N_{consumer}-\delta^{15}N_{primary\ consumer})/3.8‰]$$

where, 2 is the assumed trophic level of the primary consumers of the food web, $\delta^{15}N_{primary\text{-}consumer}$ is 9.8‰ as determined for *Calanus* spp. and 3.8‰ is the trophic

enrichment factor for $\delta^{15}N$ in an Arctic marine food web in the Bering Sea of Alaska (*23, 24*).

4.3.6 Statistical Analysis

Statistically significant ($p<0.05$) differences in trophic levels calculated from $\delta^{15}N$ values of spotted seal and sheefish muscle and liver were detected via single-factor analysis of variance (ANOVA). The significance of regressions of OC concentration versus partition coefficient (i.e., whether slopes were >0) was determined via a 1-tailed F-test ($p<0.05$). These statistical analyses were carried out using SAS statistical software (version 9.1; SAS Institute Inc.). Statistical differences between slopes of regression lines were tested using a one-tailed t-test and were performed using Minitab statistical software (version 15.1.1; Minitab Inc.).

4.4 RESULTS AND DISCUSSION

Vertebrate OC concentrations are influenced by multiple factors including location, season, year, feeding ecology, tissue, and trophic level. We minimized the effects of these variables by carefully selecting the air-breathing and water-respiring species for this study. Spotted seal (*Phoca largha*) and sheefish (*Stenodus leucicthys*) are relatively spatially, temporally and trophically matched. They were collected in Kotzebue Sound (Alaska) during the winters of 2004-2007. Both species are highly piscivorous and occupy similar trophic positions. Spotted seals had a slightly higher trophic level (4.0/4.2, as calculated from $\delta^{15}N$ values in muscle and liver, respectively) than sheefish

(3.7/3.6). Although the trophic levels were significantly different between species, the biological significance of this difference is minimal. Spatial, temporal, and trophic similarity between these species allows for a tissue matched comparison of OC dynamics in two upper trophic level organisms with vastly different respiratory physiology.

Log K_{OW} of the compounds analyzed ranged from 3.7 for γ-hexachlorocyclohexane (γ-HCH) to 8.3 for PCB 209 and log K_{OA} from 4.9 for 1,3,5-trichlorobenzene (1,3,5-TCB) to 12.8 for PCB 209 (Figure 4.1). Overall, K_{OW} and K_{OA} are significantly positively correlated ($p<0.001$), but the relationship weakens as K_{OW} and K_{OA} decrease, with no correlation present among the subset of compounds with log $K_{OW}<5$.

OCs were measured in muscle and liver of both species, and in seal blubber. Concentrations (Tables 4.2 and 4.3) were within previously reported ranges for similar arctic fish and marine mammals (*2, 25*). The species had similar ΣOC concentrations (sum of 108 analytes) in matched tissues. Mean ΣOC levels were 12.3 (±8.8) ng/g (wet weight, ww) in fish muscle, 15.7 (±9.7) ng/g in seal muscle, 15.7 (±8.8) ng/g in fish liver and 31.2 (±16.6) ng/g in seal liver. In seal blubber (no analogous tissue in fish), the mean ΣOC concentration was 837 (±245) ng/g.

The effects of K_{OW} and K_{OA} on OC concentrations were assessed independently over three K_{OW} ranges, low (log $K_{OW}<5$), moderate ($5\leq$log $K_{OW}\leq7$) and high (log $K_{OW}>7$). For compounds with $K_{OW}<5$, K_{OW} and K_{OA} are not correlated, allowing for an

independent assessment of the effects of each partition coefficient on contaminant concentrations in spotted seals and sheefish.

No significant correlation was found in any tissue between OC concentration and log K_{OW} for the subset of low K_{OW} compounds. Fish efficiently excrete these compounds to the aqueous environment across the gills, therefore they do not bioaccumulate (*11, 12*). Although these compounds may accumulate in seals, since respiratory elimination in seals is to air rather than water, concentration should not be correlated with K_{OW}, but rather K_{OA}.

We observed a significant ($p<0.05$) positive correlation between log K_{OA} and both OC concentration and ratios of OC/PCB153 in all three seal tissues for the low K_{OW} OCs (Figure 4.2, blubber data not shown). This strongly supports the hypothesis that air-breathing organisms bioaccumulate compounds with a $K_{OW}<5$ if K_{OA} is sufficiently high. Bioaccumulation of low K_{OW} compounds not known to accumulate in aquatic organisms, such as HCH, endosulfan and chlorobenzenes, has been reported in arctic marine mammals and seabirds (*26-28*).

No correlation was detected between K_{OA} and OC concentration for sheefish muscle within the low K_{OW} compounds. A significant positive correlation was observed in sheefish liver (Figure 4.2), but the slope of the correlation and the OC concentrations were significantly less than in seals. Low K_{OW} compounds with higher K_{OA}'s (i.e.,

dieldrin, heptachlor epoxide, β-HCH) were at concentrations 2-8 times higher (ww) in seals than fish for matched tissues and 5-14 times higher on a lipid weight basis. Age associated differences are unlikely since these fish are older than the seals (Table 4.1).

These findings have implications for risk assessments of new and existing commercial chemicals that do not exceed current regulatory cut-offs for defining bioaccumulative substances based on K_{OW}. Based on our current findings, low K_{OW} compounds have the potential to bioaccumulate to elevated levels in upper trophic level lunged organisms. Current bioaccumulation models that do not consider K_{OA} are therefore inappropriate for air-breathing species, including seals, polar bears and humans.

It was anticipated that concentrations of moderate K_{OW} compounds ($5 \leq \log K_{OW} \leq 7$; n=74) would increase with K_{OW} in fish and K_{OA} in seals. Since these variables are highly correlated in this range, we expected similar relationships between OC concentration and both K_{OW} and K_{OA} in spotted seals and sheefish. Although the relationship between concentration and each partition coefficient was positive for all tissues in both species, the correlation only reached or approached statistical significance for log K_{OW} versus concentration in seal liver ($p=0.04$) and blubber ($p=0.10$) and for log K_{OA} versus seal liver ($p=0.08$) and blubber ($p=0.13$).

To test whether biotransformation was overpowering the expected relationships within the moderate K_{OW} compounds, the analysis was repeated with only polychlorinated

biphenyls (PCBs) classified as non-metabolizable by cytochrome P450 enzymes (*22*). Correlations were actually weaker for this subset of compounds, suggesting that complex interactions and/or factors other than K_{OW}, K_{OA} and biotransformation are the major drivers determining chemical concentration profiles in these species.

K_{OW} and K_{OA} are highly correlated for high K_{OW} chemicals (log K_{OW}>7). For these compounds, concentrations decreased in all sheefish and spotted seal tissues with increasing K_{OW} and K_{OA}. PCB 209 was an outlier and was removed from analysis. Correlation significance between concentration and log K_{OW} ranged from p=0.08-0.14 in seal tissues and was p=0.05 for both fish tissues. Correlations with K_{OA} ranged from p=0.02-0.04 in seals and from p=0.005-0.02 in fish.

When a chemical's log K_{OW} exceeds ~7, absorption efficiency from food is reduced due to increased molecular size and decreased diffusion across the gastrointestinal tract (GIT) (*29, 30*). Thus, these compounds should exhibit decreasing bioaccumulation and increased fecal elimination.

In summary, OC concentrations and profiles differed in air-breathing versus water-respiring arctic vertebrates despite similarities in region, season, tissue type, year, trophic status and feeding habits. This can be explained in part by chemical partitioning behavior in relation to the respiratory physiology of the organisms.

In seals, low K_{OW} (log K_{OW}<5) OCs were at higher concentrations and increased in concentration in relation to K_{OA}, suggesting respiratory elimination is an important driver of bioaccumulation in air-breathing species. Fish efficiently excrete these relatively hydrophilic compounds to the aqueous environment via the gills, preventing accumulation following dietary absorption. Respiratory elimination via the lungs is driven by chemical volatility, therefore substances with a sufficiently high K_{OA} can bioaccumulate in air-breathers after absorption, even if K_{OW} is low.

Moderate K_{OW} chemicals (5≤log K_{OW}≤7) showed little correlation between concentration and partition coefficients, even when only the non-metabolizable subset of compounds was considered. Factors other than, or in combination with, partitioning behavior and biotransformation appear to be important in determining concentration profiles of these chemicals in the study species. Finally, the concentration of high K_{OW} compounds (K_{OW}>7) decreased as K_{OW} increased, presumably due to decreased absorption efficiency from food by the GIT.

A practical example of the implications of our results is that of polar bears, arctic apex predators. A lawsuit was recently brought against the U.S. EPA for failing to consider the effects of current-use pesticides on polar bears, now protected under the Endangered Species Act. Polar bears are not only air-breathers themselves, but so are ice seals, their main prey item. Consequently, there may be additional low K_{OW}, high K_{OA} POPs transported through this food web to polar bears at sufficient quantities to warrant

evaluation by the EPA and other agencies. Because subsistence hunters also sit atop the arctic food web, we must also consider the risks posed to them by this suite of chemicals.

Chemical management policies that focus only on eliminating the use of compounds with a K_{OW} greater than an assigned threshold value are too simplistic and do not apply to air-breathing species. Refined models are clearly needed which account for lipid-air chemical partitioning behavior (K_{OA}) to address risks in food webs that include birds and mammals. It is inappropriate to assess bioaccumulation potential in water- and air-respiring species using the same models.

4.5 ACKNOWLEGMENTS

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Table 4.1 Harvest date, sex, age and morphometric information for spotted seals (n=18) and sheefish (n=8) collected in Kotzebue Sound (Kotzebue, AK, USA)

Species	Animal ID	Harvest Date	Sex	Age (yrs)	Mass (kg)	Length (cm)[a]
Spotted Seal	KOTZ-SS-01-04	25-Oct-2004	Male	6	95.2	129
	KOTZ-SS-02-04	25-Oct-2004	Female	5	87.1	125
	KOTZ-SS-03-04	25-Oct-2004	Male	5	105.2	137
	KOTZ-SS-04-04	25-Oct-2004	Male	6	57.0	NA[b]
	KOTZ-SS-05-04	25-Oct-2004	Male	3	60.3	115
	KOTZ-SS-01-05	24-Oct-2005	Female	7	109.5	156
	KOTZ-SS-02-05	24-Oct-2005	Male	7	81.3	160
	KOTZ-SS-03-05	24-Oct-2005	Female	4	75.3	144.6
	KOTZ-SS-04-05	24-Oct-2005	Female	0	28.4	107.4
	KOTZ-SS-05-05	24-Oct-2005	Female	4	72.4	146.6
	KOTZ-SS-06-05	24-Oct-2005	Male	6	73.0	147.9
	KOTZ-SS-07-06	31-Oct-2006	Male	6	91.3	123.4
	KOTZ-SS-08-06	31-Oct-2006	Male	10	87.6	166.6
	KOTZ-SS-09-06	31-Oct-2006	Male	3	52.7	161.8
	KOTZ-SS-10-06	31-Oct-2006	Male	9	115.3	163.8
	KOTZ-SS-02-07	16-Oct-2007	Female	1	42.5	123.5
	KOTZ-SS-03-07	16-Oct-2007	Female	4	79.8	150.0
	KOTZ-SS-04-07	16-Oct-2007	Male	4	73.0	161.8
Sheefish	KOTZ-SF-01-05	22-Mar-2005	Male	14	5.2	83.0
	KOTZ-SF-02-05	22-Mar-2005	Male	15	5.0	79.9
	KOTZ-SF-03-05	22-Mar-2005	Female	20	5.2	81.0
	KOTZ-SF-04-05	22-Mar-2005	Male	22	5.5	83.1
	KOTZ-SF-05-05	22-Mar-2005	Female	20	6.5	87.7
	KOTZ-SF-06-05	22-Mar-2005	Female	22	6.7	90.1
	KOTZ-SF-07-05	22-Mar-2005	Female	23	4.8	86.6
	KOTZ-SF-08-05	22-Mar-2005	Female	17	5.6	79.8

[a]Spotted seal length is the straight line distance from the tip of the nose to the tip of the tail. Sheefish length is measured from the tip of the mandible to the fork of the tail.
[b]No length measurement available because body was distorted during transport and storage (could not be properly positioned for measurements).

Table 4.2 Organochlorine (OC) concentrations (ng/g *wet weight*) in spotted seal (n=18) and sheefish (n=8) tissues [mean (± 1 standard deviation), median, range][a,b]

Compound[c]	Spotted Seal Blubber	Spotted Seal Muscle	Spotted Seal Liver	Sheefish Muscle	Sheefish Liver
1,3,5-Trichlorobenzene	ND	ND	ND	0.006 (±0.009) 0.001 0.001-0.02	ND
1,2,4-Trichlorobenzene	1.10 (±0.61) 1.05 ND-2.79	ND	0.04 (±0.04) 0.05 ND-0.11	0.20 (±0.08) 0.21 0.07-0.31	0.05 (±0.05) 0.03 0.009-0.14
1,2,3-Trichlorobenzene	0.58 (±0.89) 0.33 ND-3.72	ND	ND	ND	ND
1,2,4,5-Tetrachlorobenzene	ND	ND	0.03 (±0.04) 0.03 ND-0.13	0.003 (±0.004) 0.001 0.001-0.01	ND
1,2,3,4-Tetrachlorobenzene	0.44 (±0.30) 0.39 ND-1.20	ND	0.01 (±0.01) 0.01 ND-0.04	0.08 (±0.06) 0.07 0.001-0.18	0.03 (±0.02) 0.02 0.008-0.06
Pentachlorobenzene	ND	ND	ND	0.11 (±0.08) 0.10 0.02-0.22	ND
α-Hexachlorocyclohexane	13.6 (±5.4) 12.7 6.67-23.4	0.27 (±0.27) 0.18 0.05-1.20	0.31 (±0.28) 0.32 ND-0.91	0.21 (±0.13) 0.23 0.03-0.44	0.26 (±0.15) 0.20 0.12-0.51
β-Hexachlorocyclohexane	72.5 (±32.5) 68.6 19.5-3.72	1.16 (±1.28) 0.83 0.11-5.75	1.25 (±1.33) 1.04 ND-5.24	0.16 (±0.09) 0.19 0.02-0.26	0.23 (±0.17) 0.14 0.07-0.55
Hexachlorobenzene	15.7 (±14.4) 11.3 0.13-66.5	0.36 (±0.26) 0.26 0.09-0.92	0.52 (±0.44) 0.48 0.03-1.55	0.04 (±0.04) 0.02 0.001-0.12	1.23 (±0.43) 1.08 0.82-1.93
γ-Hexachlorocyclohexane	0.94 (±0.82) 1.12 ND-2.33	ND	0.46 (±0.77) 0.03 ND-2.49	0.02 (±0.01) 0.02 0.01-0.05	0.04 (±0.03) 0.03 ND-0.09
Heptachlor	ND	ND	ND	0.001 (±1x10^{-5}) 0.001 0.001-0.001	ND
Heptachlor epoxide	24.9 (±13.6) 22.2 6.29-65.6	0.42 (±0.43) 0.34 0.07-1.99	1.17 (±0.55) 1.09 0.46-2.59	0.11 (±0.08) 0.10 0.004-0.24	0.15 (±0.08) 0.16 ND-0.23
γ-Chlordane	0.54 (±0.45) 0.58 ND-1.25	ND	ND	0.02 (±0.03) 0.01 0.001-0.09	0.09 (±0.07) 0.07 0.02-0.23
α-Chlordane	2.79 (±3.96) 1.79 0.19-18.2	0.03 (±0.04) 0.01 ND-0.12	0.09 (±0.08) 0.05 ND-0.22	0.39 (±0.35) 0.29 0.08-0.96	0.40 (±0.23) 0.33 0.24-0.94
Dieldrin	35.5 (±16.1) 32.3 10.6-72.0	0.61 (±0.72) 0.43 0.09-3.35	1.35 (±0.56) 1.29 0.40-2.43	0.27 (±0.19) 0.24 0.04-0.59	0.36 (±0.16) 0.27 0.24-0.69
p,p-DDE	137 (±66) 126 20.6-274	1.82 (±1.78) 1.46 0.32-6.76	3.33 (±3.76) 2.50 0.17-16.2	0.92 (±0.84) 0.59 0.28-2.72	0.97 (±0.55) 0.93 0.37-1.71
o,p-DDD	ND	ND	0.07 (±0.07) 0.06 ND-0.28	ND	ND
p,p-DDD	5.01 (±2.79) 3.98 1.41-11.4	0.07 (±0.10) 0.04 0.02-0.44	0.35 (±0.19) 0.31 0.15-1.02	0.38 (±0.33) 0.24 0.08-1.01	0.28 (±0.21) 0.20 0.13-0.76
o,p-DDT	ND	ND	ND	0.03 (±0.05) 0.001 0.001-0.12	ND

Table 4.2 (Continued)

Compound[c]	Spotted Seal Blubber	Spotted Seal Muscle	Spotted Seal Liver	Sheefish Muscle	Sheefish Liver
p,p-DDT	8.43 (±5.92) 10.3 0.12-20.3	0.06 (±0.07) 0.03 ND-0.23	ND	0.15 (±0.15) 0.10 0.02-0.46	ND
PCB 4-10	1.02 (±0.56) 0.89 0.43-2.09	ND	ND	ND	ND
PCB 7-9	0.37 (±0.18) 0.26 0.21-0.73	ND	ND	0.06 (±0.05) 0.04 0.02-0.18	ND
PCB 8-5	ND	ND	ND	0.04 (±0.05) 0.04 ND-0.14	0.09 (±0.09) 0.09 0.007-0.30
PCB 19	ND	ND	ND	0.16 (±0.12) 0.12 0.05-0.36	0.18 (±0.10) 0.18 ND-0.34
PCB 12-13	0.94 (±0.73) 0.64 0.27-3.21	0.05 (±0.04) 0.04 ND-0.15	ND	ND	ND
PCB 18	2.43 (±1.50) 2.40 ND-5.30	0.20 (±0.15) 0.16 0.04-0.58	ND	0.31 (±0.22) 0.35 0.05-0.73	ND
PCB 15-17	2.33 (±0.92) 2.15 1.12-4.75	ND	ND	0.16 (±0.15) 0.08 0.06-0.50	ND
PCB 24-27	0.12 (±0.10) 0.13 ND-0.28	ND	ND	0.03 (±0.02) 0.02 0.007-0.08	ND
PCB 16-32	1.12 (±0.63) 1.00 0.25-3.09	ND	ND	0.13 (±0.10) 0.09 0.04-0.34	ND
PCB 26	1.14 (±0.52) 1.20 ND-2.25	1.61 (±1.45) 1.07 0.26-5.69	ND	0.62 (±0.49) 0.52 0.06-1.38	0.07 (±0.05) 0.06 0.02-0.14
PCB 25	4.27 (±3.40) 4.76 ND-11.5	ND	0.15 (±0.15) 0.12 ND-0.41	ND	ND
PCB 31-28	3.58 (±1.02) 3.86 1.37-5.04	0.18 (±0.12) 0.14 0.04-0.41	0.32 (±0.14) 0.28 0.09-0.57	ND	0.39 (±0.41) 0.27 0.09-1.34
PCB 33-20	ND	ND	ND	0.10 (±0.07) 0.09 0.03-0.26	ND
PCB 53	ND	ND	ND	0.06 (±0.05) 0.04 0.006-0.15	ND
PCB 45	ND	ND	ND	0.06 (±0.05) 0.05 0.02-0.19	ND
PCB 52	8.35 (±2.33) 7.84 4.92-13.6	0.29 (±0.21) 0.28 0.01-0.94	0.56 (±0.40) 0.47 0.19-1.26	0.38 (±0.26) 0.24 0.16-0.89	ND
PCB 49	2.22 (±0.77) 2.08 1.15-4.11	0.07 (±0.06) 0.07 ND-0.25	ND	0.12 (±0.09) 0.08 0.03-0.32	ND
PCB 47-48	6.76 (±2.69) 7.19 1.38-13.8	0.20 (±0.13) 0.14 0.05-0.49	0.30 (±0.18) 0.24 0.10-0.63	0.22 (±0.19) 0.14 0.08-0.66	ND
PCB 44	1.26 (±0.78) 1.31 0.50-2.70	ND	ND	0.17 (±0.12) 0.12 0.04-0.41	ND

Table 4.2 (Continued)

Compound[c]	Spotted Seal Blubber	Spotted Seal Muscle	Spotted Seal Liver	Sheefish Muscle	Sheefish Liver
PCB 42	ND	0.23 (±0.28) 0.11 0.02-1.07	0.63 (±1.42) 0.09 ND-5.71	0.04 (±0.03) 0.04 0.01-0.14	ND
PCB 71-41-64	2.76 (±1.67) 2.22 1.16-7.64	ND	0.26 (±0.14) 0.28 0.12-0.57	0.16 (±0.13) 0.12 0.03-0.44	ND
PCB 100	1.16 (±0.66) 0.92 0.35-2.43	ND	ND	0.02 (±0.01) 0.01 0.003-0.04	ND
PCB 63	1.18 (±1.85) 0.27 ND-6.67	0.01 (±0.01) 0.01 ND-0.05	ND	ND	ND
PCB 74	5.22 (±1.80) 5.13 2.07-8.22	0.13 (±0.10) 0.11 0.03-0.37	0.23 (±0.14) 0.17 0.05-0.54	0.08 (±0.06) 0.06 0.02-0.20	ND
PCB 70-76-98	ND	ND	ND	0.0008 (±0.0003) 0.0009 ND-0.0009	ND
PCB 66	ND	ND	ND	0.12 (±0.08) 0.09 0.07-0.30	ND
PCB 95	2.71 (±1.19) 2.72 1.12-5.70	ND	ND	ND	ND
PCB 91	2.11 (±1.71) 1.87 0.51-7.65	ND	ND	0.08 (±0.06) 0.05 0.03-0.20	ND
PCB 56-60	1.27 (±0.60) 1.26 0.43-2.50	ND	ND	0.06 (±0.05) 0.05 0.02-0.16	ND
PCB 92	3.44 (±1.11) 3.16 1.69-5.72	0.08 (±0.04) 0.07 0.04-0.18	ND	0.08 (±0.07) 0.05 0.02-0.22	ND
PCB 101	ND	0.35 (±0.33) 0.36 ND-1.27	0.83 (±0.56) 0.86 ND-1.73	0.45 (±0.33) 0.30 0.18-1.14	0.80 (±0.48) 0.85 ND-1.68
PCB 99	26.6 (±11.1) 25.4 11.0-54.6	0.51 (±0.38) 0.41 0.09-1.40	1.04 (±0.64) 0.82 0.31-2.40	0.33 (±0.26) 0.22 0.12-0.83	0.37 (±0.25) 0.40 0.10-0.77
PCB 119	0.88 (±0.85) 0.74 0.27-4.10	ND	ND	0.02 (±0.02) 0.01 0.003-0.07	ND
PCB 97	2.58 (±1.09) 2.19 1.24-5.11	ND	ND	0.09 (±0.07) 0.06 0.02-0.25	ND
PCB 81-87	2.90 (±1.73) 2.73 ND-7.73	ND	ND	0.20 (±0.15) 0.14 0.05-0.51	ND
PCB 85	126 (±106) 125 ND-309	1.75 (±1.88) 1.47 0.13-7.47	3.12 (±3.62) 2.16 0.10-15.1	0.88 (±0.78) 0.57 0.26-2.49	0.98 (±0.55) 1.02 0.34-1.79
PCB 136	1.46 (±1.39) 0.99 ND-5.64	ND	ND	0.06 (±0.05) 0.04 0.02-0.17	ND
PCB 110	3.58 (±1.74) 3.76 ND-5.89	ND	ND	0.24 (±0.15) 0.18 0.06-0.53	ND
PCB 82	6.86 (±13.9) 3.37 0.33-61.3	0.09 (±0.08) 0.07 0.02-0.31	ND	0.22 (±0.17) 0.18 0.04-0.53	0.24 (±0.17) 0.22 0.04-0.51

Table 4.2 (Continued)

Compound[c]	Spotted Seal Blubber	Spotted Seal Muscle	Spotted Seal Liver	Sheefish Muscle	Sheefish Liver
PCB 151	2.38 (±0.96) 2.17 1.16-5.04	ND	0.16 (±0.13) 0.16 0.03-0.47	0.13 (±0.11) 0.09 0.05-0.36	0.18 (±0.11) 0.19 0.07-0.38
PCB 135-144	2.22 (±2.66) 0.96 ND-6.96	ND	ND	ND	ND
PCB 107	1.01 (±1.34) 0.57 ND-5.74	ND	ND	0.04 (±0.03) 0.04 0.004-0.11	ND
PCB 149	5.83 (±4.82) 6.53 ND-13.9	0.26 (±0.13) 0.23 0.08-0.59	0.44 (±0.29) 0.41 ND-1.20	0.28 (±0.26) 0.20 0.04-0.83	0.51 (±0.29) 0.58 ND-0.95
PCB 118	8.08 (±2.71) 8.28 3.43-14.5	0.21 (±0.14) 0.19 0.07-0.64	ND	0.24 (±0.017) 0.18 0.08-0.57	ND
PCB 114	0.22 (±0.16) 0.19 ND-0.48	ND	ND	0.009 (±0.013) 0.003 0.0009-0.04	ND
PCB 146	9.26 (±3.77) 8.18 3.90-16.4	0.15 (±0.12) 0.12 0.02-0.42	0.32 (±0.20) 0.29 0.12-0.93	0.09 (±0.08) 0.06 0.03-0.25	0.11 (±0.06) 0.12 0.04-0.19
PCB 153	66.7 (±28.0) 61.6 23.8-124	1.10 (±0.86) 0.85 0.10-3.21	2.06 (±1.37) 1.89 0.68-6.60	0.60 (±0.50) 0.42 0.21-1.66	0.65 (±0.40) 0.74 0.19-1.14
PCB 132	ND	ND	ND	0.10 (±0.09) 0.07 0.03-0.30	ND
PCB 105	4.10 (±1.32) 3.84 1.86-7.14	ND	ND	0.09 (±0.06) 0.07 0.03-0.20	ND
PCB 141	1.36 (±0.55) 1.28 ND-2.43	ND	ND	0.05 (±0.04) 0.04 0.02-0.14	ND
PCB 179	ND	ND	ND	0.03 (±0.03) 0.02 0.009-0.09	ND
PCB 137	1.65 (±0.64) 1.60 0.76-2.85	0.03 (±0.02) 0.02 0.01-0.09	0.05 (±0.04) 0.04 0.02-0.15	0.02 (±0.01) 0.01 0.008-0.05	ND
PCB 163-138	25.8 (±23.9) 24.9 ND-70.8	0.71 (±0.51) 0.56 0.14-1.78	1.59 (±0.83) 1.37 0.83-4.06	0.43 (±0.34) 0.31 0.15-1.16	ND
PCB 158	1.50 (±0.79) 1.33 0.26-3.63	ND	ND	ND	ND
PCB 129	ND	ND	ND	0.007 (±0.005) 0.007 0.002-0.02	ND
PCB 182-187	9.85 (±5.08) 8.80 4.69-24.9	0.16 (±0.12) 0.13 0.03-0.49	1.12 (±0.56) 1.02 0.37-2.19	ND	0.16 (±0.08) 0.17 0.07-0.27
PCB 183	3.93 (±1.61) 3.97 1.31-7.35	0.06 (±0.04) 0.05 0.02-0.14	0.17 (±0.12) 0.14 0.03-0.45	0.04 (±0.04) 0.03 0.01-0.12	ND
PCB 128	3.00 (±1.72) 2.61 0.93-8.45	0.05 (±0.04) 0.04 0.02-0.18	0.11 (±0.08) 0.11 0.04-0.28	0.05 (±0.04) 0.03 0.02-0.14	ND
PCB 167	0.15 (±0.09) 0.13 ND-0.30	ND	ND	0.01 (±0.02) 0.006 0.0009-0.04	ND

Table 4.2 (Continued)

Compound[c]	Spotted Seal Blubber	Spotted Seal Muscle	Spotted Seal Liver	Sheefish Muscle	Sheefish Liver
PCB 174	0.97 (±0.49) 0.88 ND-1.96	ND	ND	0.05 (±0.04) 0.04 0.01-0.13	ND
PCB 177	1.64 (±0.88) 1.35 0.74-3.78	ND	0.18 (±0.12) 0.16 0.04-0.51	ND	ND
PCB 202-171	1.44 (±0.85) 1.32 0.24-3.40	ND	ND	0.03 (±0.02) 0.02 0.009-0.07	ND
PCB 156	1.65 (±0.60) 1.68 0.59-3.00	0.02 (±0.02) 0.02 0.01-0.08	ND	0.02 (±0.01) 0.01 0.005-0.04	ND
PCB 157-200	0.44 (±0.23) 0.38 0.17-1.00	ND	ND	0.0009 ($\pm 7 \times 10^{-6}$) 0.0009 0.0009-0.0009	0.02 (±0.02) 0.02 ND-0.04
PCB 172	0.86 (±0.40) 0.77 0.24-1.73	ND	ND	0.01 (±0.01) 0.007 0.002-0.03	ND
PCB 197	ND	ND	ND	0.0009 ($\pm 7 \times 10^{-6}$) 0.0009 0.0009-0.0009	ND
PCB 180	10.2 (±4.49) 9.48 3.90-18.8	0.17 (±0.12) 0.15 0.06-0.41	0.32 (±0.24) 0.27 0.12-0.96	0.011 (±0.08) 0.08 0.05-0.28	ND
PCB 193	0.77 (±0.41) 0.68 0.22-1.79	ND	ND	0.006 (±0.008) 0.0009 0.0009-0.02	ND
PCB 191	0.25 (±0.16) 0.24 0.04-0.75	ND	ND	ND	ND
PCB 199	ND	ND	ND	0.0009 ($\pm 7 \times 10^{-6}$) 0.0009 0.0009-0.0009	ND
PCB 170-190	2.72 (±1.37) 2.48 0.53-5.69	ND	ND	0.03 (±0.01) 0.03 0.02-0.07	ND
PCB 201	1.22 (±0.68) 1.07 0.48-3.13	ND	0.09 (±0.07) 0.07 0.02-0.23	0.02 (±0.02) 0.02 0.01-0.06	ND
PCB 203-196	1.76 (±0.87) 1.64 0.43-3.57	ND	ND	0.03 (±0.02) 0.02 0.01-0.06	ND
PCB 189	ND	ND	ND	0.0009 ($\pm 7 \times 10^{-6}$) 0.0009 0.0009-0.0009	ND
PCB 208-195	0.51 (±0.27) 0.47 0.11-1.06	ND	ND	ND	ND
PCB 207	0.10 (±0.07) 0.08 ND-0.24	ND	ND	0.0009 ($\pm 7 \times 10^{-6}$) 0.0009 0.0009-0.0009	ND
PCB 194	0.85 (±0.43) 0.84 0.20-1.78	ND	ND	0.01 (±0.007) 0.009 0.005-0.03	ND
PCB 205	ND	ND	ND	0.0009 ($\pm 7 \times 10^{-6}$) 0.0009 0.0009-0.0009	ND
PCB 206	0.35 (±0.22) 0.31 0.08-0.78	ND	ND	0.003 (±0.004) 0.0009 0.0009-0.01	ND

Table 4.2 (Continued)

Compound[c]	Spotted Seal Blubber	Spotted Seal Muscle	Spotted Seal Liver	Sheefish Muscle	Sheefish Liver
PCB 209	14.6 (±16.5)	0.40 (±0.39)	0.83 (±1.02)	0.002 (±0.002)	ND
	9.48	0.40	0.62	0.0009	
	ND-50.1	ND-1.12	ND-4.03	0.0009-0.008	

[a]For the purpose of calculating summary statistics, a concentration of 1/2 MDL was assigned to samples for compounds that were detected but <MDL and a concentration of zero was assigned to compounds that were not detected. If ≥50% of samples were <MDL, no summary statistics are reported and compounds are indicated as non-detect (ND).
[b]o,p-DDE, and PCBs 6, 22, 46, 55, 83, 84, 134-131 were ND in all tissues analyzed.
[c]PCBs listed with multiple congener numbers represent co-eluting compounds.

Table 4.3 Lipid normalized organochlorine (OC) concentrations (ng/g lipid) in spotted seal (n=18) and sheefish (n=8) tissues [mean (± 1 standard deviation), median, range][a,b]

Compound[c]	Spotted Seal Blubber	Spotted Seal Muscle	Spotted Seal Liver	Sheefish Muscle	Sheefish Liver
1,3,5-Trichlorobenzene	ND	ND	ND	0.08 (±0.09) 0.05 0.01-0.23	ND
1,2,4-Trichlorobenzene	1.25 (±0.71) 1.17 ND-3.18	ND	0.84 (±0.80) 1.09 ND-2.17	4.26 (±2.16) 3.85 1.14-8.31	0.65 (±0.43) 0.66 0.17-1.35
1,2,3-Trichlorobenzene	0.65 (±1.02) 0.39 ND-4.27	ND	ND	ND	ND
1,2,4,5-Tetrachlorobenzene	ND	ND	0.70 (±0.74) 0.49 ND-2.46	0.05 (±0.05) 0.03 0.01-0.16	ND
1,2,3,4-Tetrachlorobenzene	0.50 (±0.35) 0.44 ND-1.40	ND	0.24 (±0.25) 0.23 ND-0.79	1.44 (±1.00) 1.50 0.002-2.90	0.41 (±0.17) 0.46 0.14-0.60
Pentachlorobenzene	ND	ND	ND	1.98 (±0.81) 2.01 0.40-3.29	ND
α-Hexachlorocyclohexane	15.4 (±6.3) 14.2 7.29-27.3	10.0 (±3.0) 9.72 4.88-16.6	6.20 (±5.61) 6.28 ND-17.6	3.97 (±1.58) 4.02 0.44-5.67	3.43 (±0.59) 3.50 2.53-4.24
β-Hexachlorocyclohexane	82.4 (±32.2) 77.8 24.2-164	42.9 (±17.7) 43.4 11.0-68.6	24.7 (±25.6) 20.0 ND-100	3.15 (±1.34) 3.41 0.33-4.85	2.80 (±0.85) 2.64 1.93-4.07
Hexachlorobenzene	17.9 (±16.8) 12.4 0.15-77.7	16.1 (±15.4) 12.5 7.36-76.3	10.3 (±8.5) 9.73 0.71-29.7	0.57 (±0.50) 0.54 0.01-1.35	17.9 (±3.24) 18.1 13.1-22.2
γ-Hexachlorocyclohexane	1.07 (±0.92) 1.30 ND-2.55	ND	8.52 (±13.79) 0.57 ND-42.2	0.49 (±0.22) 0.45 0.19-0.79	0.47 (±0.24) 0.44 ND-0.82
Heptachlor	ND	ND	ND	0.02 (±0.02) 0.01 0.008-0.07	ND
Heptachlor epoxide	248.3 (±16.2) 24.9 7.83-78.8	15.9 (±5.6) 15.3 6.60-24.1	23.1 (±11.7) 20.3 9.11-49.5	2.01 (±0.91) 2.07 0.07-3.04	2.01 (±1.20) 1.87 ND-4.36
γ-Chlordane	0.61 (±0.51) 0.67 ND-1.42	ND	ND	0.36 (±0.41) 0.21 0.01-1.06	1.49 (±1.35) 1.17 0.36-4.39
α-Chlordane	3.22 (±4.77) 2.01 0.21-21.9	1.09 (±1.35) 0.66 ND-5.83	1.64 (±1.55) 1.08 ND-4.50	6.89 (±3.83) 6.11 1.40-14.5	5.71 (±1.98) 5.41 2.97-9.34
Dieldrin	40.3 (±18.9) 40.5 13.2-86.4	22.6 (±8.1) 22.7 9.43-35.2	26.8 (±13.0) 24.4 8.65-59.9	4.96 (±2.00) 5.24 0.71-7.65	5.12 (±0.92) 5.06 4.17-7.13
p,p-DDE	154 (±75) 140 24.7-322	71.9 (±45.6) 52.5 26.0-179	64.3 (±71.7) 49.2 3.70-311	17.3 (±11.7) 14.5 4.98-43.1	14.7 (±10.5) 10.8 7.06-38.5
o,p-DDD	ND	ND	1.24 (±1.23) 1.04 ND-5.00	ND	ND
p,p-DDD	5.69 (±3.23) 4.49 1.76-12.9	2.76 (±1.06) 3.00 0.55-4.89	6.99 (±4.04) 5.74 3.20-19.5	6.91 (±4.23) 6.61 1.37-16.1	3.69 (±1.37) 3.45 2.15-5.67
o,p-DDT	ND	ND	ND	0.74 (±1.45) 0.05 0.01-4.22	ND

Table 4.3 (Continued)

Compound[c]	Spotted Seal Blubber	Spotted Seal Muscle	Spotted Seal Liver	Sheefish Muscle	Sheefish Liver
p,p-DDT	9.59 (±6.89) 11.5 0.13-24.4	2.49 (±2.62) 1.91 ND-8.72	ND	2.55 (±2.10) 1.83 0.38-7.31	ND
PCB 4-10	1.16 (±0.65) 0.99 0.47-2.49	ND	ND	ND	ND
PCB 7-9	0.42 (±0.22) 0.31 0.24-0.83	ND	ND	1.14 (±0.77) 1.04 0.45-2.88	ND
PCB 8-5	ND	ND	ND	0.87 (±0.81) 0.95 ND-2.05	1.83 (±2.59) 1.03 0.13-8.08
PCB 19	ND	ND	ND	2.97 (±1.45) 3.18 0.93-5.36	2.63 (±1.42) 2.52 ND-5.13
PCB 12-13	1.06 (±0.81) 0.73 0.34-3.56	3.44 (±2.90) 2.66 ND-9.09	ND	ND	ND
PCB 18	2.77 (±1.75) 2.69 ND-6.37	11.6 (±11.0) 8.91 0.66-44.9	ND	6.28 (±4.38) 4.77 1.75-12.3	ND
PCB 15-17	2.65 (±1.10) 2.48 1.29-5.70	ND	ND	3.03 (±2.27) 2.37 1.15-8.00	ND
PCB 24-27	0.14 (±0.11) 0.14 ND-0.33	ND	ND	0.57 (±0.32) 0.59 0.19-1.20	ND
PCB 16-32	1.27 (±0.71) 1.12 0.31-3.42	ND	ND	2.51 (±1.47) 2.53 0.85-5.47	ND
PCB 26	1.29 (±0.61) 1.39 ND-2.70	103 (±120) 45.9 6.01-407	ND	15.0 (±15.6) 10.8 0.97-47.7	1.12 (±1.12) 0.70 0.42-3.72
PCB 25	4.87 (±3.96) 5.36 ND-13.8	ND	3.08 (±3.31) 2.41 ND-10.7	ND	ND
PCB 31-28	4.05 (±1.17) 4.39 1.52-5.74	8.22 (±5.38) 7.02 2.08-18.3	6.20 (±2.45) 5.66 1.72-10.0	ND	7.83 (±11.85) 2.58 1.62-36.3
PCB 33-20	ND	ND	ND	2.01 (±1.31) 1.74 0.60-4.20	ND
PCB 53	ND	ND	ND	1.06 (±0.65) 0.91 0.42-2.44	ND
PCB 45	ND	ND	ND	1.27 (±0.81) 1.08 0.47-2.94	ND
PCB 52	9.47 (±2.75) 8.70 5.84-15.9	13.9 (±9.0) 9.67 3.61-31.7	10.7 (±7.4) 9.13 3.55-25.6	7.60 (±3.91) 6.65 3.43-14.2	ND
PCB 49	2.52 (±0.88) 2.31 1.44-4.55	3.55 (±2.45) 2.47 ND-8.04	ND	2.24 (±1.28) 1.91 0.99-5.03	ND
PCB 47-48	7.68 (±3.19) 7.84 1.52-16.6	9.93 (±7.47) 6.59 1.79-27.0	5.70 (±3.28) 4.90 1.24-11.8	4.24 (±2.91) 3.36 1.66-10.5	ND
PCB 44	1.43 (±0.88) 1.51 0.54-3.00	ND	ND	3.18 (±1.77) 3.06 0.71-6.49	ND

Table 4.3 (Continued)

Compound[c]	Spotted Seal Blubber	Spotted Seal Muscle	Spotted Seal Liver	Sheefish Muscle	Sheefish Liver
PCB 42	ND	15.6 (±19.7) 4.50 0.25-63.2	10.9 (±21.4) 1.82 ND-73.2	0.82 (±0.45) 0.85 0.23-1.55	ND
PCB 71-41-64	3.13 (±1.89) 2.46 1.44-8.47	ND	5.07 (±2.52) 4.71 2.36-9.32	3.07 (±1.85) 2.62 1.27-7.02	ND
PCB 100	1.32 (±0.76) 1.03 0.43-2.87	ND	ND	0.28 (±0.19) 0.23 0.11-0.70	ND
PCB 63	1.33 (±2.07) 0.30 ND-7.39	0.54 (±0.48) 0.48 ND-1.70	ND	ND	ND
PCB 74	5.93 (±2.12) 5.69 2.57-9.66	5.85 (±3.52) 6.12 1.62-13.3	4.46 (±2.54) 3.92 0.96-10.3	1.56 (±0.75) 1.42 0.77-3.12	ND
PCB 70-76-98	ND	ND	ND	0.02 (±0.02) 0.01 ND-0.07	ND
PCB 66	ND	ND	ND	2.65 (±1.60) 2.17 1.09-5.24	ND
PCB 95	3.07 (±1.39) 3.13 1.22-6.84	ND	ND	ND	ND
PCB 91	2.41 (±2.04) 2.06 0.57-9.19	ND	ND	1.60 (±0.92) 1.30 0.60-3.23	ND
PCB 56-60	1.43 (±0.68) 1.41 0.47-2.77	ND	ND	1.23 (±0.68) 1.05 0.30-2.47	ND
PCB 92	3.91 (±1.31) 3.44 1.87-6.87	3.84 (±1.81) 3.80 0.66-7.61	ND	1.53 (±0.86) 1.35 0.72-3.48	ND
PCB 101	ND	16.1 (±13.2) 15.7 ND-37.7	16.2 (±10.4) 16.9 ND-29.3	8.93 (±4.84) 7.10 4.26-18.1	13.6 (±13.9) 8.86 ND-45.5
PCB 99	30.2 (±13.1) 28.4 13.6-65.6	23.1 (±16.3) 19.6 6.26-74.1	19.8 (±11.4) 17.7 6.39-46.0	6.37 (±3.50) 5.92 2.53-13.2	5.88 (±6.31) 4.07 1.89-20.7
PCB 119	1.01 (±1.02) 0.80 0.31-4.92	ND	ND	0.42 (±0.32) 0.37 0.06-1.08	ND
PCB 97	2.93 (±1.28) 2.45 1.43-6.14	ND	ND	1.71 (±0.99) 1.52 0.77-3.91	ND
PCB 81-87	3.28 (±1.94) 3.07 ND-8.57	ND	ND	3.73 (±2.00) 3.26 1.79-8.09	ND
PCB 85	143 (±122) 140 ND-363	86.2 (±103.2) 39.5 8.49-415	62.3 (±70.9) 44.2 2.21-290	16.3 (±10.5) 14.3 4.75-39.5	14.7 (±10.3) 10.4 6.51-37.7
PCB 136	1.67 (±1.64) 1.15 ND-6.77	ND	ND	1.19 (±0.70) 1.04 0.53-2.77	ND
PCB 110	4.06 (±1.99) 4.21 ND-6.90	ND	ND	4.48 (±1.94) 4.46 2.41-8.37	ND
PCB 82	7.98 (±16.68) 4.21 0.37-73.6	4.07 (±4.23) 2.63 1.17-19.6	ND	4.12 (±2.36) 3.66 0.73-8.47	3.17 (±2.06) 3.28 0.85-7.30

Table 4.3 (Continued)

Compound[c]	Spotted Seal Blubber	Spotted Seal Muscle	Spotted Seal Liver	Sheefish Muscle	Sheefish Liver
PCB 151	2.70 (±1.14) 2.48 1.44-6.05	ND	3.09 (±2.34) 3.45 0.56-8.97	2.51 (±1.52) 2.08 0.97-5.72	3.10 (±3.07) 1.81 1.35-10.3
PCB 135-144	2.51 (±3.01) 1.09 ND-7.98	ND	ND	ND	ND
PCB 107	1.16 (±1.60) 0.66 ND-6.89	ND	ND	0.99 (±0.65) 0.95 0.06-2.17	ND
PCB 149	6.52 (±5.42) 7.31 ND-16.3	12.3 (±6.0) 11.2 3.87-25.5	8.45 (±5.63) 8.78 ND-22.9	4.90 (±3.55) 4.24 1.74-13.2	8.28 (±7.67) 5.92 ND-25.6
PCB 118	9.16 (±3.17) 9.14 4.27-17.0	10.1 (±6.2) 7.39 1.27-23.0	ND	4.55 (±2.19) 4.21 2.08-9.03	ND
PCB 114	0.25 (±0.18) 0.12 ND-0.54	ND	ND	0.14 (±0.19) 0.08 0.01-0.59	ND
PCB 146	10.5 (±4.3) 9.34 4.48-19.3	6.74 (±5.38) 5.60 1.46-23.2	6.10 (±3.65) 5.27 2.46-17.7	1.70 (±1.04) 1.50 0.62-3.96	1.73 (±1.42) 1.26 0.82-5.05
PCB 153	75.5 (±34.0) 70.2 27.3-145	49.2 (±39.6) 42.5 9.71-178	39.8 (±25.5) 37.3 14.2-127	11.4 (±7.0) 9.73 4.22-26.3	9.89 (±8.76) 7.88 3.64-29.9
PCB 132	ND	ND	ND	1.83 (±1.28) 1.24 0.97-4.75	ND
PCB 105	4.66 (±1.58) 4.30 2.06-8.57	ND	ND	1.73 (±0.82) 1.44 0.64-3.16	ND
PCB 141	1.54 (±0.62) 1.47 ND-2.69	ND	ND	1.04 (±0.62) 0.89 0.44-2.23	ND
PCB 179	ND	ND	ND	0.59 (±0.39) 0.49 0.16-1.45	ND
PCB 137	1.88 (±0.75) 1.82 0.85-3.45	1.31 (±0.99) 0.99 0.17-4.50	0.95 (±0.66) 0.90 0.37-2.95	0.36 (±0.21) 0.30 0.13-0.72	ND
PCB 163-138	29.3 (±27.5) 28.7 ND-85.0	32.5 (±21.8) 28.7 8.75-99.0	30.7 (±15.1) 26.2 16.3-17.8	8.37 (±4.83) 7.11 3.13-18.4	ND
PCB 158	1.70 (±0.91) 1.47 0.33-4.03	ND	ND	ND	ND
PCB 129	ND	ND	ND	0.15 (±0.08) 0.13 0.07-0.30	ND
PCB 182-187	11.1 (±5.7) 9.86 5.39-27.6	7.30 (±4.13) 6.16 1.63-16.3	21.6 (±9.8) 18.8 8.70-40.1	ND	2.48 (±2.02) 1.73 1.29-7.24
PCB 183	4.45 (±1.81) 4.45 1.50-8.15	2.62 (±1.81) 2.08 0.64-7.95	3.29 (±2.02) 3.08 0.64-8.63	0.77 (±0.48) 0.66 0.36-1.88	ND
PCB 128	3.41 (±2.05) 2.97 1.12-10.1	2.20 (±0.99) 1.92 0.33-4.13	2.14 (±1.44) 1.81 0.72-5.32	1.03 (±0.67) 0.81 0.41-2.25	ND
PCB 167	0.17 (±0.10) 0.15 ND-0.36	ND	ND	0.21 (±0.22) 0.14 0.01-0.60	ND

Table 4.3 (Continued)

Compound[c]	Spotted Seal Blubber	Spotted Seal Muscle	Spotted Seal Liver	Sheefish Muscle	Sheefish Liver
PCB 174	1.09 (±0.55) 0.97 ND-2.17	ND	ND	0.95 (±0.53) 0.85 0.45-2.08	ND
PCB 177	1.87 (±1.02) 1.49 0.88-4.53	ND	3.52 (±2.13) 3.17 0.83-9.60	ND	ND
PCB 202-171	1.63 (±0.96) 1.50 0.27-3.77	ND	ND	0.52 (±0.30) 0.43 0.28-1.17	ND
PCB 156	1.87 (±0.68) 1.90 0.69-3.53	1.12 (±0.70) 0.89 0.17-3.24	ND	0.33 (±0.19) 0.30 0.09-0.67	ND
PCB 157-200	0.50 (±0.26) 0.42 0.20-1.11	ND	ND	0.02 (±0.02) 0.01 0.008-0.07	0.38 (±0.37) 0.21 ND-0.87
PCB 172	0.97 (±0.45) 0.85 0.28-1.92	ND	ND	0.19 (±0.13) 0.17 0.04-0.47	ND
PCB 197	ND	ND	ND	0.02 (±0.02) 0.01 0.008-0.07	ND
PCB 180	11.6 (±5.0) 10.6 4.47-22.1	7.99 (±5.47) 5.64 1.05-22.0	6.17 (±4.29) 5.26 2.29-18.4	2.14 (±1.27) 1.87 0.80-4.43	ND
PCB 193	0.88 (±0.46) 0.75 0.26-1.98	ND	ND	0.09 (±0.12) 0.05 0.01-0.38	ND
PCB 191	0.29 (±0.19) 0.27 0.05-0.90	ND	ND	ND	ND
PCB 199	ND	ND	ND	0.02 (±0.02) 0.01 0.008-0.07	ND
PCB 170-190	3.08 (±1.56) 2.71 0.61-6.69	ND	ND	0.71 (±0.35) 0.57 0.37-1.41	ND
PCB 201	1.38 (±0.75) 1.18 0.55-3.47	ND	1.80 (±1.13) 1.41 0.42-3.79	0.52 (±0.29) 0.44 0.22-0.99	ND
PCB 203-196	1.99 (±0.97) 1.85 0.50-3.96	ND	ND	0.50 (±0.28) 0.43 0.22-0.98	ND
PCB 189	ND	ND	ND	0.02 (±0.02) 0.01 0.008-0.07	ND
PCB 208-195	0.57 (±0.30) 0.52 0.13-1.18	ND	ND	ND	ND
PCB 207	0.11 (±0.08) 0.09 ND-0.27	ND	ND	0.02 (±0.02) 0.01 0.008-0.07	ND
PCB 194	0.96 (±0.49) 0.93 0.23-2.00	ND	ND	0.24 (±0.13) 0.21 0.10-0.43	ND
PCB 205	ND	ND	ND	0.02 (±0.02) 0.01 0.008-0.07	ND
PCB 206	0.39 (±0.25) 0.35 0.09-0.92	ND	ND	0.05 (±0.07) 0.03 0.01-0.21	ND

Table 4.3 (Continued)

Compound[c]	Spotted Seal Blubber	Spotted Seal Muscle	Spotted Seal Liver	Sheefish Muscle	Sheefish Liver
PCB 209	16.4 (±18.5)	22.0 (±23.7)	16.3 (±19.4)	0.04 (±0.04)	ND
	10.8	18.5	13.4	0.02	
	ND-58.9	ND-68.3	ND-77.3	0.008-0.12	

[a]For the purpose of calculating summary statistics, a concentration of 1/2MDL was assigned to samples for compounds that were detected but <MDL and a concentration of zero was assigned to compounds that were not detected. If ≥50% of samples were <MDL, no summary statistics are reported and compounds are indicated as non-detect (ND).
[b]o,p-DDE, and PCBs 6, 22, 46, 55, 83, 84, 134-131 were ND in all tissues analyzed.
[c]PCBs listed with multiple congener numbers represent co-eluting compounds.

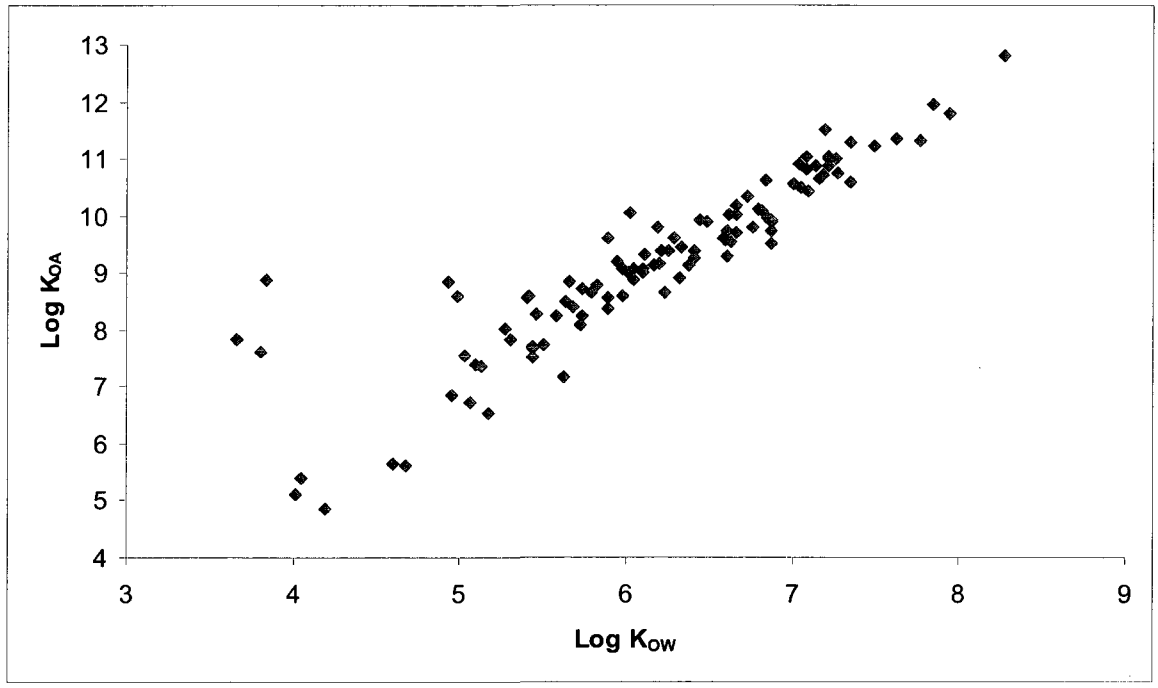

Figure 4.1. Relationship between K_{OW} and K_{OA} for organochlorines (OCs) quantified in spotted seal and sheefish tissues.

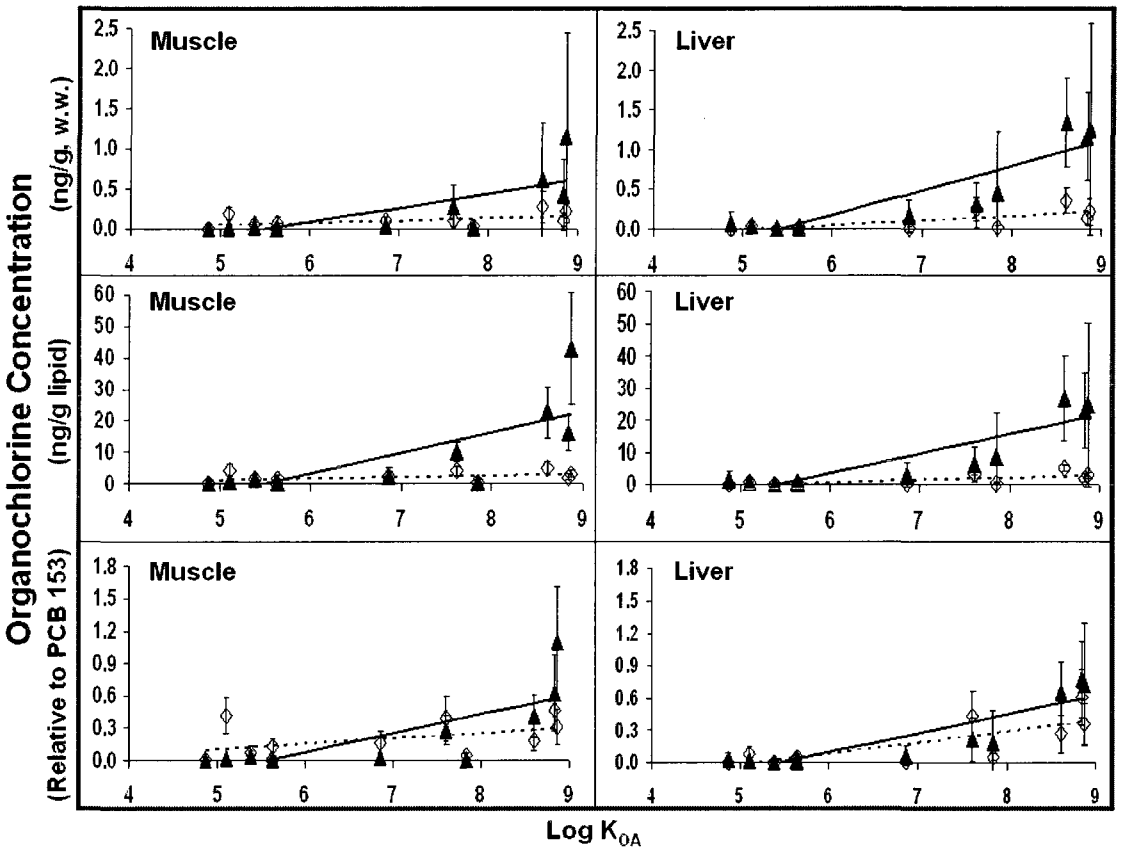

Figure 4.2. Organochlorine concentration (wet weight, lipid weight and relative to PCB 153) versus log K_{OA} in sheefish (◊, dashed line) and spotted seal (▲, solid line) tissues. Data represent geometric means ± 1 SD. The slope of the regression line is significantly >0 ($p<0.05$) in all cases for seal muscle and liver and sheefish liver.

CHAPTER 5

Blubber fatty acids in three pagophilic phocid seals of northwest Alaska: Stratification and potential applications[5]

5.1 ABSTRACT

To optimally utilize fatty acid (FA) concentrations and profiles as research and monitoring tools, variation in FA profiles within and among individuals and species must be characterized. We quantified 70 FA at three blubber depths from two body locations in ringed (*Phoca hispida*), spotted (*Phoca largha*) and bearded (*Erignathus barbatus*) seals. The three species had distinct FA signatures and exhibited a typical marine FA pattern, with high concentrations of monounsaturated FA (MUFA) and n-3 polyunsaturated FA (PUFA). No significant difference in FA composition was found between body locations, but significant variation by blubber depth was present. Stratification was assessed with regard to FA carbon chain length and number of double bonds to gain insight into potential drivers of FA variation. In general, short-chain MUFA (SCMUFA) concentrations were highest in the outer layer while saturated FA (SFA) and PUFA were higher in the inner layer. Long-chain FA (LCFA) decreased from the inner to the outer blubber layer. These FA stratification patterns are likely driven by effects of the steep temperature gradient in the blubber, with the exception of PUFA. Increased PUFA inner blubber layer may be a result of recent deposition from dietary

[5] Moses, S.K., J.M. Kennish, A.V. Whiting,T.M. O'Hara. Blubber fatty acids in three pagophilic phocid seals of northwest Alaska: Stratification and potential applications. Prepared for submission to Marine Mammal Science.

intake or preferential maintenance of certain FA in a region where they can be readily mobilized for energetic or other physiological requirements. The pagophilic ("*ice-loving*") phocid seals feed atop an ice-associated food web, making them useful indicators of the effects of climate change on polar ecosystems, such as shifts in food web community structure. Variations in the composition of blubber FA due to changing prey species abundance or physiological requirements could impact seal health if, as a result, they become nutritionally compromised or are unable to meet non-energetic FA demands. Further, the nutritional quality (i.e., essential FA) of blubber for seal consumers, such as humans and polar bears, could be impacted. Thus, the use of FA signature analysis (FASA) to monitor ice seal health could be a powerful tool for detecting the effects of climate change or other environmental stressors on arctic ecosystems.

KEY WORDS

blubber, fatty acids, stratification, phocids, ringed seal, spotted seal, bearded seal, climate change, Arctic

5.2 INTRODUCTION

Marine mammal blubber is a dynamic tissue that serves a multitude of physiological and anatomical roles. These functions include energy storage, thermoregulation, buoyancy, locomotion and body streamlining, with lipid composition varying among blubber regions that likely serve these different functions. Blubber contains, by far, the greatest mass of fatty acids (FA) within the body and serves as the main storage reserve in marine mammals. These FA can be mobilized from blubber for inclusion in cell membranes, within which they are critical for proper function and fluidity, and to serve as precursors to other important FA and biomolecules.

FA signature analysis (FASA) has provided researchers with a relatively non-invasive means with which to study marine mammal feeding ecology and health. Marine FA have a wide diversity of structures, originating in the food web through synthesis by various unicellular plankton and seaweed (Ackman 1980). Specific FA have been shown to be transferred with minimal modification through the marine food web from prey to predator (Iverson *et al.* 2004, 2007, St. John and Lund 1996). Thus, FASA has the potential to provide information on the contribution of specific prey items to the diet and illustrates the essential role of primary production in critical FA availability and ecosystem health. Since FA are of critical nutritional and physiological importance, FASA provides information on food quality and health status for the animal in which FA are measured, as well as natural predators of those species.

Differences in FA profiles among individuals, species and populations are most often explained as a direct function of the variation in FA composition of prey items. But, blubber FA variation has also been described in certain marine mammal species by sex, age, body condition (i.e., fasting), reproductive state, population, region, season and year and may be independent of diet (Andersen *et al.* 2004, Beck *et al.* 2005, 2007, Budge *et al.* 2008a, Krahn *et al.* 2008, Samuel and Worthy 2004). These differences can be explained in part by differing dietary habits among groups (e.g., Thiemann *et al.* 2007). In monogastric predators such as phocids, the greatest contribution to lipid stores is direct deposition of unmodified dietary FA, although endogenously derived FA from *de novo* synthesis as well as modified dietary FA will also be present (Budge *et al.* 2006). Due to biochemical restrictions on the types of FA that can be synthesized and the way in which dietary FA can be modified, it is possible to recognize those FA in an organism that were derived from particular prey items, thus gaining information on trophic relationships among species.

Blubber FA composition is not simply a uniform sum of dietary and endogenous FA. The FA composition of marine mammal blubber has been found to be stratified vertically from the inner layer, adjacent to the muscle, to the most outer layer. The presence of such stratification implies that blubber composition is not only a reflection of diet, but of other processes of this tissue as well. Although this stratification is generally more pronounced in cetaceans, FA variation with blubber depth has been recorded in a number of pinniped species (e.g., Best *et al.* 2003, Käkelä 1993). This stratification is most often

explained in terms of the functional demands of the tissue and the deposition and mobilization of dietary FA. FA melting point increases with increasing carbon chain length and decreasing degree of unsaturation. Thus, maintaining a particular arrangement of FA throughout likely enhances membrane fluidity and decreases heat loss in the outer-blubber, which lies adjacent to the cold arctic environment and is maintained at much lower temperatures than the body core (Pond 1998, Irving and Hart 1957). Käkelä and Hyvärinen (1996) suggested that FA stratification improves the insulative properties of blubber and is an evolutionary adaptation to cold tissue temperatures.

FA stratification is further influenced by FA deposition into and remobilization from blubber, which serves as the major energy reserve tissue in marine mammals. In phocid seals, lipid metabolism can account for >90% of energetic demands (Castellini *et al.* 1987). In these species, the blubber layer appears to be a continuous subcutaneous adipose layer from which lipid mobilization occurs uniformly across the body (Nordy and Blix 1985, Slip *et al.* 1992). The inner blubber layer, adjacent to the muscle, is the most active in terms of lipid deposition and mobilization and this layer is believed to have a higher rate of component turnover than the outer layers (Koopman *et al.*1996). It is here within the blubber that dietary FA are initially deposited and then later mobilized (Ackman *et al.* 1975, Lockyer *et al.* 1984, Koopman *et al.* 2002). Seals are known to selectively mobilize specific FA from blubber stores, particularly during periods of negative energy balance, such as during lactation or fasting (Iverson *et al.* 1995, Wheatley *et al.* 2008). Certain FA are important in physiological regulation, serving as

precursors in the synthesis of other molecules and critical components of cell membranes (Dalsgaard *et al.* 2003). For example, n-3 and n-6 PUFA are essential FA, required in the diet because they cannot be synthesized *de novo* by mammals. They are important in structural growth as well as brain and cell development (Innis 2005), and thus especially critical to the fetus, neonate and associated dam. Such FA may be preferentially stored in the inner blubber region so that they are readily available for these non-energetic functions as well.

The outer blubber layer is more stable in composition over time and has only a minor role as an energy reserve or site of essential FA deposition and mobilization, functioning primarily as a structural and thermoregulatory tissue (Aguilar and Borrell 1990). The middle blubber layer is the main lipid storage site, expanding and contracting as the animal shifts between positive and negative energy balance (Strandberg *et al.* 2008).

Dietary information gained from FA analyses provides knowledge of food web structure and shifts in the importance and abundance of various prey species across space and time, a critical step in understanding ecosystem structure and change over time. Therefore, baseline FA signatures must be established for a variety of potential indicator species of arctic marine mammals, such as the pagophilic phocid seals, to understand their diet and monitor dietary and ecosystem change. Variation in FA signatures within and among individuals and species must be characterized before this information can be applied. In addition, the influence of variables other than diet, such as biotransformation and

thermoregulatory constraints, on FA composition and stratification of blubber must be better understood to allow appropriate data interpretation.

Information on trophic relationships in marine food webs is essential for understanding flow of energy, nutrients and contaminants within these ecosystems. Top predators play a crucial role in determining both the structure and functioning of marine ecosystems (Katona and Whitehead 1988, Estes 1995, Bowen 1997). Knowledge of the foraging ecology of these species can provide integrated information on food web structure at lower trophic levels. Shifts in pinniped diets may serve as indicators of the relative abundance and distribution of certain prey species (Sinclair *et al.* 1994). In addition, because many arctic marine predators are long-lived with broad geographic distributions, they can be utilized to gain insight into ecosystem processes and structure over large temporal and spatial scales.

Defining and monitoring shifts in trophic relationships and ecosystem functioning is particularly important in the Arctic. This region is undergoing rapid environmental change (Comiso 2002, Rigor and Wallace 2004, Serreze *et al.* 2000). Changes in food web structure as a result of climate change have already been documented. For example, Gaston *et al.* (2003) linked changes in forage fish populations, inferred from changes in the diet composition of thick-billed murres (*Uria lomvia*), with warming trends in Northern Hudson Bay. Evidence suggests that changes in prey distribution in the Canadian Arctic have caused shifts in the diets of polar bears (*Ursus maritimus*) (Iverson

et al. 2006). In the Bering Sea, increased air and water temperatures are causing a reduction in sea ice cover and a general shift from arctic to subarctic conditions. Subsequently, there has been a northward progression of the pelagic-dominated ecosystem of the southern Bering Sea into more northern regions (Overland and Stabeno 2004, Grebmeier *et al.* 2006, Mueter and Litzow 2008). Such changes are expected to persist across the Arctic leading to changes in food availability for marine mammals (Bluhm and Gradinger 2008). Monitoring dietary shifts in species, such as ice seals, may aid in detecting the effects of climate change on arctic ecosystems.

Few studies exist that characterize the blubber FA composition of ice seals in Alaska. Budge *et al.* (2007) looked at FA in the blubber of Alaskan bearded seals, focusing on non-methylene interrupted (NMI) FA as biomarkers of benthic invertebrate prey items. West *et al.* (1979) measured FA in the blubber of ringed, spotted, bearded and ribbon (*Phoca fasciata*) seals from the Bering Sea, providing a basic foundation for this region. But, taking into account the known shifts in this ecosystem in the last three decades and the major advances in FA quantification and identification techniques during this time period, the results of this study cannot be assumed to be representative of the current status of these species. In addition, because the animals were collected over several seasons and a large geographic area, the data contains seasonal and spatial variation that cannot be accounted for with the small sample size of the study ($n = 2$ of each species). Cooper *et al.* (2009) looked more recently at the blubber FA signatures of these same four phocid seals, collecting samples from a larger number of animals which were

harvested in a single location (Little Diomede, Alaska) and during the same season. None of these studies attempted to characterize FA stratification throughout the blubber depth, although such stratification has been detected in the blubber of ringed seals from Scandanavia (Käkelä and Hyvärinen 1996, Strandberg *et al.* 2008) and Lake Baikal, Russia (Grahl-Nielsen *et al.* 2005). To date, blubber FA stratification has not been documented in spotted or bearded seals.

The current study quantifies FA at three blubber depths in ringed, bearded and spotted seals at two locations along the ventral midline, for a total of six blubber samples per animal. It represents only the second study of FA in these species in Alaska in recent decades. It adds to the existing literature by examining these species in a different location (Kotzebue, Alaska) and includes measures of FA concentration on blubber strata. Because the three seal species were collected at the same time of year (mid-late October) and at the same location, we have reduced seasonal and geographic variation. These data provide a baseline based on which future shifts in the marine food webs of Alaska and this arctic ecosystem could be based and aids in defining the need for standardized sampling in these species. Such information is essential for effectively monitoring ecosystem and population health.

The main objectives of the current study were to (1) expand the limited baseline data set of FA concentrations and profiles in the blubber of three Alaskan ice seals, (2) characterize the variation in FA with blubber depth (strata) and body location in these

species, and (3) investigate the role of certain chemical properties of FA (i.e., carbon chain length and number of double bonds) in determining the location of FA within stratified blubber.

5.3 METHODS

5.3.1 Sample Collection

Full thickness blubber samples were collected from subsistence harvested ringed (n = 11), spotted (n = 7) and bearded (n = 4) seals taken in October 2006 and 2007 by subsistence hunters in Kotzebue, Alaska (66.90°N, 162.59°W). Samples were collected under Marine Mammal Health and Stranding Response Program (MMHSRP) permit #932-1489-07. All animals were assessed for gross general health prior to sampling to allow for data interpretation in the context of animal condition and were deemed to be in excellent physical condition.

Animal harvest date, sex, age and morphometric information appear in Table 5.1. Seal length was measured as the straight line distance from the tip of the nose to the tip of the tail. Age was estimated by counting annual growth layers in the cementum of canine teeth as described in Moses *et al.* (2009a, b). Ages were read in triplicate by each of three independent readers and the median age is reported.

Large (≥10x10cm) blubber samples extending through the full depth of the adipose layer were taken along the ventral mid-line, one at the umbilical and one at the axillary position. Whole samples were placed in a polyethylene bag and immediately frozen at -20°C. They were then shipped to the University of Alaska Fairbanks Wildlife Toxicology Laboratory (UAF WTL) and stored at -80°C until further processing.

In the laboratory, a full-thickness blubber section (~0.5g) was removed from the center of each large blubber sample, where minimal oxidative degradation is likely to occur during frozen storage (Budge *et al.* 2006). The blubber section was then divided into three pieces of equal thickness representing the outer, central and inner portions of the entire blubber depth. Each sample was then placed in chloroform containing 0.01% butylated hydroxy-toluene (BHT) to prevent oxidation of the FA constituents.

5.3.2 Fatty Acid Analysis

FA were extracted from the blubber subsamples and derivatized to form FA methyl esters (FAME) at the UAF WTL. Lipid was extracted from each sample using a modified Folch reaction (Folch *et al.* 1957) and FA derivatized into FAME as described in Beck *et al.* (2007). Extracts were then shipped to the University of Alaska Anchorage Applied Science, Engineering and Technology Laboratory (UAA ASET) for detection and quantification. FAME were analyzed for 70 FA using gas chromatography with flame ionization detection (GC FID) as described in Dodds *et al.* (2004). Specific FA were

identified using known standard mixtures (Supelco 37 FAME Standard, Nu-check Prep) with a QA/QC tolerance of ± 20%.

Individual FA are reported as percent weight of total FA and are designated using the common shorthand nomenclature A:Bn-x, where A represents the number of carbon atoms, B the number of double bonds, and x the position of the double bond closest to the terminal methyl group. FA concentrations are not reported, but blubber in these species can reasonably be assumed to be >90% lipid.

5.3.3 Statistics

All statistical comparisons among univariate variables were computed using Friedman's test ($p<0.05$), a non-parametric alternative to ANOVA. Friedman's is a rank-based, repeated-measures test designed to compare three or more matched groups. This technique was utilized for detecting differences in individual FA and FA groups (SFA, MUFA, PUFA, *etc.*) among blubber layers within a species using SAS statistical software (version 9.1).

The double bond index (DBI), a measure of the mean number of unsaturations per FA, and the degree of unsaturation were also calculated and compared among blubber layers (Friedman test, $p<0.05$). DBI was defined as Σ [(FA_i proportion of total FA) x (# of double bonds in FA_i)]. Degree of unsaturation was defined as the ratio of

saturated:unsaturated FA. A similar analysis was carried out to determine the mean number of carbon atoms in the FA carbon chain in each blubber layer.

FA profiles from the axillary and umbilical sampling locations within a species were compared using multivariate two-group permutation, a non-parametric alternative to Hotelling's test, which utilizes the Mahalanobis squared distance measure. This analysis, which tests the equivalence of the means of two sets of multivariate data, was run with 5000 permutations using PAST statistical software (version 1.93). Comparisons were made separately for outer, central and inner blubber layers to eliminate any layer/site interactions that might be present.

Comparisons of FA signatures among species and among blubber layers within a species were made by subjecting data to multivariate principal components analysis (PCA) using PAST statistical software (version 1.93). Because no difference was detected in the blubber FA profiles between the two body locations (axillary and umbilical), analysis of FA stratification throughout the blubber column was carried out for the axillary sampling location only.

PCA has a number of assumptions that must be met prior to analysis. In order to meet the assumption of homogenous covariance matrices, the number of FA included in the PCA was reduced to ensure the analysis included more samples (*i.e.*, individual seals) in each group than the number of variables (*i.e.*, FA). The subset of FA selected were those

that were both present above trace levels (>0.5%) and exhibited the greatest variance (%RSD) within the samples. Further, to meet the assumption of multivariate normal data, proportional FA data was transformed according to the equation $x_{trans} = \ln(x_i/c_r)$, where x_i is the proportion of total FA of the FA being transformed and c_r is the proportion of total FA of the reference FA, 18:0 (Budge *et al.* 2006, Aitchison 1986).

Due to the small sample size for bearded seals ($n = 4$), this species was not subjected to the full range of statistical analyses that were carried out for ringed and spotted seals. Stratification of FA was not assessed for this species, although FA proportions are reported by strata. A basic comparison between species is also included.

5.4 RESULTS

5.4.1 Variation Among Species

Of the 70 individual FA measured in the blubber of ringed, spotted and bearded seals, only 60 were routinely identified above the quantification limit of the analytical technique (0.02%). Of these, 23 FA were consistently present above trace levels (0.5% of total FA) in one or more of the three seal species, with 16 FA above trace levels in all three species. The relative proportions (% of total lipid) of the 23 FA are shown in Table 5.2. All three species displayed a typical marine pattern of high monounsaturated FA (MUFA) and polyunsaturated FA (PUFA), particularly the long-chain n-3 PUFA 20:5n-3, 22:5n-3 and 22:6n-3.

To assess variation in FA profiles among species, PCA of the entire data set (22 total individuals from 3 species x 2 body locations x 3 blubber strata) was carried out on the basis of the 23 FA present above trace levels (0.5%) among the blubber samples (Table 5.2). Distinct differences were detected between the fatty acid profiles of the three species (Figure 5.1). PC 1 and PC 2 explained 69.6% and 9.9% of the variation in the data, respectively, or a combined total of 80% of the variation overall. The FA with the highest loadings on PC 1 were 20:1n-7, 18:1n-7, 20:5n-3 and 20:4n-6 and on PC 2 were 20:1n-7, 18:1n-9, 14:1n-5 and 20:1n-11. Samples taken from bearded seals were completely separated from those of ringed and spotted seals, which showed some degree of overlap. Species groupings were apparent despite the fact that data from multiple sampling locations and blubber depths were included in the analysis, indicating that species has a larger affect on FA profiles than either the body location or blubber depth from which the samples were collected.

In each species, the full-thickness blubber FA composition (i.e., the mean FA proportion of all three blubber depths) was dominated by eight FA present >5%, accounting for 77%, 67% and 76% of the total lipid composition in ringed, spotted and bearded seals, respectively. Six of these FA were above 5% in all three species (16:0, 16:1n-7, 18.1n-9, 20:5n-3, 22:5n-3, 22:6n-3), as well as 18:1n-7 in ringed and bearded seals and 20:1n-9 in spotted seals.

The blubber of all three species was richest in MUFA, followed by PUFA and lowest in saturated FA (SFA; Figure 5.2). Ringed seals had a significantly higher proportion of both SFA and PUFA than both spotted and bearded seals. MUFA were significantly higher in spotted than ringed seals.

Ringed seals had the greatest proportion of PUFA overall, specifically accounted for by higher levels of the n-3 PUFA 22:5n-3 and 22:6n-3. Similar to the findings of Cooper *et al.* (2009), ringed seals also had higher levels of 20:5n-3 and 16:1n-7 and lower levels of 18:1n-9, 20:1n-11, and 20:1n-9 than spotted seals, although levels were similar to those found in bearded seals. Spotted seals had relatively high levels of 18:1n-11, 18:1n-9, 20:1n-11 and 20:1n-9 relative to ringed and bearded seals and lower levels of 16:1n-7, 20:5n-3, and LCMUFA overall. Bearded seals generally had higher n-7 MUFA (18:1n-7, 20:1n-7) as well as 20:4n-6 and lower levels of 22:1n-11.

5.4.2 Variation within Individuals: Body Location

Two-group multivariate permutation revealed no significant difference in FA profiles between the axillary and umbilical sites in any of the three seal species. P-values ranged from 0.64-0.99. Since the FA profiles from the two sampling locations were not statistically different, investigations of FA stratification throughout the three blubber depths was carried out for the axillary location only.

5.4.3 Variation within Species: FA Profile by Blubber Depth

Due to the low sample size for bearded seals ($n = 4$), we did not analyze for FA stratification in the blubber of this species. PCA revealed clear evidence of stratification of FA profiles throughout the blubber depth in both ringed and spotted seals (Figure 5.3). In both species, the outer and inner layers were discretely separated into distinct groups when the first two PC were plotted. The central blubber was intermediate to and overlapped to some degree with both the inner and outer blubber samples. The separation between the three layers was more distinct in spotted seals than ringed seals despite the smaller sample size for spotted seals, suggesting a greater degree of blubber FA stratification in this species. PC 1 and PC 2 accounted for a total of 91% (76.1% and 14.9%, respectively) of the variation in the data for ringed seals and 95% (75.0% and 19.7%, respectively) for spotted seals.

Friedman's test, a non-parametric alternative to ANOVA, was utilized to test for significant differences in individual FA among the three blubber depths (Figure 5.4). Significant stratification was observed for 11 FA within the blubber of ringed seals. Nine of these were present above trace levels in all three strata, with three SCMUFA (14:1n-5, 18:1n-9, 18:1n-7) and one PUFA (22:5n-3) increasing from the inner to outer blubber layer and three SFA (14:0, 16:0, 18:0), one LCMUFA (20:1n-9) and one PUFA (18:4n-3) increasing from the outer to the inner layer. The FA showing the greatest percent variation between the inner and outer layers were 20:1n-9 (83% higher for the inner

layer), 14:1n-5 (54% higher for the outer layer), 16:0 (50% higher for the inner), and 18:4n-3 (44% higher for the inner layer).

Significant stratification was also observed for 11 FA throughout the blubber of spotted seals. Ten of these were present above trace levels, including three MUFA (18:1n-11, 18:1n-9, 20:1n-11) that increased from the inner to the outer blubber and two SFA (14:0, 16:0), one LCMUFA (22:1n-11) and four PUFA (18:4n-3, 20:5n-3, 22:5n-3, 22:6n-3) that increased from the outer to the inner blubber region. The FA showing the greatest degree of stratification between the inner and outer blubber layers were 18:1n-11 (108% higher for the outer layer), 22:1n-11 (94% higher in the inner layer) and 20:1n-11 (75% higher for the outer).

5.4.4 Variation within Species: Degree of Unsaturation by Blubber Depth

FA were placed into ten groups according to their number of unsaturations (SFA, MUFA, SCMUFA, LCMUFA, PUFA and 2-6 unsaturations). Freidman's test ($p<0.05$) was then used to determine whether these saturation groups differed by blubber depth. FA proportions of groups showing stratification are shown in Figure 5.5. Four groups, SFA, SCMUFA and LCMUFA and CX:5 FA (i.e., those FA with 5 double bonds) showed statistically significant stratification in ringed seals. SFA and LCMUFA increased from the outer toward the inner blubber while CX:5 and SCMUFA increased from the inner toward the outer layers. Spotted seals exhibited significant stratification in all of the saturation groups, with the exception of the CX:3 FA and LCMUFA. MUFA, SCMUFA

and CX:2 FA were highest in the outer blubber layer while SFA, PUFA, CX:4, CX:5 and CX:6 FA were highest in the inner blubber layer. Thus, SFA and SCMUFA show a similar statistically significant pattern in both ringed and spotted seals (Figure 5.5).

The double bond index (DBI), a measure of the mean number of unsaturations per FA in a given sample, was also calculated and compared across the three blubber layers in ringed and spotted seals. DBI increased significantly in spotted seals from the outer to the inner blubber layer, but no significant trend was detected in ringed seals. Although DBI was stratified in spotted seals, it increased only from 2.5 to 2.6 double bonds pre FA. Similarly, the ratio of DBI:carbon chain length in ringed seals was also significantly stratified, but increased only from 0.13 to 0.14 from the inner to outer blubber layer. The degree of unsaturation (% SFA:UFA) did however vary significantly by blubber strata in both species, increasing from 0.13 to 0.19 from the outer to inner layer in ringed seals and from 0.17 to 0.23 in spotted seals.

5.4.5 Variation within Species: Carbon Chain Length by Blubber Depth

FA were also grouped according to the length of their carbon chain (C14-C22) and Friedman's test ($p<0.05$) used to determine whether these groups showed significant variation with blubber depth. FA proportions of groups showing stratification are shown in Figure 5.6. In ringed seals, C14 and C20 FA were found to increase toward the inner blubber layer while C18 FA were highest in the outer layer. C18 FA also increased significantly from the inner to the outer blubber layer in spotted seals. In addition,

spotted seals showed an increase in C22 FA from the outer to the inner blubber layer. The mean number of carbons per FA significantly increased overall from the outer to the inner blubber layer in spotted seals. But, the increase was only from 18.5 to 18.7 carbons per FA and thus does not likely hold any biological significance.

5.5 DISCUSSION

5.5.1 FA Composition: Variation by Species

Distinct differences were seen between the fatty acid profiles of ringed, spotted and bearded seals. As was observed by Cooper *et al.* (2009), bearded seals had the most distinct FA signature, while ringed and spotted were more similar, showing some overlap when PC 1 and PC 2 were plotted (Figure 5.2). Ringed and spotted seals were less well resolved in the current study, perhaps due to the smaller number of individuals sampled. Our results differ from the earlier findings of West *et al.* (1979) and more recently Thiemann *et al.* (2008), who found ringed seals to have the greatest interspecific difference among these phocids. Our results also differ from Budge *et al.* (2008b), who found that the FA composition of ringed and bearded seals did not differ significantly.

Since all three species in the current study were collected in the same location and during the same season, spatial and seasonal variation in FA composition are reduced as possible sources of the observed differences in FA composition among species. Further, FA profiles have been shown not to vary by sex or age in these species (Cooper *et al.* 2009, Budge *et al.* 2007), reducing the likelihood these variables are sources of the observed

differences as well. Factors that may have contributed to the differences in FA profiles among species therefore include foraging ecology, reproductive status or body condition. In addition, there may be differential endogenous FA synthesis, dietary FA modification, or differences in structure or functioning of adipocytes due to genetic differences between these species.

All three species exhibited typical marine FA signatures overall, with high levels of MUFA and n-3 PUFA. The major FA present (16:0, 16:1n-7, 18.1n-9, 20:5n-3, 22:5n-3, 22:6n-3, 18:1n-7, 20:1n-9) were similar to those reported previously in these species in Alaska (West *et al.* 1979, Cooper *et al.* 2009). Certain FA patterns (i.e., increased n-7 MUFA and 20:4n-6 in bearded seals, increased 20:5n-3 and reduced 20:1n-9 and 20:1n-11 in ringed seals and increased 20:1n-9 and 20:1n-11 and reduced 16:1n-7 in spotted seals) were also observed by Cooper *et al.* (2009), who interpreted them as an indication of benthic feeding by bearded seals, shrimp consumption in ringed seals, and mainly foraging of pelagic fishes by spotted seals.

5.5.2 FA Variation by Body Location and Blubber Depth in Phocid Seals

FA profiles were not significantly different between the axillary and umbilical sampling sites along the ventral midline in ringed, spotted or bearded seals. Strandberg *et. al* (2008) similarly observed no significant difference in the FA profiles from dorsal or ventral sampling sites on ringed seals from Svalbard, Norway. In phocid seals, the blubber layer appears to be a continuous subcutaneous adipose layer from which lipid

mobilization occurs uniformly across the body (Nordy and Blix 1985, Slip *et al.* 1992). Blubber thickness in these species is maintained in a fairly consistent ratio relative to body radius in order to optimize the thermoregulatory capacity of this tissue (Beck and Smith 1995).

Our results indicate blubber FA stratification is present in both ringed and spotted seals, suggesting FASA interpretation would vary depending on the blubber layer analyzed. PCA analysis and comparisons of individual FA between the inner and outer blubber layers suggest that blubber stratification may be more pronounced in spotted seals. Sample sizes for ringed and spotted seals ($n = 11$ and $n = 7$, respectively) were not adequate to analyze the data by age or sex cohorts. Cooper *et al.* (2009) recently found no significant difference in the FA signature of ringed, spotted or bearded seals from northwest Alaska by age or sex, supporting the validity of pooling the data from all individuals of each species in the current study.

As is typical among pinnipeds and cetaceans, SFA, LCMUFA and PUFA were highest in the inner blubber layers while SCMUFA were highest in the outer layer. Olsen and Grahl-Nielsen (2003) pointed out that this systemic similarity across numerous species of marine mammals strongly suggests there may be a common determinant mechanism for these patterns. This mechanism likely has some genetic basis and is further driven externally by the cold polar marine environment of many marine mammals. Our results support previous theories which state that the inner blubber is the primary site of dietary

FA deposition and mobilization whereas the composition of outer layers is under stricter regulation to meet the structural and thermoregulatory demands of this tissue.

The patterns observed for SFA and SCMUFA are likely a response to the temperature gradient present throughout the blubber layer. In the cold environment of the Arctic, seal blubber temperature decreases from approximately 37°C at the core, adjacent to the muscle layer, to a temperature of only 4°C just below the skin (Irving and Hart 1957). SFA are relatively linear, allowing close molecular packing and thus increased intermolecular interactions result in relatively high melting points (m.p.). The introduction of one or more double bonds into the hydrocarbon chain causes loss of FA linearity, reducing molecular packing efficiency and the strength of intermolecular interactions. As a result, SFA have much higher m.p. than corresponding MUFA, which in turn have a higher m.p. than a PUFA of the same carbon chain length. The greater the number of unsaturation sites in a FA, the lower its m.p. Further, as the length of the carbon chain increases, FA m.p. also increases.

Overall, the double bond index (DBI) was varied significantly among blubber layers in spotted seals, but only to a very minor degree. The same was the case for the ratio of DBI:carbon chain length in ringed seals. To a much greater extent, the degree of unsaturation (% SFA:UFA) exhibited significant blubber stratification in both species. Several individual FA and subsets of FA grouped by the number of unsaturation sites present also showed significant stratification in both ringed and spotted seals. As

expected, 14:0, 16:0 and ΣSFA in ringed and spotted seals, as well as 18:0 in ringed seals, were highest in the inner blubber layer and lowest in the outer layer. Due to their higher m.p., their presence in the cool outer blubber layers at greater levels would compromise membrane fluidity and insulative capacity in this body region. In addition, a higher SFA:UFA ratio is required to keep lipids from becoming too fluid at biological temperatures, such as those found closer to the body core. One would therefore expect greater MUFA, as a result of their lower m.p., to be found at greater levels toward the more peripheral blubber. This was indeed the case in the current study for 14:1n-5, 18:1n-9 and 18:1n-7 in ringed seals and 18:1n-11, 18:1n-9 and 20:1n-11 in spotted seals.

The exceptions were 20:1n-9 in ringed seals and 22:1n-11 in spotted seals, which were actually in greater proportions in the inner blubber layer. The increased presence of this FA in the inner blubber layer may be a result of a need to retain this FA preferentially in a region where it can be readily remobilized to meet certain physiological needs. Alternately, these FA may be deposited into the inner blubber layer from the diet but then be further modified within this or other blubber regions. The 22:1 isomers have been shown to be underrepresented in adipose tissue relative to the diet due to predator metabolism (Cooper *et al.* 2006, Bremer and Norum 1982). Further, since m.p. also increases with carbon chain length this long-chain (LC) MUFA would also have a relatively high m.p. and therefore may need to be excluded from outer blubber layers to maintain fluidity and insulation. Finally, the higher levels of these FA in the inner blubber layer could be indicative of recent dietary intake since this blubber layer most

closely resembles recent diet (Andersen et al. 2004, Best et al. 2003). These seals are generally in a state of positive energy balance in the fall, thus it is reasonable that FA consumed in excess of nutritional or other demands would be deposited into the inner blubber layer at this time of year (Pond and Mattacks 1988). If the increased intake of these FA had occurred very recently, it may not yet be reflected in the outer blubber layers.

Based on theories that stratification is due primarily to temperature gradients in the blubber (e.g., Fredheim *et al.* 1995), PUFA would also be expected to increase significantly toward the outer blubber layers. The opposite trend was observed. 18:4n-3 in ringed seals and ΣPUFA, 18:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3 in spotted seals all increased from the outer to the inner blubber layers. The only exception was 22:5n-3, which was highest in the outer blubber layer of ringed seals. PUFA are not known to be produced endogenously by marine mammals, and therefore likely arise from dietary origins. Because of their biological importance, PUFA may be remobilized as an energy source or for other physiological requirements before they can be routed to the outer blubber layers. Thus, this important dietary information is less apparent in the outer blubber layer. 22:5n-3 (clupanodonic acid) is an important component of phospholipids in animal cellular membranes, plays a role in the transport and oxidation of cholesterol and is as a precursor of prostanoids which are only formed from clupanodonic acid. Recent physiological demands for this important FA in excess of dietary intake could

initiate remobilization of 22:5n-3 from the inner blubber, resulting in the observed depletion relative to outer layers.

Due to the fact that m.p. increases with increasing FA carbon chain length, long-chain FA (LCFA), those with 20 and 22 carbon chains (C20 and C22 FA, respectively), would be expected to increase in general toward the warmer inner blubber layers. Such an increase was observed for C22 FA in both ringed and spotted seals and for C20 FA in ringed seals. In spotted seals, three out of four individual C20 FA increased toward the inner blubber, but the trend was only statistically significant for 20:5n-3. Certain short-chain FA (C18) showed a decrease from the outer to the inner blubber layer in both species. The mean number of carbons per FA did increase overall from the outer to the inner layer in spotted seals. But, the increase was only from 18.5 to 18.7 carbons per FA. It is unknown whether a difference of this magnitude would hold any biological significance.

5.5.3 FA Signatures of Pagophilic Seals as Monitors of Ecosystem Change

As pointed out by Cooper *et al.* (2009), the pagophilic ("*ice-loving*") phocid seals, including ringed (*Phoca hispida*), spotted (*Phoca largha*) and bearded (*Erignathus barbatus*) seals, are well-suited as indicators of ecosystem changes resulting from climate change. These seals are top predators in the arctic marine food web and are year-round residents of polar waters, making them sensitive to shifts in prey species availability as a result of warming temperatures. For example, bearded seals, which rely heavily upon benthic prey items (Lowry *et al.* 1980, Antonelis *et al.* 1994, Pauly *et al.* 1998, Dehn *et*

al. 2007), may be particularly affected by the appearance of more pelagic-based food webs in the arctic marine ecosystem. In addition, pagophilic seals are particularly vulnerable to changes in the extent of the sea ice, which they rely on for reproduction and molting (Fay 1974). Increased temperatures along with decreased snow depth and earlier spring sea ice break-up led to decreases in reproduction and pup survival in ringed seals in the Canadian Arctic (Ferguson *et al.* 2005, Stirling and Smith 2004, Stirling 2005). Thus, monitoring changes in health status, diet, reproduction and survival in these species could be a sensitive indicator of the effects of climate change in the arctic marine ecosystem. The current study suggests that FA signature analysis can play an important role in these activities. Knowledge of the variation in these FA within and among individuals and species is an important first step in this endeavor.

5.5.4 Significance of Blubber Stratification

Overall, our results suggest FASA interpretations of diet or seal health would vary for these species depending on which blubber layer is collected and analyzed. It appears that the inner layer contains the most accurate reflection of dietary intake as well as information on the status of important PUFA used in an array of physiological functions. The composition of the outer layer is to a large degree driven by the need to maintain lipid fluidity and insulative capacity at low temperatures. Thus, shallow blubber biopsies would not be appropriate for collecting certain information in these seals. Since the composition of the outer layer is less dietary in nature and presumably under some degree of genetic control, the FA composition of this blubber layer may be more useful in stock

or population identification (Krahn *et al.* 2004). Full thickness blubber would be required to assess nutritional quality of this tissue to predators of seals, such as humans and polar bears, which typically consume the entire blubber depth.

The strata analyzed in this study (three layers of equal depth) did not provide sufficient resolution to determine conclusively if the blubber consists of a continuous gradient of FA composition from the inner to the outer layer, or if discreet strata are present. Strandberg *et al.* (2008) found evidence of three distinct strata of FA composition in ringed seals from Svalbard, Norway. Further studies incorporating a greater number of blubber depths in their analyses are required to confirm these results in this species in other regions and to determine if they hold true in other pinniped species as well.

A further implication of blubber stratification is the impact on the partitioning of lipophilic contaminants, such as organohalogens (OH), within this tissue. It is largely unknown how the variation present in blubber lipid composition affects the identity and relative concentration of contaminants found in different blubber regions. For example, if certain OH are preferentially associated with PUFA within blubber, they would likely occur in higher concentrations in the inner blubber where the highest density of PUFA are found. Because this blubber layer has the highest rate of component turnover and is most metabolically active, OH in this region may be more likely to be remobilized, increasing circulating contaminant concentrations and potentially increasing exposure at other sites within the body. Alternately, OH with a propensity for components at

relatively higher proportions in the outer blubber layers may be less systemically available. In this manner, changes in blubber FA composition could also have health implications for a marine mammal by shifting contaminant partitioning between relative sequestration in the blubber and the rest of the body.

In conclusion, FA signatures in ringed, bearded and spotted seals from Kotzebue, Alaska were similar to recent FA analyses near Little Diomede Alaska. Ringed and spotted seal blubber FA signatures did not differ between two sampling locations along the ventral midline, but did exhibit significant stratification throughout the blubber depth. Results suggest spotted seal blubber may be slightly more stratified than that of ringed seals. Stratification in both species appeared to be driven primarily by the necessity to maintain lipid fluidity and insulative capacity throughout the temperature gradient present in blubber, with additional contributions from recent dietary deposition and mobilization as well as the need to maintain essential FA available for remobilization in the innermost blubber. Monitoring health status and dietary shift in pagophilic ice seals through FASA of blubber has the potential to be a powerful tool for indicating changes in food web structure and documenting the effects of climate change on seal health and arctic ecosystems.

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Table 5.1 Harvest date, sex, age and morphometric information for ringed, spotted and bearded seals sampled from Kotzebue Sound, Alaska (2006-2007)

Species	Animal ID	Harvest Date	Sex	Age (yrs)	Mass (kg)	Length (cm)[a]	Girth (cm)[b]	Blubber Thickness (cm)[c]
Ringed Seal (*Phoca hispida*)	KOTZ-01-06	31-Oct-2006	Female	0	23.7	93.8	78.2 / 74.0	4.4 / 3.6
	KOTZ-02-06	31-Oct-2006	Male	3	29.8	107.8	79.2 / 77.2	4.4 / 3.1
	KOTZ-03-06	31-Oct-2006	Female	0	17.7	85.0	66.4 / 66.0	3.2 / 3.1
	KOTZ-04-06	31-Oct-2006	Male	0	19.5	94.0	69.6 / 63.4	3.5 / 2.7
	KOTZ-05-06	31-Oct-2006	Male	11	70.4	136.2	116.6 / 104.6	5.1 / 5.0
	KOTZ-06-06	31-Oct-2006	Male	7	47.1	123.4	98.8 / 91.2	4.2 / 4.1
	KOTZ-01-07	16-Oct-2007	Male	0	18.8	82.5	68.2 / 64.8	4.7 / 3.5
	KOTZ-07-07	18-Oct-2007	Female	1	30.3	95.1	86.1 / 79.5	4.4 / 5.0
	KOTZ-08-07	18-Oct-2007	Male	0	21.6	87.5	72.6 / 72.0	4.6 / 4.3
	KOTZ-09-07	18-Oct-2007	Female	0	13.2	68.5	63.4 / 59.1	3.5 / 2.2
	SIS-03-07	17-Oct-2007	Male	0	23.1	94.5	71.5 / 66.6	4.1 / 3.2
Spotted Seal (*Phoca largha*)	KOTZ-07-06	31-Oct-2006	Male	6	91.3	123.4	114.6 / 107.0	5.2 / 6.2
	KOTZ-08-06	31-Oct-2006	Male	10	87.6	166.6	112.2 / 107.8	6.4 / 5.6
	KOTZ-09-06	31-Oct-2006	Male	3	52.7	161.8	91.6 / 83.0	2.3 / 2.5
	KOTZ-10-06	31-Oct-2006	Male	9	115.3	163.8	128.6 / 128.0	8.5 / 5.3
	KOTZ-02-07	16-Oct-2007	Female	1	42.5	123.5	84.8 / 79.3	4.2 / 4.0
	KOTZ-03-07	16-Oct-2007	Female	4	79.8	150.0	106.9 / 96.4	6.7 / 5.6
	KOTZ-04-07	16-Oct-2007	Male	4	73.0	161.8	100.1 / 89.1	4.3 / 3.6
Bearded Seal (*Erignathus barbatus*)	KOTZ-05-07	16-Oct-2007	Female	0	80.3	159.9	102.5 / 95.5	4.7 / 4.8
	KOTZ-06-07	16-Oct-2007	Male	0	78.0	154.1	142.4 / 148.2	4.6 / 4.3
	SIS-01-07	17-Oct-2007	Male	0	87.0	150.0	104.0 / 100.5	5.3 / 4.3
	SIS-02-07	17-Oct-2007	Female	0	93.4	155.1	106.2 / 107.6	4.6 / 5.4

[a] Seal length is the straight line distance from the tip of the nose to the tip of the tail.

[b] Girth is reported at the axillary/umbilical positions.

[c] Blubber thickness is reported at the axillary/umbilical positions, measured along the ventral midline.

Table 5.2 Proportions (% of total lipid, mean ± SD) of selected fatty acids (FA) in blubber by strata (outer, central, inner) in ringed, spotted and bearded seals sampled from Kotzebue Sound, Alaska (2006-2007)[a,b]

	Ringed Seal (*Phoca hispida*)			**Spotted Seal (*Phoca largha*)**			**Bearded Seal (*Erignathus barbatus*)**		
	Outer	**Central**	**Inner**	**Outer**	**Central**	**Inner**	**Outer**	**Central**	**Inner**
14:0	2.99 ± 0.47%	3.79 ± 0.71%	4.18 ± 0.55%	3.63 ± 0.41%	3.82 ± 0.42%	4.10 ± 0.52%	2.84 ± 0.26%	2.90 ± 0.34%	2.89 ± 0.22%
14:1n-5	1.43 ± 0.29%	1.19 ± 0.28%	0.93 ± 0.30%	0.92 ± 0.20%	0.54 ± 0.11%	*<0.5%*	0.68 ± 0.10%	*<0.5%*	*<0.5%*
16:0	5.73 ± 1.14%	7.48 ± 1.48%	8.58 ± 1.54%	7.79 ± 1.50%	9.43 ± 1.06%	10.71 ± 0.99%	9.92 ± 0.68%	10.55 ± 1.39%	10.48 ± 0.66%
16:1n-7	19.17 ± 2.43%	18.81 ± 2.42%	16.68 ± 2.81%	13.74 ± 2.27%	12.06 ± 2.12%	11.21 ± 1.48%	17.34 ± 0.89%	15.62 ± 0.57%	14.30 ± 1.45%
16:2n-4	0.54 ± 0.05%	0.60 ± 0.11%	0.61 ± 0.15%	*<0.5%*	*<0.5%*	*<0.5%*	*<0.5%*	*<0.5%*	*<0.5%*
18:0	1.14 ± 0.34%	1.31 ± 0.40%	1.39 ± 0.45%	1.37 ± 0.49%	1.48 ± 0.54%	1.69 ± 0.44%	1.76 ± 0.54%	2.06 ± 0.81%	2.68 ± 0.15%
18:1n-11	1.56 ± 0.87%	1.30 ± 0.54%	1.47 ± 0.55%	3.72 ± 0.86%	2.51 ± 0.84%	1.79 ± 0.49%	0.79 ± 0.39%	0.77 ± 0.24%	0.80 ± 0.23%
18:1n-9	12.50 ± 1.50%	11.49 ± 2.14%	10.99 ± 1.82%	18.11 ± 1.72%	16.04 ± 1.58%	14.35 ± 1.68%	13.22 ± 1.91%	13.25 ± 2.16%	12.68 ± 1.70%
18:1n-7	5.84 ± 1.22%	5.35 ± 0.62%	4.94 ± 0.56%	4.70 ± 0.46%	4.67 ± 0.70%	4.46 ± 0.92%	7.99 ± 1.12%	8.30 ± 1.09%	8.40 ± 1.38%
18:1n-3	*<0.5%*	*<0.5%*	*<0.5%*	*<0.5%*	*<0.5%*	0.50 ± 0.06%	0.65 ± 0.26%	0.53 ± 0.42%	0.59 ± 0.16%
18:2n-6	0.72 ± 0.10%	0.72 ± 0.13%	0.73 ± 0.15%	0.92 ± 0.05%	0.87 ± 0.10%	0.85 ± 0.13%	0.66 ± 0.13%	0.67 ± 0.10%	0.74 ± 0.09%
18:4n-3	1.01 ± 0.13%	1.25 ± 0.23%	1.45 ± 0.38%	1.12 ± 0.14%	1.22 ± 0.18%	1.51 ± 0.15%	0.80 ± 0.14%	0.79 ± 0.09%	0.91 ± 0.10%
20:1w11	1.03 ± 0.52%	1.08 ± 0.55%	1.24 ± 0.48%	4.40 ± 1.41%	3.72 ± 1.43%	2.51 ± 0.71%	1.57 ± 0.42%	1.72 ± 0.46%	1.78 ± 0.53%
20:1n-9	2.24 ± 1.59%	2.99 ± 1.84%	4.10 ± 1.96%	5.60 ± 1.47%	5.58 ± 1.54%	6.13 ± 2.33%	1.49 ± 0.62%	1.61 ± 0.63%	1.68 ± 0.53%
20:1n-7	0.50 ± 0.44%	*<0.5%*	*<0.5%*	*<0.5%*	*<0.5%*	*<0.5%*	1.87 ± 0.53%	1.96 ± 0.60%	2.02 ± 0.59%
20:4n-6	0.54 ± 0.15%	*<0.5%*	*<0.5%*	*<0.5%*	*<0.5%*	*<0.5%*	1.30 ± 0.20%	1.21 ± 0.20%	1.19 ± 0.12%
20:4n-3	*<0.5%*	*<0.5%*	*<0.5%*	*<0.5%*	0.54 ± 0.18%	0.55 ± 0.12%	*<0.5%*	*<0.5%*	0.51 ± 0.07%
20:5n-3	9.57 ± 1.19%	9.94 ± 1.47%	9.97 ± 1.93%	5.62 ± 1.19%	6.52 ± 1.54%	7.02 ± 1.76%	9.83 ± 1.33%	9.53 ± 1.23%	8.31 ± 0.97%
22:1n-11	*<0.5%*	1.00 ± 1.28%	1.58 ± 1.43%	1.76 ± 1.14%	2.74 ± 1.43%	3.42 ± 1.73%	*<0.5%*	*<0.5%*	*<0.5%*
21:5n-3	1.13 ± 0.11%	1.11 ± 0.16%	1.10 ± 0.16%	0.92± 0.16%	0.94 ± 0.17%	0.99 ± 0.22%	1.71 ± 0.34%	2.20 ± 0.65%	1.60 ± 0.26%
22:5n-6	*<0.5%*	*<0.5%*	*<0.5%*	*<0.5%*	*<0.5%*	*<0.5%*	0.57 ± 0.20%	0.69 ± 0.14%	0.65 ± 0.21%
22:5n-3	15.56 ± 1.60%	14.07 ± 2.23%	12.66 ± 2.46%	10.30 ± 1.49%	11.35 ± 1.55%	11.39 ± 2.08%	11.26 ± 2.35%	12.05 ± 2.63%	12.33± 2.13%
22:6n-3	10.98 ± 1.33%	10.52 ± 1.97%	11.18 ± 1.93%	8.29 ± 1.04%	9.33 ± 1.08%	9.79 ± 1.02%	6.82 ± 1.50%	7.29 ± 1.38%	8.42 ± 1.44%
ΣFA[c]	93.5 ± 0.9%	94.0 ± 1.1%	93.9 ± 0.7%	93.4 ± 0.5%	93.4 ± 0.8%	92.8 ± 0.8%	92.4 ± 0.3%	93.7 ± 2.5%	92.5 ± 0.4%
ΣSFA[c]	10.56 ± 1.89%	13.28 ± 1.11%	15.05 ± 2.12%	13.70± 2.40%	15.69 ± 1.61%	17.62 ± 1.36%	15.86 ± 1.44%	16.66 ± 2.15%	17.49 ± 0.92%
ΣMUFA[c]	46.81 ± 2.21%	45.60 ± 2.89%	44.46 ± 3.66%	55.83 ± 3.61%	50.75 ± 4.17%	47.28 ± 4.07%	48.21 ± 3.42%	46.53 ± 3.23%	45.32 ± 2.84%
ΣSCMUFA[c]	42.50 ± 2.93%	39.87 ± 2.75%	36.77 ± 2.94%	43.47 ± 1.36%	37.92 ± 2.15%	34.26 ± 2.15%	42.71 ± 2.28%	40.69 ± 2.04%	39.11 ± 1.96%
ΣLCMUFA[c]	5.44 ± 2.84%	6.83 ± 4.02%	8.79 ± 3.95%	13.28 ± 3.56%	13.77 ± 4.05%	14.00 ± 4.27%	7.21 ± 0.89%	8.04 ± 0.83%	7.81 ± 1.01%
ΣPUFA[c]	42.73 ± 2.56%	41.22 ± 3.60%	40.59 ± 3.53%	30.54 ± 3.90%	33.64 ± 3.83%	35.15 ± 3.87%	35.99 ± 2.64%	36.94 ± 3.41%	37.24 ± 2.45%

[a] Fatty acid proportions from the axillary blubber sampling position are reported here. Fatty acid profiles from the axillary and umbilical blubber sampling positions were not determined to be statistically different.

[b] Summary statistics are presented only for the subset of fatty acids present at above trace levels (>0.5%). 60 fatty acids were above detection levels in at least 1 sample, but ~70% of these were present below trace levels.

[c] ΣFA = Sum of all fatty acids (FA) present above trace levels (>0.5%). ΣSFA = Sum of all saturated FA. ΣMUFA = Sum of all monounsaturated FA. ΣSCMUFA = Sum of MUFA with <20C. ΣLCMUFA = Sum of MUFA with ≥20C. ΣPUFA = Sum of all polyunsaturated FA.

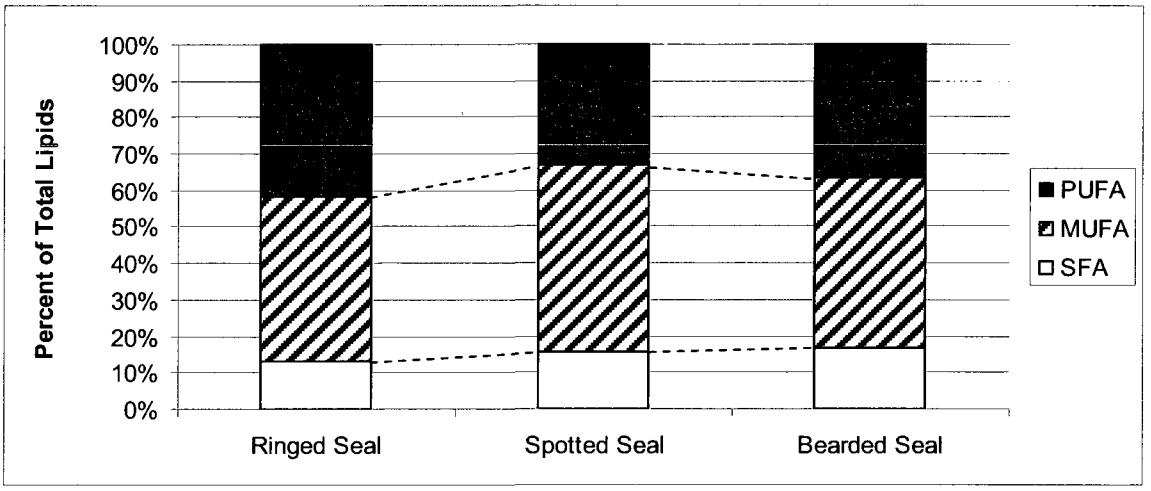

Figure 5.1 Mean proportion of fatty acid groups in full thickness blubber of ringed, spotted and bearded seals. Results from the axillary blubber sampling position are reported here. Fatty acid profiles from the axillary and umbilical blubber sampling positions were not determined to be statistically different. ANOVA indicated the following significant ($p<0.05$) differences between species: SFA (ringed>spotted=bearded), MUFA (spotted>ringed, bearded=ringed/spotted), and PUFA (ringed>spotted, bearded).

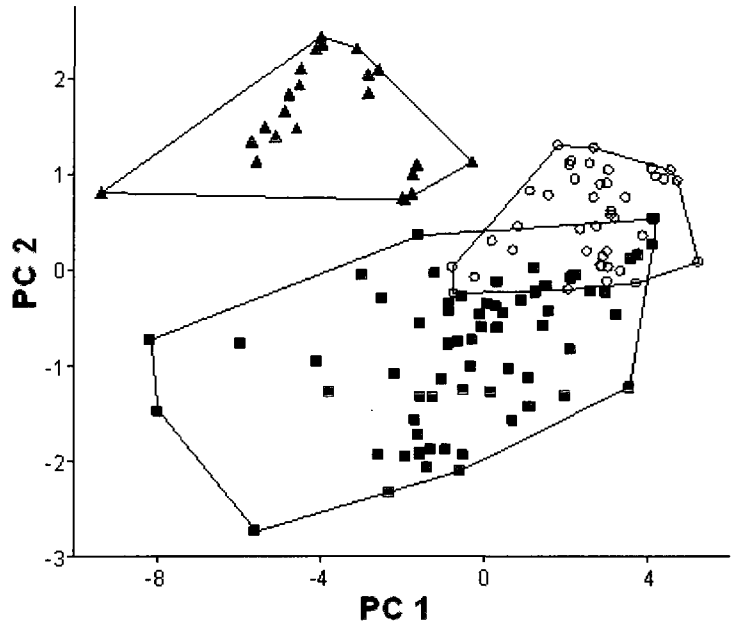

Figure 5.2 Plot of principal component scores (PC1 and PC2) derived from the analysis of FA profiles in the blubber of ringed (■), spotted (○), and bearded (▲) seals. All six blubber samples (exterior, central and interior strata from the axillary and umbilical sampling locations) from each individual were included in this analysis to incorporate the full range of inter-individual variation.

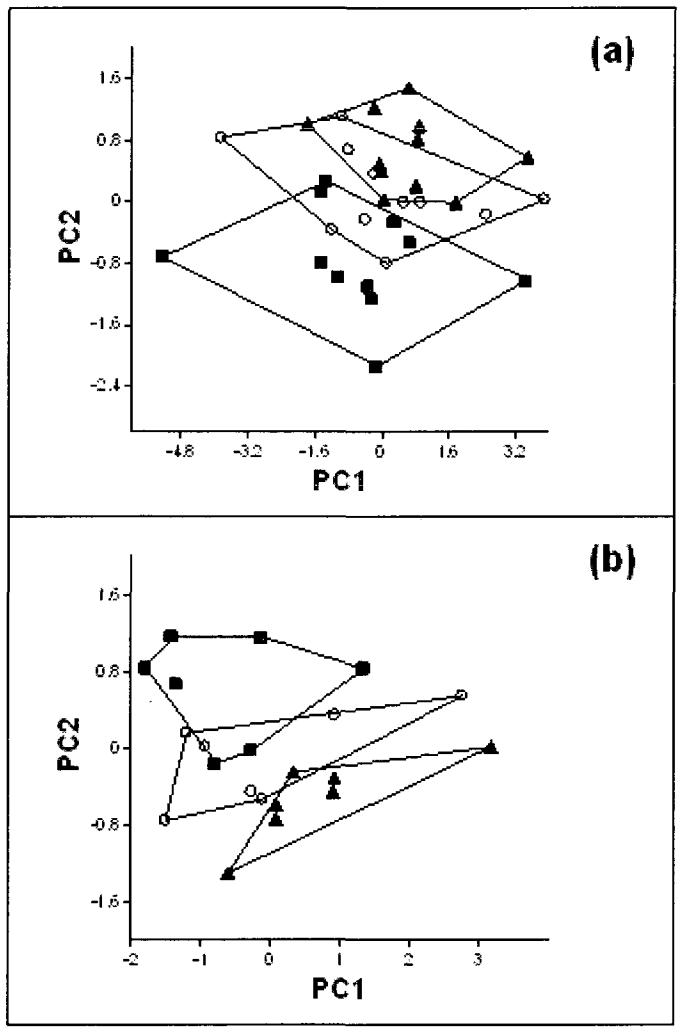

Figure 5.3 Plot of principal component scores (PC1 and PC2) derived from the analysis of FA stratification in outer (■), central (○), and inner (▲) blubber layers of a) ringed seals and b) spotted seals.

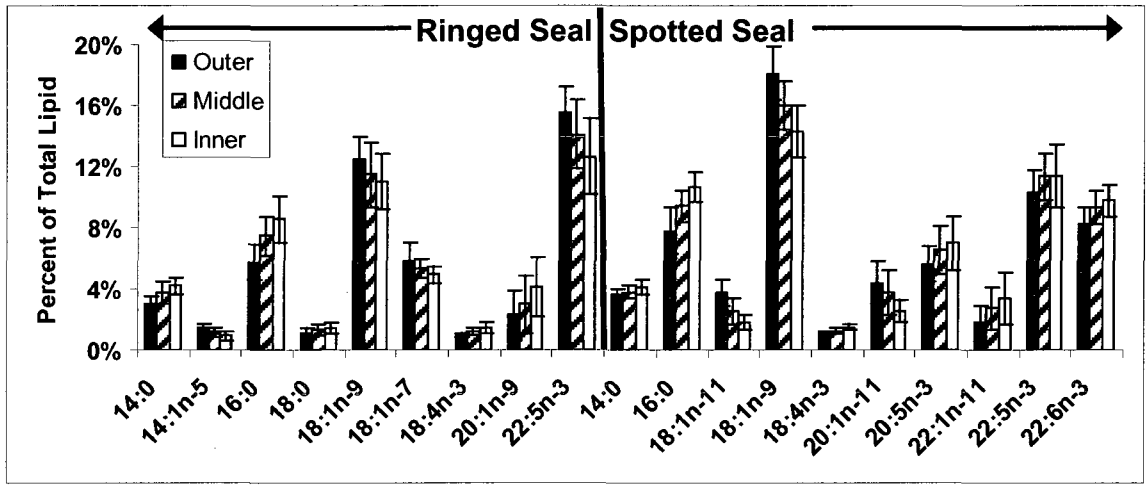

Figure 5.4 FA exhibiting statistically significant stratification (Friedman test, $p<0.05$) and an overall mean of $\geq 0.5\%$ of total lipids in ringed and spotted seals.

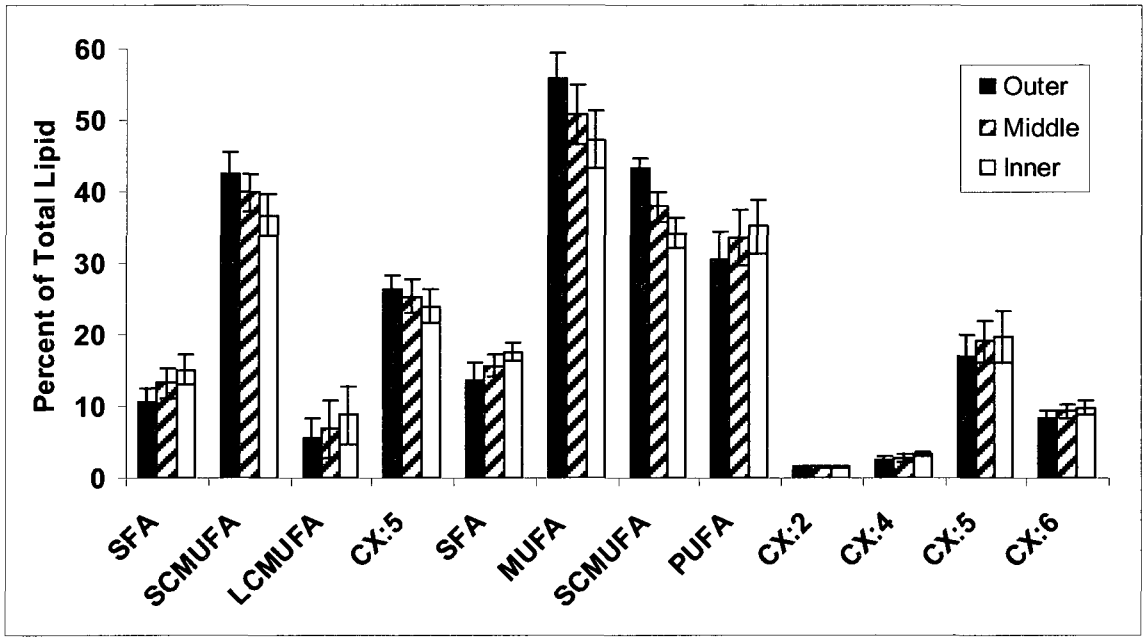

Figure 5.5 Comparison of FA proportions grouped by number of unsaturations among three blubber layers in ringed and spotted seals. Only FA groups that exhibit significant stratification among layers (Friedman test, $p<0.05$) and are present above trace levels (0.5% of total lipid) are shown.

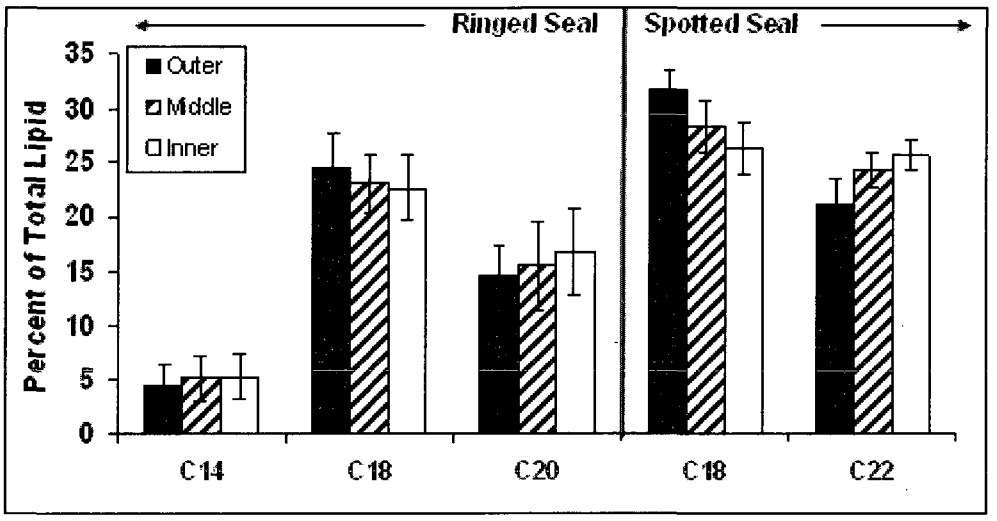

Figure 5.6 Comparison of FA proportions grouped by carbon chain length among three blubber layers in ringed and spotted seals. Only FA groups that exhibit significant stratification among layers (Friedman test, $p<0.05$) and are present above trace levels (0.5% of total lipid) are shown.

CONCLUSIONS

Subsistence foods derived from fish and seal species of northwest Alaska proved to be rich in many essential nutrients with minimal associated risk from contaminant intake. The changes in both nutrient and contaminant concentrations with food processing highlight the need to evaluate of foods in the form they are ultimately consumed when determining the risks and benefits of traditional diets. We encourage public health officials to consider these results when providing consumption advice for subsistence foods. Further research is needed in this area to account for intake of nutrients and contaminants from multiple food sources, local consumption rates for specific food items and risks and benefits to vulnerable cohorts, such as infants, children and women of child-bearing age. Human, wildlife and ecosystem health are intricately linked within the subsistence food web. Continued research on wildlife in this region will serve to provide insight into the potential future impacts of ongoing cultural and environmental change in this region.

Stable isotope ratios of carbon and nitrogen are useful tools for understanding and predicting the movement of nutrients and contaminants through the food web (Atwell *et al.*, 1998; Cardona-Marek *et al.*, 2009; Dehn *et al.*, 2006; Hobson and Welch, 1992; Horton *et al.*, 2009; Kelly, 2000; Peterson and Fry, 1987). But, our work shows that these tools and the assumptions that accompany them must be carefully scrutinized so that they can be applied appropriately. We found that stable $\delta^{13}C$ and $\delta^{15}N$ differ widely

between species and among tissues within a species. Further, food processing resulted in certain small, but statistically significant, changes in $\delta^{13}C$ and $\delta^{15}N$. Using mercury (Hg) as a model toxicant, we demonstrated that the variation in stable isotope ratios observed was not in parallel with varying contaminant concentrations. In other words, while $\delta^{13}C$ and $\delta^{15}N$ may be altered in a certain way as a result of tissue type of food processing, Hg may not be altered in the same manner. These chemical components can exhibit no or even negative correlation between tissues or processing techniques. As a result, dietary reconstructions or interpretations of contaminant pathways within food webs have the potential to be influenced by the tissue type in which $\delta^{13}C$ and $\delta^{15}N$ are measured and the processed state of that tissue. This issue is particularly relevant to species that are known to selectively consume certain tissues, such as humans, brown bears and polar bears. Care must be taken to choose the appropriate tissue from both predator and prey that should be chosen for chemical analysis. If the consumer is eating a tissue that has been processed in some manner (e.g., cooking, decomposition), the chemical components should be quantified in that tissue as it is ultimately consumed. Predators should not be assumed to simply consume whole, raw prey. Further, simple assumptions that increases in $\delta^{13}C$ or $\delta^{15}N$ will be matched by increased contaminant concentration are not always valid, as these relationships are complex and depend on a multitude of variables.

The octanol-water partition coefficient (K_{OW}) is often utilized to predict the bioaccumulation potential of lipophilic organic contaminants (U.S. EPA, 1999; Environment Canada, 1999). The relationship between K_{OW} and the bioaccumulation

factor (BAF) for a given compound was originally observed in aquatic systems, but has been applied widely across aquatic and non-aquatic taxa alike (Fisk *et al.*, 2001; Russell *et al.*, 1999; Arnot and Gobas, 2006). Our results add to a recent set of studies suggesting K_{OW} may not be an appropriate proxy for BAF in air-breathing species. We found that the relationship between contaminant concentration and K_{OW} differed when comparing an air breathing and water respiring species of similar trophic status (i.e., spotted seal and sheefish). While low K_{OW} compounds did not accumulate in sheefish, presumably due to the ability for this species to excrete these compounds over the gills into the surrounding water, they were able to accumulate in seals if the octanol-air partition coefficient (K_{OA}) was sufficiently high. Due to their respiratory physiology, seals cannot excrete these compounds via the lungs. Thus, regulatory guidelines that utilize K_{OW} based-models to predict bioaccumulation behavior are not suitable for application to air-breathing species. Updated models that incorporate K_{OA} when determining risk in such species are needed. As with the application of stable isotope ratios of carbon and nitrogen to studies of contaminant food web dynamics, the assumptions associated with the predictive use of K_{OW} do not hold true across all scenarios. It is critical that we carefully evaluate the validity of our tools to the questions at hand in order to use them appropriately and interpret them correctly.

Fatty acid (FA) profiles and concentrations are not only useful tools for feeding ecology studies, but also provide important information on animal health, nutritional statue and physiology. Fatty acid profiles within blubber can be affected by a number of dietary,

environmental and physiological factors (Andersen *et al*,. 2004; Beck *et al.*, 2005; 2007; Budge *et al*,. 2008; Krahn *et al.*, 2008; Samuel and Worthy 2004; Thiemann *et al.*, 2007). We found that FA signatures in ringed, bearded and spotted seals from Kotzebue, Alaska were similar to recent FA analyses near Little Diomede Alaska. Ringed and spotted seal blubber FA signatures did not differ between two sampling locations along the ventral midline, but did exhibit significant stratification throughout the blubber depth. The stratification patterns in both species appeared to be driven primarily by the necessity to maintain lipid fluidity and insulative capacity throughout the temperature gradient present in blubber, with additional contributions from recent dietary deposition and mobilization as well as the need to maintain essential FA available for remobilization in the innermost blubber. Monitoring health status and dietary shift in pagophilic ice seals by measuring FA in blubber has the potential to be a powerful tool for indicating changes in food web structure and documenting the effects of climate change on seal health and arctic ecosystems.

Studies of nutrients and contaminants offer a great deal of information on the health of individuals, populations and ecosystems. Further, understanding the dynamics of these components within food webs provides researchers with a means to monitor changes in food web structure and predict the potential impacts of such shifts to arctic ecosystems. Yet, we are still uncovering the the driving forces that lead to the nutrient and contaminant patterns we observe within and among individuals and species. We must be

willing to incorporate new information into our existing models as it arises in order to appropriately apply these tools in future health and risk assessments.

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