FROM HAZARD QUOTIENTS TO A BIOMARKER BASED WEIGHT OF EVIDENCE: ENHANCING THE SCIENCE IN ECOLOGICAL RISK ASSESSMENT

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FROM HAZARD QUOTIENTS TO A BIOMARKER BASED WEIGHT OF EVIDENCE: ENHANCING THE SCIENCE IN ECOLOGICAL RISK ASSESSMENT

By

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Abstract

This thesis was developed within the framework of a monitoring program for Saglek Bay, Labrador, Canada. Extensive polychlorinated biphenyl (PCB) contamination in soil and sediment associated with a former Polevault Line military station was identified at Saglek in the 1990s (ESG, 1997). PCB impacted soil remediation was carried out between 1997 and 1999 and thus, the terrestrial source of exposure has been removed. Ecological studies showed that PCBs remained in the coastal marine food web (Kuzyk *et al.*, 2005a) and the terrestrial food chain (ESG, 2005; 2007). Recent evidence indicates that the concentrations in the marine environment are decreasing (Brown *et al.*, 2009). Research for this thesis was conducted to evaluate the effects of PCBs on biomarker responses in wildlife at Saglek, and to evaluate the effectiveness of field verification of effects predicted at the screening risk assessment stage by comparing the results of a traditional food chain model approach to a biological effects based weight of evidence approach. Hazard quotients indicated that there was a potential for adverse risks to shorthorn sculpin at Saglek Beach (hazard quotient=1.6) in 1998/99 but adverse risks were unlikely (hazard quotient=0.9) in 2006/07. The biological effects based weight of evidence assessment supported the hazard quotient methodology indicating an intermediate risk to shorthorn sculpin in 1998/99 and a low risk in 2006/07. For black guillemots, the hazard quotients indicated that adverse risks were likely for both 1998/99 (hazard quotient=2.1) and 2006/07 (hazard quotient=1.2). The biological effects based weight of evidence assessment supported the hazard quotient indicating an intermediate to high risk to black guillemots for these time periods. The hazard quotients calculated for deer mice using the dietary approach (2.3) and the tissue residue approach (2.1)

indicated that adverse effects to deer mice at Saglek Beach are likely. Results of bone mineral density analysis supported this conclusion but thyroid histomorphometry and histopathology did not. Despite the predicted health effects to black guillemots and deer mice at Saglek, the populations appear to be thriving. Measurement of population and/or community indices would be helpful in confirming the predicted adverse effects. A three tier iterative approach using hazard quotients, biomarkers, and population/and or community studies is recommended for large complex sites such as Saglek, where remediation strategies are expensive and potentially destructive to the environment. This thesis emphasises field verification of adverse health effects predicted through the screening (*i.e.*, hazard quotient) assessment stage and supports an iterative tiered approach to ecological risk assessment.

Co-Authorship Statement

The student's contributions to the thesis manuscripts are as follows:

- Participant in the initial development of research projects.
- Primary researcher responsible for the successful implementation and completion of biomarker assays including:
  - bone mineral density measurements for mice and birds at the Faculty of Medicine-Endocrinology at Memorial University of Newfoundland and Labrador;
  - sorting, preparation and identification of fish parasites at Department of Biology, Memorial University of Newfoundland; and,
  - biochemical assays conducted on fish and birds at the Freshwater Institute, Winnipeg MB.
- Primary researcher responsible for successful development, implementation and completion of field work for shorthorn sculpin and deer mice during two field seasons (2006 and 2007).
- Participant in the development, implementation, and completion of the field work for sediment and black guillemot during two field seasons (2006 and 2007).
- Principal author on all papers and manuscripts.

Ken Reimer is a co-author on Chapters 2, 4, 6, and 7. Dr. Reimer provided financial support, shared in the design of the project, and provided reviews of the draft papers. David Schneider is a co-author on Chapters 2, 3, and 6. Dr. Schneider provided financial

support, aided in the study design, contributed to the conceptual development of the papers, and provided reviews of the draft papers. Bradley Park and Vincent Palace provided financial support, laboratory space and equipment, and guidance for the laboratory work involved in Chapter 4 as well as review of the draft paper. Christopher Ollson provided financial support and aided with the study design and conceptual development of Chapters 2, 4, 6, and 7 as well as reviewed the draft papers. Loren Knopper provided guidance toward the design and conceptual development of Chapter 2 as well as provided review of the draft paper. Eric Baggs provided laboratory space and equipment to conduct the parasite analyses involved in Chapter 3 as well as provided review of the draft paper.

This thesis is based on the following papers and manuscripts:

K.E. Johnson, L.D. Knopper, D.C. Schneider, C.A. Ollson, K.J. Reimer. 2009. Effects of local point source polychlorinated biphenyl (PCB) contamination on bone mineral density in deer mice (*Peromyscus maniculatus*). Science of the Total Environment, 407: 5050-5055.

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K.E. Johnson, B.J. Park, V.P. Palace, C.A. Ollson, K.J. Reimer. In prep. Biomarker responses in shorthorn sculpin (*Myoxocephalus scorpius*) at a polychlorinated biphenyl contaminated site at Saglek Bay, Labrador. Anticipated submission to Environmental Toxicology and Chemistry.

K.E. Johnson, C.A. Ollson, D.C. Schneider, K.J. Reimer. In prep. Assessment of ecological risks to a marine benthic fish (*Myoxocephalus scorpius*) and a marine piscivorous bird (*Cepphus grylle*) at Saglek Bay, a PCB contaminated site in northern Labrador. Anticipated submission to Integrated Environmental Assessment and Management.

K.E. Johnson, C.A. Ollson, K.J. Reimer. In prep. Screening assessment of the ecological risks to a terrestrial small mammal (*Peromyscus maniculatus*) exposed to

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ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ATSDR	Agency for Toxic Substances and Disease Registry
ADD	Average Daily Dose
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
BMD	Bone Mineral Density
BMC	Bone Mineral Content
BW	Body Weight
CCME	Canadian Council of the Ministers of the Environment
DXA	Dual-Energy X-Ray Absorptiometry
DEW	Distant Early Warning
EPC	Exposure Point Concentration
ERA	Ecological Risk Assessment
EROD	Ethoxyresorufin-*O*-deethylase
foc	Fraction of Organic Carbon
HRGC	High Resolution Gas Chromatography
HSI	Hepatosomatic Index
ISQG	Interim Sediment Quality Guideline
K	Condition Factor

Koc	Partition Coefficient of the Contaminant in the Organic Fraction of the Soil
LOAEC	Lowest Observed Adverse Effect Concentration
LOAEL	Lowest Observed Adverse Effect Level
LRGC	Low Resolution Gas Chromatography
MFO	Mixed Function Oxidases
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NRC	National Research Council
NWS	North Warning System
ORNL	Oakridge National Laboratory
PCBs	Polychlorinated Biphenyls
PHAHs	Polyhalogenated Aromatic Hydrocarbons
PEL	Probable Effects Level
QA/QC	Quality Assurance/Quality Control
ROI	Region of Interest
ROS	Reactive Oxygen Species
TRV	Toxicity Reference Value
UDP-GT	Uridine Diphosphate Glucuronyltransferase
USEPA	United States Environmental Protection Agency
VEC	Valued Ecosystem Component

1.0 Chapter 1 Introduction and Overview

Risk assessment is the characterization of the potential health effects on humans exposed to environmental hazards (National Research Council (NRC), 1983). According to Suter (2008), risk assessment, in the environmental context, originated first in the United States as a response to the enactment of a series of environmental laws in the 1970s: the Clean Air Act of 1970, the Federal Insecticide, Fungicide and Rodenticide Act of 1972, the Safe Drinking Water Act of 1974, the Toxic Substance Control Act of 1976, and the Clean Water Act of 1977. Initial guidance (*i.e.*, NRC, 1983), however, provided frameworks that only focussed on human health risk assessments. The need for an ecological framework was identified in 1981 when the United States Environmental Protection Agency (USEPA) commissioned the Oak Ridge National Laboratory (ORNL) to develop ecological risk assessment (ERA) methods analogous to those for human health (Suter, 2008). The ORNL developed methods that were published (Barnthouse *et al.*, 1982), applied (Suter *et al.*, 1984), and then published again as a manual after a USEPA peer-review workshop (Barnthouse and Suter, 1986). The work was further presented in two books (Bartell *et al.*, 1992; Suter, 1993) and shortly thereafter, the USEPA guidance framework was developed (USEPA, 1992a), and later expanded (USEPA, 1998). This framework has since been widely adopted in the United States and elsewhere (Power and McCarthy, 1997; Suter, 2008). In Canada, the ERA process is based on the Canadian Council of the Ministers of the Environment (CCME) document "A Framework for Ecological Risk Assessment: General Guidance" (CCME, 1996).

Ecological risk assessment is defined as the evaluation of the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or

more stressors (USEPA, 1992a). The fundamentals of human and ecological risk assessment are the same with the objective being to identify significant source-pathway-receptor linkages and to provide an assessment of risk to receptors (Figure 1-1) (Wilson, 1993). Receptors are defined by CCME (1996) as "components of the environment that can be adversely affected". Exposure is "the co-occurrence of a stressor with an ecological receptor (*e.g.,* individual, population, community or ecosystem" (CCME, 1996). Hazard refers to "the type and magnitude of effect caused by a stressor" (CCME, 1996). If all three of these conditions exist, there is a potential risk to receptors.

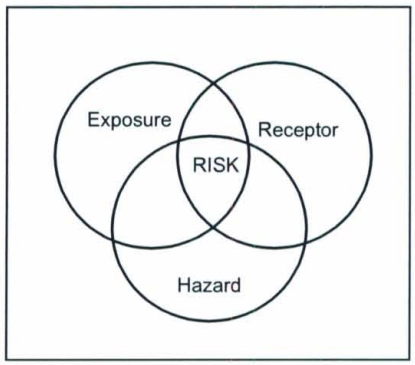

Figure 1-1. The relationship of the three components of ERA (source: CCME, 1996).

CCME (1996) defines risk as "the evaluation of whether an adverse effect will occur". The primary difference between human health and ecological risk assessment is that the former is concerned with evaluating effects on individuals while the latter is concerned with evaluating effects on populations, communities and ecosystems and is therefore a much more complex process (Parkhurst *et al.*, 1990; CCME, 1996).

The framework upon which ecological risk assessment is based (Figure 1-2) demonstrates its strength as a process and not a specific set of data collection techniques, analytical methods or results (Barnthouse, 2008; Dale *et al.*, 2008).

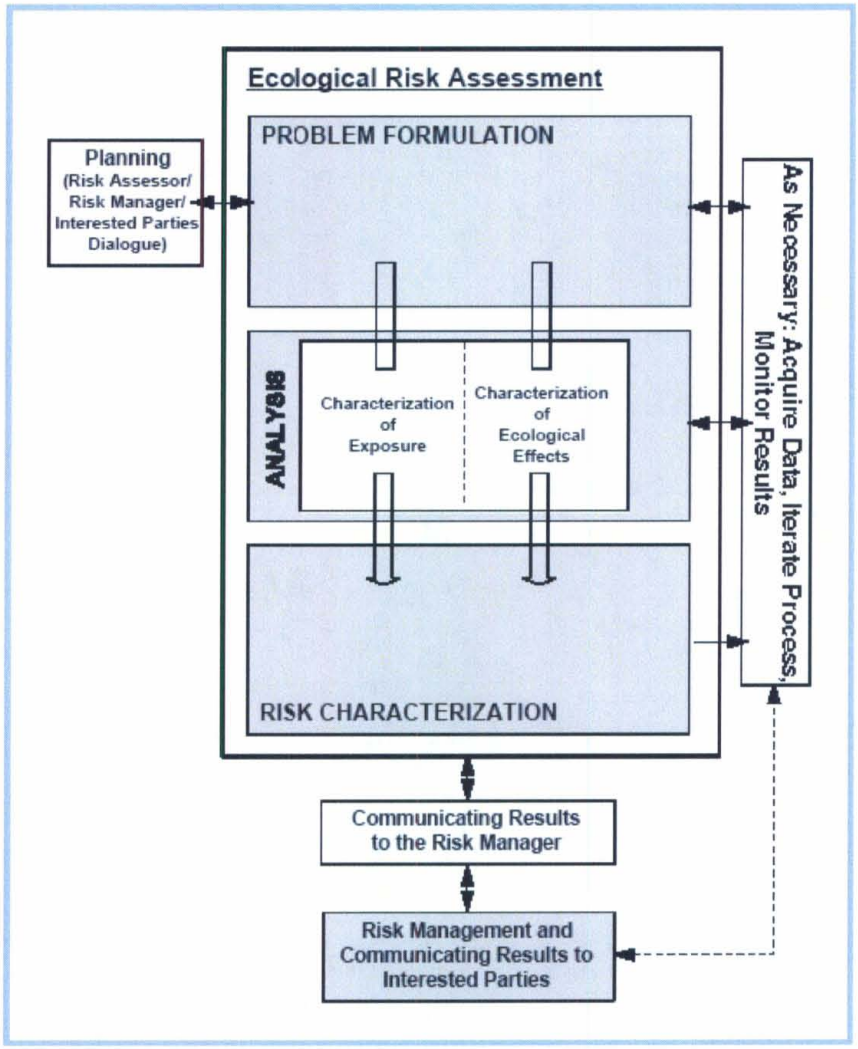

Figure 1-2. Framework for ecological risk assessment
(source: USEPA, 1992; Barnthouse, 2008). Similar frameworks are applied in federal guidance documents such as CCME (1996) and USEPA (1992).

The process begins with problem formulation which characterizes the activity followed by a preliminary characterization of exposure and effects. This includes examination of scientific data, policy and regulatory issues, and site specific factors. This information is used to define the feasibility, scope, and objectives of the risk assessment (USEPA, 1992a). Risk analysis characterizes the exposure and effects of ecological

receptors to the stressor. Risk characterization evaluates the likelihood of adverse ecological effects through the integration of exposure and stressor response profiles (USEPA, 1992a). The framework (Figure 1-2) also demonstrates the complex linkages between science and environmental decision making. This shows the central role of communication between science and management in ensuring that assessments address issues that are important to decision making (Barnthouse, 2008).

1.1 Tiered Approach of Ecological Risk Assessment

The ERA approach is iterative, that is it may be repeated one or more times until a sufficiently complete and defensible result is achieved (Suter, 2007). The iterative approach is generally manifested through multiple tiers of assessment with each successive tier composed of sequentially more sophisticated and complex evaluations. The objective of progressing to successive tiers is to reduce the uncertainty and to "provide technical support for decision making under uncertainty" (Suter, 2007). The uncertainty associated with the risk estimate determined at the completion of each tier is the motivation for moving to the next tier (CCME, 1996). The level of uncertainty is based on professional judgment by the risk assessor and can also be driven by financial and regulatory considerations (CCME, 1996). The benefits of the iterative approach are the opportunities for evaluation of progress, addition of new data to conceptual models, identification of data gaps, and the evaluation of uncertainty. These factors can aid in planning for the next tier, if deemed necessary, or for documenting that the ERA is complete (CCME, 1996). In Canada, three tiers of assessment (Figure 1-3) are described

by CCME: Screening Assessment (Tier I), Preliminary Quantitative Ecological Risk Assessment (Tier II), and Detailed Quantitative Ecological Risk Assessment (Tier III).

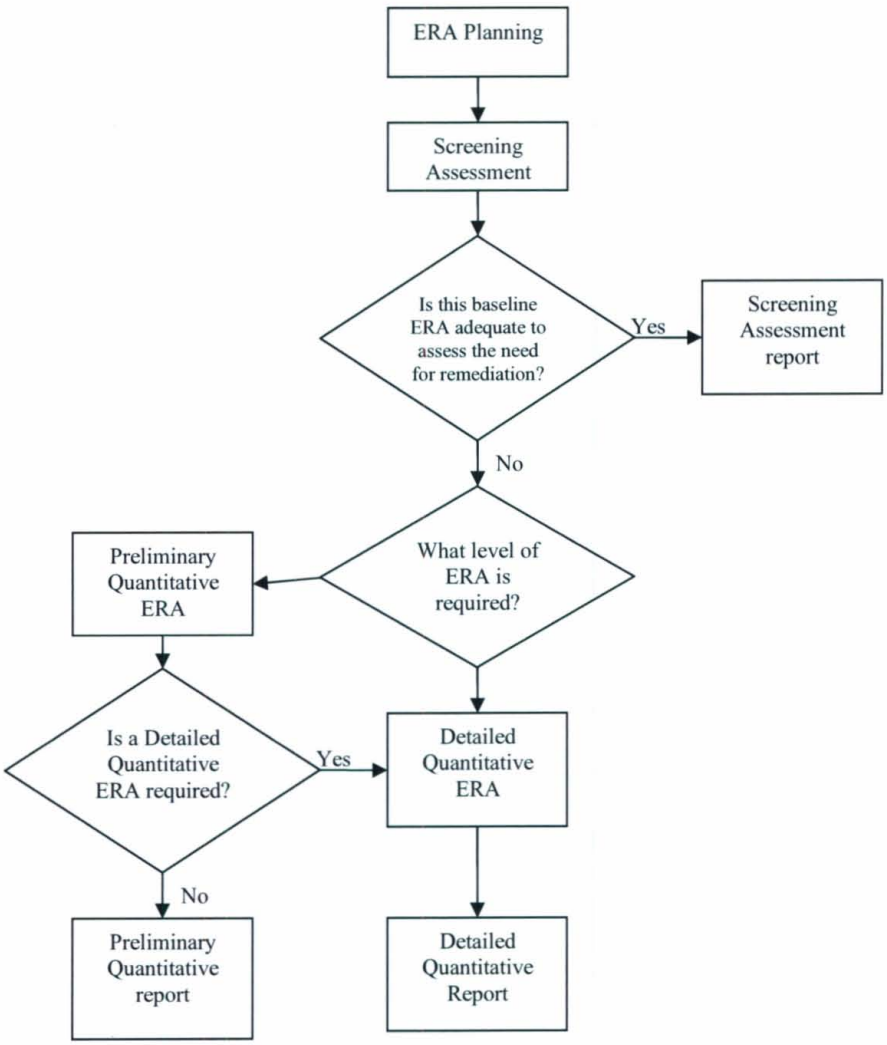

Figure 1-3. Framework for tiered ERA (from CCME, 1996).

When a lower tier cannot sufficiently define risk to support a risk informed decision, a higher assessment tier that may require additional data or applying a more refined analysis technique may be necessary.

ERA frameworks (*e.g.*, USEPA, 1992a; 1998; CCME, 1996) and literature (Suter, 2007) clearly emphasize the multi-tiered approach to ERA, however, ERAs seldom go beyond the screening assessment stage. In fact, higher tiered risk assessments are not used with any regularity by the majority of the decision makers managing chemical contaminants (Hope, 2009). Lower tiered (*i.e.*, screening) ecological risk assessments generally focus on chemical measurements with comparison to environmental quality standards for risk assessment. Perhaps the most common approach is to use chemical concentrations in abiotic and biotic components of the environment in risk models. This is referred to as the hazard quotient approach and incorporates mechanistic food chain models or tissue residue concentrations to calculate a single risk estimate by comparing the total daily exposure dose or tissue residue concentration to a toxicity reference value (Barnthouse and Suter, 1986; USEPA, 1992a). There are two primary approaches to the hazard quotient method (Millsap *et al.*, 2004). The method used most often is the "dietary approach" where the total ingested dose of a chemical consumed by a species of interest on a daily basis is predicted based on chemical concentrations in the abiotic (and/or biotic) components of the environment. This predicted daily dose is then compared to a dietary toxicity reference (TRV) value to calculate a dietary hazard quotient. The alternate approach is the "tissue based approach" where the actual concentration of a chemical in the tissue of an organism is compared to a tissue based toxicity reference value in order to calculate a hazard quotient. While the tissue residue

approach is generally presumed to be more accurate than the dietary approach, both approaches are limited by the use of laboratory based toxicity reference values and chemical concentrations for predicting toxic responses. Large uncertainties are often overlooked and descriptions of risk as upper bounds are often simplistic and misleading (NRC, 1994). Hazard quotients cannot define risk sufficiently enough to support a management decision (NRC, 1994).

1.2 Limitations of the Hazard Quotient Approach

The main uncertainties with the hazard quotient approach to ERA are associated with two key steps: 1) the use of chemical concentrations measured in abiotic and biotic components of the environment, and 2) the use of toxicity reference values derived from laboratory studies. Concentrations of a chemical in the abiotic and biotic components of the environment are used in the hazard quotient approach to predict the exposure of an organism to that chemical. Chemical analyses have the advantage of being specific, quantitative, and very sensitive, but the biological significance of chemical concentrations is not always clear. For example, the concentration of a chemical in soil often does not represent what is actually bioavailable and thus, toxic to the receptor (Ruby *et al.*, 1999). There are, however, recent studies that attempt to better estimate the fraction of the chemical that may be bioavailable (*e.g.*, Ollson *et al.*, 2009). Chemical analysis of wildlife tissues provides some insight into the actual exposure but there are limitations. For example, some contaminants such as benzo(a)pyrene are metabolised very quickly so they are not detected in body burden analyses. In this case, the metabolites are often more toxic than the parent compound. Differences between species

and between individuals as well as biotic factors such as seasons and feeding, may also affect the retention of a contaminant by the exposed organism. Exposure, therefore, cannot be accurately quantified through chemical analysis of concentrations in the environment or in biota.

The hazard quotient process makes use of screening benchmarks which are generally based on no observed adverse effect levels (NOAELs) or lowest observed adverse effects levels (LOAELs) from chronic or subchronic toxicity tests with mammals and birds (Suter, 2007). The benchmark uses reproduction, growth, or some other relevant effect as an endpoint as well as allometric equations for interspecies extrapolation and other factors to allow for shortcomings in the test design (Sample *et al.*, 1996). The toxicity tests used to derive these values are conducted using a dose of contaminant administered to the test species. For example, oral toxicity estimates for most metals are generally based on lab studies in which a soluble salt of the metal was dissolved in water or mixed in food and then ingested by an animal (Ruby *et al.* 1999). The toxicity studies do not take into account characteristics (*e.g.,* bioavailability) of the metal in soil, or the limitations these characteristics place on enteric absorption of that metal (Ruby *et al.* 1999). This can lead to overly conservative bioavailability estimates (*i.e.*, 100%) and assumptions in assessing the potential risk of exposure to a particular compound in a medium other than the ones used in the studies on which toxicity values are based.

Feeding studies in the lab are often conducted with a single chemical on one specific species in the laboratory under controlled laboratory conditions. Any extrapolation from the laboratory experiments to field situations cannot be carried out

with certainty and has many limitations (Power and McCarty, 1997). For example, toxicity data are not available for all species of wildlife that are considered in ecological risk assessments. The United States Environmental Protection Agency (1998) recommends using allometric scaling to extrapolate for interspecies variation. This is based on the observation that many biological properties vary according to body weight (Sample and Arenal, 1999). The allometric scaling of toxicity reference values is a source of uncertainty and has been debated in the literature (Mineau *et al.*, 1996; Knopper *et al.*, 2009). Application of toxicity values based on single chemical toxicity studies also does not account for the field situations where exposure to multiple chemicals is possible.

The hazard quotient approach itself is not considered a measure of risk (USEPA, 1989; Kolluru, 1996; Tannenbaum, 2003). It is a measure of the hazard and does not indicate the probability of adverse effects. If a hazard quotient exceeds one, this is merely an indicator of the level of concern (USEPA, 1989). Another issue associated with the hazard quotient includes the generation of unrealistic and toxicologically impossible values (Schmidt, 2004; Tannenbaum, 2003; Tannenbaum, 2005).

The methodology and calculations for the hazard quotient approach to screening risk assessment are well established and commonly used (USEPA, 1992a). The approach, however, incorporates conservative assumptions that attempt to err on the side of caution (*e.g.*, maximum exposure or ecological sensitivity) and reports risk as point estimates (*i.e.*, single numbers). Conservatism must be built into risk assessment calculations to avoid underestimating risk (Suter, 2007). Because of the inherent conservatism in the calculations, a hazard quotient of less than one demonstrates that adverse risks are unlikely. Over-conservatism however can lead to an unrealistic perceived

risk. According to Sample *et al.*, (1996), the hazard quotient approach was originally intended as a screening tool or as one part of a weight of evidence approach to ERA. There is, however a misconception that a hazard quotient greater than one indicates a significant risk and that this is sufficient evidence to make a risk management decision (Tannaenbaum, 2003). Guidance frameworks (USEPA, 1992a; Sample *et al.*, 1996) and the scientific literature (Tannenbaum, 2003; Hope, 2009) emphasize that hazard quotients are only a measure of concern. A hazard quotient exceeding one indicates that additional study of environmental exposure and effects is warranted. This is not a basis for making risk management decisions. In situations when the risk cannot be adequately characterized with an acceptable degree of certainty using a screening assessment, it is better to apply the iterative, tiered approach and to proceed to higher tiers of assessment. Unfortunately, while most guidance documents recommend field verification of effects predicted at lower tiers, guidance on methods is lacking.

1.3 Weight of Evidence Assessment and Biomarkers

Higher tiered ERAs are essentially weight of evidence assessments (USEPA, 1992a). Weight of evidence is defined as a risk characterization process by which measurement endpoints are related to an assessment endpoint to evaluate whether risk is posed to an organism given their environmental exposure (Menzie *et al.*, 1996). In a weight of evidence approach, all available data is examined to determine if organisms have been exposed to contaminants and if that exposure is associated with deterioration in the health status of the organism (USEPA, 1992a; Suter, 2007). The evaluation of a variety of data and tools (*e.g.*, chemical analyses, toxicity tests, biological surveys,

biomarkers) makes use of the best and most relevant science, thus providing more realistic assessments and making less conservative assumptions about exposure and effects.

One potential tool in the weight of evidence approach to ecological risk assessment is the use of biomarkers. The US National Academy of Sciences (NAS) and the National Research Council (NRC) (1987) first published the term biomarker as a result of discussions of a working group designated to study markers of ecological toxicity in 1987. Biomarkers were defined by US NAC/NRC (1987) as "xenobiotically induced variations in cellular or biochemical components or processes, structures or functions that are measurable in a biological system or samples". Early research limited the definition of biomarkers to biochemical responses (NAS/NRC, 1987; Huggett *et al.*, 1992). In 1994, however, Peakall and Shugart defined biomarkers as "a change in a biological system that can be related to an exposure to, or effect of, an environmental chemical or chemicals". This definition extended the early definitions to include biochemical, anatomical, physiological, and behavioral responses that signal exposure to and/or adverse effects of contaminants. In 1999, biomarker designations were revised again to include biological responses at the organism level or below (Peakall, 1999).

Because biomarkers indicate exposure and/or effect, it is not surprising that one of the justifications for the continued research and development of biomarkers is their potential application in ecological risk assessments. What is surprising to note, however, is that even though studies of biomarkers have been prevalent for many years, their incorporation into regulatory legislation for ecological risk assessment is severely lacking (Hagger *et al.*, 2009).

Peakall and Walker (1994) suggest that the most compelling reason for using biomarkers is that they can give information on the effect of pollutants rather than mere quantification of the contaminant levels. This being said, most biomarkers do not identify the causal agent and hence, chemical and biomarker monitoring are complementary approaches (Fossi, 1994). In a higher tiered ecological risk assessment, biomarkers can aid in building a weight of evidence case that contaminants are affecting the health of natural populations and that certain classes of chemicals are involved. Thus, while biomarkers cannot be used to directly predict ecological effects by themselves, they can be used to support other lines of inference such as chemical concentrations.

The most widely used classification of biomarkers is their division into biomarkers of exposure and biomarkers of effect (NAS/NRC, 1987; Koeman *et al.*, 1993). Biomarkers of exposure indicate an interaction between the xenobiotic and the target cell or organ (*i.e.*, exposure) has occurred but do not provide knowledge about the degree of adverse effects on the organism (Koeman *et al.*, 1993). For example, mixed function oxidases are part of an organism's natural defense mechanism against xenobiotics and their induction has been proposed as an early warning monitoring tool for some pollutants in fish (Payne *et al.*, 1987), birds (Rattner *et al.*, 1989), and invertebrates (Hyne and Maher, 2002). The induction of mixed function oxidases is regarded as a protective response to exposure of an organism to a xenobiotic but does not necessarily indicate that adverse effects are occurring in that organism.

Biomarkers of effect indicate exposure as well as a measureable alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impact or disease (NAS/NRC, 1987). An example is acetylcholinesterase

(AChE) inhibition by organophosphorous and carbamate pesticides (Lotti, 1995). Acetylcholinesterase hydrolyzes the neurotransmitter acetylcholine at neuromuscular junctions and brain cholinergic synapses, and thus terminates signal transmission. Exposure to these pesticides blocks this action leading to an increased concentration and residence time of acetylcholine at the nerve receptor and to disruption of nerve function. The inhibition of AChE in the blood reflects this inhibition in the nervous system causing accumulation of acetylcholine and toxicity. For example, 50-80% inhibition of brain AChE can cause death (Hill and Fleming, 1982). Therefore, the measured level of AChE inhibition is a biomarker of effect.

Biomarkers can further be classified as specific biomarkers and general biomarkers. Specific biomarkers represent a biological response induced only by a specific chemical or a specific group of chemicals, whereas general biomarkers may be induced by a number of different chemicals. An example of a specific biomarker is aminoalaevulinic acid dehydratase (ALAD). ALAD, a heme enzyme, is inhibited only by lead (Finlay *et al*, 1975). Determination of ALAD activity in blood samples confirms lead exposure and is considered sufficiently reliable to replace chemical analysis (Whigfield *et al.* 1986). Heat stress proteins on the other hand, respond to a variety of organism stresses ranging from heat to contaminant exposure (Lindquist, 1986) and are therefore considered a general biomarker. In fact, it is considered very difficult to even distinguish the causative stress as chemical or non-chemical (Pyza *et al.*, 1997).

1.4 Polychlorinated Biphenyls

PCBs are a group of 209 structurally similar congeners that were commercially produced as mixtures (*e.g.*, Aroclor 1254). Because of their chemical inertness, heat stability, and hydrophobic properties, PCBs were used as coolants and lubricants in transformers, capacitors, and other electrical equipment (Safe, 1994). Unfortunately, many of the properties responsible for their wide range of industrial applications, are also the same properties that are posing challenges to their global clean up.

The manufacturing of PCBs occurred from 1929 until 1977. In 1977, concern over the accumulation, persistence, and toxic effects of PCBs led to a North American ban on manufacturing and importing. PCB concentrations have since decreased but because of their persistent nature, they are still found in the environment. PCBs, particularly the higher chlorinated congeners, adsorb strongly to sediment and soil (Agency for Toxic Substances and Disease Registry (ATSDR), 2000). Because of their lipophilicity, PCBs also bioaccumulate in food chains and the highest concentrations are usually found in the adipose tissue of animals at the top of the food chain.

PCBs were used in Northern Canada during the operation of military facilities such as DEW-Line, Pinetree-Line, and Polevault stations. The subsequent abandonment of many of these sites has resulted in high levels of PCBs in soil, sediment, water, and biota in their vicinity. In other northern areas, PCBs have never been used or released and thus, their presence is attributed to transport from industrialised centres of the world via air and ocean currents. Long range transport is the primary source of PCBs in Northern environments (Muir *et al.*, 1992; 1999; Macdonald *et al.*, 2000; Braune *et al.*, 2005) and local sources are relatively small in the context of regionally distributed

contamination (Macdonald *et al.*, 2000; Stow *et al.*, 2005). PCBs originating from local sources such as former military installations (*e.g.*, Distant Early Warning (DEW)-line stations, Polevault line stations), however, have been shown to create a halo of contamination around the site caused by short range transport (Macdonald *et al.*, 2000; Pier *et al.*, 2003; Stow *et al.*, 2005). Adverse effects to the organisms living in their vicinity have been reported (Kuzyk *et al.*, 2003; Kuzyk *et al.*, 2005b; Johnson *et al.*, 2009).

Safe (1994) summarized many of the toxic responses observed in laboratory animals exposed to commercial PCBs and these effects include acute lethality, hepatomegaly, fatty liver, and other indicators of hepatotoxicity, porphyria, body weight loss, dermal toxicity, thymic atrophy, immunosuppressive effects, reproductive and developmental toxicity, carcinogenesis, genotoxic responses, modulation of diverse endocrine derived pathways, and neurotoxicity. Liver is the common target organ of PCBs and many symptoms of hepatotoxicity have been observed (*e.g.*, increased liver weight (Sanders *et al.*, 1974); fatty liver (Allen and Abrahamson, 1973)). PCBs also cause the induction of hepatic and extrahepatic detoxification enzymes (Eisler, 1986).

The toxic effects of PCBs on birds, mammals and fish have been well-doumented and reviews have been published (*e.g.*, Kimbrough *et al.*, 1978; Eisler, 1986; Safe, 1994). Acute high doses of PCBs leads to high brain tissue concentrations (300 to 400 ppm) in birds and usually results in death (Dahlgren *et al.*, 1972; Stickel *et al.*, 1984; Eisler, 1986) while prolonged chronic exposure usually results in edema related death (Vos, 1972; World Health Organization (WHO), 1993). PCB poisoning among birds includes effects such as morbidity, tremors, beak pointed upwards, and muscle in-coordination (Eisler,

1986). Sublethal effects of PCBs in birds include reproductive effects such as decreased egg hatchability and egg production (Britton and Huston, 1973; Dahlgren *et al.*, 1973; Platanow and Reinhart, 1973; Lillie *et al.*, 1975; Scott, 1977; Fernie *et al.*, 2001), embryonic mortality (Blazak and Marcum, 1975; Peakall and Peakall, 1973) as well as decreased growth of chicks (Rehfeld *et al*, 1972). Changes in behaviour such as nest inattentiveness have also been observed (Custer and Heinz, 1980). Other sublethal effects include thyroid changes (Jeffries and Parslow, 1972; Hoffman *et al.*, 1996) and porphyria (Vos *et al.*, 1971). Fatty liver and enlargement of the liver have also been observed (McCune *et al.*, 1962; Rehfeld *et al.*, 1971).

Mammals tend to be more sensitive to acute toxic effects of PCBs than birds (Eisler, 1986). The acute lethal dose required to kill 50% of the test organisms (LD50) for PCBs has been shown to range from 0.36 g/kg to 25 g/kg and depends on the PCB mixture as well as the characteristics of the test species (Safe, 1994). Young animals may be more sensitive than adults, and females may be more sensitive than males (Kimbrough *et al.*, 1978). Toxic effects also vary between species in their ability to metabolize PCBs and the different sites of action (CCME, 1999).

PCBs are strong hepatotoxic and acnegenic compounds for mammals. The gastrointestinal tract, immune system, and nervous system are also affected (WHO, 1993). Reproductive effects, which have been largely based on studies on mink, have also been widely documented in laboratory studies (Platonow and Karstad, 1973; Aulerich and Ringer, 1977). Reproductive effects of PCBs on mammals (as summarized in Safe *et al.*, (1994)) include decreased reproductive success (Arnold *et al.*, 1990; Aulerich and Ringer, 1977; Linzey, 1988), decreased fetal viability and death (Villeneuve

et al., 1971a; 1971b) and decreased litter sizes (Villeneuve *et al.*, 1971a). Effects on the immune system include increased mortality due to microbial infection (Loose *et al.*, 1978a; 1978b). Porphyria has also been observed (Bruckner 1974a; 1974b). Hepatotoxic effects of PCBs on mammals include liver enlargement and fatty liver (Kimbrough *et al.*, 1972; Kasza *et al.*, 1978).

The effects of PCBs on fish vary depending on species, developmental stage, and mode of exposure (Elonen *et al.*, 1998). The lethal body burden of Aroclor 1254 was 650 mg/kg for trout exposed to PCB 1254 via their diet for 260 days (Mayer *et al.*, 1977). Sublethal effects of PCBs on fish include increased biotransformation enzyme activity (*e.g.,* mixed function oxidases (Payne, 1987), hepatic lesions (McCain *et al.*, 1992), as well as growth inhibition and liver enlargement (Leatherland and Sonstegard, 1981)). PCBs have also been shown to cause thyroid abnormalities (Brown *et al.*, 2004) and increased thyroid activity (Mayer *et al.*, 1977). Reproductive effects of PCBs include reduced fecundity and frequency of reproduction (ACOE, 1988), reduced hatching success (Ankley *et al.*, 1991), and larval mortality (Hogan and Brauhn, 1975).

Many of the biological effects studies on PCBs were primarily carried out in the laboratory or in temperate regions such as the Great Lakes, and cannot be confidently applied to Arctic ecosystems. Arctic ecosystems may be more vulnerable to organic contaminants such as PCBs because of the dominant role of lipids resulting from the seasonality of food availability (Alexander, 1995; Riddle and Chapman, 2005) as well as shorter food chains and lack of functional redundancy (Chapman and Riddle, 2003; 2005). At the low temperatures characteristic of the Arctic, metabolic rates are slower and energy use is lower than in biota from more temperate regions (Chapman and Riddle,

2005). This means that organisms may accumulate contaminants at a slower rate but the dominant role of lipids in these animals means that they may take up more of a lipophilic contaminant than in other regions (Chapman and Riddle, 2005). Lower energy usage may mean that less energy is available for detoxification processes (Chapman and Riddle, 2005). In addition, impending climate warming may have possible effects on contaminants dynamics (Fisk *et al.*, 2003). Modifying temperate data to Arctic data would involve many assumptions and uncertainties. Therefore, the ideal method of assessing Arctic environments is to collect site specific biological effects information.

1.5 Subject Site Description and Site History

The subject site for this thesis is Saglek, a former Polevault Line military station in northern Labrador. Saglek is located on the northeast coast of Labrador (approximately 225 km north of Nain and 600 km north of Happy Valley-Goose Bay) (Figure 1-4) at the southern boundary of the Torngat Mountains National Park. Saglek is approximately 100-200 km north of the present position of the white spruce tree line and permafrost is extensive but discontinuous (Pier *et al.*, 2003). Saglek Bay is at the entrance to a series of fiords which extend west into the Torngat Mountains. Ice usually persists in the Bay from December until late June. Saglek is located within the Arctic Alpine Tundra Ecoregion which includes the Torngat Mountains and the valleys and fiords that dissect them (Meades, 1990). This ecoregion is characterised by short cool summers with long, cold winters and receives approximately 3.0 m of snow annually (Meades, 1990). The climate would be considered sub-Arctic continental with low precipitation.

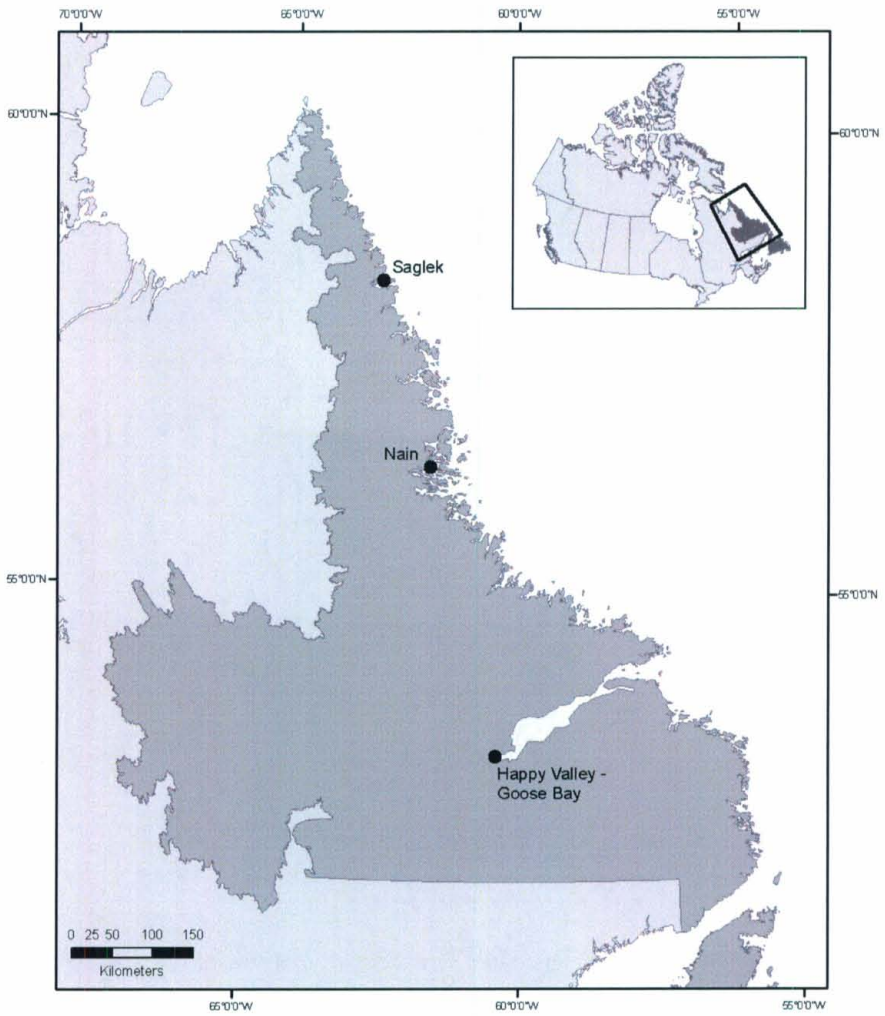

Figure 1-4. Geographic location of Saglek fiord.

Parts of Saglek Bay are included in the Labrador Inuit Land Claims Area and have traditionally been a popular hunting and fishing area for Labrador Inuit. A military radar station has operated (but not continuously) from the southern shore of the mouth of the Bay since the 1950s. The U.S. Air Force operated a Polevault Line military station at Saglek from 1951 to 1971. The site consisted of a main station on the highest summit with accommodations and operations buildings, two hilltop tropospheric antenna sites,

and a lower camp with an airstrip, several buildings and a beach landing area. The station was abandoned in 1971 and the site was destroyed by a fire in 1978 (Pier *et al.*, 2003). The Canadian Department of National Defence (DND) opened a modern North Warning System (NWS) long range radar facility in 1988. The long range radar facility is currently operated remotely and is unmanned with maintenance conducted at the site seasonally and on an as-needed basis by personnel working on contract to DND.

In 1996, an environmental site assessment of Saglek revealed concentrations of PCBs in soil exceeding the 50 ppm set out by the Canadian Environmental Protection Act (CEPA) at the site (ESG, 1997). Particularly elevated concentrations of PCBs were identified at the beach area of Saglek ranging from less than 50 mg/kg dry weight up to 1,600 mg/kg dry weight (ESG, 1997). PCB contamination was believed to be a result of the abandonment and subsequent demolition of the facility. Remediation of PCB contaminated soil was subsequently carried out in two stages during the time period 1997 to 2004. The first stage (1997-1999) involved the excavation and stockpiling of soils with PCB concentrations exceeding 50 ppm (ESG, 2005). The second phase (2002 to 2004) involved the shipment of the soil with PCB concentrations above 50 ppm off the site for treatment. Because of the proximity to the ocean, contaminated soil at Saglek Beach containing greater than 5 ppm of PCBs was also excavated and secured in areas away from the ocean and capped with clean soil (ESG, 2005). In order to evaluate the effectiveness of the terrestrial cleanup, a long term monitoring plan (LTM) has been implemented at Saglek Beach to monitor concentrations of PCBs in the terrestrial environment and biological matrices.

During the site assessment in 1996, five shorthorn sculpin were also collected from Saglek Bay to determine whether PCBs were entering the marine environment and marine food chain. Concentrations of PCBs averaging 51,000 ppb in livers and 7,000 in whole bodies (minus livers) were detected in these shorthorn sculpin. Therefore, concurrent with the soil remediation during 1997 and 1998, studies of the marine environment were also undertaken and revealed that elevated concentrations of PCBs were present in sediment in Saglek fiord throughout approximately 8 km^2 of Saglek Bay (ESG, 2005). The analysis of biota at different levels of the marine food chain (benthic invertebrates, bottom feeding fish, marine mammals and seabirds) indicated the presence of PCBs in excess of expected background concentrations. An ecological risk assessment conducted in 2002 also indicated a significant risk to marine fish and seabirds within approximately 0.65 km^2 of Saglek Bay (ESG, 2002). Because the terrestrial source of PCBs had been removed and because of the potentially destructive nature of dredging contaminated sediments from Saglek Bay, it was decided to leave the contaminated sediments in place and monitor the concentrations of PCBs in the marine ecosystem over time. A recent study indicates that concentrations of PCBs in the marine environment have decreased (Brown *et al.*, 2009).

A human health risk assessment was also conducted for the site in 2002 (Ayotte *et al.*, 2002) and indicated that harvesting over a 5 km radius of the site would result in higher long term PCB exposures. A hunting and fishing advisory was therefore implemented in the Saglek area and this advisory remains in effect today.

Since the initial discovery of PCBs at Saglek, a stakeholder group has been established for Saglek. The stakeholders include representatives from The Department of

National Defence's North Warning System Office, the Director General Environment, The Nunatsiavut Government, Environment Canada, Fisheries and Oceans Canada and the Newfoundland and Labrador Department of Environment and Conservation. The stakeholders group meets annually in St. John's, NL to discuss progress at Saglek and future initiatives. A timeline showing major events at Saglek is provided in Figure 1-5.

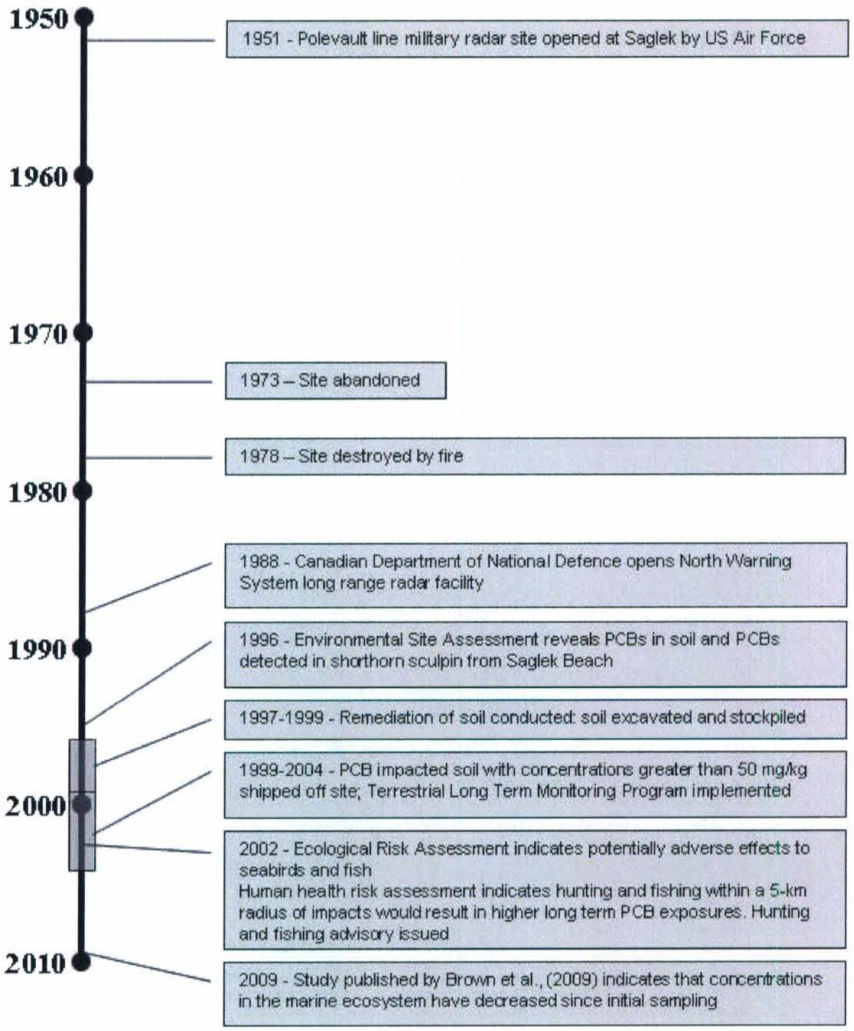

Figure 1-5. Timeline of major events at Saglek (1950-2010)

1.5.1 Valued Ecosystem Components

The Saglek area is rocky and boulder strewn. The dominant vegetation is tundra alpine heath (*Erica carnea*) with sedge meadows (*Carex bigelowii*) fed by seepage from late melting snow patches (Meades, 1990). Other plant species at the site are typical of the tundra and include dwarf shrubs such as willow (*Salix arctica*), dwarf birch (*Betula glandulosa*), Labrador tea (*Ledum groenlandicum*), and black crowberry (*Empetrum nigrum*). Wildlife expected in the tundra habitat include Arctic fox *(Alopex lagopus)*, bog lemming (*Synaptomys borealis)*, caribou (*Rangifer tarandus)*, Arctic hare (*Lepus arcticus*), and polar bear (*Ursus maritimus)*. Black bear (*Ursus americanus)* are also present in this area and represent the most northerly range of black bear in the world. Least weasel (*Mustela nivalis*), masked shrew *(Sorex cinereus)*, wolf (*Canis lupus*), meadow vole (*Microtus pennsylvanicus)*, mink (*Mustela vison),* and deer mice *(Peromyscus maniculatus)* are ubiquitous in this area. Various seals including ringed seal *(Phoca hispida)*, grey seal *(Halichoerus grypus)*, harp seal (*Phoca groenlandica)*, harbour seal (*Phoca vitulina concolor)*, and hooded seal *(Cystophora cristata*) are present as well. Common marine birds in the area include Arctic tern (*Sterna paradisaea*), glaucous gull (*Larus hyperboreus)*, great black backed gull (*Larus marinus*), and black guillemot (*Cepphus grylle*). Fish common to the area include Arctic char (*Salvelinus alpinus*), shorthorn sculpin (*Myoxocephalus scorpius*), and fourhorn sculpin (*Myoxocephalus quadricornis*).

During the initial habitat characterization studies conducted in 1998/1999, potential species of concern at Saglek were identified in consultation with Labrador Inuit, taking into consideration both human harvesting concerns and the health of the local

ecosystem (ESG, 2002). Sampling over the past ten years has thus included important food resources such as ringed seal, Arctic char, seabirds and eggs, and less socially-important species that play an ecologically significant role, such as shorthorn sculpin and benthic invertebrates. These species or communities represent valued ecosystem components (VECs), defined as resources or environmental attributes that are important to human populations, have economic and/or social value, and/or have intrinsic ecological significance (*e.g.*, rare or endangered species or communities, or an indicator that integrates the potential effects on a complex of ecological variables) (Beanlands and Duinker, 1983; CCME, 1996). Descriptions of the VECs considered in this thesis are summarized below. While it is recognized that the VECs listed here may not be considered important food resources, standards protective of the chosen VECs would be considered protective of the entire ecosystem.

- Deer mice (*Peromyscus maniculatus)* eat seeds, arthropods, and other plant material (*e.g.*, green vegetation, roots, and fruit) but they are omnivorous and highly opportunistic which leads to substantial regional and seasonal variation in their diet (USEPA, 1993). Small mammals are good monitors of environmental contaminants (*e.g.*, Talmage and Walton, 1991; Clark *et al.*, 1992; Shore and Douben, 1994,) and deer mice in particular have been recommended as a suitable sentinel species (Lower and Kendall, 1990). Deer mice and other small mammals play a significant role in northern ecosystems and their populations are often linked to habitat alterations and natural disturbances. They are also an important food source for predatory species such as fox, weasel and hawks and their population status is often a good predictor of other fur bearers at higher levels of

the food chain. Moreover, deer mice have relatively small home ranges (Bowers and Smith, 1979) and can provide valuable information about a specific site.

- Black guillemot (*Cepphus grylle*) are marine, diving, piscivorous birds that breed along rocky marine coastlines and offshore islands in shallow waters and forage in nearshore waters during breeding and non-breeding seasons (Butler and Buckley, 2002). Guillemots nest in scattered pairs at Saglek using cracks in cliffs, crevices among boulders, talus slopes at cliff bases and small islands (Kuzyk *et al.*, 2003). The feeding habits of Black guillemots vary both seasonally and geographically but the diet is composed almost solely of fish (Bradstreet, 1980; Ewins, 1986; Lonne and Gabrielsen, 1992). The species is known to be opportunistic (Butler and Buckley, 2002), but includes mainly benthic and pelagic fish in its diet and to a lesser extent, invertebrate species, including crustaceans, annelids and mollusks in shallow inshore waters. Black guillemots were chosen as a species of concern at Saglek because piscivorous birds are considered particularly vulnerable to organic contaminants due to their position at the top of aquatic food chains and because of their inshore and benthic feeding habits (Asbirk, 1978). Their limited foraging ranges (Cairns, 1987) and their habit of returning to the same nests year after year (Neil Burgess, personal communication) makes them suitable as study species. Black guillemot have also been found to tolerate investigator disturbance (Cairns, 1980).

- Shorthorn sculpin (*Myoxocephalus scorpius)* are demersal fish found on both sides of the North Atlantic Ocean (Scott and Scott, 1988). The shorthorn sculpin is a sluggish, bottom feeding, non-migratory species and is therefore suitable for monitoring of pollution exposure and effects (*e.g.*, Stephenson *et al.*, 2000; Kuzyk *et al.*, 2005b). Their diet consists mostly of benthic invertebrates and other benthic fish (Scott and Scott, 1988). A variety of amphipods, worms, crustaceans as well as other benthic fish such as gunnels, sand lance were observed in stomach contents of sculpin from Saglek. Because they are shallow water fish, they are especially abundant near Saglek Beach. Sculpin are an important link in the marine food chain at Saglek because they provide a food source for larger fish, seals, and seabirds (*e.g.*, black guillemot).

Concentrations of PCBs have been demonstrated to be elevated in each of the above species at Saglek (ESG, 2005; Kuzyk *et al.*, 2003; Kuzyk *et al.*, 2005b).

1.5.2 Previous Biomarker Studies

Black guillemot (*Cepphus grille*) was identified as a species of concern in the initial ecological risk assessment for Saglek Bay (ESG, 2002). In 1998, Black guillemot nestlings were collected from Saglek Bay by the Environmental Sciences Group (ESG) at the Royal Military College in Kingston, Ontario. Concentrations of PCBs in livers ranged from 15 to 6200 ng/g wet weight and PCBs in breast muscle ranged from 1033 ng/g to 12,100 ng/g wet weight (Kuzyk *et al.*, 2003). Concentrations of PCBs in Black guillemot eggs at Saglek ranged from 29,500 ng/g to 36,600 ng/g wet weight (Kuzyk *et al.*, 2003). Kuzyk *et al.* (2003) also examined a suite of liver biomarkers in black

guillemot from Saglek in 1999. Liver ethoxyresorufin-*O*-deethylase (EROD) activity, liver retinol, liver retinyl palmitate, malic enzyme activity and porphyrin concentrations were measured in relation to liver PCB concentrations in 31 three-week old nestlings. The nestlings were collected from three groups of guillemots: a reference group near Rose Island (PCBs = 15-46 ng/g liver, wet weight), a moderately exposed group near Big Island (PCBs = 24-150 ng/g wet weight) and the highly exposed group from the Beach area (PCBs = 170–6,200 ng/g wet weight). Results indicated that nestlings from the Beach group had enlarged livers (36%), increased EROD activity (79%) and reduced liver retinol concentrations (47%) compared to the reference group (Kuzyk *et al.*, 2003). Retinyl palmitate concentrations were reduced by 50% but only in female nestlings (Kuzyk *et al.*, 2003). Nestlings from the Big Island area also had increased EROD activity and decreased liver retinol and retinyl palmitate concentrations but to a lesser extent than in the Beach group. Other biomarkers such as malic enzyme activity and porphyrin concentrations showed no association with PCB concentrations in the nestlings.

Kuzyk *et al.*, (2005b) investigated the biological responses of sculpin in Saglek Bay to PCB concentrations. EROD activity in liver, fish body condition, lipid content and relative liver mass were examined in 35 sculpin across a wide range of PCB concentrations. The sculpin were collected from three zones of contamination: zone one – within 1.5 km of the former contamination (beach area), zone two – between 1.5 km and 4.5 km from the beach, zone three – between 4.5 km and 7.5 km from the source but still within Saglek fiord (Kuzyk *et al.*, 2005b). Results of this study indicated that significant EROD induction occurred in the highly exposed group of sculpin. Other

biological endpoints, however, did not show the same relationship and were not related to PCB concentrations or EROD induction. Kuzyk *et al.*, (2005b) concluded that the significance of the elevated EROD induction was unknown as other biological endpoints showed no relationship with the PCB concentrations or EROD induction.

The findings of the Saglek biomarker work by Kuzyk *et al.*, (2003; 2005b) showed the effects of local contamination in an Arctic ecosystem and that liver biomarkers respond to relatively low concentrations of PCBs. In addition, these results demonstrated that local contamination in the Canadian Arctic can have an important impact on the physiology of local wildlife (Fisk *et al.*, 2005).

1.5.3 Current Contaminant Concentrations at Saglek

1.5.3.1 Terrestrial Environment

Based on the 2007 study associated with the long term monitoring plan for the terrestrial environment at Saglek (ESG, 2007), concentrations of PCBs in soil at Saglek Beach in 2007 ranged from <0.5 mg/kg dry weight to 7.7 mg/kg dry weight compared to levels as high as 1,600 mg/kg dry weight in 1996 (ESG, 1997). Concentrations in three composite deer mouse samples (composed of three deer mice each) from Saglek Beach in 1998 (whole body minus liver) ranged from 25 mg/kg to 64 mg/kg wet weight while concentrations of PCBs in twenty discrete deer mice (whole body) samples from 2007 ranged from 0.38 to 23 mg/kg wet weight. No previous biomarker studies have been conducted on deer mice or other terrestrial ecological receptors at Saglek. In addition, no ecological risk assessment has been previously conducted to determine the potential effects of residual PCBs at Saglek on terrestrial ecological receptors such as deer mice.

1.5.3.2 Marine Environment

In 2006/2007, PCB concentrations were again measured in marine sediment, shorthorn sculpin and Black guillemots. This study showed that average PCB concentrations in the nearshore sediment have decreased eleven-fold, while PCB concentrations (lipid weight) in shorthorn sculpin (*Myoxocephalus scorpius*) at Saglek Beach have decreased nineteen-fold (Brown *et al.*, 2009). Concentrations of PCBs in Black guillemot (*Cepphus grylle*) nestlings (lipid weight) from the east side of Saglek Beach have decreased twenty-fold while PCB concentrations in nestlings from the west side of Saglek Beach did not show a significantly different change over time (Brown *et al.*, 2009). According to Brown *et al.*, (2009), the overall spatial trend in sculpin PCB levels indicates that elevated concentrations of PCBs in sculpin from the Beach are associated with the highest sediment concentrations which are found within 500 m of the Beach. PCB concentrations in sediment samples reported by Brown *et al.*, (2009) ranged from 1.0 to 800 ng/g dry weight and decreased with increasing distance from the former source of contamination at the beach. PCB concentrations in shorthorn sculpin liver at Saglek Beach in 2007 (reported in chapters 3 and chapters 4 of this thesis) ranged from 12 to 18000 ng/g wet weight. PCBs in black guillemot nestling livers from Saglek in 2007 (based on data from Brown *et al.*, (2009)) ranged from 120 to 1110 ng/g wet weight.

1.6 Thesis

The overall objective of this thesis was to demonstrate an improved approach for carrying out ecological risk assessments by incorporating biomarker responses into a

biological effects-based weight of evidence. This objective is achieved by first examining a suite of biomarkers in one valued ecosystem component of the terrestrial ecosystem (small herbivorous mammal) and two valued ecosystem components of the marine ecosystem (diving piscivorous bird and benthic fish) at Saglek, Labrador (chapters 2, 3, 4, 5 and 7). Evaluating the effects of contaminants on valued ecosystem components is critical to ecological risk assessments, particularly in Northern environments where information on biological effects related to contaminant exposure is lacking.

The biomarker studies are then incorporated into a biological effects-based weight of evidence assessment and results are compared to results of a traditional screening level risk assessment. The biological effects-based weight of evidence assessments presented for the marine ecosystem (chapter 6) and the terrestrial ecosystem (chapter 7) demonstrate the value of proceeding to higher tiered risk assessments at large complex sites such as Saglek where potential remediation strategies are expensive and potentially destructive to the environment.

Overall, this thesis represents a unique approach to using biomarkers to supplement chemical residue analysis in monitoring an Arctic ecosystem following removal of a contaminated source. The results of the biomarker study reported in this thesis as well as the previous biomarker studies by Kuzyk *et al.*, (2003; 2005b) represent a first step in identifying appropriate biomarkers for a future biological effects-based monitoring program at Saglek. Collectively, this work provides information on biological effects/biomarkers in Arctic wildlife that respond to PCBs and that may be applied for ecological risk assessments at other local sources (*e.g.*, DEW-Line sites) across Northern

Canada. This thesis also studies changes in the marine ecosystem at Saglek from a risk perspective by comparing risk assessment results over the two time periods.

Saglek provided a superb site to conduct an ecological risk assessment of PCB contamination because the PCBs are widespread (ESG, 1999), other inorganic and organic contaminants are low compared to ambient levels in industrialized areas (ESG, 1997; ESG, 1999), and PCBs have been confirmed to be present in the marine and terrestrial food webs (Kuzyk *et al.*, 2005a; ESG, 2005; ESG, 2007).

2.0 Chapter 2: Effects of Local Point Source Polychlorinated Biphenyl (PCB) Contamination on Bone Mineral Density in Deer Mice (*Peromyscus maniculatus*)

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2.1 Abstract

A former local source of PCBs has contaminated soil and the terrestrial food web at Saglek, Labrador. The relationship between PCB exposure and bone mineral density as a biomarker in deer mice (*Peromyscus maniculatus*) was investigated at two sites at Saglek: a contaminated beach and a reference area. Bone mineral density was measured on the femur of twenty-six deer mice using dual-energy x-ray absorptiometry (DXA) technology. Bone mineral density was significantly lower in deer mice from the high exposure site (average whole body $\sum$PCB = 5770 ng/g wet weight, n=20) than at the reference site (average whole body $\sum$PCB = 79.8 ng/g wet weight, n=7). We used T-scores from the World Health Organization to determine the degree of decreased bone mineral density in exposed mice. Assuming the same biomechanical forces apply as for humans, and using a conservative factor of 1.5 (fracture risk increases 1.5 to 3 fold for every standard deviation decrease in bone mineral density), mice from the contaminated beach are up to five folds more susceptible to fracture risk than mice from the reference area. Therefore, the PCB concentrations found locally at contaminated military sites, such as Saglek, are high enough to affect local wildlife.

Keywords: Local sources, PCBs, Labrador, Bone mineral density, Deer mouse

2.2 Introduction

Over the past thirty years, significant progress has been made in understanding the occurrence, sources, and pathways of polychlorinated biphenyls (PCBs) and other anthropogenic contaminants in Arctic environments (Muir *et al.*, 1992; Muir *et al.*, 1999; Macdonald *et al.*, 2000; Braune *et al.*, 2005). Through this research, it has generally become accepted that long range transport from industrialised centres of the world through air currents, ocean currents, and rivers is the primary source of PCBs in the Arctic. Although long range transport has been established as the primary source, local sources of PCBs also exist in the Arctic and include military installations, mining and metallurgy industries, and waste disposal sites. Of these local sources in Northern Canada, military facilities such as DEW-Line, Pinetree-Line, and Polevault Stations are believed to contribute the highest PCBs to the environment (Stow *et al.*, 2005). PCB contamination at military sites is often a result of historical disposal and maintenance practices as well as the subsequent demolition and abandonment of the sites.

The relative significance of local versus distant sources of contamination in the Arctic has been the source of some debate in the literature (Thomas, 1992; Bright *et al.*, 1995; Pier *et al.*, 2003; Stow *et al.*, 2005). It has been estimated that these military installations contribute little to the overall PCB burden of Northern Canada (Macdonald *et al*, 2000; Pier *et al.*, 2003; Stow *et al.*, 2005) but research also indicates that they influence the local environment by short range transport creating a halo of contamination (Pier *et al.*, 2003). The halo effect indicates that local PCB contamination may be a more significant source than long range transport at these military stations. Many of the military installations in Northern Canada are being remediated based upon cleanup

criteria established through human health and ecological risk assessments (Reimer *et al.*, 1993). However, because the local source redistribution of PCBs and its effects on local wildlife and ecosystem health are not well understood, it is difficult to set site specific cleanup targets. Research is therefore needed to determine the effects of primary sources of PCBs on local wildlife and ecosystem health in the Arctic.

A site where the short range transport of PCBs has been studied is Saglek, a former Pole Vault station in northern Labrador (Figure 2-1). Elevated concentrations of PCBs were identified in soil at a beach area of Saglek in 1996 ranging from less than 50 mg/kg dry weight up to 1,600 mg/kg dry weight (ESG, 1997). PCB contamination was believed to be a result of the abandonment and subsequent demolition of the facility. A major soil remediation project was carried out in two stages during the time period 1997 to 2004 (ESG, 2005; 2007). The first stage (1997-1999) involved the excavation and stockpiling of soils with PCB concentrations exceeding 50 mg/kg (ESG, 2005). The second phase (2002 to 2004) involved the shipment of the soil with PCB concentrations above 50 mg/kg off the site for treatment. Because of the proximity to the ocean, contaminated soil at Saglek Beach containing greater than 5 mg/kg of PCBs was also excavated and secured in areas away from the ocean and capped with clean soil (ESG, 2005). In order to evaluate the effectiveness of the cleanup, a long term monitoring plan has been implemented at Saglek Beach to monitor concentrations of PCBs in the terrestrial environment and biological matrices. Based on the 2007 study, concentrations of PCBs in soil at Saglek Beach in 2007 ranged from <0.5 mg/kg dry weight to 7.7 mg/kg dry weight.

The halo of contamination at Saglek due to short range transport was estimated to

be up to 27 km in diameter (Pier *et al.*, 2003). Thus, Saglek represents a maximum redistribution site due to PCB sources at or near the tops of windswept hills, with previously disturbed and sparsely vegetated areas with sandy surface soil that is low in organic carbon (ESG, 1997; Stow *et al.*, 2005). Because the levels of other organic and inorganic contaminants (*e.g.*, polycyclic aromatic hydrocarbons, metals, pesticides) in the terrestrial food web at Saglek are low compared to ambient levels in industrialized areas (ESG, 1997; ESG, 1999), the site provides a unique opportunity to study the biological effects of a local PCB source in the virtual absence of other contaminants.

To quantitatively assess biological effects, we measured bone mineral density, which could be used as a biomarker of PCBs, in deer mice (*Peromyscus maniculatus*) at two locations at Saglek: a reference area and a contaminated Beach area (Figure 2-1). Small mammals such as deer mice have been demonstrated to be good monitors of environmental contaminants in terrestrial food webs in many studies (*e.g.*, Talmage and Walton, 1991; Clark *et al.*, 1992; Shore and Douben, 1994). In laboratory settings, PCB exposure alters bone turnover mechanisms in rats (Yilmaz *et al.*, 2006) as well as bone morphometry and/or strength in rats (Andrews, 1989; Lind *et al.*, 2000a,b). The physiological mechanism is not fully known. It is believed that because PCBs are antiestrogenic, they inhibit the binding of estrogen to the estrogen receptor subsequently inhibiting estrogen-induced responses such as bone remodelling (Bonefeld-Jorgenson *et al.*, 2001). Recent studies on museum samples of polar bear and seal bones from East Greenland and the Baltic Sea, respectively, indicate that reduced bone mineral density in the time period after the 1960s is linked to organochlorine chemical exposure (Lind *et al.*, 2003; Sonne *et al.*, 2004).

The objective of this study is to examine whether the sphere of influence at PCB contaminated military sites such as Saglek, while small in the context of regionally distributed contamination from long-range transport, is large enough to affect local wildlife, specifically bone mineral density in deer mice.

2.3 Methods

2.3.1 Study Area

Saglek Bay (Figure 2-1) is located on the north east coast of Labrador at the southern extent of the Torngat Mountains. From 1951 to 1971, a Polevault Station was operated at Saglek by the U.S. Air Force. In 1971, the Station was abandoned and in 1978 the site was destroyed by a fire (Pier *et al.*, 2003). The Canadian Department of National Defence (DND) commenced construction on a modern North Warning System (NWS) long range radar facility in 1986 and the station (designated as LAB-2) opened in 1988. The LAB-2 long range radar facility is currently operated remotely and is not staffed but maintenance is conducted at the site seasonally and on an as-needed basis by DND contractors. Extensive PCB contamination in soil and marine sediment associated with the original facility was identified at Saglek in the 1990s. Soil remediation was subsequently carried out and by 2004, the terrestrial source was removed. Ecological studies show that PCBs are still present in the terrestrial and coastal marine food webs (ESG, 2005; ESG, 2007; Kuzyk *et al.*, 2005a) and there is evidence that concentrations are decreasing in the marine environment (Brown *et al.*, 2009). The long term monitoring plan being conducted by ESG indicates that the PCB concentrations in three composite deer mouse samples (composed of three deer mice each) from Saglek Beach in

1998 (whole body minus liver) ranged from 25 mg/kg to 64 mg/kg wet weight while concentrations of PCBs in twenty discrete deer mice (whole body) samples from 2007 ranged from 0.38 to 23 mg/kg wet weight.

2.3.2 Sample Collection

Two sampling locations were chosen for collection of deer mice: a reference area and the Beach (Figure 2-1). Based on terrestrial monitoring studies following remediation, residual PCB contamination in soil, plants, and deer mice exist at the Beach area (ESG, 2005; ESG, 2007). An area at the southeastern end of the airstrip was chosen for the reference site as it is not within 1,280 m^2 (maximum home range of deer mice from Bowers and Smith (1979)) of any PCB contaminated areas of the Saglek site. Therefore, the home ranges of deer mice exposed to contaminated areas of the site are not expected to overlap with the chosen reference area. The reference area is, however, still within the 27 km halo of contaminant input estimated by Pier *et al.*, (2003).

Mice were trapped in August 2007 using live traps (aluminium perforated folding traps 7.6 cm x 8.9 cm x 22.9 cm from H.B. Sherman Traps Tallahassee, Florida, USA) and snap traps (Victor® snap traps). At the Beach area, both live traps and snap traps were set in the immediate vicinity of each other. Here, mice from the live traps were used for bone mineral density measurements and mice from the snap traps were used for PCB analysis. For the purposes of the long term monitoring program being conducted at Saglek Beach, whole body samples (*i.e.*, from the live traps with appendages attached) were required for PCB analysis in order to be consistent with previous sampling. At the reference area, only live traps were set and the mice from these live traps were used for both PCB analysis and bone mineral density. The PCB analyses of mice from the snap

traps from Saglek Beach were paid for by federal funding as part of the Saglek LTM. In order to be consistent with previous years sampling, the whole mouse had to be analyzed. It was therefore necessary to collect separate mice for this thesis. It was decided to live trap the mice so that fresh livers could be immediately frozen on liquid nitrogen and biochemical assays would be possible. The live trapped mice were also used for BMD. At the reference site, only live trapped mice were caught because this is not part of the LTM.

Five live traps were set at the reference area and ten live traps and ten snap traps were set at the Beach. Traps were baited with a mixture of oatmeal and raisins and were placed approximately 30 cm apart for four successive trapping nights. The traps were set approximately one hour before sundown. Bedding material was added to the live traps to avoid trap mortality resulting from low night temperatures. Traps were checked within one to two hours of sunrise the following morning. Mice from the snap traps were placed in individually labelled sample bags and stored frozen for PCB analysis. Live traps with mice in them were placed in plastic containers and transferred back to the field base where the mice were killed by cervical dislocation. Cervical dislocation was conducted by restraining the mouse in a normal upright position on a cutting board placed on a table by holding the base of the tail firmly with the left hand. A pair of large metal forceps was used to push forward and down against the base of the skull while the base of the tail was pulled backward at the same time. The total mass of the mice was measured to the nearest 0.1 g using a top loading battery powered balance. The sex of the mouse was also recorded. The total length, snout to vent length, tail length, right hind foot length, skull length, and skull width were measured using digital callipers. It is possible that some

measurements (*i.e.*, total length and snout to vent length) may vary slightly due to the cervical dislocation procedure. The right hind legs of the live trapped mice were excised, cleaned of any remaining tendons using dissecting instruments, and stored frozen in individually labelled sample bags.

2.3.3 Bone Mineral Density

Bone mineral density (BMD) was measured using the PIXImus 2 Bone Densitometer (General Electric Lunar, Madison, WI) and analyzed with PIXImus software version 2.1 at the Faculty of Medicine-Endocrinology at Memorial University of Newfoundland and Labrador. The PIXImus II utilizes dual-energy X-ray absorptiometry (DXA) technology. A small x-ray source exposes the entire animal to a cone shaped beam of both high and low energy x-rays. A high-resolution digital picture (0.18 x 0.18 mm) is taken of an image of the x-rays hitting a luminescent panel. The ratio of attenuation of the high and low energies allows the PIXImus to separate bone from tissue and, from within the tissue samples, the lean and fat. The PIXImus measures bone mineral content (BMC) for each pixel of the scanned image and then calculates BMD by dividing the average BMC by the bone area represented by the region of interest (ROI) which is specified by the user. A standard phantom (fat 11.9% and BMC 0.063 g) was used to calibrate the PIXImus on a daily basis prior to measurements.

Bone mineral density was measured in the femur of the deer mice. The femur was selected as the ROI and an average BMD for the entire bone was obtained (*i.e.*, total femur BMD). Each bone was placed in the same position and same area of the positioning pad for each scan. Repeatability of bone mineral density measurements were

verified by blindly re-measuring a subset of bones and conducting a two way ANOVA considering sample and trial, which indicated no statistically significant difference between the datasets ($F_{1,9} = 1.91$, $p=0.20$). All bone mineral density measurements were conducted blindly without prior knowledge of the levels of PCB concentrations and all samples bore sample numbers that contained no information about their collection site or PCB exposure level.

2.3.4 PCB Analysis

The whole bodies of the snap trapped deer mice (n=20) from the Beach and the live trapped mice (n=7) from the reference site were used for PCB analysis. PCB analyses were conducted by AXYS Analytical Services in Sydney, BC using AXYS method MLA-007, "Analytical Method for the Determination of Aroclors, Total PCBs, Chlorinated Pesticides, PCB Congeners, Coplanar PCBs, Toxaphene, and Chlorobenzenes".

Briefly, concentrations of 91 PCB congeners and Aroclors 1242, 1254 and 1260 were determined using high resolution gas chromatography with detection by low-resolution mass spectrometry (HRGC/LRMS). Homogenized samples were spiked with a suite of isotopically labeled surrogate standards prior to analysis, solvent extracted, and cleaned and separated into two fractions using Florisil®. Fraction one was analyzed for PCBs and PCB Aroclors using high-resolution gas chromatography with detection by low-resolution mass spectrometry (HRGC/LRMS). Total concentrations of PCBs reported for deer mice represent the sum of the congeners. Congener concentrations less than the detection limit were replaced with one half of the detection limit. Congeners analyzed include: PCB5/8, PCB15, PCB16/32, PCB17, PCB18, PCB19, PCB20/21/33,

PCB22, PCB24/27, PCB25, PCB26, PCB28, PCB31, PCB40, PCB41/64/68/71, PCB42/59, PCB43/49, PCB44, PCB45, PCB46, PCB47/48/75, PCB51, PCB52/73, PCB56/60, PCB61/74, PCB62/65, PCB66/80, PCB70/76, PCB83/108, PCB84, PCB85/120, PCB86/97, PCB87/115/116, PCB89/90/101, PCB91, PCB92, PCB93/95, PCB99, PCB105/127, PCB106/118, PCB107/109, PCB110, PCB114, PCB123, PCB128, PCB129, PCB130, PCB131/142, PCB132/168, PCB134/143, PCB135/144, PCB136, PCB137, PCB138/163/164, PCB139/149, PCB141, PCB146, PCB151, PCB153, PCB156, PCB157, PCB158/160, PCB159, PCB170/190, PCB171, PCB172/192, PCB174/181, PCB175, PCB176, PCB177, PCB178, PCB179, PCB180, PCB182/187, PCB183, PCB185, PCB189, PCB191, PCB193, PCB194, PCB195, PCB196/203, PCB197, PCB198, PCB199, PCB201, PCB205, PCB206, PCB207, PCB208, PCB209.

AXYS uses the batch method whereby samples are worked up in batches composed of nine or fewer samples along with one QA/QC sample (certified reference material or internal spiked matrix), one analytical blank, and one analytical duplicate. Spiked material was used in cases where analytical results were expected to be outside the concentration ranges of the available certified reference materials. Recoveries of 56 congeners in four spiked samples ranged from 78% to 112%, average = 96±3.1%. Five blank samples were analyzed and results were below the detection limits for PCB Aroclors and for most congeners. Results for some congeners in the blanks (one to two from each) were reported as NDR, indicating low level peaks were detected but did not meet the quantification criteria to be identified as the congeners. One blank showed detectable concentrations of two congeners but the blank results were within the AXYS method control limit of 1 ng absolute per congener. Five deer mice were analyzed in

duplicate and average relative standard deviations ranged from 2.8% to 35% for PCB congeners with an average relative standard deviation of 12%. AXYS considers acceptable limits to be less than 30% relative standard deviation with 20% or less considered good agreement.

2.3.5 Statistical Analysis

Relationships between variables were determined using the general linear model (normal errors) which includes analysis of variance, analysis of covariance, and regression. Errors were checked to confirm normality, homogeneity, and independence by observing histograms and normal probability plots of the residuals and plotting residuals against predicted values. The significance level was set to $p \leq 5\%$.

2.3.6 T-scores

The World Health Organization operationally defines osteoporosis in humans based on bone mineral density assessment using T-scores. T-scores are determined for humans using the following formula:

$$T\ Score = (BMD - YN) / SD,$$

where *BMD* is the BMD of the patient, *YN* is the reference range of BMD using normative data from the National Health and Nutrition Examination Survey (NHANES) reference database, and *SD* is the standard deviation. Studies indicate that the risk of fragility fractures increase progressively and continuously as bone mineral density declines (Hui *et al.*, 1988; Alveblom, 2003). The WHO criteria for BMD are shown in Table 2-1 and are based on the assumption for females that fracture risk doubles for every

standard deviation below the normal reference range. The DXA method used for the deer mice in this study is widely used for determining bone mineral density in humans (WHO, 2007).

We applied this method to calculate T-scores for each mouse at the Beach area by assuming the mean of the reference population represents normative data and assuming that the DXA measurements will predict doubling of the fracture risk for every standard deviation decrease in bone mineral density. While the sample size from the reference area was small (n=7), the standard deviation was similar to that of the Beach area (n=19) and is therefore considered representative. A reference value for bone mineral density of wild populations of deer mice was not found in the literature. A study on the red backed vole (*Myodes rutilus*) in Alaska indicated that the mean summer BMD of the femur determined using DXA was 52 mg/cm^2 for males and 49 mg/cm^2 for females (Stevenson *et al.*, 2009).

2.4 Results

The body mass of the mice used for bone mineral density measurements ranged from 10.7 g to 27.9 g and the collection included twenty-one male mice and five female mice (*i.e.*, three female mice from the Beach and two female mice from the reference site). The general linear model was used to determine if body mass differed between sites. Body mass did not differ between sites ($F_{1,25}$= 0.36, p=0.55). Because of the small number of females trapped at each site, sex was not considered a factor in the statistical model.

2.4.1 PCB Concentrations

Total PCB concentrations (∑PCB; total 91 congeners measured) in the mouse whole bodies from the Beach ranged from 382 ng/g to 22,700 ng/g (wet weight). Concentrations of PCBs at the reference site ranged from 15.6 ng/g to 187 ng/g (Table 2-2). The general linear model was used to determine if PCB concentrations were related to body mass or if PCB concentrations differed between sites. The interaction term between mass and site was also considered. Total PCB concentrations were not related to body mass ($F_{1,23}$=0.10, p=0.75) and did not differ between sites ($F_{1,23}$=0.61, p=0.44). The interaction term between body mass and site was not significant ($F_{1,23}$=0.092, p=0.76).

2.4.2 Bone Mineral Density

Bone mineral density ranged from 35.4 mg/cm^2 to 64.1 mg/cm^2 at the reference site and from 23.7 mg/cm^2 to 62.3 mg/cm^2 at the Beach site. The general linear model was used to determine if BMD was related to body mass or if BMD differed between sites. Bone mineral density was related to body mass ($F_{1,22}$=15.7, p=0.001) with larger mice having a higher bone mineral density. The bone mineral density differed between sites ($F_{1,22}$=4.50, p=0.04) with mice from the reference site (56.1±9.8 mg/cm^2) having a higher mean BMD than mice from the Beach site (41.9±11.2 mg/cm^2). The interaction term between body mass and site was not significant ($F_{1,22}$=2.59, p=0.12).

2.4.3 T-scores

The average T-score calculated for mice at the Beach was -1.45 and ranged from -3.3 to 0.64. Based on the WHO classification scheme for humans, the average

classification for these mice would be osteopenia (*i.e.*, T-score ranging from -1.0 to -2.5) while the most extreme cases would be osteoporosis (T-score less than -2.5). Table 2-2 summarises the ∑PCB, bone mineral density measurements, and the T-score (Beach only) with associated classification.

2.5 Discussion

Concentrations of PCBs in deer mice whole bodies from the Beach were elevated compared to the deer mice whole bodies from the reference site. Within site variability, however, was high at both the Beach (ranged from 382 ng/g to 22700 ng/g) and the reference site (ranged from 15.6 ng/g to 187 ng/g). This may be a result of differences in home ranges of individual deer mice. Low concentrations of PCBs detected in mice from the reference site were not unexpected because the reference site falls within the halo of contamination (27 km) previously determined by Pier *et al.*, (2003).

Concentrations of PCBs in deer mice at Saglek Beach are higher than concentrations of PCBs observed in other wild populations of mice. Batty *et al.*, (1990) observed whole body concentrations of PCBs in white-footed mice (*Peromyscus leucopus*) inhabiting an area surrounding a PCB and cadmium contaminated pond ranging from 0.42 mg/kg to 4.17 mg/kg (average = 2.3 mg/kg). Effects such as decreased summer body weight, increased relative organ (*e.g.*, liver) weights and decreased testis weights were observed in the mice from the contaminated site. Fewer juvenile animals were also observed which could indicate that the population was not reproducing successfully. It was uncertain if PCBs, cadmium, or the combined effects of the two were responsible for the observed effects. No discernible effects on population density,

survival, sex ratio, reproduction or growth were observed in short-tailed shrew (*Blarina brevicauda*) inhabiting a site with soil concentrations (ranging from 1.5 mg/kg to 38.3 mg/kg) that were higher than those currently observed at Saglek (Boonstra and Bowman, 2003).

Bone mineral density was significantly lower in deer mice from the highly exposed Beach group than in mice from the reference area. No studies were found in the literature that investigated the effects of PCBs on bone mineral density in deer mice. Changes in bone composition and impaired bone strength have been found in rats (Andrews, 1989; Lind *et al.*, 2000a,b) and goats (Lundberg *et al.*, 2006) experimentally exposed to PCBs. Decreased bone mineral density in wildlife has also been shown in polar bears (Sonne *et al.*, 2004) and grey seals (Lind *et al.*, 2003) over time periods associated with increased use of endocrine disrupting chemicals such as PCBs and DDT. A subset of the polar bears collected during the supposed pollution period (1966-2002) showed that BMD was negatively correlated with levels of PCBs and chlordanes in subadult polar bears and with DDT and dieldrin in adult male polar bears. It was not possible in that study to determine which chemicals were associated with the decreased BMD or if it was a result of the interaction of the chemicals (Sonne *et al.*, 2004). The concentrations of other organic and inorganic contaminants at Saglek are low (ESG, 1997; ESG, 1999). In other studies on wildlife, differences in diet, habitat and the presence of other contaminants can confound the results. Saglek is unique as it represents an ecosystem with a single contaminant thus minimising confounding effects.

Increased circulating retinoids (vitamin A), increased cortisol, and decreased vitamin C have been suggested as mechanisms linking PCBs to decreased bone density

(Sonne *et al.*, 2004; Lind *et al.*, 2000a,b). It is considered more likely, however, that the effects of PCBs on bone are mediated through altered levels of estrogen (Bonefeld-Jorgenson, 2001; Lind *et al.*, 2004). PCBs exhibit estrogenic or anti-estrogenic properties (Lind *et al.*, 1999; Navas and Segner, 1998). The most abundant congeners at Saglek were di-ortho PCBs such as PCB 153, PCB180 and PCB138/163/164. These congeners are typical of most biotic and abiotic environments (Safe, 1994) and are antiestrogenic compounds (Pliskova *et al.*, 2005). The antiestrogenic activity of PCBs is mediated by interacting directly with the estrogen receptor, inhibiting the binding of estrogen to the estrogen receptor and subsequently inhibiting estrogen induced responses (Bonefeld-Jorgensen *et al.*, 2001; Oh *et al.*, 2007). One such process which is regulated by estrogens is bone remodeling (Price and Russell, 1992). Estrogen regulates bone mineral density by controlling bone resorption carried out by osteoclasts and formation of new bone carried out by osteoblasts (Manolagas and Jilka, 1995). The antiestrogenic activity of PCBs is believed to be associated with an upregulation of osteoclasts and thus, large increases in bone resorption leading to decreased bone mineral density (Manolagas, 2000). This upregulation of osteoclast formation is recognized as the main mechanism by which estrogen deficiency induces bone loss (Cenci, 2000). It has also been suggested that exposure to a mixture of di-ortho PCB congeners such as is the case at Saglek, may also result in a synergistic effect on antiestrogenicity through an estrogen receptor-independent pathway (Oh *et al.*, 2007). In addition to regulating bone turnover, estrogen is also essential in the final phases of skeletal maturation and bone mineralization (Bouillon *et al.*, 2004).

This study shows that terrestrial small mammals at Saglek, even at low trophic

levels in the food chain, have lower bone mineral density associated with increased exposure to PCBs. Therefore, local contamination at Saglek appears to have an impact on the physiology of wildlife. It is difficult, however, to determine if the lower bone mineral density affects survival and/or reproduction of mice at the Beach area. Studies on humans (Alveblom, 2003) and laying hens (Fleming *et al*, 1998) have associated decreased bone composition with an increased incidence of fractures. A relationship between bone mineral density and bone breaking strength has also been found in raptors (Knopper *et al.*, 2007). Osteoporosis due to lower bone mineral density has been recognized as a major public health problem for humans (Riggs and Melton, 1995). It is possible that certain wildlife exposed to elevated levels of PCBs and other endocrine disrupting chemicals may be prone to increased incidences of fractures thus affecting their ability to carry out normal functions and hence affecting their reproduction and survival. Given that PCB exposure has been shown to alter bone turnover mechanisms in rats (Yilmaz *et al.*, 2006) as well as bone morphometry and/or strength in rats (Andrews, 1989; Lind *et al.*, 2000a,b) exposed to PCBs in lab studies, it is reasonable to expect that small mammals at other sites with PCB concentrations comparable to Saglek would show similar effects as was seen in the Saglek deer mice.

In humans, the World Health Organization operationally defines osteoporosis based on bone mineral density assessment using T-scores. Based on the calculated t-scores ranging from -3.3 to 0.64, some mice from the Beach area would be classified as having osteopenia or osteoporosis. Fracture risk increases 1.5 to 3 fold for every standard deviation decrease in bone mineral density (Kanis *et al.*, 1994). For the prediction of any fracture, DXA at sites of biological relevance gives measurements of bone mineral

density (BMD) that predict fracture with an increase in fracture risk of approximately 1.5 times per standard deviation decrease in bone mineral density (termed the gradient of risk) (WHO, 2007). Assuming the same biomechanical forces apply and using a conservative factor of 1.5, mice at Saglek are up to five folds more susceptible to fracture risk than mice at the reference area. Because the t-scores for humans are based on a comparison to a reference population, they are considered applicable in this study because information of BMD at a reference site exists. It is the degree of difference between the subject organism and the normative (*i.e.*, reference) BMD that determines the t-score.

Bone mineral density in humans is dependent on age, sex, the degree of bone turnover, prior fractures, genetics, and lifestyle risk factors (WHO, 2007). The bone mineral density would also be expected to vary according to age and sexual maturity. We did not age the mice but all mice appeared to be mature based on gonad examination. The life span of a deer mouse is expected to be approximately one year (Millar and Innes, 1983). They would be expected to reach maturity at around 35 days (male) and 60 days (female) (Millar, 1994). Since deer mice generally breed around the same time of year (*i.e.*, August) in northern climates, it can be expected that the age of the mice would not vary greatly. Environmental factors such as starvation, temperature extremes, and noise disrupt bone density in laboratory studies. We have no information on environmental and nutritional factors that may affect bone mineral density but the mice appeared to be healthy and showed no visible signs of stress. Habitats at the reference site and the Beach were similar with respect to vegetation type (willow and birch; the reference area also contained some areas of moss), ground cover (some disturbed areas consisting of silty

sand with some gravel), and disturbance (the reference area is near the end of an airstrip while the Beach is near a gravel road).

The DXA method provided bone mineral density measurements (*i.e.*, mg/cm^2) which allowed for the calculation of T-scores. The DXA method is widely used for determining bone mineral density in humans (WHO, 2007). The method is considered accurate and precise (Sievanen *et al.*, 1992; Stevenson and van Tets, 2008) and has recently been validated for use in determining body composition of free living rodents (Stevenson and van Tets, 2008; Stevenson *et al.*, 2009). Other methods used in the literature to determine differences in bone densities include bone radiographs (Fleming *et al.*, 1998; Knopper and Mineau, 2004; Knopper *et al.*, 2007), bone breaking strength (Lind *et al.*, 2000a,b; Lundberg *et al.*, 2006; Knopper *et al.*, 2007), ash weight measurements (Lind *et al.*, 1999), peripheral quantitative computer tomography (pQCT) (Lind *et al.*, 2000a; Lind *et al.*, 2003; Lundberg *et al.*, 2006), and dual-energy X-ray absorptiometry (DXA) (Sonne *et al.*, 2004). Methods that use radiographs, bone breaking strength, and ash weight provide measures for comparison between individuals and/or sites but the methods do not provide a researcher with bone mineral density measurements (*i.e.*, mg/cm^2) which can be used for T-score calculations.

There are potential problems with using different individual mice from the Beach for PCBs and BMD when the measurements came from the same individual at the reference site. The approach does not allow for regression analysis between the PCB concentrations and the BMD of mice. Based on previous sampling from the long term monitoring (LTM), the average concentration of PCBs has been elevated consistently. In addition, the deer mice from the snap traps collected as part of the LTM were placed

adjacent to the live traps during the same sampling period. It is reasonable to expect that the concentrations of PCBs in deer mice from the snap traps reflect what is at the Beach site. Therefore, this approach is considered acceptable to determine if bone mineral density differs between sites.

In conclusion, deer mice exposed to PCBs at the Beach area of Saglek show a decreased bone mineral density. These results concur with laboratory studies on the effects of PCBs on bone properties in small mammals. The resulting T-scores indicate that they may be up to five folds more susceptible to fracture risk than mice from the reference area. Therefore, while the sphere of influence at PCB contaminated military sites such as Saglek is small in the context of regionally distributed contamination from long-range transport, the PCB concentrations found locally are high enough to affect local wildlife.

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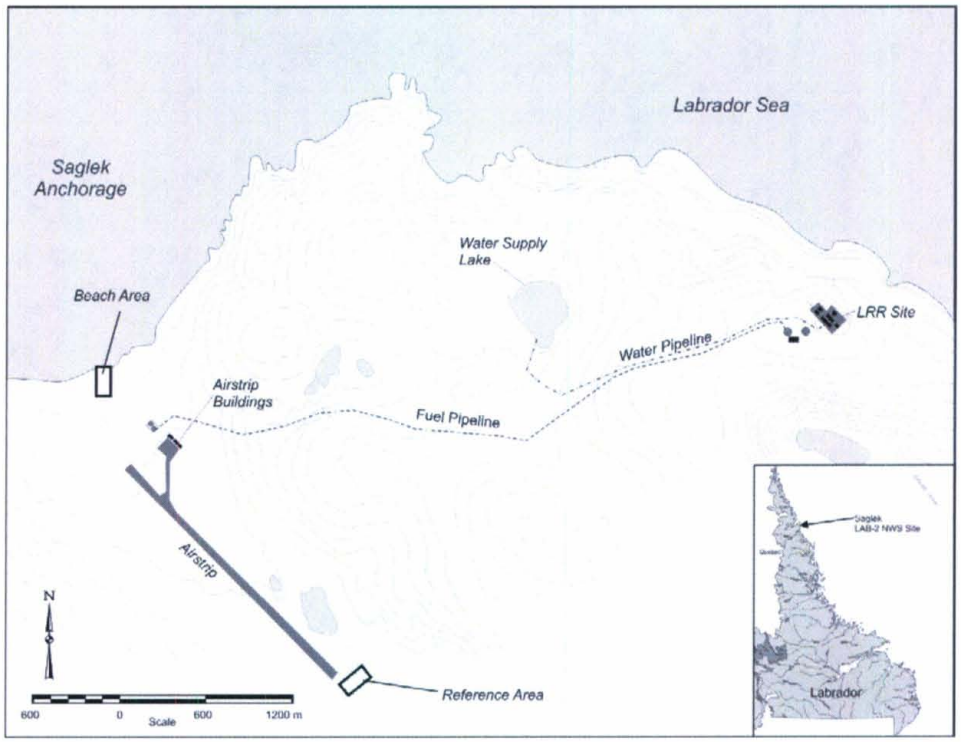

Figure 2-1. Sampling sites for deer mice (Beach and Reference) at Saglek, Labrador.

Table 2-1. World Health Organization criteria for osteoporosis based on T-scores.

T-score	Classification
>-1.0	Normal
-1.0 to -2.5	Osteopenia
<-2.5	Osteoporosis
<-2.5 and one or more fractures	Severe Osteoporosis

Table 2-2 Total PCB concentrations [average (ng/g) wet weight±sd] and bone mineral density measurements [average (mg/cm^2)±sd] in deer mice at Saglek, Labrador in 2007.

		Beach	Reference
Total PCBs (ng/g)			
Range		382-22700	15.6-187
Total PCBs	All	5770±6770	79.8±58.9
	Male	6720±7550	103±54.0
	Female	2910±2190	27.0±9.90
n	All	20	7
	Male	15	5
	Female	5	2
Most abundant PCB congeners (ng/g)			
PCB153	All	1630±2050	12.4±9.93
	Male	1920±2280	16.0±9.4
	Female	752±664	3.2±2.0
PCB138/ 163/164	All	553±637	5.76±4.74
	Male	631±714	7.3±4.8
	Female	318±225	1.9±1.7
PCB170/ 190	All	626±611	8.18±6.62
	Male	735±687	11±6.1
	Female	300±232	1.9±1.2

Table 2-2 Total PCB concentrations [average (ng/g) wet weight±sd] and bone mineral density measurements [average (mg/cm^2)±sd] in deer mice at Saglek, Labrador in 2007 (continued).

		Beach	Reference
PCB180	All	1460±1830	21.8±17.2
	Male	1730±2040	29±15
	Female	675±517	5.1±1.9
n	All	20	7
	Male	15	5
	Female	5	2
Bone Mineral Density (mg/cm^2)			
Range		23.7 – 62.3	35.4 – 64.1
BMD	All	41.9 ± 11.2	56.1 ± 9.8
	Male	40	56.1
	Female	52	56.1
t-score (average)		-1.45	-
Classification		Osteopenia	-
n	All	19	7
	Male	16	5
	Female	3	2

3.0 Chapter 3: Gastrointestinal Macroparasites as Bioindicators of Polychlorinated Biphenyls (PCBs) in Northern Labrador: Generalized Linear Model Based Analysis

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3.1 Abstract

The effects of polychlorinated biphenyls (PCBs) on the abundance and prevalence of gastrointestinal macroparasites as well as two condition indices (hepatosomatic index and body condition) of shorthorn sculpin (*Myoxocephalus scorpius*) was investigated in three marine fiords located in Northern Labrador. Saglek fiord is near the site of a former US military facility and PCBs have impacted marine sediment and the marine food chain in the area. Average PCB concentrations in shorthorn sculpin liver from Saglek fiord in 2006 were 9560±11000 ng/g wet weight. Nachvak fiord/Anaktalak fiord (average PCBs in sculpin liver (2006) = 22±11 ng/g wet weight) were used as reference fiords. This study was followed up in 2007 with a similar investigation focusing on two separate areas of Saglek fiord: Saglek Beach (average PCBs in sculpin liver = 4460±6020 ng/g wet weight) and Rose Island (average PCBs in sculpin liver = 17±7.6 ng/g wet weight). Statistical analysis was conducted using generalized linear models, a class of regression models that allows application of regression with error distributions appropriate to a wide range of dependent variables (McCullagh and Nelder, 1989). In this study, the error distributions included the negative binomial for overdispersed count data and the binomial for presence/absence data. In the 2006 study, the prevalence and abundance of the acanthocephalan *Corynosoma magdaleni* in sculpin was higher at Anaktalak/Nachvak where relatively low concentrations of PCBs were observed, compared to Saglek, where high concentrations of PCBs were observed. The relationship for abundance was observed in male sculpin but not female. In the follow up study within Saglek fiord (2007), the abundance of *C. magdaleni* in both male and female sculpin showed the same trend with lower numbers in fish collected at the PCB contaminated beach compared to the reference site, Rose Island. These results support other studies which indicate that endoparasites decrease with increasing pollution. The utility of parasites as bioindicators requires knowledge of the local area ecology as well as

the biology of the parasites being studied.

Keywords: PCBs; pollution effects; parasite; fish; shorthorn sculpin; Labrador; bioindicator; generalized linear model

3.2 Introduction

Parasite load in freshwater and marine fish has been found to be related to environmental contaminants such as PCBs (Khan, 1999; Koponen *et al.*, 2001), pulp and paper effluent (Khan, 2003; Khan, 2006), crude oil (Khan and Kiceniuk, 1983; Khan and Payne, 2004), and oil refinery/domestic waste (Kussat, 1969). In these studies, a particular species of parasite is proposed as a "biomarker" or a "bioindicator" if an association between the abundance and/or prevalence of that parasite and the presence and/or level of a particular contaminant is found. Endoparasites with complex life cycles tend to decrease with increasing levels of pollution as they may be affected directly in the free living form (Mackenzie, 1999) or in the alimentary canal within the host fish (Khan and Kiceniuk, 1983), or indirectly through adverse effects of the pollution on the intermediate hosts in the life cycle (Mackenzie, 1999). Ectoparasites on the other hand, tend to be more tolerant of environmental change and generally increase with increasing levels of pollution (Mackenzie, 1999).

In this study, the effect of polychlorinated biphenyls (PCBs) on the abundance and prevalence of gastrointestinal macroparasites as well as two condition indices (hepatosomatic index and body condition) of shorthorn sculpin (*Myoxocephalus scorpius*) was investigated in three marine fiords located in Northern Labrador. Nachvak, Saglek, and Anaktalak fiords (Figure 3-1) are currently being studied as part of a larger project addressing Inuit concerns about the impacts of climate change, modernization, and contaminants on the health of marine ecosystems and communities of Northern Labrador. Nachvak fiord is located adjacent to the Torngat Mountains National Park where human disturbance is minimal. Saglek fiord is located on the northeast coast of Labrador at the southern extent of the Park. Extensive PCB contamination associated with historical erosion from an adjacent military site was previously identified at Saglek (ESG, 1997) and there is evidence that the concentrations of PCBs the marine

environment are decreasing (Brown *et al.*, 2009). Anaktalak fiord is located near the northern community of Nain and has been traditionally used by Inuit for travel as well as subsistence and commercial harvesting. The assessment of these three marine fiord systems in Northern Labrador provides a unique opportunity to study the effects of PCBs on the gastrointestinal macroparasite fauna and condition indices of shorthorn sculpin (*Myoxocephalus scorpius*). This fish is a bottom feeding, sedentary species and has been used for monitoring of pollution exposure and effects in northern coastal areas (Kuzyk *et al.*, 2005b; Stephenson *et al.*, 2000). The initial study, carried out in 2006, examined parasite fauna of shorthorn sculpin from Nachvak fiord and Anaktalak fiord (both reference fiords) and Saglek fiord (known to be contaminated with PCBs from a former military site (Kuzyk *et al.*, 2005a)). Because these fiords are widely separated, the study was followed up in 2007 with a similar investigation focusing on two separate areas of Saglek fiord, these being Rose Island and Saglek Beach with lower and higher concentrations of PCBs, respectively. If endoparasites decrease as a result of increasing pollution as has been shown in other studies (*e.g.*, Khan, 1999; Kussat, 1969; Marcogliese and Cone, 1997), it would be expected that sculpin from Saglek Beach will have a lower abundance and prevalence of endoparasites than Nachvak and Anaktalak fiords (2006) and Rose Island (2007).

3.3 Methods

3.3.1 Sample Collection

Field work for this study was carried out in two phases. Phase one was conducted in July and August 2006 and involved the collection of fifty-nine shorthorn sculpin from various areas of Saglek fiord (n=19), Nachvak fiord (n=20), and Anaktalak fiord (n=20). All sculpin collected from Saglek in 2006 were from the Saglek Beach area. Saglek Beach is within the vicinity of the

former PCB source. Phase two was carried out in August 2007 and involved the collection of fifty sculpin from two sites within Saglek fiord, these being Rose Island (n=29) and Saglek Beach (n=21) (Figure 3-2). Rose Island is located approximately 18 km from the former PCB source but is still within Saglek fiord. Rose Island is considered a reference site based on measured concentrations of PCBs in sediment at this distance from Saglek Beach in 2006 (1.0 to 9.0 ng/g dry weight) (Brown *et al.*, 2009). Uneven sample numbers were collected from Saglek Beach (n=21) and Rose Island (n=29) in 2007 because weather conditions and delays prevented the completion of sampling at both sites.

Shorthorn sculpin were collected with hook and line over the side of a boat. Immediately after capture, the sculpin were euthanized by cutting the spinal cord at the base of the skull. Each carcass was weighed on an electronic top loading balance to the nearest 0.1 g. Body length was measured to the nearest millimetre and the fish was then dissected. A record was made of total body length, body mass, and mass of the liver, stomach and gonad. Sex was determined by gonad examination and portions of the livers were removed and stored at -20˚C for PCB analysis. The entire digestive tract was removed and stored frozen at -20˚C for stomach content analysis and parasite analysis.

3.3.2 PCB Analysis

PCB analyses were conducted on shorthorn sculpin livers by AXYS Analytical Services in Sidney, British Columbia. Due to the cost of the analysis, PCB analysis could not be performed on all individuals. Analyses were carried out on twenty-nine randomly selected sculpin from 2006: three from Nachvak, nineteen from Saglek and seven from Anaktalak. Twenty-one sculpin from 2007 were analysed for PCBs: nine from Rose Island and twelve from Saglek Beach. Briefly, concentrations of 91 PCB congeners and Aroclors 1242, 1254 and 1260 were determined

using high resolution gas chromatography with detection by low-resolution mass spectrometry (HRGC/LRMS). Samples were spiked with isotopically labelled surrogate standards, extracted, and the extract separated into fractions using Florisil® (a highly selective adsorbent also referred to as magnesium silicate). Fraction one was analyzed for PCBs and PCB Aroclors using high-resolution gas chromatography with detection by low-resolution mass spectrometry (HRGC/LRMS). Total concentrations of PCBs reported for sculpin represent the sum of the congeners. Congener concentrations less than the detection limit were replaced with one half of the detection limit. Results are reported on a wet weight basis.

AXYS uses the batch method whereby samples are worked up in batches composed of nine or fewer samples along with one QA/QC sample (certified reference material or internal spiked matrix), one analytical blank, and one analytical duplicate. Spiked material was used in cases where analytical results were expected to be outside the concentration ranges of the available certified reference materials.

For the 2006 sculpin, recoveries of 52 congeners (two samples) and 26 congeners (five samples) ranged from 71% to 116%, average = 101±6.9%. Seven blank samples were analyzed and results were below the detection limits for PCB Aroclors and for most congeners. Results for some congeners in the blanks (two to four from each) were reported as NDR, indicating low level peaks were detected but did not meet the quantification criteria to be identified as the congeners. Two blanks showed detectable concentrations of four congeners but the blank results were within the AXYS method control limit of 1 ng absolute per congener. Five sculpin were analyzed in duplicate and average relative standard deviation was 5.5 %. AXYS considers acceptable limits to be less than 30% relative standard deviation with 20% or less considered good agreement.

For the 2007 sculpin, recoveries of 53 congeners (one sample) and 38 congeners (two samples) in the spiked samples ranged from 70% to 119%, average = 94±7.6%. Three blank

samples were analyzed and results were below the detection limits for all PCB congeners. The result for one congener in one blank was reported as NDR, indicating low level peaks were detected but did not meet the quantification criteria to be identified as the congeners. Two sculpin were analyzed in duplicate and the average relative standard deviation was 6.8 % and were thus within 30%.

3.3.4 Parasite Analysis

Thirty-seven sculpin from phase one (2006) were examined for parasites and included eighteen sculpin from Saglek, nine sculpin from Nachvak and ten sculpin from Anaktalak. All sculpin analysed for PCBs in 2006 were also observed for parasites. All fifty sculpin from phase two (2007) were examined for parasites and included twenty-nine sculpin from Rose Island and twenty-one sculpin from Saglek Beach.

Gastrointestinal tracts of sculpin were examined for all visible gastrointestinal parasites using conventional parasitological techniques. The entire digestive tract of the sculpin was cut open longitudinally, folded open and contents scraped away using a scalpel. The gut and gut contents were added to water in a Petri dish and examined under a dissecting microscope. Gastrointestinal macroparasites were removed from the fish stomach, counted, and placed in a labeled vial of 70% alcohol for further processing and identification. Food items present in the gastrointestinal tract were also recorded.

Acanthocephalans, cestodes and trematodes were removed from the 70% alcohol preservative and stained in Semichon's Acetic-carmine stain for approximately ten minutes followed by de-staining in 70% acid alcohol for ten minutes. Specimens were further dehydrated in a series of 85% alcohol, 95% alcohol and 100% alcohol in Petri dishes for approximately ten minutes each and then cleared and mounted in clove oil on a microscope slide. Nematodes were removed from the 70%

alcohol preservative and were dehydrated by soaking in a series of 85% alcohol, 95% alcohol and 100% alcohol in Petri dishes for approximately ten minutes each. The nematodes were then cleared and mounted in glycerol on a microscope slide. Identifications were made using available keys.

Prevalence, abundance, mean intensity, and mean abundance were calculated according to the definitions by Bush *et al.*, (1997) as follows. Prevalence (%) is calculated as the number of hosts infected with one or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species. Abundance is the number of individuals of a particular parasite in/on a single host regardless of whether or not the host is infected. Mean intensity is the average intensity of a particular species of parasite among the infected members of a particular host species (*i.e.,* total number of a particular species of parasite found in a sample divided by the number of hosts infected with that parasite). Mean abundance is the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined (including both infected and uninfected hosts).

3.3.5 Computational Methods and Statistical Analysis

In order to increase the statistical power of the analysis, the parasite data for fish from Nachvak (n=9) and Anaktalak (n=10) were pooled. Fulton's condition factor (K) was calculated as the ratio of whole body mass (g) to length (cm) to the power of three and multiplied by 100 (Ricker, 1975). Hepatosomatic index was calculated as the ratio of liver mass (g) to body mass (g), multiplied by 100 (Heidinger and Crawford, 1977). The hepatosomatic index and condition factor were determined for all fish collected.

Relationships between variables were initially determined using the general linear model (normal errors) which includes analysis of variance (ANOVA), analysis of covariance (ANCOVA) and linear regression. In this case, errors were checked to confirm normality,

homogeneity and independence. Where these assumptions were satisfied, the F-ratio and p-value are reported. Where these assumptions were not valid, the model was revised and the generalized linear model for non-normal errors was used to estimate parameters and calculate p-values using analysis of deviance (McCullagh and Nelder, 1989) and the assumptions were verified again. For the generalized linear model, deviances (G^2) with p-values were reported. The significance level was set to 5%. The type I error was determined using the chi square distribution.

The prevalence of parasites is measured as a dichotomous variable in its raw form (*i.e.*, presence/absence of a parasite in any individual fish) and is thus a binomial variable (*i.e.*, unit scored on a nominal scale). The generalized linear model with binomial distribution was used to estimate the odds of a sculpin being infected by a parasite. The response variable was odds of infection given by the formula:

$$Odds = p\text{-}(1\text{-}p),$$

where p is the proportion of sculpin infected by a particular species of parasite. The odds ratio is a comparison of two odds to determine whether one group has higher or lower odds of some binary outcome (Hoffman, 2004).

The generalized linear model using negative binomial regression (log link) was used to determine the relationship between abundance of parasites and fiord. The negative binomial allows for overdispersion (*i.e.*, variance is greater than the mean) by fitting the model with a fixed overdispersion parameter (κ). The categorical explanatory variables included sex and fiord (Anaktalak/Nachvak or Saglek for 2006) or site (Rose Island or Saglek Beach for 2007). Body mass was also included as a continuous explanatory variable.

In some cases where prevalence was low at the fiords/sites, the binomial and negative binomial models could not be applied because the model would not converge or was expected to provide inefficient estimates (Agresti, 2007). In these cases, statistical significance could not be

calculated and therefore, was not reported. SPlus was used with the MASS library package (Venables and Ripley, 2002) for the generalized linear model computations.

The equations for the models tested as well as information on the response variables, explanatory variables, error distribution and link functions are summarized in Table 3-1.

Table 3-1. Information on the models tested for each response variable. μ is the expected value or response variable.

Response Variable (Y)	Explanatory Variables	Error Distribution	Link Function	Equation
Presence of parasite as a proportion (P)	Sex (X) Mass (M) Site (S)	Binomial	Logit $\eta = \log_e(\mu/(1-\mu))$	$P/(1-P)) = \exp(\eta)$ + binomial error $\eta = \beta_o + \beta_X*X + \beta_M*M + \beta_S*S + \beta_{XM}*XM + \beta_{XS}*XS + \beta_{MS}*MS$
Parasite counts (A)	Sex (X) Mass (M) Site (S)	Negative binomial	Log $\eta = \log_e\mu$	$A = \mu$ + negative binomial error $\eta = \beta_o + \beta_X*X + \beta_M*M + \beta_S*S + \beta_{XM}*XM + \beta_{XS}*XS + \beta_{MS}*MS$
Length (L)	Sex (X) Site (S)	Normal	Identity $\eta = \mu$	$L = \mu$ + normal error $\eta = \beta_o + \beta_X*X + \beta_S*S + \beta_{XS}*XS$
Body Mass (M)	Sex (X) Site (S)	Normal	Identity $\eta = \mu$	$M = \mu$ + normal error $\eta = \beta_o + \beta_X*X + \beta_S*S + \beta_{XS}*XS$
Hepatosomatic index (H)	Sex (X) Site (S)	Normal	Identity $\eta = \mu$	$H = \mu$ + normal error $\eta = \beta_o + \beta_X*X + \beta_S*S + \beta_{XS}*XS$
Condition Factor (K)	Sex (X) Site (S)	Normal	Identity $\eta = \mu$	$K = \mu$ + normal error $\eta = \beta_o + \beta_X*X + \beta_S*S + \beta_{XS}*XS$

3.4 Results

3.4.1 Morphometric Information

Analysis of physical information (Table 3-2) indicated that in 2006 female sculpin were significantly longer than male sculpin ($F_{1,56}$=58.3, $p<0.001$) but no significant difference was observed in length of sculpin between Saglek and Anaktalak/Nachvak ($F_{1,56}$=1.98, $p=0.165$). The interaction term between sex and site was significant ($F_{1,56}$=14.8, $p<0.001$). Analysis within sites indicated that females were significantly longer than males at Anaktalak/Nachvak ($F_{1,38}$=85.6,

$p<0.001$) as well as at Saglek ($F_{1,18} = 6.9$, $p=0.017$). Analysis within sex indicated that Anaktalak/Nachvak females were longer than Saglek females ($F_{1,33}=15.0$, $p<0.001$) but no significant difference was observed in male sculpin length between sites ($F_{1,22}=2.37$, $p=0.138$).

In 2006, female sculpin were significantly heavier than male sculpin ($F_{1,56}=35.9$, $p<0.001$) and sculpin from Anaktalak/Nachvak were significantly heavier than Saglek ($F_{1,56}=7.75$, $p=0.007$). The interaction term between sex and site was also significant ($F_{1,56}=14.3$, $p<0.001$). Analysis within sites indicated that females were significantly heavier than males at Anaktalak/Nachvak ($F_{1,38=}50.6$, $p<0.001$) and at Saglek ($F_{1,18}=6.55$, $p=0.02$). Within sexes, the Anaktalak/Nachvak females were heavier than Saglek females ($F_{1,33}=15.9$, $p<0.001$) but no significant difference was found between sites for male sculpin ($F_{1,22}=0.78$, $p=0.39$).

In 2006, no significant difference was found in condition factor between sexes ($F_{1,54}=2.25$, $p=0.14$) or sites ($F_{1,54}=1.38$, $p=0.25$) and the interaction term between sex and site was also not significant ($F_{1,54}=0.55$, $p=0.46$). In 2006, the hepatosomatic index was significantly higher in females than males ($F_{1,54}=13.9$, $p<0.001$) and was significantly higher at Saglek than at Anaktalak/Nachvak ($F_{1,54}=7.34$, $p=0.009$). The interaction term between sex and site was not significant ($F_{1,54}=0.013$, $p=0.91$).

In 2007, female sculpin were significantly longer than male sculpin ($F_{1,46}=30.5$, $p<0.001$) but no significant difference was observed in length between sites ($F_{1,46}=0.81$, $p=0.37$). The interaction term between sex and site was not significant ($F_{1,46}=2.4$, $p=0.13$). In 2007, female sculpin were significantly heavier than male sculpin ($F_{1,46}=21.5$, $p<0.001$) but no significant difference was observed in mass between sites ($F_{1,46}=0.002$, $p=0.97$). The interaction term between sex and site was not significant ($F_{1,46}=1.94$, $p=0.17$).

In 2007, no significant difference was observed in hepatosomatic index between sex ($F_{1,46}=2.34$, $p=0.13$) or site ($F_{1,46}=1.36$, $p=0.25$). The interaction term between sex and site was

not significant ($F_{1,46}$=1.94, p=0.17). In 2007, no significant difference was observed in condition factor between sex ($F_{1,46}$=0.48, p=0.49) but the condition factor at Rose Island was significantly greater than the condition factor at Saglek ($F_{1,46}$=9.23, p=0.004). The interaction term between sex and site was not significant ($F_{1,46}$=0.61, p=0.44).

3.4.2 PCB Concentrations

Total PCB congener concentrations in sculpin liver in 2006 ranged from 11 ng/g to 41,000 ng/g (Table 3-3). The highest concentrations of PCBs were from Saglek (9560±11,000 ng/g wet weight) while total PCB concentrations in sculpin liver from the reference sites (*i.e.*, Anaktalak and Nachvak) were relatively low (22±11 ng/g wet weight). Total PCB congener concentrations sculpin liver in 2007 ranged from 9.5 ng/g to 18,000 ng/g (Table 3-3). The highest concentrations of PCBs were from Saglek Beach (4460±6020 ng/g wet weight) while total PCB concentrations in sculpin liver from Rose Island were relatively low (17±7.6 ng/g wet weight).

3.4.3 Gastrointestinal Parasites

Seven species of enteric parasites were identified from the gastrointestinal tracts of sculpin from the three fiords in 2006. These included one cestode: *Pyramicocephalus anthocephalus*, three trematodes: *Derogenes varicus*, *Podocotyle atomon* and *Brachyphallus crenatus*, two acanthocephalans: *Echinorhynchus gadi* and *Corynosoma magdaleni* and one nematode: *Pseudoterranova decipiens*. Three species of enteric parasites were identified from the gastrointestinal tracts of the sculpin from the two areas of Saglek fiord in 2007. These included two acanthocephalans: *E. gadi* and *C. magdaleni* and one nematode: *P. decipiens*. Two nematodes were also identified to genus in 2006 and 2007, these being *Contracaecum sp.* and *Hysterothylacium sp.* Parasite prevalence, abundance, mean intensity, and mean abundance

(Bush *et al.*, 1997) are summarised in Table 3-4. Cestodes were recovered from sculpin from the 2006 and 2007 sampling periods but their condition did not allow for identification or quantification so abundance of cestodes was not determined. The condition of a number of trematodes (particularly in 2007) and nematodes also did not allow proper identification. Therefore, these species are included in "trematodes" and "nematodes" in Table 3-4, and are included only in data analysis where total number of trematodes and/or nematodes is evaluated. Therefore, the abundance and mean abundance of trematodes in Table 3-4 are much higher than the individual trematode species because many trematodes were not identified.

Cestodes, trematodes, acanthocephalans, and nematodes were present in at least one sculpin from each site. The prevalence of cestodes ranged from 17% to 53% with the highest prevalence at Anaktalak/Nachvak in 2006 (53%) and Saglek Beach in 2007 (39%). The prevalence of trematodes ranged from 21% to 90% with the highest prevalence at Rose Island (90%) and Saglek Beach (71%) in 2007. Most of the trematodes were not identified and the unidentified trematodes made up the majority of the prevalence as the prevalence of the individual species was lower (Table 3-4). The prevalence of acanthocephalans ranged from 28% to 68%. The highest prevalence of acanthocephalans was at Anaktalak/Nachvak in 2006 (68%) and Rose Island in 2007 (67%). The prevalence of nematodes ranged from 41% to 79% and was highest at in 2006 at Anaktalak/Nachvak (79%) and Saglek (72%).

The abundance of several parasites ranged from zero to less than ten per fish with a few exceptions. Relatively high numbers of trematodes were observed in fish from Saglek in 2006, and from Rose Island and Saglek Beach in 2007. The numbers of acanthocephalans and particularly *C. magdaleni* were particularly high at Anaktalak/Nachvak in 2006 and Rose Island in 2007. This is also reflected in the mean intensity and mean abundance. Mean intensity and mean abundance generally followed the same trends as abundances and prevalence.

3.4.4 Likelihood Ratio Tests - Nachvak, Anaktalak and Saglek Fiords (2006)

The odds of infection with cestodes (Table 3-5) were six times more likely at Anaktalak/Nachvak than at Saglek. The odds of trematode infection was three times more likely at Saglek than at Anaktalak/Nachvak. The odds of infection with the acanthocephalan *C. magdaleni* in sculpin from Anaktalak/Nachvak was seven times more likely than in sculpin from Saglek. The odds of infection with acanthocephalans and *C. magdaleni* increased with increasing body mass. No relationships were found between the odds of infection with other groups/species of parasites and fiord, body mass or sex. Odds ratios were not calculated for the trematodes *B. crenatus, D. varicus* and *P.atomon* or the nematode *P. decipiens* because the models did not converge to a maximum likelihood estimate. Prevalence of these species was low (5.3% to 11%).

The difference in total number of parasites between fiords in 2006 depended on sex as indicated by a significant interactive effect of sex and fiord (Table 3-5). When male and female sculpin were analysed separately, the abundance of parasites was significantly higher in female sculpin from Anaktalak/Nachvak fiords than at Saglek fiord but this relationship was dependent on body mass as indicated by a significant interactive effect between mass and fiord. The sample size was not large enough to conduct analysis on mass categories. No significant difference in total parasites between fiords was observed in male sculpin.

The abundance of *C. magdaleni* in the fiords and its relationship with body mass depended on host sex as indicated by a significant interactive effect of fiord and host sex as well as body mass and sex (Table 3-5). A significant interactive effect between mass and sex was also present when acanthocephalans in the fiords were compared. Further analysis of female and male sculpin separately indicated that the abundance of acanthocephalans and *C. magdaleni* was significantly higher at Anaktalak/Nachvak than at Saglek in male sculpin but not in female

sculpin. The abundance of trematodes was significantly higher at Saglek than at Anaktalak/Nachvak. The abundances of acanthocephalans, C. *magdaleni* and nematodes also increased with increasing body mass.

3.4.5 Likelihood Ratio Test Results – Rose Island and Saglek Beach (2007)

A comparison of the odds of infection with *C. magdaleni* between Rose Island and Saglek Beach depended on sex (*i.e.*, significant interactive effect between fiord and host sex) (Table 3-6). Analysis within sex (*i.e.*, male and female sculpin separately) indicated that the odds of *C. magdaleni* infection was related to site in male sculpin but not female sculpin. In this case, male sculpin from Saglek Beach were more likely to be infected with *C. magdaleni* than those at Rose Island. The prevalence of nematodes appeared to be related to body mass but a significant interactive effect between sex and body mass indicated that this relationship may depend on sex. A separate analysis of male and female sculpin indicated that the odds of infection with nematodes for both sexes increased with increasing body mass. The odds of infection at the sites increased with body mass for the individual species of nematodes as well. No relationships were found between the odds of infection of other groups of parasites and fiords, body mass and sex.

Total parasites was not related to site or sex but was related to body mass with sculpin with higher body mass having higher parasite abundance (Table 3-6). No relationship between abundance of trematodes or nematodes and site was found although the abundance of nematodes in both female and male sculpin was found to increase with body mass. A similar relationship was found between body mass and abundance of *P. decipiens*.

The abundance of *C. magdaleni* and acanthocephalans at the sites depended on sex as indicated by a significant interactive effect between site and host sex (Table 3-6). Further analysis of male and female sculpin individually indicated that the abundance of *C. magdaleni*

was significantly higher at Rose Island than Saglek Beach for female and male sculpin while in acanthocephalans, this relationship was significant for male sculpin but not female sculpin. No relationships were found between the abundance of *E. gadi* and sex, mass or site.

3.5 Discussion

Concentrations of PCBs in shorthorn sculpin liver samples from Saglek (average = 9560±11000 ng/g wet weight) were elevated compared to sculpin from Anaktalak/Nachvak (average = 22±11 ng/g wet weight) in 2006. Within site variability at Saglek was high (PCB concentrations ranged from 16 ng/g to ng/g to 41,000 ng/g wet weight). Concentrations of PCBs at Saglek Beach in 2007 were also elevated in shorthorn sculpin livers (4460±6020 ng/g wet weight) compared to sculpin from Rose Island (17±7.6 ng/g wet weight). Within site variability was also high at Saglek Beach in 2007 (PCB concentrations ranged from 12 ng/g to 18000 ng/g wet weight).

The prevalence of *C. magdaleni* and cestodes as well as the abundance of acanthocephalans and *C. magdaleni* in sculpin was higher at Anaktalak/Nachvak where relatively low concentrations of PCBs were observed, compared to Saglek, where high concentrations of PCBs were observed. The relationship for abundance was observed in male sculpin but not female. In the follow up study within Saglek fiord (2007), the abundance of acanthocephalans in male sculpin and the abundance of *C. magdaleni* in both male and female sculpin showed the same trend with lower numbers at PCB contaminated areas. A similar decreased abundance and/or prevalence of endoparasites has been observed for *Echinorhynchus gadi* and *Steringophorus furciger* in winter flounder (*Pleuronectes americanus*) in the vicinity of a pulp and paper mill (Barker *et al.*, 1994; Khan, 2006; Khan and Billiard, 2007; Khan and Payne, 1997) and near a PCB contaminated naval facility (Khan, 1999), for cestodes in brown bullhead (*Ameiurus*

nebulosus) from a river receiving industrial waste (Steyermark *et al.*, 1999), for digeneans in American eels (*Anguilla rostrata*) in acidic lakes (Marcogliese and Cone, 1997), and for acanthocephalans in white suckers (*Catostomus commersoni*) and longnose suckers (*Catostomus catostomus*) from polluted areas of the Bow River (Kussat, 1969). These studies indicate that certain species of endoparasites in fish decrease as a result of increased levels of contaminants.

Of the parasites previously demonstrated to respond to pollution, only *E. gadi* was found in fish from the current study. *E. gadi* has previously been found to have lower prevalence and abundance in winter flounder from a pulp and paper mill in Newfoundland compared to a reference site (Khan, 1999; 2006). No significant relationship was found between prevalence or abundance of *E. gadi* and PCB contamination in this study. The only parasite species for which a clear relationship was established for prevalence/abundance and fiord/site in this study, was the acanthocephalan, *C. magdaleni*. *Corynosoma magdaleni* has a complex life cycle with grey or harbour seals as the definitive host and shorthorn sculpin and halibut as the second intermediate host (Montreuil, 1958). The first intermediate hosts are crustaceans, usually amphipods (Nickol *et al.*, 2002). Analysis of sculpin stomach contents (Table 3-7) revealed the presence of the amphipod *Gammarus oceanicus* in stomachs of sculpin from all three fiords. While stomach contents may only indicate recent consumption, they can be useful when making inferences about the benthic community (Knust, 1996). Sediment grab samples in 2007 indicated that amphipods were present at Saglek fiord (*Ampelisca sp., Ampithoe sp., Gammarus sp., Haustorius canadensis*, *Hyperia sp.*) and Nachvak fiord (*Gammarus sp.* and *Hyperia sp.*) (Copeland *et al.*, in prep). Underwater video analysis, however, indicated that amphipods were present at stations from both Saglek and from Nachvak but not at the near shore anchorage area of Saglek where PCB concentrations in sediment are highest (Copeland *et al.*, in prep). Amphipods are among the first species to disappear from benthic marine communities in contaminated areas (Long *et al.*,

2001) and may be present at a lower abundance in Saglek, particularly in the near shore, compared to the other fiords due to the elevated PCBs. Decreased numbers of amphipods, the first intermediate host for *C. magdaleni*, at Saglek may explain the decreased numbers of that parasite in fish at Saglek Beach.

In order to increase the statistical power of the analysis of the initial fiord study, the parasite abundance and prevalence data for fish from Anaktalak fiord (n=10) and Nachvak fiord (n=9) were pooled. There is uncertainty in the pooling as the fiords are widely separated geographically and other factors may affect the gastrointestinal parasites present in the fiords. Because the fiord study was followed up with a similar investigation focusing on sites within Saglek fiord to support the initial study, the pooling is considered acceptable for the preliminary analysis.

The generalized linear model was applied using the binomial distribution for prevalence and the negative binomial distribution for abundance in this parasite-pollution study. This approach enabled analysis of the effects of several explanatory variables simultaneously as well as interactive effects, while avoiding the use of biologically uninterpretable transformations. In this study, the relationship between prevalence and abundance of certain endoparasites and PCB exposure was often sex dependent with a significant relationship observed in male sculpin but not female sculpin. For other species of parasites, the relationship was dependent on body mass of the host. Host sex and body mass of the host are thus key considerations for studies of endoparasites as bioindicators.

Models have the advantages of determining the strength and importance of the effects and the ability to control the effects of confounding variables (Agresti, 2007). The generalized linear model allows the error structure to be defined by a distribution that accounts for the non-normal and non-homogenous errors which are characteristic of parasite abundance data analysis. In this

way, models increase statistical power by reducing the Type I error which occurs when distributions are different in their degree of aggregation (Wilson *et al.*, 1996). In the literature, parasite abundance data is generally transformed prior to applying parametric tests. The advantage of transforming is that it is still possible to include multiple explanatory variables and interaction terms but if the data is highly aggregated (*i.e.*, many high and low values), log transforming often fails to normalize the data and can result in a bimodal distribution (Wilson *et al.*, 1996). Therefore, classical linear regression models using log transformed data are more likely to generate both Type I and II errors than with generalized linear models (O'Brien, 2008; Wilson *et al.*, 1996; Wilson and Grenfell, 1997). It is also very difficult to transform back and make a direct statement about the difference between the means themselves (Lindsey, 2000). An alternate approach in the literature is to apply non parametric tests such as Kruskal Wallis and Mann Whitney to abundance data. Non parametric tests are based on few assumptions about the distributions but Type II error is increased as a result of the reduction of data into ranks. There is loss of information in descriptive presentation of results when transforming hard earned data to ranks (Green, 1979). No control variables or interaction terms in these tests also severely restricts the analysis.

Statistical analysis on prevalence in parasite-pollution studies is generally conducted using Fisher's Exact test or Pearson's chi square contingency test. Chi square tests of independence are limited in that they only indicate the degree of evidence for an association with no determination of the strength and importance of the effects (Agresti, 2007). Studying the strength of the associations through odds ratios and residuals consistent with practices in the medical literature is recommended. The analysis of odds ratios provided biologically interpretable parameter estimates that describe the size of the effect.

Generalized linear modeling techniques in parasite-pollution studies are recommended.

Many statistical packages now have generalized linear model options (*e.g.*, SPlus, Stata, SAS) making the transition relatively easy. The negative binomial model is not included in the packages but the appropriate functions exist for applying it (*e.g.*, glm.nb function from Venables and Ripley (2002)).

This study and others (*e.g.*, Khan, 1999; Khan, 2003; Khan, 2006; Khan and Kiceniuk, 1983; Khan and Payne, 2004; Koponen *et al.*, 2001; Kussat, 1969) indicate that the abundance and prevalence of parasites are related to contaminants. Parasites are affected by pollution in a variety of ways thus making interpretation of parasite-pollution studies difficult. Increases in the abundance and prevalence of certain parasites have been attributed to suppressed immune responses of the definitive host (Barker *et al.*, 1994; Khan, 2006; Marcogliese *et al.*, 1998; Sures, 2006), and positive effects of the contaminant on the intermediate host (Khan, 2004). Decreases of certain parasites due to elevated contaminants have been associated with negative effects on the intermediate host (Khan, 1999; Kussat 1969; Marcogliese and Cone, 1997), direct effects in the parasite's free living form (Mackenzie, 1999), or direct effects on the parasite in the alimentary canal of the host fish (Khan and Kiceniuk, 1983). This study supports other studies (*e.g.*, Khan, 1999; Kussat, 1969; Marcogliese and Cone, 1997) that indicate endoparasites decrease in response to increasing levels of pollution.

The type of contaminant is also an important consideration that affects the response of the parasite. For example, winter flounder living near a PCB contaminated naval facility in Newfoundland had lower abundance of the ectoparasite *Cryptocotyle lingua* (Khan, 1999). The lower abundance of this parasite was believed to be due to toxicity to the intermediate host, the Periwinkle snail *Littorina littorea* (Khan, 1999). The organics from pulp and paper effluent, however, are believed to have created a favourable environment for the periwinkle in St. George's Bay, NL and thus, may have resulted in higher abundance of the ectoparasite *C. lingua*

on winter flounder living in its vicinity (Khan, 2004). A clear understanding of the causal relationship between parasite and pollutant is essential to make predictions and interpret results.

In addition to knowledge of the biology of the parasites and the type of contaminant being studied, it is also essential to be familiar with the local ecology of the site. The presence or absence of other intermediate and definitive hosts of the parasites can affect whether the parasites will be present and their abundances. Based on observations during field work, there are ringed seals and harbour seals present in all three fiords. These seals may act as definitive hosts for *C. magdaleni*. Other fish such as longhorn sculpin or halibut are likely present in the fiords and may act as second intermediate hosts while other species of crustaceans may act as first intermediate hosts. Therefore, knowledge of the local ecology is also essential when making predictions and interpreting results from parasite-pollution studies.

3.6 Conclusion

The prevalence and abundance of the endoparasite, *C. magdaleni,* in shorthorn sculpin were lower at PCB contaminated sites than at reference sites. A key step in using parasites as bioindicators is to identify which parasite species are particularly sensitive to environmental change and to identify which direction that change occurs (*i.e.*, increase or decrease in abundance and prevalence). Knowledge of the parasite's biology (*e.g.*, life cycle) as well as influences of the local area ecology is required in order to predict this direction. The generalized linear model enables researchers to make the best use of data in testing predictions and provides biologically interpretable parameter estimates for comparison with other studies. The acanthocephalan, *C. magdaleni*, in fish may be useful as an effects-based bioindicator.

3.7 Acknowledgements

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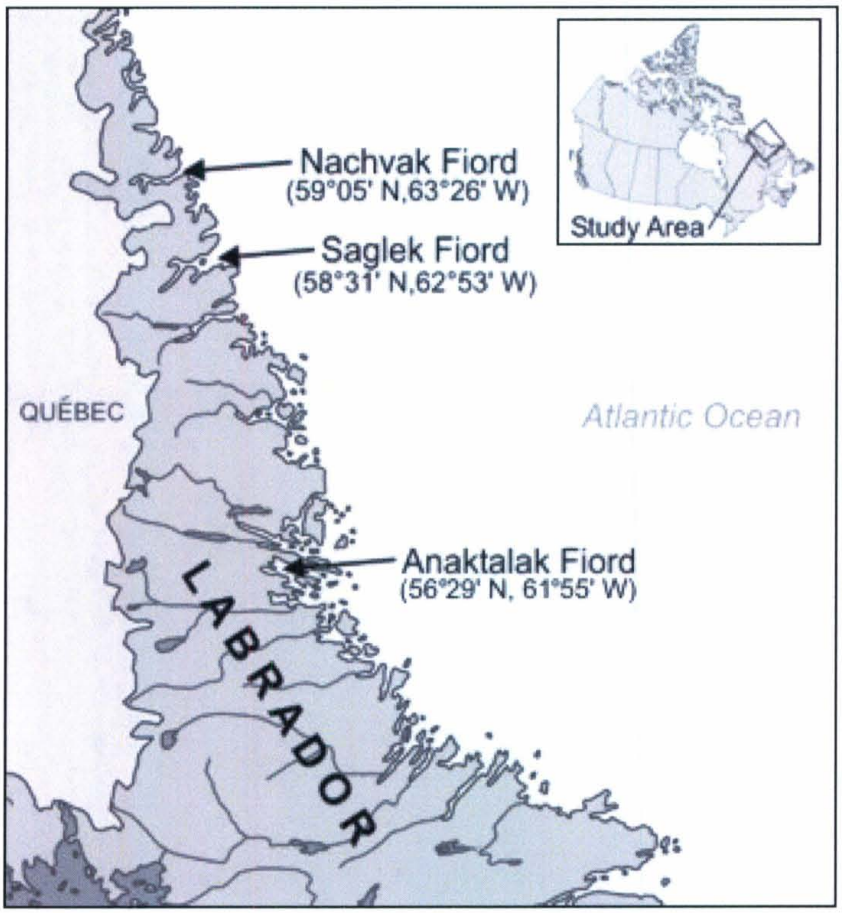

Figure 3-1. Geographic locations of Nachvak fiord, Saglek fiord and Anaktalak fiord in Labrador, Canada.

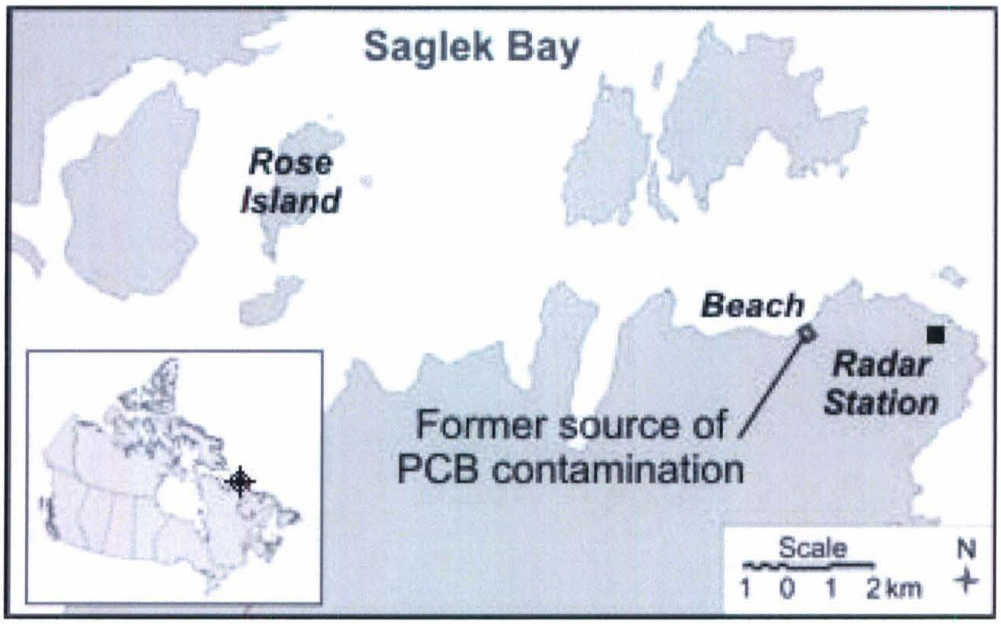

Figure 3-2. Locations of Rose Island and the Beach within Saglek fiord.

Table 3-2. Summary of physical data (mean ± standard deviation) for shorthorn sculpin

Parameter	Fiord/Site		
2006			
Anaktalak/Nachvak Fiords			
	Male (n=14)	Female (n=26)	Total (n=40)
Mass (g)	175±92.7	522±168	401±222
Length (mm)	234±39.5	337±29.8	301±59.5
Liver mass (g)	6.47±4.97	22.2±5.24	16.7±9.15
Hepatosomatic Index	3.32±1.10	4.40±0.84	4.02±1.06
Condition Factor	1.24±0.14	1.33±0.14	1.30±0.15
Saglek Fiord (Saglek Beach)			
	Male (n=9)	Female (n=10)	Total (n=19)
Mass (g)	202±66	277±70	237±74
Length (mm)	255±22	290±36	271±33
Liver mass (g)	9.5±5.4	17±7.9	13±7.3
Hepatosomatic Index	4.0±1.3	5.2±1.2	4.6±1.3
Condition Factor	1.21±0.15	1.25±0.20	1.23±0.16
2007			
Saglek Fiord, Rose Island Site			
	Male (n=10)	Female (n=19)	Total (n=29)
Mass (g)	264±93.6	389±163	346±153
Length (mm)	257±24.9	296±37.0	280±38.0
Hepatosomatic Index	2.89±0.76	2.92±1.17	2.91±1.03
Condition Factor	1.51±0.21	1.43±0.17	1.46±0.18
Saglek Fiord, Saglek Beach Site			
	Male (n=11)	Female (n=10)	Total (n=21)
Mass (g)	211±71.0	445±149	323±164
Length (mm)	250±29.0	320±38.0	283±48.1
Hepatosomatic Index	2.79±1.08	3.8±1.52	3.28±1.37
Condition Factor	1.31±0.16	1.32±0.14	1.32±0.15

Table 3-3. Total PCB concentrations (total congeners; mean ± standard deviation) in shorthorn sculpin liver samples

	2006		**2007**	
Fiord/Site	Anaktalak/Nachvak	Saglek	Rose Island	Beach
n	10	19	9	12
Total PCBs[a] (ng/g wet weight)				
Range	11-49	16-41000	9.5-34	12-18000
Average Total PCB Concentration	22±11	9560±11000	17±7.6	4460±6020
Most abundant PCB congeners[a] (ng/g wet weight)				
PCB153	6.8±3.7	2260±2690	4.8±1.6	1100±1400
PCB138/163/164	3.0±1.6	1180±1320	2.3±0.9	557±720
PCB180	1.6±0.7	1690±1040	2.5±1.2	853±1150

[a]Where congener was not detected, half the estimated quantitation limit (EQL) was used to calculate means.

Table 3-4. Parasite parameters for shorthorn sculpin

Species	Fiord (2006)		Site (2007)	
	Anaktalak/Nachvak (n=19)	Saglek (n=18)	Rose Island (n=29)	Beach (n=21)
Prevalence (%)				
Cestodes[a]	53	17	22	39
Trematodes[a]	21	44	90	71
Brachyphallus. crenatus	0	5.6	NA[a]	NA
Derogenes varicus	0	5.6	NA	NA
Podocotyle atomon	0	5.6	NA	NA
Acanthocephalans	68	28	67	59
Echinorhynchus gadi	10	11	28	38
Corynosoma magdaleni	68	22	41	43
Nematodes[a]	79	72	41	62
Pseudoterranova decipiens	5.3	0	35	48
Contracaecum sp.	79	67	10	19
Hysterothylacium sp.	16	17	17	19
Abundance				
Trematodes[a]	0-2	0-111	1-100	0-68
Brachyphallus. crenatus	0	0-1	NA	NA
Derogenes varicus	0	0-41	NA	NA
Podocotyle atomon	0	0-111	NA	NA
Acanthocephalans	0-72	0-4	0-95	0-15
Echinorhynchus gadi	0-1	0-1	0-12	0-6
Corynosoma magdaleni	0-72	0-4	0-100	0-15
Nematodes[a]	0-24	0-7	0-10	0-15
Pseudoterranova decipiens	0-1	0	0-6	0-11
Contracaecum sp.	0-24	0-7	0-1	0-6
Hysterothylacium sp.	0-4	0-1	0-3	0-1
Mean Intensity				
Trematodes[a]	1	39	26	22
Brachyphallus. crenatus	0	1	NA	NA
Derogenes varicus	0	41	NA	NA
Podocotyle atomon	0	111	NA	NA
Acanthocephalans	8	2	10	4
Echinorhynchus gadi	1	2	3	3
Corynosoma magdaleni	8	1	12	4
Nematodes[a]	8	2	4	4
Pseudoterranova decipiens	1	0	3	3

Contracaecum sp.	8	2	1	3
Hysterothylacium sp.	2	1	2	1
		Mean Abundance		
Trematodes[a]	1	11	23	15
Brachyphallus. crenatus	0	1	NA	NA
Derogenes varicus	0	2	NA	NA
Podocotyle atomon	0	6	NA	NA
Acanthocephalans	6	1	6	3
Echinorhynchus gadi	1	1	1	1
Corynosoma magdaleni	6	1	5	2
Nematodes[a]	7	2	4	4
Pseudoterranova decipiens	1	0	1	1
Contracaecum sp.	6	2	1	1
Hysterothylacium sp.	1	1	1	1

[a] Cestodes were recovered from sculpin from the 2006 and 2007 sampling periods but their condition did not allow for identification or quantification so abundance of cestodes was not determined. The condition of a number of trematodes (particularly in 2007) and nematodes also did not allow proper identification. Therefore, these species are only included in "trematodes" and "nematodes" in Table 3-4, and are included only in data analysis where total number of trematodes and/or nematodes is evaluated.

NA = not available: some specimens were not quantifiable

Table 3-5. Effect of fiord, host sex and host body mass on the odds of infection of shorthorn sculpin with parasite groups and species at three fiords in Northern Labrador (2006)

Binomial Models [a,b,c]						
Group/Species	Sex	Mass	Fiord	Sex*Mass	Sex*Fiord	Mass*Fiord
Cestodes	0.12 (0.73)	2.38 (0.12)	4.37 (0.037)*	0.38 (0.54)	0.077 (0.78)	0.051 (0.82)
Trematodes	0.57 (0.45)	0.14 (0.71)	4.34 (0.037)*	0.10 (0.75)	0.36 (0.55)	1.34 (0.25)
Acanthocephalans	0.70 (0.40)	9.07 (0.003)*	2.90 (0.088)	0.55 (0.46)	1.16 (0.28)	1.51 (0.22)
Corynosoma magdaleni	0.22 (0.64)	8.85 (0.003)*	4.51 (0.03)*	0.43 (0.51)	1.77 (0.18)	1.49 (0.22)
Echinorhynchus gadi	1.60 (0.21)	0.67 (0.41)	0.10 (0.75)	0.42 (0.52)	0.58 (0.45)	0.013 (0.91)
Nematodes	2.84 (0.092)	1.38 (0.24)	0.038 (0.84)	0.013 (0.91)	0.010 (0.92)	0.34 (0.56)
Negative Binomial Models [a,b,c]						
Total Parasites	0.21 (0.64)	4.51 (0.034)*	3.85 (0.050)*	3.10 (0.078)	4.69 (0.03)*	3.61 (0.057)
Total Parasites (males only)	-	7.90 (0.005)*	0.0003 (0.99)	-	-	1.69 (0.19)
Total Parasites (females only)	-	1.50 (0.22)	9.09 (0.003)*	-	-	7.15 (0.007)*
Trematodes	1.31 (0.25)	2.27 (0.13)	15.3 (<0.001)*	0.26 (0.61)	0.13 (0.72)	2.83 (0.09)
Acanthocephalans	7.28 (0.007)*	17.3 (<0.001)*	4.15 (0.041)*	6.79 (0.009)*	3.42 (0.064)	0.59 (0.44)
Acanthocephalans (female)	-	10.5 (0.001)*	0.096 (0.76)	-	-	0.12 (0.73)
Acanthocephalans (male)	-	13.9 (<0.001)*	7.66 (0.0056)*	-	-	1.00 (0.32)
Corynosoma. magdaleni	7.25 (0.007)*	17.5 (<0.001)*	5.31 (0.02)*	7.94 (0.005)*	5.01 (0.02)*	0.49 (0.48)
Corynosoma. magdaleni (female)	-	10.7 (0.001)*	0.10 (0.75)	-	-	0.12 (0.73)
Corynosoma. magdaleni (male)	-	14.4 (<0.001)*	10.2 (0.001)*	-	-	0.84 (0.36)
Nematodes	2.55 (0.11)	46.8 (<0.001)*	2.57 (0.11)	0.92 (0.34)	0.87 (0.35)	3.33 (0.07)

a. Values are G^2 with p-values in parentheses
b. Degrees of freedom is equal to one for each test
c. * Indicates that p-value is less than 5%

Table 3-6. Effect of site, host sex and host body mass on the odds of infection of shorthorn sculpin with parasite groups and species at two sites within Saglek fiord in Northern Labrador (2007).

Binomial Models [a,b,c]						
Group/Species	Sex	Mass	Site	Sex*Mass	Sex*Site	Mass*Site
Cestodes	0.069 (0.79)	3.49 (0.06)	2.80 (0.09)	0.0089 (0.92)	0.47 (0.49)	1.25 (0.26)
Trematodes	0.027 (0.87)	0.0023 (0.96)	2.71 (0.10)	0.77 (0.38)	0.033 (0.85)	0.46 (0.50)
Acanthocephalans	0.00014 (0.99)	1.23 (0.27)	0.32 (0.57)	0.40 (0.53)	1.74 (0.19)	0.58 (0.45)
Corynosoma magdaleni	2.73 (0.098)	0.46 (0.50)	0.15 (0.70)	0.80 (0.37)	6.87 (0.009)*	0.55 (0.46)
Corynosoma magdaleni (female)	-	0.11 (0.74)	0.98 (0.32)	-	-	0.37 (0.54)
Corynosoma magdaleni (male)	-	0.96 (0.33)	6.23 (0.01)*	-	-	0.37 (0.54)
Echinorhynchus gadi	1.95 (0.16)	0.97 (0.32)	0.31 (0.58)	0.85 (0.36)	0.023 (0.88)	3.76 (0.052)
Nematodes	0.082(0.77)	21.28 (<0.001)*	2.38 (0.12)	5.02 (0.02)*	0.22 (0.64)	2.68 (0.10)
Nematodes (female)	-	12.2 (<.001)*	1.76 (0.19)	-	-	2.45 (0.12)
Nematodes (male)	-	12.6 (<0.001)*	2.41 (0.12)	-	-	0.51 (0.47)
Pseudoterranova decipiens	0.68 (0.41)	15.2 (<0.001)*	1.24 (0.26)	0.66 (0.42)	0.17 (0.68)	1.94 (0.16)
Negative Binomial Models [a,b,c]						
Total Parasites	1.54 (0.21)	4.14 (0.042)*	0.78 (0.38)	0.59 (0.44)	3.0 (0.083)	0.54 (0.46)
Trematodes	0.35 (0.55)	0.074 (0.79)	0.76 (0.38)	0.46 (0.50)	1.22 (0.27)	0.10 (0.74)
Acanthocephalans	4.98 (0.03)*	23.6 (<0.001)*	0.71 (0.40)	0.36 (0.55)	8.02 (0.005)*	7.31 (0.007)*
Acanthocephalans (female)	-	22.4 (<0.001)*	1.71 (0.19)	-	-	8.36 (0.004)*
Acanthocephalans (male)	-	0.98 (0.32)	7.78 (0.005)*	-	-	0.0063 (0.94)
Corynosoma magdaleni	6.22 (0.01)*	19.0 (<0.001)*	0.081 (0.78)	0.035 (0.85)	14.6 (<0.001)*	2.87 (0.09)
Corynosoma magdaleni (female)	-	18.2 (<0.001)*	5.03 (0.02)*	-	-	2.45 (0.12)
Corynosoma magdaleni (male)	-	1.93 (0.16)	8.99 (0.003)*	-	-	0.90 (0.34)
Echinorhynchus gadi	0.28 (0.59)	3.20 (0.07)	2.35 (0.12)	0.83 (0.36)	0.000084 (0.99)	6.92 (0.008)*
Nematodes	2.44 (0.12)	52.3 (<0.001)*	3.23 (0.07)	5.17 (0.02)*	0.0067 (0.93)	1.20 (0.27)
Nematodes (female)	-	30.9 (<0.001)*	3.29 (0.07)	-	-	1.32 (0.25)

Nematodes (male)	-	23.3 (<0.001)*	1.71 (0.19)	-	-	0.032 (0.86)
Pseudoterranova decipiens	2.98 (0.084)	32.45 (<0.001)*	2.01 (0.16)	5.10 (0.024)*	0.17 (0.67)	2.24 (0.13)
Pseudoterranova decipiens (female)	-	15.2 (<0.001)*	1.91 (0.17)	-	-	0.79 (0.37)
Pseudoterranova decipiens (male)	-	20.7 (<0.001)*	1.98 (0.16)	-	-	1.58 (0.21)

a. Values are G^2 with p-values in parentheses
b. Degrees of freedom is equal to one for each test
c. * Indicates that p-value is less than 5%

Table 3-7. Stomach contents of shorthorn sculpin from Saglek, Anaktalak and Nachvak fiords in Northern Labrador.

2006			2007	
Anaktalak Fiord	Saglek Fiord	Nachvak Fiord	Rose Island	Saglek Beach
Shrimp Nereid worms (*Nereis sp.*) Sand lance (*Ammodytes sp.*) Amphipod (*Gammarus oceanicus*) Gunnel (Family Pholidae) Toad crab (*Hyas coarctatus*)	Crustaceans Moon snails (Family Natacidae) Amphipod (*Gammarus oceanicus*) Clams Nereid worms (*Nereis sp.*) Sand lance (*Ammodytes sp.*) Gastropods Periwinkle snail (*Littorina littorea*)	Shrimp Gunnel (Family Pholidae) Amphipod (*Gammarus oceanicus*)	Clams Nereid worms (*Nereis sp.*) Shrimp Gunnel (Family Pholidae) Amphipod (*Gammarus oceanicus*)	Nereid worms (*Nereis sp.*) Gunnel (Family Pholidae) Clams Shrimp Amphipods (*Gammarus oceanicus*)

4.0 Biomarker Responses in Shorthorn Sculpin (*Myoxocephalus scorpius)* at a Polychlorinated Biphenyl Contaminated Site at Saglek Bay, Labrador

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4.1 Abstract

A local source of polychlorinated biphenyls (PCBs) has contaminated marine sediments and the coastal food web at Saglek, a former Polevault line military station in Northern Labrador. Following the remediation of impacted soils from Saglek Beach in 1997-1999, concentrations of PCBs in sediment and biota have decreased significantly in Saglek Bay. A suite of biomarkers including biotransformation enzyme activity, vitamins, organ histopathology/histomorphometry, body condition, liver lipid content, and hepatosomatic index were measured in shorthorn sculpin (*Myoxocephalus scorpius*) in 2007, eight years after remediation of PCB impacted soil. The hepatic concentrations of vitamin E in sculpin at Saglek Beach were significantly lower than the reference site which may indicate that vitamin E is involved in protecting the Beach sculpin against contaminant induced oxygen radical production. Body condition factor was also significantly higher at the reference site compared to Saglek Beach. Despite the elevated concentrations of PCBs at Saglek Beach compared to the reference site, other biological endpoints examined (hepatosomatic index, liver lipid content, biotransformation enzyme activity, vitamin A, and thyroid histopathology/histomorphometry) did not differ between the sites nor did these biomarker responses show significant relationships to PCB concentrations. Studies in temperate areas have shown that biomarker responses change over time at sites where remediation has occurred. The biomarker study at Saglek represents a first step in the identification of appropriate biomarkers for a biological effects-based monitoring program.

4.2 Introduction

The occurrence of highly persistent pollutants such as polychlorinated biphenyls (PCBs) in Arctic environments is a concern because of their ability to accumulate in tissues of organisms and magnify within food webs (Skaare *et al.*, 2002; Fisk *et al.*, 2005). Long range transport is the primary source of PCBs in Northern environments (Muir *et al.*, 1992; 1999; Macdonald *et al.*, 2000; Braune *et al.*, 2005). Local sources are relatively small in the context of regionally distributed contamination (Macdonald *et al.*, 2000; Stow *et al.*, 2005). PCBs originating from local sources such as former military installations (*e.g.*, Distant Early Warning (DEW)-line stations, Polevault line stations), however, have been shown by short range transport to create a halo of contamination around the site (Macdonald *et al.*, 2000; Pier *et al.*, 2003; Stow *et al.*, 2005). Adverse effects to organisms living in their vicinity have been reported (*e.g.*, decreased bone mineral density in deer mice (*Peromyscus maniculatus*) (Johnson *et al.*, 2009), increased liver biotransformation enzyme activity and decreased liver retinol concentrations in Black guillemot (*Cepphus grylle*) (Kuzyk *et al.*, 2003) and increased liver biotransformation enzyme activity in shorthorn sculpin (*Myoxocephalus scorpius*) (Kuzyk *et al.*, 2005b)).

In 1985, Canada and the United States reached an agreement to replace the DEW-line with a modernized, unattended system. Coincident with this, a decision was made to remediate the old sites (Poland *et al.*, 2001). Subsequently, many of the military sites across Canada have undergone cleanup, and concentrations of contaminants have been shown to be decreasing in some areas (*e.g.*, Brown *et al.*, 2009). Essential to these clean-up efforts is an effective post-remediation monitoring program to document the continued

recovery and protection of the marine and terrestrial ecosystems. Early monitoring programs generally consisted of temporal monitoring of physical and chemical parameters that were compared over time. In recent years, there has been a shift to using biological responses (*i.e.*, biomarkers) in organisms to assess and monitor environmental change. Biomarkers have been widely used as a measure of the deterioration of the health of an ecosystem and most frequently document the effects of contaminated sites (*e.g.*, Martin and Black, 1996; Schreiber *et al.*, 2006). Comparatively, there are fewer biomarker monitoring programs designed to measure changes in an ecosystem following remediation. Moore *et al.*, (2005) found a reduction in liver tumours and pre-carcinogenic lesion hydropic vacuolation in winter flounder (*Pseudopleuronectes americanus*) after a series of improvements to a sewage outfall (cessation of sludge discharge, primary and secondary treatment, diversion of an outfall to an offshore outfall). Similarly, Myers *et al.*, (2008) found decreases in hepatic DNA adducts and lesions in English sole (*Parophrys vetulus*) approximately four years after the capping of polycyclic aromatic hydrocarbon (PAH) contaminated sediments at Eagle Harbor, Washington. These studies indicate that biomarkers may be useful for environmental monitoring after remediation has occurred. There is however, little long-term data available on how biomarkers can be used in environmental monitoring following remediation in Arctic environments.

Saglek, Labrador is a former Polevault Line military station in Northern Labrador, Canada (Figure 4-1). The abandonment and demolition of the original facility resulted in extensive PCB contamination in soil at levels ranging from less than 50 µg/g dry weight up to 1,600 µg/g dry weight (ESG, 1997). PCBs adsorbed to soil particulates had also

eroded from a beach ravine and accumulated in nearshore sediments (ESG, 2000). A major remediation project, conducted between 1997 and 1999, removed much of the PCB contaminated soils. Initial residue analysis work conducted concurrent with the remediation at Saglek found extensive PCB contamination in the marine sediments and the coastal food web in Saglek Bay. This was particularly documented in those species that fed on or near the seabed and had limited foraging ranges (Kuzyk *et al*, 2005a). To support an effects-based study of the marine foodweb in 1999, Kuzyk *et al.*, (2003; 2005b) measured biological endpoints in Black guillemot at Saglek (ethoxyresorufin-*O*-deethylase (EROD), vitamin A, malic enzyme, and porphyrin in liver) and shorthorn sculpin (EROD, lipid content, and relative liver mass). Increased EROD activity and decreased vitamin A concentrations in Black guillemot liver as well as increased EROD activity in shorthorn sculpin liver were found in association with the elevated concentrations of PCBs.

In 2006/2007, PCB concentrations were again measured in sediment, shorthorn sculpin and Black guillemots. Results indicated that concentrations of PCBs have decreased significantly in sediment, shorthorn sculpin, and Black guillemots (Brown *et al.*, 2009). In addition to the tissue analysis, a suite of biomarkers was analyzed in shorthorn sculpin and Black guillemot at Saglek Bay in 2007. This chapter examines the associations between PCB exposure and the following biomarkers in shorthorn sculpin from Saglek Bay: hepatosomatic index, body condition, liver lipid content, biotransformation enzyme activity, vitamins, and organ histopathology/histomorphometry. Biomarkers measured in shorthorn sculpin by Kuzyk *et al.*, (2005b) that were also repeated in this study are hepatosomatic index and body

condition. The biomarker data from Kuzyk *et al.*, (2005b) was not available. Therefore, no temporal comparisons were conducted.

The hepatosomatic index has been traditionally used as a biomarker of various contaminants based on its sensitivity to liver hypertrophy and/or hyperplasia due to detoxification properties of the liver as well as increased liver weight due to fat content and liver necrosis (Goede and Barton, 1990; Hinton and Laurén, 1990; Gul *et al.*, 2004). The condition factor is a generalized indicator of the physical and physiological status of the fish and can reflect the integrated effect of both nutrition and metabolic costs induced by stress (Adams *et al.*, 1990; Encina and Granado-Lorencio, 1997). While these indices have traditionally been accepted for monitoring, they have also been found to vary seasonally in fish (Yang and Bauman, 2006) and between sexes (Khan and Billiard, 2007; Yang and Baumann, 2006).

Uridine diphosphate glucuronyltransferase (UDP-GT) is an enzyme that is commonly used in biomonitoring programs (Halliwell and Gutteridge, 1999). UDP-GT catalyzes the process of glucuronidation and represents a major pathway of conjugative biotransformation metabolism in fish. Initially, the phase I enzyme cytochrome P4501A (CYP1A) increases the solubility of hydrophobic molecules such as PCBs by a reduction reaction which introduces an oxygen atom into the molecule (Andersson and Forlin, 1992; Palace *et al.*, 1996; Schlezinger *et al.*, 1999). This leads to an increase in reactive oxygen species (ROS) production or the generation of reactive redox cycling intermediates thus eliciting oxidative stress (Lehtinen, 1990; Palace *et al.*, 1996; Palace *et al.*, 1998; Senthil kumar *et al.*, 2004). With PCBs, the result is toxic metabolites called hydroxylated (OH)-PCBs which are more water soluble (Kawano *et al.*, 2005). The OH-

PCBs can be further converted through this process of glucuronidation – a phase II conjugation reaction where a glycosyl group from a nucleotide sugar is added to an acceptor compound at a nucleophilic functional group of oxygen, nitrogen, sulphur, or carbon (King *et al.*, 2000). UDP-GT catalyzes the process of glucuronidation and the product is ultimately excreted as β-glucuronide product.

The reactive oxygen species mentioned in the previous paragraph can cause lipid peroxidation, decreased glutathione content, decreased hepatic membrane fluidity, increased DNA damage in cells (Stohs, 1990) as well as testicular degeneration and infertility (Koksal *et al.*, 2003). Vitamin E is known as one of the major antioxidants protecting membranes against oxidation (Kawai-Kobayashi and Yoshida, 1986; Packer, 1991; Latchoumycandane and Mathur, 2002; Yun *et al.*, 2005) and thus, has also been proposed as a biomarker for contaminant induced oxygen radical stress (Packer, 1991). While other vitamins such as vitamin A and vitamin C have many physiological roles, the only function of vitamin E is as an antioxidant so it is a good indicator of oxidative stress (Packer, 1991). Vitamin E is particularly important in fish because fish have a higher amount of polyunsaturated fatty acid lipids that are susceptible to lipid peroxidation (Palace *et al.*, 1996).

Vitamin A has been proposed as a biomarker of contaminant exposure because levels of stored vitamin A in the liver and circulating levels of vitamin A in the plasma are often depleted in organisms exposed to PCBs (Zile, 1992; Palace and Brown, 1994; Ndayibagira *et al*, 1995; Palace *et al.*, 1996; Doyon *et al.*, 1998; Rolland, 2000). In fish, vitamin A is stored in the liver as esterified retinoids, in particular retinyl palmitate. When plasma concentrations of retinol, the circulating form of vitamin A decrease,

retinyl esters in the liver are hydrolyzed by hydrolytic enzymes to form retinol. Retinoid stores can vary with age, reproductive state, diet and other external conditions but retinol levels in circulation are kept relatively constant (Higashi, 1961) except in instances of excessive depletion of stores (Palace *et al.*, 2001). The mechanism by which circulating retinol and retinyl ester reserves are depleted by exposure to PCBs is not fully understood. In birds and mammals, a theory is that hydroxylated PCBs compete for the binding sites on transthyretin, the blood carrier protein for retinol and thyroxine resulting in urinary excretion of retinol and thyroxine (Brouwer and van den Berg, 1986; Letcher *et al.*, 2000; Skaare *et al.*, 2001). Other theories suggest loss of appetite (Spear *et al.*, 1994), increased use as an antioxidant due to oxidative stress (Palace *et al.*, 1996) and effects on the activity of retinyl ester hydrolase, the enzyme responsible for liberating free retinol from storage sites in the stellate cells of the liver (Chen *et al.*, 1992). Vitamin A is important in the normal differentiation of epilthelial structures, reproduction, vision, and immune system function (Zile, 1983). Decreases in vitamin A storage levels have been related to developmental deformities in lake sturgeon exposed to co-planar PCBs in the St. Lawrence (Doyon *et al.*, 1998) and lethal viral infections and reproductive disorders in seals in the Baltic, North and Wadden Seas (Brouwer *et al.*, 1989).

Abnormalities in the thyroid have also been reported as a result of exposure to PCBs (Brown *et al.*, 2004). In fish, the thyroid tissue mainly occurs in the pharyngeal region and consists of follicles formed of a single layer of epithelial cells surrounding an extracellular lumen containing proteinaceous colloid (Brown *et al.*, 2004; Fournie *et al.*, 2005). If the thyroid undergoes stimulation, predictable increases in epithelial cell height occur as well as effects such as vascularity, follicle irregularities, and decreases in colloid

density occur (Eales and Brown, 1993). The epithelial cell height as well as the histopathology of thyroid cells can be measured as a biomarker of PCB exposure.

PCBs may result in the increase of lipid content in the liver, which also increases the retention of PCBs in the liver (Matsusue *et al.,* 1999). Therefore, liver lipid was measured as a biomarker in shorthorn sculpin liver.

These biomarkers were chosen for this study because they have previously been shown to respond to exposure to PCBs in other studies. The results of the current biomarker study as well as the results of the biomarker study by Kuzyk *et al.*, (2005b) will be used to determine suitable biomarkers for the implementation of a biological effects-based monitoring plan for shorthorn sculpin at Saglek Bay. This study and the study by Kuzyk *et al.*, (2005b) represent a unique approach to using biomarkers to supplement chemical residue analysis in monitoring an Arctic ecosystem following removal of a source of contamination.

4.3 Methods

4.3.1 Study Area

Saglek Bay is located on the northeast coast of Labrador at the southern extent of the Torngat Mountains (Figure 4-1). From 1951 to 1971, a Polevault Line military radar station was operated from Saglek by the U.S. Air Force. In 1971, the Station was abandoned and in 1978 the site was destroyed by a fire (Pier *et al.*, 2003). The Canadian Department of National Defence (DND) commenced construction of a modern North Warning System (NWS) long range radar facility in 1986 and the station (designated as LAB-2) opened in 1988. The LAB-2 long range radar facility is currently operated remotely and is unmanned with site maintenance conducted seasonally and on an as-

needed basis by DND contractors. Extensive PCB contamination in soil and sediment associated with the original facility was identified at Saglek in the 1990s (ESG, 1997). Soil remediation was carried out between 1997 and 1999 and thus, the terrestrial source of exposure has been removed. Ecological studies showed that PCBs remained in the coastal marine food web (Kuzyk *et al.*, 2005a) but recent evidence indicates that the concentrations are decreasing in sediment and biota (Brown *et al.*, 2009).

4.3.2 Sample Collection

Shorthorn sculpin (n=68) were collected from three locations within Saglek Fiord in August 2007: Rose Island (29 sculpin), Big Island (18 sculpin), and Beach (21 sculpin) (Figure 4-1). It should be noted that the sculpin used in this study are the same sculpin used in Chapter 3 for parasite analysis. Uneven sample numbers of sculpin were collected because weather conditions and delays prevented the completion of sampling at the sites. Rose Island is considered a reference site as it is located approximately 18 km from the former source but is still within Saglek fiord. Big Island is located 6 km from the former source and is considered an intermediate site. The Beach is within the vicinity of the former PCB source and is considered the most contaminated site. Shorthorn sculpin were collected with hook and line over the side of a boat. Immediately after capture, the sculpin were euthanized by cutting the spinal cord and were then dissected. A record was made of total body length, body mass, and mass of the liver, stomach and gonad. Sex was determined by macroscopic gonad examination and confirmed histologically. Livers were subsampled and a portion was finely minced on ice to a homogenous consistency. Approximately 2-g portions were placed in two individual 2

mL cryovials and immediately flash frozen on liquid nitrogen. The remaining liver was stored frozen at -20°C for PCB analysis.

4.3.3 PCB and Lipid Content Analysis

PCB and lipid content analyses were conducted on shorthorn sculpin livers by AXYS Analytical Services in Sidney, BC. Due to the cost of the analysis, PCB analysis could not be performed on all individuals. Analyses were carried out on twenty-eight randomly selected fish: nine from Rose Island, seven from Big Island and twelve from Beach. Samples were selected for PCB analysis based on variance in PCB concentrations observed at each site during previous investigations. In addition, the use of the PCB analytical results was also considered. The variance in PCB concentrations was expected to be higher at Saglek Beach so more samples were analysed from there. In addition, because sculpin from Saglek Beach were expected to have the highest concentrations of PCBs, the biomarker responses were expected to be greater from this site.

Briefly, concentrations of 91 PCB congeners and Aroclors 1242, 1254 and 1260 were determined using high resolution gas chromatography with detection by low-resolution mass spectrometry (HRGC/LRMS). Samples were spiked with isotopically labelled surrogate standards, extracted, and the extract separated into fractions using Florisil®. Fraction one was analyzed for PCBs and PCB Aroclors using high-resolution gas chromatography with detection by low-resolution mass spectrometry (HRGC/LRMS). The procedures are summarized in Appendix 1. Total concentrations of PCBs reported for sculpin represent the sum of the congeners. Congener concentrations less than the detection limit were replaced with one half of the detection limit. Results are

reported on a wet weight basis. AXYS uses the batch method whereby samples are worked up in batches composed of nine or fewer samples along with one QA/QC sample (certified reference material or internal spiked matrix samples), one analytical blank, and one analytical duplicate. Spiked matrix samples was used in cases where analytical results were expected to be outside the concentration ranges of the available certified reference materials. AXYS prepares spiked matrix samples using a clean matrix (canola oil) that is spiked with a suite of native analytes in a known concentration.

For the 2007 sculpin, recoveries of 53 congeners (one sample) and 38 congeners (two samples) ranged from 70% to 119%, average = 94±7.6%. Three blank samples were analyzed and results were below the detection limits for all PCB congeners. The result for one congener in one blank was reported as NDR, indicating low level peaks were detected but did not meet the quantification criteria to be identified as the congeners. Two sculpin were analyzed in duplicate and the average relative standard deviation was 6.8 %. AXYS considers acceptable limits to be less than 30% relative standard deviation with 20% or less considered good agreement.

4.3.4 Biochemical Assays

4.3.4.1 Microsome Preparation

To prepare the microsomes, liver samples were homogenized in 4 mL of cold 0.1 M Tris hydrogen chloride (HCl) homogenization buffer (pH = 7.6 at 25°C) using a polytron homogenizer. Centrifugation of 3.5 mL of homogenate at 4°C for ten minutes at 28,000 *g* yielded a supernatant that was recovered and recentrifuged at 103,000 *g* for ninety minutes at 4°C. After this centrifugation, the supernatant was aspirated off the

microsomal pellet which was resuspended in 1.2 mL of cold 0.05 M Tris-HCl resuspension buffer (pH = 7.4 at 25°C). All processing was performed on ice and resuspended microsomes were frozen at -90°C in 1.5 mL microcentrifuge tubes until assays were performed (less than three months).

4.3.4.2 Phase II Enzyme - Uridine diphosphate glucuronyltransferase (UDP-GT) Activity

UDP-GT activity was assayed in duplicate for each sample using a modification of the method by Clarke *et al.* (1992). A blank was prepared for each sample set and every tenth sample was assayed twice for QA/QC purposes. It was ensured that variability in the duplicates varied by less than 10%. UDP-GT assays were carried out at room temperature in 0.1 M tris buffer at pH 7.2, in the presence of magnesium chloride hexahydrate. Microsomes were preincubated for 10-15 minutes on ice with the non-ionic detergent Lubrol (0.24 mg/mg microsomal protein) to disrupt microsomal vesicles. The assay was initiated by the addition of 25 µL of 4-nitrophenol (pNP) (600 nM) and run for twenty minutes at room temperature in a metabolic shaker. The reaction was stopped by the addition of 0.5 mL of ice cold trichloroacetic acid (TCA) followed by centrifugation at 2,000 *g* for ten minutes. The supernatant was recovered and recentrifuged at 10,000 *g* for ten minutes followed by alkalinisation with 2M sodium hydroxide. The remaining pNP was measured spectrophometrically at 405 nm. Protein content of the microsomes was determined using the Bradford method (Bradford, 1976).

4.3.4.3 Vitamins

Vitamin E (tocopherol) and vitamin A (retinol, retinyl palmitate and five other

retinyl esters) were measured in the sculpin liver samples using an extraction and reverse phase high performance liquid chromatography (HPLC) detection method described in Palace and Brown (1994) except that 1% proprionic acid was included in the mobile phase to facilitate separation of retinoids and retinyl esters. Standard retinol, retinyl palmitate and tocopherol standards from Sigma Chemical Co. were used for HPLC analysis. The standards used for HPLC analysis for other vitamin A esters were synthesised using a modification of a cholesterol ester synthesis method as outlined in Palace *et al.* (1998). Every tenth sample was assayed twice for QA/QC purposes and it was ensured that variability was less than 10%. If the variability exceeded 10%, the samples were repeated until a consistent result was achieved. Each sample also has two internal standards incorporated into them to ensure that the differences in extraction efficiencies are corrected. Peaks are also quantified based on authentic commercial standards which are positively identified according to retention time and verified against a spectral library constructed with the authentic standards. The recovery of tocopherol ranged from 70-102%, average = 90±7.5%. The recovery of retinol ranged from 61-104%, average = 84±8.4%.

4.3.5 Thyroid Histopathology and Histomorphometry Assessment

Pharyngeal tissue samples were fixed in 10% neutral buffered formalin for 48 hours and were then rinsed three times in 70% ethanol. The operculum and gill arches were trimmed off, and the anterior portion of the remaining tissue was halved in sagittal plane and decalcified. The tissues were dehydrated in graded alcohols, cleared in toluene, and infiltrated with molten paraffin using a Sakura Tissue Tek VIP 5 tissue

processor. Tissues were then embedded in paraffin blocks for routine sectioning. Sculpin thyroids were oriented for sagittal/parasagittal sectioning. Tissues were stepsectioned (5 μm) and five to eight representative sections per tissue were affixed to slides, and stained with Harris' hematoxylin and eosin. Slides were analyzed using a Leitz compound microscope, an Olympus Qcolor3 digital camera, and ImagePro Express v.5.1.0.12 software.

Sculpin thyroids were examined for evidence of follicular cell proliferative lesions as described by Fournie *et al.* (2005) (*i.e.* follicular cell hyperplasia, adenoma, and carcinoma). In addition, thyroid epithelial cell heights were measured on one representative section from each fish. The thyroid follicle-containing region was photographed (two separate fields of view per fish), and a random point overlay was applied to the photographs to determine 10 random follicles to measure. For each follicle, the epithelial cell height was measured at the four cardinal points. The values were averaged (n=40) to determine the mean epithelial cell height for each fish.

4.3.6 Computational Methods and Statistical Analysis

Fulton's condition factor (K) was calculated as the ratio of whole body mass (g) to length (cm) to the power of three and multiplied by 100 (Ricker, 1975). Hepatosomatic index was calculated as the ratio of liver mass (g) to body mass (g), multiplied by 100 (Heidinger and Crawford, 1977).

Relationships between variables were initially determined using the general linear model. Errors were checked to confirm normality, homogeneity and independence. For the models involving thyroid epithelial cell height, UDP-GT, vitamin A and lipid as

response variables, the assumptions of the general linear model (normal error distribution) were satisfied and the F-ratio and p-value were reported. The assumptions of the general linear model (normal error distribution) were not satisfied for the models with vitamin E as the response variable. In this case, the model was revised and the generalized linear model for non-normal errors (gamma distribution with log link) was used and the assumptions were verified again. The deviance (G-statistic) and p-value are reported for the models involving vitamin E. The G-statistic follows a chi-square distribution with the degrees of freedom equal to the number of parameters in the model minus one. Initially the effects of the explanatory variables (sculpin body mass, sex, and liver PCB concentration) on the response variables (thyroid epithelial height, UDP-GT activity, liver tocopherol (vitamin E) concentration, liver vitamin A (retinol, retinyl palmitate, retinyl esters) concentration, and liver lipid) were modelled. Because only twenty-eight of the sixty-eight sculpin were analysed for PCBs, a separate model for sculpin body mass, sex, and collection site (Rose Island, Big Island and Beach) was developed to increase the sample size (n=68) and hence, the power of the analysis. An overall test was conducted to detect differences in response variables between sites. Where a significant difference was detected between sites, two *a priori* tests were set up (*i.e.*, Rose Island vs. Beach and Rose Island vs. Big Island) and a one-tailed test was conducted to detect differences between individual sites. A priori tests are considered more powerful because they use external information to set up tests.

Multivariate analysis of variance (MANOVA) was used to model the effects of body mass, sex, collection site, and PCB concentration on the concentrations of retinol, retinyl palmitate and retinyl esters. MANOVA carries out a generalization of an

ANOVA for cases in which several variables have been measured for two or more samples (Sokal and Rohlf, 1995). The F-ratio (estimated from the Pillai Trace value) and p-value are reported for the results of the MANOVA. The significance level was set to $p \leq 5\%$.

The equations for the models tested as well as information on the response variables, explanatory variables, error distributions and link functions are summarized in Table 4-1. In the table, μ is the expected variable or response variable. Interaction terms were examined before tests were interpreted. As per examples in Sokal and Rolf (1995), interaction terms were not removed from the models if they were not significant.

Table 4-1. Information on the models tested for each response variable.

Response Variable (Y)	Explanatory Variables	Error Distribution	Link Function	Equation
PCB Models				
Thyroid epithelial cell height (μm) (T)	Body mass (M) Sex (S) PCB (P)	Normal	Identity $\eta = \mu$	$T = \mu$ + normal error $\eta = \beta_o + \beta_M*M + \beta_S*S + \beta_P*P + \beta_{MS}*MS + \beta_{MP}*MP + \beta_{SP}*SP$
UDP-GT (nmol min^{-1} mg^{-1}) (U)	Body mass (M) Sex (S) PCB (P)	Normal	Identity $\eta = \mu$	$U = \mu$ + normal error $\eta = \beta_o + \beta_M*M + \beta_S*S + \beta_P*P + \beta_{MS}*MS + \beta_{MP}*MP + \beta_{SP}*SP$
Vitamin E (μg/g) (E)	Body mass (M) Sex (S) PCB (P)	Gamma	Log $\eta = \log_e\mu$	$E = \mu$ + gamma error $\eta = \beta_o + \beta_M*M + \beta_S*S + \beta_P*P + \beta_{MS}*MS + \beta_{MP}*MP + \beta_{SP}*SP$
Vitamin A (μg/g) (A)	Body mass (M) Sex (S) PCB (P)	Normal	Identity $\eta = \mu$	$A = \mu$ + normal error $\eta = \beta_o + \beta_M*M + \beta_S*S + \beta_P*P + \beta_{MS}*MS + \beta_{MP}*MP + \beta_{SP}*SP$
Lipid (%) (L)	Body mass (M) Sex (S) PCB (P)	Normal	Identity $\eta = \mu$	$L = \mu$ + normal error $\eta = \beta_o + \beta_M*M + \beta_S*S + \beta_P*P + \beta_{MS}*MS + \beta_{MP}*MP + \beta_{SP}*SP$
Site Models				
Thyroid epithelial cell height (μm) (T)	Body mass (M) Sex (S) Site (Z)	Normal	Identity $\eta = \mu$	$T = \mu$ + normal error $\eta = \beta_o + \beta_M*M + \beta_S*S + \beta_z*Z + \beta_{MS}*MS + \beta_{MZ}*MZ + \beta_{SZ}*SZ$
UDP-GT (nmol min^{-1} mg^{-1}) (U)	Body mass (M) Sex (S) Site (Z)	Normal	Identity $\eta = \mu$	$U = \mu$ + normal error $\eta = \beta_o + \beta_M*M + \beta_S*S + \beta_z*Z + \beta_{MS}*MS + \beta_{MZ}*MZ + \beta_{SZ}*SZ$
Vitamin E (μg/g) (E)	Body mass (M) Sex (S) Site (Z)	Gamma	Log $\eta = \log_e\mu$	$E = \mu$ + gamma error $\eta = \beta_o + \beta_M*M + \beta_S*S + \beta_z*Z + \beta_{MS}*MS + \beta_{MZ}*MZ + \beta_{SZ}*SZ$
Vitamin A (μg/g) (A)	Body mass (M) Sex (S) Site (Z)	Normal	Identity $\eta = \mu$	$A = \mu$ + normal error $\eta = \beta_o + \beta_M*M + \beta_S*S + \beta_z*Z + \beta_{MS}*MS + \beta_{MZ}*MZ + \beta_{SZ}*SZ$
Lipid (%) (L)	Body mass (M) Sex (S) Site (Z)	Normal	Identity $\eta = \mu$	$L = \mu$ + normal error $\eta = \beta_o + \beta_M*M + \beta_S*S + \beta_z*Z + \beta_{MS}*MS + \beta_{MZ}*MZ + \beta_{SZ}*SZ$

4.4 Results

4.4.1 Morphometric Information

Physical information as well as hepatosomatic indices (HSI) and condition factors (K) for sculpin collected during 2007 are presented in Table 4-2. Female sculpin were significantly longer ($F_{1,62}$=26.1, $p<0.001$) than male sculpin and a significant difference in length was detected between sites ($F_{2,62}$=490, $p<0.001$). The interaction term between sex and site was also significant ($F_{2,62}$=6.1, $p=0.004$) so analyses were done within both

sex and site. When analysis was done within sexes, a significant difference was found between sites ($F_{2,37}$=301, $p<0.001$) for female and male sculpin ($F_{2,25}$=251, $p<0.001$). When analysis was done within sites, females were significantly longer than male sculpin at Rose Island ($F_{1,27}$=8.8, p=0.006), Big Island ($F_{1,16}$=7.7, p=0.013), and Saglek Beach ($F_{1,19}$=22.6, $p<0.001$). Female shorthorn sculpin generally grow faster than male sculpin and attain larger sizes than males (Ennis, 1970).

Female sculpin were also significantly heavier than male sculpin ($F_{1,62}$=23.9, $p<0.001$) and a significant difference in sculpin body mass was detected between sites ($F_{2,62}$=3.4, p=0.04). The interaction term between sex and site was not significant ($F_{2,62}$=1.9, p=0.15). Comparisons between Beach and Rose Island indicated that no significant difference was observed between mass of sculpin at these sites ($F_{1,46}$=0.002, p=0.97) but female sculpin at these sites were heavier than male sculpin ($F_{1,46}$=21.5, $p<0.001$) and the interaction term between sex and site was not significant ($F_{1,46}$=1.94, p=0.17). A comparison between sculpin at Rose Island and Big Island indicated that the mass of sculpin at Rose Island was significantly higher than at Big Island ($F_{1,43}$=5.2, p=0.03) and female sculpin at these two sites were heavier than male sculpin ($F_{1,43}$=7.9, p=0.008) and the interaction term was not significant ($F_{1,43}$=0.23, p=0.63). A comparison between sculpin at Saglek Beach and Big Island indicated that the mass of sculpin at Saglek Beach was significantly higher than sculpin at Big Island ($F_{1,35}$=7.6, p=0.009) and females at these two sites were heavier than males ($F_{1,35}$=24.8, $p<0.001$). The interaction term between sex and site was significant ($F_{1,35}$=5.0, p=0.032). Analysis within sexes indicated that female sculpin at Saglek Beach were significantly heavier than females at

Big Island ($F_{1,19}$=9.2, p=0.007) but no significant difference was found in male body mass between Saglek Beach and Big Island ($F_{1,16}$=0.31, p=0.59).

No significant difference was found in condition factor between female and male sculpin ($F_{1,62}$=1.0, p=0.31) but a significant difference was found between sites ($F_{2,62}$=5.6, p=0.006). The interaction term between sex and site was not significant ($F_{2,62}$=0.35, p=0.7). A comparison between Saglek Beach and Rose Island indicated that the condition factor in sculpin from Rose Island was significantly higher than for sculpin at Saglek Beach ($F_{1,46}$=9.4, p=0.004). No significant difference was observed between sexes in this comparison ($F_{1,46}$=0.47, p=0.50) and the interaction term between sex and site was not significant ($F_{1,46}$=0.60, p=0.44). A comparison between Rose Island and Big Island indicated that the condition factor in sculpin from Rose Island was significantly higher than for sculpin at Big Island ($F_{1,43}$=4.3, p=0.04). No significant difference was observed between sexes in this comparison ($F_{1,43}$=1.6, p=0.2) and the interaction term between sex and site was not significant ($F_{1,43}$=0.03, p=0.9). No significant difference was observed in condition factor between Saglek Beach and Big Island ($F_{1,35}$=0.3, p=0.6). No significant difference was observed between sexes in this comparison ($F_{1,35}$=1.0, p=0.3) and the interaction term between sex and site was also not significant ($F_{1,35}$=0.42, p=0.5).

No significant difference was observed in HSI between sexes ($F_{1,62}$=0.48, p=0.49) or sites ($F_{2,62}$=1.2, p=0.32). The interaction term between sex and site was also not significant ($F_{2,62}$=2.1, p=0.13).

4.4.2 PCB Concentrations

Total PCB congener concentrations in sculpin liver from 2007 ranged from 9.5 ng/g to 18,000 ng/g (Table 4-3). The highest concentrations of PCBs were from Saglek Beach (4460±6020 ng/g wet weight) while total PCB concentrations in sculpin liver from Rose Island were relatively low (17±7.6 ng/g wet weight). Intermediate concentrations of PCBs were detected in sculpin from Big Island (99±70 ng/g wet weight).

4.4.3 UDP-GT, Vitamins and Liver Lipid Content

The activity of UDP-GT and the percentage liver lipid content (Table 4-4) were not related to body mass and did not differ between sexes or between sites (Table 4-5). These parameters were also not related to PCB concentrations measured in the livers (Table 4-5). Figure 4-2 presents a graph of PCB concentrations in sculpin liver and UDP-GT activity.

The concentration of vitamin E in sculpin liver (Table 4-4) was significantly different between the three sites (Table 4-5). Results of two one-tailed tests indicate that the concentration of vitamin E in sculpin liver was significantly lower at the Beach than at Big Island (G^2=5.95, p=0.005, df=1) and Rose Island (G^2=3.55, p=0.03, df=1). The relationship between sculpin sex and liver vitamin E concentration was affected by sculpin body mass as indicated by a significant interaction between mass and sex (Table 4-5). When male sculpin and female sculpin were analysed separately, it was found that vitamin E concentrations increased significantly with body mass in male sculpin (G^2=6.36, p=0.01) but this relationship was not found in female sculpin (G^2=0.49, p=0.49, df=1). The effect of PCB concentrations on vitamin E concentrations in the liver

depended on body mass as indicated by a significant interactive effect (Table 4-5). Because both body mass and PCB concentrations are both continuous explanatory variables, further analysis could not be conducted.

The concentrations of vitamin A measured as retinol, retinyl palmitate, and total retinyl esters in the livers of the sculpin (Table 4-4) decreased with body mass but did not differ between sexes or between sites (Table 4-5). Similarly, the concentrations of these vitamins were not related to PCB concentrations measured in the liver (Table 4-5).

4.4.4 Thyroid Histopathology and Histomorphometry

Simple follicular cell hyperplasia occurred in one fish from Rose Island (n=29; 3.4 % incidence) and in two fish from each of Big Island (n=18; 11.1% incidence) and Beach (n=21; 9.5% incidence). Hyperplastic tissue was comprised of extensive proliferating follicles, typically containing colloid, and with basophilic epithelial cells (Figure 4-3B). Papillary follicular cell adenoma occurred in two fish (n=21; 9.5% incidence) from Beach, but not in fish from Rose Island and Big Island. The lesions were characterized by abnormal follicular structure with pronounced involutions in a discrete nodule (Figure 4-3C). No other proliferative lesions were detected. The incidence of papillary follicular cell adenoma and simple follicular cell hyperplasia were not associated with liver PCB concentrations. The Beach sculpin with hyperplasia had concentrations of 220 ng/g and 719 ng/g while the sculpin with adenoma had liver concentrations of 12.5 ng/g and 981 ng/g. Sculpin from the Beach with particularly high concentrations of PCBs (*e.g.*, two sculpin with PCB concentrations of >10,000 ng/g) showed no evidence of thyroid adenoma or hyperplasia.

Mean thyroid epithelial cell height (Table 4-4) was not related to body mass or sex of sculpin, nor did the height differ between sites (Table 4-5). The height also was not related to PCB concentrations in the fish livers (Table 4-5).

4.5 Discussion

Shorthorn sculpin from the Beach and Big Island at Saglek Bay had elevated liver PCB concentrations compared to those from the reference site, however, within-site variability was high (*e.g.*, ranged from 12 ng/g to 18,000 ng/g at the Beach). This is consistent with historical analytical data from Saglek and is likely a reflection of differences in individual home ranges and foraging behaviours as well as influences of gender and age (Kuzyk *et al.*, 2005ab; Brown *et al.*, 2009). According to Brown *et al.*, (2009), the overall spatial trend in sculpin PCB concentrations at Saglek indicates that elevated concentrations of PCBs in sculpin from the Beach are associated with the highest sediment concentrations which are found within 500 m of the Beach.

According to Brown *et al.*, (2009), concentrations of PCBs in shorthorn sculpin at Big Island and the Beach have decreased significantly since sampling was conducted in 1998/1999. Average PCB concentrations in shorthorn sculpin, however, are still higher than what is normally observed in fish across the Arctic (Fisk *et al.*, 2005 and references within). A direct comparison is difficult because fish sampled in other areas of the Arctic (*e.g.*, Arctic charr (*Salvelinus alpinus*), Greenland cod (*Gadus ogac*)) have larger home ranges than shorthorn sculpin, which are sedentary. Measured concentrations of PCBs in longhorn sculpin liver samples from ten sites within Cambridge Bay, Northwest Territories ranged from less than 50 ng/g up to approximately 2,000 ng/g (Bright *et al.*,

1995). PCB concentrations were highest at the northern shore of Cambridge Bay where an active military radar installation (CAM-M, a former DEW-Line site) and the hamlet dump exist. The range of concentrations reported by Bright *et al.* (1995) is lower than concentrations measured at Saglek Beach.

The hepatosomatic index in this study did not differ between sites nor was there a relationship to PCB concentrations in the liver of sculpin. The lack of observed effects on hepatosomatic index is consistent with sculpin from Saglek in 1999 (Kuzyk *et al.*, 2005b). In the current study (2007), the condition factor for sculpin at Rose Island was significantly higher than the condition factor in sculpin from Big Island or Saglek Beach. Kuzyk *et al.*, (2005) did not find a significant difference in condition factor between sites in the 1999 sculpin data. While these indices have traditionally been accepted for monitoring, they have also been found to vary seasonally in fish (Yang and Bauman, 2006) and between sexes (Khan and Billiard, 2007; Yang and Baumann, 2006). Shorthorn sculpin in this study were collected at the same time of year to minimize seasonal effects. No significant difference was found in condition factors between sexes in this study.

Increased hepatosomatic index and/or decreased condition factor have been observed in winter flounder (*Pseudopleuronectes americanus*) exposed to PCBs and PAHs from a contaminated naval facility (liver $\sum$PCBs ranged from 13,500 ng/g to 83,200 ng/g) (Khan, 1999; Khan, 2004), male English sole (*Parophrys vetulus*) exposed to PCBs and PAHs (mean liver $\sum$PCBs = 2,200 ng/g) (Sol *et al.*, 2008) and rabbitfish (*Siganus oramin*) exposed to a mixture of PCBs, metals and other contaminants (Fang *et al.*, 2009a; Fang *et al*, 2009b) (muscle $\sum$PCBs ranged from 45.1 to 76.9 ng/g). These

PCB concentrations are comparable or less than concentrations observed in sculpin from Saglek. In each case, however, fish were also exposed to elevated concentrations of other contaminants such as metals and PAHs. The levels of other organic and inorganic contaminants (*e.g.*, polycyclic aromatic hydrocarbons, metals, pesticides) in the marine food web at Saglek were low compared to ambient levels in industrialized areas (ESG, 1997; ESG, 1999) so it is likely that PCBs are the sole contaminant impacting the sculpin. PCBs were not adversely affecting hepatosomatic index in sculpin at Saglek Bay.

PCBs may result in the increase of lipid content in the liver, which also increases the retention of PCBs in the liver (Matsusue *et al.,* 1999). No relationship was found between PCB exposure and liver lipid content in this study. Similarly, no relationship between body lipid and PCB exposure was observed in 1999 (Kuzyk *et al.*, 2005b).

In this study, no relationship was found between UDP-GT activity and PCBs in sculpin, nor did UDP-GT activity differ between sites. Compared to the well-studied phase I enzyme system, less research has been done on phase II transformation enzymes, particularly in Arctic biota (Fisk *et al.*, 2005). In some studies, the response of UDP-GT and Phase II enzymes has been found to be less sensitive and less responsive than Phase I enzymes (Sturm *et al.*, 1999; Solé *et al.*, 2003; Schreiber *et al.*, 2006; Verreault *et al.*, 2009). In other studies, fish exposed to PCBs had increased levels of UDP-GT (Andersson *et al.*, 1985; Ankley *et al.*, 1986; Celander *et al.*, 1994; Forlin *et al.*, 1996; Viganò *et al.*, 2001; Brown *et al.*, 2004).

A review of lab and field studies by Niimi (1996) indicates that PCBs cause cellular and biochemical changes in fish at tissue concentrations in the 1,000 ng/g range.

It is therefore surprising that induction of UDP-GT was not observed at the concentrations found in this study. This could be attributable to the number of alternate mechanisms that may compete with UDP-GT such as excretion, protection via protein binding, and secondary oxidation (Verreault *et al.*, 2009). The large number of constitutive levels of UDP-GT than EROD also complicates the relationship with contaminant levels (Schreiber *et al.*, 2006). This means that at a basal level, UDP-GT activity is higher than EROD thus making increases in activity more difficult to detect.

In this study, vitamin E was measured in sculpin liver as alpha-tocopherol, which is the major dietary and antioxidant component of vitamin E (Packer, 1991). Concentrations in sculpin livers from the Beach were significantly lower than concentrations in sculpin from the reference site. These results support other studies that indicate that vitamin E levels in fish are depressed after exposure to PCBs (*e.g.*, Palace *et al.*, 1996; Hennig *et al.*, 1999; Palace *et al.*, 2001; Brown *et al.*, 2002). The significantly lower vitamin E levels at the contaminated Beach may indicate that vitamin E is involved in protecting the sculpin against contaminant induced oxygen radical production.

No significant relationships between retinol, retinyl palmitate, and total retinyl esters and PCB exposure were observed in this study, however, there was a tendency toward decreased concentrations of retinyl esters at the more contaminated sites compared to the reference site.

The effects of PCBs on thyroid status in birds and mammals are fairly consistent resulting in an observed increase in the thyroid (*e.g.*, Sonstegard and Leatherland, 1979; Leatherland and Sonstegard, 1980; Moccia *et al.*, 1986). The mode of action in fish however is less understood. In the wild, observations of enlarged thyroids are rare with

the exception of the coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) from the Great Lakes in the 1970s to the 1990s. These fish had evidence of thyroid gland hypertrophy and hyperplasia, however, no correlation to tissue levels of PCBs was found nor did salmon or rainbow trout (*Oncorhynchus mykiss*) fed diets containing PCBs develop any thyroid enlargement (Leatherland and Sonstegard, 1982; Leatherland, 1992; Leatherland, 1993).

Experimentally, hyperplasia has been observed in lake trout (*Salvelinus namaycush*) exposed to PCBs (Brown *et al.*, 2004) but not in PCB exposed rainbow trout (*Oncorhynchus mykiss*) (Brown *et al.*, 2002), mink (*Mustela vison*) (Martin *et al.*, 2006), or Arctic grayling (*Thymallus arcticus*) (Palace *et al.*, 2001). Because many of the effects of PCBs on the thyroid are considered transitory, it is often difficult to determine if minor proliferation has occurred (Brown *et al.*, 2004). Using measurements of a sample of follicles of varied sizes to be representative of the entire gland when the heterogenous nature of fish thyroid tissue is known can also present errors (Eales and Brown, 1993).

The results of this study indicated no relationship between thyroid epithelial cell height and PCB exposure. It is likely that the PCBs are not adversely affecting the thyroid and this is supported by the lack of relationships with other biomarkers. Evidence from wildlife field and laboratory studies support a relationship between alterations in UDP-GT and thyroid status. Glucuronidation catalyzed by UDP-GT inactivates thyroid hormones, increases their solubility, and facilitates their excretion in bile and urine (Visser, 1990). Exposure to PCBs has been shown to increase UDP-GT activity and thus increase glucuronidation of hepatic thyroxine (Barter and Klaasen,

1994; Hood *et al.*, 2003; Brown *et al.*, 2004). The decrease in circulating thyroxine causes an increase in overall demand for thyroxine which leads to stimulated thyroid function and growth (hypertrophy and hyperplasia), endocytosis of collagen, and increased secretion of thyroxine (Capen and Martin, 1989). The lack of any effect on thyroid epithelial cell height in this study is consistent with the lack of effects on UDP-GT.

Sculpin from the Beach showed a slightly higher incidence of simple follicular cell hyperplasia and papillary follicular cell adenoma in thyroid, however, the incidence of adenoma and hyperplasia was not associated with the highest PCB concentrations. Histology of the thyroid provides a sensitive index of chronic change in the thyroid function at the level of the thyroid tissue itself (Eales and Brown, 1993), however, a xenobiotically induced change in fish thyroid function has yet to be causally linked to decreased fitness or survival (Brown *et al.*, 2004). Even the salmon from the Great Lakes with enlarged thyroids appeared to have normal growth and development (Rolland, 2000). Elevated concentrations of PCBs at Saglek Bay are not affecting thyroid form or function in sculpin.

4.5.1 Conclusions

A suite of biomarkers at the biochemical level (UDP-GT, vitamin A, vitamin E), the tissue level (thyroid histomorphometry, liver lipid, hepatosomatic index), and the organism level (body condition index) were used to assess changes in an ecosystem following remediation. An integrated monitoring program needs to consider biomarkers at different levels of organization, thus providing early detection of environmental stress

as well as insight into causal relationships between exposure to contaminants and effects at higher levels of organization (Adams *et al.*, 1990). Changes at the molecular, biochemical, or physiological level can be used as early warnings of contaminant exposure. Effects observed at the individual or population level are less reversible, more detrimental, and of greater ecological relevance (de Wit *et al*, 2004). The importance of considering multiple species in monitoring studies is also emphasized. Black guillemot were also studied at Saglek in 1999 and again in 2007. Findings on biomarkers in these birds will be reported elsewhere.

Differences in biomarkers in sculpin at Big Island and the Beach compared to the reference site are absent or minor with the exception of liver vitamin E concentrations and body condition. It is interesting that the relatively higher concentrations of PCBs at the Beach are not having adverse effects on the tissue level and organism level biomarkers studied here when other studies indicate biological effects in fish with comparable concentrations of PCBs. There are a number of other sites in the Canadian Arctic (*e.g.*, Lake Laberge) where levels of organochlorine contaminants are higher than threshold levels for possible effects but population level effects have not been observed (Fisk *et al.*, 2005). The decrease in concentrations of vitamin E in highly exposed sculpin at the Beach and Big Island may indicate that vitamin E is involved in protecting the sculpin against contaminant induced oxygen radical production.

It is recommended that future monitoring at Saglek include a suite of biomarkers supported with chemical residue analysis in sediment and biota. Based on the results of this biological effects study as well as the study by Kuzyk *et al.*, (2005b), measures of oxidative stress and antioxidant defense mechanisms should be included in future

monitoring of the marine ecosystem at Saglek Bay. This would include mixed function oxidases, enzymatic antioxidants (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic antioxidants (vitamin A and vitamin E) important in the detoxification of oxygen radicals.

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Table 4-2. Summary of physical data (mean ± standard deviation) for shorthorn sculpin

Parameter	Site		
2007			
Saglek Fiord, Rose Island Site			
	Male (n=10)	Female (n=19)	Total (n=29)
Mass (g)	264±93.6	389±163	346±153
Length (mm)	257±24.9	296±37.0	280±38.0
Hepatosomatic Index	2.89±0.76	2.92±1.17	2.91±1.03
Condition Factor	1.51±0.21	1.43±0.17	1.46±0.18
Saglek Fiord, Big Island			
	Male (n=7)	Female (n=11)	Total (n=18)
Mass (g)	194±47.5	283±91.0	249±87.8
Length (mm)	240±19.5	275±29.9	262±31.3
Hepatosomatic Index	3.0±0.79	2.55±1.17	2.72±1.03
Condition Factor	1.39±0.085	1.33±0.15	1.36±0.13
Saglek Fiord, Saglek Beach Site			
	Male (n=11)	Female (n=10)	Total (n=21)
Mass (g)	211±71.0	445±149	323±164
Length (mm)	250±29.0	320±38.0	283±48.1
Hepatosomatic Index	2.79±1.08	3.8±1.52	3.28±1.37
Condition Factor	1.31±0.16	1.32±0.14	1.32±0.15

[a]K = Fulton's Condition Factor
[b]HSI = Hepatosomatic Index

Table 4-3. Total PCB concentrations (total congeners; mean ± standard deviation) in shorthorn sculpin liver samples

	2007		
Fiord/Site	Rose Island	Big Island	Beach
n	9	7	12
Total PCBs[a] (ng/g wet weight)			
Range	9.5-34	29- 241	12-18000
Average Total PCB Concentration	17±7.6	99±70	4460±6020
Most abundant PCB congeners[a] (ng/g wet weight)			
PCB153	4.8±1.6	24±19	1100±1400
PCB138/163/164	2.3±0.9	13±9.8	557±720
PCB180	2.5±1.2	19±16	853±1150

[a]Where congener was not detected, half the estimated quantitation limit (EQL) was used to calculate means.

Table 4-4. Summary of biomarker results for shorthorn sculpin from three sites within Saglek fiord: Rose Island, Big Island and Beach in August 2007.

Parameter	Sex	Rose Island	Big Island	Beach
Thyroid epithelial cell height (μm)[a]	Total	12.3 ± 2.9 (27) [7.0 – 17.8]	11.6 ± 2.1 (17) (6.5 – 17.0)	12.6 ± 3.6 (19) [6.9 – 20.2]
	Male	11.1 ± 3.43 (10) (7.0 – 17.7]	10.2 ± 1.9 (6) [6.49 – 11.5]	11.0 ± 2.8 (9) [6.9 – 15.3]
	Female	13.1 ± 2.4 (17) [9.2 – 17.8]	12.3 ± 2.0 (11) [10.1 – 17.0]	14.1 ± 3.7 (10) [8.4 – 20.2]
UDP-GT (nmol min^{-1} mg^{-1})[a]	Total	0.14 ± 0.09 (26) [0.007 – 0.36]	0.12 ± 0.07 (15) [0.01 – 0.26]	0.13 ± 0.11 (19) [0.0008 – 0.43]
	Male	0.17 ± 0.11 (10) [0.02 – 0.36]	0.11 ± 0.08 (6) [0.02 – 0.26]	0.14 ± 0.14 (9) [0.0008 – 0.43]
	Female	0.13 ± 0.08 (16) [0.007 – 0.31]	0.13 ± 0.07 (9) [0.013 – 0.26]	0.12 ± 0.07 (10) [0.007 – 0.22]
Tocopherol[b] (μg/g)	Total	25.8 (26) [1.4 – 262.0]	14.9 (18) [2.7 – 53.4]	11.7 (17) [1.1 – 298]
	Male	38.8 (10) [1.4 – 262]	12.6 (7) [2.7 – 53.4]	15.7 (6) [3.5 – 298]
	Female	20.0 (16) [1.9 – 92.5]	16.6 (11) [3.6 – 49.3]	9.1 (9) [1.1 – 85.5]
Retinol (μg/g)[a]	Total	2.6 ± 1.4 (26) [0.7 – 7.0]	2.1 ± 0.9 (18) [0.6 – 4.1]	2.3 ± 1.7 (17) [0.5 – 6.7]
	Male	3.0 ± 1.6 (10) [1.4 – 7.0]	1.8 ± 0.6 (7) [1.0 – 2.7]	3.2 ± 2.0 (6) [1.5 – 7.0]
	Female	2.4 ± 1.3 (16) [0.7 – 5.0]	2.2 ± 1.0 (11) [0.6 – 4.1]	1.4 ± 0.5 (9) [0.5 – 2.3]
Retinyl palmitate (μg/g)[a]	Total	38.6 ± 38.2 (26) [6.6 – 214]	30.4 ±14.6 (18) [9.9 – 61.6]	29.8 ± 17.0 (17) [10.3 – 73.0]
	Male	48.7 ± 58.9 (10) [17.4 – 214]	28.7 ± 17.6 (7) [9.9 – 54.8]	30.6 ± 20.3 (6) [10.3 – 73.0]
	Female	32.3 ± 15.4 (16) [6.6 – 57.4]	31.5 ± 13.1 (11) [16.5 – 61.6]	29.2 ± 14.6 (9) [12.6 – 57.6]
Total retinyl esters (μg/g)[a]	Total	90.8 ± 86.4 (26) [13.4 – 432]	61.9 ± 28.7 (18) [21.6 – 124]	61.3 ± 34.8 (17) [19.2 – 146]
	Male	106 ± 118 (10) [35.9 – 432]	56.0 ± 33.7 (7) [21.6 – 105]	61.6 ± 39.3 (6) 19.2 – 146]
	Female	81.6 ± 61.9 (16) [13.4 – 261]	65.7 ± 26.0 (11) [36.3 – 124	60.9 ± 32.8 (9) [25.3 – 125]
Lipid (%)[a]	Total	12.9 ± 6.1 (9) [6.8-22.8]	12.7 ± 2.0 (7) [9.8-16.1]	11.0 ± 3.6 (12) [5.8-17.7]
	Male	17.9 ± 5.7 (4) [9.6-22.8]	14.7 ± 1.9 (2) [13.4-16.1]	13.3 ± 2.8 (4) [9.5-16.0]
	Female	8.9 ± 2.0 (5) [6.8-12.1]	11.9 ± 3.0 (5) [9.8-13.5]	9.9 ± 3.6 (8) [5.8-17.7]

[a] Values are arithmetic mean ± standard deviation with sample size in parentheses and range in square brackets

[b] Geometric mean

Table 4-5. Effect of body mass, sex, site and liver PCB concentration on biomarker responses of shorthorn sculpin from three sites within Saglek fiord: Rose Island, Big Island and Beach in August 2007.

Source	df	Thyroid Epithelial Cell Height [a] (μm)	UDP-GT [a] (nmol min^{-1} mg^{-1})	Vitamin E [b] (μg/g)	Vitamin A[a,c] (μg/g)	Lipid [a] (%)
PCB Models						
Mass	1	0.27 (0.61)	1.44 (0.25)	2.63 (0.10)	3.75 (0.032)*	0.49 (0.49)
Sex	1	0.004 (0.95)	2.36 (0.14)	5.30 (0.021)*	1.14 (0.36)	1.12 (0.3)
PCB	1	0.11 (0.74)	0.001 (0.97)	2.22 (0.14)	1.29 (0.31)	0.84 (0.37)
Mass*Sex	1	0.13 (0.73)	2.50 (0.13)	4.32 (0.038)*	0.17 (0.91)	0.08 (0.78)
Mass*PCB	1	0.034 (0.85)	0.093 (0.76)	8.30 (0.004)*	1.32 (0.30)	0.30 (0.59)
Sex*PCB	1	0.025 (0.87)	0.11 (0.75)	0.001 (0.97)	1.36 (0.29)	0.15 (0.70)
Site Models						
Mass	1	0.93 (0.34)	0.25 (0.62)	0.034 (0.85)	0.034 (0.59)	0.025 (0.88)
Sex	1	0.073 (0.79)	3.07 (0.086)	7.56 (0.006)*	0.085 (0.18)	1.71 (0.21)
Site	2	0.89 (0.42)	2.07 (0.14)	6.32 (0.042)*	0.11 (0.36)	0.21 (0.81)
Mass*Sex	1	1.30 (0.26)	2.14 (0.15)	4.64 (0.031)*	0.048(0.43)	0.17 (0.69)
Mass*Site	2	1.01 (0.37)	1.48 (0.24)	5.32 (0.07)	0.070 (0.67)	0.051 (0.95)
Sex*Site	2	0.076 (0.93)	0.009 (0.99)	4.09 (0.13)	0.91(0.49)	1.47 (0.25)

[a] Values are F-ratios with p-values in parentheses
[b] Values are deviance with p-values in parentheses
[c] Vitamin A includes retinol, retinyl palmitate and total retinyl esters
* indicates p-value is less than 5%

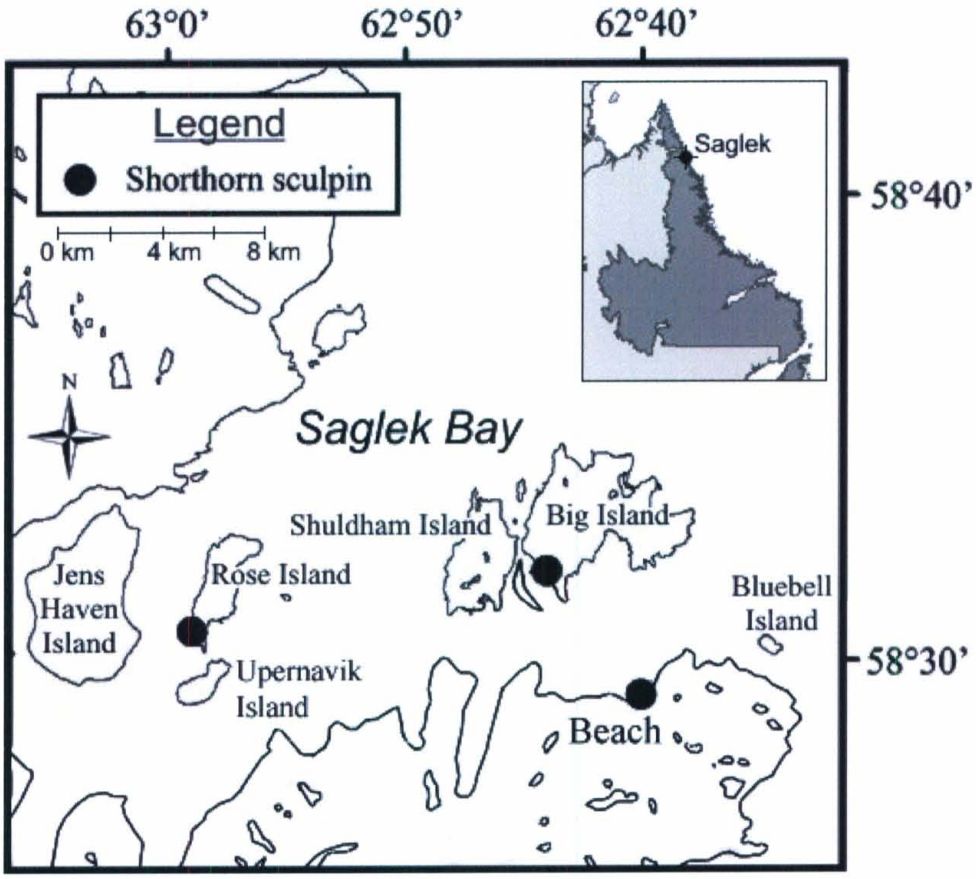

Figure 4-1. Map of Saglek Bay, Labrador showing locations of shorthorn sculpin collections.

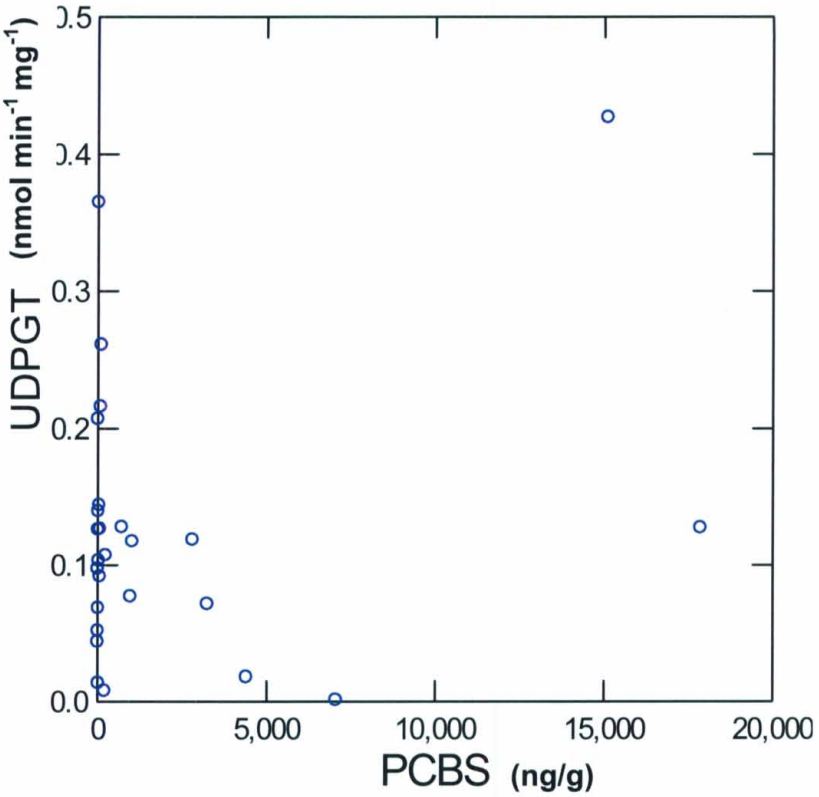

Figure 4-2. Graph of UPD-GT activity (nmol min^{-1} mg^{-1}) and liver PCB concentrations (ng/g) in shorthorn sculpin at Saglek Bay (2007).

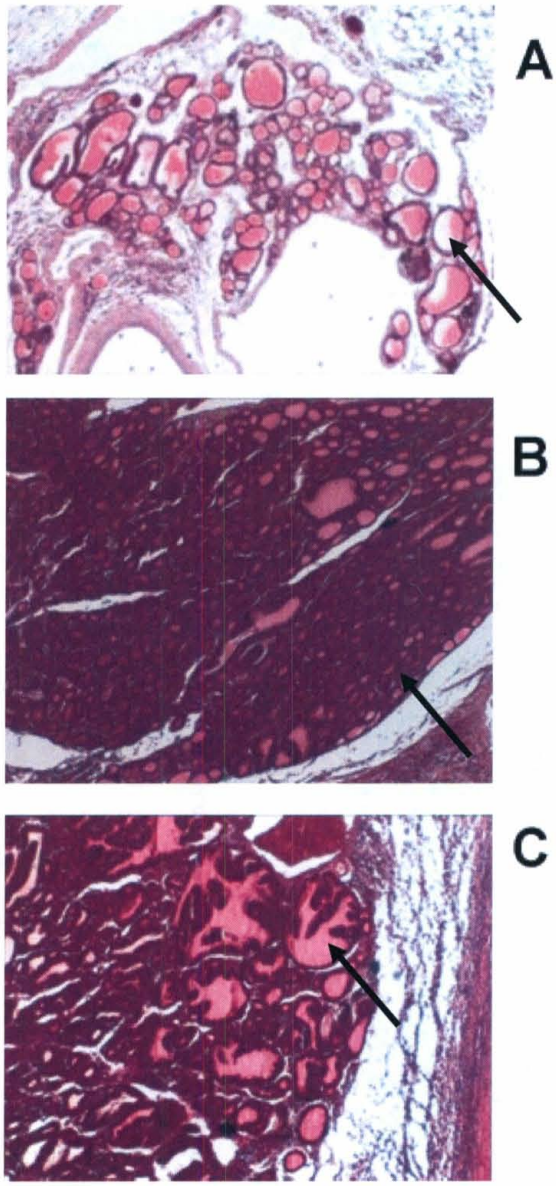

Figure 4-3A. Photomicrographs of a thyroid section of a shorthorn sculpin from the Rose Island reference site (magnification = 100x). Arrow indicates an example of a normal thyroid follicle which are comprised of a single layer of cuboidal to columnar epithelial cells arranged in colloid-filled spheroids.

Figure 4-3B. Photomicrograph of simple follicular cell hyperplasia in the thyroid of a shorthorn sculpin from Saglek Beach. Arrow indicates where the lesion is characterized by an increased number of colloid-containing follicles that are proliferating in the pharyngeal region. Epithelial cells are strongly basophilic (magnification = 100x).

Figure 4-3C. Photomicrograph of papillary cell follicular adenoma in the thyroid of a shorthorn sculpin from Saglek Beach. Arrow indicates where the lesion is characterized by structurally complex atypical follicle growth in a nodular form (magnification = 100x).

5.0 Chapter 5: Biological Effects of Point Source Polychlorinated Biphenyl Contamination on Black Guillemot (*Cepphus grylle*) at Saglek, Labrador

5.1 Abstract

In Northern Canada, PCBs were used during the operation of military facilities such as DEW-Line, Pinetree-Line, and Polevault stations. PCBs in northern environments are a particular concern due to their ability to accumulate in tissues of organisms and magnify within food webs. The effects of PCBs on Black guillemots (*Cepphus grylle*) is currently being studied at Saglek Bay, a former Polevault Line military radar facility in northern Labrador where PCBs have contaminated marine sediments and the coastal food web. A suite of biomarkers including bone mineral density, phase I biotransformation enzyme activity, and phase II biotransformation enzyme activity were studied in Black guillemot at three sites in Saglek Bay: the Beach Group (n=12) within 4 km of the beach (geometric mean PCBs in guillemot liver = 130 ng/g wet weight), the Islands Group (n=10) located on two unnamed islands approximately 5-6 km offshore from the beach (geometric mean PCBs in guillemot liver = 16 ng/g wet weight), and a Reference Group (n=13) located on three islands approximately 16-18 km northwest of the beach (geometric mean PCBs in guillemot liver = 8.0 ng/g wet weight). Despite the elevated concentrations of PCBs in Black guillemot liver at the Beach, bone mineral density and phase I and II biotransformation enzymes did not show a significant relationship to PCBs nor did biomarker responses differ between sites.

5.2 Introduction

Polychlorinated Biphenyls (PCBs) are a class of non-polar, semi-volatile chemicals that were manufactured for use in electrical transformers, capacitors, paint plasticizers, and insulating fluids (Safe, 1994). The production of PCBs is now prohibited in many countries due to their adverse effects on humans and wildlife, but because of their chemically stable and persistent properties, they are still found in the environment. In Northern Canada, PCBs were used during the operation of military facilities such as DEW-Line, Pinetree-Line, and Polevault stations. Historic waste disposal practices and the subsequent abandonment of many of these sites have resulted in high levels of PCBs in soil, sediment, water, and biota in their vicinity. Military stations are believed to be the most significant local sources of PCBs to Northern Canada (Stow *et al.*, 2005). Short range transport creates a halo of contamination around the point sources (Bright *et al.*, 1995; Dushenko *et al.*, 1996) and augments impacts due to long range atmospheric transport, and to a lesser extent, via ocean currents and rivers (Fisk *et al.*, 2005).

The occurrence of highly persistent pollutants such as PCBs in Arctic environments is a concern due to their ability to accumulate in tissues of organisms and magnify within food webs (Skaare *et al.*, 2002; Fisk *et al.*, 2005). Over the past thirty years, considerable knowledge has accumulated on the occurrence, concentrations, and distribution of contaminants (*e.g.*, Muir *et al.*, 1992; Muir *et al.*, 1999; Braune *et al.*, 2005) but less is known about the toxicology and biological effects of contaminants in Northern and Arctic environments (Chapman and Loehr, 2003; Fisk *et al.*, 2003; Riddle and Chapman, 2003; Snape *et al.*, 2003; Fisk *et al.,* 2005). Research on avian species and

contaminants in particular has generally focused on determination of contaminant levels in the tissue and the eggs of birds. While these studies are essential to establish the concentrations present, clearly linking the contaminants in birds and eggs to specific biological effects is also necessary. The biological effects of contaminants such as dichlorodiphenyltrichloroethane (DDT) on avian species have been well-studied, partly due to the observation of eggshell thinning and the subsequent reduction in the populations of birds of prey (Blus *et al.*, 1996). The reproductive effects of PCBs and dioxin-like compounds are also well studied in birds. Like many other biological effects investigations, however, these studies were primarily carried out in temperate regions such as the Great Lakes, and cannot be confidently applied to Arctic ecosystems. Arctic ecosystems may be more vulnerable to organic contaminants such as PCBs because of the dominant role of lipids resulting from the seasonality of food availability (Alexander, 1995; Riddle and Chapman, 2005) as well as shorter food chains and thus, lack of functional redundancy (Chapman and Riddle, 2003; 2005). At the low temperatures characteristic of the Arctic, metabolic rates are slower and energy use is lower than in biota from more temperate regions (Chapman and Riddle, 2005). This means that organisms may accumulate contaminants at a slower rate but the dominant role of lipids in these animals means that they may take up more of a lipophilic contaminant than in other regions (Chapman and Riddle, 2005). Lower energy usage may mean that less energy is available for detoxification processes (Chapman and Riddle, 2005). In addition, impending climate warming may have possible effects on contaminants dynamics (Fisk *et al* 2003).

A site in Northern Canada where the biological effects of PCBs on avian receptors are being studied is Saglek Bay, a former Polevault Line military radar facility in northern Labrador (Figure 5-1). Extensive polychlorinated biphenyl (PCB) contamination in soil and sediment associated with the original facility was identified at Saglek in the 1990s (ESG, 1997). Soil remediation was carried out between 1997 and 1999 and thus, the terrestrial source has been removed. Initial residue analysis work conducted concurrently with the remediation at Saglek found extensive PCB contamination in the marine sediments and the coastal food web in Saglek Bay, particularly in those species that feed on or near the seabed, and with limited foraging ranges (Kuzyk *et al*, 2005a). At Saglek Beach in 1999, concentrations of PCBs were particularly elevated in shorthorn sculpin (*Myoxocephalus scorpius*) ranging from 3954 to 41000 ng/g wet weight (based on data published by Brown *et al*., (2009)) and black guillemot (*Cepphus grylle*) nestlings ranging from 170 to 6200 ng/g wet weight (Kuzyk *et al*., 2003). To support an effects-based study of the marine foodweb in 1999, Kuzyk *et al*., (2003; 2005b) measured biological endpoints in Black guillemot at Saglek (ethoxyresorufin-*O*-deethylase (EROD), vitamin A, malic enzyme, and porphyrin in liver) and shorthorn sculpin (EROD activity in liver, lipid content, and relative liver mass). Increased EROD activity and decreased vitamin A concentrations in Black guillemot liver as well as increased EROD activity in shorthorn sculpin liver were found in association with the elevated concentrations of PCBs.

A follow-up study was carried out in 2006/07 and showed that average PCB concentrations in the nearshore sediment have decreased eleven-fold, while PCB concentrations (lipid weight) in shorthorn sculpin (*Myoxocephalus scorpius*) at Saglek

Beach have decreased nineteen-fold (Brown *et al.*, 2009). Concentrations of PCBs in Black guillemot (*Cepphus grylle*) nestlings (lipid weight) from the east side of Saglek Beach have decreased twenty-fold while PCB concentrations in nestlings from the west side of Saglek Beach did not show a significantly different change over time (Brown *et al.*, 2009). In addition to the PCB analysis, a suite of biomarkers was analyzed in Black guillemots at Saglek Bay in 2007. This chapter examines the associations between PCB exposure and the following biomarkers in Black guillemot at Saglek Bay in 2007: bone mineral density, phase I biotransformation enzymes (mixed function oxidases), and phase II biotransformation enzymes (uridine diphosphate-glucuronyltransferase (UDP-GT). Phase I biotransformation enzymes were measured in black guillemots during previous biomarker work in 1999 (Kuzyk *et al.*, 2003) while bone mineral density and phase II biotransformation enzymes are being studied at Saglek for the first time. The black guillemot biomarker data from Kuzyk *et al.*, (2003) was not available. Therefore, no temporal comparisons of mixed function oxidase activity could be conducted. Other biomarkers were measured in black guillemots and will be reported elsewhere (Burgess *et al.*, in prep).

Black guillemots were chosen as a species of concern at Saglek because piscivorous birds are considered particularly vulnerable to organic contaminants due to their position at the top of aquatic food chains and because of their inshore and benthic feeding habits (Asbirk, 1978). Their limited foraging ranges (approximately 0.4 to 4 km) (Cairns, 1987) and their habit of returning to the same nests year after year makes them suitable as study species. Black guillemot have also been found to tolerate investigator disturbance (Cairns, 1980).

The results of this study and those of Burgess *et al.*, (in prep) provide a basis for assessing the ecological risk of PCBs to black guillemot and other marine piscivorous birds at Saglek. The study also provides information on biological effects/biomarkers in Arctic wildlife that respond to PCBs and that may be applied for ecological risk assessments at other sites in Northern Canada.

5.3 Methods

5.3.1 Sample Collection

Active black guillemot nests were identified in and around Saglek Bay during July and August, 2007. A total of thirty-five nests were visited every two to three days, weather permitting, to monitor hatching success and establish hatching dates. The distribution of nests fell into three groups as per Kuzyk *et al.* (2003), these being the Beach Group (n=12) within 4 km of the beach (*i.e.*, the source of PCBs - highly contaminated), the Islands Group (n=10) located on two unnamed islands approximately 5-6 km offshore from the beach (moderately contaminated), and a Reference Group (n=13) located on three islands approximately 16-18 km northwest of the beach (Figure 5-1). The foraging range of a nesting adult guillemot is estimated to be approximately 0.4 to 4.0 km and the diet of their nestlings is primarily fish (Bradstreet and Brown, 1985). The PCB concentrations in black guillemot nestlings at Saglek Bay have been shown to correspond closely to the sediment concentrations collected within a 500 m radius (estimated home range of black guillemots at Saglek (ESG, 2002)) (Brown *et al.*, 2009). Therefore, it is unlikely that the home ranges of the guillemot from the Beach Group, the Islands Group, and the Reference Group overlap.

Hatching dates were established and nestlings were identified with leg bands bearing a unique number. Body size measurements were collected at the time of nest visits. Nestling ages were also estimated using linear regression of head-bill measurements from known-age nestlings (Burgess, unpublished).

When the nestlings were three to four weeks of age, one individual was removed from each nest, decapitated using a guillotine, allowed to bleed out, and immediately dissected. Sex was determined by gonad examination. The liver was removed and weighed to the nearest 0.1 g on a top loading battery powered balance. The left lobe of the liver was finely minced on ice to a homogenous consistency. Two 2-g portions were placed in separate 2 mL cryogenic vials and preserved immediately on liquid nitrogen for biochemical assays. The right lobe of the liver was frozen for PCB analysis. The femur was excised and cleaned of any remaining tendons using dissecting instruments, and frozen for bone mineral density measurements.

5.3.2 PCB Analysis

PCB analyses were conducted on all black guillemot liver samples by AXYS Analytical Services Ltd. in Sidney, British Columbia. Methods are summarized in Appendix 1. Briefly, concentrations of 91 PCB congeners were determined using high resolution gas chromatography with detection by low-resolution mass spectrometry (HRGC/LRMS). Homogenized samples were spiked with isotopically labelled surrogate standards, extracted, and the extract separated into fractions using Florisil® (a highly selective adsorbent also referred to as magnesium silicate). Fraction one was analyzed for PCBs using high-resolution gas chromatography with detection by low-resolution

mass spectrometry (HRGC/L RMS). Sample-specific detection limits were determined from the analysis data by converting the minimum detectable signal to a concentration by the same procedures used to convert target peak responses to concentrations. Total concentrations of PCBs reported for black guillemot are reported on a wet weight basis and represent the sum of the congeners. Congener concentrations less than the detection limit were replaced with one half of the detection limit.

AXYS uses the batch method whereby samples are worked up in batches composed of nine or fewer samples along with one QA/QC sample (certified reference material or internal spiked matrix), one analytical blank, and one analytical duplicate. Spiked material was used in cases where analytical results were expected to be outside the concentration ranges of the available certified reference materials. Recoveries of 53 congeners (three samples) ranged from 84% to 107%, average = 97±3.9%. Three blank samples were analyzed and results were below the detection limits for PCB Aroclors and for most congeners. Results for some congeners in the blanks (two to five from each) were reported as NDR, indicating low level peaks were detected but did not meet the quantification criteria to be identified as the congeners. One blank showed detectable concentrations of three congeners but the blank results were within the AXYS method control limit of 1 ng absolute per congener. Five guillemot liver samples were analyzed in duplicate and the average relative standard deviation was 3.7%. AXYS considers acceptable limits to be less than 30% relative standard deviation with 20% or less considered good agreement.

5.3.3 Bone Mineral Density

Bone mineral density (BMD) was measured using the PIXImus 2 Bone Densitometer (General Electric Lunar, Madison, WI) and analyzed with PIXImus software version 2.1 at the Faculty of Medicine-Endocrinology at Memorial University of Newfoundland and Labrador. The PIXImus II utilizes Dual Energy X-ray Absorptiometry (DXA) technology. A small x-ray source exposes the entire animal to a cone shaped beam of both high and low energy x-rays. A high-resolution digital picture (0.18 x 0.18 mm) is taken of an image of the x-rays hitting a luminescent panel. The ratio of attenuation of the high and low energies allows the PIXImus to separate bone from tissue and, from within the tissue samples, the lean and fat. The PIXImus measures bone mineral content (BMC) for each pixel of the scanned image and then calculates BMD by dividing the average BMC by the bone area represented by the region of interest (ROI) which is specified by the user. A standard phantom (fat 11.9% and BMC 0.063 g) was used to calibrate the PIXImus on a daily basis prior to measurements.

Bone mineral density was measured in the femur of the black guillemots. The entire femur was selected as the ROI so that the BMD represents the average bone density of the entire bone. Each bone was placed in the same position and same area of the positioning pad for each scan. Repeatability of bone mineral density measurements were verified by blindly re-measuring a subset of bones and conducting a two-way ANOVA considering sample and trial, which indicated no statistically significant difference between datasets ($F_{1,9} = 1.91$, $p=0.20$). All bone mineral density measurements were conducted without prior knowledge of the levels of PCBS and all samples bore

sample numbers that contained no information about their collection site or PCB exposure level.

5.3.4 Biochemical Assays

5.3.4.1 Microsome Preparation

To prepare the microsomes, liver samples were homogenized in 4 mL of cold 0.1 M Tris hydrogen chloride (HCl) homogenization buffer (pH = 7.6 at 25°C) using a polytron homogenizer. Centrifugation of 3.5 mL of homogenate at 4°C for ten minutes at 28,000 *g* yielded a supernatant that was recovered and recentrifuged at 103,000 *g* for ninety minutes at 4°C. After this centrifugation, the supernatant was aspirated off the microsomal pellet which was resuspended in 1.2 mL of cold 0.05 M Tris-HCl resuspension buffer (pH = 7.4 at 25°C). All processing was performed on ice and resuspended microsomes were frozen at -90°C in 1.5 mL microcentrifuge tubes until assays were performed. Protein content of the microsomes was determined using the Bradford method (Bradford, 1976).

5.3.4.2 Phase I Enzyme (Mixed Function Oxidase) Activity

Mixed function oxidase enzymes activity was measured as 7-ethoxyresorufin deethylase (EROD) activity. Analyses were conducted by National Wildlife Research Centre in Hull, Quebec. EROD activity (pmol resorufin/mg protein min) was measured using an adaptation of the method of Kennedy and Jones (1994), optimized for avian microsomes (Trudeau and Maisonneuve, 2001). The assays were conducted in 48 well plates and the reaction product (resorufin) was measured with a fluorescence plate reader.

5.3.4.3 Phase II Enzyme (Uridine diphosphate glucuronyltransferase (UDP-GT)) Activity

UDP-GT activity was assayed in duplicate for each sample using a modification of the method by Clarke *et al.* (1992). A blank was prepared for each sample set. A blank was prepared for each sample set and every tenth sample was assayed twice for QA/QC purposes. It was ensured that variability in the duplicates varied by less than 10%. UDP-GT assays were carried out at room temperature (19-21˚C) in 0.1 M tris buffer at pH 7.2, in the presence of magnesium chloride hexahydrate. Briefly, microsomes were preincubated for 10-15 minutes on ice with the non-ionic detergent Lubrol (0.24 mg/mg microsomal protein) to disrupt microsomal vesicles. The assay was initiated by the addition of 25 µL of 4-nitrophenol (pNP) (600 nM) and run for twenty minutes at room temperature in a metabolic shaker. The reaction was stopped by the addition of 0.5 mL of ice cold trichloroacetic acid (TCA) followed by centrifugation at 2,000 *g* for ten minutes. The supernatant was recovered and recentrifuged at 10,000 *g* for ten minutes followed by alkalinisation with 2M sodium hydroxide. The remaining pNP was measured spectrophometrically at 405 nm.

5.3.5 Statistical Analysis

Relationships between variables were initially determined using the general linear model which includes analysis of variance (ANOVA), analysis of covariance (ANCOVA), and linear regression. In this case, errors were checked to confirm normality, homogeneity and independence. For the models involving bone mineral density and UDP-GT as response variables, the assumptions of the general linear model (normal error distribution) were satisfied and the F-ratio and p-value were reported. The

assumptions of the general linear model (normal error distribution) were not satisfied for the models with EROD as the response variable. In this case, the model was revised and the generalized linear model for non-normal errors (gamma distribution with log link) was used and the assumptions were verified again. The deviance (G-statistic) and p-value are reported for the models involving EROD. The G statistic follows a chi-square distribution with the degrees of freedom equal to the number of parameters in the model minus one. The effects of the explanatory variables (body mass, age, sex, and liver PCB concentration) on the response variables (bone mineral density, EROD activity, and UDP-GT activity) were initially modelled. A separate model for body mass, age, sex, and collection site (Reference, Islands and Beach) was also developed to determine differences between sites. Interaction terms were also included in the model (refer to results Table 5-2 for summary of interaction terms included in models). Interaction terms were examined before tests were interpreted. As per examples in Sokal and Rolf (1995), interaction terms were not removed from the models if they were not significant. Body mass and age are expected to be correlated but including both variables in the model will remove variation associated with both.

5.4 Results

A total of thirty-five black guillemot nestlings were collected: thirteen from the Reference group, twelve from the Beach group, and ten from the Islands group. Fourteen nestlings were male and twenty-one were female. The ages of the nestlings ranged from 17 days to 36 days. The age of the guillemot nestlings did not differ between sex ($F_{1,22}$=2.61, p=0.12) or site ($F_{2,22}$=0.16, p=0.85) but age was related to body mass ($F_{1,22}$=25.7, p=<0.001). The interaction terms between sex and mass ($F_{1,22}$=2.91,

p=0.10), site and sex ($F_{2,22}$=1.43, p=0.26) and site and mass ($F_{2,22}$=0.22, p=0.80) were not significant. The body mass of the nestlings ranged from 225 g to 372 g. The mass of the guillemot nestlings did not differ between sex ($F_{1,22}$=2.95, p=0.10) or site ($F_{2,22}$=1.53, p=0.24) but mass was related to age ($F_{1,22}$=28.4, p=<0.001). The interaction terms between sex and age ($F_{1,22}$=2.45, p=0.13), site and age ($F_{2,22}$=1.20, p=0.32) and site and sex ($F_{2,22}$=0.11, p=0.89) were not significant.

5.4.1 PCB Concentrations

The concentrations of total PCBs in the livers of the guillemots in 2007 ranged from 4.0 ng/g (wet weight) to 1110 ng/g (wet weight) (Table 5-1). The concentrations of PCBs in guillemots from 2007 are based on concentrations reported by Brown *et al.*, (2009) but the statistical analysis that follows was generated by the student specifically for this thesis. The concentration of total PCBs did not differ significantly between sex ($F_{1,17}$=0.068, p=0.80) but a significant relationship was found between PCB concentrations and age ($F_{1,17}$=14.3, p=0.001) as well as PCB concentrations and mass ($F_{1,17}$=16.8, p=0.001). A significant difference in PCB concentrations was also found between sites ($F_{2,17}$=5.60, p=0.014). The interaction terms between sex and age ($F_{1,17}$=1.47, p=0.24), sex and mass ($F_{1,17}$=1.28, p=0.27) as well as site and sex ($F_{2,17}$=0.83, p=0.45) were not significant. The interaction terms between mass and age ($F_{1,17}$=15.6, p=0.001), site and age ($F_{2,17}$=8.68, p=0.003) and site and mass ($F_{2,17}$=8.67, p=0.003) were significant. Therefore, the sites were analysed separately.

Analysis of the reference site indicated that PCB concentrations were related to age ($F_{1,5}$=10.2, p=0.024) and mass ($F_{1,5}$=7.54, p=0.041) but did not differ between sexes

($F_{1,5}$=1.49, p=0.28). The interaction terms between sex and age ($F_{1,5}$=0.003, p=0.95) and between sex and mass ($F_{1,5}$=0.16, p=0.71) were not significant. The interaction term between mass and age was significant ($F_{1,5}$=9.53, p=0.027). No further analysis of the interaction between mass and age could be conducted because both are continuous variables.

Analysis of the Islands site indicated that PCB concentrations were not related to age ($F_{1,2}$=1.65, p=0.33), mass ($F_{1,2}$=0.42, p=0.58) or sex ($F_{1,2}$=1.16, p=0.39). The interaction terms between mass and age ($F_{1,2}$=0.86, p=0.45) and sex and mass ($F_{1,2}$=12.6, p=0.071) were not significant. The interaction term between sex and age was significant ($F_{1,2}$=29.1, p=0.033). Further analysis among sexes at the Islands was not conducted due to the small sample size (6 females and 3 males).

Analysis of the Beach site indicated that PCB concentrations were not related to age ($F_{1,4}$=0.27, p=0.63), mass ($F_{1,4}$=0.73, p=0.44) or sex ($F_{1,4}$=0.27, p=0.63). The interaction terms between sex and age ($F_{1,4}$=1.09, p=0.36) and mass and age ($F_{1,4}$=0.27, p=0.63) and sex and mass ($F_{1,4}$=1.09, p=0.35) were not significant.

Concentrations of PCBs detected in guillemots in 1999 are also presented in Table 5-1. This analytical data was previously published by Kuzyk *et al.*, (2003) and is included in Table 5-1 for comparison purposes.

5.4.2 Biomarkers

Bone mineral density in black guillemots in 2007 ranged from 91 mg/cm^2 to 120 mg/cm^2 at the reference group, 80 mg/cm^2 to 117 mg/cm^2 at the Islands group and 90 mg/cm^2 to 118 mg/cm^2 at the Beach group (Table 5-1). No significant difference was detected in BMD between sites or sex, nor was BMD related to mass, age, or hepatic

PCB concentration (Table 5-2). Interaction terms as presented in Table 5-2 were also not significant.

The EROD activity in black guillemot livers in 2007 ranged from 5.5 pmol min^{-1} mg^{-1} to 48.9 pmol min^{-1} mg^{-1} at the reference site, 6.1 pmol min^{-1} mg^{-1} to 18.5 pmol min^{-1} mg^{-1} at the Islands group, and from 7.0 pmol min^{-1} mg^{-1} to 14.6 pmol min^{-1} mg^{-1} at the Beach (Table 5-1). The hepatic activity of UDP-GT in guillemot livers in 2007 ranged from 33.2 pmol min^{-1} mg^{-1} to 278 pmol min^{-1} mg^{-1} in the reference group, from 42.0 pmol min^{-1} mg^{-1} to 296 pmol min^{-1} mg^{-1} in the Islands group, and from 71.7 pmol min^{-1} mg^{-1} to 201 pmol min^{-1} mg^{-1} in the Beach group (Table 5-1). The hepatic activity of UDP-GT and EROD were also not related to body mass, age, or PCB concentrations and did not differ between sexes or between sites (Table 5-2). Interaction terms as presented in Table 5-2 were also not significant. The EROD results from 1999 presented in Table 5-2 are from Kuzyk *et al.*, (2003) and are presented here for comparison. Figure 5-2 presents a graph of the PCB concentration in guillemot liver and the EROD activity in guillemot liver.

5.5 Discussion

The wet weight concentrations of ΣPCBs (Table 5-1) in black guillemots at the Reference site, Islands site, and Beach site have decreased three-, four-, and six-fold, respectively since initial sampling was conducted in 1999. As discussed in Brown *et al.*, (2009), this result is consistent with the observed temporal pattern of decreased PCB concentrations observed in surface sediment and shorthorn sculpin at Saglek between 1999 and 2007.

Despite the elevated concentrations of PCBs detected in black guillemot liver at the Beach and the Islands group compared to the Reference site, the elevated PCB exposure was not reflected in the biomarkers studied here. No significant differences in bone mineral density, EROD activity, or UDP-GT activity were found between black guillemot nestlings from the high exposure Beach group, medium exposure Islands group, and low exposure Reference group. In addition, no significant relationship was found between the concentrations of PCBs in the liver of the guillemots and the biomarkers.

Deer mice exposed to elevated concentrations of PCBS at Saglek Beach had lower bone mineral density than mice at a reference area where PCB concentrations in liver were low (Johnson *et al.*, 2009, chapter 2 of this thesis). Altered bone composition and impaired bone strength has also been observed in rats (Andrews, 1989; Lind *et al.*, 2000) and goats (Lundberg *et al.*, 2005) experimentally exposed to PCBs. Decreased bone mineral density in wildlife has also been shown in polar bears (Sonne *et al.*, 2004) and grey seals (Lind *et al.*, 2003) over time periods associated with increased use of endocrine disrupting chemicals such as PCBs and DDT. While studies exist on the effects of PCBs on bone composition of wildlife and laboratory animals, studies investigating the effects of PCBs on composition of bones of birds were not found. In a study on UK raptors exposure to second generation anticoagulant rodenticides, no relationship was found between bone density and bone breaking strength and concentrations (Knopper *et al.*, 2007). Decreased growth of bones was observed in American Kestrel nestlings as a result of laboratory exposure to PCB126 (Hoffman *et al.*, 1996) but bone composition was not considered.

The mechanism linking PCB exposure and lowered bone mineral density is described in detail in Johnson *et al.*, (2009; chapter 2 of this thesis). Briefly, PCBs are antiestrogenic and this activity is mediated by interacting directly with the estrogen receptor and subsequently inhibiting estrogen induced responses (Bonefeld-Jorgensen *et al.*, 2001; Oh *et al.*, 2007). Estrogen regulates bone mineral density by controlling bone resorption carried out by osteoclasts and formation of new bone carried out by osteoblasts (Manolagas and Jilka, 1995). The antiestrogenic activity of PCBs is believed to be associated with an upregulation of osteoclasts and thus, large increases in bone resorption leading to decreased bone mineral density (Manolagas, 2000). This upregulation of osteoclast formation is recognized as the main mechanism by which estrogen deficiency induces bone loss (Cenci, 2000).

The lack of effects on bone mineral density observed in this study may be due to the young age of the nestlings. The first secretion of ovarian hormones estrogens and androgens in birds occurs with ovarian follicle maturation and coincides with initial formation of medullary bone (Miller, 1992). In a commercial laying strain of birds, medullary bone did not occur until sometime between twelve weeks and twenty-five weeks (Wilson *et al.*, 1992). Histological differences between male and female birds were also visible in bones at this age. Based on internal examination, Institut de Selection Animale (ISA) Brown chicks were just on the point of sexual maturity at fifteen weeks and some birds were showing signs of ovarian development at this age (Fleming *et al.*, 1998). The birds at Saglek were between 17 and 36 days old and it is therefore unlikely that the remodelling of bones is taking place whereby the cells responsible for resorbing bone remove bone matrix and the loss is balanced by an equal amount of bone

formation. In young developing birds, bone mineral density is regulated by bone production which is influenced by hormones such as growth hormone and the processes of bone formation and resorption are accelerated under genetic influences. This scenario highlights the importance of considering the developmental stage of the organism being studied when biomarker studies are being interpreted. Tanhuanpää *et al.* (1999) also found developmental stage dependent responses of biomarkers in three wild passerine bird species and emphasized the same consideration.

It should be noted that while no effects on bone mineral density were observed in black guillemot nestlings, early exposure to PCBs may lead to adverse effects in adult life. Goats exposed perinatally to PCB 153 had impaired bone composition seven months later (Lundberg *et al.*, 2006). Maternal exposure to low levels of PCBs also suppressed immunity and delayed the onset of puberty in the female goat offspring (Lyche *et al.*, 2006; Lundberg *et al.*, 2006). Male adult offspring exposed perinatally to PCBs had reduced pre-pubertal gonadotrophin concentrations, decreased testosterone in the breeding season, a slight reduction in testicular diameter and an increase in the percentage of sperm cells with DNA damage (Oskam *et al.*, 2005). A previous investigation at Saglek Bay identified elevated concentrations of PCBs in breast muscle of adult black guillemots ranging from 1,030 ng/g to 12,100 ng/g (ESG, 1999). Based on conversations with Neil Burgess of Environment Canada, collecting adult Black guillemots at Saglek Bay for bone mineral density measurements may have a negative impact on the population. Therefore, the measurement of biomarkers in adult guillemots was not pursued.

In 1999, the hepatic EROD activity in black guillemots at Saglek Beach was

approximately two times that of the Reference group (Table 5-1) and a strong positive relationship was observed between EROD activity and liver PCB concentrations (Kuzyk *et al.*, 2003). Most of the EROD induction in nestlings, however, occurred between a narrow range of PCB exposure (15 to 100 ng/g ww in nestling livers) above which no relationship was found (Kuzyk *et al.*, 2003). The biomarker study conducted on black guillemots in 2007 found no significant relationship between PCB concentrations and EROD activity nor was any difference observed between sites (Table 5-2). It should be noted however, that the EROD activity at the reference group is twice as high in 2007 as it was in 1999 (Table 5-1). In fact, with the exception of two values (5.4 pmol min^{-1}mg $protein^{-1}$ and 5.6 pmol min^{-1}mg $protein^{-1}$), all EROD measurements for the reference site in 2007 were greater than the geometric mean recorded for 1999 and six of the thirteen measurements in 2007 actually exceeded 10 pmol min^{-1}mg $protein^{-1}$.

It is difficult to determine if the apparent lack of effects on EROD in 2007 reflects the decreased PCB concentrations found by Brown *et al.*, (2009) because the EROD activity at the Island and Beach groups do not appear to have changed substantially since 1999 (Table 5-1). Data from Kuzyk *et al.*, (2003) was not available for statistical comparison with data from 2007. Based on the geometric means, the EROD activity at the reference group is twice as high in 2007 as it was in 1999 (Table 5-1). Kuzyk *et al.*, (2003) questioned the use of EROD as an indicator of PCB exposure in the guillemots because prefledging gulls with similar concentrations of PCBs experienced up to eight-fold higher EROD activity than the reference site in that study (Kennedy *et al.*, 2003). The EROD induction in that gull study may have been due to other polyhalogenated aromatic hydrocarbons (PHAHs) that are not present in Saglek. Several other studies,

however, have failed to find relationships between PCB exposure and EROD activity in many gull species (Yamashita *et al.*, 1992; Henriksen, 1998; 2000; Kennedy *et al.*, 2003).

Both CYP1A and UDP-GT biotransform the same classes of compounds. The expectation is that exposures to chemicals that induce CYPIA generally also trigger UDP-GT induction (Schreiber *et al.*, 2006). In theory, a balance between cytochrome P450 enzymes and conjugative biotransforming enzymes such as UDP-GT is responsible for the detoxification or bioaccumulation of toxic metabolites in the body (Bock, 1991). No significant relationship between UDP-GT activity and PCB exposure (Table 5-2) was found in black guillemots at Saglek. Less research has been done on UDP-GT activity in avian species compared to mammals but increased UDP-GT activity has been observed in Japanese quail exposed to PCBs (Riviere *et al.*, 1978; Webb *et al.*, 2008). The enzyme however, was not as responsive as the phase I biotransformation enzymes (Riviere *et al.*, 1978) and the magnitude of the response in birds was much less than that observed in mammals (Webb *et al.*, 2008). Other studies have found no clear relationship between PCB exposure and UDP-GT activity in birds (Murk *et al.*, 1994; McCleary, 2001). The lack of a significant relationship between UDP-GT activity and PCB exposure in black guillemots at Saglek supports the results of the EROD study. A study of shorthorn sculpin from Saglek Bay in 2006 also found no relationship between PCB exposure and hepatic UDP-GT activity (Chapter 4 of this thesis).

Elevated PCB exposure at Saglek Beach does not appear to be influencing the biomarkers studied in black guillemots. A clear relationship between PCB concentrations in liver and EROD activity was evident in 1999 (Kuzyk *et al.*, 2003) but this relationship was not present in 2007. The results of this chapter will be published as part of a

manuscript by Burgess *et al.*, (in prep) which reports the several biomarkers studied in Black guillemot during 2007. The results of this chapter as well as the results of other biomarkers studied by Burgess *et al.*, (in prep) will be used in an ecological risk assessment conducted in Chapter 6 of this thesis.

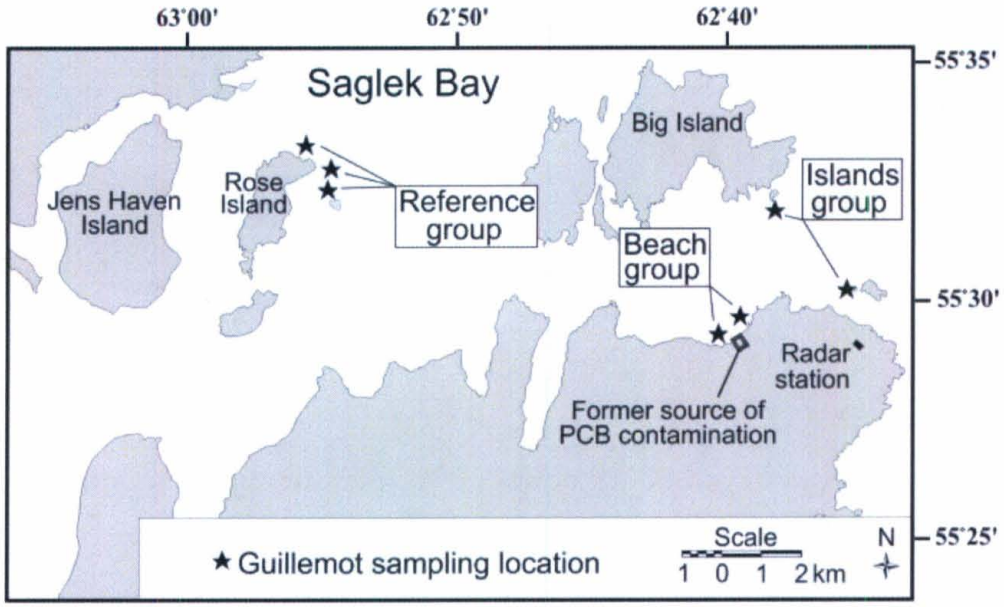

Figure 5-1. Sampling sites (Beach, Islands and Reference) for black guillemot nestlings at Saglek Bay in 2007.

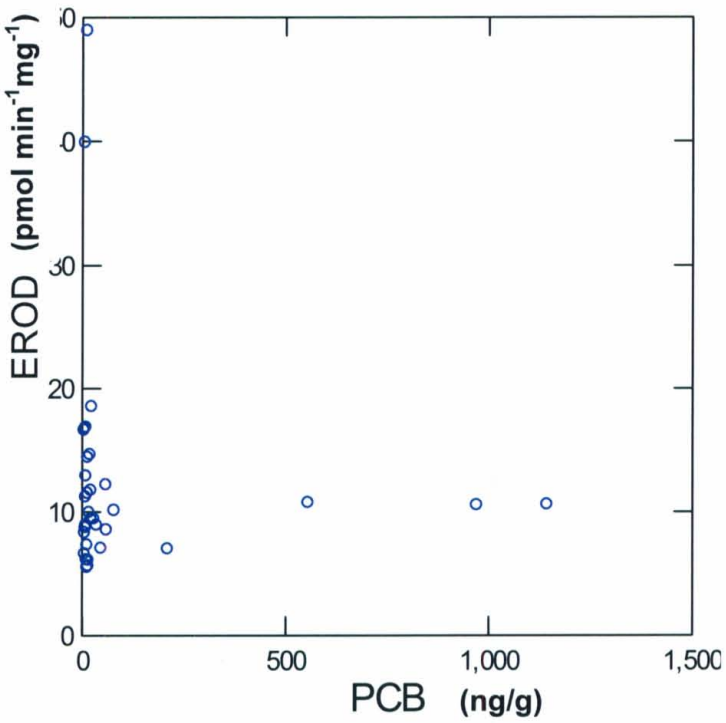

Figure 5-2. Graph of EROD activity (pmol min^{-1} mg^{-1}) and liver PCB concentrations (ng/g) in black guillemot enstlings at Saglek Bay in 2007.

Table 5-1 Total hepatic PCB concentrations and biomarker measurements in black guillemot from three exposure groups sampled in Saglek Bay, Labrador.

	Reference Group	Islands Group	Beach Group
Total PCBs (ng/g) wet weight with range in parentheses			
1999: Total PCB in nestling liver[a, b]	25 (15-46)	73 (24-150)	830 (170-6200)
n	10	10	11
2007: Total PCB in nestling liver[a,b]	8.0 (4.0-14)	16 (6.0-77)	130 (120 – 1110)
n	13	10	12
Bone Mineral Density (mg/cm^2) with range in parentheses			
Bone Mineral Density[c]	108 (91 – 120)	100 (80 – 117)	105 (90 – 118)
n	13	10	12
Phase I biotransformation enzymes (pmol $min^{-1}mg^{-1}$) with range in parentheses			
1999: EROD activity[a, d]	5.8 (4.1-10.2)	9.1 (5.6-17.1)	10.4 (7.1-14.0)
n	10	10	11
2007: EROD activity[a]	11.2±13.7 (5.5-48.9)	11.3 ±4.29 (6.1-18.5)	9.88 ±2.3 (7.0-14.6)
n	13	10	12
Phase II biotransformation enzymes (pmol $min^{-1}mg^{-1}$) with range in parentheses			
UDP-GT activity[c]	158±76.1 (33.2 - 278)	124 ±70.7 (42.0 - 296)	144 ±38.0 (71.7 - 201)
n	13	10	12

a. geometric mean
b. PCB concentrations in guillemot in 1999 are from Kuzyk *et al.*, (2003) and in 2007 are based on data presented in Brown *et al.*, (2009)
c. arithmetic mean
d. EROD activity in guillemot in 1999 is from Kuzyk *et al.*, (2003)

Table 5-2. Effect of body mass, age, sex, site and liver PCB concentration on biomarker responses of black guillemot from three sites within Saglek fiord: Reference, Islands and Beach in August 2007.

Source	df	Bone Mineral Density[a] (mg/cm^2)	EROD[b] (pmol $min^{-1}mg^{-1}$)	UDP-GT[a] (pmol $min^{-1}mg^{-1}$)
PCB Models				
Mass	1	0.16 (0.69)	0.42 (0.52)	0.83 (0.37)
Age	1	0.14 (0.71)	0.042 (0.84)	0.39 (0.54)
Sex	1	2.25 (0.15)	0.25(0.62)	0.59 (0.45)
PCB	1	0.065 (0.80)	0.06 (0.81)	0.62 (0.44)
Mass*Age	1	0.012 (0.91)	1.72 (0.19)	0.45 (0.51)
Mass*Sex	1	1.25 (0.28)	0.0000038 (0.99)	0.076 (0.78)
Mass*PCB	1	0.061 (0.81)	0.15 (0.70)	0.54 (0.47)
Age*Sex	1	0.14 (0.71)	0.018 (0.89)	0.061 (0.81)
Age*PCB	1	0.035 (0.85)	0.011 (0.92)	0.29 (0.59)
Sex*PCB	1	0.020 (0.89)	0.14 (0.71)	1.26 (0.27)
Site Models				
Mass	1	0.29 (0.59)	0.42 (0.52)	0.024 (0.88)
Age	1	0.14 (0.72)	0.042 (0.84)	0.019 (0.89)
Sex	1	2.27 (0.15)	0.25 (0.62)	0.13 (0.72)
site	2	0.92 (0.42)	0.99 (0.61)	0.90 (0.42)
Mass*Age	1	0.53 (0.48)	1.00 (0.32)	0.055 (0.82)
Mass*Sex	1	0.32 (0.58)	0.023 (0.88)	0.024 (0.88)
Mass*site	2	0.30 (0.74)	0.43 (0.81)	0.17 (0.84)
Age*Sex	1	0.13 (0.72)	0.0011 (0.97)	0.0020 (0.97)
Age*Site	2	0.68 (0.52)	0.35 (0.84)	0.043 (0.96)
Sex*Site	2	1.45 (0.26)	0.12 (0.94)	0.93 (0.41)

[a] Values are F-ratios with p-values in parentheses
[b] Values are deviance with p-values in parentheses
* indicates p-value is less than 5%

6.0 Chapter 6: Assessment of Ecological Risks to a Marine Benthic Fish (*Myoxocephalus scorpius*) and a Marine Piscivorous Bird (*Cepphus grylle*) at Saglek Bay, a PCB Contaminated Site in Northern Labrador

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6.1 Abstract

An ecological risk assessment was conducted for shorthorn sculpin (*Myoxocephalus scorpius*) and black guillemot (*Cepphus grylle*) living near a polychlorinated biphenyl (PCB) contaminated beach at Saglek, Labrador. The ERA was conducted based on information collected from two time periods (1998/1999 and 2006/2007) during which total PCBs in sediment and biota tissues were observed to decrease substantially. A screening level hazard quotient approach was initially applied. Site-specific biological effects including biomarkers and condition indices were further examined in a weight of evidence approach to evaluate the effectiveness of the lower tiered assessment. Hazard quotients from both time periods indicated that black guillemots could be experiencing adverse health effects as a result of their exposure to PCBs. This was supported by the biomarker based weight of evidence approach which indicated an intermediate to high risk to black guillemots. Hazard quotients for the shorthorn sculpin on the other hand indicated potentially adverse health effects in 1998/1999 but not in 2006/2007. The weight of evidence supported the hazard quotients indicating an intermediate risk in 1998/1999 and a low risk in 2006/2007. Despite the predicted health effects to black guillemots at Saglek, the population appears to be thriving. Measurement of population indices would be required to confirm the predicted adverse effects on black guillemots. A three tier iterative approach utilizing hazard quotients, biomarkers, and population and/or community studies is recommended for large complex sites such as Saglek, where remediation strategies are expensive and potentially destructive to the environment. This study emphasises field verification of adverse health effects predicted through the screening (*i.e.*, hazard quotient) assessment stage and supports an iterative tiered approach to ecological risk assessment.

6.2 Introduction

Ecological risk assessment (ERA) evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors (United States Environmental Protection Agency (USEPA), 1992). The process is iterative; that is, it may be repeated one or more times until a sufficiently complete and defensible result is achieved (Suter, 2007). The iterative approach is generally manifested through multiple tiers of assessment with each successive tier composed of sequentially more sophisticated and complex evaluations. There are no standards to define "sufficiently complete and defensible results" but generally, the objective of progressing to successive tiers is to reduce the uncertainty and to "provide technical support for decision making under uncertainty" (Suter, 2007). The level of uncertainty is based on professional judgment by the risk assessor and can also be driven by financial and regulatory considerations (CCME, 1996). The uncertainty associated with the risk estimate determined at the completion of each tier is the motivation for moving to the next tier (CCME, 1996).

Lower tiers of ERA typically incorporate mechanistic food chain models or tissue residue concentrations to calculate a single risk estimate by comparing the total daily exposure dose or tissue residue concentration to a toxicity reference value (*i.e.*, hazard quotient approach) (Barnthouse and Suter, 1986; USEPA, 1992a). The methodology and calculations for this type of risk assessment are well-established and commonly used (USEPA, 1992a). The hazard quotient approach, however, incorporates conservative assumptions that attempt to err on the side of caution (*e.g.*, maximum exposure or ecological sensitivity) and reports risk as point estimates (*i.e.*, single numbers). Conservatism must be built into risk assessment calculations to avoid underestimating risk

(Suter, 2007). Because of this conservatism, a hazard quotient of less than one demonstrates that adverse risks are unlikely. Over-conservatism however can lead to an unrealistic perceived risk if the hazard quotient exceeds one.

The hazard quotient approach was originally intended as a screening tool or as one part of a weight of evidence for conducting ERA (Sample *et al.*, 1996). Unfortunately, there is a misconception that a hazard quotient greater than one indicates a significant risk and that this is sufficient evidence to make risk management decisions (Tannaenbaum, 2003). Risk assessors seldom proceed with more detailed, higher tiered studies to confirm the results of the hazard quotient; that is, they seldom go beyond the screening assessment stage. In fact, higher tiered risk assessments are not used with any regularity by the majority of the decision makers managing chemical contaminants (Hope, 2009).

An alternate approach to making risk management decisions is to use higher tiers of assessment. Higher tiered ERAs are essentially a weight of evidence (USEPA, 1992a), a risk characterization process by which measurement endpoints are related to an assessment endpoint to evaluate whether risk is posed to an organism given their environmental exposure (Menzie *et al.*, 1996). In the weight of evidence approach, all available data is examined to determine if organisms have been exposed to contaminants and/or if that exposure is associated with deterioration in the health status of the organism (Suter, 2007; USEPA, 1992a). Evaluating a variety of data and tools (*e.g.*, chemical analyses, toxicity tests, biological surveys, biomarkers) allows a researcher to make use of the best and most relevant science, thus providing realistic assessments and making site-specific assumptions about exposure and effects.

This chapter investigates the value of a biological effects-based weight of evidence to support a hazard quotient approach to ecological risk assessment. The study was conducted at Saglek, a former Polevault Line military station in northern Labrador (Figure 6-1). Extensive polychlorinated biphenyl (PCB) contamination in soil and sediment associated with the original facility was identified at Saglek in the 1990s (ESG, 1997). PCB impacted soil remediation was carried out between 1997 and 1999 and thus, the terrestrial source of exposure has been removed. Ecological studies showed that PCBs remained in the coastal marine food web (Kuzyk *et al.*, 2005a), but recent evidence indicates that the concentrations are decreasing (Brown *et al.*, 2009).

A site-specific ERA of Saglek Beach will be conducted using information from pre-remediation (1998/99) and post-remediation (2006/07). A hazard quotient approach will be initially applied. Site-specific biological effects data including biomarkers and condition indices will be further examined in a weight of evidence assessment to evaluate the effectiveness of the hazard quotient assessment.

Saglek provides a superb site to conduct an ERA of PCB contamination because the PCBs are widespread (ESG, 1999), other inorganic and organic contaminants are low compared to ambient levels in industrialized areas (ESG, 1997; ESG, 1999), and PCBs have been confirmed to be present in the marine food web (Kuzyk *et al.*, 2005a).

6.3 Methods

6.3.1 Study Area

Saglek Bay is located on the north east coast of Labrador (Figure 6-1). The U.S. Air Force operated a Polevault Line military station at Saglek from 1951 to 1971. The

station was abandoned in 1971 and the site was destroyed by a fire in 1978 (Pier *et al.*, 2003). The Canadian Department of National Defence (DND) opened a modern North Warning System (NWS) long range radar facility in 1988. The long range radar facility is currently operated remotely and is unmanned with maintenance conducted at the site seasonally and on an as-needed basis by DND.

The abandonment of the original military facility at Saglek resulted in extensive PCB contamination in soil near Saglek Beach at concentrations up to 1600 µg/g (ESG, 1997). Prior to soil remediation in 1997-1999, PCBs adsorbed to soil particulates had eroded from the beach ravine and accumulated in nearshore sediments at Saglek Beach (ESG, 2000). Initial residue analysis work in 1998/99 conducted concurrently with the remediation at Saglek found extensive PCB impacts in the marine sediments and the coastal food web in Saglek Bay. This was particularly documented in those species that feed on or near the seabed and with limited foraging ranges (Kuzyk *et al*, 2005a). A follow up study in 2006/2007 found that PCB concentrations in marine sediment, as well as in tissue of shorthorn sculpin (*Myoxocephalus scorpius*) and black guillemot (*Cepphus grylle*) near Saglek Beach had decreased significantly since the chronic terrestrial source was removed (Brown *et al.*, 2009). To supplement the tissue residue analysis, a suite of biomarkers in shorthorn sculpin and black guillemot was also studied at Saglek during both time periods (Table 6-1). Johnson *et al.*, (in prep) (chapters 4 and 5 of this thesis), Burgess *et al.*, (in prep), and Kuzyk *et al.*, (2003; 2005b) provide detailed methods and results for the biomarkers. It should be noted that because of uncertainty surrounding the use of parasites as bioindicators of pollution, the results from the sculpin parasite study (presented in Chapter 3) were not included in this ERA.

Table 6-1. Biomarkers assessed at Saglek in 1998/99 and 2006/07 in shorthorn sculpin and black guillemot (√ indicates that the biomarker was studied during that time period).

Biomarker	Shorthorn sculpin		Black guillemot	
	1998/99[a]	2006/07[b]	1998/99[c, d]	2006/07[e, f]
Phase I biotransformation enzyme activity in liver (ethoxyresorufin-*O*-deethylase (EROD))	√		√	√
Phase II biotransformation enzyme activity in liver (Uridine diphosphate glucuronyltransferase (UDP-GT))		√		√
Adaptive Organ (liver) enlargement	√	√	√	
Adaptive Organ (thyroid) enlargement		√		
Liver/Body lipid	√	√		
Vitamin A concentration in liver		√	√	√
Vitamin E concentration in liver		√		
Thyroid histopathology		√		
Condition index	√	√		
Malic enzymes			√	
Thyroid function			√	√
Immune function			√	√
Steroid hormones			√	√
AcHE/MAO in brain				√
Hatching success			√	

a Data are from Kuzyk *et al.*, (2005b)
b Data are from Chapter 4 of this thesis
c Data for EROD, liver enlargement, vitamin A, malic enzymes are from Kuzyk *et al.*, (2003)
d Data for thyroid function, immune function, steroid hormones, and hatching success are from ESG (2002)
e Data for EROD and UDP-GT are from Chapter 5 of this thesis
f Data for vitamin A, thyroid function, immune function, steroid hormones, AcHE/MAO are from Burgess *et al.*, (in prep)

This paper presents the results of the ecological risk assessment for shorthorn sculpin and black guillemot at Saglek Beach, where the highest concentrations of PCBs were observed during both time periods (Kuzyk *et al.*, 2005a; Brown *et al.*, 2009). Sediment sampling for both time periods followed the methods described in Brown *et al.*, (2009). Shorthorn sculpin were collected by hook and line from Saglek Beach (adjacent to the former PCB source) and Rose Island (reference site located approximately 18 km from

the former source but still within Saglek fiord) (Figure 6-1). Shorthorn sculpin sampling and analysis methods are documented in Johnson *et al.*, (chapter 4 of this thesis). Black guillemot nestlings used in this risk assessment were collected from the Saglek Beach area as well as the reference site in 2006/07. Methods for sampling and analysis are documented in chapter 5 of this thesis.

6.3.2 Ecological Risk Assessment

This ecological risk assessment considers risk to marine fish and piscivorous birds based on information for shorthorn sculpin and black guillemot. Concentrations of inorganic and organic contaminants other than PCBs are low at Saglek compared to ambient levels in more industrialized regions of North America (ESG, 1999; Kuzyk *et al.*, 2003). Therefore, PCBs are the sole chemical of concern at Saglek based on measured concentrations in sediment and biota.

This ecological risk assessment was carried out in four steps: 1) problem formulation, 2) exposure assessment, 3) toxicity/hazard assessment, and 4) risk characterization (Gaudet, 1994). Problem formulation includes the identification and preliminary characterization of stressors, the ecosystem potentially at risk, and ecological effects (USEPA, 1992a). Ecologically based endpoints are also chosen and the results of the three steps above are then incorporated into a conceptual model. A conceptual model in problem formulation is a written description and visual representation of predicted relationships between ecological entities and the stressors to which they may be exposed (USEPA, 1998). The key task in exposure assessment is to investigate the level of exposure for the receptors of concern by determining the sources, pathways, and

distribution of chemicals of concern around the contaminated site (Gaudet, 1994). For the toxicity/hazard assessment, toxicity information for the contaminant is obtained from the literature and the relationship between the contaminants of concern and the most important ecological endpoints is described (Gaudet, 1994; CCME, 1996). Risk characterization combines the results of the exposure assessment and the hazard assessment to describe the nature and magnitude of potential environmental risks (USEPA, 1992a; Gaudet, 1994).

6.3.2.1 Exposure Assessment- Black Guillemot

For black guillemot, the dietary hazard quotient method approach was used to assess the total estimated ingested daily intake of PCBs. Life history parameters for black guillemot used in this exposure and effects assessment are summarized in Table 6-2. Calculations for these parameters are presented in Appendix 2.

Table 6-2. Life history parameters used in the dietary model for black guillemot at Saglek Beach.

Parameter	Value	Reference/Notes
Body weight (kg)	0.38	Dunning, 2008 (based on mean body weight, range = 0.35 kg to 0.44 kg)
Food ingestion rate (kg wet weight/day)	0.15	Calculated from allometric equations in Nagy (1987)
Water ingestion rate (L/day)	0.031	Calculated from allometric equations in Calder and Braun (1983)
Sediment ingestion rate (kg dry weight/day)	0.00062	Calculated based on sediment in diet of merganser (<2%), a diving piscivorous bird (Beyer *et al.*, 2008); 2% of total food ingestion rate (dry weight) is used to be conservative
Fish ingestion rate (kg wet weight/day)	0.13	Based on 1) dry weight dietary composition of black guillemots from Svalbard (Lonne and Gabrielsen, 1992) and 2) 75% moisture content of fish (USEPA, 1993)
Benthic invertebrate ingestion rate (kg wet weight/day)	0.025	Based on 1) dry weight dietary composition of black guillemots from Svalbard (Lonne and Gabrielsen, 1992) and 2) 80% moisture content of benthic invertebrates (USEPA, 1993)
Home range (km)	0.50 km	Ranges from 0.5 km to 30 km (Bradstreet and Brown, 1985); Low end of foraging range was found appropriate based on observations of foraging guillemots at Saglek (Brown *et al.*, 2009)
Temporal use factor (unitless)[a]	0.50	Based on migration to open water and pack ice edges in winter (Brown, 1985)

a Temporal use factor is the fraction of time spent at the site by the receptor

Prediction of the total dietary PCB intake for black guillemot was based on a food chain model. Site specific concentrations of PCBs in sediment and fish as well as predicted concentrations in benthic invertebrates (based on uptake factors) were used to predict the total ingestion daily intake for black guillemot. The total ingestion daily intake was then compared to a toxicity reference value to determine the hazard quotient.

To determine representative exposure point concentrations (EPCs) for ∑PCBs in sediment, ProUCL 4.0 (USEPA, 2007) was used to determine the 95% upper confidence limit (UCL) given the specific distribution of the site specific analytical results data (*i.e.*, normal, log normal, or gamma).

The sediment pore water concentrations may be used as a conservative estimate of the exposure of fish, such as sculpin that spend much of their lives near the sediment (Suter, 1996). The following equation (Di Toro and McGrath, 2000; Suter, 2007) was used to determine the concentration of PCBs in the sediment pore water (C_{pw}) based on total PCB concentrations in sediment:

$$C_{pw} = C_s/(K_{oc}*foc),$$

where:

C_{pw} = concentration of PCBs in pore water (mg/L)

C_s = concentration of total PCBs in sediment (mg/kg) (site specific; refer to Table 6-8)

K_{oc} = partition coefficient (L/kg) (Hansen *et al.*, (1990); refer to Table 6-3)

foc = fraction of organic carbon (kg/kg) (site specific; refer to Table 6-3)

Body burdens of invertebrates exposed to contaminated sediments were determined using biota sediment accumulation factors (BSAFs). The BSAF was determined using the following equation modified from Di Toro *et al.*, (2000):

$$Log_{10}BSAF = -0.00028 + 0.038*Log_{10}K_{ow},$$

where:

BSAF = biota sediment accumulation factor

K_{ow} = octanol-water partitioning coefficient for PCBs (ATSDR, 2000; refer to Table 6-3).

In order to apply the BSAF as an uptake factor for sediment to benthic invertebrates, it must be normalized for the amount of lipid in the benthic invertebrates. In addition, the concentration of PCBs in sediment must be normalized for the amount of organic carbon in sediment. The equation for concentration in benthic invertebrates becomes,

$$C_{bi} = BSAF * (C_{sed}/foc_{sed}) * lipid_{bi}$$

where

C_{bi} = concentration of PCBs in benthic invertebrates (mg/kg)

$BSAF$ = biota sediment accumulation factor (calculated above)

C_{sed} = concentration of total PCBs in sediment (mg/kg) (site specific; refer to Table 6-8)

foc_{sed} = fraction of organic carbon in sediment (site specific; refer to Table 6-3)

$lipid_{bi}$ = fraction of lipid in benthic invertebrates (Gewurtz *et al.*, 2000; refer to Table 6-3)

Some deviations between empirical and theoretical BSAFs using this formula exist when used for persistent substances with high K_{ow} such as PCBs. Di Toro *et al.*, (2000) recommend this method for chemicals with $\log_{10}K_{ow}$ values below 6.5. Any deviations are expected to be of greater magnitude at higher trophic level organisms. Table 6-3 lists the values utilized in the above equations and their sources.

Table 6-3. Values utilized in calculations of PCB concentrations in pore water and benthic invertebrates.

Parameter	Value	Source
foc_{sed}	Beach 0.012 unitless	Average for site
$lipid_{bi}$	0.017 unitless	Gewurtz *et al*, 2000
$log\ K_{oc}$	5.8 unitless	Hansen, 1990
$log\ K_{ow}$	6.5 unitless	ATSDR, 2000

Site specific data for PCBs in shorthorn sculpin liver were used to calculate whole body concentrations. Based on previous sampling at Saglek, PCB concentrations in the liver are approximately five times higher than the whole body (minus the liver) (ESG, 1999). Whole body concentrations in each fish were therefore calculated based on the mass of the liver and mass of the whole body minus the liver. ProUCL 4.0 (USEPA, 2007) was used to determine the 95% UCL given the specific distribution of the data (*i.e.*, normal, log normal, or gamma).

The following equation consistent with USEPA's wildlife exposure guidance (USEPA, 1993) was used to calculate the average daily dose (ADD_{PCBs}) for black guillemot from ingestion (mg kg^{-1} day^{-1}):

$$ADD_{PCBs} = \frac{[\sum(C_{food}*I_{food}*F_{food})+(C_{water}*I_{water}*F_{water})+(C_{sed}*I_{sed}*F_{sed})]}{BW}$$

where:

C_{food} = concentration of PCBs in food (mg/kg) (refer to Table 6-8)

I_{food} = food ingestion rate (kg/day) (refer to Table 6-2)

F_{food} = fraction of food from the site (100%, see below)

C_{water} = concentration of PCBs in water (mg/L) (calculated) (refer to Table 6-8)

I_{water} = water ingestion rate (L/day) (refer to Table 6-2)

F_{water} = fraction of water from the site (100%, see below)

C_{sed} = concentration of PCBs in sediment (mg/kg) (site specific; refer to Table 6-8)

I_{sed} = sediment ingestion rate (kg/day) (refer to Table 6-2)

F_{sed} = fraction of sediment from the site (100%, see below)

BW = body weight of the receptor (kg). (refer to Table 6-2)

It was assumed that the entire diet for black guillemot during the time the organism is at the site comes from the area and that 100% of the PCBs are absorbed (*i.e.*, absorption factor is equal to one). A temporal use factor of 0.5 (*i.e.*, fraction of time spent exposed to the site) was used in the risk assessment for the black guillemot to account for migration to ice edges during the winter. A hazard quotient of one or more indicates that an adverse effect could occur (USEPA, 1992a; Gaudet, 1994).

6.3.2.2 Toxicity Assessment – Black Guillemot

A literature search of PCB feeding studies for avian species was conducted to determine an appropriate toxicity reference dose for black guillemots exposed to PCBs at Saglek. Choice of lowest observed adverse effect level (LOAEL) was based on chronic exposure of sensitive life stages, measure of ecologically relevant endpoints (*e.g.*, reproduction), close relatedness of the test species to the receptor species, and minimal impact of other contaminants (Table 6-4).

Table 6-4. Toxicity reference values for avian species exposed to PCBs.

Species	Duration	PCB	Effects	LOAEL ($mgkg^{-1}day^{-1}$)	NOAEL ($mgkg^{-1}day^{-1}$)	Reference
White leghorn chickens (*Gallus domesticus*)	6 weeks (subchronic)	1242	Hatchability	0.67	0.34	Britton and Huston, 1973
White leghorn hens (*Gallus domesticus*)	8 weeks (subchronic)	1248	Hatchability	0.67	-	Scott, 1977
White leghorn chickens (*Gallus domesticus*)	39 weeks (chronic)	1254	Egg production	0.34	-	Platonow and Reinhart, 1973
Ring-necked pheasant (*Phasianus colchicus*)	16 weeks (chronic)	1254	Hatchability	1.8	-	Dahlgren *et al.*, 1972
Mallard ducks (*Anas platyrhynchos*)	1 month (subchronic)	1254	Reproductive success, nest attentiveness	-	1.5	Custer and Heinz, 1980
American kestrel (*Falco sparverius*)	100 days (chronic)	1248:1254:1260	Hatchability, nestling survival, clutch size	7		Fernie *et al.*, 2001

In the absence of appropriate toxicity studies on black guillemots, studies on the effect of PCBs on other avian species were considered (Table 6-4). In the absence of appropriate studies on PCB1260, other Aroclor studies were used. The endpoints most commonly studied include reproductive success measured in a variety of ways including number of hens laying, date of first egg laid, survival of hatchlings, egg production and egg weight, shell thickness and shell weight, and hatchability. PCBs did not affect eggshell weight or thickness (Britton and Huston, 1973; Lillie *et al.*, 1975), however, egg hatchability and egg production were sensitive endpoints (Britton and Huston, 1973; Dahlgren *et al.*, 1973; Platonow and Reinhart, 1973; Lillie *et al.*, 1975; Scott, 1977; Fernie *et al.*, 2001).

The sensitivity of avian species to PCBs varies. Chickens (i.e, white leghorn

hens) have been shown to be most sensitive followed by pheasants, turkeys, ducks and gulls (Bosveld and Van den Berg, 1994). The bobwhite (*Colinus virginianus*) was shown to be more sensitive to PCB 1254 and PCB 1260 than the ring-necked pheasant *(Phasianus colchicus*), followed by the mallard duck (*Anus platyrhynchos*), and with the Japanese quail (*Coturnix coturnix japonica*) being least sensitive (Heath *et al.*, 1972; Hill *et al.*, 1975). A study by Fernie *et al.*, (2001) indicates that American Kestrels (*Falco sparverius*) may be the least sensitive with an estimated LOAEL of 7 mg/kg/day.

Both gulls and guillemots are in the Order Charadriiformes (*i.e.*, the auks). Gulls have been shown to be less sensitive than chickens (Bosveld and Van den Berg, 1994) and pheasants (Brunstrom and Reutergardh, 1986). Jeffries and Parslow (1972; 1976), however, showed that common guillemots (*Uria aalge*) exposed to PCB1254 were more sensitive to thyroid changes than greater black backed gulls (*Larus fuscus*).

The TRV for black guillemots is based on the study by Dahlgren *et al.*, (1972) which studied the chronic effects (*i.e.*, 16 weeks) of PCBs on a representative endpoint (*i.e.*, hatchability) in a wildlife species (*i.e.*, pheasant). Three types of uncertainty factors need to be considered: interspecies uncertainty factors for extrapolating the LOAEL from the pheasant to the guillemot, a subchronic-to-chronic uncertainty factor and a LOAEL-to-NOAEL uncertainty factor. The study was a chronic study and the LOAEL derived from the study was used so subchronic to chronic uncertainty factors or LOAEL to NOAEL uncertainty factors were not applied. An interspecies uncertainty factor of 10 is considered too conservative since pheasants have been shown to be more sensitive than many other avian species, including gulls. Because guillemots have been shown to be more sensitive than gulls, an uncertainty factor of 3 (intermediate to 1 and 10) was

applied to the TRV to account for interspecies differences. It should be noted that allometric scaling factors to extrapolate toxicity data between species with different body masses were not considered here because the test species body weight was greater than the wildlife species (*i.e.*, allometric scaling of the TRV for pheasant would produce a higher TRV for the black guillemot). As recommended by Knopper *et al.*, (2009), the TRV was not scaled upward. Therefore, a TRV of 0.6 mg/kg is considered protective for the guillemot.

6.3.2.3 Exposure Assessment – Shorthorn Sculpin

Concentrations of PCBs in whole body shorthorn sculpin were used as a measure of exposure to PCBs. Concentrations were determined as described previously in the risk assessment for black guillemots (*i.e.*, measured concentrations). To calculate potential risks to sculpin, a hazard quotient approach was used to compare concentrations of PCBs in sculpin to effects concentrations reported in the literature.

6.3.2.4 Toxicity Assessment – Shorthorn Sculpin

A literature search of PCB concentrations in fish associated with adverse effects was conducted to determine an appropriate toxicity reference concentration. Choice of lowest observed adverse effect concentration (LOAEC) was based on chronic exposure of sensitive life stages, measure of ecologically relevant endpoints (*e.g.*, reproduction), close relatedness of the test species to the receptor species, and minimal impact of other contaminants (Table 6-5). The studies by Hansen *et al.*, (1976) and ACOE (1988) were considered more relevant mainly because the study by Hansen *et al.*, (1974) was not considered a chronic study. The lowest concentration (13.7 mg/kg) of the relevant

studies was used as the LOAEC. To establish the TRV for shorthorn sculpin, three types of uncertainty factors need to be considered: interspecies uncertainty factors for extrapolating the LOAEC from the minnow to the sculpin, a subchronic-to-chronic uncertainty factor and a LOAEC-to-NOAEC (*i.e.*, no observed adverse effect concentration) uncertainty factor. An uncertainty factor of 3 (intermediate to 1 and 10) was applied to the TRV to account for interspecies differences. The study was a chronic study and the LOAEC derived from the study was used so subchronic to chronic uncertainty factors or LOAEC to NOAEC uncertainty factors were not applied. Monosson (1999) indicated that liver concentrations ranging from 25 mg/kg to 70 mg/kg interferes with the proper functioning of the reproductive system of fish while Niimi (1996) indicated concentrations exceeding 100 mg/kg in females. Using the factor of 5 to convert from whole body to liver concentrations, 4.6 mg/kg corresponds to a liver concentration of 23 mg/kg in the liver. Therefore, the TRV of 4.6 mg/kg is considered protective for the sculpin.

Table 6-5. Toxicity concentrations for sculpin exposed to PCBs at Saglek.

Test species	Exposure	Effect	Concentration (mg/kg)	Reference	Uncertainty factors	TRV (mg/kg)
Channel catfish (*Ictalurus punctatus)*	Exposed to PCB 1242 in diet for 20 weeks (chronic)	Growth	14.3 (LOAEC)	Hansen *et al.*, 1976	3	4.8
Fathead minnow *(Pimephales promelas)*	Exposed to PCBs 1254 in sediment for 16 weeks (chronic)	Reproduction (reduced fecundity and frequency)	5.3 (NOAEC) 13.7 (LOAEC)	US Army Corps Of Engineers (ACOE), 1988	3	4.6
Sheepshead minnow (*Cyprinodon variegates*)	Exposed to PCBs in water for 28 days (subchronic)	Reproduction (fry mortality)	1.9 (NOAEC) 9.3 (LOAEC)	Hansen *et al.*, 1974	3	3.1[a]

a Because the study by Hansen *et al.*, (1974) was not a chronic study, the TRV of 3.1 mg/kg was not considered relevant.

6.3.3 Risk Characterization

Risk characterization was initially carried out using a hazard quotient approach and then using a weight of evidence approach. The weight of evidence approach was based on biomarker studies on shorthorn sculpin (Johnson *et al.*, in prep (chapter 4 of this thesis); Kuzyk *et al.*, 2005b) and black guillemot (Chapter 5 of this thesis; Burgess *et al.*, in prep; Kuzyk *et al.*, 2003).

6.3.3.1 Hazard Quotients

The hazard quotient for black guillemot exposed to PCBs is calculated as ADD_{PCBs}/TRV. For sculpin, measured concentrations of PCBs in sculpin were compared to the effects concentration from the literature (Table 6-5) to calculate a hazard quotient.

6.3.3.2 Weight of Evidence

A qualitative weight of evidence analysis was performed using the methods of Menzie *et al.* (1996) which are based on the Massachusetts Weight of Evidence Working Group (1995). The weight of evidence was defined in the Introduction as the process by which measurement endpoints are related to an assessment endpoint to evaluate whether risk is posed to an organism given their environmental exposure (Menzie *et al.*, 1996). A qualitative approach is suitable when there are relatively few lines of evidence (Mcdonald *et al.*, 2007). The approach by Menzie *et al.*, (1996) has been recommended by Suter (2007) and has been successfully applied to field collected data (Johnston *et al.*, 2002). The assessment endpoints for fish and birds are reduction in species richness, abundance, and reproduction. The measurement endpoints (*i.e.*, biomarkers) are related to an

assessment endpoint to evaluate whether risk is posed to an organism given their environmental exposure (Menzie *et al.*, 1996).

Menzie *et al.*, (1996) identify three critical characteristics of each measurement endpoint: 1) a weight assigned to each measure, 2) the magnitude of response observed in the measures, and 3) the concurrence among multiple measures. A weight (low, medium, high) was initially assigned to each measurement endpoint based on data quality, strength of association between measurement endpoint and assessment endpoint, and study design and execution (Appendix 3). Based on the weights assigned to the three categories, professional judgment was used to determine an overall weight (low, medium, high) for each measurement endpoint.

Each line of evidence was initially grouped into exposure type lines or effects based lines of evidence. The magnitude of the response in the measurement endpoint was then ranked based on an assigned score of whether the measurement added weight to the conclusion of risk or no risk (Table 6-6). These rankings are based on Johnston *et al.*, (2002). Modifications made here are as follows. The assessment endpoint measures of exposure considered by Johnston *et al.*, (2002) were primarily concentrations or toxicity data which have conservative and non-conservative benchmarks for comparison. In this study, we applied biomarkers of exposure as assessment endpoint measures of exposure. No benchmarks exist for these biomarkers of exposure (*e.g.*, phase I biotransformation enzyme activity). Therefore, the scheme used for interpretation of magnitude of response of measurement endpoints was modified. The definitions of negligible and low used here are the same as Johnston *et al.*, (2002). The evidence of exposure is classified in this study as moderate or high based on differences in responses from the reference site

(Table 6-6) whereas in Johnston *et al.,* (2002), the evidence of exposure was classified as elevated, high, or adverse based on comparison to benchmarks.

The concurrence among measurement endpoints was examined using a graphical method that plots the symbol designation of the measurement endpoint within a graph with weight of measurement endpoint on the y-axis and the degree of response as the x-axis. A visual examination was performed to determine agreements, divergences among measurement endpoints along with the weights assigned to the endpoints (Menzie *et al*, 1996).

Table 6-6. Scheme used for interpretation of magnitude of response of measurement endpoints.

Type of measure	Degree of response	Interpretation
Exposure	Biomarker response is less than or similar to reference condition Concentration is below conservative benchmark concentration (*e.g.*, ISQG for sediment)	Negligible
	Biomarker response is greater than the reference condition but not statistically greater Concentrations is greater than the conservative benchmark (*e.g.*, ISQG for sediment)	Low
	Biomarker is statistically greater than reference condition but mean response is less than 2 times the mean reference response Concentration is greater less than 2 times the non-conservative benchmark (*e.g.*, PEL for sediment)	Moderate
	Biomarker is statistically greater than reference and mean response is greater than 2 x the mean reference response Concentrations is 2x the non-conservative benchmark (*e.g.*, PEL for sediment)	High
Effect	Similar to reference condition or below ecologically relevant threshold (*e.g.*, hazard quotient <1)	No effect
	Worse than reference condition but not statistically significant or similar to ecologically relevant threshold (*e.g.*, hazard quotient~1)	Potential effect
	Statistically worse than reference condition or substantially greater than ecologically relevant threshold (*e.g.*, hazard quotient>1)	Probable effect

Definitions of risk (based on modifications described previously) were also used to interpret the results of the exposure and effects information in a matrix approach (Table 6-7).

Table 6-7. Interpretation of exposure and effect evidence in determining risk.

Evidence of Effect	Evidence of Exposure			
	Negligible	**Low**	**Moderate**	**High**
None	Negligible	Negligible	Low	Intermediate
Potential	Negligible	Low	Intermediate	High
Probable	Low	Intermediate	High	High

6.4 Results

6.4.1 Problem Formulation

The conceptual model for this ecological risk assessment is shown in Figure 6-2. The conceptual model indicates that there are potentially significant exposure pathways for several receptor species in the Saglek marine ecosystem. These receptors are benthic invertebrates (have a direct association with sediment) and bottom-feeding fish and diving seabirds (linked to sediment via the benthic based food chain). This study considers risk to the benthic fish and diving seabirds.

Other potential marine receptors such as seals and polar bears have not been evaluated in this ERA. Work is currently underway at Saglek Bay as well as along other parts of the Labrador coast to determine PCB exposures and biological effects information for ringed seals. Larger receptors at Saglek Bay such as seals have not been considered further in this ecological risk assessment, however, will likely be evaluated once additional information is available.

6.4.2 Risk Analysis and Exposure and Effects Assessment

Results of analytical testing for PCBs in sediment are from Brown *et al.*, 2009. Concentrations of PCBs in shorthorn sculpin and Black guillemot are presented in

Chapters 4, and 5 respectively. Concentrations of PCBs in shorthorn sculpin from 1998/1999 are based on concentrations that were reported as lipid weight concentrations in Brown *et al.*, (2009). Concentrations of PCBs in Black guillemot from 1998/99 are from Kuzyk et al., (2003) and in 2007 are based on data from Brown *et al.*, (2009).

The calculated exposure point concentrations for sediment (calculated from data published by Brown *et al.*, (2009), surface water (calculated from sediment concentrations), benthic invertebrates (calculated from pore water concentrations) and sculpin (calculated from data from Brown *et al.*, (2009) and from chapters 3 and 4 of this thesis) used in this ecological risk assessment are reported here (Table 6-8).

Table 6-8. Exposure point concentrations for sediment, pore water, benthic invertebrates, and sculpin (values in parentheses are sample sizes).

Media/biota	1998/99	2006/07
Sediment (mg/kg)	6.65[a] (54)	0.33[c] (30)
Sculpin liver (mg/kg)	32.77[b] (10)	18.13[c] (19)
Sculpin whole body (mg/kg)[d]	7.34	4.06
Benthic invertebrates (mg/kg)[e]	1.26	6.20E-02
Marine water (mg/L)[f]	8.80×10^{-4}	1.57×10^{-5}

a. 95% H-statistic UCL (lognormal)
b. 95% student's t-distribution UCL (normal)
c. 95% gamma distribution UCL
d. Calculated based on liver:whole body (minus liver) ratio of 1:5 (ESG, 1999) and liver percentage of body
e. Calculated from pore water concentrations
f. Calculated from sediment concentrations

6.4.3 Risk Characterization – Hazard Quotients

Hazard quotients were calculated for sculpin at Saglek Beach by comparing concentrations of PCBs in sculpin to the lowest observed adverse effect concentration from the literature (Table 6-9). At Saglek Beach in 1998/99, the hazard quotient calculated from measured concentrations of PCBs in sculpin was 1.6 indicating that there was a potential risk to sculpin at Saglek Beach. In 2006/07, the hazard quotient was 0.9

indicating that there was unlikely an adverse risk to sculpin at Saglek Beach. The hazard quotient did decrease over the 1998-2007 time period. This likely reflects the decrease in sediment and sculpin concentrations during the period between sampling events.

Table 6-9. Hazard quotients for shorthorn sculpin

Year	Concentration in sculpin (mg/kg ww)	TRV (mg/kg ww)	Hazard Quotient
1998/99	7.3	4.6	1.6
2006/07	4.1		0.9

The hazard quotients for black guillemot in 1998/99 and 2006/07 (Table 6-10) were 2.1 and 1.2, respectively. This indicates a potential for adverse risks to black guillemot at Saglek Beach. An overall decrease in hazard quotient for black guillemot was evident over the 1998/99 and 2006/07 time periods. This likely reflects the decrease in both sediment and sculpin concentrations during the period between sampling events.

Table 6-10. Hazard quotients for black guillemot

Site	Average Daily Dose	1998/99	2006/07
	Sediment	0.011	5.4×10^{-4}
	Water	7.2×10^{-5}	1.3×10^{-6}
	Benthic Inverts	0.08	0.004
	Sculpin	2.5	1.4
	Total	2.5	1.4
	TRV (mg/kg/day)	0.6	
	Occupancy Factor	0.5	
	Hazard Quotient	2.1	1.2

6.4.4 Risk Characterization – Weight of Evidence

Results of the biomarkers assays (*i.e.*, measurement endpoints) for shorthorn sculpin and black guillemot are detailed in Johnson *et al.* (in prep; chapters 4 and 5 of this thesis), Burgess *et al.* (in prep), and Kuzyk *et al.*, 2003; 2005b. Biomarker responses were compared to Rose Island, a reference site located approximately 18 km from the

former source (but still within Saglek fiord). The hazard quotient was also included as a line of evidence as recommended by Sample *et al.*, (1996).

Based on the assessment endpoints and criteria, all measures of exposure were assigned an overall weight of either medium or high (Tables 6-11 and 6-12). Data quality was considered high for all assessment endpoints based on sensitivity, precision, accuracy, completeness, representativeness, and comparability (Menzie *et al.*, 1996). An explanation of the overall endpoint weight assignment for each assessment endpoint measure is provided following Tables 6-11 and 6-12.

Table 6-11. Weights assigned to each measurement endpoint[a] for shorthorn sculpin at Saglek Beach.

Assessment endpoint measure	Data quality	Strength of association between measurement and assessment endpoints	Study design	Overall endpoint weight	Symbol On Figure 6-3
Measures of exposure					
Concentration of PCBs in sediment	H	L	M	M	A
Concentrations of PCBs in sculpin liver	H	M	M	M	B
Phase I biotransformation enzyme activity in sculpin liver (EROD)	H	M	M	M	C
Phase II biotransformation enzyme activity in sculpin liver (UDP-GT)	H	M	M	M	D
Adaptive Organ (liver) enlargement	H	L	L	L	E
Adaptive Organ (thyroid) enlargement	H	L	L	L	F
Liver/Body lipid	H	L	L	L	G
Measures of Effect					
Vitamin A concentration in sculpin liver	H	M	M	M	1
Vitamin E concentration in sculpin liver	H	M	M	M	2
Thyroid histopathology	H	M	H	M	3
Condition index	H	H	L	M	4
Hazard quotient	H	H	M	M	5

a. Refer to Table 6-1 for source of data for each measurement endpoint.

Table 6-12. Weights assigned to each measurement endpoint[a] for black guillemot at Saglek Beach.

Assessment endpoint measure	Data quality	Strength of association between measurement and assessment endpoints	Study design	Overall endpoint weight	Symbol On Figure 6-4
Measures of effect					
Concentration of PCBs in sediment	H	L	M	M	A
Concentrations of PCBs in sculpin liver (sculpin)	H	L	M	M	B
Concentrations of PCBs in nestling liver	H	M	M	M	C
Phase I biotransformation enzyme activity in bird liver (EROD)	H	M	M	M	D
Phase II biotransformation enzyme activity in bird liver (UDP-GT)	H	M	M	M	E
Malic enzymes	H	M	M	M	F
Adaptive Organ (liver) enlargement	H	L	L	L	G
Measures of Effect					
Vitamin A concentration in bird liver	H	M	M	M	1
Gonad histopathology	H	M	H	M	2
Thyroid function	H	M	M	M	3
Immune function	H	M	M	M	4
Steroid hormones	H	M	M	M	5
AcHE/MAO in brain	H	M	M	M	6
Hatching success	M	H	M	M	7
Hazard quotient	H	H	M	M	8

a. Refer to Table 6-1 for source of data for each measurement endpoint.

Measured concentrations of PCBs in sediment and biota are specific, quantitative, and sensitive. Concentrations in sediment and dietary items may not be good measures of effects on receptors because toxicity often depends on bioavailability, receptor characteristics, and exposure duration (*e.g.*, home ranges). The study design quality was considered medium and benchmarks (*e.g.*, CCME interim sediment quality guideline (ISQG) and probable effects level (PEL) are available for sediment. An overall weighting of medium was therefore assigned to sediment concentrations. Concentrations

of PCBs in sculpin and black guillemot nestlings were correlated to effects on the receptor. Because the study design may not account for temporal and natural variability, an overall weighting of medium was assigned to PCB concentrations in biota.

Phase I and II biotransformation enzymes are involved in a series of reactions that metabolize PCBs and make them more readily eliminated from the body. The activity of phase I and II biotransformation enzymes is a well-accepted, sensitive measure of PCB exposure and can be correlated well to PCBs. These enzymes are possible predictors of pathologies through mechanistic links but the relationship between this measurement endpoint and effects at higher levels of organization is not always clear. The study design for this measure also did not account for temporal and natural variability, so an overall weight of medium was assigned to both phase I and II biotransformation enzyme activity.

If the thyroid undergoes stimulation, predictable increases in epithelial cell height occur as well as effects such as vascularity, follicle irregularities, and decreases in colloid density occur (Eales and Brown, 1993). The hepatosomatic index has been traditionally used to indicate liver hypertrophy and/or hyperplasia because of detoxification properties of the liver as well as increased liver weight because of fat content and liver necrosis (Goede and Barton, 1990; Gul *et al.*, 2004; Hinton and Laurén, 1990). Adaptive liver and thyroid enlargement and liver lipid may, however, be affected by other factors and their correlation to PCBs is not as clear as for the enzyme activities. For example, hepatosomatic index has been found to vary seasonally in fish (Yang and Bauman, 2006) and between sexes (Khan and Billiard, 2007; Yang and Baumann, 2006). In addition, a biological link to assessment endpoints and significant environmental harm at higher

levels of organization is considered low for these measures. For example, a xenobiotically induced change in fish thyroid function has yet to be causally linked to decreased fitness or survival (Brown *et al.*, 2004). The study design also did not consider temporal and natural variations that are known to affect these measures. An overall weight of low was assigned to adaptive liver and thyroid enlargement and liver/body lipid.

Vitamin E is known as one of the major antioxidants protecting membranes against oxidation (Kawai-Kobayashi and Yoshida, 1986; Latchoumycandane and Mathur, 2002; Packer, 1991; Yun *et al.*, 2005) and thus has been proposed as a biomarker for contaminant induced oxygen radical stress (Packer, 1991). Vitamin E is particularly important in fish which have a higher amount of polyunsaturated fatty acid lipids that are susceptible to lipid peroxidation (Palace *et al.*, 1996). Vitamin A is important in the normal differentiation of epilthelial structures, reproduction, vision, and immune system function (Zile, 1983). Decreases in vitamin A storage levels have been related to developmental deformities in lake sturgeon exposed to co-planar PCBs in the St Lawrence (Doyon *et al.*, 1998) and to lethal viral infections and reproductive disorders in seals in the Baltic, North and Wadden Seas (Brouwer *et al.*, 1989). Relationships between levels of vitamin A and E and PCB exposure have been well studied and established and have a strong scientific basis. Temporal variation in diet and natural variation may also contribute to variations in vitamin A and E, however, because they can indicate harm to the individual organism (*e.g.*, oxidative stress), measures of vitamin A and E were assigned an overall weight of medium.

Pathologies such as lesions, tumors and other signs of disease in tissue have a

strong correlation to PCB toxicity and are excellent indicators of toxicity but they are still considered a lower level of organization than the assessment endpoint. Histopathology is a general response and is not contaminant specific. The study design was considered high for this measurement endpoint and histopathology was therefore assigned an overall medium weight.

The condition factor is a generalized indicator of the physical and physiological status of the fish. The condition factor can reflect the integrated effect of both nutrition and metabolic costs induced by stress (Adams *et al.*, 1990; Encina and Granado-Lorencio, 1997) but has been found to vary seasonally in fish (Yang and Bauman, 2006) and between sexes (Khan and Billiard, 2007; Yang and Baumann, 2006). Other factors may affect this endpoint and the temporal variability was not accounted for in the study design. Therefore, the endpoint was assigned an overall weight of medium.

The hazard quotient was assigned a high strength of association because it represents a direct measure of the assessment endpoint. This is because the concentration of PCBs in fish was directly compared to the concentrations causing reproductive/growth effects (*i.e.*, assessment endpoints). The overall weight of the hazard quotient was assigned a weight of medium relative to other measures because temporal and natural variability were not accounted for in the study design.

For black guillemots, phase I and II enzyme activity, concentrations of PCBs in sediment, adaptive organ enlargement, vitamin A, and histopathology were assigned the same weight as for these measurements in the sculpin (Table 6-12). Another measure for the guillemot includes malic enzymes. Malic enzymes are considered similar to the phase I and II enzymes as they measure exposure but may not be biologically linked to effects

at higher levels of organization. An overall designation of medium was assigned to this endpoint.

Measures of immune function, thyroid function, steroid hormones and brain AcHE/MAO are considered to have a strong biological linkage to the assessment endpoints but they are still at lower levels of biological organization and may not indicate adverse effects at the population/community level. The study design for these measures also did not account for temporal and natural variability so an overall weighting of medium was assigned. Hatching success is considered to be strongly associated with the assessment endpoints because it directly measures reproductive effects but the study design was considered to have medium quality due to a low sample size (n=54). Hatching success may also be expected to vary from year to year depending on environmental factors such as food availability and weather.

The hazard quotient has a high strength of association with assessment endpoints as the daily dose is compared to a laboratory dose known to cause reproductive effects. The study design, however, was considered medium because site specific measures of dietary preferences, body weights, and other information were based on studies conducted elsewhere. An overall weight of medium was therefore assigned to the hazard quotient.

The degree of response for each exposure and effect measurement endpoint (Table 6-13) was ranked using objectives that are based on existing conservative and non-conservative environmental benchmarks and comparisons to reference concentrations/effects. Rankings varied from negligible (*i.e.*, no effect was observed or concentrations were below the conservative benchmarks) to high (*i.e.*, the biomarker

response was statistically higher than the response at the reference site or the concentration/hazard quotient substantially exceeded the non-conservative benchmark).

Table 6-13. The magnitude of response observed in measurement endpoints for shorthorn sculpin at Saglek Beach.

Measure	Year			
	1998/1999		2006/2007	
	Magnitude of Response	**Justification**	**Magnitude of Response**	**Justification**
Measures of Exposure				
Concentration of PCBs in sediment (A)	High	Concentrations of PCBs in sediment exceed the CCME PEL (0.189 mg/kg) by more than more than 2x	Moderate	Concentrations of PCBs in sediment exceed the CCME PEL (0.189 mg/kg) by less than 2x
Concentrations of PCBs in sculpin liver (B)	High	Concentrations of PCBs in sculpin were statistically greater than the reference concentrations (>2x)	High	Concentrations of PCBs in sculpin were statistically greater than the reference concentrations (>2x)
Phase I biotransformation enzyme (EROD) activity (C)	High	MFO activity at the Beach was statistically greater than the reference activity (>2x)	-	-
Phase II biotransformation enzyme (UDP-GT) activity (D)	-	-	Negligible	No trends were observed
Adaptive Organ (liver) enlargement (E)	Negligible	No trends were observed	Negligible	No trends were observed
Adaptive Organ (thyroid) enlargement (F)	-	-	Negligible	No trends were observed
Liver/Body lipid (G)	Negligible	No trends were observed	Negligible	No trends were observed
Measures of Effect				
Vitamin A concentration in sculpin liver (1)	-	-	Potential	A trend toward decreasing liver concentrations of retinyl esters was observed in the Beach sculpin compared to the Reference site but this trend was not statistically significant (<2x)
Vitamin E concentration in sculpin	-	-	Probable	Liver vitamin E concentrations were

liver (2)				statistically lower in sculpin from the Beach compared to the reference site
Thyroid histopathology (3)	-	-	None	No evidence of histopathology was observed
Condition index (4)	None	No trends were observed	Probable	The condition factor at the Reference site was significantly higher than the condition factor at Saglek Beach (<2x)
Hazard quotient (5)	Probable	Hazard quotient was greater than one (hazard quotient = 1.6)	None	Hazard quotient was less than one (hazard quotient = 0.9)

Table 6-14. The magnitude of response observed in measurement endpoints for black guillemot at Saglek Beach.

Measure	Year			
	1998/1999		2006/2007	
	Magnitude of Response	**Justification**	**Magnitude of Response**	**Justification**
Measures of Exposure				
Concentration of PCBs in sediment (A)	High	Concentrations of PCBs in sediment exceed the PEL by more than more than 2x	Moderate	Concentrations of PCBs in sediment exceed the PEL by less than 2x
Concentrations of PCBs in sculpin (B)	High	Concentrations of PCBs in sculpin were statistically greater than the reference concentrations (>2x)	High	Concentrations of PCBs in sculpin were statistically greater than the reference concentrations (>2x)
Concentrations of PCBs in guillemot nestlings(C)	High	Concentrations of PCBs in guillemot nestlings were statistically greater than the reference concentrations (>2x)	High	Concentrations of PCBs in guillemot nestlings were statistically greater than the reference concentrations (>2x)
Phase I biotransformation enzyme (EROD) activity (D)	Moderate	MFO activity was statistically greater than the reference site but by less than 2x	Negligible	No trends observed in MFO activity
Phase II biotransformation enzyme (UDP-GT) activity (E)	-	-	Negligible	No trends observed in UDP-GT activity
Malic Enzyme activity (F)	Negligible	No significant differences between exposure groups	-	-

Adaptive Organ (liver) enlargement (G)	Moderate	Relative liver mass was significantly higher at the Beach but by less than 2x	Pending	Pending
Measures of Effect				
Vitamin A concentration in bird liver (1)	Probable	Vitamin A was significantly lower than the reference site but less than 2x	None	No trends observed
Gonad histopathology (2)	-	-	Pending	Pending
Thyroid function (3)	Potential	No difference between exposure groups but a significant relationship between T4 and PCB concentrations was observed	Potential	No difference between exposure groups but a significant relationship between T4 and PCB concentrations was observed
Immune function (4)	Probable	Beach nestlings had lower immune response than the Reference nestlings	-	-
Steroid hormones (5)	Probable	Beach nestlings had higher concentrations of estradiol and testosterone than reference birds (~2x)	None	No trends observed
ChE/MAO activity in the brain (6)	-	-	Probable	Activity levels were statistically higher at the Beach than at the reference site (ChE:~2x; MAO: less than 2x)
Hatching success (7)	None	No trends observed. Reference = 64%, Islands = 73% and Beach = 51%	-	-
Hazard quotient (8)	Probable	Hazard quotient >1 (hazard quotient = 2.1)	Potential	Hazard quotient ~1 (hazard quotient = 1.2)

6.4.5 Concurrence among Measurement Endpoints and Estimate of Risk

The concurrence among measurement endpoints was illustrated graphically by plotting each measurement endpoint in a graph of the endpoint weight and degree of risk as axis as recommended by Menzie *et al.*, (1996) (Figures 6-3 and 6-4). The centroid (*i.e.*, geometric center) was used to determine magnitude of risk (Table 6-15). Refer to Table

6-7 for interpretation of exposure and effect evidence in determining risk.

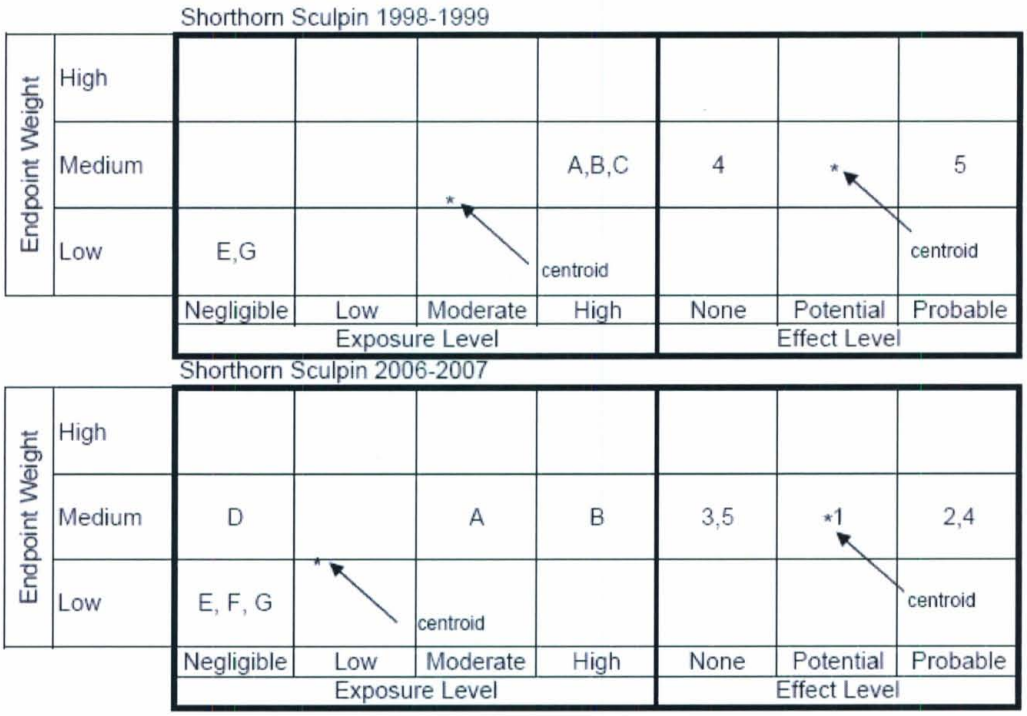

Figure 6-3. Concurrence among measurement endpoints for shorthorn sculpin from Saglek Beach in 1998/99 and 2006/07.

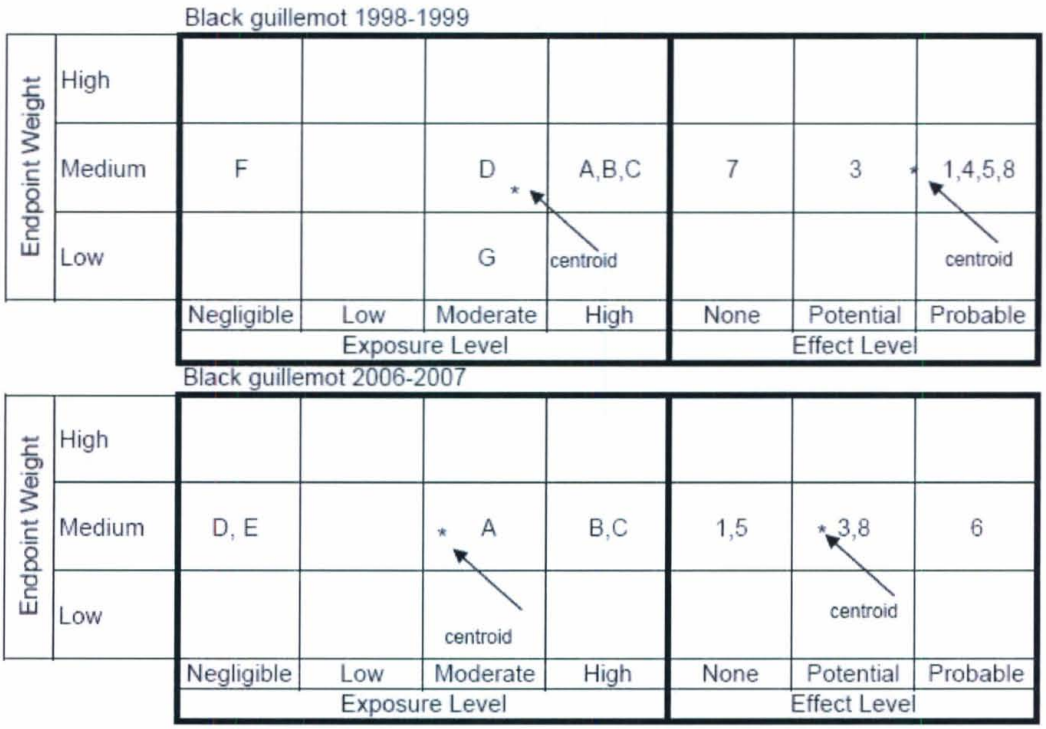

Figure 6-4. Concurrence among measurement endpoints for black guillemot from Saglek Beach in 1998/99 and 2006/07.

Table 6-15. Magnitude of risk for shorthorn sculpin and black guillemot at Saglek Beach (based on definitions in Table 6-7).

Organism	Sampling period	Evidence of exposure	Evidence of effect	Magnitude of risk	Confidence
Shorthorn sculpin	1998/99	moderate	potential	intermediate	medium
	2006/07	low	potential	low	medium
Black guillemot	1998/99	moderate	potential to probable	intermediate to high	medium
	2006/07	moderate	potential	intermediate	medium

The concurrence among measurement endpoints (Figure 6-3) indicated an intermediate risk for sculpin in 1998/1999 and a low risk for sculpin in 2006/2007. The concurrence among endpoints (Figure 6-4) predicted an intermediate to high risk for black guillemots in 1998/99 and an intermediate risk for black guillemot in 2006/07.

6.5 Discussion

Measured concentrations of PCBs in shorthorn sculpin were compared to tissue based toxicity reference values from the literature to calculate a hazard quotient for sculpin at Saglek Beach. For the black guillemots, an average daily dose was calculated using dietary models and this dose was compared to dietary toxicity reference values from the literature to calculate hazard quotients. Hazard quotients indicated that there was a potential for adverse risks to shorthorn sculpin at Saglek Beach (hazard quotient=1.6) in 1998/99 but adverse risks were unlikely (hazard quotient=0.9) in 2006/07. The weight of evidence assessment supported the hazard quotient methodology indicating an intermediate risk to shorthorn sculpin in 1998/99 and a low risk in 2006/07. For black guillemots, the hazard quotients indicated that adverse risks were likely for both 1998/99 (hazard quotient=2.1) and 2006/07 (hazard quotient=1.2). The weight of evidence assessment supported the hazard quotient indicating an intermediate to high risk to black guillemots for 1998/99 and an intermediate risk for 2006/07.

The hazard quotient approach can be strengthened by the collection of site specific measurements such as body burdens but the approach is still limited in its ability to define risk sufficiently enough to support a management decision (National Research Council, 1994). Sample *et al.*, (1996) recommend that the hazard quotient be used solely for screening purposes or as part of a weight of evidence. Because the calculations for hazard quotients are well developed and commonly used, and because there is little guidance on how field verification or higher tiers of assessment should be conducted, hazard quotients are often used as a basis for making risk management decisions.

Interpretation of the risk assessment for the sculpin in 2006/07 is relatively

straightforward, that is, if significant risk to sculpin populations were occurring at Saglek Beach, then the individual sculpin would display suborganismal and organismal signs of the toxic effects of PCBs (*e.g.*, histopathology, decreased growth). This was not the case and with the exception of vitamin E, no significant differences in biomarker responses were observed between Saglek Beach and the reference site. The results of the weight of evidence for the black guillemot, however, are more difficult to interpret from an ecological risk standpoint. Although changes in biomarkers such as increased circulating thyroid levels may be considered adverse to an individual bird, the biological significance of these findings for the health of the population is not clear. It would therefore be difficult to determine the presence of adverse risk as the biomarkers do not have clear implications for properties of the population or community. It is possible that although biological effects are occurring at the organism and suborganism level, compensatory processes that act at the individual, population, and community level may have allowed the population to persist (Suter *et al.*, 1999).

Some ecological risk assessments base their risk decisions on the presence of biomarker responses at the suborganism and organism level (*e.g.* Galloway *et al.*, 2004; Morales-Caselles *et al.*, 2008). For non-endangered species, ecological risk assessment decisions should be based on the goal of protecting populations rather than on effects at the individual level (Suter, 2007, USEPA, 1998). Therefore, any risk assessment based on biomarker responses that lack a clear link to population or community effects may overestimate risk resulting in unwarranted remediation of a chemical that may not be causing harm to the population. It would therefore violate the basic premise of ecological risk assessment to recommend remediation of impacts based on potential risks to black

guillemot at Saglek Beach predicted using biomarker responses. Although organism and suborganism level biomarkers respond, they do not have clear implications for black guillemot populations or communities. Population and community level measurements would support a clear understanding of the risks to black guillemot populations.

With the exception of a measurement of hatching success in 1998/99, no quantitative measures were available for assessing population level effects to black guillemots at Saglek. Hatching success indicated that no adverse effects were occurring. It is still difficult to use the hatching success as a measure of population level effects because it is uncertain if the success is sufficient to maintain the population. Black guillemots have been observed at Saglek beach during site visits to Saglek over the past fifteen years. The presence of highly mobile species such as birds, however, indicates almost nothing about risk because immigration replaces losses from mortality or reduced reproduction (Suter, 2007). Because black guillemots nest at the same places each year at Saglek and there is evidence that a majority of the birds are returning to the area, there may be some justification to conclude that the local population is not being affected. There is uncertainty in this justification as well since observations supply evidence that PCBs are not high enough to be lethal, but it may not address whether exposure is sufficient to limit reproduction and result in population declines (Spromberg, 1998).

PCB contamination at Saglek is believed to be associated with the abandonment of the facility which likely occurred in the 1980s so it is possible that enough time has elapsed to have brought on toxicological responses in organisms that live there. Estimates of the life span of a black guillemot are about 14 years (Environment Canada, 2005) although twenty year old adults have been found in Shetland (Ewins, 1989) and

Iceland (Fredericson, 1998). Therefore, the birds being assessed have likely only lived in the vicinity of the contaminated sediments. If the population is persisting despite the predicted adverse exposure and effects of PCBs to individual black guillemot at Saglek Beach, it is possible that the effects are not being manifested at the population level. It is also possible that the bioavailability and toxicity of PCBs in sediment has become greatly compromised with time and this has decreased the toxicity to the exposed organisms (Tannenbaum, 2003). Physiological adaptation, genetically based adaptation, and ecological processes unrelated to chemicals may also influence responses of populations to chemical exposure (Barnthouse *et al.*, 2009b). These processes do not occur in the lab and toxicity reference values derived from such lab studies are therefore very conservative. Additional information at the population level would be required to determine if the risk to black guillemots is adverse or not. The lack of reliable population and community measures limits the ability of this ecological risk assessment to determine whether or not a risk exists for the black guillemot populations at Saglek.

This assessment demonstrates the advantages of an iterative weight of evidence approach to ecological risk assessment. Because food chain models have a natural tendency toward conservatism because of the use of dietary assumptions, a tissue residue based approach is preferable. Inherent conservatism in the use of toxicity reference values, 95% UCL concentrations, as well as the variability of the parameters in Table 6-2, however, decreases the certainty of both these methods. Hazard quotients therefore should only be used in screening level assessments or as part of a weight of evidence as recommended by Sample *et al.*, (1996) (*i.e.*, a hazard quotient exceeding one is not a basis for making risk management decisions).

A screening level assessment utilizing the hazard quotient approach may be useful for initial preliminary risk estimates. Incorporation of biomarkers, chemical information, and hazard quotients into a weight of assessment may be useful for field verification of adverse health effects predicted through the screening (*i.e.*, hazard quotient) assessment stage. Often at this point, no effects are seen at the individual level such as the case of the shorthorn sculpin at Saglek and so, additional population effects studies would not be necessary if there is certainty of this lack of effects. If individual effects are observed and the extrapolation to population effects is uncertain as is the case of black guillemots at Saglek, the risk assessment may proceed to the third tier which would involve a detailed assessment of population and/or community level effects. Empirical population level assessments such as habitat assessments, biological surveys, demographic data collection, and field manipulations (Carlsen *et al.*, 2008), or modelling approaches such as individual based models and metapopulation models (Munns *et al.*, 2008) have been recommended.

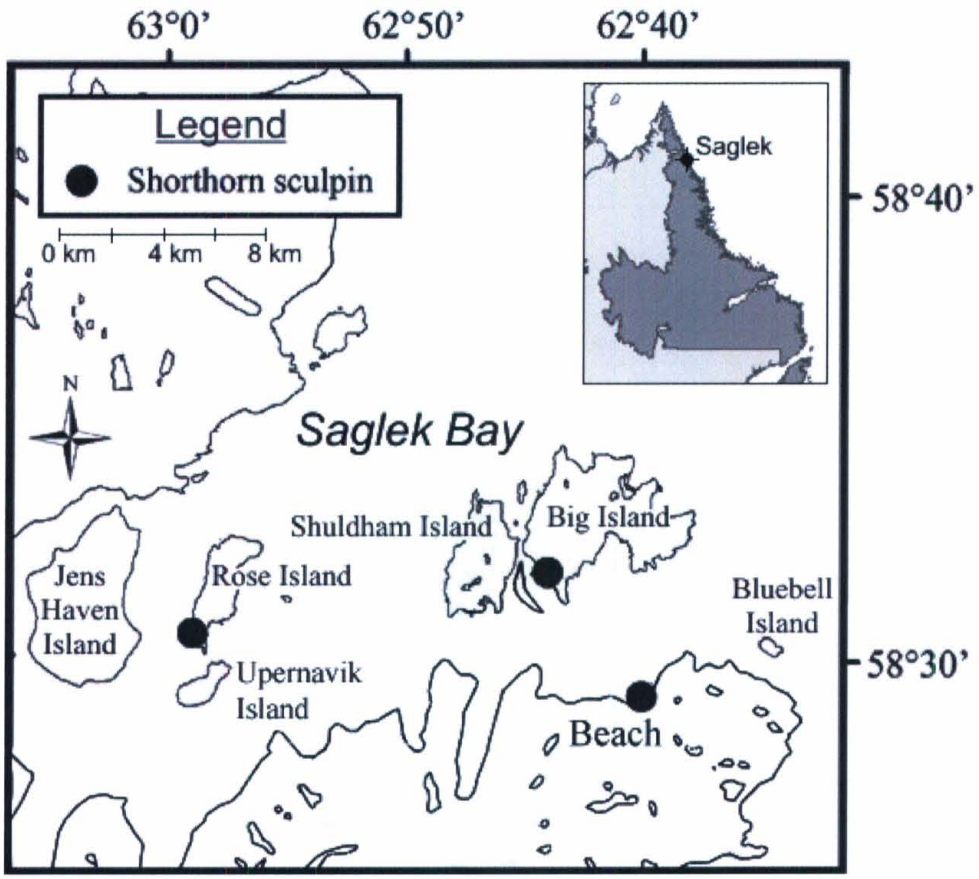

Figure 6-1. Map of Saglek showing sampling locations

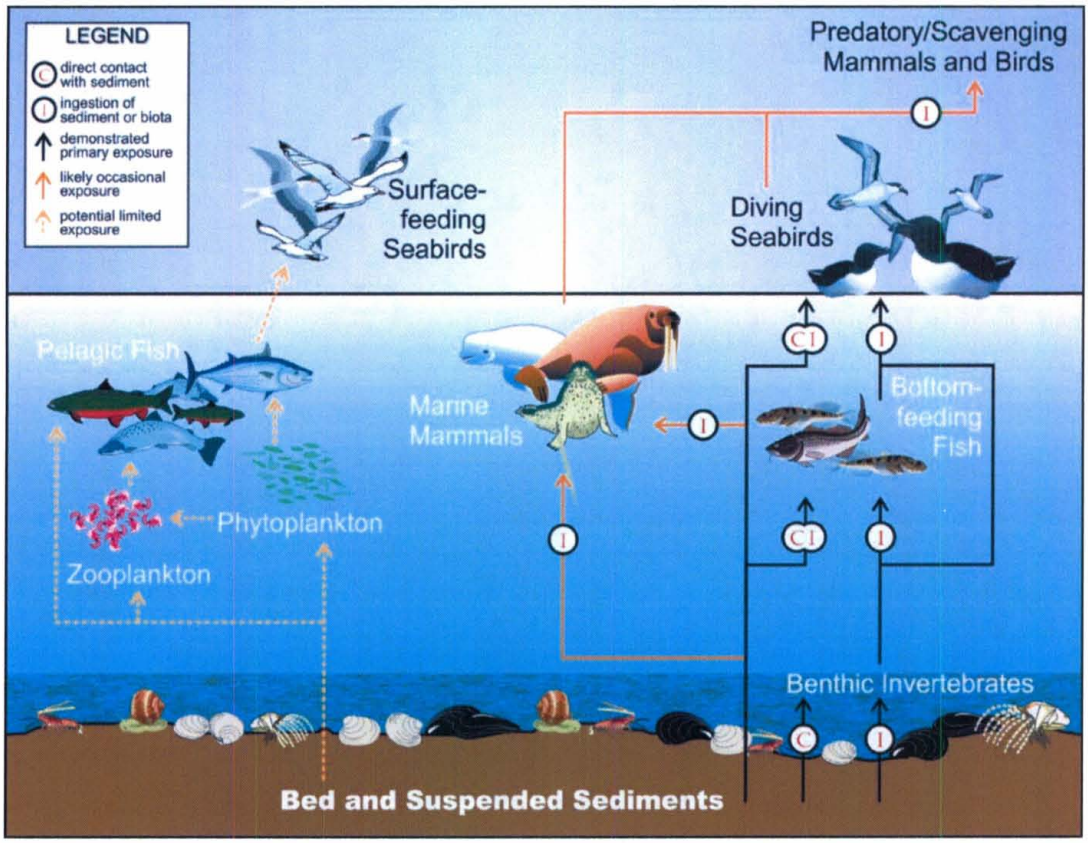

Figure 6-2. Conceptual Site Model (from ESG, 2002). The conceptual site model indicates that benthic invertebrates may be exposed to PCBs in sediments via direct exposure (symbol C) as well as ingestion of sediment or biota (symbol I). Bottom feeding fish (middle right side of Figure 6-2) are exposed via direct contact (C) and ingestion of sediment (I) as well as ingestion of benthic invertebrates (I). Marine mammals (middle of Figure 6-2) may be exposed to PCBs via ingestion of sediment (I) as well as ingestion of bottom feeding fish (I). Diving seabirds (top right hand corner) are exposed via direct contact (C) with sediment and ingestion (I) of sediment as well as ingestion (I) of bottom feeding fish. Predatory and scavenging birds and mammals are subsequently exposed via ingestion (I) of diving seabirds and marine mammals.

On the left hand side of Figure 6-2, some potential exposure pathways include the exposure of zooplankton and phytoplankton to PCBs in sediment with pelagic fish that feed on the plankton being exposed via ingestion as well as direct contact/ingestion of sediment. Surface feeding birds may be exposed via ingestion of the pelagic fish.

7.0 Chapter 7: Screening Assessment of the Ecological Risks to a Terrestrial Small Mammal (*Peromyscus maniculatus*) Exposed to Polychlorinated Biphenyls at Saglek, Labrador.

7.1 Abstract

Hazard quotients were calculated for deer mice (*Peromyscus maniculatus*) exposed to PCBs at Saglek Beach in Labrador using the dietary approach and the tissue based approach. Hazard quotients were comparable and indicated that adverse effects to deer mice at Saglek Beach are likely. This is further supported by the results of a study which indicated that bone mineral density in deer mice was lower at Saglek Beach than at a reference site. Thyroid histopathology and histomorphometry of deer mice at Saglek Beach, however, were not affected by the elevated PCB exposure. Despite the elevated hazard quotients and the effects on bone mineral density at Saglek Beach, the deer mouse population appears to be thriving based on high capture rates. Considering the short life span of the deer mouse (<1 year) and that enough time has elapsed (>20 years) for chronic population effects to be evident, there is no evidence that the deer mouse populations are being negatively affected. Other field studies on small mammals exposed to similar or higher concentrations of PCBs show a similar lack of population effects.

Key words: deer mouse; *Peromyscus maniculatus*; PCBs; ecological risk assessment; hazard quotient; biomarker

7.2 Introduction

Laboratory studies indicate that polychlorinated biphenyl (PCB) exposure can adversely affect reproduction and growth in small mammals such as *Peromyscus spp.* (Linzey, 1988; Shore and Doubin, 1994; McCoy *et al.*, 1995). The laboratory experiments that report these adverse effects differ from field situations in the use of single species of small mammals, the mode of administration, exposure to single chemicals (*i.e.*, no mixtures), and the use of laboratory-reared animals. The extent to which the laboratory studies can be used to predict adverse effects in field situations is therefore unclear. It is uncertain if concentrations of PCBs that cause sublethal effects in the lab have similar adverse effects on wild populations (Linzey and Grant, 1994; Boonstra and Bowman, 2003).

Traditionally, the evaluation of adverse effects on wild populations of terrestrial mammals is conducted using an ecological risk assessment. Ecological risk assessment (ERA) is defined as the evaluation of the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors (USEPA, 1992a). The traditional approach to ERA for terrestrial mammals is to incorporate mechanistic food chain models or tissue residue concentrations to calculate a single risk estimate. This is done by comparing the total daily ingested dose or tissue residue concentration to a toxicity reference value (*i.e.*, hazard quotient approach) (Barnthouse and Suter, 1986; USEPA 1992a). There are two primary approaches to the hazard quotient method (Millsap *et al.*, 2004). The method used most often is the "dietary approach" where the total ingested dose of a chemical consumed by a species of interest on a daily basis is predicted based on chemical concentrations in the abiotic (and/or biotic) components of

the environment. This predicted daily dose is then compared to a dietary toxicity reference (TRV) value to calculate a dietary hazard quotient. The alternate approach is the "tissue based approach" where the actual concentration of a chemical in the tissue of an organism is compared to a tissue based toxicity reference value in order to calculate a hazard quotient. While the tissue residue approach is generally presumed to be more accurate than the dietary approach, both approaches are limited by the use of laboratory based toxicity reference values and chemical concentrations for predicting toxic responses. As discussed in chapter 6 of this thesis, the hazard quotient approach was originally intended solely for screening purposes or as part of a weight of evidence approach (Sample *et al.*, 1996). Because of the conservatism in hazard quotients, a hazard quotient of less than one demonstrates that adverse risks are unlikely. Over-conservatism however can lead to an unrealistic perceived risk if the hazard quotient exceeds one. Unfortunately, there is a misconception that a hazard quotient greater than one indicates a significant risk and that this is sufficient evidence to make a risk management decision (Tannenbaum, 2003).

Another approach to assessment of the exposure and effects of small mammals to PCBs in field settings is to use biomarkers. Biomarkers are defined as a change in a biological system that can be related to an exposure to, or effect of, an environmental chemical or chemicals (Peakall and Shugart, 1994). The biological system referred to generally includes biological responses at the organism level or below (Peakall, 1999). Because biomarkers indicate exposure or effect, it is not surprising that one of the justifications for the continued research and development of biomarkers is their potential application in ecological risk assessments. Unfortunately, even though studies of

biomarkers have been conducted for many years, their incorporation into regulatory legislation for ecological risk assessment is severely lacking (Hagger *et al.*, 2006).

In this paper, hazard quotients will be calculated for deer mice (*Peromyscus maniculatus*) exposed to PCBs in a field setting. Biomarkers (including bone mineral density as examined in Johnson *et al.*, (2009) as well as effects on the thyroid which will be presented for the first time in this chapter) will also be examined to determine if the results support the hazard quotient results. The study was conducted at Saglek, a former Polevault Line military station in northern Labrador (Figure 7-1). Extensive PCB contamination in soil associated with the original facility was identified at Saglek Beach in the 1990s at concentrations ranging from less than 50 µg/g up to 1,600 µg/g dry weight (ESG, 1997). Soil remediation was carried out between 1997 and 1999 and thus, the terrestrial source has been removed. After remediation, a long term terrestrial monitoring plan was implemented and included the measurement of PCB concentrations in soil, plants, and deer mice from the site on an annual basis.

Both the dietary approach and the tissue based approach are applied to calculate hazard quotients for deer mice at Saglek Beach and the results are compared. Three biomarkers (bone mineral density, thyroid histopathology, and thyroid epithelial height) are further investigated to determine whether biomarker responses support the findings of the hazard quotient approach. A weight of evidence approach to ecological risk assessment has been advocated (Menzie *et al.*, 1996; Cook *et al.*, 1999), however, these methods are seldom applied at the same location. Weight of evidence is defined as a risk characterization process by which measurement endpoints are related to an assessment endpoint to evaluate whether risk is posed to the environment (Menzie *et al.*, 1996).

7.3 Methods

7.3.1 Study Site

Saglek Bay is located on the northeast coast of Labrador (approximately 225 km north of Nain and 600 km north of Happy Valley-Goose Bay) (Figure 7-1) at the southern extent of the Torngat Mountains National Park. The U.S. Air Force operated a Polevault Line military station at Saglek from 1951 to 1971. The site consisted of a main station on the highest summit with accommodations and operations buildings, two hilltop tropospheric antenna sites, and a lower camp with an airstrip, several buildings and a beach landing area. The station was abandoned in 1971 and the site was destroyed by a fire in 1978 (Pier *et al.*, 2003). The Canadian Department of National Defence (DND) opened a modern North Warning System (NWS) long range radar facility in 1988. The long range radar facility is currently operated remotely and is unmanned with maintenance conducted at the site seasonally and on an as-needed basis by DND.

7.3.2 Soil, Plant and Deer Mouse Sample Collection

The soil and plant samples used in this study were collected from Saglek Beach in 2005 (soil n=7; plants n=4), 2006 (soil n=20; plants n=10), and 2007 (soil n=10; plants n=10) as part of the long term monitoring plan implemented after remediation at Saglek (ESG, 2005; 2006; unpublished data). In 2007, as part of the field work for this thesis, mice were sampled from the reference site. No soil or plants were collected or analysed from the reference site in 2007 as the reference site was not part of the long term monitoring.

Soil and plant samples were collected in August of each respective sampling year. Soil samples were collected from depths ranging from 0 to 20 cm. The soils were collected using a scoop that was cleaned before and after each sample was taken. Each soil sample (approximately 200 g) was stored in a polyethylene bag and stored on ice packs in coolers until they could be frozen.

Plant samples were collected within the vicinity of the soil samples at each site. An aluminium foil envelope (approximately 10 cm × 10 cm) was prepared and at least 15 g of material was collected for each sample. Because of the small stature of Arctic vegetation, several plants (same species) were sampled during the collection of one individual sample. Considerable effort was invested to ensure that each individual plant was represented in the sample. Care was taken to ensure that woody stems were not included in the samples, and that leaf surfaces were not touched during the sampling process as this could remove PCBs from the samples and potentially result in the cross-contamination of samples. Once a sample had been collected, the foil package was closed and placed in a labelled polyethylene bag. Samples were stored frozen until analysis. Plant samples collected consisted of willow (*Salix arctica*) (n=1) and dwarf birch (*Betula glandulosa*) (n= 23)

Deer mice were collected from Saglek Beach as well as a reference area located at the southeastern end of the airstrip (Figure 7-1) in August 2007. Mice were collected using live traps (aluminium perforated folding traps 7.6 cm x 8.9 cm x 22.9 cm from H.B. Sherman Traps Tallahassee, Florida, USA) and snap traps (Victor® snap traps) placed in the same general area as the soil and plant sample collection. Mice from the live traps were killed by cervical dislocation. At the Beach area, both live traps and snap traps

were set in the immediate vicinity of each other. Mice from the live traps were used for biomarker measurements (bone mineral density, thyroid histopathology and thyroid morphometrics) and mice from the snap traps were used for PCB analysis. At the reference area, only live traps were set and the mice from these live traps were used for PCB analysis and biomarkers. Each mouse was placed in a labelled polyethylene bag and stored frozen until analysis.

7.3.3 PCB Analysis

PCB analyses were conducted by AXYS Analytical Services in Sidney, British Columbia. Thirty-seven surface (*i.e.*, 0 - 20 cm depth) soil samples collected from the Beach in 2005 (n=7), 2006 (n=20), and 2007 (n=10) were analysed for PCBs. Twenty-four plant samples collected from the Beach in 2005 (n=4), 2006 (n=10), and 2007 (n=10) were analysed for PCBs. The whole bodies of the snap trapped deer mice (n=20) from the Beach and the live trapped mice (n=7) from the reference site were used for PCB analysis. The PCB concentrations reported and analysed here are total congeners. Analytical methods for soil, plant, and deer mouse samples are summarized in Appendix 1.

7.3.4 Statistical Analysis

Relationships between variables were determined using the general linear model (normal errors) which includes analysis of variance, analysis of covariance, and regression. Errors were checked to confirm normality, homogeneity, and independence

by observing histograms and normal probability plots of the residuals and plotting residuals against predicted values. The significance level was set to $p \leq 5\%$.

7.3.5 Hazard Quotients

For the dietary approach, the representative exposure point concentration (EPC) for ∑PCBs in soil and plants were determined using ProUCL 4.0 (USEPA, 2007). The EPC was determined as the upper confidence limit (UCL) given the specific distribution of the site specific analytical results data (*i.e.*, normal, log normal, or gamma). For data that do not follow a distribution or for highly skewed log normal distributions, other non-parametric UCLs (*e.g.*, Chebyshev (mean, standard deviation) UCL) were used. USEPA (1992b) recommends that the 95 percent UCL be used as the concentration term because it provides reasonable confidence that the true site average will not be underestimated. Concentrations of PCBs in invertebrates were not available for the site so appropriate models were applied. The relationship between concentrations of PCBs in earthworms and concentrations of PCBs in soil was developed by Sample *et al.*, (1998) using a regression model as follows:

$$ln\ (C_{earthworm}) = (1.41 + 1.361 * ln(C_{soil}))$$

where:

$C_{earthworm}$ = concentration of PCBs in earthworm tissue (mg/kg dry weight)

C_{soil} = concentration of PCBs in soil (mg/kg dry weight) from Table 7-1

The dry weight concentration of PCBs in invertebrates is converted to wet weight by multiplying by 0.16 (*i.e.*, moisture content for earthworms is 84% (USEPA, 1993)).

It is unlikely that deer mice would feed on earthworms at Saglek but the concentrations calculated for this animal are used to represent other invertebrates that the deer mouse may feed on. The following equation consistent with USEPA's wildlife exposure guidance (USEPA, 1993) was used to calculate the average daily dose (ADD_{PCBs}) for deer mice from ingestion:

$$ADD_{PCBs} = \sum(C_y * IR_y * F_y)/BW$$

Where C_y is the concentration of PCBs (mg/kg) in each food item (*i.e.*, soil, plants, or invertebrates), IR_y is the food ingestion rate of each food item (kg/day), F_y is the fraction of food from the site and BW is the body weight of deer mice (kg). Values used in this calculation are presented in Table 7-1.

Table 7-1. Values used in the calculation of the average daily dose for deer mice at Saglek Beach.

Parameter	Value	Reference
C_{soil} (mg/kg)	8.95	Site specific, 99% Chebyshev (mean, sd) UCL (data do not follow a discernible distribution)
C_{plants} (mg/kg)	0.019	Site specific, 95% student's t-distribution UCL (normal distribution)
$C_{invertebrates}$ (mg/kg)	13.0	Calculated
Body weight (kg)	0.017	Site data (average)
food ingestion rate (kg/d)	0.0046	Based on normalized food ingestion rate of 0.27 g.g $bw^{-1}d^{-1}$ (arithmetic mean of means (EPA 1993))
Plant fraction of food intake	0.50	Based on data from Whitaker (1966), Batzli (1977) and Wolff *et al.* (1985), diet is considered to be the reproductive portions of the plants
Animal fraction of food intake	0.46	Arthropods, based on data from Whitaker (1966), Batzli (1977) and Wolff *et al.* (1985)
Soil fraction of food intake	0.02	Beyer *et al* (1994)

It was assumed that the entire diet of the deer mice is from the site (*i.e.*, F_y =1) and that 100% of the PCBs are absorbed (*i.e.*, absorption factor is equal to one). The hazard quotient for PCBs is then calculated as ADD_{PCBs}/TRV.

A literature search of PCB feeding studies for *Peromyscus* species was conducted to determine an appropriate toxicity reference dose for deer mice exposed to PCBs at Saglek. Choice of lowest observed adverse effect level (LOAEL) was based on chronic exposure of sensitive life stages, measure of ecologically relevant endpoints (*e.g.*, reproduction), close relatedness of the test species to the receptor species, and minimal impact of other contaminants (Table 7-2).

Table 7-2. Toxicity reference value derivation for deer mice exposed to PCBs at Saglek Beach.

Test species	Duration	PCB	Effect	Reference	Daily dose or effects concentration ($mg\ kg^{-1}\ day^{-1}$)
White-footed mouse (*Peromyscus leucopus*)	60 days laboratory exposure to PCB1254 orally in diet (subchronic)	PCB1254	Reproduction (decreased number of litters)	Merson and Kirkpatrick (1976)	18 $mg\ kg^{-1}day^{-1}$ (LOAEL)
White-footed mouse (*Peromyscus leucopus*)	28 days laboratory exposure PCB 1254 orally in diet (subchronic)	PCB1254	Reproduction (first exposure at 16 weeks of age: smaller numbers of young; first exposure at 12 weeks of age: longer intervals between births, smaller litter sizes at birth and at weaning)	Linzey, 1987	0.83 $mg\ kg^{-1}day^{-1}$ (LOAEL)
Oldfield mouse (*Peromyscus poliontus*)	Chronic laboratory (12 months) exposure to PCB1254 orally in diet (chronic)	PCB1254	Reproductive effects (reduced number of litters, offspring weights, and offspring survival)	McCoy *et al.* (1995)	0.68$mg\ kg^{-1}day^{-1}$ (LOAEL)
					7.51 $mg\ kg^{-1}$ (LOAEC)

White-footed mouse (*Peromyscus leucopus*)	4 months laboratory exposure to 2:1 PCB1242: PCB1254 orally in diet (chronic)	PCB1242: PCB1254	Reproductive effects (decreased number of litters)	Voltura and French (2007)	6.2 mg $kg^{-1}day^{-1}$ (LOAEL)

In the absence of appropriate studies on PCB1260, other Aroclor studies were used. The endpoint most commonly studied include reproductive success measured in a variety of ways including litter size, number of litters, pup growth rate, and number of young weaned. The number of litters appeared to be a sensitive endpoint (Merson and Kirkpatrick, 1976; Linzey 1988; McCoy *et al.*, 1995; Voltura and French, 2007) while the offspring survival was also sensitive (Linzey, 1987; Linzey, 1988; McCoy *et al.*, 1995).

White footed mice exposed to 200 ppm PCB1254 in their diet (18 mg $kg^{-1}day^{-1}$) had decreased numbers of litters but the litter size was not affected (Merson and Kirkpatrick, 1976). Linzey (1987) found that white footed mice exposed to 10 mg/kg of PCB1254 in their diet starting at 16 weeks of age had decreased survival of the offspring but also showed no changes in litter size. In the same study, mice exposed staring at 12 weeks of age had longer intervals between births, smaller sizes at birth, and smaller litter sizes at weaning (Linzey, 1987). The second generation of these mice had decreased reproduction and smaller offspring (Linzey, 1988). McCoy *et al.* (1995) studied the reproductive effects in oldfield mice at half the exposure concentration as Linzey (1987; 1988) (*i.e.*, 5 ppm PCB1254) for twelve months. The first and second generation offspring had lower body mass than that of controls. In addition, reduced numbers of litters and decreased offspring survival were observed. Voltura and French (2007)

observed decreased numbers of litters in white footed mice exposed to 25 ppm (6.2 mg $kg^{-1}day^{-1}$) of a 2:1 mixture of PCB1242:PCB1254 but no effects on litter size at birth or at weaning were observed. The male mice in this study were not exposed to PCBs until a few days before mating with the female mice whereas in other studies the male mice were exposed to PCBs for the duration of the studies. Decreased testicular mass (Batty *et al.*, 1990), decreased male accessory organ and female uteri and ovary mass (Linzey, 1988), as well as histological degeneration of testicular tissue has been shown in mice exposed to PCBs (Alston *et al.*, 2003). Voltura and French (2007) therefore suggest that a decrease in fertility of the male mice may be responsible for the decreased litter sizes observed in other studies.

The TRV chosen for deer mice is based on the study by McCoy *et al.*, (1995). In that study, the chronic effects of PCBs on a representative endpoint (*i.e.*, reproduction) in a wildlife species (*i.e.*, oldfield mice) are studied. To establish the TRV for deer mice, three types of uncertainty factors were considered: interspecies uncertainty factors for extrapolating the lowest observed adverse effect level (LOAEL) from the oldfield mouse to the deer mouse, a subchronic-to-chronic uncertainty factor, and a LOAEL-to-no observed adverse effect level (NOAEL) uncertainty factor. Both the oldfield mouse and the deer mouse are from the genus *Peromyscus* so no interspecies uncertainty factor was applied. The study was a chronic study and the derived LOAEL was used so subchronic to chronic uncertainty factors or LOAEL to NOAEL uncertainty factors were not applied. A hazard quotient of one or more indicates that an adverse effect is likely to occur (USEPA, 1992a). It should be noted that allometric scaling factors to extrapolate toxicity data between species with different body masses were not considered here because the

test species body mass (0.014 kg) was similar to the body masses observed in this study (average = 0.017 kg).

The tissue residue approach was also used to compare concentrations of PCBs in mice to effects concentrations reported in the literature. The representative exposure point concentration (EPC) for ∑PCBs in deer mice whole body at Saglek Beach was determined using ProUCL 4.0 (USEPA, 2007). The EPC was determined as the 95% upper confidence limit (UCL) on the log normal distribution of the site specific analytical results data. The calculated exposure point concentration of PCBs in deer mice (16.2 mg/kg) was then compared to the effects concentration from the literature (Table 7-2) to calculate a hazard quotient. The study by McCoy *et al.* (1995) (used to derive the dietary TRV) also report the mean whole body concentration (7.51 mg/kg) of the mice that experienced the reproductive effects upon which the dietary TRV was based. This concentration was therefore used as the lowest observed adverse concentration (LOAEC) in the tissue based hazard quotient calculations.

7.3.6 Biomarkers

7.3.6.1 Bone Mineral Density

Methods for bone mineral density are detailed in Johnson *et al.* (2009) which is Chapter 2 of this thesis. Briefly, bone mineral density (BMD) was measured on the femur of the deer mice using the PIXImus 2 Bone Densitometer (General Electric Lunar, Madison, WI) and analyzed with PIXImus software version 2.1 at the Faculty of Medicine-Endocrinology at Memorial University of Newfoundland and Labrador. All bone mineral density measurements were conducted without prior knowledge of the

levels of PCB concentrations and all samples bore sample numbers that contained no information about their collection site or PCB exposure level.

7.3.6.2 Thyroid Histopathology and Morphometry

The throat (containing the thyroid gland) of each live-trapped deer mouse was excised at the time of dissection and fixed in 10% neutral buffered formalin for several days. Tissues were then rinsed three times in 70% ethanol and dehydrated in graded alcohols, cleared in toluene, and infiltrated with molten paraffin using a Tissue Tek VIP 5 tissue processor. Tissues were then embedded in paraffin blocks for routine sectioning. Mouse thyroids were oriented in frontal plane and examined for evidence of proliferative lesions. Thyroid epithelial cell heights were measured on one representative section per mouse. One lobe of the thyroid gland was photographed at low magnification, and a transect was overlaid on the longest axis. The first 10 colloid-containing follicles on the transect were measured (*i.e.* epithelial cell heights were measured at the four cardinal points). The values were averaged (n=40) to determine the mean epithelial cell height for each mouse.

7.4 Results

Thirty-seven soil and twenty-four plant samples were collected from Saglek Beach and were analysed for PCBs during 2005, 2006, and 2007. Snap-trapped mice (n=20) and live-trapped mice (n=19) were collected from the Beach area in 2007. Snap trapped mice from the Beach area were used for PCB analysis while live trapped mice were used for biomarker measurements. Only live-trapped mice (n=7) were collected

from the reference area and these mice were used for both PCB analysis and biomarker measurements.

The body mass of the mice used for biomarkers ranged from 10.7 g to 27.9 g and the collection included twenty-one male mice and five female mice (*i.e.*, three female mice from the Beach and two female mice from the reference site). As discussed in Chapter 2, the general linear model was used to determine if body mass differed between sites. Body mass did not differ between sites ($F_{1,25}$= 0.36, p=0.55). Because of the small number of females trapped at each site, sex was not considered a factor in the statistical model.

7.4.1 PCB Concentrations

Total PCB (∑PCB) concentrations in soil samples from Saglek Beach ranged from 0.05 mg/kg to 20 mg/kg (Table 7-3). In plant samples from Saglek Beach, ∑PCB concentrations ranged from 0.003 mg/kg to 0.03 mg/kg. No soil samples or plant samples were collected from the reference area. Total PCBs (∑PCB; total 91 congeners measured) in deer mouse whole bodies from the Beach ranged from 0.4 mg/kg to 23 mg/kg (Table 7-3).

As discussed in Chapter 2, the general linear model was used to determine if PCB concentrations were related to body mass or if PCB concentrations differed between sites. Total PCB concentrations were not related to body mass ($F_{1,23}$=0.10, p=0.75) and did not differ between sites ($F_{1,23}$=0.61, p=0.44). The interaction term between body mass and site was not significant ($F_{1,23}$=0.092, p=0.76).

7.4.2 Bone Mineral Density

Bone mineral density was initially presented in Chapter 2. Bone mineral density in mice from ranged from 24 mg/cm^2 to 62 mg/cm^2 at Saglek Beach and from 35 mg/cm^2 to 64 mg/cm^2 at the reference area (Table 7-3). As discussed in Chapter 2, the general linear model was used to determine if BMD was related to body mass or if BMD differed between sites. Bone mineral density was related to body mass ($F_{1,22}$=15.7, p=0.001) with larger mice having a higher bone mineral density. The bone mineral density differed between sites ($F_{1,22}$=4.50, p=0.04) with mice from the reference site (56±10 mg/cm^2) having a higher mean BMD than mice from the Beach site (42±11 mg/cm^2). The interaction term between body mass and site was not significant ($F_{1,22}$=2.59, p=0.12).

7.4.3 Thyroid Histopathology and Histomorphometry

Thyroid epithelial cell height and histopathology for deer mice is presented for the first time in this chapter. Thyroid epithelial cell height ranged from 3.8 µm to 6.9 µm at Saglek Beach and from 4.7 µm to 6.2 µm at the reference site (Table 7-3). The general linear model was used to determine if thyroid epithelial cell height was related to body mass or if thyroid epithelial cell height differed between sites. The interaction term between body mass and site was also examined. Thyroid epithelial cell height was not related to body mass ($F_{1,22}$=0.006, p=0.94) nor did thyroid epithelial cell height differ between sites ($F_{1,22}$=1.60, p=0.22). The interaction term between body mass and site was not significant ($F_{1,22}$=1.76, p=0.20).

The thyroid follicles contained eosinophilic colloid with varying degrees of vacuolation (Figure 7-2). There was no consistent difference among sites in the histological appearance of mouse thyroids.

Table 7-3. Total PCB concentrations (total congeners) in soil, plants and deer mice and biomarker measurements in deer mice at Saglek, Labrador. Note that soil and plant concentrations were reported previously in ESG (2005; 2007; unpublished data).

	Beach	**Reference**
Total PCBs (mg/kg)		
Soil		
Range	0.05 – 20	-
average	2.2 ± 4.1	-
n	37	-
Plants		
Range	0.003 – 0.03	-
average	0.02±0.007	-
n	24	-
Deer Mice		
Range	0.4-23	0.02-0.2
average	5.8±6.8	0.08±0.06
n	20	7
Bone Mineral Density		
BMD [average (mg/cm^2)±sd]	42 ± 11	56 ± 10
range	24 - 62	35 - 64
t-score (average)	-1.45	-
Classification	Osteopenia	-
n	19	7
Thyroid Epithelial Cell Height		
Cell height [average (µm)±sd]	5.5 ± 0.8	5.5 ± 0.7
range	3.8 - 6.9	4.7 – 6.2
n	19	7

7.4.4 Hazard Quotients

The hazard quotients calculated for deer mice at Saglek Beach using the tissue based approach and the dietary approach were 2.1 and 2.3, respectively (Table 7-4) thus indicating that adverse risks to deer mice at Saglek Beach are likely. For the dietary approach, ingestion of invertebrates contributed the highest daily dose of ingested PCBs (Table 7-4).

Table 7-4. Hazard quotients calculated using the tissue based approach and the dietary approach for deer mice at Saglek Beach.

Tissue Based Approach		
Concentration in deer mice (mg/kg)	TRV (mg/kg)	Hazard Quotient
16	7.51	2.1
Dietary Approach		
Average Daily Dose	Soil (mg $kg^{-1}day^{-1}$)	0.05
	Invertebrates (mg $kg^{-1}day^{-1}$)	1.6
	Plants (mg $kg^{-1}day^{-1}$)	0.002
	Total (mg $kg^{-1}day^{-1}$)	1.6
	TRV (mg $kg^{-1}day^{-1}$)	0.68
	Hazard Quotient	2.3

7.5 Discussion

The hazard quotients calculated using the dietary approach (2.3) and the tissue residue (2.1) indicated that adverse effects to deer mice at Saglek Beach are likely. Bone mineral density was significantly lower in deer mice from Saglek Beach (liver PCB concentrations ranging from 0.05 mg/kg to 23 mg/kg) than in mice from the reference area where PCBs in deer mice liver were relatively low (liver PCB concentrations ranging from 0.02 mg/kg to 0.2 mg/kg). Thyroid histopathology on the other hand indicated no consistent differences in the histological appearance of thyroids from Saglek Beach deer mice and reference deer mice. Similarly, there was no significant difference in mean thyroid epithelial cell height between mice from the reference site and Saglek Beach.

There was considerable agreement in the hazard quotient calculated using the dietary approach and the hazard quotient calculated using the tissue based approach. The tissue residue approach is considered preferable to the dietary approach because the lower uncertainty in the estimated risk and the fact that less information is needed for the estimate (Millsap *et al.*, 2004; Leonards *et al.*, 2008). Obtaining contaminant

concentrations in tissues is rare however, because of legal and ethical issues which may prevent tissue collection and analysis. Other animals may be difficult to sample as they may be solitary, nocturnal, or occupy a large territory. In rare cases when body burdens are available for a site, the lack of toxicological information for certain species (particularly top predators) and the fact that regular animal toxicity studies are based on dose and rarely report the internal tissue residue levels, makes interpretation difficult (Leonards *et al.*, 2008). This study indicates that the results of both methods are comparable thus supporting the findings of Millsap *et al.* (2004) who recently found good agreement between the hazard quotients from a tissue based approach and a dietary approach for mink exposed to PCBs.

As discussed in the previous paragraph, the dietary approach has higher uncertainty in the estimated risk than the tissue based approach (Millsap *et al.*, 2004; Leonards *et al.*, 2008). The uncertainty of the dietary approach is mainly due to the variability in parameters used in the calculations such as the parameters in Table 7-1. Conservatism is introduced by using the lowest applicable LOAEL as well as by using conservative models such as the earthworm uptake factor. It is unlikely that deer mice would feed on earthworms at Saglek as they would be expected to feed on other invertebrates that would take up less PCBs than an earthworm. In addition, PCB concentrations in soil, plants and deer mice were variable. The use of the 95% UCL concentrations and the lowest LOAEL in the calculations also introduces conservatism.

It should be noted that no formal weight of evidence assessment was conducted for the deer mice at Saglek because of the low numbers of lines of evidence. Therefore, the biomarkers will be discussed here as well as whether or not they support the results of

the hazard quotients. Bone mineral density in deer mice was lower at Saglek Beach where exposure to PCBs is elevated compared to deer mice at the reference site where exposure to PCBs is minimal. Other studies have found similar results for polar bears (Sonne *et al.*, 2004) and grey seals (Lind *et al.*, 2003) over time periods associated with increased use of endocrine disrupting chemicals such as DDT and PCBs. For a discussion of the mechanisms linking PCB exposure to reduced bone mineral density, refer to Johnson *et al* (2009) which is chapter 2 of this thesis. T-scores (based on World Health Organization methodology) were also calculated for mice at the Beach area and indicated that the average classification of bone mineral density status for these mice would be osteopenia while the most extreme cases would be osteoporosis (Johnson *et al.*, 2009). As discussed in chapter 2 of this thesis, it is unknown if lower bone mineral is having an adverse effect on survival or reproduction of the deer mice from Saglek Beach. Studies on humans (Alveblom, 2003) and laying hens (Fleming *et al*, 1998) have associated decreased bone composition with an increased incidence of fractures. A relationship between bone mineral density and bone breaking strength has also been found in raptors (Knopper *et al.*, 2007). Osteoporosis because of lower bone mineral density has been recognized as a major public health problem for humans (Riggs and Melton, 1995). It is possible that deer mice exposed to elevated levels of PCBs at Saglek Beach may be prone to increased incidences of fractures thus affecting their ability to carry out normal functions and hence affecting their reproduction and survival.

PCB exposure has been shown to affect thyroid hormone secretion and thyroid gland histology in small mammals (Bastomsky *et al.*, 1976; Sonstegard and Leatherland, 1979; Ness *et al.*, 1993; French *et al.*, 2001). If the thyroid undergoes stimulation,

predictable increases in epithelial cell height occur as well as effects such as vascularity, follicle irregularities, and decreases in colloid density (Eales and Brown, 1993). In this study, there was no significant difference in mean thyroid epithelial cell height between mice from the reference site and Saglek Beach. Similarly, thyroid histopathology indicated no consistent differences in the histological appearance of thyroids in deer mice from Saglek Beach and the reference site. Johnson *et al.*, (in prep) (Chapter 4 of this thesis) also found a similar lack of effects on thyroid histopathology and histomorphometry for shorthorn sculpin (*Myoxocephalus scorpius*) exposed to PCBs at Saglek Beach.

An ecological risk assessment for the marine ecosystem at Saglek Bay indicated that black guillemot (*Cepphus grylle*), a diving piscivorous bird, are at elevated risk because of PCB exposure (chapter 6 of this thesis). Despite the indications (*i.e.*, hazard quotients and biomarkers), the species appears to be thriving (see chapter 6 of this thesis). Observational studies indicate that black guillemots are nesting and reproducing at Saglek Beach and that the same birds are returning to their nests year after year. There is uncertainty here because observational studies only supply evidence that PCBs are not high enough to be lethal, but do not address whether exposure is sufficient to limit reproduction and result in population declines (Spromberg, 1998). PCB contamination at Saglek is believed to be associated with the abandonment of the facility which took place in the 1980s. Therefore, enough time has likely elapsed to have brought on toxicological responses in organisms that live there. The potential life span of black guillemot is 14 years (Environment Canada, 2005) and so it is possible that black guillemot being studied have been living in the vicinity of the contaminated sediments for their entire lives, but it

is difficult to determine if enough time has elapsed for adverse reproductive effects to have occurred and to be evident in the population. Deer mice on the other hand have a short life span (< 1 year) (Millar and Innes, 1983). They are expected to reach sexual maturity at 35 days for male and 60 days for female (Millar, 1985) and average approximately 2 litters per year (Millar, 1989; Millar, 1985). Therefore, if PCBs at Saglek are associated with the abandonment of the facility which occurred in the 1980s, then elevated PCB concentrations have existed there for at least 20 generations. If adverse effects on reproduction are occurring, then enough time has elapsed for the effects to be seen in the population. The capture rates for four successive trapping nights at Saglek Beach ranged from 30% to 70% (average = 48%) while the capture rates for the same four successive trapping nights at the Reference site ranged from 0% to 80% (average = 35%).

Other studies on similar species of small mammals exposed to PCBs in the field do not provide evidence of adverse effects on populations (*e.g.*, Linzey and Grant, 1994; Boonstra and Bowman, 2003). Boonstra and Bowman (2003) did not observe any population effects in a study of short-tailed shrew (*Blarina brevicauda*) exposed to soil with average PCB concentrations higher than what is observed at Saglek Beach (ranging from 1.5 mg/kg up to 38.3 mg/kg). Linzey and Grant (1994) also did not observe adverse population effects in white footed mice (*Peromyscus leucopus*) exposed to PCBs but the soil concentrations were lower (average = 0.30 mg/kg, range = 0.00 to 0.68 mg/kg, n=25) than what has been observed at Saglek Beach.

The measurement of population related indices of deer mice was not an objective of this study. However, from the information collected in the field, it was determined

that body mass of the deer mice did not differ significantly between sites. Of the live trapped mice from Saglek Beach, the male to female ratio was 16:3 while at the reference area it was 5:2. Sex ratios may give insight into the breeding structure and whether there are differential effects of PCBs on one sex (Boonstra and Bowman, 2003). The higher proportions of males compared to females at both sites is consistent with other studies of *Peromyscus spp.* (Manneville, 1956; Batty *et al.*, 1990). It has been suggested that the higher proportion of males caught is because of their greater movement resulting in greater exposure to the traps (Townsend, 1935) or that females are less inclined to approach novel objects, especially during the breeding season (Batty *et al.*, 1990).

7.6 Conclusions

Although PCBs are elevated at Saglek Beach and although bone mineral density in deer mice appears to be lower at Saglek Beach as a result of the PCB exposure, it is possible that effects are not being manifested at the population level. Physiological adaptation, genetically based adaptation, and ecological processes unrelated to chemicals may also be influencing responses of populations to chemical exposure (Barnthouse *et al.*, 2009b). These processes do not occur in the lab and toxicity reference values derived from such lab studies are therefore very conservative. Additional information at the population level (*e.g.*, density estimates, reproductive conditions, age-sex ratios) would be required to confirm if the risk to deer mice is adverse or not. Based on capture rates, Saglek Beach appears to support a healthy and abundant population of deer mice. Given that the population continues to persist after over 20 years of elevated exposure to PCBs, it is considered unlikely that population effects would be observed.

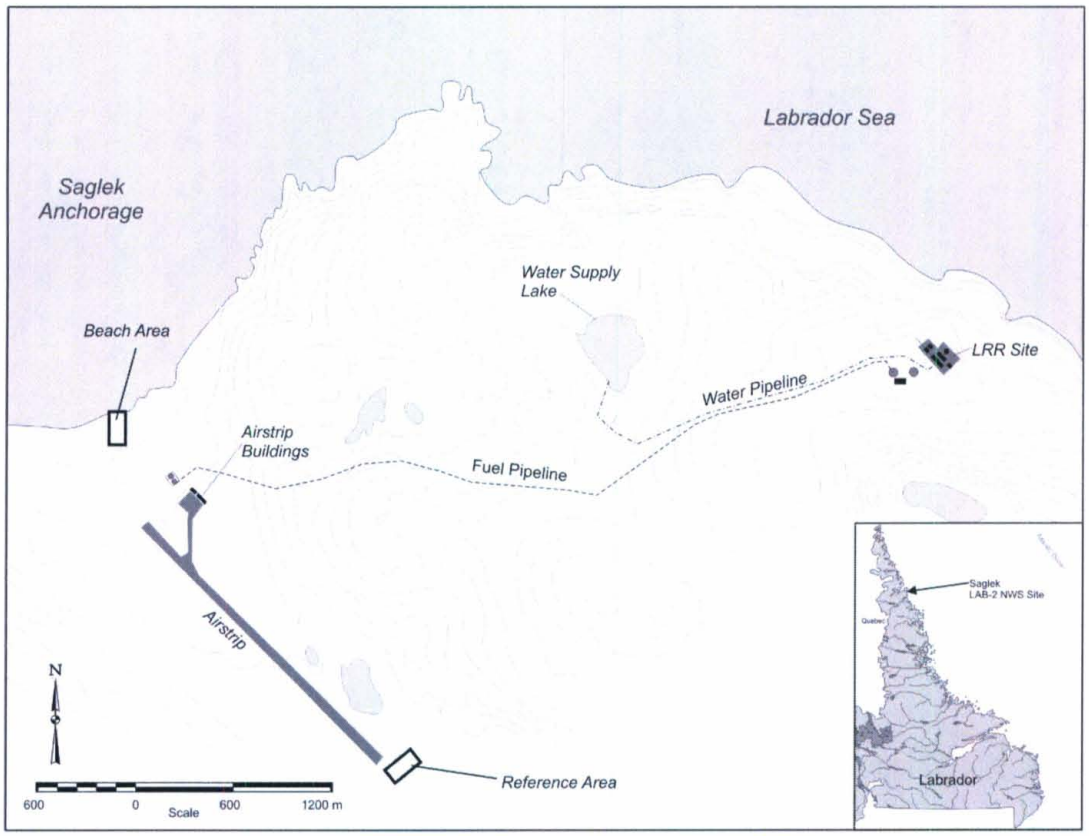

Figure 7-1. Sampling sites for soil, plants and deer mice (Beach and Reference) at Saglek, Labrador.

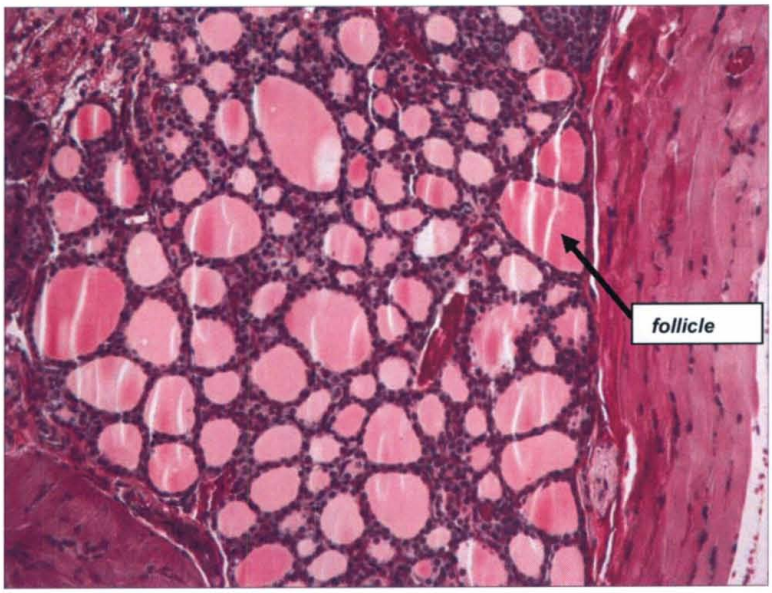

Figure 7-2. Photomicrograph of a thyroid section of a deer mouse from the reference site (magnification = 200x).

8.0 Chapter 8 Summary and Conclusions

8.1 Biomarkers at Saglek

Localised sediment and soil PCB contamination at Saglek, a former military radar site in Northern Labrador, provided a unique opportunity to study biomarker responses in marine and terrestrial ecological receptors and to use these biomarker responses in a biological effects based ecological risk assessment. Saglek provided a superb site to conduct an ecological risk assessment of PCB contamination because the PCBs are widespread (ESG, 1999), other inorganic and organic contaminants are low compared to ambient levels in industrialized areas (ESG, 1997; ESG, 1999), and PCBs have been confirmed to be present in the marine and terrestrial food webs (Kuzyk *et al.*, 2005a; ESG, 2005; ESG, 2007).

Biomarker studies presented in chapters 2, 3, 4, 5 and 7 of this thesis are consistent with previous studies (Kuzyk *et al.*, 2003; 2005b), which indicate that PCBs at Saglek are affecting certain biomarker responses but not others. In shorthorn sculpin (chapter 4), hepatosomatic index, liver lipid content, biotransformation enzyme activity, vitamin A, and thyroid histopathology/histomorphometry did not differ between the sites nor did these biomarker responses show significant relationships to PCB concentrations. The body conditions as well as the hepatic concentrations of vitamin E, however, were significantly lower at Saglek Beach compared to the reference site. The prevalence and abundance of certain gastrointestinal parasites in shorthorn sculpin (chapter 3) were also lower at Saglek Beach compared to the reference site. In Black guillemot (chapter 5), bone mineral density as well as phase I and phase II biotransformation enzymes did not show a significant relationship to PCBs nor did these biomarker responses differ between sites. In deer mice, thyroid histopathology and histomorphometry (chapter 7) did not differ between the sites, however, bone mineral density

(chapter 2) was lower at Saglek Beach than at the reference site.

8.2 Biomarkers and Ecological Risk Assessment

As discussed in chapter 1, ERA frameworks (*e.g.*, USEPA, 1992a; 1998; CCME, 1996) and literature (Suter, 2007) clearly emphasize a multi-tiered approach to ERA. However, ERAs, seldom go beyond the screening assessment stage and are not used with any regularity by the majority of the decision makers managing chemical contaminants (Hope, 2009). This thesis provides useful information on the value of a tiered approach to ERA using field verification of adverse health effects predicted through the screening (*i.e.*, hazard quotient) assessment stage.

Ecological risk assessments typically involve assessment endpoints that are generally not measurable in a practical sense. Measurement endpoints such as biomarkers are measurable environmental characteristics that can be related to assessment endpoints. Biomarker information is relatively easy to collect in the field and can be easily interpreted. As shown in Chapters 6 and 7, biomarkers can provide unique field exposure and effects information for contaminants and wildlife but because of the uncertainty of cause-effect linkages and the complexity of interpreting biomarker responses from a population/community perspective, it is difficult to draw confident conclusions about risk and to make risk management decisions. The quantitative relationship to the assessment endpoint is often lacking and a subjective qualitative assessment of the relationship is required. This makes the interpretation of biomarkers from an ecological risk assessment perspective difficult. If biomarker responses are absent as was the case with shorthorn sculpin at Saglek Beach and there is certainty of the lack of effects (*i.e.*, no missed effects or effects below the detection limit), there are likely no effects at the population or community level. For situations where biomarker responses indicate potential adverse effects, as

is the case with black guillemots and deer mice at Saglek Beach, however, the interpretation is more difficult from an ecological risk assessment perspective. Although PCBs may be having adverse effects on individuals at Saglek, it is difficult to extrapolate these effects to the population or community level.

The assessment of measurement endpoints at higher levels of organization such as the population level (*e.g.*, abundance, biomass) or community level (*e.g.*, richness, diversity) may provide more information to make clear site specific risk management decisions for remediation. Population level effects, however, are difficult to interpret because the relationship to the contaminant may be obscured because of movements of individuals within the population (CCME, 1996). Population measurement endpoints may be more appropriate for very large contaminated sites or for populations with small ranges (CCME, 1996). Community level effects present difficulties in establishing a relationship between the community effect and the contamination (CCME, 1996). It may therefore be necessary at sites such as Saglek to pursue measurement endpoints from several levels of organization.

8.3 Tiered Approach to Ecological Risk Assessment

Based on this thesis, a three tiered approach to assessing large complex sites such as Saglek is recommended. A screening level assessment utilizing the hazard quotient approach may be useful for initial preliminary risk estimates. Often at the hazard quotient stage, no effects are identified. Because of the inherent conservatism in the calculations, a hazard quotient of less than one demonstrates that adverse risks are unlikely. Incorporation of biomarkers, chemical information, hazard quotients and other lines of evidence into a weight of assessment may be useful for field verification of adverse health effects predicted through the screening (*i.e.*, hazard

quotient) assessment stage. If no effects are observed at this stage, additional population effects studies may not be necessary if there is certainty of this lack of effects. If individual effects are observed and the extrapolation to population effects is uncertain, the risk assessment may proceed to the third tier which would involve a detailed assessment of population or community level effects. Empirical population level assessments such as habitat assessments, biological surveys, demographic data collection, and field manipulations (Carlsen *et al.*, 2008), or modelling approaches such as individual based models and metapopulation models (Munns *et al.*, 2008) have been recommended. The development and application of population-level assessment methods is now accelerating rapidly as evidenced by a recent workshop and publication on population level ecological risk assessment, and these methods are being applied more often in the literature (Barnthouse *et al.*, 2008; Barnthouse *et al.*, 2009a). It is possible that population and community level effects indicate that no adverse effects are occurring. For example, the presence of apparently healthy populations of mummichog (*Fundulus heteroclitus*) at New Bedford Harbour, largemouth bass (*Micropterus salmoides*) in Housatonic River (Reiser *et al.*, 2004), and white perch (*Morone americana*) and striped bass (*Morone saxatilus*) at the Hudson River (Barnthouse *et al.*, 2009b) where levels of PCBs should have theoretically caused adverse effects.

These three tiers of ecological risk assessment support the iterative approach to ecological risk assessment recommended by regulatory agencies in Canada and the United States (CCME, 1996; USEPA, 1992a). With the iterative approach, each successive estimate becomes more realistic and if the risk cannot be adequately characterized with an acceptable degree of uncertainty, then the risk assessment moves to the next tier. The weight of evidence approach also provides greater diagnostic power by combining chemical information, biological responses,

and population properties. It is a familiar concept for stakeholders, which is particularly beneficial at Saglek where a variety of stakeholders and interested regulatory agencies make it necessary to develop a clear and transparent approach. The use of multiple lines of evidence also provides assurance to stakeholders that evidence is not being ignored or covered up (National Research Council, 1994). It would be far easier to demonstrate the presence or absence of risk through consideration of multiple lines of evidence than based on one line of evidence using conservative assumptions (*i.e.*, hazard quotient).

It is not always practical to implement the three tiered approach at some smaller industrial sites. In these situations, it may be feasible to make informed decisions based on lower tiers (Hope, 2009) but the uncertainty must also be communicated (Barnthouse *et al.*, 2009a). Barnthouse *et al.*, 2009 emphasize the importance of proceeding to population level risk assessments at large complex sites such as Saglek where potential remediation strategies are expensive and potentially destructive to the environment.

8.4 Current Risk Levels and Future Work

In this study, certain biomarkers in fish and wildlife living in the vicinity of Saglek Beach were affected by exposure to PCBs. Measuring biological effects of contaminants on local wildlife is critical for ecological risk assessments in Northern environments where information on biological effects related to contaminant exposure is lacking. Biomarkers do not identify the causal agent, however, and chemical monitoring is thus also necessary.

A comparison of the ecological risk assessment results from 1998/1999 and 2006/2007 indicated that the potential risk to the VECs examined in this thesis have decreased. This likely reflects the decrease in sediment and biota concentrations during the period between sampling

events as documented by Brown *et al.*, (2009). Hazard quotients calculated in this thesis for 2006/2007 indicated that adverse risks to shorthorn sculpin at Saglek Beach are unlikely (hazard quotient=0.9). The weight of evidence assessment supported the hazard quotient methodology indicating a low risk. For black guillemots, the hazard quotients indicated that adverse risks are likely (hazard quotient=1.2). The weight of evidence assessment supported the hazard quotient indicating an intermediate risk to black guillemots. Hazard quotients calculated using the dietary approach (2.3) and the tissue residue (2.1) for deer mice indicated that adverse effects to deer mice at Saglek Beach are likely. Bone mineral density was significantly lower in deer mice from Saglek Beach than in mice from the reference area where PCBs in deer mice liver were relatively low. Thyroid histopathology and histomorphometry on the other hand indicated no consistent differences in the thyroids from Saglek Beach deer mice and reference deer mice. A subjective assessment of deer mouse risk was conducted because the number of lines of evidence was not considered sufficient to do a weight of evidence assessment.

It is difficult to draw conclusions regarding the potential for adverse risks to populations at Saglek based on this risk assessment because of the difficulties in interpreting biomarker responses from a population/community perspective. Because it cannot be said with certainty whether populations of deer mice or black guillemots are being adversely affected by current levels of PCBs, additional information at the population or community level is required. The lack of reliable population and community measures limits the ability of this ecological risk assessment to determine whether or not a risk exists for the black guillemot and deer mouse populations at Saglek. Because of the uncertainty, a higher tiered assessment would be beneficial.

The marine ecosystem at Saglek Bay should be monitored by measuring concentrations

of PCBs in biota and sediment as well as measuring a suite of biomarkers and population indices. Based on the results of this biological effects study as well as the studies by Kuzyk *et al.*, (2003; 2005b), measures of oxidative stress and antioxidant defense mechanisms should be included in future monitoring of the marine ecosystem at Saglek Bay. This would include mixed function oxidases, enzymatic antioxidants (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic antioxidants (vitamin A and vitamin E) important in the detoxification of oxygen radicals. A long term terrestrial monitoring program is already in place at Saglek to monitor concentrations of PCBs in soil and deer mice. This thesis represents the first study of biomarkers in terrestrial ecological receptors at Saglek. Given, the results of the bone mineral density testing conducted in Chapter 2 and the screening ERA conducted in Chapter 7, measuring additional biomarkers and population indices could be considered for deer mice at Saglek as well.

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Appendix 1

Methods for Sediment and Biota Analyses

Sediment Analysis. Sediment samples with expected concentrations of PCBs greater than 500ppb were analysed by the Analytical Services Group at the Royal Military College in Kingston, Ontario, while all low-level analyses were conducted by the AXYS Analytical Services Group in Sidney, BC for Aroclor equivalents. Sediment samples analyzed at AXYS were assayed by GC/MS using similar methods described below, or an HP 5890 Series II Plus GC equipped with a ^{63}Ni electron capture detector (GC/ECD). All sediment samples were thoroughly homogenized before analysis. Sediment samples were subsampled for the determination of wet/dry weight ratio, spiked with surrogate standard, and then 10-15 g were extracted on a shaker table with 1:1 dichloromethane/methanol followed by dichloromethane. Cleanup was conducted on a Florisil® column using the same solvent system as was used for biological samples. The samples were analyzed by gas chromatography (GC) with mass selective detection (MS), using an Agilent 6890 Plus Gas Chromatograph equipped with an Agilent 5973 MS Detector, an SGE HT-8 fused silica capillary column or equivalent (50 m, 0.22 mm i.d. x 0.25 μm film thickness) and MSD ChemStation software. The instrument was calibrated daily using solvent blanks and a standard of Aroclor 1260. A serial isotope dilution mixture was used for quantification and relative response factors (RRFs) were used to further quantify all identified and unidentified analytes.

Tissue Analysis. Samples were analyzed by GC/MS using AXYS in-house methods modified from EPA 608/ 1625. The method uses C-13 labeled PCB standards added at the commencement of analysis. Those compounds quantified against the C-13 labeled standard, added at the beginning of the analysis procedure, are recovery corrected in the samples by the method of quantification. Analytes that are quantified against a recovery standard are not

recovery corrected by the C-13 labeled PCB standard. Surrogate recoveries are determined similarly against the recovery (internal) standard. Homogenized wet tissue (3-10 g), anhydrous sodium sulphate and surrogate standard were ground with motor and pestle and then extracted by chromatographic elution with dichlorimethane. Tissue extracts were eluted through a gel permeation column to remove lipids and then fractionated and cleaned up on a florisil column. An Agilent 5973 mass spectrometer equipped with an Agilent 6890 gas chromatograph was used for instrumental analysis. High resolution gas chromatography/low resolution mass spectrometry (HRGC/LRMS) analysis is performed on a gas chromatograph (GC) equipped with a quadrupole mass spectrometer (MS). A J&W DB-5 chromatography column (60 m, 0.25 mm i.d., 0.10 μm film thickness) is coupled directly to the MS source. The MS is operated at a unit mass resolution in the electron ionization (EI) mode using multiple ion detection (MID) acquiring two characteristic ions for each target analyte and surrogate standard. A splitless/split injection sequence was used.

Summary of congeners for shorthorn sculpin and deer mice: PCBs 3, 4/10, 5/8, 15, 16/32, 17, 18, 19, 20/21/33, 22, 23/34, 24/27, 25, 26, 28, 31, 37, 40, 41/64/68/71, 42/59, 43/49, 44, 45, 43/49, 44, 45, 46, 47/48/75, 51, 52/73, 54, 56/60, 61/74, 62/65, 66/80, 70/76, 77, 81, 83/108, 84, 85/120, 86/97, 87/115/116, 89/90/101, 91, 92, 93/95, 99, 104, 105/127, 106/108, 107/109, 110, 114, 123, 126, 128, 129, 130, 131/142, 132/168, 134/143, 135/144, 136, 137, 138/163/164, 139/149, 141, 146, 151, 153, 155, 156, 157, 158/160, 159, 167, 169, 170/190, 171, 172/190, 171, 172/192, 174/181, 175, 176, 177, 178, 179, 180, 182/187, 183, 185, 188, 189, 191, 193, 194, 195, 196/203, 197, 198, 199, 201, 202, 205, 206, 207, 208, 209

Summary of congeners for Black Guillemot: PCBs 1, 2, 3, 4/10, 5/8, 6, 7/9, 11, 12/13, 14, 15, 16/32, 17, 18, 19, 20/21/33, 22, 23/34, 24/27, 25, 26, 28, 29, 30, 31, 35, 36, 37, 38, 39, 40, 41/64/68/71, 42/59, 43/49, 44, 45, 46, 47/48/75, 50, 51, 52/73, 53, 54, 55, 56/60, 57, 58, 61/74, 62/65, 63, 66/80, 67, 69, 70/76, 72, 77, 78, 79, 81, 82, 83/108, 84, 85/120, 86/97, 87/115/116, 88/121, 89/90/101, 91, 92, 93/95, 94, 96, 98/102, 99, 100, 103, 104, 105/127, 106/118, 107/109, 110, 111/117, 112, 113, 114, 119, 122, 123, 124, 125, 126, 128, 129, 130, 131/142, 132/168, 134/143, 135/144, 136, 137, 138/163/164, 139/149140, 141, 145, 146, 147, 148, 150, 151, 152, 153, 154, 155, 156, 157, 158/160, 159, 161, 162, 165, 166, 167, 169, 170, 190, 171, 172/192, 173, 174/181, 175, 176, 177, 178, 179, 180, 182/187, 183, 184, 185, 186, 188, 189, 191, 193, 194, 195, 196/203, 197, 198, 199, 200, 201, 202, 204, 205, 206, 207, 208, 209

Summary of Congeners for Plant Samples: PCBs 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12/13, 14, 15, 16, 17, 18/30, 19, 20/28, 21/33, 22, 23, 24, 25, 26/29, 27, 31, 32, 34, 35, 36, 37, 38, 39, 40/41/71, 42,43,44/47/65, 45/51, 46, 48, 49/69, 50/53, 52, 54, 55, 56 57, 58, 59/62/75, 60, 61/70/74/76, 63, 64, 66, 67, 68, 72, 73, 77, 78, 79, 80, 81, 82, 83/99, 84, 85/116/117, 86/87/97/108/119/125, 88/91, 89, 90/101/113, 92, 93/95/98/100/102, 94, 96, 103, 104, 105, 106, 107/124, 109, 110/115, 111, 112, 114, 118, 120, 121, 122, 123, 126, 127, 128/166, 129/138/160/163, 130, 131, 132, 133, 134/143, 135/151/154, 136, 137, 139/140, 141, 142, 144, 145, 146, 147/149, 148, 150, 152, 153/168, 155, 156/157, 158, 159, 161,162, 164, 165, 167, 169, 170, 171/173, 172, 174, 175, 176, 177, 178, 179, 190/193, 181, 182, 183/185, 184, 186, 187, 188, 189, 190, 191, 192, 194, 195, 196, 197/200, 198/199, 201, 202, 203, 204, 205, 206, 207, 208, 209

Appendix 2
Calculations for life history parameters used in the dietary model for black guillemot (from Table 6-2, Chapter 6)

Parameter	Equation	Parameters	Calculations
Food ingestion rate (kg wet weight/day)	FI (kg/day) = 0.0582 (mass (kg))$^{0.651}$ (from USEPA (1993) based on Nagy, 1987)	Mass = 0.38 kg (Dunning (2008)) 80% moisture of prey items (USEPA, 1993)	0.0582 (0.38)$^{0.651}$ =0.031 kg/day dw FI (ww) = 0.031 kg/day dw/0.2 **=0.15kg/day ww**
Water ingestion rate (L/day)	WI (L/day) = 0.059 (mass (kg))$^{0.67}$ (from USEPA (1993) based on Calder and Braun (1983))	Mass = 0.38 kg	0.059(0.38)$^{0.67}$ **=0.031 L/day**
Sediment ingestion rate (kg dry weight/day)	Based on Beyer et al., (2008) sediment ingestion rate is 2% of total food ingestion rate (dry).	FI = 0.031 kg/day dw	0.02 * 0.031 kg/day dw = **0.00062 kg /day dw**
Fish ingestion rate (kg wet weight/day)	Based on Lonne and Gabrielsen (1992), fish is 83.5% of guillemot's total food ingestion (dw)	FI = 0.031 kg/day dw 80% moisture of prey items (USEPA, 1993)	Fish Ing (dw) = (0.835)(0.031 kg/day dw) =0.026 kg/day dw 0.026 kg/day dw/0.2 **=0.13 kg/day ww**
Benthic invertebrate ingestion rate (kg wet weight/day)	Based on Lonne and Gabrielsen (1992), benthic invertebrates are 16.5 % of guillemot's total food ingestion (dw)	FI = 0.031 kg/day dw 80% moisture of prey items (USEPA, 1993)	BI (dw) = (0.165)(0.031kg/day dw) = 0.0051 kg/day dw 0.0051 kg/day dw/0.2 = **0.025 kg/day ww**

Notes:
ww = wet weight
dw = dry weight

Appendix 3

Measurement attributes, evaluation criteria, and weighting score values used to weight measures of exposure and effects (from Johnston *et al.*, 2002).

Attribute	Evaluation criteria	Weighting Score [a]
Data Quality	Did data from the measure attain data quality objectives for sensitivity, precision, accuracy, completeness, representativeness, and comparability?	H = data met all data quality objectives M = one data quality objective not met L = data failed to meet two or more data quality objectives, not included in the risk characterization
Strength of Association	Is there a biological linkage between the measure and the assessment endpoint, a correlation between the measure's response and stressor levels, and is there a scientific basis for using the measure to judge environmental harm?	H = the measure is equivalent or similar to the assessment endpoint, a statistically significant correlation exists between stressor levels, and the measure's response there is a high to moderate scientific basis for inferring environmental harm, and sensitive benchmarks are available. M=the measure is linked to the assessment endpoint but the level of biological organization is different there is a quantitative relationship between assessment response and stressor levels, and although benchmarks may not be available there is a moderate scientific basis for inferring harm. L = the measure is affected by factors unrelated to the stressor levels, a correlation between stressor levels and measurement of response is expected but not demonstrated benchmarks are not available, and a relationship between the ,measure has been suggested or is expected but the scientific basis for inferring harm is weak or lacking
Study Design	As the study designed to account for (1) specifics of the site, (2) spatial variation, (3) temporal changes; was the measure (4) sensitive to changes due to stressor levels; was the measure able to (5) provide quantitative data, and was the measure (6) reproducible, applicable, suitable, and acceptable for assessing environmental harm?	H = the data obtained from the measure met five or six of the evaluation criteria M = the data obtained from the measure met four or five of the evaluation criteria L= the data obtained from the measure was unable to meet three or more of the evaluation criteria

a Measures were assigned an endpoint weight score of high (H), medium (M), or low (L) relative to their ability to assess harm to the assessment endpoint.