PCB-related exposure and effects in ringed seals (*Pusa hispida*) frequenting a locally-contaminated marine environment in Labrador

by

Tanya Brown
B.Sc., University of Guelph, 2004
M.Sc., Memorial University, 2007

A Dissertation Submitted in Partial Fulfillment
of the Requirements for the Degree of

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in the Department of Biochemistry and Microbiology

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Supervisory Committee

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Abstract

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The release of 260 kg of polychlorinated biphenyls (PCBs) by a military radar station into Saglek Bay (Labrador) in the eastern Canadian Arctic contaminated adjacent marine sediments, and some fish, seabirds, and ringed seals. However, attributing the PCBs found in high trophic level and highly mobile marine mammals to any point source is, in most cases, impossible. This thesis demonstrated the extent to which a local PCB source at Saglek Bay led to the contamination and health effects in ringed seals. The dominance of PCBs at this contaminated marine site afforded a unique opportunity to evaluate the effects of this single class of industrial chemical in a manner that has not been previously possible in marine mammals.

We used a variety of tools to characterize the contribution of local PCB contamination in the Labrador ringed seal food web. These tools included: 1) univariate and multivariate statistical exploration of contaminant patterns; 2) stable isotope ratios and fatty acid

signatures to describe feeding ecology; and 3) satellite telemetry to track the movements of seals on the coast.

Divergent PCB congener profiles and contaminant ratios enabled an assignment of seals into either 'local' or 'long-range' categories, with up to 60% of ringed seals sampled exhibiting patterns consistent with the local source. PCB concentrations in locally-contaminated adult males were 2-fold higher than in those exposed only to long-range PCB sources. Seals with smaller home ranges had an increased likelihood of feeding on prey contaminated by the local PCB source.

Similar fatty acid profiles between those seals with 'local' PCB profiles and those with 'long-range' or background profiles indicate little support for the possibility that differential feeding ecologies explained the divergent PCB profiles. Ringed seals fed predominantly on zooplankton (*Mysis oculata* and *Themisto libellula*), dusky snailfish (*Liparis gibbus*) and arctic cod (*Boreogadus saida*). Heavier PCB profiles in the Saglek food web, compared to the same species exposed to only background contaminants, provided additional insight into the mechanisms of localized PCB contamination of some Labrador ringed seals.

In addition to ascertaining the importance of a point source to contamination in ringed seals, we assessed the effects of PCBs on their health through quantitative real-time polymerase chain reaction (qPCR) assay. Levels of mRNA transcripts for five gene targets, including aryl hydrocarbon receptor (*Ahr*), interleukin-1 beta (*Il1b*), estrogen receptor alpha (*Esr1*), insulin-like growth factor receptor 1 (*Igf1*) and glucocorticoid receptor alpha (*Nr3c1*), correlated with increasing levels of PCBs, indicating an effect of this persistent organic pollutant (POP) in these seals. Threshold values were calculated

for these five genes, with the most conservative value being 1,380 ng/g lipid weight (lw). Approximately 14% of the seals sampled exceeded this threshold, suggesting a risk of adverse effects in a proportion of the local population attributed to PCBs. While the implications for these sublethal molecular changes at the individual or population level are unclear, contaminant-related changes in endocrine, immune, and molecular endpoints have been observed in ringed seals from the Baltic Sea exhibiting reproductive and developments abnormalities, and virus epizootics. Results of this study improve our understanding of the effects of PCBs in free-ranging marine mammals and provide new information needed to inform mitigation and monitoring efforts, both for ringed seals in the north and other seals around the world.

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Finally, I would like to express my deepest appreciation to my husband, Peter, for his love and steadfast support throughout.

List of Abbreviations

ANOSIM	Analysis of similarity
ANOVA	Analysis of Variance
Ahr	Aryl hydrocarbon receptor
CCME	Canadian Council of the Ministers of the Environment
CYP	Cytochrome P450 enzymes
DDT	Dichlorodiphenyltrichloroethane
DSL	Domestic Substance List
Esr1	Estrogen receptor α
Nr3c1	Glucocorticoid receptor α
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
Igf1	Insulin like growth factor receptor 1
Il1b	Interleukin-1 β
Kow	Octanol-water partition coefficient
MDS	Multidimensional scaling
OCP	Organochlorine pesticides
PBT	Persistent, bioaccumulative and toxic
PCA	Principal components analysis
POP	Persistent organic pollutants
PCB	Polychlorinated biphenyl
qPCR	Quantitative real-time polymerase chain reaction

Rara	Retinoic acid receptor α
TEQ	Toxic equivalency factor
Thra	Thyroid hormone receptor α
Tshb	Thyroid stimulating hormone β
Ugt1a1	Uridine 5’-diphospho-glucuronosyltransferase

Thesis Format and Manuscript Claims

The thesis is presented in a manuscript format. Chapters 2, 3, 4 and 5 are written in a manuscript style containing an Abstract, Introduction, Materials and Methods, Results and Discussion and Conclusions. Chapter 1 provides background information and introduces the overall theme of the thesis. Chapter 6 summarizes and concludes the major findings of the papers and provides suggestions for future directions.

Chapter 2: **Tanya M. Brown**, Aaron T. Fisk, Caren C. Helbing, Ken J. Reimer. 2014. Polychlorinated biphenyl profiles in ringed seals (*Pusa hispida*) reveal historical contamination by a military radar station in Labrador, Canada. *Environmental Toxicology and Chemistry* **33**, 592-601. Tanya Brown and Tom Sheldon co-ordinated the ringed seal sample collection. Tanya Brown prepared all field samples for submission to analytical laboratories and ran all statistical analyses. Tanya Brown wrote the manuscript with the participation of the co-authors.

Chapter 3: **Tanya M. Brown**, Sebastian Luque, Becky Sjare, Aaron T. Fisk. 2014. Satellite telemetry informs PCB source apportionment in a mobile, high trophic level marine mammal – the ringed seal (*Pusa hispida*). This manuscript has been submitted to *Environmental Science and Technology*. Tanya Brown with the assistance of Sebastian Luque and Becky Sjare live-captured and tagged the ringed seals. Tanya Brown directed the analysis of the ringed seal tracking data. Sebastian Luque performed the ringed seal tracking data analysis. Tanya Brown prepared all field samples for submission to analytical laboratories. Tanya Brown ran all statistical analyses. Tanya Brown wrote the manuscript with the participation of the co-authors.

Chapter 4: **Tanya M. Brown**, Sara J. Iverson, Aaron T. Fisk, Robie W. Macdonald, Caren C. Helbing, Ken J. Reimer. 2014. Local contamination, and not feeding preferences, explains elevated PCB concentrations in Labrador ringed seals (*Pusa hispida*). This manuscript has been submitted to Science of the Total Environment. Tanya Brown with the assistance of Joey Angnatok and the crew of the Motor Vessel (M/V) *Whats Happening*, D. Angnatok, M. Carpenter, and the science and technical crew of the CCGS *Amundsen* collected numerous prey species in four fjords of Labrador. Tanya Brown prepared all field samples for submission to analytical laboratories. Tanya Brown ran all statistical analyses. Tanya Brown wrote the manuscript with the participation of the co-authors.

Chapter 5: **Tanya M. Brown**, Peter S. Ross, Ken J. Reimer, Nik Veldhoen, Neil J. Dangerfield, Aaron T. Fisk, Caren C. Helbing. 2014. PCB related effects thresholds as derived through gene transcript profiles in locally contaminated ringed seals (*Pusa hispida*). This manuscript has been accepted by *Environmental Science and Technology*. Tanya Brown with the assistance of Joey Angnatok and the crew of the Motor Vessel (M/V) *Whats Happening*, D. Angnatok, and M. Carpenter collected the ringed seal samples for the study. Tanya Brown prepared all ringed seal blubber and muscle samples for submission to analytical laboratories. Tanya Brown performed the qPCR laboratory analysis for the ringed seal liver samples. Tanya Brown ran all statistical analyses with assistance from Taka-Aki Ichu. Tanya Brown wrote the manuscript with the participation of the co-authors.

Chapter 1: Introduction

1.1 Contaminants of concern in the marine environment

Oceans receive chemical inputs from a myriad of sources including run-off from land, waste disposal, pollution from shipping, oil drilling, and inadvertent incidents, such as spills and dumping. As a result, the global marine ecosystems are continuously under pressure from point source and non-point source pollutants. Increasing demands have led to expanding modern technologies and anthropogenic activities and the development and release of new chemicals. Approximately 8 million chemical substances are commercially available and about 30,000 are reported to be in wide commercial (marketed in amounts above 1 tonne per year) use globally (Muir and Howard, 2006). The vast majority of these have never been measured in the environment and their transport, and fate remains unknown (Muir and Howard, 2006). In Canada alone, over 23,000 chemical substances are used commercially and are listed on the Domestic Substance List (DSL) (Muir and Howard, 2006).

Chemical substances of particular concern biomagnify, reaching highest concentrations in animals at the top of the food chain, are typically ones classified as being persistent, bioaccumulative and toxic (PBT) and have the potential for long-range transport. This class of chemicals tend to degrade slowly and persist in the environment for extensive periods of time. When these chemicals are consumed they bioaccumulate in the tissues of organisms and can elicit chronic forms of toxicity, notably those mediated through the disruption of endocrine processes (a.k.a. hormone mimics or endocrine disrupting substances). Additionally, their physiochemical properties allow them to be

transported over great distances *via* environmental processes, deposited, and incorporated into aquatic food webs.

Persistent organic pollutants (POPs) are an important part of the PBT group, which additionally includes trace metals and organo-metal compounds. International action on POPs culminated in the signing of the Stockholm Convention in 2001. This global agreement, which entered into force in 2004, identified 12 chlorinated chemical groups (commonly known as the 'dirty dozen) for global bans or reduced emissions (Muir and Howard, 2006). In the present thesis, I have investigated polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs). PCBs were produced commercially starting in 1929 and used mainly as electrical transformer fluids, flame retardants, hydraulic lubricants, and sealants (Tanabe, 1988). Restrictions on their production and use were put in place in the 1970s in most industrialized countries, resulting in a decrease in their environmental concentrations (Muir et al., 1999a). PCBs consist of a biphenyl molecule with 1-10 chlorine atoms distributed around the phenyl rings (Hutzinger et al., 1974) (Figure 1). Different patterns of chlorine substitutions can create 209 individual compounds (or congeners) with unique physical, chemical, and biological properties (Safe, 1987).

OCPs are synthetic, non-polar, and toxic compounds that were previously used extensively in agriculture, forestry, and for domestic pest control. OCPs were produced commercially in the 1940s and 1950s and due to their persistence and toxicity were banned or restricted in most countries in the 1970s and 1980s. Representative compounds in this group include dichlorodiphenyltrichloroethane (DDT), dieldrin, chlordane,

toxaphene, mirex, aldrin, endrin, heptachlor, hexachlorobenzene (HCB) and hexachlorocyclohexane (HCH).

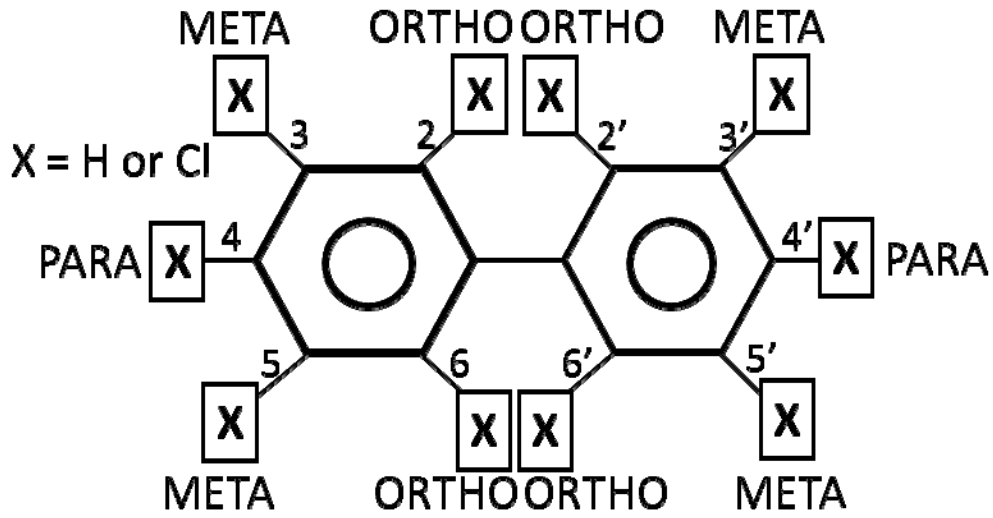

Figure 1. PCB molecule indicating the ten possible sites of chlorine substitution and the *ortho*, *meta*, and *para* positions.

1.2 Persistent Organic Pollutants in the Arctic

Although POP concentrations in the Canadian Arctic are approximately 10-50 times lower than in temperate more industrialized areas, their occurrence in such a remote environment has vexed decision makers and scientists for decades. A notable driver of federal government policies nationally and internationally relates to the consequences of POPs in the country foods of Inuit peoples that rely heavily on fish and marine mammals (Dewailly and Furgal, 2003). Organochlorine contamination of marine mammals in the Arctic was first documented by Holden (Holden, 1970) who measured PCBs and OCPs (dieldrin and DDT) in the blubber of ringed seals (*Phoca hispida*) from Baffin Island. Since then, POPs have been measured in virtually every component of the arctic marine environment including seawater (Bidleman et al., 1989; Hung et al., 2005), zooplankton (Braune et al., 2005), benthic and pelagic invertebrates (Braune et al., 2005), fish (Muir et

al., 1992; Muir et al., 1988), seabirds (Braune et al., 2001), and marine mammals (Muir et al., 1990; Muir et al., 1992).

The Arctic has been described as a sink for POPs that are transported through a process known as "global distillation" *via* prevailing atmospheric and oceanic currents from warmer southern regions (Wania and Mackay, 1995; Wania and McLachlan, 2001). These compounds are carried to the Arctic by cycles of evaporation, transport by air and condensation. Once POPs reach the Arctic, they are constrained from removal through atmospheric processes, due to low evaporation rates in polar regions. Cool temperatures also favour greater adsorption of these compounds to atmospheric particulate matter and slower decomposition rates (Wania, 1996). Atmospheric deposition is the dominant contributor to POP contamination in the arctic marine environment (Barrie et al., 1997; Macdonald et al., 2000).

Differences in the physicochemical properties and environmental half-lives of different POPs lead to a process known as 'fractionation', where more volatile compounds would be transported more rapidly and over larger distances than less volatile compounds (Wania and Mackay, 1993; Wania, 1996). In the case of PCBs, 'lighter' congeners which are highly volatile tend to remain airborne and migrate faster, whereas 'heavier' less volatile congeners partition into environmental media (e.g. water, soil, vegetation, ice, snow) making their transport slower and more resistant to travelling large distances. The logarithm of the octanol-water partition coefficient (log K_{OW}), can serve as a proxy for characterizing important aspects of the fractionation and partitioning of different compounds in different environmental compartments (e.g. water and lipid)

(Ross et al., 2004). This parameter is therefore commonly used for predicting the transport and fate of POPs in the environment.

POPs are removed from the atmosphere to surfaces by three processes (Barrie et al., 1997): diffusive gas exchange between the atmosphere and the ocean, scavenging by rain or snow (either from gas or particulate phases), and dry particle deposition of aerosol-bound POPs. In the water column, POPs can be found either dissolved or adsorbed to particulate organic matter (Muir et al., 1999a). Once adsorbed, they become available for uptake by biota (Dickson et al., 1994).

Uptake of POPs at lower trophic levels occurs through a passive process driven by a fugacity gradient, whereby contaminants move towards matrices with lower fugacity until equilibrium is attained. Rapid adsorption to the surface of phytoplankton and/or zooplankton is followed by diffusion through membranes into their cellular matrix (Del Vento and Dachs, 2002; Swackhamer and Skoglund, 1993). In addition, contaminants adsorbed onto particle surfaces are taken up by zooplankton and other filter feeding invertebrates. In fish, contaminants are accumulated *via* the gills, dermis, and gastro-intestinal tract. POPs enter the organism by diffusion across the gill surfaces into the blood (Connell, 1988).

In larger marine organisms, bioaccumulation and biomagnification of POPs occurs as a result of a sequence of solvent depletion and solvent switching steps (Macdonald et al., 2002). Solvent depletion occurs in the digestive tract when lipids are hydrolysed by digestive enzymes. As digestion continues the increased loss of dietary lipids forces contaminants to redistribute from lipid to other organic matter (solvent-switching process). The products of lipid hydrolysis then diffuse into the cells lining the intestine,

where triglycerides are resynthesized to form packets called chylomicrons. These packets represent a solvent pool that enables the contaminant to diffuse into the cell (solvent-switching process). The final step in biomagnification occurs in every tissue, where all assimilated lipids are metabolized for energy (solvent depletion process). These depleting steps result in POP accumulation from dietary food items against a fugacity gradient, which results in a fugacity amplification or biomagnification (Macdonald et al., 2002). These steps, coupled with the slow rate of excretion and metabolism result in the increase in concentrations of a contaminant from prey to predator, the net effect of which is biomagnification (Figure 2).

Some marine mammals are particularly vulnerable to elevated levels of POPs as a result of their high trophic level, low detoxification capacity, large lipid reserves, and long life span (Boon et al., 1994; Ross, 2000). The ringed seal, the most abundant and widely distributed Arctic pinniped, has been continuously monitored for POPs across the Arctic since the 1970s. Concentrations of PCBs, DDT and chlordane-related compounds, as well as toxaphene, tend to be elevated in Eastern compared to the Western Canadian Arctic consistent with circumpolar trends (Braune et al., 2005). PCBs and most OCPs declined in Canadian Arctic biota, including ringed seals, from the 1970s to the 1990s (Braune et al., 2005). Despite these declines, concentrations of PCBs in ringed seals continue to exceed the Canadian PCB tissue residue guidelines for the protection of mammalian wildlife consumers of aquatic biota (0.79 ng TEQ/kg diet wet wt, CCME 2001), U.S. National Academy of Sciences/National Academy of Engineering ∑PCB guideline (500 µg/kg diet wet wt) and the New York State Department of Environmental Conservation ∑PCB guideline (110 µg/kg diet wet wt) for fish-eating wildlife (Wong et

al., 2000). In cases where predators consume ringed seal blubber (e.g. polar bears), for example, they would be exposed to PCB concentrations that readily exceed these guidelines.

Since marine mammals, specifically ringed seals, are important food items consumed by Inuit, they represent an important pathway for the transfer of contaminants to humans. With diets that include Arctic top predators, Inuit are more exposed to POPs than are populations living in more southern regions. Dietary surveys conducted in the Canadian Arctic and contaminant measurements taken in breast milk and blood have provided a large amount of information regarding Inuit exposure to POPs (Dewailly and Furgal, 2003). For example, Kuhnlein et al. (2000) showed that approximately 55% of Inuit consume a daily diet containing PCBs and OCPs, which are often in quantities exceeding established tolerances. The breast milk of Nunavik Inuit women has five to ten-fold greater POP concentrations than milk of women in southern Quebec (Dewailly, 1989). In addition, 43% of Inuit women have blood PCB concentrations above the "level of concern" established by Health Canada for women of child bearing age (5 μg/L) (Dewailly and Furgal, 2003). The main source of POPs in the Inuit diet is marine mammal fats, including ringed seal blubber, narwal blubber, beluga blubber, and walrus blubber. In addition to studying sources of exposure, researchers have been trying to determine if POPs are having an effect on the health of Inuit. Studies conducted on Inuit in the Arctic have found adverse health effects on the immune system and with the incidence of infections, osteoporosis, and neurodevelopment (Dewailly and Furgal, 2003).

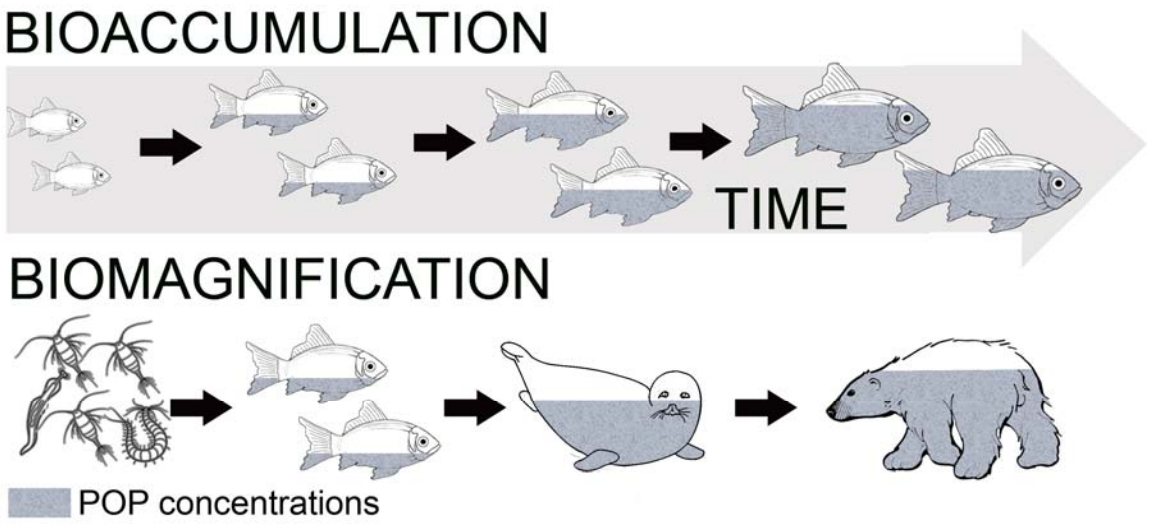

Figure 2: A simplified schematic of persistent organic pollutant (POP) uptake processes in an Arctic marine ecosystem. Bioaccumulation is the accumulation of a contaminant in an organism over time; biomagnification is an increase in concentration of a contaminant as it moves up the food chain.

1.3 Marine mammals as indicators of food web contamination

While marine mammals are often used as useful indicators of food-web contamination, they are typically poor indicators of point sources of environmental contaminants. Their complex feeding ecologies and often extensive use of large habitat inevitably mask the contributions of specific inputs. Researchers have used a variety of statistical techniques and study designs (e.g. PCB profiles and POP ratios (Jarman et al., 1996; Krahn et al., 1999; Krahn et al., 2007; McKinney et al., 2011; Ross et al., 2004) to infer the contributions of regional and/or local POP sources (Table 1). However, without an understanding of movement, foraging behaviour, and diet it remains challenging to adequately explain the contributions of point sources to the body burden of a given species.

Satellite tracking data has been used to document seasonal movements, haul-out and dive patterns, and habitat use of marine mammals by providing detailed records of their

geographical locations (Heide-Jorgensen et al., 1992; Kelly et al., 2010) (Table 2). Studies involving tagging of marine mammals are increasing and advances in satellite tag technology, have led to improvements in both their performance and size. However, such studies are expensive and logistically challenging.

Stable isotopes of carbon and nitrogen have been used to assess food web transfer of PCBs in aquatic food webs (Fisk et al., 2001a). The ratio of the heavier to lighter stable isotopes of nitrogen (e.g. $^{15}N/^{14}N$), expressed relative to a standard, generally increases with trophic position in aquatic food webs, providing a continuous variable with which to assess both trophic level and food web transfer (Fisk et al., 2001a). In contrast, the ratios of stable carbon isotopes ($^{13}C/^{12}C$) in biota can help to elucidate trophic interactions by establishing the relative contribution of marine (or pelagic) versus coastal (or benthic) carbon sources (France and Peters, 1997). The use of stable isotopes has advantages over traditional methods, such as analysis of stomach contents, as it averages dietary assimilation over a longer period of time (Hoekstra et al., 2003) (Table 2). However, stable isotopes do not allow for specific identification of prey and relative proportion of ingestion (Hoekstra et al., 2003).

In addition to stable isotopes, fatty acids have emerged as a powerful tool for quantitative assessment of predator diets (Budge et al., 2006; Iverson et al., 2004). Studies that have compared the fatty acids found in predator fat stores with those found in their prey have allowed both qualitative and quantitative comparisons of the spatial and temporal scales of foraging (Falk-Petersen et al., 2004; Iverson et al., 2004). This is possible because the fatty acids consumed by predators with single-chambered stomachs are deposited into adipose tissue with little modification or in a predictable pattern, thus

providing an integrated record of dietary intake over time (Budge et al., 2006). Fatty acid profiles or "signatures" can be used to answer qualitative questions about spatial or temporal variations in diets both among and within individual marine mammals or marine mammal populations (Iverson et al., 1997).

Information obtained using telemetry and feeding ecology tracers (i.e., stable isotope and fatty acid profiles) combined with contaminant residues in prey and/or predator can provide important insight to contributions of different pollutant sources. For example, Elliott et al. (Elliott et al., 2007) used satellite tracking of osprey to demonstrate that the northern breeding grounds of this fish-eating bird represented the principal source of contaminant exposure. Satellite telemetry has also been used to investigate geographical differences in contaminant concentrations and patterns in loggerhead sea turtles (*Carella caretta*) and polar bears (*Ursus maritimus*) (Olsen et al., 2003; Ragland et al., 2011).

Fatty acid profiles and stable isotopes have been used to describe dietary processes for contaminants in beluga whales (Loseto et al., 2008) and dietary differences which resulted in regional contaminant level differences (McKinney et al., 2011) and temporal contaminant burden declines (McKinney et al., 2013) in polar bears. These studies have typically used only one of these feeding ecology and habitat use techniques (Table 2) in combination with contaminants to better understand regional contaminant sources, trends, or food web transfer in upper trophic level marine species, but no studies have applied multiple techniques (Tables 1 and 2) to assess the impacts of point sources in marine ecosystems.

Table 1: A variety of contaminant measurement strategies have been employed to characterize sources of contamination in marine mammals and seabirds and their prey.

Technique used	Advantages	Disadvantages	References
Basic contaminant concentration analyses	Cost effective	Only useful as a crude measure of contamination	Addison and Smith 1974 Addison and Brodie 1977 Muir et al. 1995 Muir et al. 2000
Contaminant ratios	Cost effective; provides first tier indication of dominant contaminants	Limited ability to infer source or fate	Elliott et al., 2007 Krahn et al., 2007 Calambokidis &Barlow, 1991 Krahn et al, 1999 Muir et al., 1990
High resolution contaminant profiles	Can explore complex signatures using multivariate statsitcial tools	More costly; requires high resolution instrumentation	Ross et al., 2004

Table 2: A variety of feeding ecology and habitat use strategies have been employed to characterize diet in marine mammals, semiaquatic mammals and seabirds and their prey.

Technique used	Advantages	Disadvantage	References
Gut analysis	- cost effective - allows for specific identification of prey	- identifies only recent prey consumed - inaccuracies due to digestion - underestimates low trophic level consumption	McLaren, 1958 Lowry et al., 1980 Gjertz & Lydersen, 1986 Holst et al., 2001 Hammill et al., 2005
Scat analysis	- cost effective - allows for specific identification of prey	- identifies only recent prey consumed - underestimates low trophic level consumption - overestimates fish consumed	Andersen et al., 2004 Browne et al., 2002 Da Silva & Neilson, 1985 Guertin et al. 2010 Guertin et al. 2012
Stable isotopes	- cost effective - averages dietary assimilation over a long period of time	- does not allow for specific identification of prey, or - relative proportion of ingestion	Hobson et al., 1996 Lawson & Hobson 2000 Hammill et al., 2005 Bentzen et al., 2007
Fatty acids	- cost effective - allows for specific identification of prey	- requires extensive prey database	Iverson et al., 1997 Iverson et al., 2004 Andersen et al., 2004 Loseto et al., 2009
Telemetry	- provides information on space-use, and - foraging depths	- expensive - logistically challenging	Gjertz et al. 2000 Olsen et al., 2003 Elliott et al., 2007 Harwood et al., 2012

1.4 Local contamination by a military radar station in Labrador, Canada

Concerns regarding food safety with respect to ringed seals (*Pusa hispida*) and general health of the ringed seal population in northern Labrador have arisen following reports of elevated polychlorinated biphenyl (PCB) concentrations in some ringed seals. Ringed seals are a source of country food to local Inuit and as such play an integral role in their diet culture. These marine mammals feed opportunistically on a variety of fish and crustaceans and are important prey of polar bears and arctic foxes (Wolkers et al., 2008; Wolkers et al., 1998). As a result, ringed seals play a critical role in the dynamics of arctic marine ecosystems. High trophic level marine mammals are particularly vulnerable to POPs, such as PCBs (Cullon et al., 2005). Ringed seals along the Labrador coast are exposed to various stressors, including contaminants, changing sea ice conditions, disease and local industrial activities. These factors make ringed seals an excellent species of choice for assessing and monitoring contaminant accumulation, contaminant-related health effects at the top of the food web, and/or to evaluate the status of the marine ecosystem (Wolkers et al., 2008). Although long range transport is the primary source of contaminants to Labrador, local sources of PCBs also exist at Saglek Bay due to historical operations at the former military radar site (Figure 3).

During a preliminary site investigation in 1996, PCB contamination in excess of 50 parts per million (ppm), the maximum allowable amount specified in the Canadian Environmental Protection Act (CEPA) PCB material storage regulations, was discovered in three areas (Site Summit, Antenna Hill and beach area) at the site, along with evidence that PCBs had entered the marine ecosystem of Saglek Bay (ESG, 2000). Over three seasons (1997 to 1999), the contaminated soil was cleaned up by the Department of

National Defence (DND) (ESG, 2000). The beach area was identified as the first priority because of the vulnerability of marine ecosystems to PCB contamination. As a result, 28,500 m^3 of soil (enough to cover a Canadian football field to a depth of approximately 7 m) with PCB concentrations greater than 5,000 ppb was excavated and removed from the beach area. In conjunction with the excavation and removal of the PCB-contaminated soil from the terrestrial environment at Saglek, an extended study was undertaken to assess the contamination in the marine sediments and associated PCB uptake and accumulation in species representing various trophic levels of the local food web (Kuzyk et al., 2005a) (Figure 4). An effects-based ecological risk assessment (ERA) was completed to identify risks to the local biota (Brown et al., 2013). The PCB-contaminated marine sediments were widely distributed (10 km^2, Figure 3) between the mainland, where the radar station is situated, and Big Island, located approximately 6 km to the north (Brown et al., 2009; Kuzyk et al., 2005a). The PCB contamination was largely localized to the Saglek Anchorage area and decreased exponentially with increasing distance from the source beach (Brown et al., 2009; Kuzyk et al., 2005a). Average PCB concentrations in the nearshore marine sediments exceeded the Canadian sediment quality guideline (21.5 ng/g dry weight (dw)) by 41-fold and PCB concentrations in benthic invertebrates, shorthorn sculpin, and black guillemots were exceptionally high (Kuzyk et al., 2005a). Results of the ERA indicated that the survival and reproduction of shorthorn sculpin and black guillemot nestlings were at risk (Brown et al., 2013).

Relatively high PCB concentrations were also measured in some ringed seals and great black-backed gulls (*Larus marinus*), and an alarmingly high PCB concentration (9,400 ng/g wet wt) was measured in the blubber of a 10-yr-old male ringed seal from

Saglek Bay (Kuzyk et al., 2005a). Comparable concentrations had never been previously measured in ringed seals in the Canadian Arctic (Kuzyk et al., 2005a). Concentrations of PCBs in surface sediments and the marine benthic-associated food web clearly reflected the input of the local source, but no conclusions could be drawn for the high PCB concentrations measured in the ringed seals (Kuzyk et al., 2005a).

More recently, preliminary data collected from environmental monitoring between 2008 and 2011 indicate that ringed seals from the Labrador coast have the highest PCB concentrations in the Canadian Arctic possibly due to both long range "background" and local "hotspot" PCB contamination (Figure 5). Understanding the degree to which the local source at Saglek contributes to the PCB levels in ringed seals is needed to inform mitigation and monitoring efforts for the Saglek Bay area. Age and sex do not appear to be contributing factors for the elevated concentrations, as seals of the same sex and approximately the same age have concentrations significantly lower than those of 'hot' seals. Ringed seals have a home range (2,138 km, average migration distance, 706-6,140 km, range, for ringed seals in Beaufort and Chukchi Seas (Harwood et al., 2012) that is far greater than the spatial extent (~10 km^2) of the contaminated sediments and thus were not expected to be strongly influenced by the local PCB contamination. It is possible, however, that the seals with elevated PCB concentrations are ranging over a more local area within Saglek Bay for longer periods of time. Another possible explanation for these elevated seals is a difference in diet.

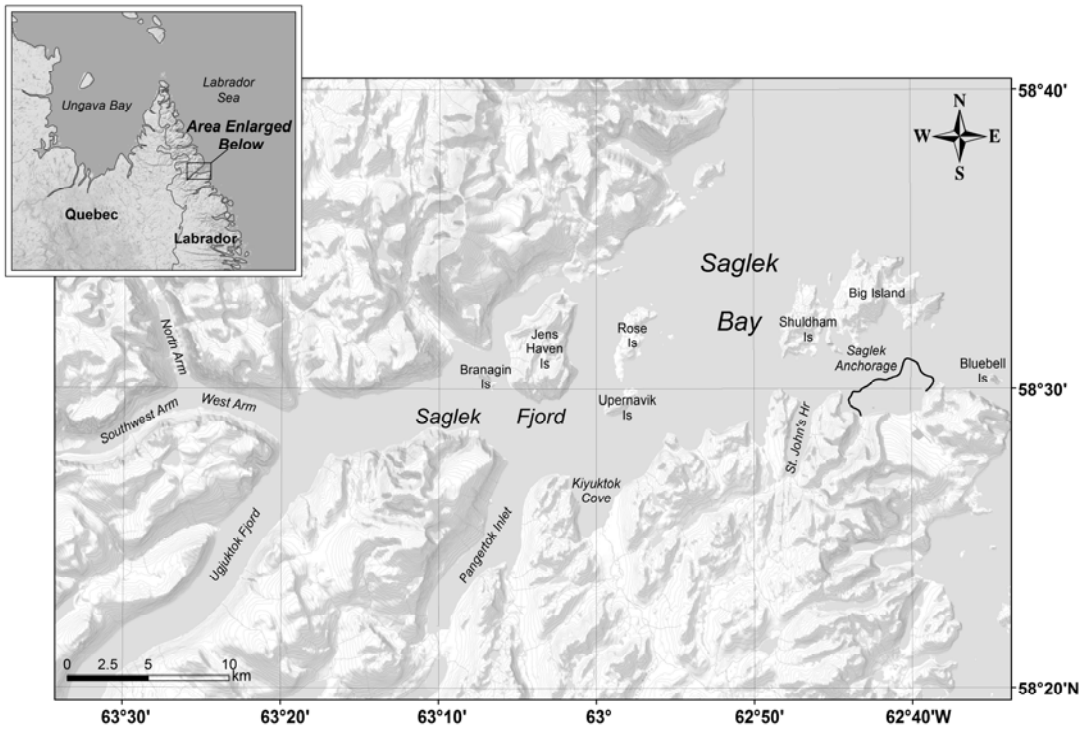

Figure 3: Map of Saglek Fjord, showing the extent of marine sediment PCB contamination (solid line) in the Saglek Anchorage area.

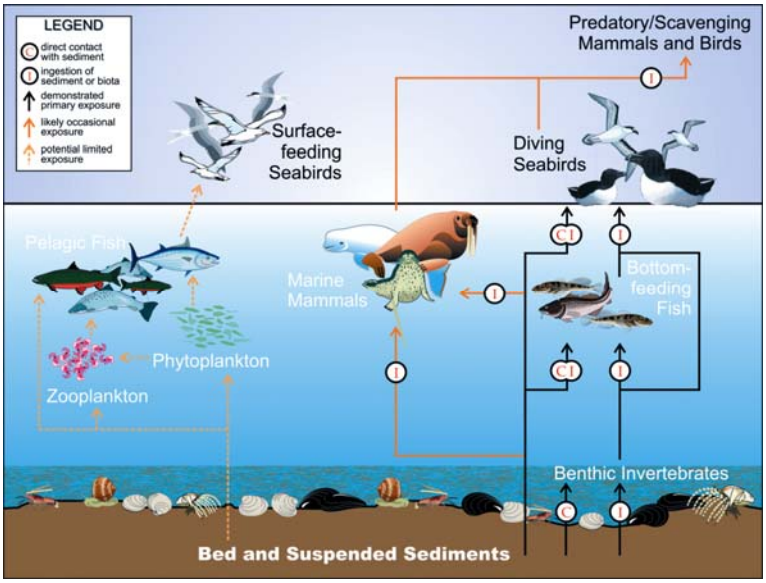

Figure 4: Conceptual model depicting the primary polychlorinated biphenyl (PCB) exposure routes from contaminated sediments in Saglek Bay, Labrador, Canada (adapted from Brown et al., 2013).

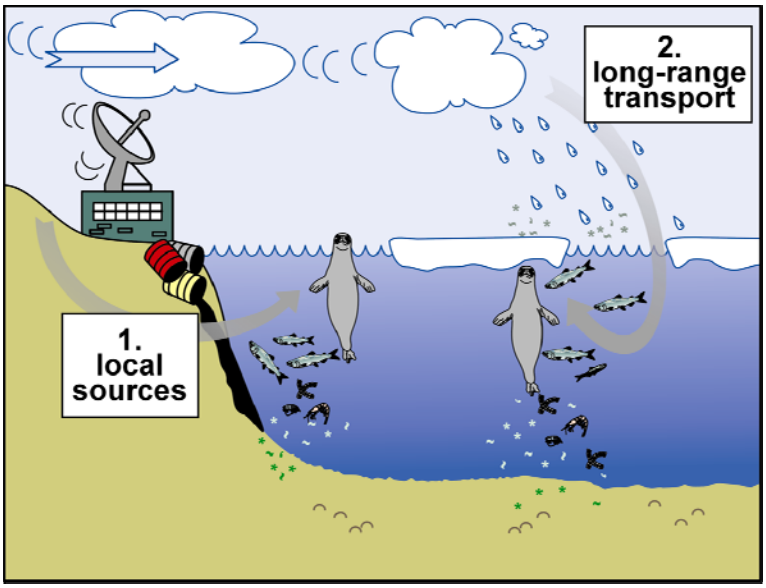

Figure 5: A simplified schematic showing the two possible polychlorinated biphenyl (PCB) sources (local sources from Saglek Bay and long-range atmospheric transport sources from southern industrial regions) delivered to ringed seals in Labrador.

1.5 Health risks related to contaminants of concern in marine mammals

POPs pose a risk to the health of marine mammals with studies revealing a wide range of endocrine disruptive effects including failures in reproduction, thyroid hormone and vitamin homeostasis, adrenal function, bone formation, and tumours in populations inhabiting contaminated areas (Beland et al., 1993; Bergman and Olsson, 1985; Bergman et al., 1992; Helle et al., 1976b; Jenssen et al., 2003; Mos et al., 2007; Olsson et al., 1994; Routti et al., 2010a; Tabuchi et al., 2006). PCB-related immunosuppression has been associated with PCBs in polar bears (*Ursus maritimus*) (Lie et al., 2005), ringed seals (Routti et al., 2010a), bottlenose dolphins (*Tursiops truncatus*) (Lahvis et al., 1995) and captive feeding studies of harbour seals (*Phoca vitulina*) (Ross et al., 1996). Positive relationships between the aryl hydrocarbon receptor (*Ahr*) and PCB concentrations have also been observed in killer whales (*Orcinus orca*) (Buckman et al., 2011), striped

dolphins (*Stenella coeruleoalba*) (Panti et al., 2011), and fin whales (*Balaenoptera physalus*) (Fossi et al., 2010).

The complexity of ecological systems greatly complicates toxicological studies involving free-ranging marine mammals. This is due to the presence of hundreds of different chemicals in the tissues of marine mammals (even in remote waters), the confounding influence of multiple ecological and biological factors, and a limited suite of methods to measure health endpoints in marine mammals. While numerous field studies have shown associations between individual POPs and adverse health effects in marine mammals, these studies have been confounded by other co-occurring contaminants. The combination of long range "background" and a local PCB "hotspot" on the Labrador coast provides an invaluable opportunity to evaluate the effects of PCBs on the health of a free-ranging marine mammal as the concentration of this chemical dominates those of other POPs.

Transcriptomics offers a particularly powerful tool for evaluating the effects of contaminants on marine mammals (Buckman et al., 2011; Mollenhauer et al., 2009; Mos et al., 2007; Tabuchi et al., 2006). Exposure to different contaminants can create unique patterns of gene expression which can reflect contaminant-related alterations at the molecular level (Ankley et al., 2006). While a variety of methods have been used for gene expression quantification, quantitative real-time polymerase chain reaction (qPCR) and DNA microarrays have been the most widely used techniques in recent years. qPCR is considered the "gold standard" method with the primary advantages being the relative simplicity of experiments, cost-effective, precise quantification of mRNA levels, and its ability for the analysis of hundreds of samples (or genes) simultaneously. qPCR analyses

are particularly useful for low abundance transcripts and for studies where limited tissue sample is available (Pfaffl et al., 2004).

Monitoring the changes in mRNA transcripts encoding detoxification proteins can be used to study the biological response of an animal to contaminant exposure. Contaminants can modulate receptor activity and change the pattern of mRNA expression (Veldhoen et al., 2012). For example, dioxin-like PCBs bind to the aryl hydrocarbon receptor (AHR) and modulate the activity of the transcriptional regulator which, in turn, induces expression of phase 1 and 2 xenobiotic detoxification enzymes, including cytochrome P450 enzymes (*Cpy1a1*, *Cyp1a2*, and *Cpy1b1*) and uridine 5'-diphospho-glucuronosyltransferase (*Ugt1a1*) (Stejskalova et al., 2011). These molecular indicators can therefore provide early warning signals of toxicant exposure and potential for adverse biological effects.

This molecular biomarker approach has been identified as a valuable, non-lethal, and effective means of evaluating the health and toxicity of other marine mammals (Buckman et al., 2011; Mos et al., 2007; Routti et al., 2010a; Tabuchi et al., 2006). For example, PCB-related increases have been observed in harbour seal blubber transcriptome constituents, retinoic acid receptor α (*Rara*) and thyroid hormone (*Thra*) receptor α mRNA (Mos et al., 2007; Tabuchi et al., 2006). In addition, associations between PCBs and levels of mRNA transcripts related to adverse effects have been shown in killer whales inhabiting the Northeastern Pacific (Buckman et al., 2011), ringed seals from the Baltic Sea (Routti et al., 2010b), striped dolphins and fin whales from the Mediterranean Sea (Fossi et al., 2010; Panti et al., 2011), and beluga whales from the Western Canadian Arctic (Noel et al., 2014). In all these cases, however, correlations between health effects

and PCB concentrations are based on the premise that PCBs are either the putative contaminant driving the relationship or represent a proxy for the other co-correlating POPs.

In the present thesis, I investigate the relationship between ringed seal blubber PCB burden and hepatic mRNA abundance profiles of gene transcripts encoding proteins that play an important role in animal health with respect to chemical detoxification, the immune and endocrine systems, and the regulation of growth, development, and metabolism. The dominance of PCBs in the seals from northern Labrador enabled an assessment of the effects of this chemical on the health of a highly mobile predator, something that is rarely possible in the world of complex mixtures.

1.6 Objectives

The main objectives of this thesis are:

1) to characterize the contribution of a major point source of PCBs to the background burden in ringed seal food webs in Labrador using a multi-faceted approach (i.e. stable isotope of $\delta^{13}C$ and $\delta^{15}N$, fatty acid profiles, telemetry and contaminant pattern analysis).

2) *to evaluate PCB-related health effects in Labrador ringed seals*

The first three chapters address the first objective. In Chapter 2, contaminant levels, patterns and ratios are evaluated in ringed seal blubber samples from four marine inlets in northern Labrador, Canada. This enables an evaluation of the importance of a local PCB source to an upper trophic level marine mammal in the Arctic. By evaluating a variety of persistent organic pollutants not associated with the PCB point source in this study (e.g. organochlorine pesticides), I am able to distinguish between local and long-range contaminant sources on the basis of the contaminant profiles.

Habitat use measures derived from satellite telemetry and stable isotope ($\delta^{13}C$ and $\delta^{15}N$) profiles for ringed seals from Saglek Fjord in Chapter 3 provide indirect and direct measures related to feeding and foraging ecology, the basis of exposure to persistent pollutants including PCBs. Information obtained from these measures complement the contaminant profiles characterized in Chapter 2 and heighten the ability to distinguish the importance of the PCB source in Labrador to ringed seal contamination.

Fatty acid signatures and PCB profiles in ringed seals and their prey from Labrador help to characterize the feeding preferences of ringed seals in Chapter 4, and offer insight into the ways in which local PCBs are entering the food web and accumulating in ringed seals. This confirms the divergent contaminant profiles observed in ringed seals in

Chapter 2 can indeed be attributed to feeding on locally contaminated prey, and not to a difference in feeding preferences between two groups of seals.

Because of the highly complex contaminant profiles in free-ranging, high trophic level marine mammals, no studies exist that have been able to elucidate the effects of a single contaminant class on seals. Some ringed seals inhabiting areas in and around Saglek Fjord have been found to exceed an established endocrine and immunotoxicity effects threshold (1,300 ng/g lipid wt; blubber) established for harbour seals (Mos et al., 2010), highlighting a unique opportunity to examine the effects associated with this one industrial chemical. Chapter 5 addresses objective 2 in which I investigate the relationship between ringed seal hepatic mRNA abundance profiles of gene transcripts and PCB concentrations in blubber. Gene transcripts selected for study encode proteins that play an important role in animal health with respect to chemical detoxification, the immune and endocrine systems, and the regulation of growth, development, and metabolism. The local PCB 'hotspot' on the Labrador coast provided an invaluable opportunity to evaluate the effects of this one chemical on the health of a free-ranging marine mammal. Evaluating the health risks to ringed seals is particularly important as they are a valued component of the traditional diet of Inuit in Nunatsiavut.

Chapter 2: Polychlorinated biphenyl profiles in ringed seals (*Pusa hispida*) reveal historical contamination by a military radar station in Labrador, Canada

Abstract

Significant amounts of soil contaminated with polychlorinated biphenyls (PCBs) were discovered at a military radar station in Saglek Bay, Labrador (Canada) in 1996, and subsequent work found elevated PCB concentrations in local marine sediments, the benthic-associated food web and in some ringed seals (*Pusa hispida*). The benthic-associated food web clearly reflected local PCB contamination, but the high PCB concentrations found in some ringed seals remained unexplained. In this study, we assess the extent to which this local PCB source at Saglek Bay is contributing to the contamination of ringed seals in northern Labrador. Of 63 ringed seals sampled along the northern Labrador coast, five (8%) had PCB levels that were higher than recorded anywhere else in the Canadian Arctic. In addition, compared to seals exhibiting a long-range signal, 45% and 60% of sub-adults and adult males, respectively exhibited heavier PCB congener profiles as characterized by Principal Components Analysis, >1.6-fold higher PCB/OCP ratios, and higher PCB concentration-weighted average log K_{ow} values, consistent with a local source. Despite the spatially confined nature of contaminated sediments in Saglek Bay, the influence of this PCB source is not inconsequential; PCB concentrations in locally contaminated adult males are two-fold higher than those exposed only to long-range PCB sources, and exceed an established threshold of 1.3 mg/kg lw for adverse health effects in seals.

2.1 Introduction

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) bioaccumulate to very high concentrations in high trophic level Arctic marine mammals (Borga et al., 2004; Muir et al., 2000). While long-range atmospheric transport is the primary pathway by which POPs reach the Arctic (Macdonald et al., 2000; Muir et al., 1999a), local sources within the Arctic (e.g. military sites) also exist and can contaminate local food-webs (Bright et al., 1995; Kuzyk et al., 2005b). A PCB source (commercial Aroclor 1260 mixture) associated with a military radar station in Saglek Bay, Labrador has contaminated the local benthic food-web (Kuzyk et al., 2003; Kuzyk et al., 2005a; Kuzyk et al., 2005b). Saglek Bay has been the site of a military radar station since the late 1950s, however it wasn't until 1996 that the PCB contamination, in excess of 50 parts per million (ppm), the maximum allowable amount specified in the Canadian Environmental Protection Act (CEPA) PCB material storage regulations, was discovered in three areas (Site Summit, Antenna Hill and beach area) at the site, along with evidence that PCBs had entered the marine ecosystem of Saglek Bay (Kuzyk et al., 2005b).

From 1997 to 1999, concurrent with the terrestrial remediation of PCBs, a study was undertaken to assess PCB contamination in the marine sediments and associated uptake and accumulation in species representing various trophic levels of the local marine food web (Kuzyk et al., 2005b). An effects-based ecological risk assessment (ERA) was completed to identify risks to the local biota (Brown et al., 2013). The PCB-contaminated marine sediments were widely distributed between the mainland, where the radar station is situated, and Big Island, located approximately 6 km to the north (Kuzyk et al., 2005b).

Average PCB concentrations in the nearshore marine sediments exceeded the Canadian sediment quality guideline (21.5 ng/g dry weight, CCME 1999) by 41-fold and PCB concentrations in benthic invertebrates, a bottom feeding fish (shorthorn sculpin, *Myoxocephalus scorpius*), and a diving seabird (black guillemots, *Cepphus grylle*) were exceptionally high (Kuzyk et al., 2005b). Results of the ERA indicated that the survival and reproduction of shorthorn sculpin and black guillemot nestlings were at risk (Brown et al., 2013). Relatively high PCB concentrations were also measured in some ringed seals (*Pusa hispida*) and great black-backed gulls (*Larus marinus*), and one alarmingly high PCB concentration (9,400 ng/g ww) was measured in the blubber of a 10-year old male ringed seal from Saglek Bay (Kuzyk et al., 2005b). Comparable concentrations had never been previously found in ringed seals in the Canadian Arctic (Kuzyk et al., 2005b). Concentrations of PCBs in surface sediments and the marine benthic-associated food web clearly reflected the input of the local source but no conclusions could be made for the high PCB concentrations measured in the ringed seals (Kuzyk et al., 2005b). These marine mammals have a home range (2,138 km, average migration distance for ringed seals in the Beaufort and Chukchi Seas, (Harwood et al., 2012)) that is far greater than the spatial extent (~10 km^2) of the contamination at Saglek Bay (Born et al., 2004; Brown et al., 2009; Kuzyk et al., 2005b) and thus were not expected to be strongly influenced by the local PCB contamination.

Ringed seals are especially vulnerable to elevated exposure of PCBs as a result of their high trophic level, low detoxification capacity, large lipid reserves, and long life span (Boon et al., 1992; Nyman et al., 2003). Elevated PCB concentrations in ringed seals from the northern Labrador coast could represent a serious threat to the health of ringed

seals and the local wildlife that prey on them (e.g. polar bears). Furthermore, since ringed seals are an important part of the local Inuit diet (Laird et al., 2013), PCB exposure of local communities harvesting along the northern Labrador coast may be of significant concern. By understanding these PCB sources to ringed seals and their concentrations, we can better understand and manage the potential health risks and/or effects.

Marine mammals are exposed to complex mixtures of POPs, thus researchers have used a variety of tools to pinpoint regional (i.e. local) POP sources. POP ratios is one approach which has been used to assess regional sources of POPs transferred to predators from their prey (Calambokidis and Barlow, 1991; Krahn et al., 2007; Muir et al., 1990). As a consequence of the heavy use of DDTs in California before their ban in the 1970s, the ∑DDTs/∑PCBs ratio is generally higher in Californian marine species than in species from other North Pacific locations, thereby providing a "California signature" (Calambokidis and Barlow, 1991; Jarman et al., 1996). PCB congener pattern analysis is another approach which has been used to determine a 'regional' signature (i.e. a result of heavier congeners from regional sources adhering to organic particles and remaining closer to source) versus 'long-range' signature (i.e. a result of the volatilization of PCBs away from distant sources) (Ross et al., 2004). For example, due to long-term discharges of PCBs into Puget Sound, harbour seals from this area were found to have a 'regional' or 'heavy' (i.e. increased degree of chlorination) PCB signature relative to harbour seals from more remote waters which had a 'global' or 'long-range' signature with a greater contribution of lighter PCB congeners (Ross et al., 2004). The objective of the present study was to assess whether the local source at Saglek Bay is contributing to the elevated PCB levels in ringed seals from the north Labrador coast. To this end, we investigate not

only PCBs in ringed seals but also five OCPs which usually exhibit similar patterns of transport and fate to that of PCBs. Ringed seals were collected from four marine inlets in northern Labrador, Canada (Figure 6): Nachvak Fjord, a pristine fjord surrounded by Torngat Mountains National Park (TMNP); Saglek Fjord, a fjord with a PCB point source of contamination; Okak Bay, a fjard in central Labrador frequently used for harvesting and travel by Inuit from Nain, the nearest community approximately 100 km to the south; and Anaktalak Bay, another fjard in central Labrador that is the shipping route to a mine and concentrator at the head of the bay (Figure 6). A 'fjard' is a type of inlet which is similar to a fjord, however it has shallow, irregularly shaped and glacially formed inlets with more gently sloping sidewalls and large intertidal zones (Brown et al., 2012).

No known local sources of OCPs exist along the northern Labrador coast; therefore seals that have been influenced by Saglek's local source would likely show elevated levels of PCBs relative to OCPs. We also evaluate the patterns of PCB congeners in ringed seals to determine if there is a difference in congener composition, signifying more than one PCB source. Condition and diet indices were evaluated to eliminate the ecological and biological factors that confound the interpretation of contaminant data in marine mammals.

2.2 Materials and Methods

Sample Collection

Tissue samples (muscle and blubber) from ringed seals (n=63, Table 3) were obtained from Inuit hunters at several locations in the four marine inlets during the fall (September and October) of 2008. Prey species: shorthorn sculpin (*Myoxocephalus* scorpius), sand

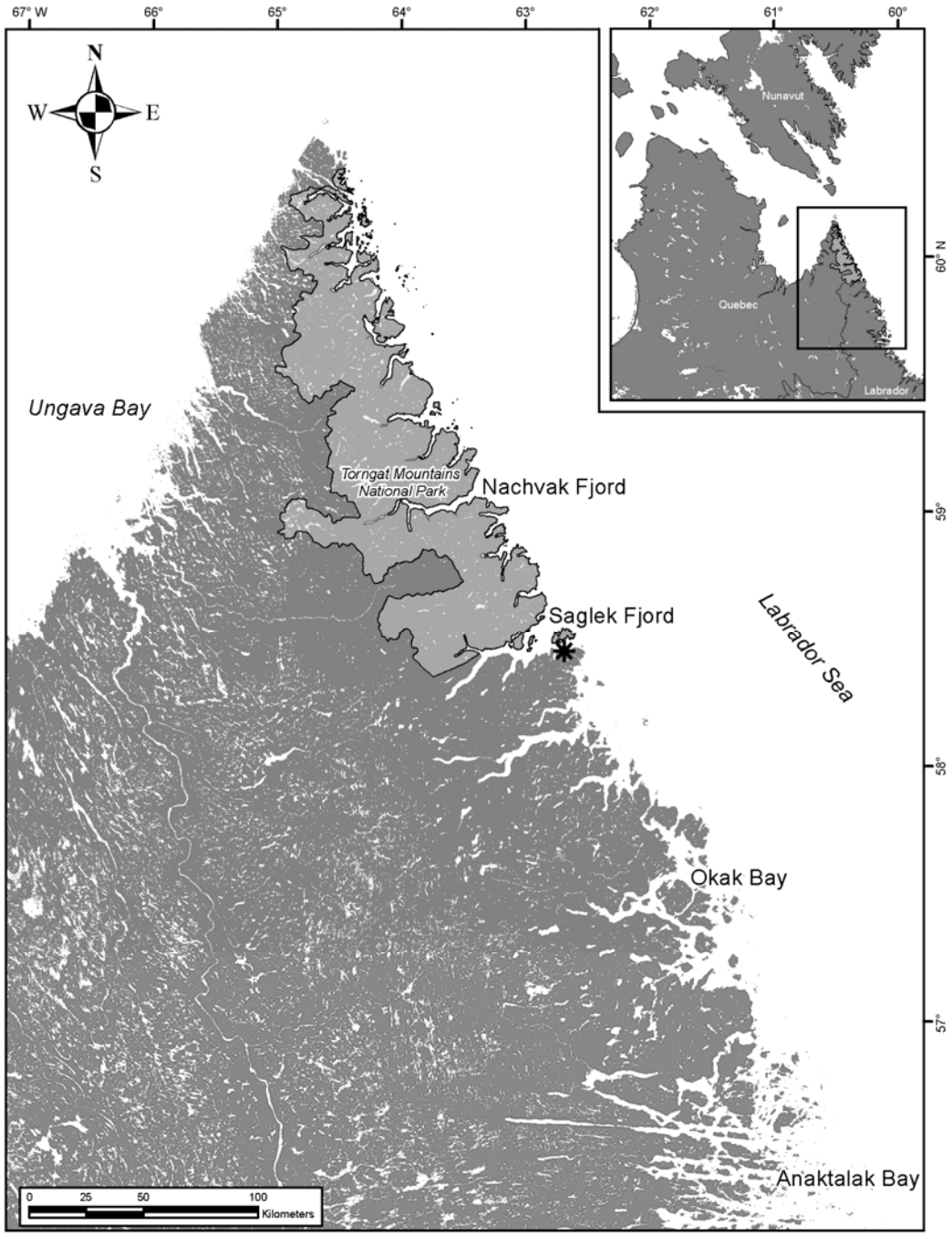

Figure 6: Map of northern Labrador, Canada showing the location of the four fjords where ringed seal collections were taken. Asterisk (*) shows the location of the former PCB source and PCB contaminated sediments at Saglek Bay.

lance (*Ammodytes* spp.), daubed shanny (*Leptoclinus maculatus*), rock cod (*Gadus ogac*), northern astarte (*Astarte borealis*), and chalky macoma (*Macoma calcarea*), were collected from 2008-2011 from the zone of contamination in Saglek Bay (i.e. Saglek Anchorage) and from several locations in the three reference inlets. All samples were placed in aluminum foil and Whirl Pak bags and frozen at -20 °C until analysis. Sex, weight, length, girth and blubber thickness (at the sternum) were determined in the field for all ringed seals. Ages were determined by Matson's Laboratory, USA, by longitudinal thin sectioning a lower canine tooth and counting the annual growth layers in the cementum using a compound microscope and transmitted light (Stewart, 1996). All samples were stored at -20 ^{o}C until analyzed for stable isotopes (muscle) and organochlorines (blubber) within 1 year of sample collection.

For all samples collected, appropriate permits and community approval was obtained from the Nunatsiavut Government, Nunatsiavut Health and Environment Review Committee and Department of Fisheries and Ocean Canada.

Chemical Analysis

Concentrations of 53 PCB congeners (19, 18/17, 16/32, 26, 28/31, 33/20, 22, 45, 46, 52, 49, 47/48, 44, 64/41/71, 74, 70/76, 66/95, 60/56, 92/84/101, 99, 87, 85, 110, 151/82, 144/135, 149, 118, 146, 153, 105/132, 141, 137, 130/176, 138/163, 158, 178, 187/182, 183, 128/167, 185, 174, 177, 156/171/202, 157/201, 172, 180, 170/190, 199, 203/196, 195/208, 207, 194, 206) and OCPs: α-, β-, γ-hexachlorocyclohexane, α- and γ-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, heptachlor epoxide, *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT, dieldrin, hexachlorobenzene (HCB) and percent lipid were measured in ringed seal blubber samples and prey by the Great Lakes Institute for Environmental Research's

accredited organic analytical laboratory, Windsor, ON (Canadian Association for Laboratory Accreditation and ISO17025 certified). OCP (Quebec Ministry of Environment Congener Mix) and PCB standard mixtures were supplied by Sigma-Aldrich (Oakville, ON, Canada) and AccuStandard (New Haven, CT, USA), respectively. 1,3,5-tribromobenzene (TBB) was used as the OCP/PCB recovery efficiency standard.

Homogenized wet tissue (0.5 – 1 g), anhydrous sodium sulphate and surrogate standard were ground with motor and pestle and then extracted following the micro-extraction technique described in (Daley et al., 2011; Daley et al., 2009). Sample lipid contents were determined gravimetrically using 1 mL of sample extract and a microbalance (GD, 2004). The remaining extract was concentrated to 2 mL under vacuum with sample cleanup performed by Florisil (F100-500, Fisher Scientific, Ottawa, ON) chromatography as described by Lazar et al. (GD, 1992), followed by collection of the first (50 mL hexane; ACP, Montreal, Quebec, Canada) and second fractions (50 mL; hexane/dichloromethane 85/15 v/v; Fisher Scientific, Fair Lawn, NJ). After Florisil chromatography, extracts were concentrated to 1 mL under vacuum and transferred to 1.8 mL gas chromatography (GC) vials. Samples were analyzed for individual PCB congeners and OCPs by GC electron capture detection (GC-ECD).

For each batch of six samples, an in house reference homogenate tissue (carp muscle), method blank, and the external TBB recovery standard were analyzed. All PCB congeners and OCPs were detected with sufficient frequency to be included in the data analysis. Recoveries of individual PCB congeners in the homogenate reference tissue with each batch of samples were within 2 standard deviations from the mean laboratory database value derived from laboratory control charts. Recovery efficiencies for the TBB

standard were 89 ± 0.9% (mean ± SE). Procedural blanks (n = 18) were below detection for all PCB congeners and OCPs. Sample PCB congener and OCP concentrations were recovery corrected.

Hereinafter ∑PCBs refers to the sum of the 53 PCB congeners, ∑HCH refers to the sum of α-, β-, γ-hexachlorocyclohexane, ∑chlordanes refers to the sum of α- and γ-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, heptachlor epoxide, and ∑DDT refers to the sum of *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT.

Stable Isotope Analysis

Muscle tissue was freeze-dried and homogenized. Lipid was extracted from all samples by agitating the dried powered muscle tissue in a 2:1 chloroform-methanol solution for 24 h. The tissue and solvent were then filtered and the resulting residue-filter paper dried at 60°C for 48 h to evaporate the remaining solvent. Five hundred µg of lipid extracted tissue was weighted into tin capsules, and stable carbon and nitrogen isotope ratios were analyzed by Continuous Flow Ion Ratio Mass Spectrometer (CFIR-MS) (Finnigan MAT Delta^{plus}, Thermo Finnigan, San Jose, California, USA). Stable isotope abundances are expressed in delta (δ) values as the deviation from standards in parts per thousand (‰) using the following equation:

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

where R is the ratio of heavy to light isotope ($^{15}N/^{14}N$ or $^{13}C/^{12}C$) in the sample and standard. The nitrogen stable isotope standard was atmospheric nitrogen; Pee Dee Belemnite limestone formation was the standard for the carbon stable isotope. Precision based on two standards (bovine muscle (NIST 8414) and an internal lab standard (tilapia fish muscle) n =144 for each) were <0.17 and <0.09‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively.

Accuracy of isotope analysis, based on NIST standards (sucrose (NIST 8542) and ammonia sulphate (NIST 8547); n = 3 for each) analyzed during the study were within <0.1‰ of certified $\delta^{15}N$ and $\delta^{13}C$ values.

Data Analysis

Total PCB and OCP concentrations and PCB congener composition in the blubber of phocids is influenced by age, sex, and reproductive status (Addison and Smith, 1974; Shaw et al., 2005; Wolkers et al., 2004). To control for these confounding factors, we separated the data into three groups for statistical exploration: sub-adults (<6 years; male and females combined), adult males (≥ 6 years) and adult females (≥ 6 years). Univariate statistical analyses were performed using the IBM SPSS 20.0 for Windows. The level of statistical significance used was $p \leq 0.05$. Data were log transformed when necessary to meet the normality assumptions for parametric analyses. Any PCB congeners less than the detection limit were replaced with a random number between the detection limit and zero. Geometric means and ranges were calculated and tissue concentrations were converted to a lipid-weight (lw) basis by dividing wet-weight (ww) values by the proportion of lipid in the samples. ∑PCB and OCP: ∑DDT, ∑chlordanes, ∑HCH, dieldrin, HCB concentrations are expressed on a lw basis. Linear regression analysis was used to determine relationships between contaminant concentrations and condition indices (length, girth, blubber thickness, % lipid) and stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) and between stable isotopes and condition indices. One-way ANOVA was used to compare the mean of contaminant concentrations and stable isotopes among sub-adult and adult ringed seals by gender and differences between locations. A Tukey's *post hoc* test was

performed to determine differences among the locations when significant differences were found by ANOVA.

Principal components analysis (PCA) was used to elucidate differences in PCB patterns in sub-adults and adult male ringed seals. Females seals were not used to study the differences in PCB patterns or ratios, because females can transfer individual persistent organic pollutants and congeners at differential rates to their offspring (Borga et al., 2004; Desforges et al., 2012). Samples were standardized to the total concentration total before PCA to remove artefacts related to concentration differences between samples. The centered log ratio transformation (division by the geometric mean of the concentration-normalized sample followed by log transformation) was then applied to these datasets to produce a dataset that was unaffected by negative bias or closure (Ross et al., 2004). Data were auto-scaled before PCA. One outlier was removed from the adult male PCA model.

Linear regression was used to assess the relationships between PCA projections and ∑PCB/OCP ratios, log K_{ow} values (log of the octanol-water partition coefficient, a proxy for particle affinity), and location. Log K_{ow} values for the PCB congeners were taken from Hawker and Connell (Hawker and Connell, 1988).

The log K_{ow} for each PCB congener was used to calculate concentration-weighted average log K_{ow} values (Grant et al., 2011) for each sub-adult and adult male ringed seal according to the following equation:

Concentration-weighted average log K_{ow} value

$$= \frac{\sum_{\text{all congeners}} \textit{concentration of individual congener x (log Kow) value for that congener}}{\left[\textit{total concentration of all congeners}\right]}$$

A one-way ANOVA was used to compare the mean of contaminant concentrations, ∑PCB/OCP ratios, and concentration-weighted average log K_{ow} values among sub-adults and adult ringed seals for differences between 'local' and 'long-range' seals.

2.3 Results and Discussion

PCBs and organochlorine pesticide concentrations

The average concentrations for condition indices (length, girth, blubber thickness, % lipid) (Table 3) and the average concentrations of PCBs and OCPs found in ringed seals in the present study (Table 4) generally fell within the range reported previously for ringed seals in the Canadian Arctic (Addison et al., 2005; Fisk et al., 2002; Muir et al., 1999a; Muir et al., 2000). However, two adult females (1,090 ng/g lw, 6-years-old from Okak Bay and 1,050 lw, 9-years-old from Nachvak Fjord) and three adult males (3,160 ng/g lw, 22-years-old from Saglek Bay, 3,380 ng/g lw, 28-years-old from Saglek Bay, and 8,770 ng/g lw, 20-years-old from Nachvak Fjord) exceeded the highest values for PCBs reported for adult female (821 ng/g lw) and adult male (3,060 ng/g lw) ringed seals at other Canadian Arctic locations (Holman, Resolute, Eureka, Arctic Bay, Grise Fjord, Pangnirtung) over the past 20 years (Fisk et al., 2002; Gaden et al., 2012; Hoekstra et al., 2002; Muir et al., 2000). OCP concentrations in four (three adult males and the 6-year old female) of the five seals were not elevated relative to the other ringed seals in the present study and were similar to OCP concentrations reported in ringed seals from other Canadian Arctic locations (Fisk et al., 2002; Hoekstra et al., 2002; Muir et al., 1999b; Muir et al., 2000). As a result, concentrations of ∑PCBs relative to the five OCPs measured (i.e. ∑PCB/OCP ratios) in these four seals were higher than the mean ∑PCB/OCP ratios for adult males and females collected from the area (Figure 7). The

elevated levels of PCBs relative to OCPs in these seals are most likely explained by the influence of different PCB sources (e.g. Saglek's local PCB contamination to the marine environment). The 9-year old adult female from Nachvak, however, had relatively high concentrations of all five OCPs, and as a result the ∑PCB/OCP ratio was lower than the mean ∑PCB/OCP ratio for adult females. A possible explanation for this is that this female seal, which was not pregnant at the time of sampling, may be infertile and as a result maintains a higher PCB and OCP burden.

Only two of the five ringed seals with elevated ∑PCB concentrations were from Saglek Fjord. While these two adult males may display some degree of site fidelity to this particular fjord, the other three seals from reference fjords suggest that a portion of the population may roam more widely. From our entire dataset, average PCB concentrations in adult male ringed seals from Saglek Fjord (1,751 ng/g lw; n=8) were higher (p=0.01) than concentrations detected in those sampled in Nachvak Fjord (448 ng/g lw; n=6) (Appendix 1). No differences ($p \geq 0.05$) were found among the four fjords for average concentrations of OCPs for adult males (Appendix 1). No differences ($p \geq 0.05$) were found among the four fjords for average concentrations of ∑PCBs and OCPs for adult female and sub-adult ringed seals (Appendix 2, 3). The results indicate that location influences PCB exposure more in adult males than in adult females or sub-adults.

The five seals with exceptionally high PCB concentrations from the present study support the previous observation of an anomalous 10-year-old male seal with unusually high PCB levels in the Saglek Bay area (Kuzyk et al., 2005b). To our knowledge, comparable PCB concentrations have not been found previously in ringed seals from other areas in the Canadian Arctic. Muir et al. (Muir et al., 1995) did however, find a

surprisingly high concentration (4,500 ng/g) in a 7-year-old female seal from Inukjuak (Eastern Hudson Bay), which was collected sometime between 1989-1991, but the concentration was still not as high as the concentrations reported in the 20-year old male from Nachvak from the present study and the 10-year-old male ringed seal from Saglek collected during the 1997-1999 site investigations (Kuzyk et al., 2005b). The PCB congener pattern was examined for the 7-year old female seal from Inukjuak, but the authors concluded that there was no evidence to suggest the influence of different PCB sources or altered metabolism (Muir et al., 1995).

Table 3: Morphometric, percent lipid, and stable isotope data for sub-adult (<6 years; male and female combined), adult male (≥ 6 years) and adult female (≥ 6 years) ringed seals collected from northern Labrador. Values represent mean ± standard error (SE). Stable isotope data were obtained from muscle tissue.

	Sub-adults	Adult male	Adult female
N	22	20	21
age (yr) (range)	0.45 (0-4)	16.1 (6-28)	13.6 (6-25)
sex (male:female)	6:16	NA	NA
length (cm)	110 ± 2.0	129 ± 1.9	141 ± 3.0
girth (cm)	81 ± 1.7	111 ± 2.2	107 ± 2.4
blubber thickness (cm)	3.8 ± 0.2	5.3 ± 0.2	5.6 ± 0.2
% lipid (blubber)	93.3 ± 0.7	93.7 ± 0.5	91.5 ± 1.6
$\delta^{13}C$ (‰)	-18.6 ± 0.1	-18.0 ± 0.1	-17.3 ± 0.9
$\delta^{15}N$ (‰)	13.7 ± 0.1	14.6 ± 0.2[a]	14.2 ± 0.2[a]

[a] $p<0.05$ compared to sub-adults

Table 4: Geometric means and ranges [ng/g lipid weight (range, 95% upper confidence limit)] of ∑PCB and organochlorine pesticide concentrations in blubber tissue of sub-adult (<6 years; male and females combined), adult male (≥ 6 years) and adult female (≥ 6 years) ringed seals collected from northern Labrador.

	Sub-adults	Adult male	Adult female
∑PCBs	356 (180-886, 494)[a]	818 (139-8770, 2380)	337 (69-1090, 543)[a]
∑DDTs	142 (51-522, 202)[a]	309 (71-1010, 571)	133 (30-851, 297)[a]
∑Chlordanes	79 (40-162, 100)[a]	163 (30-616, 303)	73 (16-307, 139)[a]
∑HCHs	49 (29-83, 57)	49.7 (18.5-118, 70)	45 (21-85, 58)
Dieldrin	25 (11-95, 40)	32 (10-78, 45)	25 (10-112, 40)
HCB	4.4 (2.4-6.5, 4.9)[a]	5.1 (2.8-8.0, 5.9)	4.7 (3.1-22, 7.2)

[a] $p<0.05$ compared to adult males

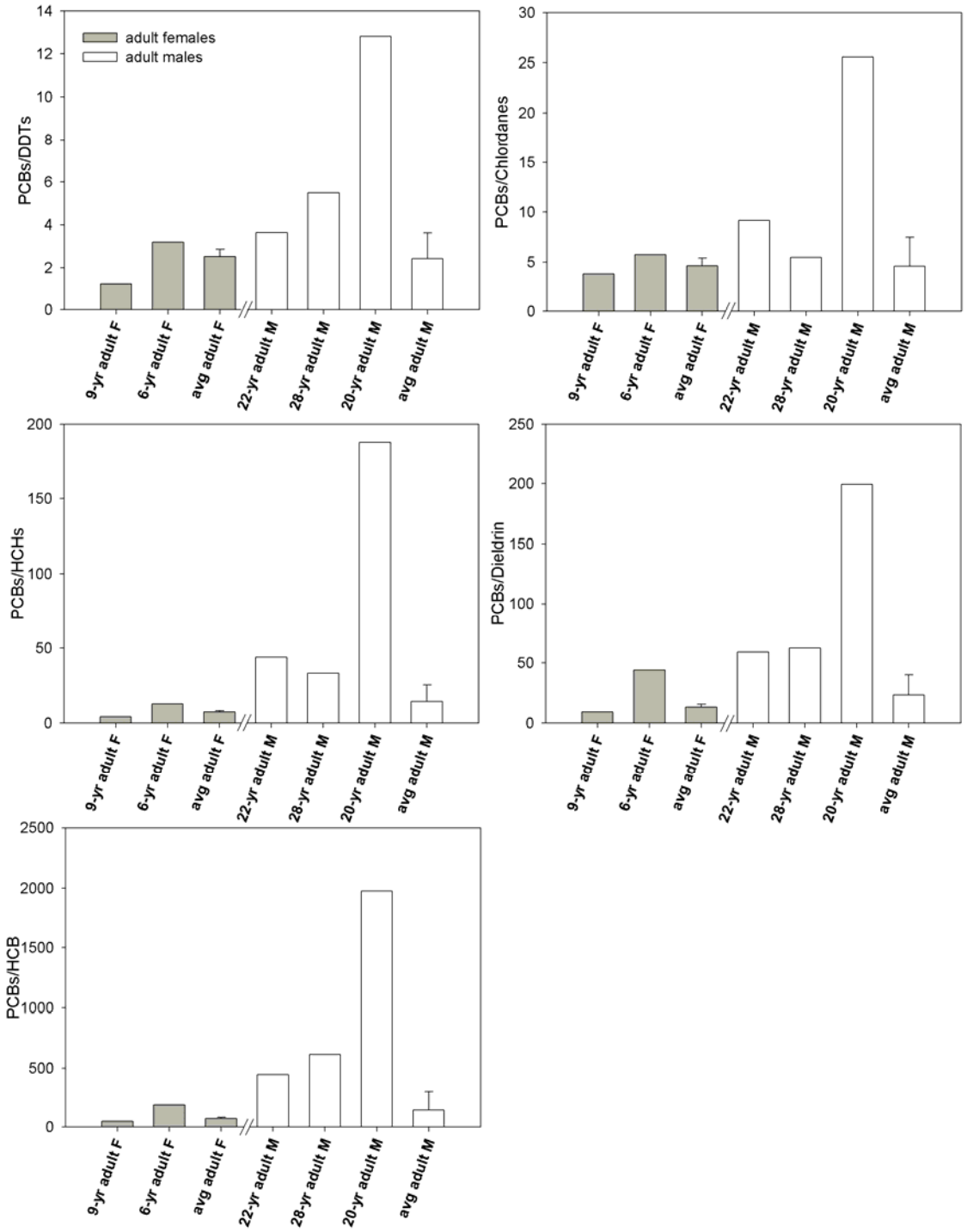

Figure 7: ∑PCB/OCP ratios in adult female and adult male ringed seals. Means and standard errors are shown for adult females (avg adult F) and adult males (avg adult M). Values are shown for the five ringed seals with elevated ∑PCB concentrations.

PCB congener patterns in sub-adult and adult male ringed seals

The first principal component (p1: sub-adults=47.4% and adult males=53.1%) clearly differentiates ringed seals with a greater proportion of the lighter (less-chlorinated) congeners from ringed seals with a greater proportion of the more heavily chlorinated congeners (Figure 8). The light PCB signature is typical of a long-range atmospheric transport signal (Wania and Mackay, 1993), which is a result of more volatile (e.g. lighter) congeners traveling great distances. Whereas, the heavy signature is more characteristic of a local source signal, where more chlorinated congeners (e.g. heavier) dominate the composition of PCBs (Ross et al., 2004). These divergent PCB signatures in ringed seals are consistent with observations in harbour seals where proximity to regional sources explained their heavier PCB profiles compared to those in more remote locations (Ross et al., 2004).

The ∑PCBs/OCP ratios correlated ($p<0.05$) with t1 (the sample scores of the first principal component) for sub-adult and adult male ringed seals (Figure 9; Table 5), indicating that ringed seals with a heavier PCB signature had a higher PCB concentration relative to the five different OCPs. These results are consistent with our observations above for four of the five ringed seals with elevated PCB concentrations. To further validate our results we assessed whether t1 correlated with ∑PCBs/p,p'-DDE and ∑PCBs/trans-nonachlor ratios; all of which have been shown to biomagnify in seals. The ∑PCB/p,p'-DDE and ∑PCB/ trans-nonachlor ratios correlated ($p<0.05$) with t1 (Table 5). These relationships support previous reports of regional sources of POPs being transferred to top predators from their prey, whereby the concentration of a regional source POP would be elevated relative to POPs with no known regional or local sources (Calambokidis and Barlow, 1991; Krahn et al., 2007; Muir et al., 1990).

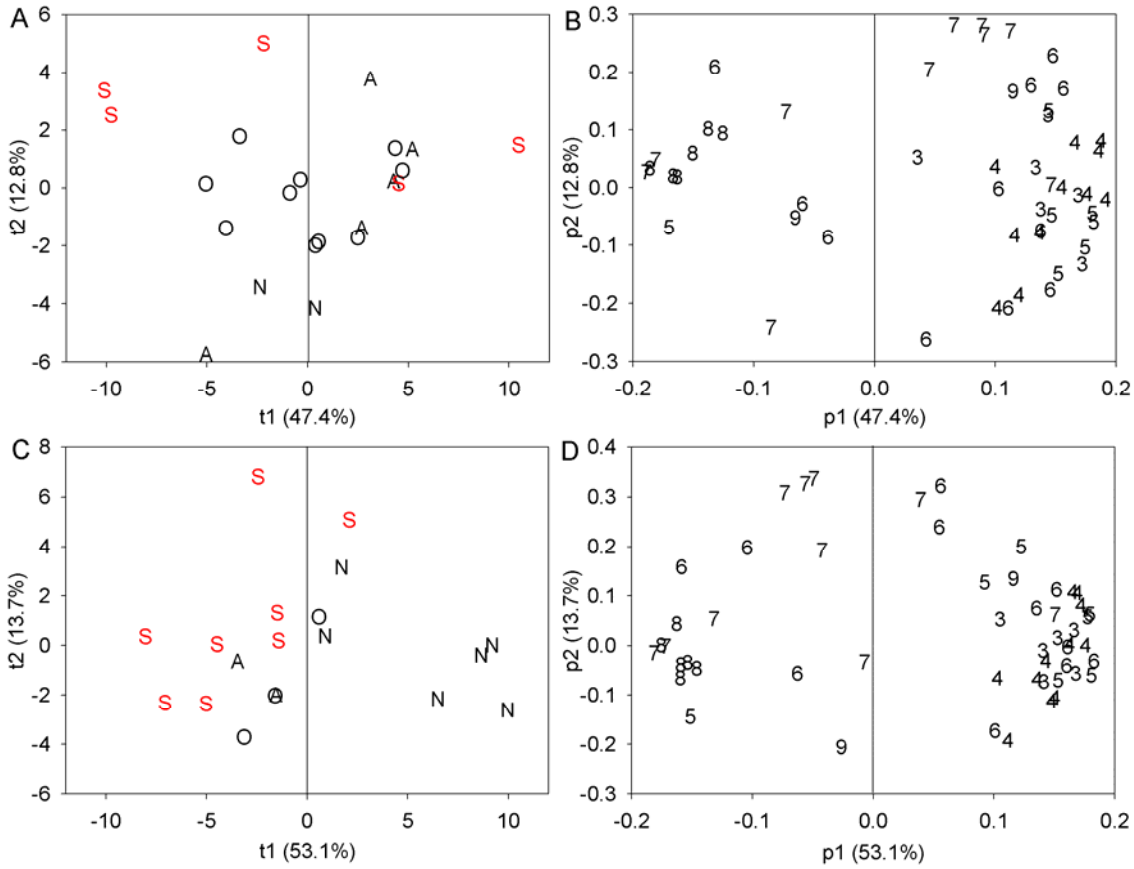

Figure 8: Principal Components Analysis (PCA) of PCB patterns (53 congeners) in sub-adult (A,B) and adult male (C,D) ringed seals reveals that seals to the left of each scores plot (A,C) are dominated by heavier congeners (B,D), consistent with exposure of a local PCB source. Panels A & C are PCA scores for individual seals, while panels B & D are PCA loadings for the individual PCB congeners. Symbols represent seals from four fjords in Labrador. (N = Nachvak; S = Saglek; O = Okak; A = Anaktalak). (B,D) Numbers identify the degree of chlorination of each PCB congener (i.e. number of chlorines per congener). One adult male outlier removed: 20-year-old ringed seal from Nachvak Fjord with the elevated PCB concentration (8,770 ng/g lw). This seal was dominated by heavier congeners (PC1= -16) and fell to the far left of the PCA.

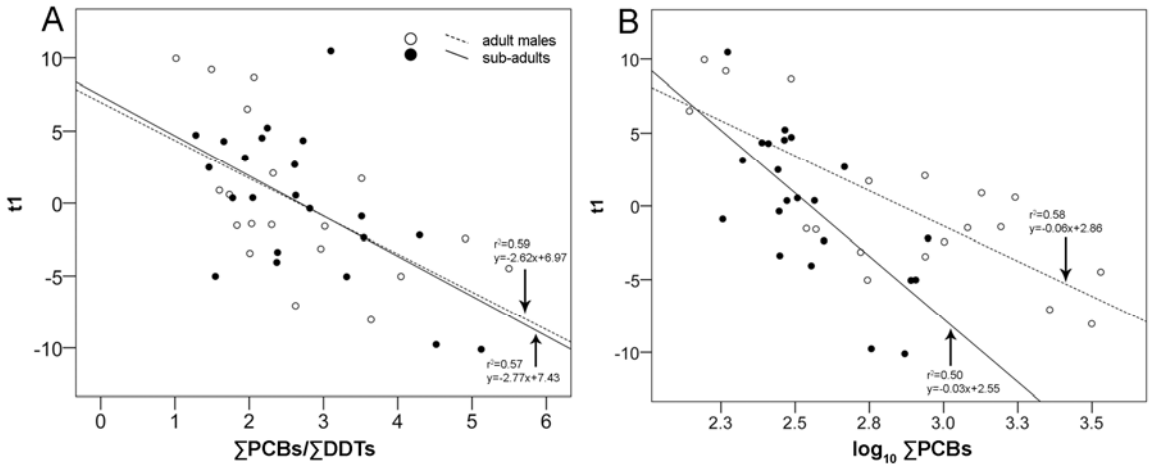

Figure 9: The first principal component (t1) is correlated with (A) ∑PCBs/OCPs ratios (∑PCBs/∑DDTs shown here; see Table 5 for other ∑PCBs/OCP ratio results) and (B) log_{10} ∑PCBs ng/g lipid weight for adult male and sub-adult ringed seals.

Table 5: Regression analyses revealed that t1 correlated with all ∑PCBs/organochlorine pesticide ratios (∑PCBs/∑DDTs, ∑PCBs/p,p'-DDE, ∑PCBs/∑Chlordanes, ∑PCB/ trans-nonachlor, ∑PCBs /∑HCHs, ∑PCBs/Dieldrin, ∑PCBs/HCB).

t1 versus	**Sub-adults**	**Adult males**
∑PCBs/∑DDTs	r^2=0.57; p=0.006	r^2=0.57; p=0.008
∑PCBs/p,p'-DDE	r^2=0.53; p=0.01	r^2=0.52; p=0.02
∑PCBs/∑Chlordanes	r^2=0.83; p<0.001	r^2=0.68; p<0.001
∑PCB/ trans-nonachlor	r^2=0.83; p<0.001	r^2=0.73; p<0.001
∑PCBs /∑HCHs	r^2=0.79; p<0.001	r^2=0.79; p<0.001
∑PCBs/Dieldrin	r^2=0.29; p=0.01	r^2=0.75; p<0.001
∑PCBs/HCB	r^2=0.82; p<0.001	r^2=0.63; p=0.004

The log of total PCBs correlated with t1 for sub-adult and adult male ringed seals (p<0.001; Figure 9). No relationship was found between the five OCPs (∑DDTs, ∑HCHs, ∑chlordanes, dieldrin, HCB) and t1 (p≥0.05). These results are consistent with the observations presented above, with the more heavily chlorinated seals having higher

PCB concentrations relative to the OCP concentrations (i.e. higher ∑PCB/OCP ratios) than the lighter, less chlorinated 'long-range' seals. A correlation was found between log K_{ow} for the PCBs and p1 (the variable loadings of the first principal component of the individual PCB congeners) for sub-adult and adult male ringed seals ($p<0.001$; Figure 10). Our results are consistent with Ross et al. (2004) which showed a PCB profile that strongly correlated with both total PCB concentrations and log H (Henry's law constant, which describes the partitioning between surface waters and the atmosphere). Log K_{ow} values and log H constants can be used to characterize the partitioning of PCB congeners across different environmental interfaces (e.g. air-water or lipid-water) (Hawker and Connell, 1988). As such, they play a key role in affecting the environmental fate and bioaccumulation of POPs in top predators. PCB concentrations from ringed seal prey species were added to a second PCA to provide a more complete view of the regional food web, and to further illustrate the influence of the Saglek PCB source on adult male seals sampled (Appendix 4). This PCA clearly shows that Saglek prey species to the left of the score plot are dominated by heavier congeners (i.e. 'local' signature), whereas reference fjord species to the right of the score plot are dominated by lighter congeners (i.e. 'long-range' signature).

T1 was correlated with location (four marine inlets) for adult males ($p=0.015$). No correlation was found between t1 and location for sub-adults ($p\geq0.05$). Among the sub-adult and adult male ringed seals with a heavy 'local' PCB signature, 30% and 58 % respectively, were from Saglek Fjord (Figure 8). These results suggest that these seals may have shown some site fidelity to this fjord, and therefore had a greater chance of exposure to the local PCB contamination. The higher percentage observed for adult male

ringed seals is consistent with other studies (Kelly et al., 2010; Kelly and Quakenbush, 1990; Smith and Hammill, 1981), where adult ringed seals were more likely to demonstrate signs of site fidelity than younger sub-adults that roam more widely. These observations with PCB patterns complement the finding of higher total PCB concentrations in adult males sampled in Saglek. The remaining 70% and 42% of sub-adult and adult male ringed seals, respectively, with a heavy 'local' PCB signature were from reference fjords (Figure 8; Nachvak, Okak, Anaktalak), which were sampled within 250 km of the source (Figure 6). The 20-year-old adult male ringed seal from Nachvak, which had the highest PCB concentration (8,770 ng/g lw) in the study, had the heaviest PCB profile (PC1= -16). A possible explanation for these reference seals with a local PCB signature is that these seals have fed previously in the contaminated area of Saglek Bay. The distance to which these seals may have travelled away from Saglek is not uncommon, as ringed seals are known to carry out extensive migrations (Smith, 1987), in some instances travelling thousands of kilometres (Kapel et al., 1988). Further research is needed to characterize the role of movement and foraging patterns in influencing POP concentrations in ringed seals for the area.

Collectively, our results strongly suggest that some ringed seals from the north Labrador coast have been influenced by the PCB exposure at Saglek Bay. Since no known local PCB sources other than Saglek exist along the Labrador coast, these results reflect the exposure of two distinct PCB sources (i.e. long-range atmospheric transport via the effect of a volatilization of PCBs away from southern sources and a local signal, a result of heavier congeners from Saglek Bay adhering to organic particles and remaining closer to source). It is possible that another local source of PCBs may exist on the

Labrador coast but is likely to be much smaller (Aivek-Stantec Ltd. 2012). In addition, ringed seal very rarely frequent this area (W. Piercy, pers. comm. 2013).

Although depuration and metabolic processes can deplete ringed seals and their prey of, for example, lighter PCBs (Boon et al., 1994), a PCA analysis using only recalcitrant congeners revealed no differences from the results presented in Figure 8 (data not shown), suggesting that metabolism was not overtly shaping the pattern differences observed in sub-adult and adult male ringed seals.

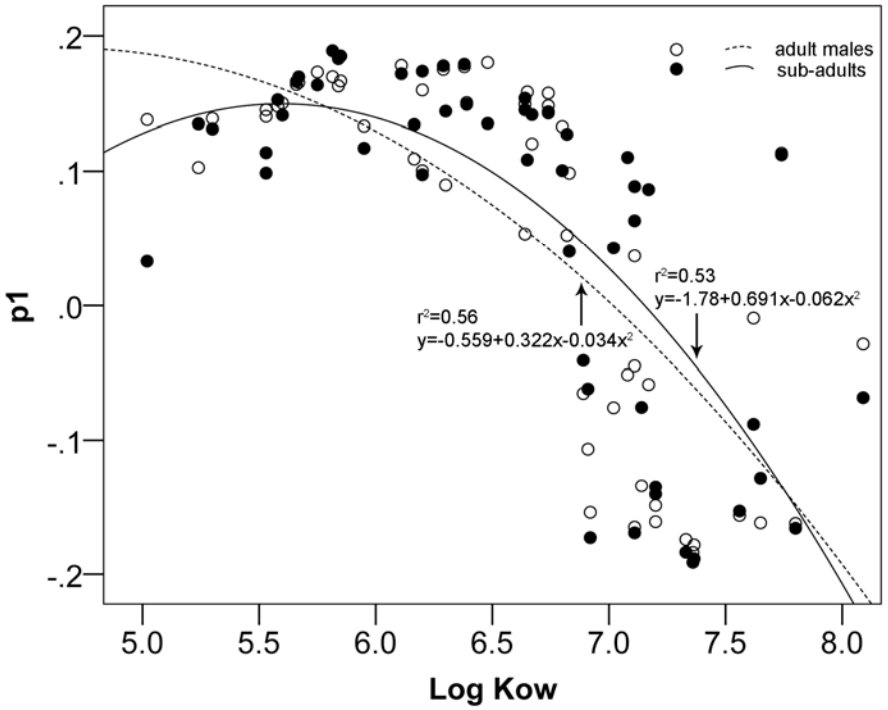

Figure 10: The first principal component (p1) for adult males (solid line) and sub-adults (dashed line) correlated with the octanol-water partition coefficient (Kow) for PCBs. The data pattern for adult males and sub-adults suggests that there are two groups of ringed seals along the Labrador coast; seals which have been exposed to a heavier PCB mixture from a local source (e.g. Aroclor 1260, local Saglek signature) and seals that have been exposed to a lighter PCB mixture from long-range atmospheric sources.

Local contribution to ringed seal PCB body burden

Based on the divergent PCB congener profiles (Figure 8; Appendix 5) and PCB/OCP ratios (Figure 9; Table 5), we divided the sub-adults and adult males into two groups. Ringed seals to the left of the t1-axis hereafter, will be referred to as 'local' and ringed seals to the right of the t1-axis hereafter, will be referred to as 'long-range'. Our data indicate that 45% and 60% of the sub-adult and adult male ringed seals, respectively, have been influenced by the local PCB source at Saglek Bay. ∑PCB concentrations in 'local' sub-adults (527 ± 82 ng/g lw) and adult males (1,386 ± 329) were two-fold higher ($p < 0.05$, Figure 11) than concentrations in 'long-range' seals (sub-adults: 293 ±21 ng/g lw; adult males: 662 ± 214 ng/g lw). No significant differences in OCPs were found between 'local' and 'long-range' sub-adults or adult males (Figure 11).

The five ∑PCB/OCP contaminant ratios were significantly higher in 'local' sub-adult and adult male ringed seals than in 'long-range' seals (Table 6). These results are consistent with the observations presented above, with the more heavily chlorinated 'local' seals having higher PCB concentrations relative to the OCP concentrations (i.e. ∑PCB/OCP ratios) than the lighter, less chlorinated 'long-range' seals. Furthermore, the lack of difference found between the 'local' and 'long-range' seals for all OCP concentrations (Table 4) further supports the use of these contaminants as a reference to identify the sources of PCBs to northern Labrador ringed seals. The concentration-weighted average log K_{ow} values for PCBs in 'local' ringed seals (sub-adults=6.8 ± 0.02; adult males = 6.9 ± 0.01) was higher than 'long-range' seals (sub-adults=6.7 ± 0.01; adult males = 6.7 ± 0.03; $p < 0.001$ in both cases).

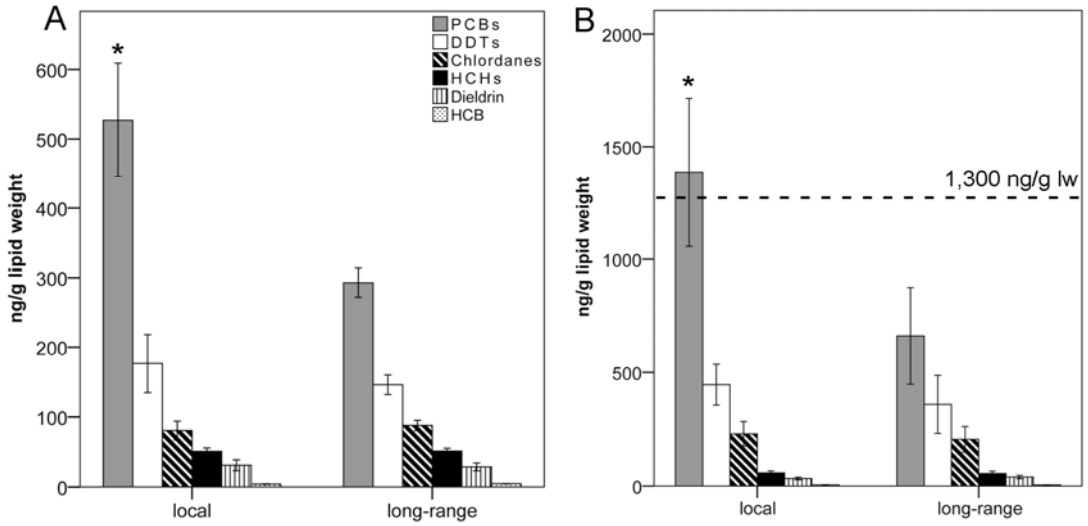

Figure 11: Local and long-range PCB and OCP concentrations measured in the blubber of sub-adults (A) and adult male (B) ringed seals. Mean (±SE) PCB concentrations in sub-adult (A) and adult male (B) ringed seals are higher in 'local' ringed seals compared to 'long-range' seals whereas organochlorine pesticide (∑DDTs, ∑Chlordanes, ∑HCHs, Dieldrin, HCB) concentrations did not differ between groups. Average PCB concentrations in adult male ringed seals exceed the effects threshold (1,300 ng/g (ppb), Mos et al. 2010; indicated by a dashed line) in a similar pinniped species, the harbour seal. An asterisk (*) above a column indicates significant difference between the 'local' and 'long-range' seals for that compound.

Table 6: Arithmetic means ± SE of the ratios of ∑PCBs/legacy organochlorine pesticides (∑DDTs, ∑Chlordanes, ∑HCHs, Dieldrin, HCB) in blubber tissue of 'local' and long-range' sub-adult and adult male ringed seals.

	Sub-adults		Adult male	
	local	**long-range**	**local**	**long-range**
∑PCBs/∑DDTs	3.3 ± 0.3	2.1 ± 0.2[a]	3.2 ± 0.4	1.9 ± 0.3[a]
∑PCBs/∑Chlordanes	6.7 ± 0.7	3.5 ± 0.3[a]	6.9 ± 0.8	3.0 ± 0.3[a]
∑PCBs/∑HCHs	10 ± 1.2	5.8 ± 0.3[a]	23 ± 3.0	11 ± 2.8[a]
∑PCBs/ Dieldrin	23 ± 3.7	13 ± 1.5[a]	39 ± 3.9	16 ± 4.2[a]
∑PCBs/ HCB	125 ±15	63 ± 5.6[a]	247 ± 50	128 ± 41[a]

[a] $p<0.05$ compared to local group

Variations with sex, condition, and diet

No significant differences (p >0.05) were found between sub-adult males and females for ∑PCBs and the five OCPs (∑DDT, ∑chlordanes, ∑HCHs, HCB and dieldrin). Concentrations of ∑PCBs, ∑DDT, ∑chlordanes and HCB were significantly higher in adult male ringed seals than in sub-adult ringed seals ($p \leq 0.05$). No significant differences (p >0.05) were found between sub-adult and adult female ringed seals for ∑PCBs and the five OCPs. Concentrations of ∑PCBs, ∑DDT, and ∑chlordanes were significantly higher in adult male ringed seals than in adult female ringed seals ($p \leq 0.05$). These differences between males and females are typical for pinnipeds, whereby females are able to maintain a lower OC burden by transferring a proportion of their contaminant load to offspring via placental and lactational transfer (Addison and Brodie, 1977; Fisk et al., 2002). ∑HCHs, HCB and dieldrin concentrations in adult ringed seals did not differ (p >0.05) between sexes. No relationship between sex for ∑HCHs and HCB has been observed previously in adult ringed seals (Fisk et al., 2002). The average concentrations for condition indices (length, girth, blubber thickness, % lipid) found in ringed seals from this study (Table 3) fell within the range reported previously for ringed seals in the Canadian Arctic (Addison et al., 2005; Fisk et al., 2002; Muir et al., 1999a; Muir et al., 2000). Concentrations of ∑PCBs and all five OCPs in sub-adults, adult males and adult females did not differ with any of the condition indices (p >0.05).

Values of $\delta^{15}N$ and $\delta^{13}C$ did not influence PCB and OCP concentrations in sub-adult, adult female, and adult male ringed seals (p >0.05; Table 3). We also evaluated whether or not feeding ecology was being influenced by sex, age, and/or condition indices. For sub-adult and adult ringed seals, there were no sex-related difference in $\delta^{15}N$ or $\delta^{13}C$ values (p >0.05; Table 3). Similarly, no sex-related differences were found in the diet of

ringed seals from other Canadian Arctic locations (Bradstreet and Finley, 1983; Holst et al., 2001), Svalbard (Gjertz and Lydersen, 1986), or Alaska (Johnson et al., 1966). Contrary to other observations for ringed seals (Dehn et al., 2007; Holst et al., 2001), adult females and males were enriched in $\delta^{15}N$ over sub-adults (p=0.05 and p=0.001, respectively; Table 3). No differences were found between adult females and males and sub-adults for $\delta^{13}C$ (p=0.17 and p=0.69, respectively; Table 3). Similarly, no age related differences were found for $\delta^{13}C$ for ringed seals from the Canadian Arctic (Dehn et al., 2007). $\delta^{15}N$ and $\delta^{13}C$ in sub-adults, adult males and adult females were not found to differ with any of the condition indices measured (p >0.05; Table 3).

We evaluated whether diet indices or age was influencing the contaminant concentrations and ∑PCB/OCP ratios in 'local' and 'long-range' ringed seals. No significant differences were found between 'local' and 'long-range' sub-adult and adult male ringed seals for $\delta^{15}N$. No significant differences were found between 'local' and 'long-range' adult male ringed seals for $\delta^{13}C$. However, 'local' sub-adult seals had slightly higher $\delta^{13}C$ values than 'long-range' seals (local = -18.4 ± 0.1; long-range = 18.8 ± 0.1; p=0.03), suggesting that 'local' seals were consuming more benthic or inshore prey than 'long-range' seals. A benthic or inshore feeding behaviour could increase a seals chance of being influenced by the PCB contamination at Saglek Bay, such that these seals would be more likely to forage directly within the Saglek Anchorage area and/or Saglek fjord than seals feeding more pelagically or offshore. No significant differences were found between 'local' and 'long-range' adult male (p=0.45) and sub-adult (p=0.31) ringed seals for age.

Health risks for northern Labrador ringed seals

Numerous field studies have shown associations between PCBs and adverse health effects (e.g. impaired reproduction, endocrine disruption, bone lesions, reduced immune function, and tumour incidence) in ringed seals (Bergman and Olsson, 1985; Helle et al., 1976b; Nyman et al., 2003; Olsson et al., 1994; Routti et al., 2010a; Routti, 2008). However, due to confounding factors (e.g. complex mixtures) inherent in field studies, evidence of a 'cause-effect' linkage between an adverse health effect and a single contaminant has been difficult to achieve. As a result, effects thresholds and/or toxicity reference values (TRV) for marine mammal species are virtually non-existent (Mos et al., 2010). To our knowledge, only three toxicity thresholds for PCBs exist for marine mammals. These include a TRV for immunotoxicity (17 mg/kg lw; blubber) determined during a captive feeding study of harbour seals (*Phoca vitulina*; (Ross et al., 1996)), a TRV for reproductive effects (10 mg/kg lw; blubber) established in an epidemiological study of free-ranging bottlenose dolphins (*Turisiops truncatus*; (Hall et al., 2006)), and a TRV for immunotoxicity and endocrine disruption (1.3 mg/kg lw; blubber) in juvenile free-ranging harbour seals (*Phoca vitulina*; (Mos et al., 2010)).

Average PCB levels in adult male seals from the 'local' group exceed the toxicity threshold for immunotoxicity and endocrine disruption established in harbour seals (1.3 mg/kg lw; (Mos et al., 2010)) but are lower than the other two thresholds established for marine mammals (Figure 11). The average PCB levels in the 'long-range' adult male and 'local' and 'long-range' sub-adult ringed seals were below all thresholds established for marine mammals. These results collectively suggest that adult male ringed seals which have been exposed to local sources of PCBs may be at risk for toxic effects including the alteration of vitamin A levels and its receptor (retinoic acid receptor RARα), altered

thyroid hormone physiology and receptor function, and impaired immune function (Mos et al., 2007).

2.4 Conclusions

The present study illustrates how a contaminated site can become a source of PCBs to a wide-ranging, upper-trophic level species. We found elevated PCB concentrations in a proportion of individual ringed seals from Labrador, relative to those observed in ringed seals from across the Canadian Arctic. Divergent PCB patterns and PCB/OCP ratios provided further evidence that seals from both within Saglek Fjord as well as outside this area had been exposed to PCBs from the former radar station. We estimate that approximately 50% of the PCB burden of ringed seals in the region originates from the local source. Despite their ecology (i.e. wide-ranging foraging behavior), our research suggests that ringed seals could be used as an indicator of local marine contamination and to model contaminant accumulation in Arctic food webs. Furthermore, our study shows that locally contaminated adult male ringed seals sampled in 2008 exceed an adverse health effects threshold, despite documented reduction in sediment, bottom-feeding fish (shorthorn sculpin) and seabird (black guillemot) contamination during the period of 1999-2006 (Brown et al., 2009; Kuzyk et al., 2005b).

Chapter 3: Satellite telemetry informs PCB source apportionment in a mobile, high trophic level marine mammal – the ringed seal (*Pusa hispida*)

Abstract

Marine mammals are typically poor indicators of point sources of environmental contaminants as a consequence of their often complex feeding ecologies and extensive movements, all of which mask the contributions of specific inputs. The release of polychlorinated biphenyls (PCBs) by a military radar station into Saglek Bay, Labrador (Canada) has contaminated marine sediments, bottom-feeding fish, seabirds, and some ringed seals, but attributing the PCBs in the latter highly mobile animals to this source is exceedingly difficult. In addition to the application of such tools as stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) and univariate and multivariate statistical exploration of contaminant patterns and ratios, we used satellite telemetry to track the movements of 13 seals in their transient use of different feeding areas. Reduced size of home range and core area (i.e. areas of concentrated use), as well as increased time in coastal inlets, were important determinants of increased PCB concentrations in seals reflecting the contribution of Saglek Bay. While the PCB source at Saglek provided a strong local signal in a remote environment, this first use of satellite telemetry demonstrates the utility of evaluating space-use strategies to better understand contaminant exposure, and more specifically the contribution of contaminant hotspots to mobile predators.

3.1 Introduction

Source apportionment for persistent contaminants in high trophic level marine species is notoriously difficult to assess, yet these animals are often the most contaminated and at risk to these bioaccumulative and toxic chemicals (Buckman et al., 2011). These species generally have a large home range, such that exposure to biomagnifying persistent organic pollutants (POPs) can be attributed to the consumption of prey contaminated by a combination of distant, regional and/or local point sources. Researchers have used a variety of statistical techniques and study designs (e.g. PCB profiles and POP ratios (Calambokidis and Barlow, 1991; Jarman et al., 1996; Krahn et al., 2007; Mckninney et al., 2011; Ross et al., 2004)) to infer the contributions of regional and/or local POP sources. However, without an understanding of movement and foraging behavior, it remains challenging to adequately explain the contributions of point sources to the body burden of a given species.

Satellite telemetry offers a novel way of assessing behavior and movement patterns for marine species by providing detailed records of geographical location . Such information, combined with contaminant residues in prey and/or predator, can provide important insight to the contributions of different source regions. For example, Elliott et al. (2007) used satellite tracking of osprey to demonstrate that the northern breeding grounds of this fish-eating bird represented the principal source of contaminant exposure. Satellite telemetry has also been used to investigate geographical differences in contaminant concentrations and patterns in loggerhead sea turtles (*Caretta caretta*) and polar bears (*Ursus maritimus*) (Olsen et al., 2003; Ragland et al., 2011), but this method has never been used to assess the implications of point sources related to spills or discharge of contaminants in marine ecosystems.

Polychlorinated biphenyls (PCBs) are a POP of concern in arctic food webs, where biomagnification of these persistent and lipophilic contaminants leads to relatively high concentrations in upper trophic level species (Muir et al., 2000). PCB contamination in Arctic marine ecosystems is largely attributed to atmospheric deposition following long-range transport from southern industrial source regions, but local sources have been documented in the Arctic (e.g. military radar stations) (Bright et al., 1995; Macdonald et al., 2000). Saglek Bay, Labrador, Canada has been the site of a military radar station since the late 1950s; however, it was not until 1996 that PCB contamination was discovered at the site, along with evidence that PCBs had entered the marine environment (Kuzyk et al., 2005b). Approximately 260 kg of PCBs had been released into the marine environment (ESG, 2000), contaminating adjacent marine sediments, benthic invertebrates, bottom-feeding fish, diving seabird, and some ringed seals (Kuzyk et al., 2005b). While the elevated PCBs in the benthic associated food-web could be causally attributed to the Saglek PCB source, ascertaining the contribution of this local source to the body burden of the highly mobile ringed seals was fraught with uncertainty (Kuzyk et al., 2005b). 'Heavier' PCB profiles and higher PCB:organochlorine pesticide (OCP) ratios provided a recent basis to identify up to 60% of ringed seals sampled in the central and northern Labrador coast as being exposed to the local PCB source at Saglek (Brown et al., 2014a). This chemocentric approach enabled a classification of seals as either 'local' or 'long-range', reflecting their respective exposure to these two types of sources.

Given the varying degrees to which different ringed seals move (and therefore feed), the determination of PCBs in biopsy-sampled individuals will yield results reflecting their lifelong exposure *via* prey in different areas. Some ringed seals have a home range

that involves movement over large distances (Freitas, 2008; Harwood et al., 2012; Ridoux et al., 1998; Teilmann et al., 1999), whereas other ringed seals stay within much smaller areas and exhibit strong site fidelity (Freitas, 2008; Harkonen et al., 2008; Kapel et al., 1988; Lydersen et al., 2014; Smith and Hammill, 1981).

A number of variables can be used to describe space-use and foraging behavior in ringed seals. For example, "home range" captures the area that each ringed seal utilizes, or covers during its normal activities, including feeding, travelling, resting, and breeding (Burt, 1943). Very little is known about the home range of ringed seals along the Labrador coast; studies indicate that they remain in Labrador waters throughout the year and undergo relatively short migrations northward during the summer and fall foraging months (Boles, 1980; McLaren, 1958b). Other important variables that describe the space-use of ringed seals include the extent of latitudinal and longitudinal movements, time spent in coastal inlets or local areas such as Saglek Fjord, and areas of concentrated use, commonly termed core areas (Burt, 1943). Core areas represent sites of greater ecological significance to the animal and therefore are a better representation of preferred foraging sites. Measures of time spent feeding benthically and stable isotope ratios (carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$)) are important variables that describe foraging behavior in relation to prey choice. For example, enrichment of $\delta^{15}N$ ratios increases with trophic position in marine food chains providing a continuous variable with which to assess both trophic level and food web transfer (Fisk et al., 2001b). Ratios of $\delta^{13}C$ can elucidate trophic interactions by establishing the relative contribution of inshore/benthic versus offshore/pelagic feeding preferences (France and Peters, 1997).

Herein, we use satellite telemetry to evaluate whether contaminant profiles can indeed be used to assign seals to either 'local' or 'long-range' categories. We previously documented divergent PCB signatures in ringed seals in the region, and concluded that 'heavy' signatures reflected localized exposure to the Saglek Bay PCB source, whereas 'light' PCB signatures reflected background (Brown et al., 2014a). We hypothesize here that satellite telemetry and $\delta^{13}C$ and $\delta^{15}N$ can be used as measures of habitat use (hence, feeding ecology) that will validate our PCB signature assignment in ringed seals from the Labrador coast. The mobility of marine mammals, coupled with the dearth of major sources of contaminants in the Arctic, highlight the unique opportunity here to evaluate the utility of satellite telemetry in support of exposure attribution to a high trophic level predator in the marine environment.

3.2 Materials and Methods

Seal capture and handling

During August and September (2008-2011), thirteen ringed seals (2008: *n*=3; 2009: *n*= 3; 2010: *n*=5; 2011: *n*=2) were captured from various locations in Saglek Fjord, Labrador, Canada (Table 7). Following the methods of Smith et al. (Smith et al., 1973), we captured ringed seals using green monofilament floating nets, with 279 mm (11") stretched mesh, 12 and 16 meshes deep and 50 m in length. The nets were set in shallow water (up to 8 m) and were anchored to shore on one end and to the bottom on the other.

Captured seals were manually restrained, and a satellite-linked Platform Transmitter Terminal (PTT; Wildlife Computers Splash; dimensions: 5 cm by 6 cm; weight: 65 g in air) was glued to the fur mid-dorsally between the scapulae using fast-setting two-component epoxy glue, after drying and cleaning the fur with acetone. Sex, weight, girth,

and length (nose to tail, with belly down) were recorded. Age class was estimated by counting annuli on the claws of the forelimbs, observing each animal closely, and taking morphometric measurements (i.e. length and weight) in the field. The determined age class for each seal was further validated by comparing their length and weight measurements to measurements taken previously for aged Labrador ringed seals (Appendix 6). A blubber biopsy (6-mm diameter), consisting of the entire blubber column down to muscle was sampled from each seal prior to release and kept frozen at -80 °C until contaminant analyses. Fur samples were obtained from 10 of the 13 seals for stable isotope analysis. Fur was selected for analysis, since it provides a non-invasive way to investigate stable isotopes of live-captured seals.

The Nunatsiavut Government, Nunatsiavut Health and Environment Review Committee and The Animal Use Protocol (AUP) administered by Fisheries and Ocean Canada approved all animal-handling and sampling procedures.

Transmitters

PTTs were programmed to send up to 250 transmissions per day. The transmission repetition rate was 45 s in the water and 90 s when the seal was hauled out. Location data was collected using the ARGOS system (System Argos, Toulouse, France). Seal locations were estimated following uplinks when the PTT communicated with ARGOS satellites while the seal was at the surface. PTTs are also equipped with pressure transducers that record depth every 10 seconds whenever the instrument was wet. Dives were defined as any excursion from the surface to depths exceeding 2 m. Water depth at each location was obtained from 1°-resolution General Bathymetric Chart of the Oceans (GEBCO) data (IOC et al., 2003).

Home range and core areas

We fit the two-state "switching" state space model described by Jonsen et al. (Jonsen et al., 2005) to the time series of each seal's ARGOS locations to account for observation error and infer behavioural changes along each track. The model was fit using a 12 hour time step to estimate locations at regular intervals, given the autocorrelation and turning angles between consecutive locations.

The modelled regular time series was used to study spatial distribution by calculating utilization distribution (UD) maps. UDs were estimated using a Brownian bridge kernel method (Horne et al., 2007), implemented in R package adehabitatHR (Calenge, 2007), which requires specification of two smoothing parameters: one for uncertainty in displacement distance between successive locations per unit time, and another for uncertainty in location estimates. We determined the former (30 m) using a maximum likelihood method (Horne et al., 2007), and the latter (12 km) from published estimates (Silva et al., 2014). This procedure, as opposed to the classical kernel estimator, has the advantage of accounting for non-linear movement between successive locations. Before estimating the UDs, locations were projected to an Albers Equal Area coordinate system with local central meridians and standard parallels chosen using the "one-sixth rule" (Snyder, 1987).

The projected data were used to build a spatial raster composed of 1 x1 km cells, where the value in each cell indicates the percentage level of home-range that it belongs to. We estimated home range (95%) and core area (70%) contour intervals, removing areas over land as determined by the Global Self-consistent Hierarchical High-resolution Shorelines database (http://www.ngdc.noaa.gov/mgg/shorelines/gshhs.html). UDs were

estimated using this procedure on locations for each individual and for each group of seals (i.e. 'local' versus 'long-range').

The average ocean depth of the core areas for each seal was defined as the mean depth from the GEBCO grid within each core area (IOC et al., 2003). Although there are data gaps using this method, it provides the most extensive coverage for the area.

Index of benthic diving

PTTs reported 6 h histograms of dives performed within 0-4 m, 4-10 m, at 10 m intervals from 10 m to 100 m, at 40 m intervals from 100 m to 220 m, 220-300 m, and deeper than 300 m.

Mean dive depth (x) was calculated as for the i^{th} 6-h period as:

$$x_i = \frac{\sum_{j=1}^{n} x_j f_{ij}}{\sum_{j=1}^{n} f_{ij}}$$

Where x_j is the mid-range value of the jth histogram bin, f_{ij} is the number of dives in the jth histogram bin and n is the number of bins. The difference between mean dive depth and average ocean depth throughout each individual's track was calculated. The proportion of dives where the difference was smaller than 50 m was used as an index of benthic activity; i.e. if the difference was smaller than 50 m, the seal was considered to be in the benthic layer.

Tissue analysis

The three 2008 blubber biopsies were analyzed by the Great Lakes Institute for Environmental Research's (GLIER) accredited organic analytical laboratory, Windsor, ON, Canada (Canadian Association for Environmental Analytical Laboratories Accreditation and ISO17025 certified). The other 10 blubber biopsies collected from

2009 to 2011 were analyzed by the Laboratory for Expertise of Aquatic Chemical Analysis (LEACA) at the Institute of Ocean Sciences, Sidney, BC, Canada.

PCBs and organochlorine pesticides (OCPs): α-, β-, γ-hexachlorocyclohexane, α- and γ-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, heptachlor epoxide, *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT, dieldrin, hexachlorobenzene (HCB) were measured by GLIER and LEACA using gas chromatography electron capture detection (GC-ECD) and high-resolution gas chromatography/high resolution mass spectrometry (HR-GCMS), respectively. The detailed methodology for extraction, cleanup, and quantification of target analytes has been reported elsewhere [GLIER: (Brown et al., 2014a; Drouillard et al., 2004; Lazar et al., 1992); LEACA (Ikonomou et al., 2001)]. Percent lipid was determined using gravimetric lipid determination by weight of extract method with dichloromethane.

Sample batches submitted for analysis at GLIER consisted of six samples, an in-house reference homogenate tissue, a method blank, and the external recovery standard (2-ethylhexyl-2,3,4,5-tetrabromobenzoate; TBB). 62 PCB congeners and 14 OCPs were detected with sufficient frequency to be included in the data analysis. Recoveries of individual PCB congeners in the homogenate reference tissue with each batch of samples were within 2 standard deviations from the mean laboratory database value derived from laboratory control charts. Recovery efficiencies for the TBB standard were 95 ± 5.1% (mean ± SE). Procedural blanks (n = 18) were below detection for all PCB congeners and OCPs.

Sample batches submitted for analysis at LEACA consisted of eight samples, a procedural blank, certified reference material, and a random duplicate sample. Recovery

efficiencies based on the internal standards were 79.8 ± 1.1% (mean ± SE). Procedural blanks (n = 5) had concentrations slightly above detection (<20 pg/g) for 80 ± 11 (mean ± SE) of the 182 PCB congeners measured. Analytical duplicates were within 10% (n = 3).

All samples were recovery-corrected for concentrations of PCBs and OCPs. The smaller congener suite (n=62) detected by GLIER was used as the primary reference so as to synchronise the two data sets (versus n=182 for LEACA). An inter-laboratory comparison between GLIER and LEACA was completed for ten ringed seal blubber samples (data not shown), with 47 individual congeners having <30% (r^2 =0.96 ± 0.03; p < 0.05) difference and ∑PCBs having <15% difference between the laboratories. These congeners were therefore chosen for inclusion in the final data-set. Any PCB congeners less than the detection limit were replaced with a random number between the detection limit and zero.

Stable isotope analysis

Prior to analysis, fur from each individual was washed in a diluted standard detergent, rinsed in Milli-Q water baths, and dried for 24 hours at room temperature. Fur samples were homogenized, weighted into tin capsules, and stable carbon and nitrogen isotope ratios were analyzed by Continuous Flow Ion Ratio Mass Spectrometer (CFIR-MS) (Finnigan MAT Delta^{plus}, Thermo Finnigan, San Jose, California, USA). Stable isotope abundances are expressed in delta (δ) values as the deviation from standards in parts per thousand (‰) using the following equation:

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

where R is the ratio of heavy to light isotope ($^{15}N/^{14}N$ or $^{13}C/^{12}C$) in the sample and standard. The nitrogen stable isotope standard was atmospheric nitrogen; Pee Dee

Belemnite limestone formation was the standard for the carbon stable isotope. Precision based on two standards (bovine muscle (NIST 8414) and an internal lab standard (tilapia fish muscle); n =65 for each) were <0.16 and <0.08‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. Accuracy of isotope analysis, based on NIST standards (sucrose (NIST 8542) and ammonia sulphate (NIST 8547); n = 3 for each) analyzed during the study were within <0.1‰ of certified $\delta^{15}N$ and $\delta^{13}C$ values.

Data analysis

Data pretreatment, methods and rationale for dividing the ringed seals into two groups: 'local' and 'long-range', are described elsewhere (Brown et al., 2014a). Briefly, principal components analysis (PCA) was used to elucidate differences in PCB patterns in the thirteen tagged ringed seals. PCA ordinations have been widely used to capture differences in PCB patterns for both physical (i.e. sediment) and biological environments (Brown et al., 2014a; Christiansen et al., 2007; Cullon et al., 2009; Kuzyk et al., 2010; Ross et al., 2004; Yunker et al., 2011). In particular, PCB congener pattern analysis has been used in harbour seal and ringed seal blubber to determine a regional 'local' signature versus long-range signature (Brown et al., 2014a; Ross et al., 2004). This established approach was applied to the present study to determine which ringed seals had been influenced by the local PCB source compared to seals which had only come into contact with long-range 'distant' sources. Samples were standardized to the concentration total before PCA to remove artifacts related to concentration differences between samples.

Linear regression was used to assess the relationships between PCA projections and ∑PCB/OCP ratios, log K_{ow} values (log of the octanol-water partition coefficient, a proxy

for particle affinity), $\delta^{15}N$, and $\delta^{13}C$. Log K_{ow} values for the PCB congeners were taken from literature values (Hawker and Connell, 1988). The log K_{ow} for each PCB congener was used to calculate concentration-weighted average log K_{ow} values (Grant et al., 2011) for each ringed seal according to the following equation:

Concentration-weighted average log K_{ow} value

$$= \frac{\sum_{\text{all congeners}} \textit{concentration of individual congener x (log Kow) value for that congener}}{\left[\textit{total concentration of all congeners}\right]}$$

To further validate the division of ringed seals into two groups ('local' versus 'long-range'), a one-way ANOVA was used to compare the mean of contaminant concentrations, ∑PCB/OCP ratios and concentration-weighted average log K_{ow} values for ringed seals in the two groups (Brown et al., 2014a).

Univariate statistical analyses were performed using the IBM SPSS 20.0 for Windows. Data were log transformed when necessary to meet the normality assumptions for parametric analyses. Contaminant concentrations are expressed on a lipid-weight (lw) basis.

A one-way ANOVA was used to examine the effects of space use and foraging behaviour variables and biological variables (length, weight, age class, gender, year) on 'local' and 'long-range' seals.

Table 7: Sex, age-class, PCB source ('local' versus 'long-range'), and transmission period for thirteen ringed seals tagged at Saglek Fjord, Labrador, Canada.

ID[a]	PCB source	Sex	Age-class	Weight (kg)	Length (cm)	Axillary girth (cm)	Deployment date	Last signal received
08-1	long-range	male	sub-adult	25	90	83	2008 August 11	2009 April 29
08-2	long-range	male	adult	84	124	127	2008 August 14	2009 January 24
08-3	local	female	sub-adult	37	96	87	2008 August 19	2008 November 02
09-4	long-range	male	sub-adult	32	94	85	2009 September 1	2009 October 29
09-5	long-range	male	sub-adult	23	91	72	2009 September 1	2009 October 20
09-8	long-range	male	sub-adult	24	95	79	2009 September 3	2009 December 10
10-9	long-range	female	sub-adult	26	83	76	2010 September 1	2011 April 22
10-10	local	male	sub-adult	31	96	85	2010 September 1	2011 January 15
10-11	local	male	sub-adult	27	91	83	2010 September 1	2011 March 7
10-12	local	female	sub-adult	34	113	85	2010 September 2	2011 June 4
10-13	long-range	female	sub-adult	29	95	84	2010 September 2	2011 April 7
11-14	local	male	adult	73	128	84	2011 August 11	2012 January 28
11-15	local	female	sub-adult	38	90	82	2011 August 11	2011 December 16

[a] Numbers depict two digit year of sampling, followed by seal ID.

3.3 Results and Discussion

PCB patterns and POP ratios determine 'local' and 'long-range' groupings

A principal components analysis of ringed seal PCB congener profiles identified seals with a heavier (more chlorinated) 'local' PCB signature from seals with a lighter (less-chlorinated) 'long-range' PCB signature (Figure 12). The first principal component (PC1: 45.8%) differentiates ringed seals with a greater proportion of the lighter congeners from seals with a greater proportion of the more heavily chlorinated congeners (Figure 12). The light PCB signature found in seals to the right of the t1-axis is characteristic of a long-range atmospheric transport signal, whereas the heavy PCB signature found in seals to the left of the t1-axis is characteristic of a local source signal. The divergent PCB profiles shown in Figure S1 (Supporting Information) further corroborate these findings

and are consistent with the PCB profiles observed in 'local' and 'long-range' ringed seals from northern Labrador (Brown et al., 2014a).

The ∑PCBs/OCPs ratios were correlated ($p<0.05$) with t1 (the sample scores of the first principal component) for ringed seals (Appendix 7) indicating that ringed seals to the left of the t1-axis with a heavier PCB signature (i.e. local signature) have a higher PCB concentration relative to the five different OCPs. These relationships support previous observations for ringed seals in Labrador (Brown et al., 2014a), with the local source POP (i.e. PCBs) being elevated relative to OCPs.

The log of total PCBs was correlated with t1 for ringed seals ($r^2=0.52$; $p=0.005$). No relationship was found between the five OCPs (∑DDTs, ∑HCHs, ∑chlordanes, dieldrin, HCB) and t1 ($p\geq0.05$). These results are consistent with the observations presented above, with the more heavily chlorinated seals having higher PCB concentrations relative to the OCP concentrations (i.e. higher ∑PCB/OCP ratios) than the lighter, less chlorinated 'long-range' seals. The log K_{ow} for the PCBs was correlated with p1 (the variable loadings of the first principal component) for ringed seals ($r^2=0.58$; $p<0.001$), suggesting that seals to the right of the t1-axis are exposed to lighter PCB mixtures, consistent with a long-range source, whereas seals to the left of the t1-axis are exposed to more heavily chlorinated congeners (e.g. hepta-, octa-. and nona- PCBs) consistent with a local source.

The second principal component (PC2: 26.5%) was positively correlated ($r^2=0.58$; $p<0.001$) with $\delta^{15}N$, with the 2010 seals (local and long-range sub-adult males and females, Figure 12), located below the t2-axis, feeding at a much lower trophic level ($\delta^{15}N=12.5\pm0.5$) than the 2009 and 2011 sub-adult and adult male seals, located above

the t2-axis ($\delta^{15}N$=15.0±0.5). The stable isotope profiles in fur reflect the isotopic elements deposited during the annual moult (Aubail et al., 2011). The low $\delta^{15}N$ levels in the 2010 seals could be due to changes in their foraging ecology in response to unfavorable ice conditions (i.e. below normal extent of coverage and earlier spring breakup) reported for that year (Colbourne et al., 2011). These factors may have influenced the abundance and/or availability of key prey species that year such that seals may have been forced to feed lower on the food chain. For example, the 2010 ice conditions and timing of the spring bloom were factors linked to low capelin abundance that year (Mowbray, 2013); other key prey species may have been affected as well. Collectively our PCA results reveal the strong influence of a local PCB source on some of the seals sampled (i.e. seals to the left along the PC1 axis), with trophic level (i.e. $\delta^{15}N$) dietary choices resigned to a secondary role influencing the PCB patterns in both the local and long-range seals.

Based on these divergent PCB profiles and PCB/OCP ratios, we hereafter refer to ringed seals to the left of the p1-axis as 'local' and seals to the right of the p1-axis as 'long-range'. Our data indicate that 46% of the tagged ringed seals have been influenced by the local PCB source at Saglek Bay (Figure 12; Table 7).

A second PCA, which included PCB concentrations for 84 congeners for LEACA ringed seals (*n*=10), confirmed the PCB pattern (Figure 12) and group classification (i.e. 'local' versus 'long-range') for each of the 2009 to 2011 seals (Appendix 8). Lastly, a PCA, which included the GLIER 2008 harvested seals from Brown et al. (2014a) and the three GLIER 2008 tagged seals (*n*=3) from the present study confirmed the group classification for the three 2008 tagged GLIER seals.

Age class, sex, length, weight and year were similar ($p > 0.05$; Table 7) between the two groups and did not influence contaminant concentrations and patterns (data not shown).

The geometric mean ∑PCB concentrations (1,930 ± 1,640 ng/g lw) in 'local' ringed seals were four-fold higher (p=0.02, Table 8) than 'long-range' seals (538 ± 89 ng/g lw), suggesting that 75% of the PCB burden of ringed seals sampled originated from the local PCB source at Saglek Bay. OCP concentrations did not differ between 'local' and 'long-range' seals (Table 8). The five ∑PCB/OCP contaminant ratios were higher ($p \geq 0.05$) in 'local' ringed seals than in 'long-range' seals (Table 9). These results are consistent with the observations presented above, with the 'local' seals having higher PCB concentrations relative to the OCP concentrations (i.e. higher ∑PCB/OCP ratios) than the 'long-range' seals. The concentration-weighted average log K_{ow} value for PCBs in 'local' ringed seals (7.2 ± 0.04) was higher than 'long-range' seals (6.9 ± 0.05, p=0.005). Overall these results are consistent with Brown et al. (2014a) and further support the grouping of the seals with regard to PCB source. These results also demonstrate that regional contaminant hot spots can have a larger influence on contaminant concentrations than factors (e.g. age and sex) that have been established as drivers of contaminant accumulation (Lydersen et al., 2002; Muir et al., 2000; Nakata et al., 1995; Routti et al., 2010b).

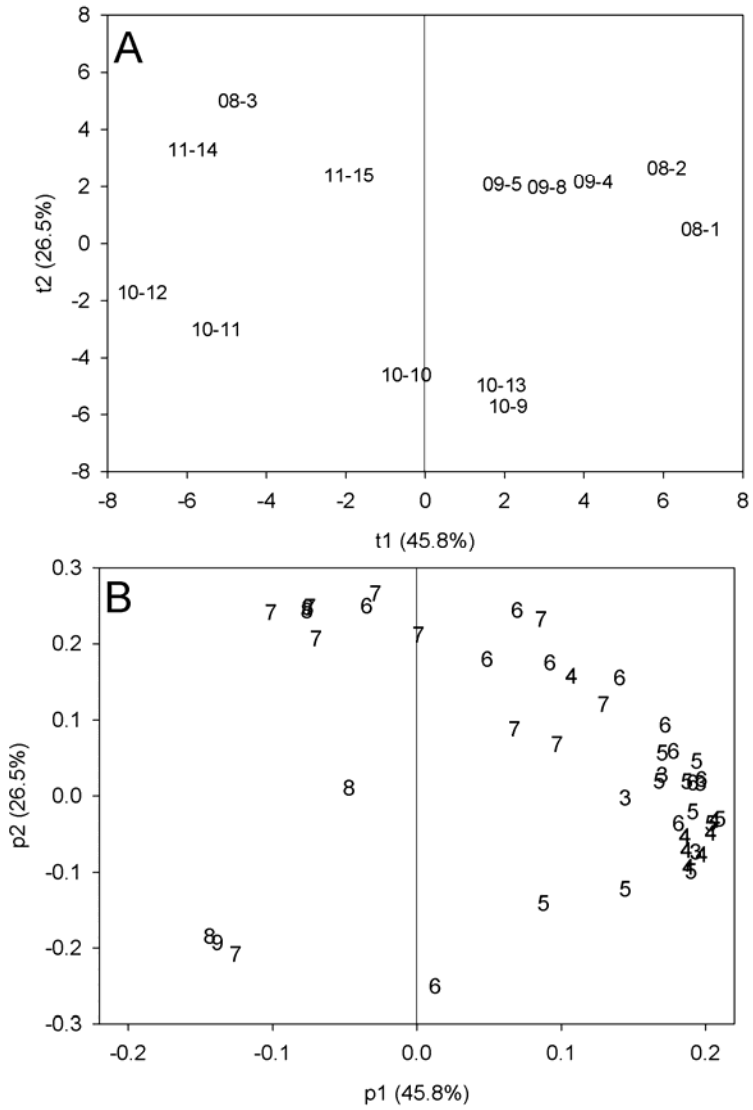

Figure 12: A Principal Components Analysis (PCA) of PCB patterns (47 congeners) in ringed seals reveals that individual seals (numbers depict two digit year of sampling, followed by seal ID) to the left of the scores plot (A) are dominated by heavier congeners (B), consistent with exposure of a local PCB source. Numbers in (B) identify the degree of chlorination of each PCB congener (i.e. number of chlorines per congener).

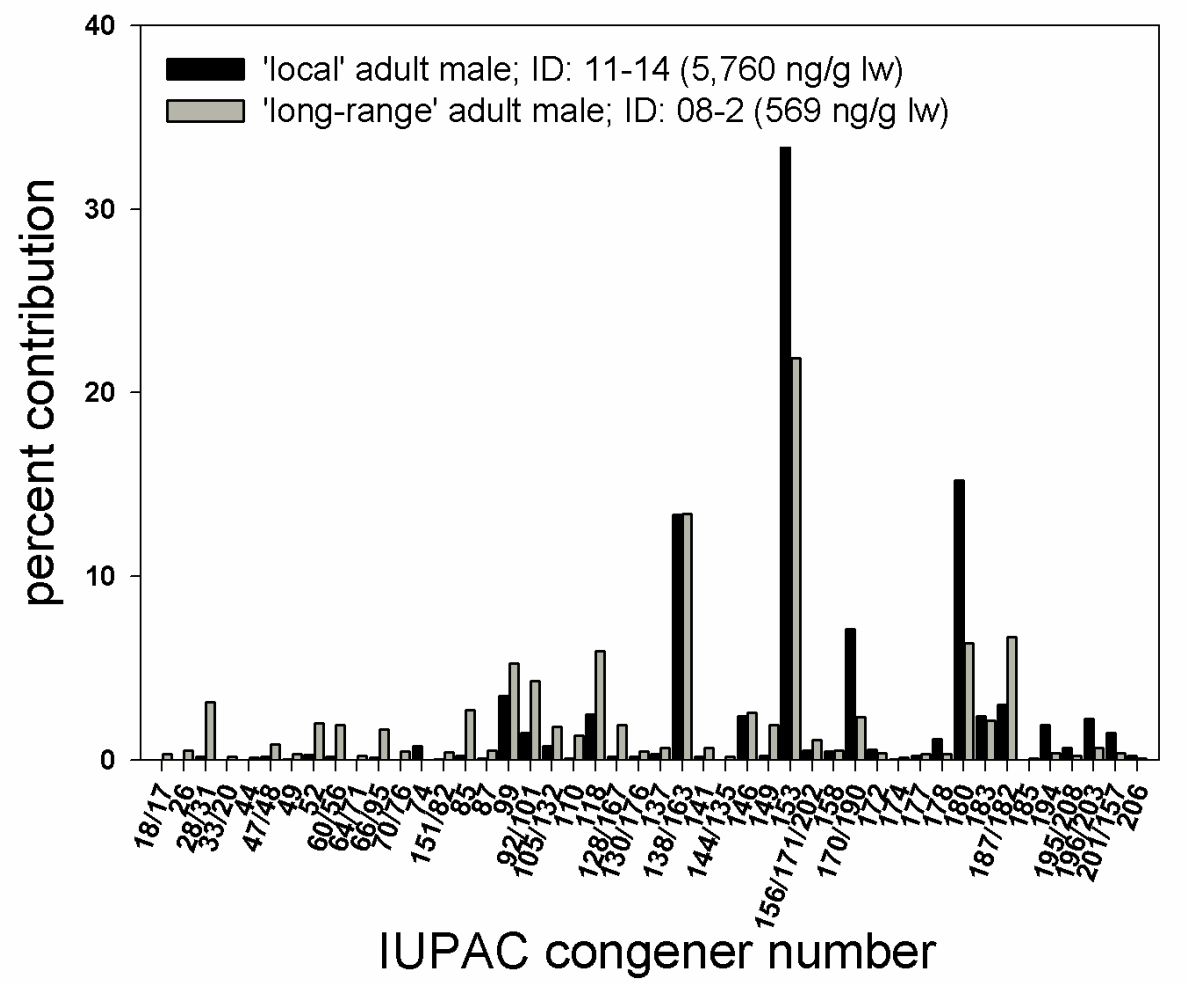

Figure 13: Variation in PCB profiles as a percentage of total PCB concentrations in a representative 'local' adult male ringed seal and a 'long-range' adult male seal. Values in the legend represent their ∑PCB concentration.

Table 8: Geometric means, standard error and ranges (ng/g lipid weight) of ∑PCB and organochlorine pesticide concentrations in blubber biopsies of ringed seals collected from Saglek Fjord.

	local	long-range
∑PCBs[a]	1,930 ± 1,640 (421-10,700)[e]	485 ± 89 (300-1,010)
∑DDTs[b]	389 ± 241 (135-1,600)	192 ± 23 (123-258)
∑Chlordanes[c]	50 ± 32 (18-206)	74 ± 14 (19-110)
∑HCHs[d]	48 ± 5.8 (37-71)	67 ± 8.9 (50-108)
Dieldrin	78 ± 42 (13-272)	47 ± 7.2 (22-69)
HCB	8.7 ± 2.8 (2.0-19)	5.8 ± 1.1 (3.6-9.8)

[a] ∑PCBs refers to the sum of the 47 PCB congeners. [b]∑DDT refers to the sum of *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT. [c]∑chlordanes refers to the sum of α- and γ-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, heptachlor epoxide. [d]∑HCH refers to the sum of α-, β-, γ-hexachlorocyclohexane. [e]$p \leq 0.05$ compared to long-range seals.

Table 9: Arithmetic means ± SE for the ratios of ∑PCBs/organochlorine pesticides (∑DDTs, ∑Chlordanes, ∑HCHs, Dieldrin, HCB) in blubber tissue of 'local' and long-range' ringed seals.

	Local	long-range
∑PCBs/∑DDTs	6.0 ± 1.5[a]	2.7 ± 0.3
∑PCBs/∑Chlordanes	44 ± 9.7[a]	8.4 ± 1.9
∑PCBs/∑HCHs	60 ± 22[a]	7.7 ± 1.1
∑PCBs/ Dieldrin	62 ± 20[a]	12 ± 2.1
∑PCBs/ HCB	400 ± 96[a]	90 ± 15

[a] $p \leq 0.05$ compared to long-range seals

Comparison to PCB concentrations in ringed seals in the Canadian Arctic

The PCB and OCP concentrations in the 'long-range' seals were generally within the range reported previously for ringed seals in Labrador (Brown et al., 2014a) and the Canadian Arctic (Addison et al., 2005; Fisk et al., 2002; Muir et al., 1999a; Muir et al., 1999b; Muir et al., 2000). However, four of the six (67%) 'local' ringed seals exceeded the highest PCB values reported for ringed seals in the Canadian Arctic over the past 20 years (Fisk et al., 2002; Hoekstra et al., 2002; Muir et al., 2000). The sub-adult female (ID: 08-3, 10,700 ng/g lw; Table 10) that had the highest ∑PCB concentration in the present study, also exceeded the highest values reported in ringed seals in Labrador (9,400 ng/g lw, 10-year old male seal (Kuzyk et al., 2005b)) and in a 7-year old female seal (4,500 ng/g lw) that was collected sometime between 1989-1991, from Inukjuak, Eastern Hudson Bay. The PCB concentration in the 'local' sub-adult female was also two-fold greater than the 'local' adult male (ID: 11-14, 5,760 ng/g lw; Table 10). This result confirms that this female is pre-reproductive and has been feeding more in the contaminated area or on locally contaminated prey than the 'local' adult male ringed seal. This sub-adult female spent more time (2-fold) in Saglek Fjord than the 'local' adult male, as indicated below, which suggests that this female has a stronger affinity to the fjord, and demonstrates the value of using satellite telemetry to assess contaminant exposure.

While none of our long-range seals exceeded established effects thresholds for marine mammals (Hall et al., 2006; Mos et al., 2010; Ross et al., 1996), some of the 'local' seals exceeded endocrine and immune thresholds (67%) and reproductive thresholds (17%). Despite the improvements observed in local marine sediments and lower food web-levels (Brown et al., 2009), these observations highlight the risks in long-lived ringed seals.

Table 10: Total PCB concentrations (ng/g lw), $\delta^{15}N$ and $\delta^{13}C$ ratios for thirteen ringed seals tagged at Saglek Fjord, Labrador, Canada.

ID[a]	PCB source	Sex	Age-class	∑PCBs	$\delta^{15}N$	$\delta^{13}C$
08-1	long-range	male	sub-adult	441	N/A	N/A
08-2	long-range	male	adult	569	N/A	N/A
08-3	local	female	sub-adult	10,700	N/A	N/A
09-4	long-range	male	sub-adult	407	16.1	-15.8
09-5	long-range	male	sub-adult	300	15.3	-17.0
09-8	long-range	male	sub-adult	1,010	17.4	-16.5
10-9	long-range	female	sub-adult	730	15.3	-17.1
10-10	local	male	sub-adult	421	12.4	-18.0
10-11	local	male	sub-adult	1,080	12.8	-17.0
10-12	local	female	sub-adult	2,040	13.2	-16.4
10-13	long-range	female	sub-adult	581	12.8	-16.6
11-14	local	male	adult	5,760	17.1	-15.7
11-15	local	female	sub-adult	892	15.0	-17.1

[a] Numbers depict two digit year of sampling, followed by seal ID.

Space-use and foraging behavior determine the exposure of ringed seals to a pollution hotspot

Mean home range was smaller (p=0.03; Table 11; Figure 14) for 'local' seals (2,281 ± 616 km^2) than for 'long-range' seals (11,854 ± 3,642 km^2). 'Local' ringed seals maintained a relatively consistent pattern of home range dispersion which was generally within 100 km of Saglek Bay (Figure 14A), indicating that 'local' seals were able to secure sufficient prey resources within a small geographic area located within and directly surrounding Saglek Fjord. Home range dispersion of 'long-range' seals was much larger (6-fold) (Figure 14B), varied geographically, and displayed little, if any, site fidelity to one area. For example, one of the 'long-range' sub-adult males (ID: 09-5; home range of 16,493 km^2; Appendix 9) travelled north of Saglek and west into Ungava Bay, where he remained for the duration of his tagging period. A 'long-range' sub-adult female (ID: 10-13; home range=27,141 km^2) travelled offshore north of Saglek, across

Hudson Strait, and remained along the south-western coastline of Baffin Island (Appendix 9). While, the other 'long-range' sub-adult female (ID: 10-9; home-range= 16,675 km^2; Appendix 9) and one of the 'long-range' sub-adult males (ID: 09-8; home-range= 6,722 km^2; Appendix 9) travelled south of Saglek, towards the southern tip of Labrador. One of the 'long-range' sub-adult males (ID: 08-1; home-range= 15,491 km^2; Appendix 9), remained in Saglek Bay for 1% of his time, then travelled south to Okak Bay and then moved offshore (~150 km) and remained there till the end of his tagging period. The two (28%) remaining 'long-range' seals (sub-adult and adult male) displayed similar home ranges (ID: 09-4; home range=1,035 km^2 and ID: 08-2; home range=420 km^2; Appendix 9) and behaviors to that of the locally contaminated seals, whereby they remained closer to shore (<52 km E-W) and to Saglek Fjord (<40 km N-S). The similar space-use measures for these two 'long-range' seals may reflect the fact that these seals had not yet come into contact with the contaminated prey at Saglek. The adult male (ID: 08-2) displayed extreme site fidelity to the south-western part of Saglek Fjord, but didn't approach or swim through the zone of PCB contaminated sediments in the Saglek Anchorage area. This observation is consistent with large adult ringed seals in Svalbard which displayed extreme site fidelity to areas in front of tidewater glaciers (Lydersen et al., 2014). The minimum distance travelled by these two seals may be the reason for the lack of difference ($p > 0.05$) between the two groups for mean longitude and mean latitude (Table 11).

Mean core areas were smaller (p=0.03) for 'local' seals (487 ± 128 km^2) than 'long-range' seals (1,912 ± 722; Table 11, Figure 14). All core areas for the 'local' seals were located within Saglek Bay and other nearby surrounding inlets (Appendix 10), consistent

with exposure to contaminated prey. In contrast, the core areas for the 'long-range' seals were generally located further offshore and at great distances from Saglek Bay (e.g. Ungava Bay, Baffin Island, and central and southern Labrador coast; Appendix 9).

Time spent in coastal inlets was greater (p=0.02) for 'local' seals (31 ± 6 %) than 'long-range' seals (9 ± 0.4; Table 11, Figure 14). This observation is consistent with the locations of the 'local' seal core areas which were generally located within coastal inlets compared with 'long-range' seals which were further offshore (Appendix 9 and 10).

Although there was no significant difference ($p > 0.05$) in the average water depth of the core areas between the two groups, the data suggests (Table 11) that the water depth for 'local' seals tended to be shallower (51 m; range 1-102 m; n=5) than that of the 'long-range' seals (95 m; range 15-196; n=6). The lack of significance between the two groups could be due to the data being underpowered: the sample size may not be large enough to pick up a statistically significant difference (values for two of the seals could not be calculated due to the current GEBCO bathymetric data coverage). The observation of 'local' seals feeding at shallower depths than the 'long-range' seals is consistent with the locations of their core areas (Figure 14A); such that 'local' seal core areas were located in shallow bay areas and at the heads of the inlets rather than towards the mouths of the inlets and/or further offshore.

Although time spent within Saglek Fjord did not differ ($p > 0.05$) between the two groups, the data suggests (Table 11) that the 'local' seals tended to spend more time in Saglek Fjord (17%; range 1-47%; n=6) than did the 'long-range' seals (10%; range 0-52%; n=7). The lack of difference between the two groups is likely due in part to the 'long-range' adult male seal (ID: 08-2) that spent (52%) of his time in the inner part of

Saglek Fjord. Four (67%) of the 'local' tagged ringed seals, including the highly contaminated adult female, surfaced at least once within the zone of PCB contaminated sediments in the Saglek Anchorage area (Figure 14A). The tracks of the other two 'local' seals (ID: 10-12 and 11-15; sub-adult females) suggest that they swam through the contaminated zone, with locations recorded just west and east of Saglek Anchorage. Only two (29%) of the 'long-range' seals (ID: 09-5 and 10-13) were recorded to have surfaced within the Saglek Anchorage area. The remaining five (79%) 'long-range' seals left Saglek Fjord via the north entrance (Figure 14A).

No differences ($p > 0.05$) in mean time spent feeding benthically and stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) were found between the two groups (Table 11). Even though no significant differences were found, it is however still possible that seals from the two groups have distinct preferences for particular prey species which influence their exposure to the local source. However, prior to drawing conclusions on the importance of particular prey species in delivering local contaminants to ringed seals, more research is needed on feeding ecology, using such methods as fatty acid analysis (Iverson et al., 1997). Furthermore, stable isotope measurements ($\delta^{15}N$) obtained from fur reflect the isotopic elements deposited during the annual moult (preceding spring), which is prior to when the animal was tagged.

These results establish that space-use, as defined by home range, core area, and time spent in coastal inlets is an important determinant of PCB accumulation in ringed seals. The 'local' seals tend to have a smaller home range and displayed a strong preference and/or fidelity to a number of core areas located mostly within inlets both within and directly surrounding Saglek Fjord. In contrast, the 'long-range' seals had a larger home

range with a more dispersed offshore distribution and displayed little, if any, site fidelity to one area.

Strong site fidelity has been previously observed in ringed seals. Kelly et al. (2010) observed fidelity to breeding sites among adult Arctic ringed seals. In two different years, Smith and Hammill (1981) observed the same ringed seal resting on the ice of a Baffin Island fjord. Krafft et al. (2007) marked and subsequently recaptured adult ringed seals (unidentified number) in a fjord on Svalbard in the following year or after many years at sites only a few 100 m from where they were originally marked. Lydersen et al. (2014) observed large adult ringed seals showing strong site fidelity in front of tidewater glaciers, compared with sub-adults which were found to be highly mobile. In the Baltic Sea, Harkonen et al. (2008) monitored ringed seal activity over ten months and found strong spatial fidelity in animals tagged from different areas. Fidelity to small ranges, in general, have numerous biological implications (e.g. foraging ecology, breeding biology, and population structure) for ringed seals (Kelly et al., 2010), and with respect to ringed seals along the northern Labrador coast, increased exposure to a local PCB source.

The fidelity of 'local' seals to Saglek Fjord and its surrounding marine inlets may be related to preferred prey species and/or density of prey in these areas. Saglek Fjord and the marine inlets surrounding Saglek (e.g. Okak Bay) support a diverse and productive benthic and pelagic feeding ground for ringed seals, with a number of diverse habitats including productive nearshore areas dominated by boulders and kelp (Carpenter, 2011; Copeland, 2008). Sculpin are one of the most common benthic fish species found in these nearshore environments and are also the most locally contaminated fish species in the Saglek Anchorage area (Brown et al., 2013; Kuzyk et al., 2005b). It is possible that seals

with a strong affinity to these marine inlets are preferentially feeding on sculpin than less contaminated prey. There may also be genetic differences between the two groups of seals, with 'local' and 'long-range' seals representing two subpopulations with divergent biological and ecological characteristics. Guertin et al. (2012; 2010) used fecal genotyping in river otters to show that time spent in a contaminated harbour influenced POP levels.

The satellite telemetry data from the tagged ringed seals provide a forward-looking representation of their movements subsequent to the attachment of tags at capture. In this manner, we can only assume that the biopsy-based PCB, OCP, and stable isotope values are representative of their prior feeding ecology and habitat use. In addition, stable isotope measurements ($\delta^{15}N$) obtained from fur reflect the isotopic elements deposited during the annual moult (Aubail et al., 2011), whereas the contaminant measurements obtained from blubber reflect total lifetime organic contaminant exposure and accumulation. Despite the temporal and pharmacokinetic differences between these two measurements, the present study shows that the feeding ecology of the seals (i.e. $\delta^{15}N$) influenced PCB profiles, and that the satellite telemetry data were generally consistent with the notion of a distinct point source PCB contribution in 'local' seals. It is important to note that the tagging period varies among seals as a function of moult, polar bear predation, or detachment, such that the longer the seal has been tagged, the more confidence we have regarding its space-use behavior.

This first study applying space-use to inform the source apportionment of pollutants in a marine mammal complements the results of stable isotopes and contaminant profiles and ratios. Using this coupled approach, we were able to explain the mode of exposure as

a function of habitat use and reaffirm our previous assertion that divergent PCB patterns, PCB/OCP ratios, and PCB concentration-weighted average log K_{ow} values observed in Labrador ringed seals can be attributed to the Saglek PCB source (Brown et al., 2014a). We suggest that those seals in Labrador that display reduced home range and core area, as well as an increased time spent in coastal inlets, exhibit an increased likelihood of coming into contact with prey contaminated by the local PCB source at Saglek Bay. The present study demonstrates that satellite telemetry can be used to inform the impact of regional or point source pollution exposure on mobile marine animals. We suggest that space-use studies, such as this one, can be used to inform mitigation and monitoring efforts for contaminated sites, and may well be useful in the exploration of other regional stressors.

Table 11: Summary of space use and foraging ecology information for thirteen ringed seals tagged at Saglek Fjord, Labrador, Canada. Values represent mean (standard error).

	local	**long-range**
Home range (km^2)	2,281 ± 616 (1,145-4,341)[a]	11,854 ± 3,642 (420-27,141)
Longitude (east – west) (UTM)	102 ± 20 (62-173)	306 ± 88 (34-614)
Latitude (north – south) (UTM)	129 ± 25 (71-233)	344 ± 99 (24-710)
Average size of core use areas (km^2)	487 ± 128 (245-1,101)[a]	1,912 ± 722 (143-5,102)
Average depth of core use areas (m)[b]	51 ± 17 (1-102)	95 ± 33 (15-196)
Time spent in coastal inlets (%)	31 ± 6 (14-56)[a]	9 ± 0.4 (1-44)
Time spent in Saglek Fjord (%)	17 ± 1 (1-47)	10 ± 8 (0-52)
Time spent feeding benthically (%)	49 ± 0.7 (45-50)	47 ± 1.4 (41-50)
$\delta^{15}N$	14.1 ± 0.9 (12.5-17.1)	15.4 ± 0.7 (12.8-17.4)
$\delta^{13}C$	-16.8 ± 0.4 (-18.0-(-)15.7)	-16.6 ± 0.2 (-17.1-(-)15.8)

[a] $p \leq 0.05$ compared to long-range seals

[b] values for two of the seals (long-range seal 0802, local seal 1010) could not be calculated due to the current GEBCO bathymetric data coverage

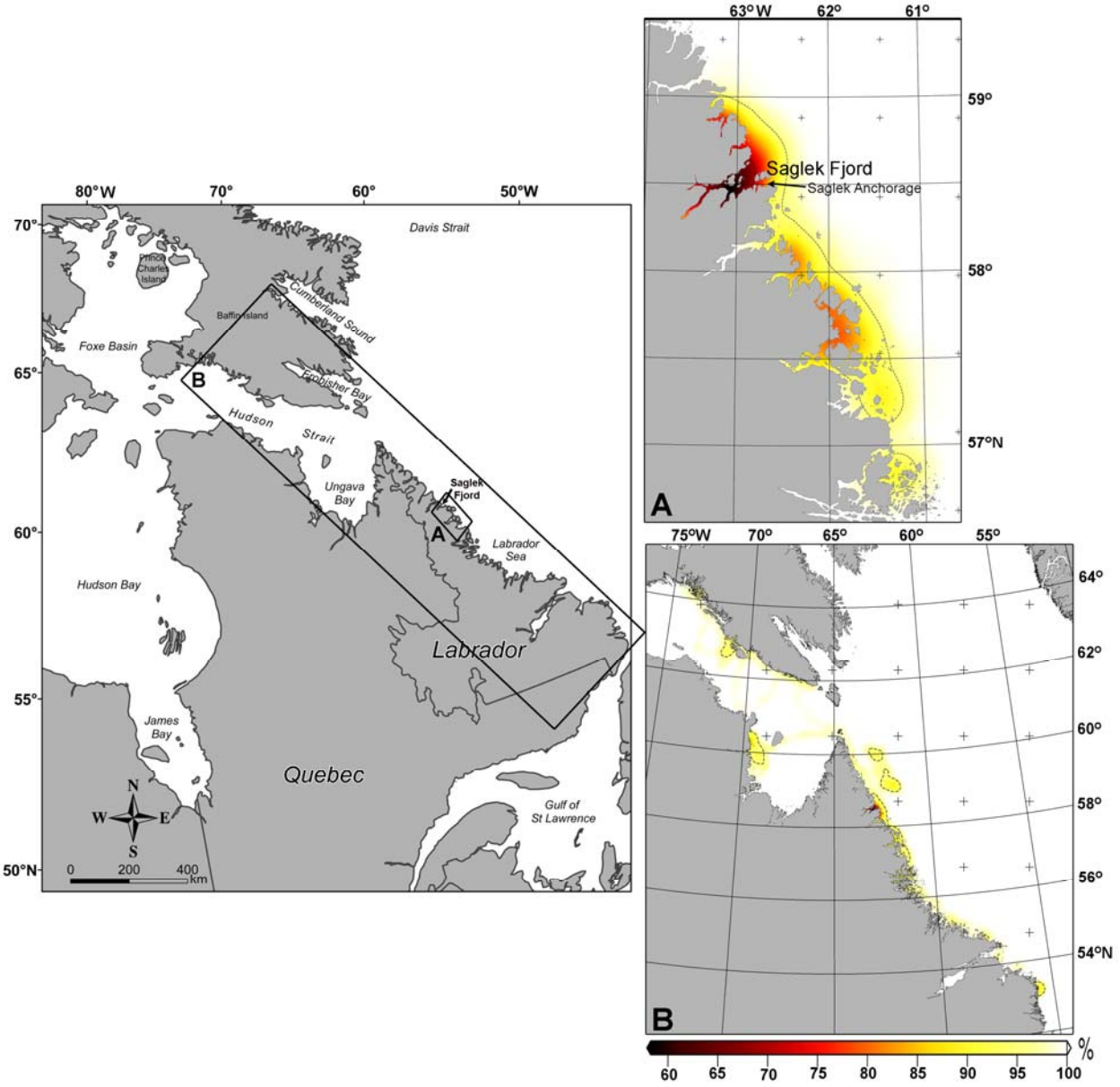

Figure 14: Divergent space-use indices are evident in Labrador ringed seals, as determined by home range and core area. Home range (95%) is defined as the contour intervals for (A) six local and (B) seven long-range ringed seals tagged with satellite transmitters and deployed in Saglek Fjord between 2008 and 2011. Core area (70%) is defined as the contour intervals delineated by the red outline. The outlined boxes in the map of eastern Canada show the area included in the colour utilization distribution maps to the right.

Chapter 4: Local contamination, and not feeding preferences, explain elevated PCB concentrations in Labrador ringed seals (*Pusa hispida*)

Abstract

Polychlorinated biphenyls (PCBs) in high trophic level species typically reflect the contributions of myriad sources, such that source apportionment is rarely possible. The release of PCBs by a military radar station into Saglek Bay, Labrador contaminated the local marine food web. For instance, while heavier (higher chlorinated) PCB profiles in some ringed seals (*Pusa hispida*) were previously attributed to this local source, differences in feeding preferences among seals could not be ruled out as a contributing factor. Herein, similar fatty acid profiles between those seals with 'local' PCB profiles and those with 'long-range' or background profiles indicate little support for the possibility that differential feeding ecologies underlay the divergent PCB profiles. Ringed seals appeared to feed predominantly on zooplankton (*Mysis oculata* and *Themisto libellula*), followed by the dusky snailfish (*Liparis gibbus*) and arctic cod (*Boreogadus saida*). Principal Components Analysis (PCA) and PCB homolog profiles illustrated the extent of contamination of the Saglek food web, which had very different (and much heavier) PCB profiles than those food web members contaminated by 'long-range' sources. Locally contaminated prey had PCB levels that were higher (2- to 544-fold) than prey contaminated by 'long-range' sources and exceeded wildlife consumption guidelines for PCBs. The application of multivariate analyses to two distinct datasets, including PCB congeners (n=62) and fatty acids (n=74), afforded the opportunity to clearly distinguish the contribution of locally-released PCBs to a ringed seal food web from those delivered *via* long-ranged transport. Results from the present study strongly suggest that habitat use

rather than differences in prey selection is the primary mechanism explaining the divergent PCB patterns in Labrador ringed seals.

Introduction

The often complex feeding habits and extensive movements of marine mammals can present a considerable challenge when attempting to attribute the contribution of specific contaminant inputs to the body burden of a given species. Researchers have used a variety of statistical techniques and study designs (e.g., polychlorinated biphenyl (PCB) profiles and persistent organic pollutant (POP) ratios) to infer the contributions of regional and/or local POP sources (Brown et al., 2014a; Calambokidis and Barlow, 1991; Jarman et al., 1996; Krahn et al., 2007; McKinney et al., 2011; Ross et al., 2004). However, without an understanding of the diet of a given species, it remains difficult to account for the contribution of a point source contaminant to the body burden.

Fatty acids, which represent a large group of molecules that comprise the majority of lipids found in all organisms, have emerged as a powerful tool for the assessment of predator diets (Budge et al., 2006; Iverson et al., 2004). The comparison of fatty acids found in predator fat stores with those found in their prey have allowed both qualitative and quantitative assessments of the spatial and temporal scales of foraging of a given species (Falk-Petersen et al., 2004; Iverson et al., 2004) and have been used to characterize trophic links within and among species (Budge et al., 2002; Iverson et al., 1997; Richoux et al., 2005; Stevens et al., 2004a; Stevens et al., 2004b). The inference of diet in predators is possible because many fatty acids transfer from prey to predator adipose tissue with little modification (Budge et al., 2006). Fatty acid analysis has been used to describe dietary processes for contaminants in beluga whales (*Delphinapterus*

leucas) and dietary differences which resulted in regional contaminant level differences (McKinney et al., 2011) and temporal contaminant burden declines (McKinney et al., 2013) in polar bears (*Ursus maritimus*), but has never been used to assess the impacts of point sources in marine ecosystems.

Polychlorinated biphenyls (PCBs) are mixtures of chlorinated hydrocarbons that were banned in the late 1970s in most industrial countries due to their persistent, bioaccumulative, and toxic properties. PCBs are contaminants of concern in arctic food webs, where they can biomagnify to high levels in top predators (Muir et al., 2000). PCB contamination in Arctic marine ecosystems is largely attributed to atmospheric deposition following long-range transport from southern industrial regions (Macdonald et al., 2000; Muir et al., 1999b), but local sources within the Arctic (e.g., military sites) have also contaminated local marine food webs (Brown et al., 2009; Kuzyk et al., 2005a). Saglek Bay, Labrador, Canada has been the site of a military radar station since the late 1950s; however, it was not until 1996 that PCB contamination was discovered at the site, along with evidence that PCBs had contaminated the adjacent marine environment (Kuzyk et al., 2005b). Average PCB concentrations in the nearshore marine sediments exceeded the Canadian sediment quality guideline (21.5 ng/g dry wt) by 41-fold and PCB concentrations in benthic invertebrates, bottom-feeding fish, diving seabird, and some ringed seals were exceptionally high (Kuzyk et al., 2005b). While the elevated PCBs in the benthic-associated food-web closely reflected the concentrations of PCBs in the sediments, determining the contribution of the local source to the body burden of the highly mobile ringed seals proved challenging. ‘Heavier’ PCB profiles and higher PCB:organochlorine pesticide (OCP) ratios recently provided a basis to identify up to

60% of ringed seals sampled in the central and northern Labrador coast as being exposed to the local PCB source at Saglek (Brown et al., 2014a). This chemocentric approach enabled a classification of seals as either ‘local’ or ‘long-range’, reflecting their respective exposure to these two types of sources.

Ringed seals typically feed on a variety of fish, amphipods, euphosiids, mysids, shrimp, bivalves, and cephalopods (Holst et al., 2001; Lowry et al., 1980; McLaren, 1958a; Smith, 1987). Spatial and temporal differences have been detected in the diet of ringed seals (Yurkowski et al., 2014), and some studies have shown diet variability due to age, sex, and season (Holst et al., 2001; Lowry et al., 1980; Thiemann et al., 2007). Contaminant levels and patterns among prey species can differ due to differences in trophic positions, foraging strategies, metabolic transformations, along with other biological factors (Bang et al., 2001; Hoekstra et al., 2003). Thus, two mechanisms could explain the divergent PCB pattern and increased PCB concentrations in locally contaminated ringed seals (Brown et al., 2014a): differences in prey selection between the two groups or feeding on similar prey items that are more contaminated in Saglek Bay.

Herein, we use fatty acid and PCB signature analysis in ringed seals and their prey to determine which mechanism may be involved. In doing so, we evaluate whether contaminant profiles can indeed be used to assign seals to either ‘local’ or ‘long-range’ categories. For this, we obtained samples of adult male and sub-adult ringed seals and their prey from the central and northern Labrador coast and measured them for 62 PCB congeners and 65 fatty acids.

Materials and Methods

Sample collection

Ringed seal blubber samples (*n*=63) were obtained from Inuit hunters in four marine inlets (Nachvak Fjord, Saglek Fjord, Okak Bay, and Anaktalak Bay; Figure 15) along the northern Labrador coast during the fall season (September and October) of 2008. Sex, length, girth, and blubber thickness (at the sternum) were recorded for each ringed seal. Ages were determined at Matson's Laboratory, USA by longitudinal thin sectioning a lower canine tooth and counting annual growth layers in the cementum using a compound microscope and transmitted light. Prey species were collected from 2008 to 2011 from the zone of contamination in Saglek Bay (i.e., Saglek Anchorage) and from several locations in the 3 reference inlets (Table 12). Most of the fish (arctic cod (*Boreogadus saida*); dusky snailfish (*Liparis gibbus*); slender eelblenny (*Lumpenus fabricii*); fish doctor (*Gymnelus viridis*) and pelagic invertebrates (Striped pink shrimp (*Pandalus montagui*); greenland shrimp (*Eualus macilentus*); hyperiid amphipod (*Themisto libellula*); *Mysis oculata*) were collected from the 4 inlets from the CCGS *Amundsen* using integrated vertical tows taken with a double Tucker Trawl (200 µm mesh and 500 µm mesh) and from depth-stratified samples taken with a Hydorbios multinet (200 µm mesh). Nearshore fish (shorthorn sculpin (*Myoxocephalus scorpius*); rock cod (*Gadus ogac*); sand lance (*Ammodytes* spp.); daubed shanny (*Leptoclinus maculatus)*; capelin (*Mallotus villosus*)) and the benthic invertebrates (Arctic argid (*Argis dentata*); Iceland cockle (*Clinocardium ciliatum*); northern astarte (*Astarte borealis*) were collected from the 4 inlets from the long-liner vessel M/V *Whats Happening*. Arctic char (*Salvelinus alpinus*) were collected using gill nets from shore. For all samples collected, appropriate permits and community approval were obtained from the Nunatsiavut

Government, Nunatsiavut Health and Environment Review Committee and Department of Fisheries and Oceans Canada.

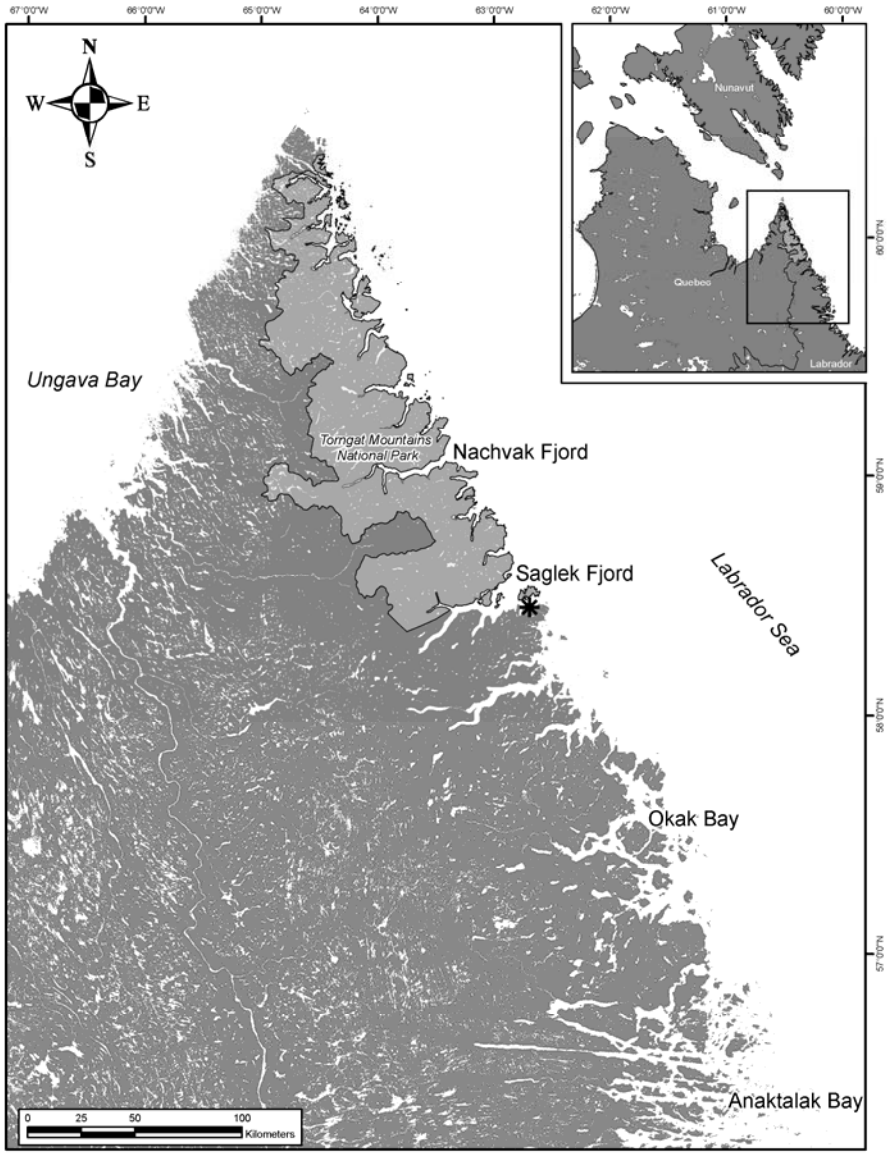

Figure 15: Map of northern Labrador, Canada, showing the location of the four fjords where ringed seals and prey collections were taken. Asterisk shows the location of the former polychlorinated biphenyl (PCB) source and PCB-contaminated sediments at Saglek Bay.

Table 12: Latin and common names including abbreviations for prey items evaluated for fatty acid and PCB congener analyses in the present study. The sample size (*n*), length (±SD), and sample location sites in Labrador (N=Nachvak; S=Saglek; O=Okak; A=Anaktalak) are indicated. n.a., not applicable.

Latin name	Common name	Abbrev	*n*	Length (cm)	Sample Location
Fish					
Ammodytes spp.	Sand lance	SDL	3	7.6 ± 1.1	N, S
Boreogadus saida	Arctic cod	ACD	5	5.5 ± 2.9	N, O
Gadus ogac	Rock cod	GDO	9	33.4 ± 5.2	N, S
Gymnelus viridis	Fish doctor	GMV	2	7.6 ± 2.5	S
Leptoclinus maculatus	Daubed shanny	DBD	21	6.5 ± 2.4	N, S, O, A
Liparis gibbus	Dusky snailfish	LPG	3	8.8 ± 0.6	N
Lumpenus fabricii	Slender eelblenny	LPF	8	5.1 ± 0.8	A, N
Mallotus villosus	Capelin	CAP	9	10.9 ± 4.1	N, S
Myoxocephalus scorpius	Shorthorn sculpin	SSC	12	23.5 ± 3.7	N, S, O
Pelagic invertebrates					
Eualus macilentus	Greenland shrimp	ELM	12	n.a.	S, O, A
Mysis oculata	none	MYS	23	n.a.	N, S, O, A
Pandalus montagui	Aesop shrimp	PDM	7	n.a.	N, S, O, A
Themisto libellula	none	TL	28	n.a.	N, S, O, A
Benthic invertebrates					
Argis dentata	Arctic argid	AGD	21	n.a.	N, S, O, A
Astarte borealis	Northern astarte	AB	15	n.a.	N, S, O, A
Clinocardium ciliatum	Iceland cockle	CCC	12	n.a.	N, S, O
Macoma calcarea[a]	Chalky macoma	MC	4	n.a.	S, O

[a] Only analyzed for PCB congeners.

Fatty acid analysis

Lipid was extracted from the inner blubber layer of ringed seals and from whole homogenized fish and invertebrate samples according to Iverson et al. (2001). Whole homogenized fish and invertebrate samples were used to provide the best representation of the prey fatty acid signatures in ringed seal diets (Budge et al., 2002). Fatty acid methyl esters (FAME) were prepared from the extracted lipid using an acidic catalyst (H_2SO_4 in methanol, (Thiemann et al., 2004)). Duplicate analyses and identification of FAME were performed using temperature-programmed gas-liquid chromatorgraphy according to (Iverson et al., 1997; Iverson et al., 2001) and (Budge et al., 2002; Budge et

al., 2006). Samples were analyzed on a VarianCP-3800 gas chromatograph with a flame ionization detector fitted with a flexible fused silica capillary column (30 m x 0.25 mm inner diameter) coated with 50% cyanopropyl polysiloxane (0.25-um film thickness) (DB-23; Agilent Technologies, Palo Alto, California, USA). Sixty-five fatty acid methyl esters were identified using known standard mixtures, silver nitrate chromatography and mass spectroscopy, and all chromatograms and identifications were individually examined for accuracy in identification and integration of peak areas and corrected and reintegrated as necessary. Fatty acid data are expressed as the mass percentage of total fatty acids. Individual fatty acids are referred to by the shorthand nomenclature of carbon-chain length: number of double bonds, and position of the first double bond relative to the terminal methyl group.

PCB analysis

Concentrations of 62 PCB congeners were analyzed in ringed seal blubber samples by the Great Lakes Institute for Environmental Research's organic analytical laboratory (Windsor, ON, Canada) (Canadian Association for Environmental Analytical Laboratories Accreditation and ISO17025 certified). The detailed methodology for extraction, cleanup, and quantification of target analytes has been reported elsewhere (Brown et al., 2014a; Drouillard et al., 2004; Lazar et al., 1992). Percent lipid was determined using gravimetric lipid determination by weight of extract method with dichloromethane. For each batch of six samples, an in-house reference homogenate tissue, method blank, and the external 1,3,5-tribromobenzene (TBB) recovery standard were analyzed for 62 PCB congeners. All PCB congeners and OCPs were detected with sufficient frequency to be included in data analysis. Recoveries of individual PCB

congeners in the homogenate reference tissue with each sample batch run were within 2 standard deviations from the mean laboratory database value derived from laboratory control charts. Recovery efficiencies for the TBB standard were 89 ± 0.9% (mean ± standard error). Procedural method blanks (n = 18) were below detection for all PCB congeners. All study samples were recovery corrected for PCB congener concentrations. Hereinafter, ∑PCBs refers to the sum of the 62 PCB congeners.

Concentrations of 91 PCB congeners were measured in individual *Macoma calcarea* (n=4) and *Gadus ogac* (n=7) by AXYS Analytical Services Ltd, Syndey, BC to calculate Toxic equivalent concentrations (TEQs) to 2,3,7,8-tetrachlorodibenzo-p-dioxin for PCBs for a sub-set of prey items. Samples were analyzed by GC/MS using AXYS in-house methods (see Brown et al. 2009 for detailed methods). Detection limits for PCB congeners were sample specific and ranged from 0.01 to 1.0 ng/g. Recoveries of 29 PCB congeners from 2 samples of spiked reference materials averaged 96 ± 3.3. Procedural blanks (n=2) were below detection for all congeners. Analytical duplicates were within 10% (n=2). TEQs to 2,3,7,8-tetrachlorodibenzo-p-dioxin were calculated for PCBs using World Health Organization International toxic equivalent factors for humans and wildlife (Van den Berg et al., 1998).

Stable isotope analysis and trophic level calculations

Stable nitrogen isotope ratios were analyzed by Continuous Flow Ion Ratio Mass Spectrometer (CFIR-MS) (Finnigan MAT Delta^{plus}, Thermo Finnigan, San Jose, California, USA). Stable isotope abundances are expressed in delta (δ) values as the deviation from standards in parts per thousand (‰) using the following equation:

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

where R is the ratio of heavy to light isotope ($^{15}N/^{14}N$) in the sample and standard. The nitrogen stable isotope standard was atmospheric nitrogen. Precision estimated from replicate analyses for two standards (bovine muscle (NIST 8414) and an internal lab standard (tilapia fish muscle); n =149 for each) was <0.2 for $\delta^{15}N$. Accuracy of isotope analysis, estimated from NIST standards (sucrose (NIST 8542) and ammonia sulphate (NIST 8547); n = 3 for each) analyzed during the study was within <0.1‰ of certified $\delta^{15}N$ values.

Trophic levels relative to the copepod Calanus hyperboreus, which we assumed occupied trophic level 2 (i.e. primary herbivore), were determined using equations modified from Hobson et al (1995). For each individual sample of pelagic and benthic invertebrates, fish, and ringed seals trophic level was determined using the following relationship:

$$TL_{consumer}=2 + (\delta^{15}Nconsumer - \delta^{15}N_{C.\ hyperboreus})/3.8 \quad (2)$$

Where $TL_{consumer}$ is the trophic level of the organism, $\delta^{15}N_{C.\ hyperboreus}$ is equal to 9.4 ± 0.2 (mean ± SE, $\delta^{15}N$ for *C. hyperboreus*), and 3.8 is the isotopic enrichment factor (Hobson et al., 2002).

Trophic magnification factors were determined from the slope (b) of the linear relationship between log10-transformed PCB 153 concentrations (lipid-normalized) and TL (Fisk et al., 2001a):

$$TMF=10^{b}$$

Data analysis

Fatty acid composition in the blubber of ringed seals can be influenced by age and sex, given potential differences in feeding patterns among demographic groups (Thiemann et al., 2007). To control for these confounding factors, we separated the data into 2 groups

for statistical exploration: sub-adults (<6 yr, male and females combined) and adult males (≥6 yr). Although 65 fatty acids were identified, only the fatty acids known to transfer from prey to predator (Thiemann et al., 2004) were analyzed in the present study. The percent fatty acid values were subjected to centered log ratio transformation (division by the geometric mean of the sample followed by log transformation) prior to multivariate analyses. Multivariate analyses were carried out using Pirouette 4.0 software and the Primer v6 package. Univariate statistical analyses were performed in SPSS 20.0 for Windows.

Ringed seal and prey fatty acid profiles were explored using a principal component analysis (PCA) of the covariance matrix. Multivariate ordination techniques such as PCA have been successfully used for qualitative diet analyses (Bradshaw et al., 2003; Budge et al., 2002; Dahl et al., 2000; Iverson et al., 1997; Loseto et al., 2009). This qualitative analysis shows similarities and differences among ringed seal diet profiles and among prey items, which provide insight into their habitat use and feeding ecology. Qualitative analysis is completed on the fatty acid PCA ringed seal and prey plot by examining the positioning of prey in relation to the ringed seals. For example, prey items positioned closer to the ringed seals have similar fatty acid profiles and likely represent an important prey species. Linear regression was used to assess the relationships between PCA projections and biological variables (e.g., length) and ∑PCBs.

Fatty acid composition of ringed seal blubber for adult males and sub-adults was compared between the two PCB source apportionment groupings ('local' versus 'long-range') and across the four sample location using multidimensional scaling (MDS) and analysis of similarity (ANOSIM) on Bray-Curtis distances (Clarke and Warwick, 1994).

Data pretreatment, methods, and rationale for dividing the ringed seals into two source apportionment groupings: 'local' and 'long-range', are described elsewhere (Brown et al., 2014a). A stress value tending towards zero (>0.1) indicates that there is good separation between the groups with high reliability (Clarke and Warwick, 1994).

PCA was used to elucidate differences in PCB patterns in prey species. Samples were standardized to total PCB concentration before multivariate analyses to remove artifacts related to concentration differences between samples. The centered log ratio transformation was then applied to the data set to produce a data set that was unaffected by negative bias or closure (Ross et al., 2004). Data were autoscaled before PCA.

Results and Discussion

Fatty acid composition of ringed seals

The 10 most abundant fatty acids in ringed seal blubber included the saturated fatty acids 14:0, 16:0, and 18:0, the monounsaturates 16:1n7, 18:1n9, 20:1n9, and 22:1n9 and the essential polyunsaturates 20:5n3, 22:5n3, and 22:6n3. Abundant levels of those fatty acids were similar to those found in ringed seals from across the Canadian Arctic (Thiemann et al., 2007), and other pinnipeds (Iverson et al., 1997) and marine mammals (Dahl et al., 2000; Loseto et al., 2008). The fatty acid composition of adult male and sub-adult ringed seal blubber did not differ between 'local' and 'long-range' groupings (ANOSIM, $p > 0.05$), but varied significantly across locations (ANOSIM, $p < 0.001$). MDS and Pairwise ANOSIM tests indicated that adult male ringed seals from the two northern inlets (Saglek and Nachvak: ANOSIM p=0.07) and two southern inlets (Okak and Anaktalak: ANOSIM p=0.10) tended to have the most similar fatty acid signatures, whereas those separated by greater distance (northern inlet vs southern inlet) had more

distinct signatures (Figure 16A, Table 13). Principal components analysis confirmed these findings for adult males with the first principal component (p1: 50.8%) clearly differentiating ringed seals from the northern inlets from ringed seals from the southern inlets (Appendix 11). The fatty acids explaining the northern ringed seal distribution to the right of the score plot included 20:1n11, 20:1n9, 14:0, 22:1n9, 20:1n11. Whereas, the fatty acids explaining the southern ringed seal distribution to the left of the score plot included 16:2n4, 18:3n1, 20:4n6, 22:4n6, 22:6n30 (Appendix 11).

Significant fine-scale variability was also evident in the sub-adults where seals from Saglek had a distinct signature from seals from the two southern inlets (Okak and Anaktalak) (Figure 16B, Table 13). The fatty acids contributing most to the Saglek sub-adult ringed seals included 22:1n7, 20:1n7, 20:1n11, 22:1n9, 20:1n9 (Appendix 11). These results are consistent with observations in ringed seals from other areas across the Arctic in which fine-scale (<500 km) regional variability (i.e. location) explained the fatty acid signature pattern (Thiemann et al., 2007). There was no relationship ($p > 0.05$) between either t1 or t2 and length for adult male and sub-adult ringed seals. Other marine mammal studies have detected a relationship between diet and length, possibly due to habitat selection relating to a particular size requirement of the animal (Loseto et al., 2008). There was no relationship ($p > 0.05$) between either t1 or t2 and ∑PCBs for adult male and sub-adult ringed seals. These results are consistent with observations above where locally contaminated ringed seals showed no dietary differences from 'long-range' seals.

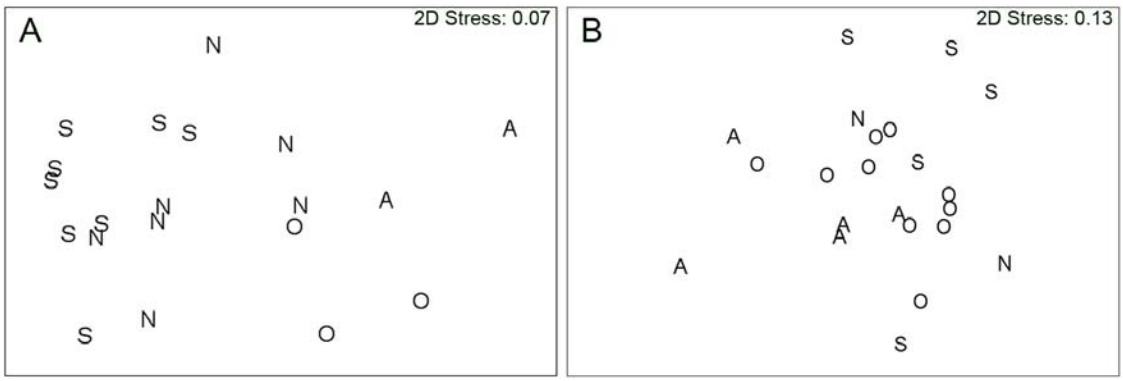

Figure 16: Multidimensional scaling (MDS) plot of Bray-Curtis similarities of fatty acid profiles of (A) adult male and (B) sub-adult ringed seals revealed that location had a significant effect on ringed seal fatty acid signatures at the four sites examined along the northern Labrador coast (see Table 13 for R and *p* values). N=Nachvak; S=Saglek; O=Okak; A=Anaktalak.

Table 13: One-way analysis of similarities (ANOSIM) tests comparing adult male or sub-adult ringed seal fatty acid compositions across the four marine inlet locations. R-values are presented with *p*-value in parentheses. An asterisk (*) indicates significant difference (α = 0.05).

Seal group	Location	Nachvak	Saglek	Okak
Adult male	Saglek	0.155 (0.710)		
	Okak	0.475 (0.036)*	0.772 (0.006)*	
	Anaktalak	0.667 (0.036)*	0.935 (0.022)*	0.750 (0.10)
Sub-adult	Saglek	0.091 (0.76)		
	Okak	0.274 (0.136)	0.425 (0.005)*	
	Anaktalak	0.055 (0.476)	0.396 (0.008)*	0.234 (0.076)

Ringed seal dietary preference

Fifty-seven percent of the variance in the prey and ringed seal fatty acid profiles was explained by the first two PCA axes (PC1: 39.1%, PC2: 17.9%) (Figure 17). The ringed seals in the prey PCA (Figure 17) maintained the same positioning as the ringed seals in the seal PCA (Appendix 11). The placement of the mysid *Mysis occulata* and the amphipod *Themisto libellula* close to the cluster of adult male ringed seals suggests strong similarities among their fatty acid profiles (Figure 17). Arctic cod, Arctic char, and

the dusky snailfish were the next closest prey items to the seals and were plotted on the positive side of the first PCA axis. Prey items furthest from the ringed seals were the two bivalves (*Astarte borealis* and *Clinocardium ciliatum*), which project together on the top right side of the PCA score plot, and the benthic shrimp *Argis dentata*. The sub-adult ringed seal food web PCA showed a similar distribution to the adult male ringed seal food web PCA (Appendix 12), further revealing that *Mysis occulata* and *Themisto libellula* were the most important prey items to ringed seals from coastal Labrador. These findings are consistent with stomach content data (B. Sjare pers. comm.) and stable isotope mixing model and isotopic niche size results (Yurkowski et al., 2014) for the same seals analyzed in the present study with one exception being Arctic cod appeared to dominate the diet more than *Themisto libellula* and the mysid *Mysis occulata*. A possible explanation for this exception could be due to the small size (5.5 ± 2.9 cm, Table 12) and young year-class (1-2 years, (Matley et al., 2013)) of Arctic cod collected in the present study, such that this year-class (and therefore fatty acid composition) may not have been representative of the year-class the seals were feeding on. Generally, adult Arctic cod are distributed deeper in the water column than small, juvenile Arctic cod, which tend to dominate the pelagic zone and shallow areas (Falk-Petersen et al., 1986; Lonne and Gulliksen, 1989). Overall, the fatty acid results from the present study are in partial agreement with previous ringed seal dietary studies that show subadults mainly foraging on zooplankton (e.g. *Themisto libellula* and *Mysis occulata*) and adults consuming primarily Arctic cod (Bradstreet and Cross, 1982; Chambellant et al., 2013; Dehn et al., 2007; Holst et al., 2001; Lowry et al., 1980; Siegstad et al., 1998; Wathne et al., 2000; Weslawski et al., 1994).

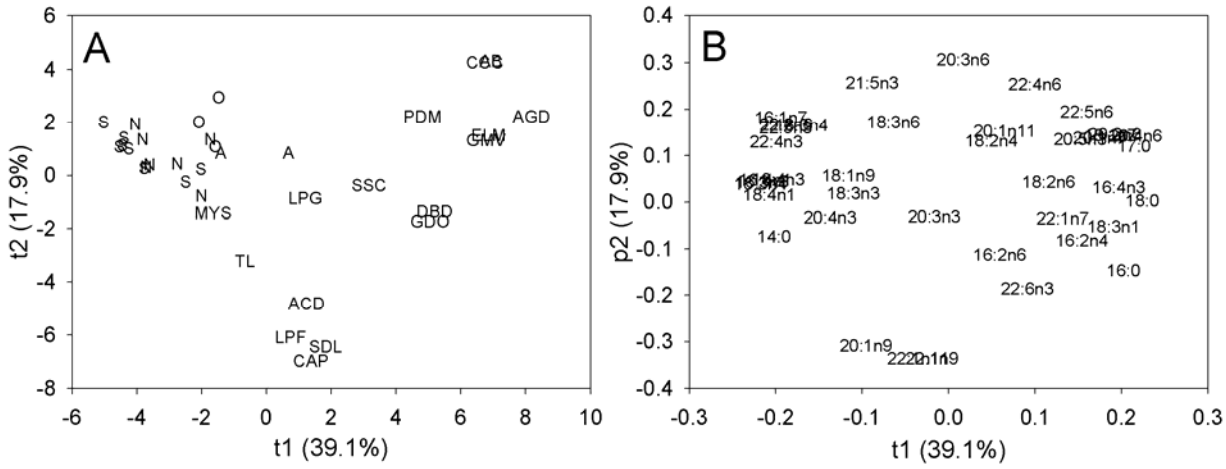

Figure 17: Principal component analysis (PCA) of the 40 dietary fatty acids measured in adult male ringed seals and their prey. The scores plot (A) reveals that seals from four fjords in Labrador (N=Nachvak; S=Saglek; O=Okak; A=Anaktalak) grouped together, with important prey items identified as a function of their proximity to seals. B) Loadings identify those fatty acids that explain seal and prey profiles. Species abbreviations: (AB=*Astarte borealis*; ACD= *Boreogadus saida*; AGD= *Argis dentata*; CCC= *Clinocardium ciliatum*; DBD= *Leptoclinus maculatus*; ELM= *Eualus macilentus*; GDO= *Gadus ogac*; GMV= *Gymnelus viridis*; LPG= *Liparis gibbus*; LPF= *Lumpenus fabricii*; MYS= *Mysis oculata*; PDM= *Pandalus montagui*; SDL= *Ammodytes* spp.; SSC= *Myoxocephalus scorpius*; TL= *Themisto libellula*).

Prey item contaminant patterns

PCB congener patterns reveal strong differences between the Saglek prey items and the reference fjord prey items, with the first principal component explaining 52% of the total variance (Figure 18). The Saglek fjord prey items have a greater proportion of the more heavily chlorinated congeners than the reference fjord prey items, which have a greater proportion of the lighter (less-chlorinated) congeners. The heavy PCB signature is typical of a local source signal, in which more chlorinated congeners dominate the composition of PCBs, whereas the light signature is more characteristic of a long-range atmospheric transport signal (Wania and Mackay, 1993), a result of the favoring of lighter, more volatile congeners during volatilization and atomoshperic transport. These divergent PCB signatures are consistent with the PCB profiles observed in 'local' and 'long-range' ringed seals from northern Labrador (Brown et al., 2014a) and further corroborate previous conclusions that the local contamination is the dominant source for the 'heavier' signature observed in 'local' ringed seals. Based on the divergent PCB congener profiles, the prey were divided into the 'local' and 'long-range' groups. Prey items to the right of the t1 axis, which were collected in the Saglek Bay area, hereafter will be referred to as 'local', and prey items to the left of the t1 axis, which were collected in the reference inlets, hereafter will be referred to as 'long-range'.

We compared the homologue PCB patterns in five prey species to the adult male ringed seals to further investigate the pattern similarities among different species and trophic levels. As expected, the homologue patterns observed in the 'local' and 'long-range' contaminated prey strongly resembled that of the ringed seals (Figure 19). Although depuration and metabolic processes can deplete organisms of lighter PCBs (Boon et al., 1994) and has been found to be the key driver of PCB patterns in farmed and

wild salmon (Yunker et al., 2011) and Arctic beluga whales (Desforges et al., 2013b), the divergent profiles observed between the two groups and the similarities in pattern observed across species and trophic levels strongly suggests that metabolism was not shaping the pattern differences observed in the prey items.

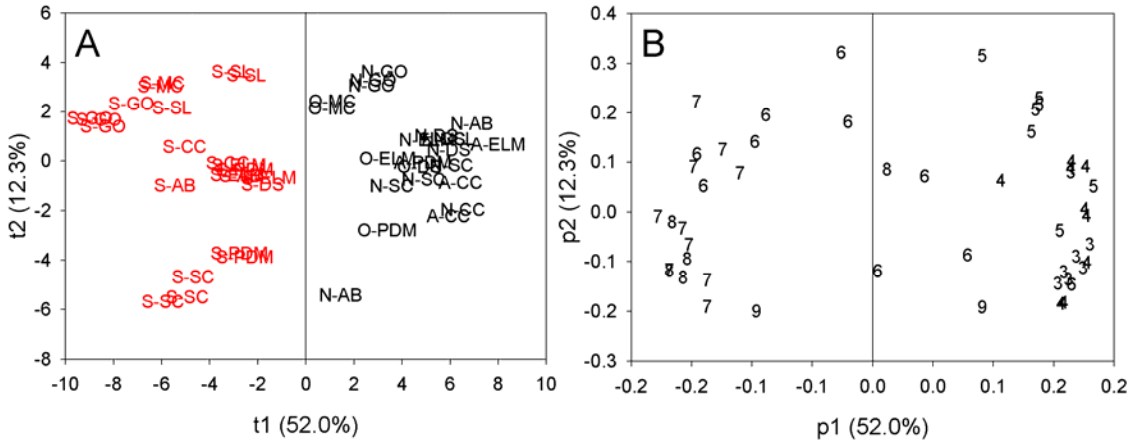

Figure 18: Principal components analysis (PCA) of the 53 polychlorinated biphenyl (PCB) congeners reveal that prey on the right of the scores plot are dominated by heavier congeners, consistent with exposure to the local Saglek source (A: Symbols represent prey from the four fjords in Labrador: N=Nachvak; S=Saglek; O=Okak; A=Anaktalak). Numbers in the loadings plot identify the degree of chlorination of each PCB congener (B). Species abbreviations: (AB=*Astarte borealis*; CCC= *Clinocardium ciliatum*; DBD= *Leptoclinus maculatus*; ELM= *Eualus macilentus*; GDO= *Gadus ogac*; MC=*Macoma calcarea*; PDM= *Pandalus montagui*; SDL= *Ammodytes* spp.; SSC= *Myoxocephalus scorpius*).

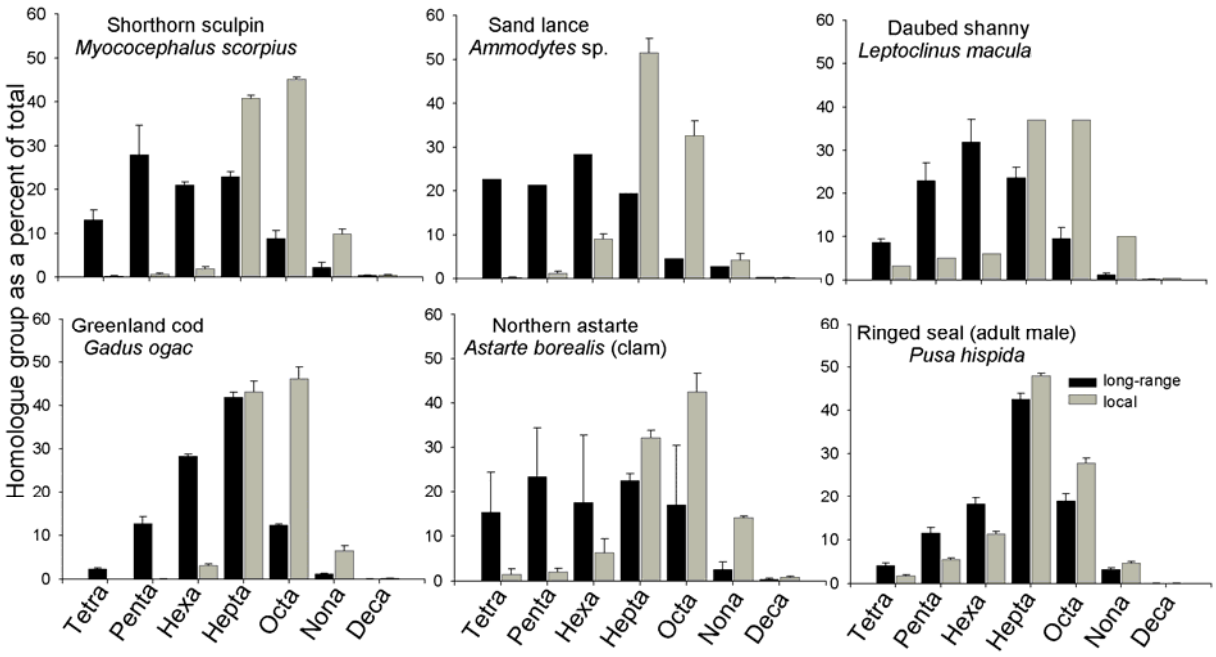

Figure 19: Polychlorinated biphenyl (PCB) homologue group (i.e. degree of chlorination of congeners) patterns in 'local' (grey bars) and 'long-range' (black bars) adult male ringed seals and their prey are similar across food web members.

Prey PCB concentrations

Despite temporal (1998-2006) declines in PCB concentrations in the marine sediments and two indicator species (shorthorn sculpin; *Myoxocephalus scorpius* and black guillemot; *Cepphus grylle*) at the Saglek Anchorage (Brown et al., 2009), PCB concentrations in seven of the nine ringed seal prey species collected in the present study were greater in the 'local' group compared with the 'long-range' group (Table 14). There was no differences (p>0.05) in average PCB concentrations between the two groups for the sand lance (*Ammodytes* sp.) and the bivalve, *Astarte borealis*, however this is likely attributable to the small sample size (Table 14). Overall, these results are consistent with previous observations in ringed seals, with the 'local' seals having higher PCB concentrations relative to the 'long-range' seals (Brown et al., 2014a).

The deposit feeding bivalve (*Macoma calcarea*) from the 'local' group had the highest PCB concentrations compared with all other benthic invertebrates and fish and pelagic species (Table 14). Average PCB concentrations in the 'local' *Macoma calcarea* were up to 10- and 2-fold greater than concentrations in the two suspension feeding bivalves (*Astarte borealis* and *Clinocardium ciliatum*) and the two most contaminated benthic feeding fish (*Myoxocephalus scorpius* and *Gadus ogac*), respectively. The elevated levels in the deposit feeding bivalve *Macoma calcarea* compared with all other species, is likely due to different feeding strategies. Deposit feeders take up PCBs passively through physical contact with porewater (bioconcentration) as well as actively through ingestion of sediment particles (Zhang et al., 2013), whereas suspension feeders take up PCBs *via* physical contact with porewater as well as by pumping water to obtain particulate organic matter utilised as food (Bjork, 1995). Lower concentrations in the benthic feeding fish were likely due to them feeding on invertebrates located both within the contaminated sediments and in areas with lower concentrations of PCBs. PCB concentrations in the two 'local' pelagic shrimp species (*Eualus macilentus* and *Pandalus montagui*) were elevated above background levels in the shrimp and most of the fish and bivalve species from the 'long-range' group (Table 14). Pelagic shrimp accumulate PCBs both from water and from food and in some instances do this more rapidly than fish (Borga et al., 2005). Furthermore, these species tend to respond more quickly than their predators to fluctuations of organochlorine contaminants in the water column (Bettinetti et al., 2012) due largely to a comparatively short life span and the ability to equilibrate with contaminant concentrations in the water column (Larsson, 1989). These observations suggest that the sediment contamination at Saglek continues to impact the benthic-

associated biota and early-warning indicator species of the pelagic food web. These results also suggest that the 'local' pelagic food web, which includes the two dominant ringed seal prey species: *Themisto libellula* and *Mysis oculata* has contributed to the elevated PCB levels in 'local' ringed seals.

Within the Saglek Anchorage area, average PCB concentrations in the *Myoxocephalus scorpius* have decreased approximately 5-fold ($\Delta_{2007\text{-}2011}$=2,380) since the last marine investigation in 2007 (Table 14; (Brown et al., 2009)). Average PCB concentrations in the benthic invertebrates have decreased 10- to -50-fold since the initial marine investigations conducted in 1998 (Table 14; (Kuzyk et al., 2005b)). Despite these declines and documented declines in the sediment (Brown et al., 2009), biota across all trophic levels continue to exceed background concentrations.

Table 14 Arithmetic means ± SE and ranges [ng/g wet weight (range, *n*)] of PCB concentrations in local and long-range prey items.

Latin name	Abbrev	Local	Long-range	Fold Difference
Fish				
Ammodytes spp.	SDL	115 ± 58	8	14
(n=3, 1)[b]		*(32-227)*[c]	n.a.	
Gadus ogac	GDO	599 ± 105	10 ± 2*[,a]	60
(n=4,3)[b]		*(389-887)*[c]	*(7-14)*[c]	
Leptoclinus maculatus	DBD	71	31 ± 2*[,a]	2
(n=1, 2)[b]		n.a.	*(29-35)*[c]	
Myoxocephalus scorpius	SSC	560 ± 149	12 ± 1*[,a]	47
(n=3, 3)[b]		*(409-859)*[c]	*(10-14)*[c]	
Pelagic invertebrates				
Eualus macilentus	ELM	20 ± 3	3 ± 0.5*[,a]	7
(n=4, 4)[b]		*(14-30)*[c]	*(2-4)*[c]	
Pandalus montagui	PDM	22 ± 0.03	5 ± 3*[,a]	4
(n=2, 2)[b]		*(22)*[c]	*(2-8)*[c]	
Benthic invertebrates				
Astarte borealis	AB	239 ± 115	27 ± 9	9
(n=2, 2)[b]		*(124-354)*[c]	*(18-36)*[c]	
Clinocardium ciliatum	CCC	97 ± 58	12 ± 1*[,a]	8
(n=2, 4)[b]		*(39-155)*[c]	*(9-14)*[c]	
Macoma calcarea	MC	1,090 ± 4	2 ± 0.06*[,a]	544
(n=2, 2)[b]		*(1,080-1,090)*[c]	*(2, 2)*[c]	

[a,]*p<0.05 compared with 'local' group.
[b] number of samples in the 'local' group followed by the number of samples in the 'long-range' group.
[c] PCB concentration range

The ∑PCB TEQs of two of the 'local' prey items were approximately 55- to 70-fold higher than the 'long-range' prey items (Table 15). These results may indicate a PCB-related induction *via* the aryl hydrocarbon receptor (AhR) of detoxifying enzymes in the more contaminated seals. These findings are in agreement with a previous study that found a relationship between hepatic *Ahr* mRNA levels and PCB concentrations in ringed seals from this study area (Brown et al., 2014c).

To further assess potential health risks associated with dietary exposure in seals feeding on contaminated prey from Saglek, we compared prey item ∑PCB (Table 14) and ∑PCB TEQs (Table 15) concentrations with published estimated dietary threshold concentrations. Long-range prey PCB concentrations were below all dietary thresholds. Whereas, 45% of the average 'local' prey (*Gadus ogac*, *Myoxocephalus scorpius*, *Macoma calcarea*, *Astarte borealis*) PCB concentrations were above a PCB dietary threshold concentrations of 140 ng/g diet wet wt for immune and reproductive impairment in seals (Kannan et al., 2000) based on semi-field feeding studies (Boon et al., 1987; Brower et al., 1989; De Swart et al., 1994; Reijnders, 1986; Ross et al., 1995) (Table 14). Three (33%) of the 'local' prey items (*Gadus ogac*, *Myoxocephalus scorpius*, *Macoma calcarea*) were above the 250 ng/g wet wt threshold for reproductive toxicity in mink (*Mustela vison*) established in laboratory feeding studies (Kannan et al., 2000). All of the 'local' prey items and two of the 'long-range' prey items (*Astarte borealis* and *Leptoclinus maculatus*) exceeded the dietary threshold of 20 ng/g wet wt for vitamin A disruption in the European otter (*Lutra lutra*) (Kannan et al., 2000) established in semi-field studies (Leonards et al., 1997; Murk et al., 1998; Smit et al., 1996).

The 'long-range' ∑PCB TEQs for the bivalve *Macoma calcarea* and the benthic-feeding fish *Gadus ogac* fell below the Canadian PCB tissue residue guidelines for the protection of mammalian wildlife consumers of aquatic biota (0.79 ng TEQ/kg diet wet wt, CCME 1999; CCME 2002). Whereas, the 'local' ∑PCB TEQs calculated for these two species exceed this guideline by 6-7-fold (Table 15). These observations substantiate previous findings of adverse effects in some ringed seals from this study area (Brown et al., 2014c).

Trophic magnification factors were calculated for the 'local' and 'long-range' food webs to evaluate the PCB accumulation in each of the two food webs. Log PCB 153 concentrations increased with increasing trophic level in the 'long-range' food web (r^2=0.2, p=0.02), but not in the 'local' food web (r^2=0.01, p=0.67; Figure 20). In the 'local' food web the ringed seals had lower PCB concentrations than the benthic-feeding fish species, *Gadus ogac* and *Myoxocephalus scorpius*. This observation is consistent with previous results with the benthic associated food-web at Saglek being more contaminated than the locally contaminated ringed seals (Table 14, (Brown et al., 2014a; Kuzyk et al., 2005b)). These marine mammals have a home range that is far greater than the spatial extent (~10 km^2) of the contamination at Saglek Bay and are therefore feeding on contaminated prey in Saglek but also on less contaminated 'long-range' prey from elsewhere. These findings are consistent with observations from a recent space-use study which showed 'locally' contaminated ringed seals from Labrador travelling and feeding (2,281 km^2) only within the marine inlets located within and directly surrounding Saglek Fjord (Brown et al., 2014b). The TMF calculated for the 'long-range' food web was similar to that observed in other arctic food webs (Borga et al., 2011). Using the slope

from the 'long-range' food web and a conversion factor (25%) for PCB 153 to ∑PCBs, we would predict that adult male seals that restrict their feeding to the Saglek Anchorage would have an average ∑PCB concentration of approximately 16,300 ng/g lipid wt (Figure 20). This concentration is similar to those measured in ringed seals from the contaminated Baltic Sea where increased phase I enzyme activity and endocrine effects have been reported (Routti et al., 2010a; Routti et al., 2008) and where a history of reproductive and developmental abnormalities exist (Bergman and Olsson, 1985; Helle et al., 1976a; Helle et al., 1976b).

Table 15: Toxic equivalents (TEQs) to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for polychlorinated biphenyls (PCBs) in local and long-range prey items. Arithmetic means ± SE in the local prey items exceed the Canadian tissue residue guideline (0.79 ng TEQ kg^{-1} wet weight) for PCBs for the protection mammalian consumers of aquatic biota, whereas the long-range prey items do not.

Latin name	Abbrev	Local prey items	Long-range prey items
		ng TEQ kg^{-1} wet weight	
Gadus ogac *(n=4,3)*[a]	GDO	5.44 ± 2.6	0.10 ± 0.03
Macoma calcarea *(n=2,2)*[a]	MC	4.81 ± 0.39	0.07 ± 0.04

[a] number of samples in the 'local' group followed by the number of samples in the 'long-range' group.

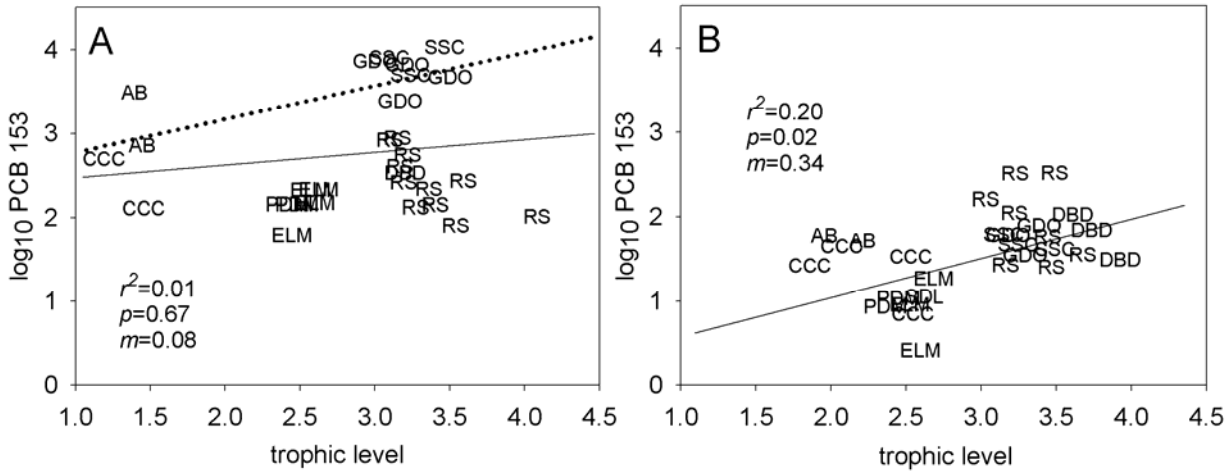

Figure 20: PCB 153 concentration (ng/g, lipid corrected) trophic level relationships for the local (A) and long-range (B) food web. Solid lines are linear regressions for each group. Using the slope from the 'long-range' group we were able predict (A, dotted line) PCB 153 concentrations in adult male ringed seals feeding exclusively in the Saglek Anchorage. Species abbreviations: Species abbreviations: (AB=*Astarte borealis*; CCC= *Clinocardium ciliatum*; DBD= *Leptoclinus maculatus*; ELM= *Eualus macilentus*; GDO= *Gadus ogac*; MC=*Macoma calcarea*; PDM= *Pandalus montagui*; RS=*Pusa hispida*; SDL= *Ammodytes* spp.; SSC= *Myoxocephalus scorpius*).

Conclusions

The results from the present study strongly suggest that habitat use (i.e., geographic foraging range) rather than differences in prey selection is the primary mechanism explaining the divergent PCB patterns in Labrador ringed seals. These findings are consistent with a previous study that explained the mode of exposure as a function of space use (i.e. habitat use), whereby locally contaminated seals displayed a strong preference to inlets located both within and directly surrounding Saglek Fjord (Brown et al., 2014b). To our knowledge, this is the first time that fatty acid signatures have been used to determine regional or local source apportionment of a contaminant in a marine mammal. The present study demonstrates that foodweb tracers, such as fatty acids and

contaminant pattern analysis on prey, can be used to inform the contribution of point source pollution to exposure in mobile marine animals. Furthermore, our study shows that locally contaminated prey exceeded wildlife consumption guidelines for PCBs, which further supports the previous finding that ringed seals foraging within the contaminated area are at risk of adverse health effects, notwithstanding recent temporal declines in sediment PCB concentrations (Brown et al., 2009; ESG, 2013).

Chapter 5: PCB related effects thresholds as derived through gene transcript profiles in locally-contaminated ringed seals (*Pusa hispida*)

Abstract

Causal evidence linking adverse effects to polychlorinated biphenyl (PCB) exposure is typically confounded by the complexity of real-world contaminant mixtures to which aquatic wildlife are exposed. A local PCB 'hotspot' on the Labrador coast provided a rare opportunity to evaluate the effects of PCBs on the health of a marine mammal as this chemical dominated their persistent organic pollutant (POP) burdens. The release of approximately 260 kg of PCBs by a military radar facility over a 30 year period (1970-2000) contaminated some local marine biota, including the ringed seal (*Pusa hispida*). The abundance profiles of eight health-related gene transcripts were evaluated in liver samples collected from 43 ringed seals in the affected area. The mRNA transcript levels of five gene targets, including aryl hydrocarbon receptor (*Ahr*), interleukin-1 β (*Il1b*), estrogen receptor α (*Esr1*), insulin like growth factor receptor 1 (*Igf1*), and glucocorticoid receptor α (*Nr3c1*) correlated with increasing levels of blubber PCBs. PCB threshold values calculated using best-fit hockey-stick regression models for these five genes averaged 1,680 ± 206 ng/g lw, with the lowest, most conservative, being 1,370 ng/g lw for *Il1b*. Approximately 14% of the seals in the region exceeded this threshold. The dominance of PCBs in the seals studied enabled an assessment of the effects of this chemical on gene transcripts involved in regulating the health of a highly mobile predator, something that is rarely possible in the world of complex mixtures.

Introduction

While marine mammals occupying high trophic levels in arctic food webs have been found to be contaminated with moderately high concentrations of persistent organic pollutants (POPs) (Borga et al., 2004; Muir et al., 2000), it remains unclear whether current levels represent a risk to their health. Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs) in the Arctic have been largely attributed to long range transport through atmospheric processes, although some point sources do exist (e.g. military radar stations) (Bright et al., 1995; Macdonald et al., 2000).

The highly complex mixtures of POPs to which marine mammals are exposed in Arctic and sub-Arctic regions render it difficult to ascribe cause-and-effect relationships to any single contaminant. However, PCBs are widely considered a priority contaminant at the top of food webs with impacts on the biology of a variety of northern wildlife species. For example, endocrine disruption, reproductive impairment and immunotoxicity in polar bears (*Ursus maritimus*) have been associated with PCBs (Bechshoft et al., 2012; Braathen et al., 2004; Lie et al., 2005; Sonne et al., 2004; Villanger et al., 2011a). An association between thyroid hormone disruption and some PCB congeners was found in beluga whales (*Delphinapterus leucas*) from Svalbard, Norway (Villanger et al., 2011b). Phase I enzyme and glutathione-S-transferase (GST) activities, which biotransform group III and IV PCBs, were positively associated with PCBs in ringed seals (*Pusa hispida*) from the Baltic Sea (Routti et al., 2008). Furthermore, PCBs were considered to be the cause of uterine occlusions which resulted in reproductive failure in grey seals (*Halichoerus grypus)* in the Baltic Sea (Bergman and Olsson, 1985; Helle et al., 1976b).

More recently, changes in hepatic and circulatory vitamin A levels and hepatic *Ahr* and *Cyp1a1* mRNA levels in beluga whales from the western Canadian Arctic have been associated with PCBs (Desforges et al., 2013a; Noel et al., 2014).

In southern, more contaminated areas, chemical detoxification enzymes and altered endocrine and immune functions in several marine mammal populations have also been associated with PCBs (Beineke et al., 2007; Buckman et al., 2011; Lahvis et al., 1995; Mos et al., 2007; Tabuchi et al., 2006). In all these cases, however, correlations between health effects and PCB concentrations are based on the premise that PCBs are either the putative contaminant driving the relationship, or represent a proxy for the other co-correlating POPs.

A PCB point source associated with a military radar station in Saglek Bay, Labrador, Canada, has contaminated the adjacent marine food web (Brown et al., 2014a; Brown et al., 2009; Kuzyk et al., 2005b). This radar facility has been in operation since the late 1950s; however, it was not until 1996 that extensive contamination was discovered in three areas (Site Summit, Antenna Hill, and beach area) at the site, and that PCBs were found to have entered the marine environment (Kuzyk et al., 2005b). Very high PCB concentrations were measured in the local marine sediments, the benthic food web, and ringed seals (Brown et al., 2014a; Brown et al., 2009; Kuzyk et al., 2005b). Results of an ecological risk assessment indicated that shorthorn sculpin (*Myoxocephalus scorpius*) and black guillemot (*Cepphus grylle*) nestlings from the area were at increased risk of impaired reproduction or death (Brown et al., 2013). Until now, no studies have assessed health risks to ringed seals, despite the findings that up to 60% of ringed seals sampled in the region were exposed to the local PCB source and that average PCB concentrations in

adult male ringed seals exceeded a 1,300 ng/g (lipid wt; blubber) threshold for endocrine and immunotoxic effects derived for harbor seals (*Phoca vitulina*; (Mos et al., 2010)).

The combination of a long-range POP 'background' and a local PCB 'hotspot' on the Labrador coast provides an invaluable opportunity to evaluate the effects of PCBs on the health of a free-ranging marine mammal. The present study investigates the relationship between ringed seal blubber PCB burden and hepatic mRNA abundance profiles of gene transcripts encoding proteins that play an important role in animal health with respect to chemical detoxification, the immune and endocrine systems, and the regulation of growth, development, and metabolism. Further, this transcriptomic approach provides quantitative information at the molecular level, which could serve as an early detection indicator for higher-level health effects (Tabuchi et al., 2006).

Materials and Methods

Sample collection

All tissue samples from adult (≥ 6 years) and sub-adult (< 6 years) ringed seals (n=43) were obtained from Inuit hunters in four marine inlets (Nachvak Fjord, Saglek Fjord, Okak Bay, and Anaktalak Bay) along the northern Labrador coast during the fall season (September and October) from 2009-2011 (see Figure 1 in [23]). Males/females ratio was 10/11 and 11/11 for sub-adult and adult seals, respectively. Sex, weight, length, girth and blubber thickness (at the sternum) were recorded for each ringed seal. Ages were determined at Matson's Laboratory, USA, by longitudinal thin sectioning a lower canine tooth and counting annual growth layers in the cementum using a compound microscope and transmitted light. Samples used for stable isotopes (muscle) and organochlorines (blubber) were stored at -20 ^{o}C prior to the analyses. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$)

isotope signatures were evaluated because altered mRNA transcript levels have been associated with changes in feeding ecology in another marine mammal species, the arctic beluga whale [15]. Liver samples (~ 1 g) collected for the measurement of mRNA abundance profiles were preserved directly in the field in RNA*later* tissue preservation solution as per the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA) and stored at -20 °C until isolation of total RNA. All tissue samples were obtained within one hour of harvesting.

Contaminant analysis

Ringed seal blubber samples were analyzed by the Great Lakes Institute for Environmental Research's organic analytical laboratory (Windsor, ON) (Canadian Association for Environmental Analytical Laboratories Accreditation and ISO17025 certified) for concentrations of 62 PCB congeners and organochlorine pesticides (OCPs): α-, β-, γ-hexachlorocyclohexane, α- and γ-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, heptachlor epoxide, dichlorodiphenyldichloroethane [*p,p'*-DDD], dichlorodiphenyldichloroethylene [*p,p'*-DDE], *p,p'*-DDT, dieldrin, hexachlorobenzene (HCB). The detailed methodology for extraction, cleanup, and quantification of target analytes has been reported elsewhere (Brown et al., 2014a; Drouillard et al., 2004; Lazar et al., 1992). Briefly, homogenized wet tissue (0.5-1 g), anhydrous sodium sulfate, and surrogate standard were ground with motor and pestle and then extracted following a micro-extraction technique (Lazar et al., 1992). Samples were analyzed for individual PCB congeners and OCPs by gas chromatography electron capture detection (GC-ECD). Percent lipid was determined using gravimetric lipid determination by weight of extract method with dichloromethane. For each batch of six samples, an in-house reference

homogenate tissue, method blank, and the external PCB-34 recovery standard were analyzed for 62 PCB congeners. All PCB congeners and OCPs that were detected in 90% of the samples were included in the data analysis, in samples where an individual congener was not detected it was replaced with a random number between the detection limit (0.011 to 0.150 ng/g) and zero. Recoveries of individual PCB congeners in the homogenate reference tissue with each sample batch run were within 2 standard deviations from the mean laboratory database value derived from laboratory control charts. Recovery efficiencies for the PCB34 standard were 99 ± 0.95% (mean ± standard error). Procedural method blanks (n = 11) were below detection for all PCB congeners and OCPs. All study samples were recovery corrected for PCB congener and OCP concentrations.

Hereinafter, ∑PCBs refers to the sum of the 62 PCB congeners, ∑HCH refers to the sum of α-, β-, γ-hexachlorocyclohexane, ∑chlordanes refers to the sum of α- and γ-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, heptachlor epoxide, and ∑DDT refers to the sum of *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT. ∑PCBs was used in the data analysis since both co-planar and non-coplanar PCBs have been shown to elicit toxic effects. Further, less than half (42%) of the dioxin-like PCBs were analyzed in the present study using GC-ECD.

Hepatic RNA isolation and cDNA synthesis

Detailed procedures on total RNA extraction and cDNA synthesis are described elsewhere (Buckman et al., 2011; Noel et al., 2014; Tabuchi et al., 2006). Briefly, each sample was homogenized in a 1.5 mL microcentrifuge tube using a Retsch MM301 mixer mill (Thermo Fisher Scientific, Ottawa, ON, Canada) following the addition of 700 µL

TRIzol reagent (Thermo Fisher Scientific) and a 3 mm diameter tungsten-carbide bead. Samples were homogenized in two 3 min intervals at a frequency of 20 Hz with a cooling period of 2-3 minutes on ice and 180° rotation of the mixing chambers between intervals. Isolated hepatic total RNA was resuspended in 40 µL of diethyl pyrocarbonate-treated distilled deionized water and stored at -80°C. RNA purity and concentration were assessed by spectrophotometry at A_{260} and A_{280} and 1 µg of each sample was subsequently used to prepare cDNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA). Each cDNA sample was diluted 40-fold with PCR-grade water prior to gene-specific analysis.

Quantitative real time polymerase chain reaction (qPCR) assay

Eight genes were selected to provide an overview of the biological consequences of exposure to PCBs, based in large measure on past mechanistic studies of this chemical class. These include aryl hydrocarbon receptor (*Ahr*), thyroid hormone receptor α (*Thra*), estrogen receptor α (*Esr1*), thyroid stimulating hormone β (*Tshb*), retinoic acid receptor α (*Rara*), interleukin-1 β (*Il1b*), insulin like growth factor receptor 1 (*Igf1*), and glucocorticoid receptor α (*Nr3c1*). These protein-encoding genes play critical roles in detoxification pathways, immune and endocrine systems, and the regulation of growth, development, and metabolism. Three additional transcripts were chosen as normalizers for correction of input variation and assessment of sample quality: ribosomal protein L8 (*Rpl8*), β-like 2 actin (*Actbl2*), and eukaryotic translation elongation factor-1 α (*Eef1a1*).

Expressed gene sequences were isolated from ringed seal by PCR-directed cloning using degenerate primers designed against aligned gene sequences from different mammal species as described elsewhere (Buckman et al., 2011; Noel et al., 2014) and

deposited in NCBI GenBank (Appendix 13 and 14). The obtained species-specific cDNA sequences were used to evaluate previously established marine mammal qPCR primer sets for efficacy towards ringed seal as well as perform species-specific *de novo* design with Primer Premier 5 (PREMIER Biosoft International, Palo Alto CA, USA). All primers were obtained from Integrated DNA Technologies Inc. (Coralville, IA, USA). Gene-specific qPCR was assessed in liver using a three-tier quality control process as outlined in detail elsewhere (Veldhoen et al., 2014). All eleven primer pairs satisfied the criteria for use of the comparative $\Delta\Delta C_t$ quantification method (Livak and Schmittgen, 2001). Ringed seal qPCR primer pair sequences used in the present study are presented in Appendix 13.

qPCR assays using SYBR Green I-based detection were conducted on a MX3005P Real-Time PCR System (Agilent Technologies Canada Inc., Mississauga, ON, Canada) as described previously (Veldhoen et al., 2011). For all expressed gene sequences investigated, quadruplicate amplification reactions (15 µL) were performed for each liver sample. The amplification thermocycle program for each transcript consisted of an initial enzyme activation step at 95°C (9 min) followed by 40 cycles of 95°C denaturation (15 sec), 60°C (or 57°C for *Rara*, *Tshb*, *Il1b*) annealing (30 sec), and 72°C elongation (45 sec). A subsequent thermodenaturation profile was included from 55°C to 95°C to evaluate qPCR amplification quality. Inter-run variation was assessed by the use of a standard control amplification reaction that included a pooled ringed seal cDNA sample. Specificity of target amplification was assessed by including a no DNA template control. Replicate cycle threshold (C_t) data for each sample were averaged and transformed to fold change values using the comparative $\Delta\Delta C_t$ method (Livak and Schmittgen, 2001).

Data normalization, which accounted for variation in cDNA input, used a geometric mean calculated from the three normalizer genes (*Rpl8*, *Actbl2*, *Eef1a1*). These three genes displayed strong intercorrelation in abundance across the qPCR data set with a Cronbach's alpha of 0.852 and demonstrated strong normalizer scores in RefFinder (http://www.leonxie.com/referencegene.php?type=reference).

Stable isotope analyses

Muscle tissue (0.5-1 g) was freeze-dried and homogenized. Lipid was extracted from all samples using a chloroform/methanol extraction and then dried for analysis. Carbon and nitrogen isotopic analyses were performed using Continuous Flow Ion Ratio Mass Spectrometer (CFIR-MS) (Finnigan MAT Delta^{plus}, Thermo Finnigan, San Jose, CA, USA). Detailed methodology on the procedure has been reported elsewhere (Brown et al., 2014a). Stable isotope abundances are expressed in delta (δ) values as the deviation from standards in parts per thousand (‰) using the following equation:

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

where *R* is the ratio of heavy to light isotope ($^{15}N/^{14}N$ or $^{13}C/^{12}C$) in the sample and standard. The standards used for carbon and nitrogen analyses were Pee Dee Belemnite limestone formation and atmospheric nitrogen, respectively. Precision based on two standards (bovine muscle (NIST 8414) and tilapia fish muscle internal laboratory standard; n =65 for each) were <0.16‰ and <0.08‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. Accuracy of isotope analysis, based on the NIST standards sucrose (NIST 8542) and ammonia sulphate (NIST 8547) analyzed during the present study (n=3 for each) were within <0.1‰ of certified $\delta^{15}N$ and $\delta^{13}C$ values.

Data analysis

Unless otherwise stated, univariate statistical analyses were performed in SPSS Version 20.0 (IBM Software, Armonk, NY, USA). Data were log-transformed when necessary to meet the normality assumptions for parametric analyses. Linear and non-linear "hockey-stick" regression analyses were used to determine relationships between blubber PCB concentrations and hepatic mRNA abundance. Hockey-stick regression analyses were carried out using R version 2.9.0 (http://cran.r project.org/bin/windows/base/old/2.9.0/). The "hockey-stick" regression assumes a constant background mRNA abundance up to a threshold tissue concentration of PCBs, above which mRNA abundance increases in concert with PCB concentrations reflective of a threshold response (Horness et al., 1998). The two segments meet at the change-point (or "threshold") value. The derived equation is expressed as:

mRNA abundance = a when [PCB]< $[PCB]_T$

and mRNA abundance = $a + b$ ([PCB]-$[PCB]_T$) when ([PCB]>$[PCB]_T$), where the estimated parameters are: a= background mRNA abundance of seals not eliciting a response; b = the slope of the relationship between mRNA abundance and tissue PCB concentration [PCB] above $[PCB]_T$, the threshold PCB concentration. The model generates best estimates for a, b, and $[PCB]_T$.

The best variable or combination of variables (biological: age, sex, year, weight, length, girth, blubber thickness, $\delta^{15}N$ and $\delta^{13}C$; contaminant: ∑PCBs, ∑DDTs, ∑chlordanes, ∑HCH, HCB, dieldrin) to describe the mRNA abundance profile of each gene was selected using the lowest Akaike information criteria (AIC) (SYSTAT Version 13, Systat Software Inc., San Jose, CA, USA). The Akaike differences (Δi) and normalized Akaike weights (w_i) were calculated to select the best variable or variables.

The models with a ΔAIC of zero and up to two were considered to have the most support (Ferguson et al., 2006).

Significant relationships were further evaluated using Principal Component Analysis (PCA) (Pirouette, Infometrix, Bothell, WA, USA). Gene transcript fold change values were autoscaled (scaled to variable mean and standard deviation) before PCA. The PCA scores from axis one and two were regressed by each of the biological or contaminant variables.

Results and Discussion

Contaminant concentrations in ringed seals

Concentrations of ∑PCBs, ∑DDTs, and ∑chlordanes were higher in the blubber of adult male ringed seals than in subadult and adult female ringed seals ($p \leq 0.05$; Table 16). No differences ($p > 0.05$) were found between subadult males and females for ∑PCBs and the 5 OCPs (∑DDT, ∑chlordanes, ∑HCHs, dieldrin and HCB) measured. No differences ($p > 0.05$) were found between subadult and adult female ringed seals for ∑PCBs and the 5 OCPs. Such results are typical for pinnipeds, where females reduce their POP burden by transferring fat-soluble contaminants to offspring *via* placental and lactational transfer (Addison and Brodie, 1977; Fisk et al., 2002). The ∑HCHs, HCB, and dieldrin concentrations did not vary ($p > 0.05$) between sexes or age classes. Similar results between sexes have been observed previously in ringed seals for these contaminants (Brown et al., 2014a; Fisk et al., 2002).

While average ∑PCB and OCP concentrations for ringed seals at the Labrador sites fell within the range observed across the Canadian Arctic (Addison et al., 2005; Brown et al., 2014a; Fisk et al., 2002; Muir et al., 1999b; Muir et al., 2000), three features stood out

in the present study. Firstly, some individuals (14%) exhibited much higher PCB levels than expected for ringed seals in the Arctic, something we previously attributed to feeding in the contaminated Saglek Bay (Brown et al., 2014a). Secondly, PCBs accounted for 58-68% of the total POP profile in Labrador ringed seals (Table 16), which far exceeds the contribution observed in ringed seals elsewhere in the Canadian Arctic (~25-40%) (Fisk et al., 2002; Gaden et al., 2012; Muir et al., 2000). DDT was consistently ranked as the second most prevalent POP, with some variation (∑Chlordanes or ∑HCHs) across the three age/sex categories for the third POP (Table 16). This variation is likely due to differing biological status related to seal age and sex that influence POP uptake and metabolism. Thirdly, some seals in the area were previously shown to have heavier PCB congener profiles than others, consistent with exposure to a local source (Brown et al., 2014a). Polybrominated diphenyl ethers in blubber and perfluoroalkyl compounds in liver have been detected in ringed seals across the Canadian Arctic at lower or similar concentrations, respectively (de Whit et al. 2006; Butt et al. 2008), to the legacy OCP concentrations in the present study. These compounds were not included in our study but would likely show similar trends to the legacy OCPs.

Labrador ringed seals offer a unique opportunity to examine PCB effects in a marine mammal species as PCBs dominate and concentrations of other POPs are extremely low compared to background levels in more industrialized regions. While none of the adult female seals exceeded proposed PCB effects thresholds for marine mammals (Hall et al., 2006; Mos et al., 2010; Ross et al., 1996), nearly half (46%) of the adult males and 1 (5%) of the subadults (<1 yr) exceeded endocrine and immune thresholds. Overall, these results suggest that ringed seals inhabiting the northern Labrador coast are at increased

risk for endocrine and immune disruption from PCBs compared to seals inhabiting other areas in the Arctic. This increased risk can be attributed to the exposure to the 'local' PCB source at Saglek Bay.

Table 16: Blubber contaminant concentrations (ng/g lipid weight) for subadult (<6 yr; male and female combined), adult male (≥6 yr), and adult female (≥6 yr) ringed seals collected from northern Labrador, Canada (mean ± standard error and range).

	subadults (A)	adult female (B)	adult male (C)	Anova	Tukey's
n	21	11	11	N/A	N/A
∑PCBs[a]	477 ± 62	630 ± 296	1,290 ± 231	p<0.001*	(AC: p<0.001; BC: p=0.005)
	(130-1,390)	(142-1,090)	(464-2,430)		
∑DDTs	235 ± 24	196 ± 174	555 ± 96	p<0.001*	p<0.001 (AC; BC)
	(36-444)	(31-682)	(189-1,110)		
∑Chlordanes	23 ± 2	28 ± 14	54 ± 13	p=0.003*	(AC: p=0.002; BC: p=0.03)
	(9-42)	(8-50)	(11-161)		
Dieldrin	27 ± 6	30 ± 11	36 ± 6	p=0.557	
	(12-70)	(16-52)	(14-59)		
∑HCHs	52 ± 4	38 ± 13	52 ± 5	p=0.080	
	(18-99)	(21-56)	(36-85)		
HCB	13 ± 6	7 ± 1	5 ± 1	p=0.550	
	(3-133)	(3-16)	(2-10)		

* $p < 0.05$ among subadults (A), adult females (B), and adult males (C).
[a]∑PCBs include the following: 19, 18/17, 24/27, 16/32, 26, 28/31, 33/20, 22, 45, 46, 52, 49, 47/48, 44, 42, 64/41/71, 40, 74, 70/76, 66/95, 91, 60/56, 92/84/101, 99, 97, 87, 85, 136, 110, 151/82, 144/135, 149, 118, 134, 146, 153, 105/132, 141, 179, 137, 130/176, 138/163, 158, 178, 187/182, 183, 128/167, 185, 174, 177, 156/171/202, 157/173/200/204, 172, 180, 201, 170/190, 199, 203/196, 207, 194, 206.

PCB-related changes in hepatic mRNA abundance

We know of no other case of a marine mammal population that has been solely exposed to a PCB point source, such that this study afforded us with the opportunity to investigate the association between liver mRNA abundance profiles and PCB concentrations.

A correlation was observed between 5 of the 8 gene transcripts assessed (Aryl hydrocarbon receptor (*Ahr)*, interleukin-1 β (*Il1b*), estrogen receptor α (*Esr1*), insulin like

growth factor receptor 1 (*Igf1*) and glucocorticoid receptor α (*Nr3c1*)) and ∑PCB concentrations; with both linear and non-linear regressions being significant (Appendix 15; Figure 22; Table 17; $p < 0.05$). Coefficients of determination (r^2) were highest for the non-linear regressions compared with the linear regressions. We therefore present the non-linear 'hockey stick' regressions (Figure 22; Table 17). Modeling of data using a 'hockey-stick' regression has been previously used to establish sediment quality thresholds and effects thresholds in biota (Horness et al., 1998; Kuzyk et al., 2005a). Furthermore, this approach provided a model that incorporates a change point representative of a contaminant level (threshold) below which an effect is not expected (Horness et al., 1998). The correlation between hepatic *Ahr*, *Il1b*, *Esr1*, *Igf1* and *Nr3c1* mRNA abundance and ∑PCB concentrations and their associated effects thresholds for ringed seals from Labrador (Table 17) suggest that contaminant-associated responses in molecular endpoints can be detected at levels considerably lower (~10- to 20-fold) than those currently observed in ringed seals from the contaminated Baltic Sea where increased phase 1 enzyme activity and endocrine effects have recently been reported (Routti et al., 2010a; Routti et al., 2008) and where a history of reproductive and developmental anomalies exists (Helle et al., 1976b).

For ringed seals, ∑PCB threshold values estimated for the five genes averaged 1,680 ± 206 ng/g lw, with the lowest being 1,370 ng/g lw for *Il1b* (Table 17). Three of the five genes (*Ahr*, *Il1b* and *Nr3c1*) had a threshold similar to those derived for endocrine and immune effects in harbor seals (1,300 ng/g lw, (Mos et al., 2010)) and vitamin A disruption in beluga whales (1,600 ng/g lw, (Desforges et al., 2013a)), but lower than most other thresholds reported for marine mammals (Hall et al., 2006; Ross et al., 1996).

In contrast, *Esr1* (2,460 ng/g lw ∑PCBs) and *Igf1* (1,740 ng/g lw ∑PCBs) had the highest thresholds, suggesting that these parameters may be less sensitive to PCBs compared with the other molecular endpoints. Thus, the most conservative threshold value identified (1,370 ng/g lw ∑PCBs) may be considered as the most protective among those thresholds for ringed seals.

Adult males had the highest ∑PCB levels and exceeded our proposed threshold concentration of 1,370 ng/g lw (Table 16; Figure 22). One sub-adult seal had a PCB concentration (1,390 ng/g lw) that just slightly exceeded the effects threshold concentration. This is consistent with previous findings where the majority of adult males and a minority of sub-adult ringed seals exceeded the effects threshold (1,300 ng/g lw, (Mos et al., 2010)) for immunotoxicity and endocrine disruption in harbor seals (Brown et al., 2014a).

The ligand-induced aryl hydrocarbon receptor (AHR) mediates the metabolism of many POPs, including dioxin-like PCBs (Mimura and Fujii-Kuriyama, 2003). Such compounds bind to the AHR and modulate the activity of the transcriptional regulator which, in turn, induces expression of phase 1 and 2 xenobiotic detoxification enzymes, including cytochrome P450 enzymes (*Cyp1a1*, *Cyp1a2*, and *Cyp1b1*) and UDG-glucuronosyltransferases (*Ugt1a1*) (Stejskalova et al., 2011). Consistent with the results of the present study, hepatic *Ahr* mRNA levels were correlated with non-*ortho* planar PCB concentrations in Baikal seals (*Pusa sibirica*) (Kim et al., 2005) and with total PCB concentrations in arctic beluga whales (*Delphinapterus leucas*) (Noel et al., 2014). In addition, positive relationships between blubber *Ahr* and PCB concentrations have been observed in heavily contaminated killer whales (*Orcinus orca*) from the Northeastern

Pacific (Buckman et al., 2011), striped dolphins (*Stenella coeruleoalba*) from the Mediterranean (Panti et al., 2011), and fin whales (*Balaenoptera physalus*) from the Mediterranean Sea and Gulf of California (Fossi et al., 2010).

A weight of evidence suggests that elevated PCBs and other POPs (e.g. polychlorinated dibenzo-*p*-dioxins and furans) may have contributed to marine mammal epizootics through reduced immunocompetence (Ross, 2002). *Il1b* encodes a pro-inflammatory cytokine that is involved in the pathological process of a number of diseases, including cancer (Moots et al., 1999; Soria et al., 2011; Yano et al., 2008). Similar to our present observations, hepatic *Il1b* mRNA levels in ringed seals from the Baltic Sea were associated with increased hepatic PCB concentrations (Routti et al., 2010a). Expression of *Il1b* is additionally induced by dioxins *via* AHR (Monteiro et al., 2008; Sutter et al., 1991). We observed that *Il1b* and *Ahr* mRNA levels in the liver of ringed seals from the Labrador coast are positively correlated with PCB concentrations in blubber, suggesting that dioxin-like PCBs may be playing an active role in this animal population through AHR-dependent induction of both genes.

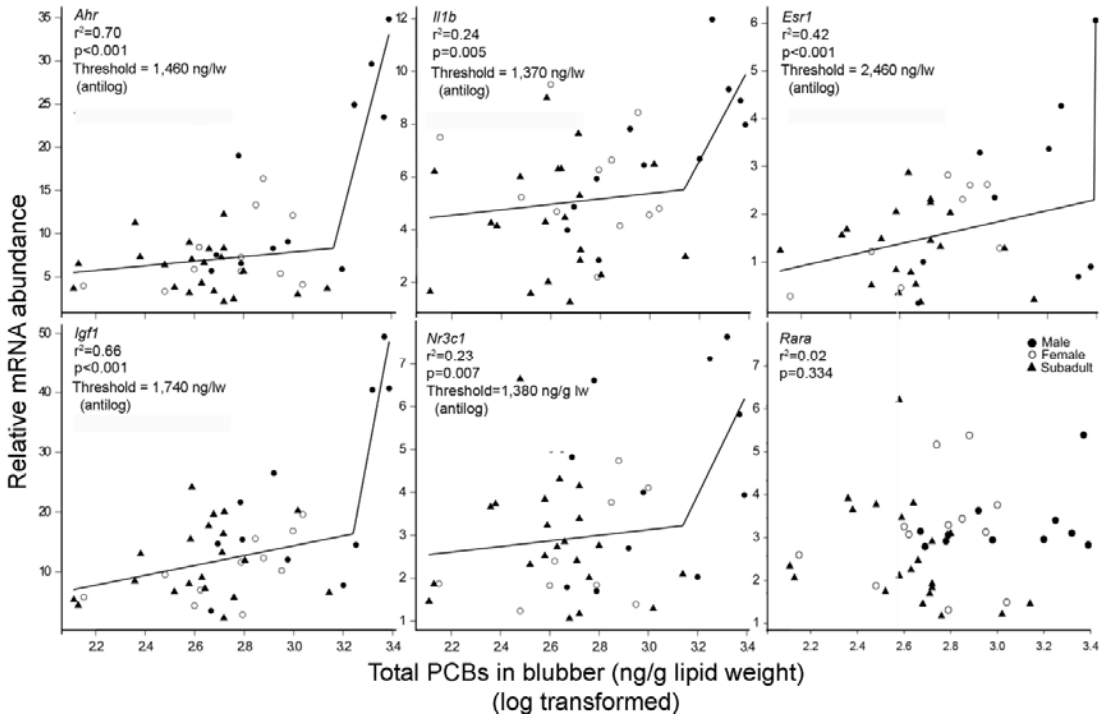

Figure 21: Hepatic mRNA transcript levels for five genes (*Ahr*, *Il1b*, *Esr1*, *Igf1* and *Nr3c1*) correlated with ∑PCBs (ng/g lipid) in the blubber of ringed seals (*n*=43). The data are best described by a non-linear or "hockey-stick" regression. The threshold PCB concentration or "change-point" for each gene is indicated by the antilog. *Rara* is included as a representative of the three other transcripts examined that exhibited no association with ∑PCBs.

Table 17: Effects threshold estimates for the hockey stick regression of the relationship between hepatic mRNA gene transcripts and blubber PCB concentrations in ringed seals (*n*=43).

Gene	Threshold (log ng/g lw)	Threshold (antilog ng/g lw)
Ahr	3.16	1,460
Il1b	3.14	1,370
Esr1	3.39	2,460
Igf1	3.24	1,740
Nr3c1	3.14	1,380
Average ± SE	3.23 ± 2.31	1,682 ± 206

PCBs exhibit both estrogenic and anti-estrogenic activity (Warner et al., 2012). For example, PCBs can interfere with endocrine signaling by mimicking endogenous hormone action through binding to estrogen receptors and modulating their function with resultant impact on estrogen-dependent processes, such as steroid metabolism (Warner et al., 2012). A few studies have looked for a possible link between disruption of estrogen-dependent processes and/or estrogen levels and PCBs in marine mammals. Female polar bears did not show a significant relationship between estradiol and PCB levels in plasma although a borderline negative relationship was observed between estradiol and PCB-118 in females with offspring (Haave et al., 2003). Harbor seals fed PCB/DDE-contaminated fish from the Wadden Sea, The Netherlands, exhibited a perturbation in the estrus cycle consistent with the reduced reproductive success of seal populations from this geographic area (Reijnders, 1986). Killer whales (*Orcinus orca*) from the Northeastern Pacific showed elevated blubber *Esr1* mRNA levels that was positively correlated with blubber ∑PCB concentrations (Buckman et al., 2011). This latter observation is consistent with the results of the present study. Further, previous studies have reported a decrease in circulating levels of estrogen and PCBs in marine mammals from contaminated areas (Haave et al., 2003; Reijnders, 1986).

Igf1 plays an important role in regulating cellular differentiation and proliferation, as well as a number of tissue-specific functions (Laviola et al., 2007). *Igf1* is also involved in the development of a number of diseases, including cancer, diabetes, and growth disorders (Dunger et al., 2003; Renehan et al., 2004; Sherlock et al., 2011). Previous studies have suggested that exposure to environmental contaminants, such as PCBs and other aromatic hydrocarbons, may alter *Igf1* homeostasis in rats and humans (Croutch et

al., 2005; Dickerson et al., 2011; Luzardo et al., 2012). Contaminant-related variation in *Igf1* mRNA levels has not been reported previously in marine mammals. However, *Igf1* mRNA levels were altered by prenatal PCB exposure in rats (Dickerson et al., 2011) and a negative correlation was observed between circulating levels of *Igf1* and PCB concentrations in human serum (Luzardo et al., 2012).

The glucocorticoid receptor (NR3C1) is a steroid hormone receptor involved in regulating growth, development, metabolism, and apoptosis. Contaminant-related effects on *Nr3c1* mRNA levels have not been reported previously in marine mammals. However, there is evidence that PCBs can reduce the number of brain glucocorticoid receptors and that some PCB metabolites can bind competitively to glucocorticoid receptors (Johansson et al., 1998; Xu et al., 2006). In addition, a reduced or delayed cortisol response was observed in fish experiencing stress in heavily polluted waters (Hontela et al., 1997; Hontela et al., 1992; Jorgensen et al., 2002), suggesting that PCBs and other organochlorine contaminants may alter cortisol secretion. Further, gray seal (*Halichoerus grypus*) and ringed seal populations in the heavily polluted Baltic Sea suffered from a disease syndrome which is thought to have been caused by increased glucocorticoid hormones concentrations (Bergman and Olsson, 1985).

The remaining 3 gene transcripts (Thyroid hormone receptor α (*Thra*), Retinoic acid receptor α (*Rara*), Thyroid-stimulating hormone β (*Tshb*)) in ringed seals exhibited no relationship with PCBs. These transcripts are involved in hormone signaling pathways, regulation of growth and metabolism, and development (Mos et al., 2007; Routti et al., 2010a; Tabuchi et al., 2006). These results differ from those of more contaminated marine mammals, which may reflect the lower PCB dose to which our Labrador ringed

seals were exposed. For example, *Thra* mRNA transcripts increased with PCB concentrations in harbor seals (Tabuchi et al., 2006) and killer whales from the Northeastern Pacific Ocean (Buckman et al., 2011), and the liver of ringed seals from the Baltic Sea (Routti et al., 2010a). These marine mammals have concentrations 6 to 300-fold greater than ringed seals in the present study. Higher *Rara* mRNA transcripts with increasing PCB concentrations were observed in the liver of ringed seals from the Baltic Sea (Routti et al., 2010a) and harbor seals from the Pacific Ocean (Mos et al., 2007). Lastly, higher *Tshb* mRNA transcripts were reported with increasing PCB concentrations in the liver of ringed seals from the Baltic Sea (Routti et al., 2010a).

While univariate statistical approaches in the present study provided evidence of PCB-related increases in five hepatic gene transcripts (*Ahr*, *Il1b*, *Esr1*, *Igf1* and *Nr3c1*), best-fit models using AIC (Table 18) confirmed that PCBs explained the variation in the five endpoints. In addition, age, sex, and weight (a proxy for age) contributed to the final model for 3, 2, and 2 of the 5 significant gene targets, respectively (Table 18). These statistical results build on the observations above, which showed that the adult males were most contaminated and were driving the up-regulation of the five PCB-correlated molecular endpoints (Figure 22).

Age and sex-class vs vulnerability to PCB effects

The females in the present study were far less contaminated than the adult males, which can likely be attributed to transfer of PCBs through the placenta and nursing to their young (Addison and Brodie, 1977; Fisk et al., 2002), In this way, our findings that the females and sub-adults exhibited no relationship (p>0.05) with PCBs, while the adult males did for 3 of the 5 significant gene transcripts (*Ahr*: r^2 =0.76; p=0.007; *Il1b*: r^2

=0.62; p=0.004; *Igf1*: r^2 =0.45; p=0.025) illustrates the way in which the contaminant burdens of our study animals straddle our derived effects threshold. The lack of relationship between *Esr1* and *Nr3c1* and ∑PCBs in adult males may be due to the small sample size (*n*=11) and increased variation for these two transcripts. The previously demonstrated endocrine and immune threshold of 1,300 ng/g lw (Mos et al., 2010) is remarkably similar to the inflection point of our most conservative effects threshold (1,370 ng/g lw), providing strong support for an effects threshold in phocid seals for PCBs in this range. In transcriptomic studies of more contaminated marine mammal populations, PCBs drive gene expression response with secondary, limited contributions from age and sex (Buckman et al., 2011; Routti et al., 2010a) further substantiating our threshold derivation for relatively low levels of PCBs.

PCA was used to further explore the factors underlying these patterns in adult males (Figure 23). Forty-three percent of variance in mRNA levels was explained by the first PCA factor (Figure 23a). The PCA variables plot of mRNA transcripts revealed a divergence of the three mRNA gene transcripts (*Ahr*, *Il1b* and *Igf1*) which correlated with ∑PCBs and the other 5 gene transcripts (Figure 23b). ∑PCBs were correlated (r^2 =0.42; p=0.04) with the sample scores of the first principal component (t1) (Figure 23c). None of the other biological variables correlated with t1 or t2 ($p > 0.05$). Despite the lack of relationship between *Nr3c1* and ∑PCBs for adult males, *Nr3c1* was positioned relatively close to these three transcripts, suggesting that it too may be affected by ∑PCBs. These observations corroborate with our univariate findings for adult males with mRNA transcript levels for three of the eight genes being driven by ∑PCBs. Collectively our findings suggest that while some Labrador ringed seals have low PCB levels which do

not elicit detectable effects, others, notably the adult males are affected by elevated PCBs.

This present study provides a unique opportunity to evaluate the effects of PCBs on the health of a wild marine mammal, as this chemical class dominated tissue residues over other POPs. Despite declining PCB concentrations and associated effects in bottom-feeding fish (shorthorn sculpin) and seabirds (black guillemots) at Saglek Bay following remedial action (ESG, 2013), we show here protracted effects in a long-lived high trophic level pinniped.

Table 18: Akaike information criterion (AIC) in combination with backwards stepwise regression confirmed that PCB concentrations was the best variable to explain the variations of hepatic mRNA levels for the five genes which correlated with blubber PCB concentrations in ringed seal liver. Models with ΔAIC_c below 2 are presented.

Gene	Predictors	r^2	p-value	AIC	AIC_c	ΔAIC_c	w_i
Ahr	**PCBs, Age**, $\log_{10}$Weight	0.49	<0.001	256.5	264.9	0	0.71
	PCBs, Age, $\log_{10}$Weight, HCH	0.50	<0.001	257.8	260.3	1.3	0.53
	PCBs, Age, $\log_{10}$Weight, HCH, chlordanes	0.52	<0.001	258.2	262.9	1.7	0.11
Il1b	**PCBs, Age**	0.26	0.005	178.3	179.4	0	0.48
	PCBs, Age, $\log_{10}$Blubber	0.26	0.014	179.3	181.2	1.1	0.28
Esr1	**PCBs,** HCH	0.27	0.007	112.8	114.2	0	0.25
	PCBs, HCH, $\log_{10}$Weight	0.30	0.012	113.6	115.7	0.8	0.17
	PCBs, HCH, $\log_{10}$Weight, Age	0.33	0.014	114.3	119.9	1.5	0.12
Igf1	**PCBs, Sex**	0.24	0.007	328.3	329.5	0	0.42
	PCBs, Sex, Age	0.25	0.014	329.6	331.4	1.3	0.53
	PCBs, Sex, Age, HCH	0.28	0.017	329.9	332.4	1.5	0.47
Nr3c1	**PCBs, Sex, Age**, $\log_{10}$Weight, $\delta^{13}C$	0.35	0.009	148.9	151.5	0	0.29
	PCBs, Sex, Age, $\log_{10}$Weight, $\delta^{13}C$, HCH	0.37	0.015	149.3	154.0	0.42	0.24

[a] AICc = second order AIC $n\log(\sigma 2) + 2K$) bias adjusted AIC for small sample size = AIC + ($2K(K + 1/(n - K - 1)$ where K is the total number of estimated regression parameters including $\sigma 2$ (no intercept) and n is sample size. [b] Δ_i = AIC differences computed as AIC_i-AIC_{min}. [c] $w_i = \exp(-1/2\Delta_i)/\Sigma\exp(-1/2\Delta_r)$.

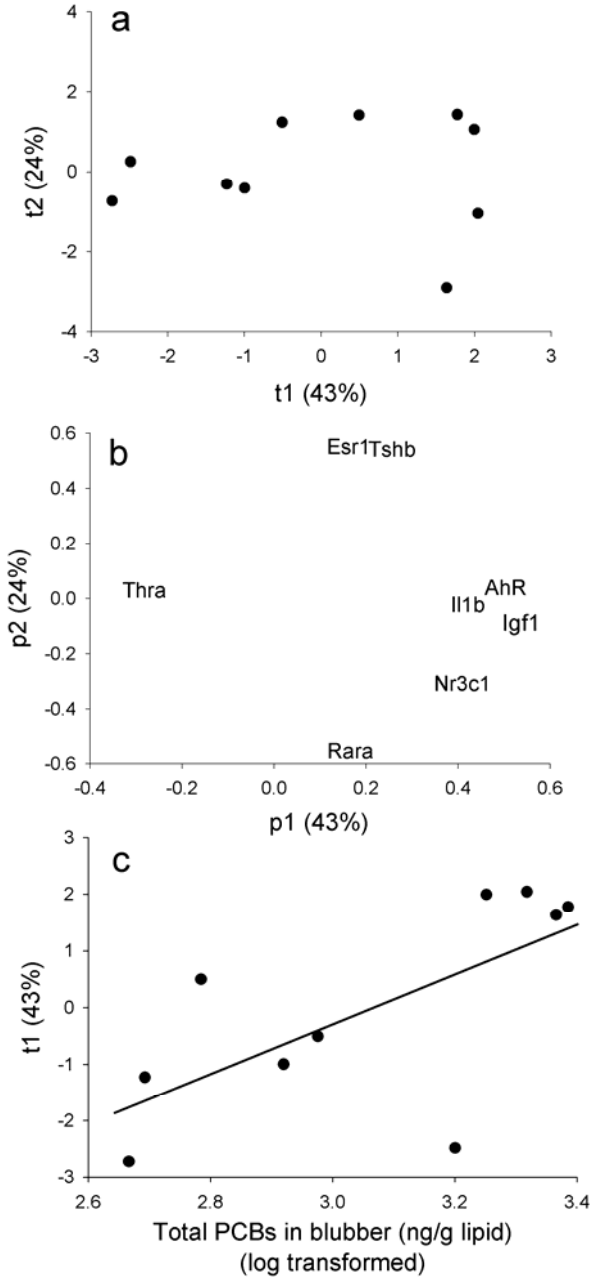

Figure 22: (a) Principal component analysis (PCA) of hepatic mRNA transcript levels in liver of Labrador ringed seals (*n*=11). Each circle on the scores plot represents an adult male seal. (b) Eight mRNA gene transcripts were included in the PCA; a divergence between three gene transcripts (*Ahr*, *Il1b* and *Igf1*) which correlated with ∑PCBs in the blubber and the other 5 gene transcripts was observed along the variable loadings of the first principal component (p1). (c) Sample scores of the first principal component (t1) was correlated with ∑PCBs.

Chapter 6: Conclusions

This thesis demonstrated the extent to which a local PCB source at Saglek Fjord led to contamination and health effects in a long-lived, mobile, and high trophic level marine mammal, the ringed seal. The dominance of PCBs at this site afforded a unique opportunity to evaluate the effects of this single class of industrial chemical in a manner that has not been possible in marine mammals. It also improves our understanding of the effects of PCBs in free-ranging marine mammals and provides new information needed to inform mitigation and monitoring efforts, both for ringed seals in the north and other phocid seals around the world.

6.1 The contribution of a local PCB source to the PCB burden in ringed seals from the northern Labrador coast

Contaminant concentrations and patterns

Source apportionment for persistent contaminants in high trophic level marine species is notoriously difficult to assess, yet these animals are often the most contaminated and at risk to these bioaccumulative and toxic chemicals. Although long-range atmospheric transport is the primary mechanism by which POPs reach the Arctic (Macdonald et al., 2000; Muir et al., 1999a), local sources within the Arctic (e.g. military sites) also exist and can contaminate local food webs (Brown et al., 2009; Kuzyk et al., 2005b). The PCB source associated with a military radar station in Saglek Bay, Labrador offered a unique opportunity to investigate exposure attribution to a high trophic level predator in the marine environment.

In chapter 2, I demonstrate for the first time the contribution of a local PCB source to an upper trophic level marine mammal in the Arctic. The use of contaminant pattern analysis enabled the determination that up to 60% of ringed seals frequenting the

Labrador coast have been influenced by the military radar station PCB spill at Saglek Bay. This chemocentric approach enabled a classification of seals as either ‘local’ or ‘long-range’ reflecting their respective exposure to these two types of sources. Despite the spatially confined nature of contaminated sediments in Saglek Bay, the influence of this PCB source is not inconsequential; approximately 50% of the seals PCB burden was contributed by the local source. In addition, average concentrations in locally contaminated adult male seals exceed an established threshold (1.3 mg/kg lw, Mos et al. 2010) for adverse effects in seals suggesting a notable proportion of the population may be at risk for toxic effects.

Habitat use and feeding ecology

In chapter 3, satellite telemetry was used to evaluate whether contaminant profiles can be used to assign seals to either ‘local’ or ‘long-range’ categories. In this first application of space use measurements in a marine mammal, I demonstrate that habitat use plays a critical role in shaping the exposure of ringed seals to a pollution hotspot. The results suggest that a reduced size of home range and core area (i.e. areas of concentrated use), as well as increased number of core areas, are important determinants of increased PCB concentrations in seals reflecting the contribution of Saglek Bay. In addition, the local contribution from long-range (background) sources were further distinguished and are consistent with the divergent PCB patterns observed in the ‘local’ and ‘long-range’ seals from chapter 2. This research offers a novel approach to determining the source apportionment in a mobile marine species and can be used to inform mitigation and monitoring efforts for contaminated sites.

Results from Chapter 2 and 3 strongly suggest that there are two groups of Labrador seals reflecting 'local' versus 'long-range' contaminant sources. Although this appears to be valid, it could not be proven until the feeding preferences are identified in these two groups of seals. In Chapter 4, a combination of fatty acid signatures and PCB profiles in ringed seals and their prey from Labrador were used to characterize the feeding preferences of ringed seals and offer insight into the ways in which local PCBs are entering the food web and accumulating in ringed seals. Similar fatty acid profiles between those seals with 'local' PCB profiles and those with 'long-range' or background profiles provide little support for the possibility that differential feeding ecologies underlay the divergent PCB profiles. Ringed seals feed predominantly on zooplankton (*Mysis oculata* and *Themisto libellula*), followed by the dusky snailfish (*Liparis gibbus*) and arctic cod (*Boreogadus saida*). PCB congener and homologue profiles illustrated the extent of contamination of the Saglek food web, which had very different (and much heavier) PCB profiles than those food web members contaminated by long-range background. Results from this chapter confirmed that the divergent contaminant profiles observed in ringed seals can indeed be attributed to feeding on locally contaminated prey, and not to a difference in feeding preferences between two groups of seals.

6.2 PCB-related health risks in ringed seals

Despite the extensive laboratory animal evidence establishing the toxic nature of PCBs, the complexity of ecological systems greatly confounds such studies involving free-ranging marine mammals. This is due to the presence of hundreds of different chemicals in the tissues of marine mammals (even in remote waters), the influence of multiple ecological and biological factors, and a limited suite of methods to measure

health endpoints in marine mammals. While numerous field studies have shown associations between PCBs and adverse health effects in marine mammals, differentiating between the effects caused by PCBs and other co-occurring contaminants has not been possible. The combination of a long-range POP 'background' and a local PCB 'hotspot' on the Labrador coast provides an invaluable opportunity to evaluate the effects of PCBs on the health of a free-ranging marine mammal as the concentration of this chemical dominates those of other POPs.

In Chapter 5, I investigated the relationship between ringed seal hepatic mRNA abundance profiles of eight health-related gene transcripts and PCB concentrations in blubber. Gene transcripts selected for study encode proteins that play an important role in animal health with respect to chemical detoxification, the immune and endocrine systems, and the regulation of growth, development, and metabolism. Five mRNA transcript levels correlated with increasing levels of PCBs in seals enabling confirmation and strengthening of a previously-derived effects threshold of 1,300 ng/g lw in pinnipeds (Mos et al., 2010) (Table 1). Approximately 14% of the seals sampled exceeded this threshold, suggesting a risk of adverse health effects in a proportion of the local population. While the implications for these sublethal molecular changes at the individual or population level are unclear, contaminant-related changes in endocrine, immune, and molecular endpoints have been observed in ringed seals exhibiting reproductive and developments abnormalities, and serious virus epizootics (Helle et al., 1976a; Helle et al., 1976b; Routti et al., 2010a).

The dominance of PCBs in the seals studied enabled an assessment of the effects of this chemical on the health of a highly mobile predator; something that is rarely

possible in the world of complex mixtures. While the population-level consequences of changes observed at the molecular level are unclear, these PCB associated alterations in these gene endpoints could lead to reduced endocrine and immune function and adverse effects on growth and development in ringed seals exposed to from the North Labrador coast.

Table 19: Threshold concentrations of PCBs (ng/g, lipid wt) in marine mammals for endocrine and immune toxicity and reproductive effects.

Value	Endpoint	Species	References
1,300	immune and endocrine endpoints	juvenile free-ranging harbour seals	Mos et al. 2010
1,370	interleukin-1 β *(Il1b)*	free-ranging ringed seals	this study
1,380	glucocorticoid receptor α *(Nr3c1)*	free-ranging ringed seals	this study
1,460	aryl hydrocarbon receptor *(Ahr)*	free-ranging ringed seals	this study
1,600	disruption of vitamins A and E	free-ranging beluga whales	Desforges et al. 2013a
1,740	insulin like growth factor 1 *(Igf1)*	free-ranging ringed seals	this study
2,460	estrogen receptor α *(Esr1)*	free-ranging ringed seals	this study
10,000	reproductive effects	free-ranging bottlenose dolphins	Hall et al. 2006
17,000	immunotoxicity	captive harbour seals	Ross et al. 1996

6.3 Future contaminant trends in ringed seals

The concentrations and spatial extent of PCBs in the sediments of Saglek Bay are significantly decreased (up to 100 fold reduction) compared to 1998 (Figure 23). In 2006-07, PCB concentrations were measured in the sediments and the benthic-associated biota of Saglek to determine whether the local ecosystem had recovered since the removal of the PCB source on the beach and to gain a better understanding of the extent of PCB contamination in the adjacent bay (Brown et al., 2009). PCB concentrations in the

sediment and two at-risk species (shorthorn sculpin and black guillemot nestlings and eggs) showed a significant decline between 1999 and 2006-07 (Brown et al., 2009). More recently, sediment PCB concentrations in the ecological risk zone decreased rapidly in 2006 – 2011 (Figure 23), with PCB concentrations being below the site-specific threshold (77 ng/g dw, Brown et al., 2013) established for effects in black guillemot nestlings (ESG, 2013). Moreover, average PCB concentrations in shorthorn sculpin and black guillemot nestlings in 2011 were below concentrations previously associated with risks of impaired reproduction and survival (Brown et al., 2009; ESG, 2013). A screening-level Hazard Quotient (HQ) approach and a biomarker-based weight-of-evidence approach was applied to shorthorn sculpins and black guillemots in 2007 and confirmed that the PCB-related risks to both indicator species had decreased over time.

Despite declining PCB concentrations and associated effects in bottom-feeding fish and seabirds at Saglek Bay following remedial action, impacts persist in long-lived high trophic level ringed seals. The rate of PCB decline for ringed seals is inevitably protracted as a function of their long-lives (or age at sampling) relative to these two indicator species. Black guillemots were sampled at 21 to 36 days, whereas ringed seals were sampled at ages between 1 and 28 years. While the long lives of seals predispose them to a slow recovery time for PCB concentrations (Hickie et al., 2005), life history also plays a role in the case of sculpins. These bottom-feeding fish have a shorter life span (~15 years) and feed lower in the food chain, with a diet consisting mainly of benthic invertebrates. In this manner, sculpins are likely to more rapidly reflect changes in sediment PCB levels, whereas ringed seals reflect the more protracted condition of the food web.

Based on earlier sediment transport predictions (Solomon, 2000) and trends reported in more recent studies (Brown et al., 2009; ESG, 2013), it is expected that residual PCBs from Saglek Anchorage will first move toward the western end of the shallow Saglek Bay area, where some will be deposited under the influence of oceanic transport processes. Some will continue to move to the north into the deep muddy basin, where they will simultaneously be diluted and buried with "clean" sediment. This will result in an overall decrease in the surface sediment PCB levels in the area. Modeling would be a useful tool to monitor future declines of PCB concentrations in Labrador ringed seals, which would provide important information needed to inform mitigation and monitoring efforts.

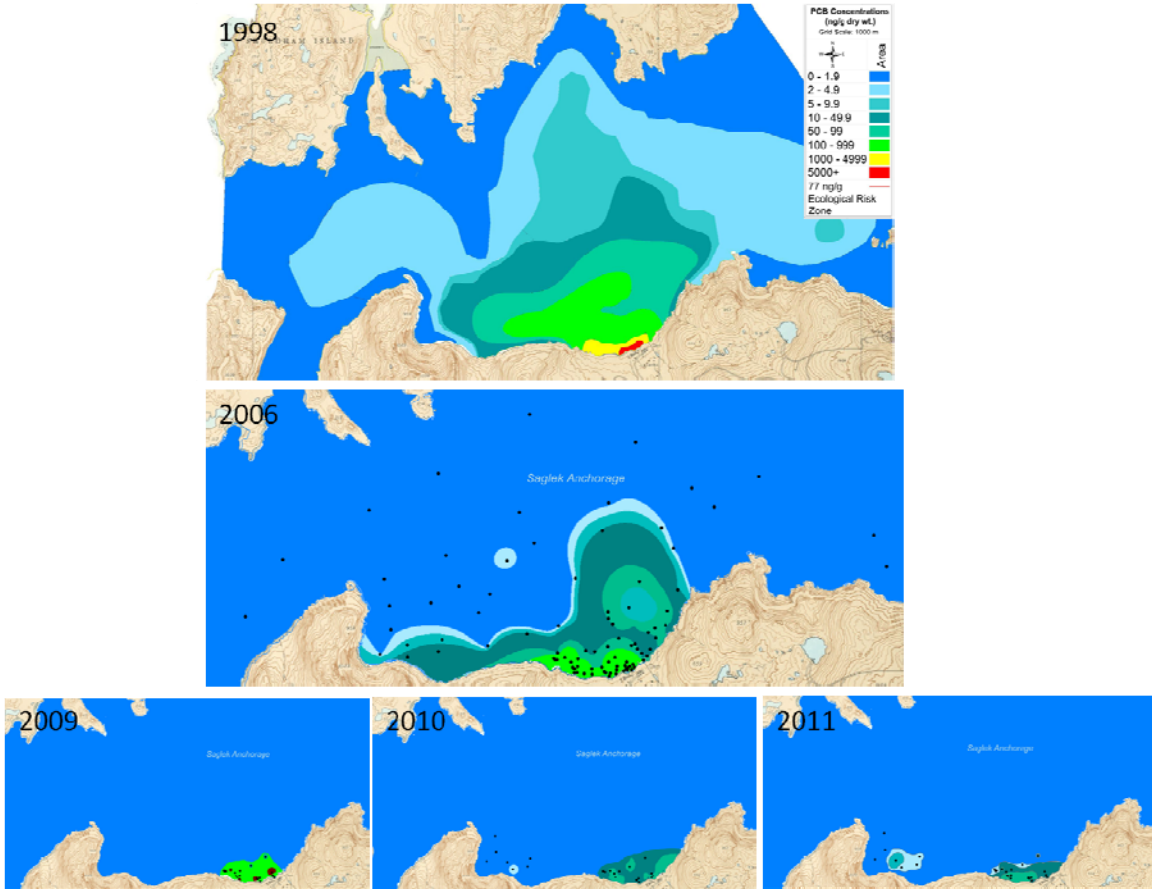

Figure 23: PCB concentrations and spatial extent of contamination in Saglek Anchorage decreased from 1998 to 2011, reflecting a recovery from historical contamination. While PCB levels also declined in the benthic-associated food web, the long-lived and high trophic

level ringed seals continue to face high levels, and a protracted recovery. The black dots indicate sampling locations.

The Arctic is undergoing considerable changes associated with climate warming, where air temperatures are increasing by 1.35°C per decade (Bekryaev et al., 2010), sea ice extent is declining at a rate of 13.7% per decade (NSIDC 2013), snow fall is declining (Iacozza and Ferguson, 2014), and sea surface temperatures are increasing (Hinzman et al., 2013). Ringed seals are heavily dependent on sea ice for reproduction and feeding (Ferguson et al., 2005) and a shift in their feeding ecology or migration patterns due to climate warming could have a dramatic effect on ringed seal health. Furthermore, diminishing sea ice and snowfall will likely result in declining pup production and survival.

Contaminant pathways in the Arctic are also expected to change as a result of a changing climate. These changes will likely affect PCB transport and fate in arctic environments, as well as uptake by marine mammal food webs (Burek et al., 2008). The consequences for Labrador ringed seals are unclear. A lack of integrated long-term studies on health, diseases, and contaminant related-effects in Arctic marine mammals greatly reduces our ability to predict and monitor the effects of climate change on marine mammal health (Burek et al., 2008). Establishing baseline conditions for ringed seal contaminant concentrations, habitat use and health in Northern Labrador is an important foundation future assessment and monitoring efforts. Any change in the health of Labrador ringed seal populations will have important impacts on polar bears and the people of this region.

6.4 Future directions

Next Generation Sequencing methods are a powerful way to obtain large amounts of transcriptome data with the intent to identify all expressed transcripts. Unlike microarrays, RNA-Seq is not limited to the detection of known transcripts (Wang et al., 2009). However, one of the current challenges with RNA-Seq data is the reconstruction of all full-length transcripts from short reads. Computational strategies for transcriptome reconstruction have been developed and are being used to create transcript assemblies from these short reads. Trans-ABySS is an assembly-first (*de novo*) method which uses the reads to assemble transcripts directly rather than relying on genome scaffolds. Given the paucity of seal genome and transcript information currently available, RNA-Seq could be used to perform a de novo assembly of the ringed seal transcriptome, to identify genes involved in responses to elevated PCB levels in ringed seals. These genes could serve as likely targets for selection to monitor PCB associated health effects and for further development of species-specific molecular tools. In addition to identifying new gene targets, future studies should be directed toward improving our understanding of the mechanism of receptor action for various genes targets affected by PCB exposure.

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Appendix

Appendix 1: Geometric means ± SE and ranges of ∑PCB and organochlorine pesticide concentrations (ng/g lipid weight) in blubber tissue of adult male (≥6 years) ringed seals collected from Nachvak Fjord, Saglek Fjord, Okak Bay, and Anaktalak Bay.

	Nachvak Fjord *n*=6	Saglek Fjord *n*=8	Okak Bay *n*=3	Anaktalak Bay *n*=2
∑PCBs	448 ± 190 (139-1,342) [b]	1,751 ± 379 (554-3,384)[a]	880 ± 433 (372-1,742)	606 ± 261 (345-868)
∑DDTs	249 ± 118 (71-840)	544 ± 101 (137-868)	435 ± 285 (123-1,005)	310 ± 122 (188-432)
∑Chlordanes	155 ± 62 (46-455)	317 ± 55 (84-616)	161 ± 119 (29-400)	107 ± 45 (62-153)
∑HCHs	51 ± 14 (29-118)	76 ± 7 (45-102)	39 ± 11 (24-61)	24 ± 6 (19-30)
Dieldrin	39 ± 10 (18-78)	43 ± 4 (19-54)	16 ± 3 (13-18)	16 ± 3 (13-19)
HCB	5 ± 0.5 (3-6)	6 ± 0.4 (5-8)	5 ± 1 (3-6)	5 ± 1 (3-6)

[a] $p<0.05$ compared to Nachvak Fjord
[b] one outlier removed

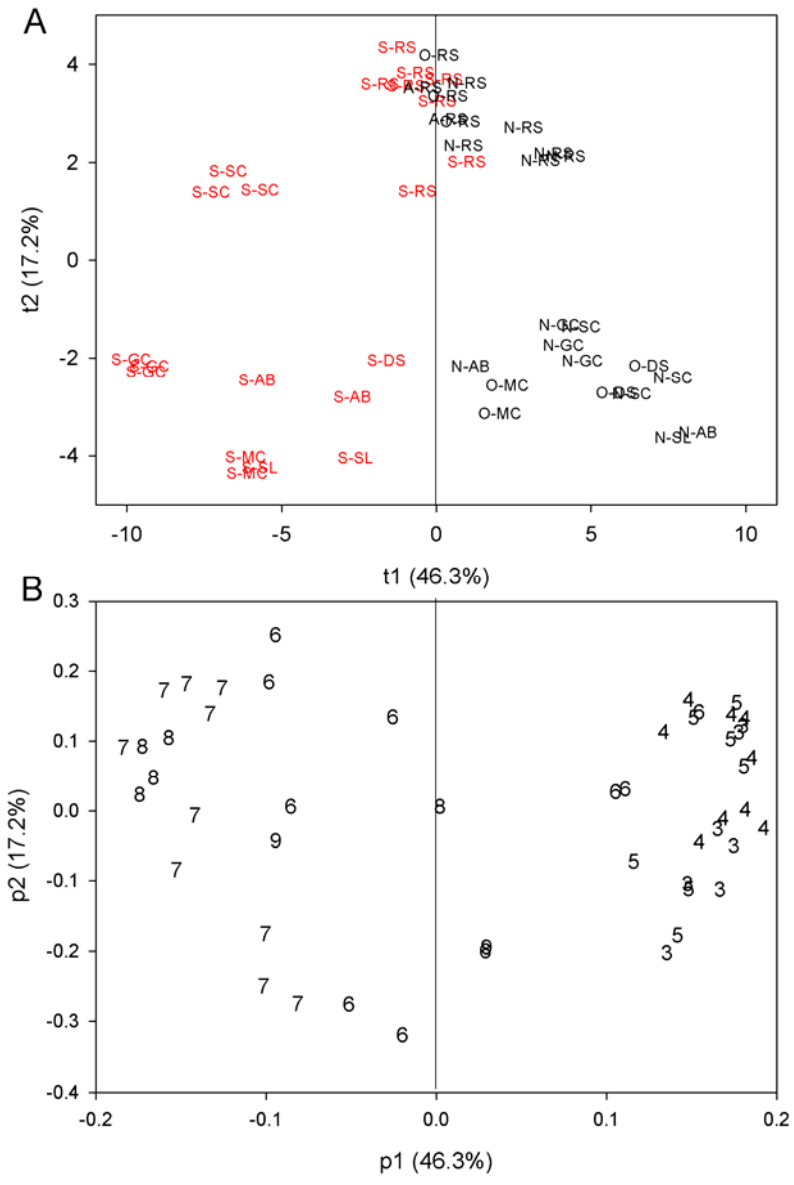
Appendix 2: Geometric means ± SE and ranges of ∑PCB and organochlorine pesticide concentrations (ng/g lipid weight) in blubber tissue of adult female (≥6 years) ringed seals collected from Nachvak Fjord, Saglek Fjord, Okak Bay, and Anaktalak Bay.

	Nachvak Fjord *n*=6	Saglek Fjord *n*=8	Okak Bay *n*=3	Anaktalak Bay *n*=4
∑PCBs	415 ± 90 (152-1,050)	302 ± 55 (137-365)	540 ± 142 (69-1,090)	308 ± 219 (88-528)
∑DDTs	257 ± 96 (43-851)	88 ± 23 (55-154)	222 ± 51 (34-341)	131 ± 100 (30-231)
∑Chlordanes	115 ± 35 (16-307)	50 ± 7 (33-62)	107 ± 30 (25-238)	113 ± 84 (29-197)
∑HCHs	59 ± 6 (35-84)	31 ± 2 (26-35)	53 ± 7 (35-85)	27 ± 6 (21-33)
Dieldrin	41 ± 10 (16-112)	16 ± 4 (10-28)	25 ± 2.4 (15-31)	18 ± 1 (17-19)
HCB	6 ± 2 (3-22)	4 ± 0.3 (4-5)	5 ± 0.3 (4-6)	4 ± 0.6 (3-5)

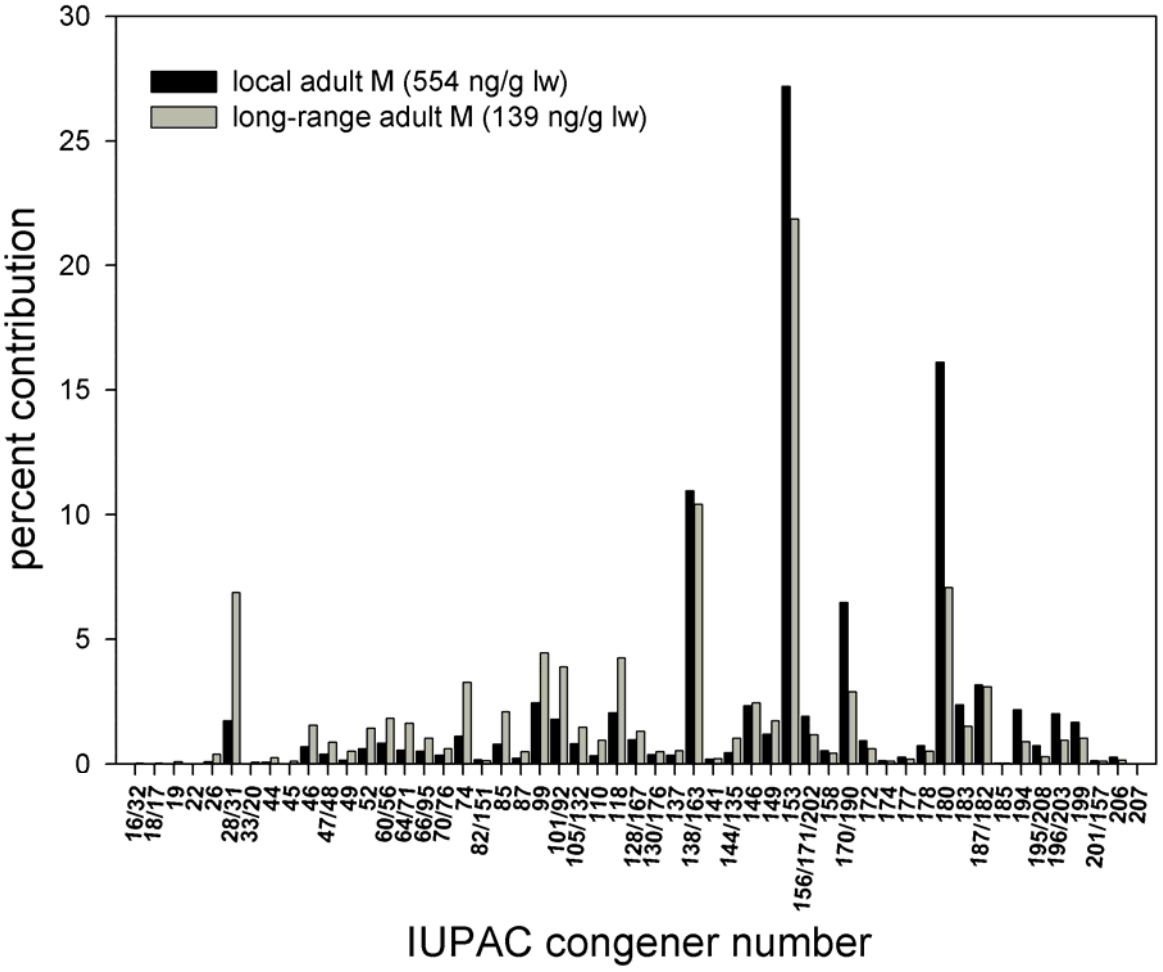
Appendix 3: Geometric means ± SE and ranges of ∑PCB and organochlorine pesticide concentrations (ng/g lipid weight) in blubber tissue of sub-adult (<6 years) ringed seals collected from Nachvak Fjord, Saglek Fjord, Okak Bay, and Anaktalak Bay.

	Nachvak Fjord *n*=2	Saglek Fjord *n*=5	Okak Bay *n*=10	Anaktalak Bay *n*=5
∑PCBs	346 ± 49 (297-395)	535 ± 132 (187-886)	339 ± 52 (180-777)	406 ± 109 (210-806)
∑DDTs	139 ± 28 (112-167)	134 ± 23 (60-206)	147 ± 20 (51-240)	218 ± 77 (108-521)
∑Chlordanes	57 ± 17 (40-73)	94 ± 14 (64-148)	72 ± 6 (43-109)	113 ± 19 (61-162)
∑HCHs	62 ± 6 (57-68)	51 ± 6 (39-69)	47 ± 4 (29-66)	55 ± 10 (31-83)
Dieldrin	22 ± 2 (20-24)	18 ± 3 (11-27)	34 ± 8 (13-96)	37 ± 11 (18-74)
HCB	4 ± 0.1 (3.9-4)	5 ± 0.5 (3-7)	4 ± 0.3 (2-5)	5 ± 0.9 (5-5.5)

Appendix 4: A Principal Components Analysis (PCA) of PCB patterns (53 congeners) in adult male ringed seals and their prey species (shorthorn sculpin (SC), sand lance (SL), daubed shanny (DS), greenland cod (GC), Astarte borealis (AB), Macoma calcarea (MC)) reveal that Saglek (S=Saglek) species to the left of the score plot are dominated by heavier congeners, whereas reference fjord (N = Nachvak; O = Okak; A = Anaktalak) species to the right of the score plot are dominated by lighter congeners. (B) Numbers identify the degree of chlorination (i.e. number of chlorines per congener).



Appendix 5: Percent congener composition of a representative 'local' ringed seal (age 7) and that of a representative 'long-range' ringed seal (age 8). Values in the legend represent their ∑PCB concentration.



Appendix 6: Morphometric and age data for harvested subadult (<6 yr; male and female combined), adult female (≥6 yr) ringed seals, and adult male (≥6 yr) ringed seals collected from northern Labrador, Canada.

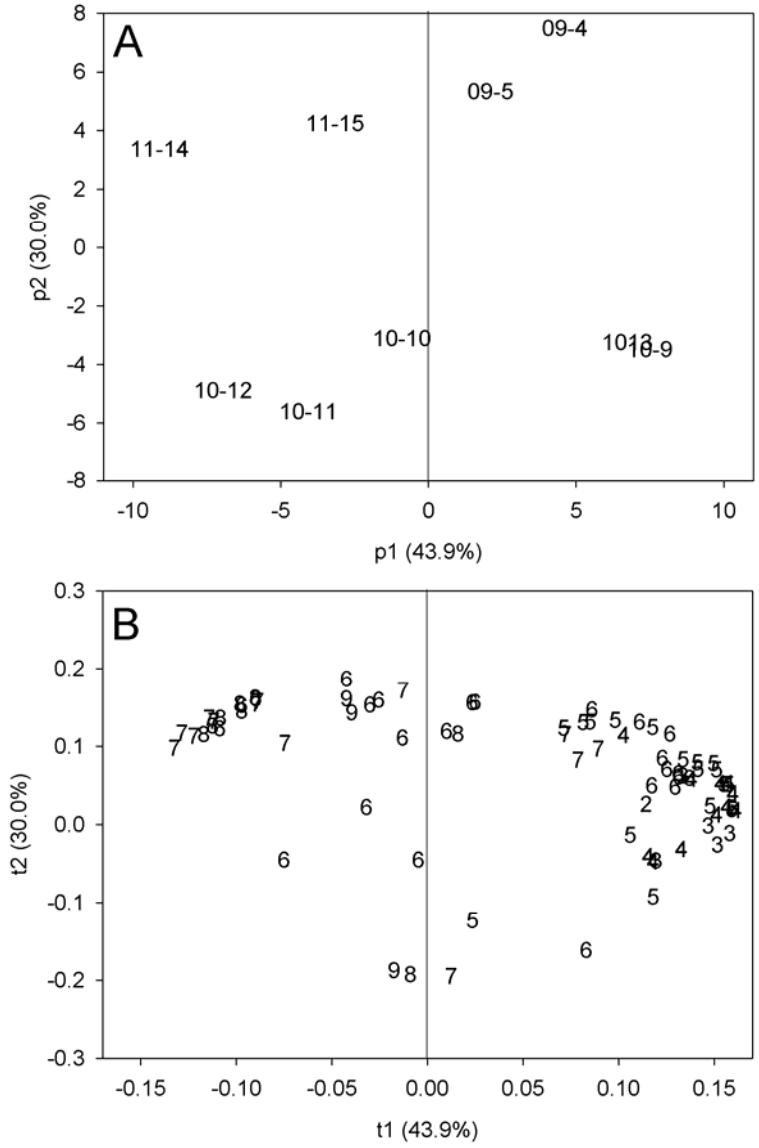
Age-class	subadults	Adult female	Adult male
n	21	20	14
age [a]	1.2 ± 1.4 (0-4)	16 ± 5 (6-25)	18 ± 7 (6-32)
weight (kg)	30 ± 6 (28-36)	68 ± 15 (46-107)	65 ± 15 (46-98)
length (cm)	95 ± 9 (83-114)	128 ± 8 (113-140)	128 ± 12 (113-156)
girth (cm)	75 ± 12 (64-90)	106 ± 12 (80-127)	99 ± 18 (86-130)

[a] Ages were determined by Matson's Laboratory, USA, by longitudinal thin sectioning a lower canine tooth and counting the annual growth layers in the cementum using a compound microscope and transmitted light.

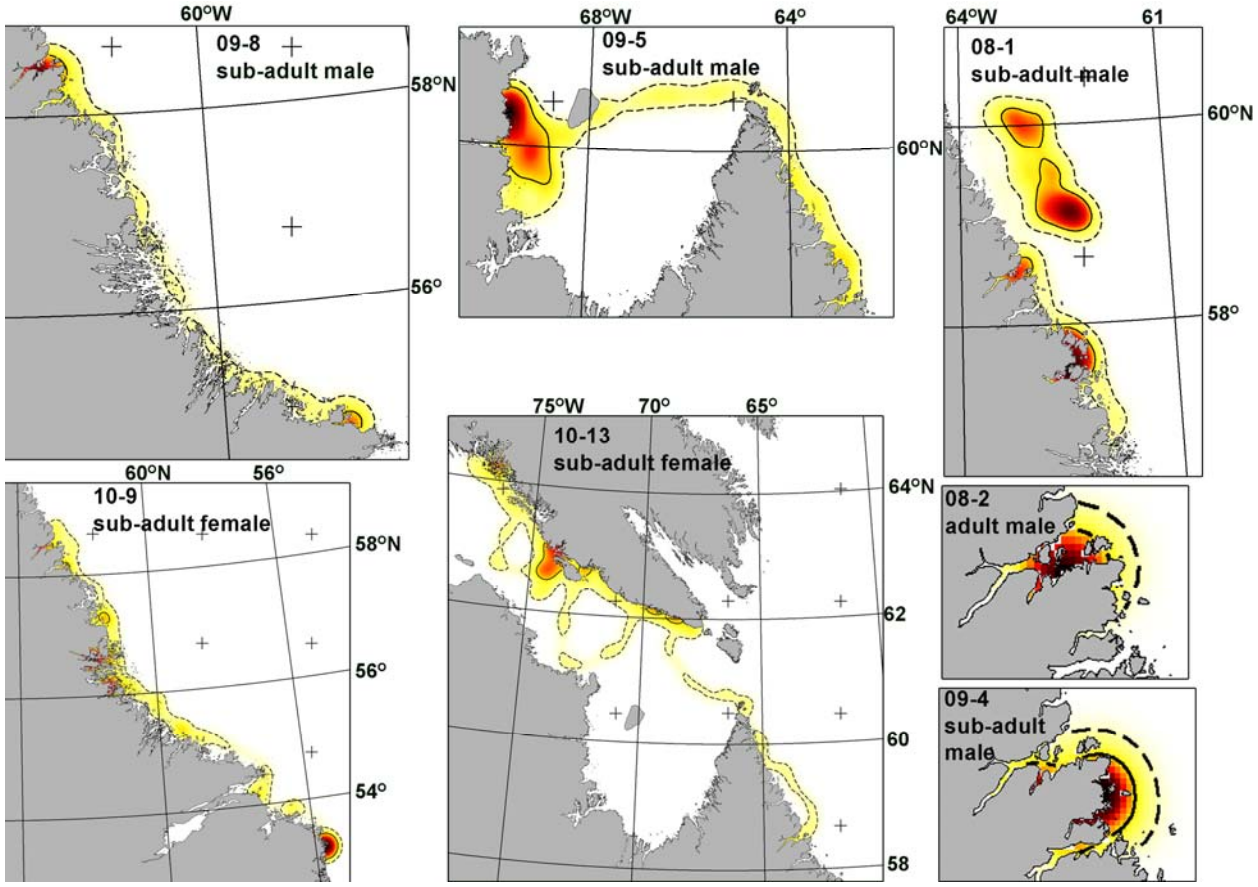
Appendix 7: Regressions revealed that t1 correlated with all ∑PCBs/organochlorine pesticide ratios (∑PCBs/∑DDTs, ∑PCBs/∑Chlordanes, ∑PCBs /∑HCHs, ∑PCBs/Dieldrin, ∑PCBs/HCB).

∑PCBs/∑DDTs	∑PCBs/∑Chlordanes	∑PCBs /∑HCHs	∑PCBs/Dieldrin	∑PCBs/HCB
r^2=0.50; p=0.01	r^2=0.65; p=0.002	r^2=0.50; p=0.017	r^2=0.60; p=0.003	r^2=0.74; p<0.001

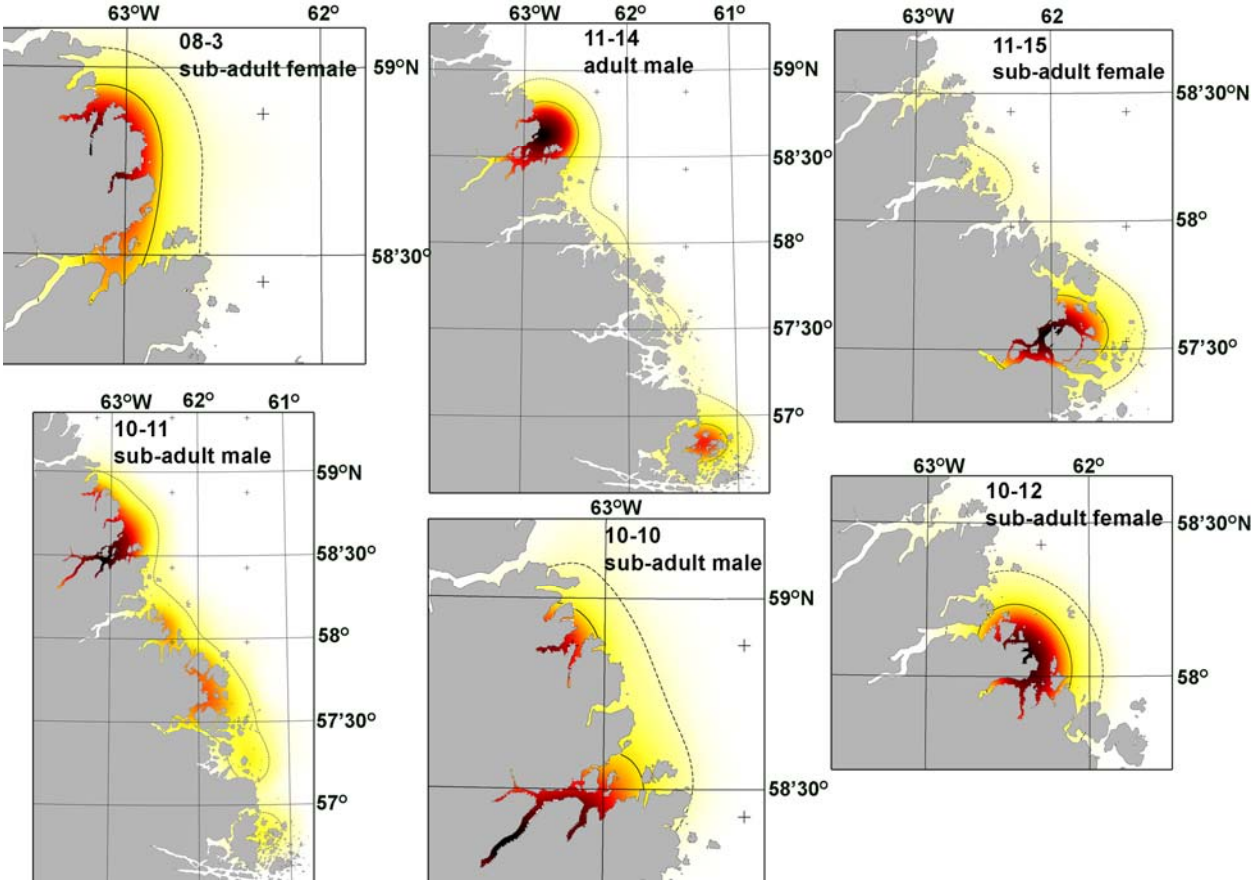
Appendix 8: A Principal Components Analysis (PCA) of PCB patterns (84 congeners) in ringed seals reveal the same group classification (i.e. 'local' versus 'long-range') as Figure 2. (A) Numbers depict two digit year of sampling followed by seal ID. (B) Numbers identify the degree of chlorination (i.e. number of chlorines per congener).



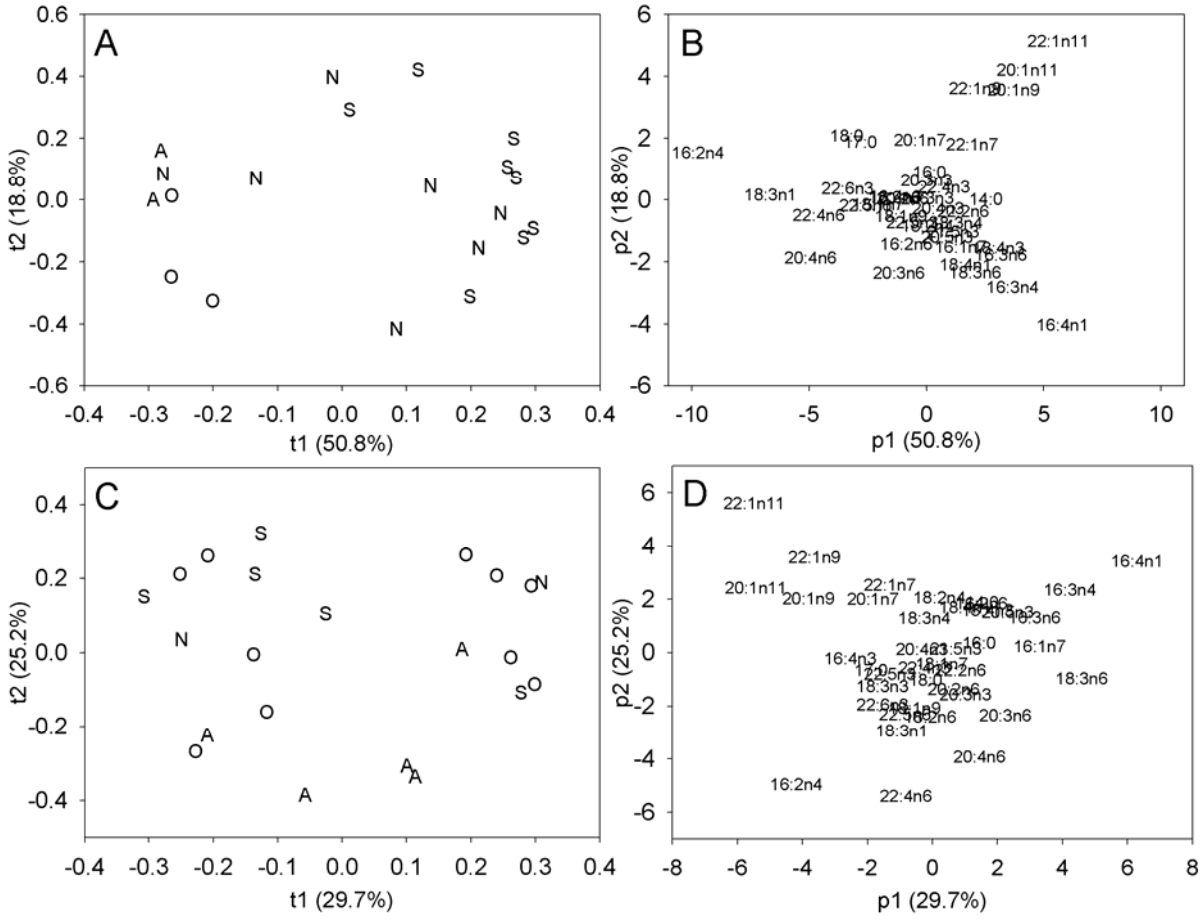
Appendix 9: Utilization distribution maps for 'long-range' ringed seals. Home range (95%) is defined as the contour intervals for each ringed seal tagged with satellite transmitters and deployed in Saglek Fjord between 2008 and 2011. Core area (70%) is defined as the contour intervals delineated by the red outline. The seals ID and age class is presented on each map.



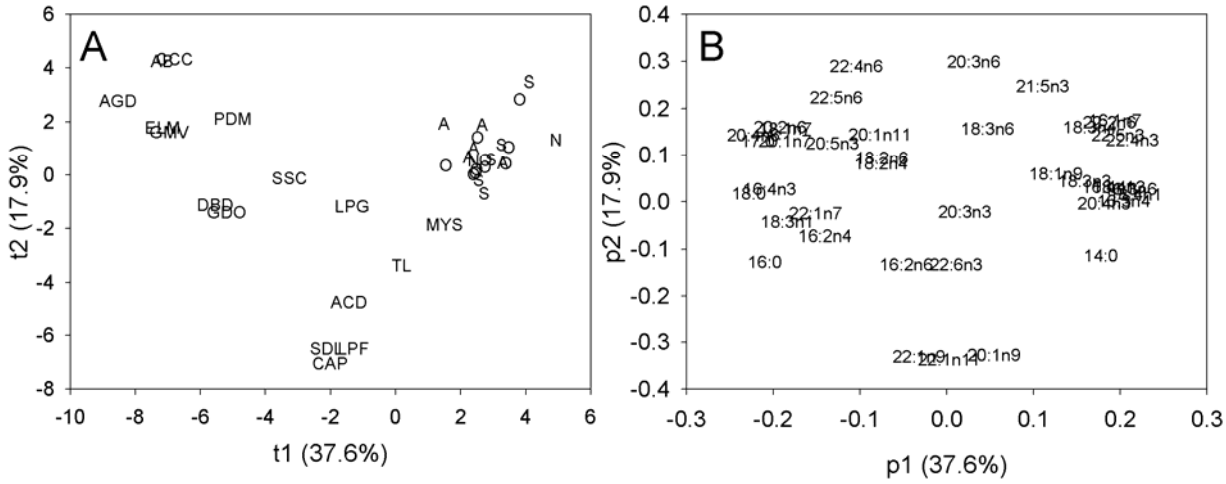
Appendix 10: Utilization distribution maps for 'local' ringed seals. Home range (95%) is defined as the contour intervals for each ringed seal tagged with satellite transmitters and deployed in Saglek Fjord between 2008 and 2011. Core area (70%) is defined as the contour intervals delineated by the red outline. The seals ID and age class is presented on each map.



Appendix 11. Principal component analysis of the 40 dietary fatty acids in adult male (A,B) and subadult (C,D) ringed seals revealed that location had a significant effect on ringed seal FA signatures along the northern Labrador coast. The fatty acid pattern in the factor loading plots describes the patterns observed in the ringed seal score plots. Fatty acids towards the center of the plot have the lowest importance relative to those furthest from the origin.



Appendix 12. Principal component analysis of the 40 dietary fatty acids in prey items and subadult ringed seals. A) PCA score plot of seals from 4 fjords in Labrador (N=Nachvak; S=Saglek; O=Okak; A=Anaktalak) and prey. B) Factor loadings of individual fatty acids. Prey items located closest to the ringed seals indicate similarities among their fatty acid profiles, compared with prey items located furthest from the seals.



Appendix 13. qPCR primer pair sequences for genes used in ringed seal (Pusa hispida) liver.

Gene transcript	Abbr.	Major roles	NCBI Genbank Accession No.	Primer name	Primer Sequence	Amplicon size	Annealing temperature
Ribosomal protein L8	*Rpl8*	Normalizer gene representative of sample quality	KJ633276	UL8up L8-2dn	GGTGTGGCTATGAATCCTGT ACGACGAGCAGCAATAAGAC	126	55
Cytoplasmic β Actin	*Actbl2*	Normalizer gene representative of sample quality	KJ633278	ORQ16 up ORQ16 dn	CCTGGACTTCGAGCAGGAG GCACCGTGTTGGCATAGAG	236	60
Eukaryotic translation elongation factor 1 α 1	*Eef1a1*	Normalizer gene representative of sample quality	KJ633282	ORQ28 up ORQ28 dn	ATTACAGGCACATCTCAGGCT CTTTACGGGTGACTTTCCATC	352	60
Aryl hydrocarbon receptor	*Ahr*	Induction of metabolizing enzymes	KJ633272	HIQ3 up HIQ3 dn	ACCCACTGCTTGTGATGC TTCGCTTTCGTAAATGYTCT	308	60
Thyroid hormone receptor α	*Thra*	Development, cell differentiation, metabolism	KJ633279	PV19/20 up PV19/20 dn	CGACGGAAGGAGGAAATG GATCTTGGTAAACTCGCTGAA	231	60
Estrogen receptor α	*Esr1*	Cell differentiation, growth, development, reproduction	KJ633271	ORQ2 up ORQ2 dn	CCGAGCCCACTCTTGATT CCTCTTTGCCCAGTTGAT	213	60
Insulin-like growth factor 1	*Igf1*[a,b]	Cell differentiation & proliferation, disease development	NS	ORQ25 up ORQ25 dn	TTTATTTCAACAAGCCCACG TACATCTCCAGCCTCCTCA	112	60
Glucocorticoid receptor	*Nr3c1*[a,b]	Growth, development, metabolism, immune function	NS	ORQ12 up ORQ12 dn	GCCCAGTTTATTGTCAGG TGTTGAGAAAGGGATGCT	139	60
Thyroid-stimulating hormone β	*Tshb*[b]	Development, cell differentiation, metabolism	NS	Tshb forward Tshb reverse	TCCATGCTTTTTGGCCTTAC AGCACAGATGGTGGTGTTGA	125	57
Retinoic acid receptor α	*Rara*[b]	Cell differentiation & proliferation, immune function	NS	Rara forward Rara reverse	CAGTACTGCCGGCTGCAGAA TGTAGCTCTCGGAGCACTCG	115	57
Interleukin-1β	*Il1b*[b]	Immune function	NS	Il1b forward Il1b reverse	GCTGCTTCCAAGACCTGAAC CTGACACGAAATGCCTCAGA	120	57

[a] NS=not submitted to NCBI GenBank due to size limitations; see Appendix 14 and Routti et al., 2010 for sequence.
[b] QPCR primers were obtained from Routti et al., 2010.

Appendix 14. Isolated ringed seal (Pusa hispida) expressed gene sequences not submitted to NCBI GenBank.

Gene Transcript	Cloning PCR Primers	cDNA Sequence	Conceptual Protein Sequence
Glucocorticoid receptor (*Nr3c1)*	ORQ12 up; GCCCAGTTTATTGTCAGG ORQ12 dn; TGTTGAGAAAGGGATGCT	Base pairs;139 GCCCAGTTTATTGTCAGGCAAGCTTTTCTGGG GCAAATATAATTGGTAATAAAATGTCTGCCA TTTCTGTTCATGGTGTGAGTACCTCTGGGGGA CAGATGTACCACTATGACATGAATACAGCAT CCCTTTCTCAACA	Frame; +3 PVYCQASFSGANIIGNKMSAISVHGVSTSGGQM YHYDMNTASLSQ
Insulin-like growth factor 1 (*Igf1)*	ORQ 25 up: TTTATTTCAACAAGCCCACG ORQ 25 dn: TACATCTCCAGCCTCCTCA	Base pairs;54 GGAGGGCACCTCAGACAGGCATCGTGGACGA GTGCTGCTTCCGGAGCTGTGATT	Frame; +3 RAPQTGIVDECCFRSCD

Appendix 15. Linear regressions revealed that abundance for five mRNA transcripts (Ahr, Il1β, Esr1, Igf1, Gra) correlated with ∑PCBs.

Ahr	*Il1β*	*Esr1*	*Igf1*	*Gra*
r^2=0.60; p<0.001	r^2=0.23; p=0.012	r^2=0.20; p=0.017	r^2=0.60; p<0.001	r^2=0.20; p=0.018