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Elements and stable isotopes as tracers for sleeper shark biology and the Iceland marine food web

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ELEMENTS AND STABLE ISOTOPES AS TRACERS FOR SLEEPER SHARK
BIOLOGY AND THE ICELAND MARINE FOOD WEB

by

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ABSTRACT

Stable isotope studies often rely on only two tracers (usually $\delta^{13}C$ and $\delta^{15}N$) to study marine ecosystems, which are inherently complex. The ability of elements to act as additional tracers of ecological processes in marine organisms and in a marine food web was investigated. The element analysis of two sleeper shark species, Pacific (*Somniosus pacificus*) and Greenland (*Somniosus microcephalus*), collected from different ecosystems demonstrated that elements are useful indicators of physiological and exposure differences between closely related species. The Greenland shark's food web about Iceland was more clearly resolved concerning trophic links and carbon sources by combining mercury data with stable isotope and stomach content data. Mercury also indicated that *Lycodes* potentially belonged to a different food web than the other fishes. Results from this research demonstrated the value of elemental tracers in food web studies and generated new questions about the application and interpretation of trophic position designations.

CO-AUTHORSHIP

The following thesis contains material from a manuscript that was published in Environmental Pollution (2007), volume 148, pages 281-290. The manuscript titled "Essential and non-essential element concentrations in two sleeper shark species collected in arctic waters" is co-authored by B.M. McMeans, K. Borgå, W.R. Bechtol, D. Higginbotham and A.T. Fisk. This thesis also contains material from a manuscript titled "Establishing trophic links in a marine ecosystem through integration of established and novel tracers" that will be submitted to the Canadian Journal of Fisheries and Aquatic Sciences in the near future. The manuscript is co-authored by B.M. McMeans, J. Svavarsson, Å. Bergman, and A.T. Fisk. Laboratory work presented within this thesis was performed by the author, with help and guidance from A.T. Fisk.

DEDICATION

I dedicate this thesis to my dad, Dennis McMeans, and my uncle, Ellis Darby, who would have loved to have seen it finished, and to my mom, Wilda Darby McMeans for her unwavering strength and support in everything I have ever done.

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STATEMENT OF ORIGINALITY

I certify that this thesis, and the research to which it refers, are the product of my own work and that any ideas or quotations from the work of other people, published or otherwise, are fully acknowledged in accordance with the standard referencing practices of this discipline. I acknowledge the helpful guidance and support of my supervisors, Dr. Aaron Fisk (University of Windsor) Dr. Brian Fryer (University of Windsor) and Dr. Trevor Pitcher (University of Windsor).

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CHAPTER 1

INTRODUCTION

FOOD WEB THEORY

Food webs describe the feeding interactions among organisms in an ecosystem and are one of the most central and unifying concepts in ecology (Lindeman 1942; Martinez 1995). Knowledge of food web structure is important for understanding species interactions (Vander Zanden and Rasmussen 1996; Post 2002) and the flow of energy and contaminants through ecosystems (Fisk et al. 2002; Hobson et al. 2002) and is now considered critical for ecosystem management and conservation purposes, especially due to impending climate changes. The study of food webs has evolved from compiling ball and stick diagrams from published literature (DeAngelis et al. 1982) to drawing elaborate webs based on species lists and the presence or absence of feeding interactions (Cohen and Briand 1984), to investigating the dynamics of populations utilizing biochemical analysis techniques, which provide a time integrated view of an organism's diet (Vander Zanden and Rasmussen 1999).

The development of food web theory began with the idea of the "food-cycle" by Elton (1927) and was furthered by the recognition that feeding interactions between organisms represent the movement of energy, which Lindeman (1942) referred to as "trophic dynamics". Feeding interactions between organisms are referred to as trophic linkages (Cohen and Briand 1984). Initially food webs were created by grouping all members of a food web into a series of discrete trophic levels, where trophic level one, the primary producers, provide all

the energy for the organisms in trophic level two, the primary consumers, and trophic level two then provides all the energy to trophic level three and so on (Elton 1927; Lindeman 1942).

Food web theory characterizes feeding links between species using a binary system (presence or absence) and several other properties to describe food web structure, including the fraction of species in a web that are top species, intermediate species and basal species (Cohen and Briand 1984) and the strength of feeding links (Paine 1980). These classical food web studies often produced elaborate diagrams in an attempt to uncover general patterns across a variety of different ecosystems and to estimate various food web parameters including stability, which indicates the ability of a community to recover after perturbations, and the relationship between species diversity and stability (Cohen and Briand 1984; Cohen et al. 1993; Pimm et al. 1991; Martinez 1994) . However, it is often impossible to collect all species present in a food web and to identify all feeding interactions among species, such that food web theory has come under criticism for its oversimplification of real food webs and lack of ability to describe omnivorous and/or opportunistic feeding (Polis 1991; Polis and Strong 1996; Vander Zanden and Rasmussen 1996).

Quantitative stomach content data provide an alternative to food web diagrams by assigning organisms a continuous measure of trophic position, which more adequately represents complexity and omnivory in food webs than discrete trophic levels (Vander Zanden and Rasmussen 1996). The application of stomach content data in diet studies is based upon accurate estimates of

trophic positions, which has often been limited by an inability to collect quantitative dietary data for all species interacting in a food web (Vander Zanden and Rasmussen 1996). It is often stated that stomach contents only provide a "snapshot" of an organism's diet and several factors affect the accuracy of stomach content data in predicting trophic positions including different rates of prey digestion, periodic feeding, misidentification of partially digested material, the inability to quantify material such as blood and mucus in the diet of an organism and empty stomachs (Layman et al. 2005; Arrington and Winemiller 2002).

Although quantitative stomach content analysis has been useful for freshwater and marine fisheries management purposes, aquatic food webs are not entirely understood, which has contributed to disagreement concerning the state of global fish stocks (Burgess et al. 2005) and the degree of omnivory (Cohen 1994; Vander Zanden and Rasmussen 1996), among other things. Naturally occurring stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopes have been used with increasing frequency to study aquatic food webs as they have proven useful for investigating trophic positions and sources of carbon (e.g., benthic vs. pelagic), respectively. Unlike food web studies based on species lists and the presence/absence of feeding interactions or estimates of discrete trophic levels based on stomach contents, stable isotopes provide a continuous, time integrated view of an organism's diet that is capable of identifying the occurrence of omnivory (Cabana and Rasmussen 1994; Vander Zanden and Rasmussen 1996), within-population variation (Vander Zanden et al. 2000), and community

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wide measures of trophic position (Layman et al. 2007) without having detailed taxonomic information for all species present in a food web.

STABLE ISOTOPES AS TOOLS IN FOOD WEB ECOLOGY

Isotopes are different forms of the same element that vary in their number of neutrons and therefore atomic masses. Stable isotopes are those that are non-radioactive and that do not decay with time. Isotopes of an element are often referred to as "heavy" (e.g., for carbon ^{13}C is the heavy isotope) or "light" (e.g., for carbon ^{12}C is the light isotope) and the heavier stable isotope is generally present in lower concentrations in the environment (Ehleringer and Rundel 1989). Different stable isotopes of an element will form the same bonds, and hence compounds, because they contain the same number of electrons and protons. However, lighter isotopes are preferentially selected during chemical reactions (i.e., those associated with growth and/or metabolism) because heavier isotopes form stronger bonds and thus react at slightly slower rates than their lighter counterparts (Sacks 1953; Peterson and Fry 1987). Therefore, an organism's tissues will have different isotope ratios relative to its food (Minagawa and Wada 1984; Peterson and Fry 1987). Ratios of carbon (^{13}C: ^{12}C) and nitrogen (^{15}N: ^{14}N) stable isotopes are frequently used in diet/food web studies for structuring food webs because they are assumed to increase in a predictable manner between an organism and its food.

Stable isotopes are expressed as a ratio of heavy to light isotopes in a sample relative to a standard reference material, which is referred to as delta (δ)

notation and is a means of standardizing results between labs. Delta values are calculated using the equation:

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

where X is ^{15}N or ^{13}C and R is the ratio ^{15}N/ ^{14}N or ^{13}C/ ^{12}C. The standard reference material for carbon was Pee Dee Belemnite and for nitrogen atmospheric N_2.

Ratios of carbon stable isotopes (δ^{13}C) have been reported to not increase at all (Hobson and Welch 1992) or by only ~1‰ (Peterson and Fry 1987) with each stepwise increase in trophic position in aquatic food webs. However, inshore/benthic organisms are typically enriched in ^{13}C (i.e., more positive or greater δ^{13}C value) relative to offshore/pelagic organisms (France, 1995), such that carbon isotope ratios are useful for identifying carbon sources in aquatic food webs (Hobson and Welch 1992). The discrepancy of δ^{13}C between inshore and offshore consumers is thought to result from the presence of a stagnant boundary layer surrounding benthic algae that limits the diffusion rate of CO_2 relative to planktonic algae. Benthic algae therefore utilize ^{13}C for photosynthesis that would normally be discriminated against (as it is in planktonic/offshore algae) and thus have a higher δ^{13}C that is substantial enough to be reflected in the tissues of benthic/inshore consumers (France 1995). For example, two fishes within the same food web displaying distinct δ^{13}C values would suggest multiple sources of primary productivity within that food web (Pinnegar and Polunin 2000).

Nitrogen isotope ratios (δ^{15}N) increase to a greater degree than those of carbon between an organism and its food in an assumedly consistent manner,

such that $\delta^{15}N$ is used to calculate trophic positions within aquatic food webs (Peterson and Fry 1987). A consumer becomes enriched in ^{15}N relative to its diet due to the preferential selection and catabolism of amino acids containing isotopically light nitrogen (i.e., the excretion of isotopically light nitrogenous wastes and retention of isotopically heavy amino acids) (Minagawa and Wada 1984). The difference in relative stable carbon and nitrogen isotope ratios between an organism and its food (e.g., $\delta^{15}N_{predator} - \delta^{15}N_{prey}$) can be expressed as $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$, respectively. Food web studies most commonly report $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ values between 3-4‰ for $\delta^{15}N$ in aquatic food webs (DeNiro and Epstein 1981; Minagawa and Wada 1984; Post 2002). Values of $\delta^{15}N$ can be used to calculate relative trophic positions for consumers based on their relative $\delta^{15}N$ to that of a basal organism of known trophic level (usually a primary producer at trophic level 1 or an obligate filter feeder at trophic level 2). The equation for calculating trophic position using stable isotopes of nitrogen is:

$$TP_i = \lambda + (\delta^{15}N_{consumer} - \delta^{15}N_{baseline}) / \Delta\delta^{15}N$$

Where $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ of the species of interest, $\delta^{15}N_{baseline}$ is the $\delta^{15}N$ of a known trophic level species and λ is the trophic level of the baseline species. The increase in $\delta^{15}N$ is assumed to be constant for all members within a food web, however, concerns have been raised regarding a constant $\Delta\delta^{15}N$ as several factors have been shown to influence the $\delta^{15}N$ of fishes including form of nitrogen excretion (Vanderklift and Ponsard 2003) and physical condition (Gannes et al. 1997; Jardine et al. 2006).

Stable nitrogen and carbon isotopes have proven useful for investigating trophic positions in various types of aquatic systems including lakes (Vander Zanden et al. 1999; Post 2002), estuaries (Herzka 2005) and marine environments (Hobson and Welch 1992; Fisk et al. 2002), however, several concerns and assumptions surround their use. For example, due to considerable variation in $\delta^{15}N$ and $\delta^{13}C$ among ecosystems, trophic position calculations must be based on an isotopic baseline unique to the ecosystem of interest, such that variation in $\delta^{15}N$ and $\delta^{13}C$ can be contributed to changes in food web structure and carbon flow, and not just to variation in the isotopes' baseline values (Post 2002; Jardine et al. 2006). Because the calculation for trophic position depends on the baseline $\delta^{15}N$ value, choosing an isotopic baseline that captures the temporal variation in all energy sources (e.g., primary producers, detritus) for the consumer is crucial for an accurate trophic position measurement (Post 2002). Trophic position estimates also rely on the assumption that $\Delta\delta^{15}N$ with each trophic level is between 3-4‰, which is a major concern considering that $\Delta\delta^{15}N$ are known to vary outside of this range (Gannes et al. 1997). Concerning the generation of an appropriate isotopic baseline, mussels and snails have been shown to be good integrators of the isotopic baseline at the base of pelagic and littoral food webs (Post 2002). The assumption of $\Delta\delta^{15}N = 3$-4‰ is still a concern for stable isotope research, and although many laboratory studies have addressed this concern for various fishes, it is impossible to investigate the true nitrogen isotope fractionation in large marine fishes through feeding studies. Therefore, the calculation of specific trophic positions should be done with care in

organisms where isotope fractionation and nutrient allocation data is lacking, but stable carbon and nitrogen isotopes are still powerful tools for identifying different and potentially important sources of carbon and nutrients to consumers, for investigating the degree of omnivory in a food web and for investigating the increase of contaminants between a consumer and its diet (i.e., biomagnification).

Several non-essential elements found in fish are often considered contaminants and have been the focus of much research due to their toxicity, potential effects on human health and ability to biomagnify (Hg, Cd, Pb, As) (Dietz et al., 1996; Campbell et al. 2005a, 2005b; Riget et al. 2007). However, the biomagnifying elements, especially mercury, are now being investigated because they have potential as tracers of feeding behavior, where the essential elements could be indicative of physiological processes, a technique which has not been used frequently used. The use of elements could help support/dispute the data gathered from stable isotopes and there is a need to asses the potential of elements as ecological tracers.

NON-ESSENTIAL AND ESSENTIAL ELEMENTS

Essential elements

Certain elements are required for life processes, and all organisms must obtain the elements they need for normal functioning. Elements required by an organism are referred to as "essential". Currently 29 of the 90 naturally occurring elements are known to be essential to animal life (Lall 2002). Fish derive their

essential elements from their diets or from ambient water. Examples of "major" essential elements in fish include carbon, hydrogen, nitrogen, oxygen, phosphorus, sulfur, calcium, magnesium, sodium, potassium and chlorine (Lall 2002). Elements required in very small amounts are described as "trace" elements. Some of the trace essential elements important to mammals have not been described in fish, such that the role of certain elements in fish is unclear. Based on research for the purposes of aquaculture, the most important trace elements to fish are iron, copper, manganese, zinc, cobalt, selenium and iodine (Watanabe et al. 1997). Elements with limited data concerning their function in fish include: arsenic, molybdenum, fluorine, lead, nickel, silicon, vanadium and lithium (Watanabe et al. 1997).

Essential elements are maintained within narrow concentrations by homeostatic mechanisms, which regulate fluctuating element concentrations incoming from the diet (Bury et al. 2003). The function, cellular uptake and homeostatic control of several important essential elements to teleost fish are discussed in detail by Bury et al. (2003), Lall (2002) and Watanabe et al. (1997). The regulation of essential elements within narrow ranges could be useful for making inferences about an organism's physiology.

Non-essential elements

Elements referred to as non-essential serve no *known* purpose for fish (although some elements referred to as non-essential lack sufficient data to definitively classify them as non-essential in fish). Although almost any element can be toxic at high enough concentrations, non-essential elements are typically

toxic at smaller concentrations than essential elements, which has contributed to much research focusing on the concentrations of non-essential elements in fish. Mercury, for example, which has been associated with neurological damage in humans and biota (World Health Organization 1990) and is known to biomagnify, has received much attention and study (Dietz et al. 1996; Campbell et al. 2005a). Interest has also been expressed in rubidium, which has also been shown to biomagnify (Dietz et al. 1996; Campbell et al. 2005b), and lead and cadmium, which have reached concentrations in many Arctic biota that exceed World Health Organization guidelines for human consumption (Muir et al. 2005). Concentrations of non-essential elements in an organism's tissues could be useful for determining environmental exposure, or when combined with stable isotope analysis, for investigating feeding interactions.

Elements as tools in food web ecology

Biomagnifying anthropogenic contaminants such as polychlorinated biphenyls (PCBs) have frequently been combined with stable nitrogen and carbon isotopes in ecotoxicological studies to investigate feeding interactions and the movement of energy and contaminants through food webs (Fisk et al. 2002; Hobson et al. 2002). Non-essential elements such as mercury, cadmium and lead have also been analyzed in food webs to investigate their potential to biomagnify (Power et al. 2002; Kidd et al. 1995; Atwell et al. 1998; Cabana and Rasmussen 1994; Riget et al. 2007). Although assessing element concentrations because of potential toxicicity issues is a valid research objective, non-essential elements could have further use as ecological tracers of feeding interactions in

aquatic food webs. For example, mercury has proven especially useful because it has been shown to increase with trophic position ($\delta^{15}N$) at a similar rate across food webs from several different types of ecosystems, including a tropical freshwater lake (Kidd et al. 2003), several lakes in northern Canada (Kidd et al. 1995; Power et al. 2002) and several marine food webs (Atwell et al. 1998, Campbell et al. 2005; Riget et al. 2007). Therefore, the combination of stable isotopes with mercury data could have great potential for investigating biological processes in individual organisms, and on a larger scale as a tracer of energy pathways and food web structure.

The use of element and stable isotope data to structure food webs could be especially useful in marine ecosystems that are generally not well understood (Burgess et al. 2005). Of the studies that report non-essential and essential element concentrations along with stable isotope data in marine food webs, all have marine mammals as the top predator (Riget et al. 2007; Campbell et al. 2005; Dehn et al. 2006). There is a need to asses the utility of elements as ecological tracers of feeding interactions and physiological processes in a marine food web with several different feeding strategies (e.g., carnivores, omnivores) and with a cold-blooded top predator.

SLEEPER SHARKS

Somniosidae is a family of sharks referred to as "sleeper sharks" due to their general lethargic nature and slow movements. The Greenland shark, *Somniosus microcephalus*, and the Pacific sleeper shark, *Somniosus pacificus*

are megafaunal vertebrates that typically inhabit northern latitudes in the Atlantic and Pacific oceans, respectively (Bigelow and Schroeder 1948; Skomal and Benz 2004), although Greenland sharks have been reported as far south as about the coast of Georgia, United States (Herdendorf and Berra, 1995) and Pacific sleeper sharks have been reported as far south as approximately the southern coast of California, United States (Compagno 1984). Recently, a species of *Somniosus* was also reported in the Gulf of Mexico (Benz et al. 2007), and thus these sharks could have a wide global distribution. Based on stomach content data, the Greenland and Pacific sleeper sharks feed on a wide variety of taxa including invertebrates, fish, and marine mammals (Bigelow and Schroeder 1948; Bright 1959; Yang and Page 1999). Very little data exists for these species because of the logistical difficulty in studying them, as they often inhabit areas seasonally covered by ice (Bigelow and Schroeder 1948; Skomal and Benz 2004) and because they lack commercial importance. Therefore, little is known concerning the population size, age, physiology or role in the food web of Greenland sharks and Pacific sleeper sharks. Non-essential and essential elements could have utility in investigating similarities and/or differences between the species and, when combined with stable isotopes, could be used to study the flow of energy and sources of carbon in the sharks' food webs. Because Greenland sharks and Pacific sleeper sharks are omnivorous and therefore have a complicated feeding behavior, they are a good test species for the applicability of elements as ecological tracers in real marine food webs.

RATIONALE

This thesis investigates the concentrations of elements in two species of sleeper shark (Greenland sharks and Pacific sleeper sharks) and in the food web of Greenland sharks about Iceland. By utilizing multiple stable isotopes and essential and non-essential elements, this research will aid in the understanding of marine trophic structure and carbon flow and will help in determining the potential of elements as ecological tracers, which could be useful for the conservation and management of aquatic organisms. Further, this work provides the first report for element concentrations in any sleeper shark species and is the first attempt at assessing the food web of a sleeper shark with element concentrations.

OBJECTIVES

The goal of this research is to determine the utility of non-essential and essential elements as ecological tracers of food web structure, carbon flow and physiological processes. The two main objectives of this research are:

1) to compare element profiles between two closely related species of sleeper shark (Greenland and Pacific sleeper sharks) to investigate patterns of non-essential elements, which could indicate exposure differences and of essential elements, which could be indicative of physiological requirements, and

2) to integrate stable isotope and mercury data to establish trophic positions of and sources of carbon to Greenland sharks in relation to other members of its food web, which will be useful in fitting the Greenland shark into a food web and

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in determining the ability of stable isotopes and mercury to structure a marine food web with an omnivorous top predator.

The first objective is dealt with in Chapter 2-*Essential and non-essential elements in two sleeper sharks collected in arctic waters*. The second objective is addressed in Chapter 3- *Establishing trophic links in a marine ecosystem through integration of established and novel tracers*.

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CHAPTER 2

ESSENTIAL AND NON-ESSENTIAL ELEMENT CONCENTRATIONS IN TWO
SLEEPER SHARK SPECIES COLLECTED IN ARCTIC WATERS

INTRODUCTION

Certain elements[1] have been highly studied in arctic invertebrates, freshwater fish, birds, and marine mammals due to concern over their toxicity and potential to biomagnify in arctic wildlife (Dietz and Johansen 1996; Riget et al. 2000). Mercury (Hg), for example, is frequently analyzed in arctic ecosystems, as it is associated with neurological damage in humans and biota (World Health Organization 1990), is known to biomagnify (Campbell et al. 2005a; Dietz et al. 1996) and has achieved concentrations in some biota that warrant concern for both the wildlife and for humans whose diet includes these wildlife species (Fisk et al. 2003). Cesium, zinc, and rubidium have also been shown to biomagnify (Campbell et al. 2005a; 2005b; Dietz et al. 1996). Despite concern over elements in arctic biota, there are limited data on elements in arctic marine fish, and no data for the only sharks, the Greenland shark, *Somniosus mircocephalus*, and the Pacific sleeper shark, *Somniosus pacificus*, that routinely inhabit arctic waters (Bigelow and Schroeder 1948; Benz et al. 2004). As high concentrations of organic contaminants (e.g., poly-chlorinated biphenyls) have been previously measured in the Greenland shark, likely due to the shark's high trophic level (Fisk et al. 2002), there is a need to address the concentrations of elements in the Greenland shark and its Pacific congener, the Pacific sleeper shark.

The Greenland and Pacific sleeper sharks are megafaunal vertebrates of Somniosidae, the sleeper sharks (Compagno 2005). To date, Greenland sharks

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[1] The term "elements" is considered more appropriate than the more commonly used terms "metals" or "heavy metals" because the large suite of elements generated by modern analytical methods, and reported in this study, include both metallic and non-metallic members (Duffus, 2002).

have been primarily reported from areas seasonally covered by ice in the North Arctic (Bigelow and Schroeder 1948; Skomal and Benz 2004), but have also been reported through more temperate areas in the Atlantic (Bigelow and Schroeder 1948) and as far south as about the coast of Georgia, United States (Herdendorf and Berra 1995). Pacific sleeper sharks have been reported from areas as far north as the Chukchi Sea (Benz et al. 2004) to as far south as approximately the southern coast of California, United States (Compagno 1984). Based on stomach content data, the Greenland and Pacific sleeper sharks feed on a wide variety of taxa including invertebrates, fish, and marine mammals (Bigelow and Schroeder 1948; Bright 1959; Yang and Page 1999), although the recent use of biochemical tools suggests that the species composition of the sharks' diet could vary between the species. For example, using stable isotope and stomach content analysis, the Greenland shark was shown to consume piscivorous fishes (e.g., turbot, *Reinhardtius hippoglossoides*) and piscivorous pinnipeds (e.g., ringed seal, *Phoca hispida*) (Fisk et al. 2002), whereas a recent study utilizing fatty-acid analysis suggested that Pacific sleeper sharks off the coast of Alaska feed heavily on zooplanktonivore fishes (e.g., Pacific herring, *Clupea pallasi*) and occasionally consume the blubber of filter-feeding cetaceans (i.e., baleen whales) (Schaufler et al. 2005). Although more detailed studies on the feeding behavior of these sharks are needed, these studies suggest that Pacific sleeper sharks could be feeding at a lower trophic level than Greenland sharks. The Greenland and Pacific sleeper sharks are the largest fish in arctic waters, are apparently abundant (Bigelow and Schroeder 1948; Hansen 1963),

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and likely ecologically significant (Fisk et al. 2002; Taggart et al. 2005), however, there is a lack of data concerning their biology and life history. Further, the potentially high trophic level (Fisk et al. 2002) and long life (Hansen 1963) of the Greenland shark could make it an excellent sentinel for contaminants in the arctic marine ecosystem.

Few previous studies have assessed element concentrations in sharks (Marcovecchio et al. 1991; Storelli et al. 2003; Domi et al. 2005), and most have only focused on four or five elements of concerns due to their toxicity. Elements differ from most organic contaminants in that they occur naturally in the environment and can be classified as either essential (e.g., copper, zinc, manganese), which are necessary to an organism for life, or non-essential (e.g., arsenic, cadmium, mercury), which may be present in an organism, but serve no known purpose. Non-essential elements can sometimes behave like essential elements if regulated through the same processes, but are generally believed to be regulated less efficiently than essential elements (Kraemer et al. 2005). Therefore, interpreting concentrations of non-essential and biomagnifying elements (Hg and Rb) could provide information concerning diet and trophic level, whereas concentrations of non-essential elements that have not been shown to biomagnify could provide information on geographical exposure variations. Essential elements, which should be highly regulated, could be used to infer the physiological requirements of an organism. Very little is known about element regulation in marine fishes, and even less information exists concerning elasmobranchs (Grosell et al. 2003). Although the lack of data requires extreme

conservation when drawing conclusions about shark physiology, further investigations into concentrations and patterns of certain essential elements in the Greenland shark and Pacific sleeper shark, or any species of shark, is a valid effort with potentially important implications.

This study addresses a data gap for elements in sharks and provides the first hepatic element data for the Greenland shark (collected in Cumberland Sound, Nuavut) and the Pacific sleeper shark (collected in Prince William Sound, Alaska) (Figure 2.1), and has two main goals: 1) To assess within-species relationships of individual element concentration vs. shark length for all non-essential elements (which could be useful in determining the occurrence of any biomagnification) and essential elements (which could provide information concerning changes in physiological requirement with size), and 2) To make between-species comparisons of non-essential element concentrations to determine environmental and/or dietary exposure differences, and essential element concentrations to infer physiological differences between the species. The Greenland sharks were expected to exhibit higher concentrations of non-essential, biomagnifying elements (i.e., mercury and rubidium) based on the assumption that they feed at a higher trophic level than the Pacific sleeper sharks. Variances in other non-essential elements that are not known to biomagnify are expected to reflect environmental exposure differences. The Greenland and Pacific sleeper sharks were expected to have similar concentrations of essential elements based on their close phylogenetic relationship and therefore potentially similar physiologies.

METHODS

Sampling

Greenland shark samples (n= 24, 14 males and 10 females) were collected as by-catch in the bottom long-line commercial turbot fisheries in Cumberland Sound in April of 1999 and 2000 and ranged in size from 234 cm to 322 cm (fork length) with a mean fork length of 278.0 ± 4.8 (mean ± 1 SE). All Pacific sleeper shark samples (n=14, 7 males and 7 females) were collected as part of an Alaska Department of Fish and Game bottom long-line survey in Prince William Sound in September of 2000. The Pacific sleeper sharks ranged in size from 139 cm to 244 cm (fork length) with a mean fork length of 201.3 ± 8.9. The low sample sizes are due to the enigmatic nature of these sharks, in that they often occur at high latitudes and in deep water (Compagno 1984; Herdendorf and Berra 1995). Liver samples (left lobe) were collected from each shark shortly after death and were stored frozen in plastic bags until element analysis. Liver tissue typically has higher concentrations of elements relative to muscle tissue (Dietz et al. 1998), and was therefore chosen for element analysis in an attempt to maximize the number of quantifiable elements.

Element Analysis

Tissue samples were analyzed for elements at the National Laboratory for Environmental Testing (NLET) at the National Water Research Institute in Burlington, Canada (National Laboratory for Environmental Testing, 2003). Total mercury (Hg) in liver tissue (NLET Method 02-2802) was analyzed by cold

vapour atomic absorption spectrometry (CVAAS). Analyses of 24 elements in liver samples (NLET Method 02-2705) were analyzed by inductively coupled plasma-Sector Field spectrometry (ICP-SFMS), with 22 elements analyzed at low resolution (silver (Ag), barium (Ba), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), gallium (Ga), lanthanum (La), lithium (Li), manganese (Mn), molybdenum (Mo), nickel (Ni), palladium (Pd), lead (Pb), platinum (Pt), rubidium (Rb), antimony (Sb), strontium (Sr), thallium (Tl), uranium (U), vanadium (V) and zinc (Zn) and the rest at high resolution (arsenic (As) and selenium (Se)). Instrumental detection limits (DL) for most elements is 0.001 µg/g, except for Sr (0.05 µg/g), Pt (0.01 µg/g), Pd (0.1 µg/g), Li (0.1 µg/g) and Sb (0.01 µg/g). Of the analyzed elements, Co, Cr, Cu, Mn, Mo, Ni, Se, and Zn were considered essential for the sharks based on their known necessity in the diets of fish (Lovell, 1998), and therefore, the remaining elements (Ag, As, Ba, Cd, Ga, Hg, La, Li, Pd, Pb, Pt, Rb, Sb, Sr, Tl, U, V) were considered non-essential. All elements were analysed with wet tissue, and therefore are expressed as µg/g wet weight (µg/g, ww). NLET is certified by the Canadian Environmental Analytical Laboratory program of the Canadian Standards Association and participates in the QA program for the Northern Contaminants Program, with good results (Stokker 2003).

Data analyses

Elements that were below DL in more that 30% of all samples (Ba, Cr, La, Li, Ni, Pb, Pd, Pt, Sb, Tl, U, and V) were excluded from the within-species (linear regression) and between-species (univariate and multivariate) statistical

analyses. The data for Ag and Mn were arcsine transformed and concentrations of As, Cd, Co, Cu, Hg, and Zn were logarithmically transformed to reduce skewness and heterogeneity prior to analyses. Concentrations of Ga, Mo, Rb, Se, and Sr were left untransformed.

To investigate length/element relationships within-species, linear regressions were performed on individual element concentrations vs. length for each shark species separately.

Element concentrations between-species were compared using univariate and multivariate statistics. Univariate analyses (general linear methods GLM, Type III Sum of Squares, SAS, version 8.2, Cary, NC) were performed on absolute element concentrations to determine the effect of individual element concentrations as a function of species and sex of the shark, and any interaction between species and sex (GLM: ELEMENT = SPECIES + SEX + SPECIES * SEX). Length was left out of the GLM expression because linear regression analysis did not reveal any significant relationships between element concentration and length for either species, and also because length and species were confounding variables, due to the almost exclusive separation of the generally larger Greenland sharks and smaller Pacific sleeper sharks into two size classes. It should also be noted that because all Greenland sharks were collected in Cumberland Sound and all Pacific sleeper sharks were collected in Prince William Sound, the variable "species" could have also been defined as "location" in the GLM expression.

Multivariate ordinations (Principal component analyses PCA, CANOCO 4.5 for Windows, Ter Braak and Šmilauer 1998) were performed both on logarithmically transformed absolute element concentrations and on element pattern (sample standardized by norm, similar to [element]/ [☐elements]). ☐Elements only include the 13 elements quantified above the DL. The PCA assigns scores to the individual samples that are linear combinations of 13 elements, and are presented relative to their ordination axes (Figure 2.2). Elements are presented as arrows pointing to the direction of increasing value. Details for diagram interpretation are described elsewhere (Ter Braak 1995; Van Wijngaarden et al. 1995; Van den Brink and Ter Braak 1999).

Molar Hg and Se relationships were investigated more closely, due to their known biochemical relationship (Dietz et al. 2000). Hg and Se concentrations (µg/g, ww) were converted to nmol/g as explained in Dietz et al. (2000). The means and standard deviation of molar Se:Hg ratios were calculated for each shark.

RESULTS

Among all shark samples, Zn had the highest mean concentration, followed by As (Table 2.1). Means of element concentrations that were below detection limit, along with the number of samples above detection limit, are shown in Table 2.2. The within-species comparisons (linear regression) revealed that no element concentration was related to shark length for either the Greenland sharks (r^2 = 0.0009 – 0.0856, p = 0.291- 0.890), which had a fork

length range of 234 cm – 322 cm, or the Pacific sleeper sharks (r^2 = 0.0076 –

0.2320, p = 0.088 – 0.767), which had a fork length range of 139 cm - 244 cm.

The Hg vs. length relationship is shown in Figure 2.2A, B.

The GLM (univariate statistics) revealed sex was only significant for Rb (F

$_{1,34}$ = 4.98, p = 0.0323), with higher levels in females than males (0.44 ± 0.11,

0.38 ± 0.07 µg/g, ww, respectively), and there was no interaction between

species and sex for any element. Several elements differed between the species

based on GLM analysis of concentrations (Table 2.1): the non-essential elements

Ag, As, Cd and Hg ($F_{1,34}$ = 4.47 – 56.35, p = <0.0001 – 0.0420) and the

essential element Cu ($F_{1,34}$ = 13.17, p = 0.0009) were higher in Greenland

sharks (Cumberland Sound) than Pacific sleeper sharks (Prince William Sound).

Concentrations of the non-essential element Rb (F $_{1,34}$ = 6.05, p = 0.0192), and

the essential elements Co, Mn and Zn (F $_{1,34}$ = 6.35 – 122.72, p = <0.0001–

0.0166) were higher in Pacific sleeper sharks (Prince William Sound) than

Greenland sharks (Cumberland Sound). The other elements (essential: Ga, Sr;

non-essential: Mo, Se) did not differ between the species (all p > 0.05).

The PCA (multivariate statistics) of absolute element concentrations

(Figure 2.3A) provided similar results to the GLM analysis. One exception is that

Zn did not differ between species based on the PCA of concentrations (Figure

2.3A) but was significantly higher in Pacific Sleeper sharks based on the GLM

results. The PCA of element concentrations also revealed four Pacific sleeper

sharks that grouped out with the Greenland sharks, but overall, the variance of

element concentrations between the species that were suggested by the GLM are supported by the PCA of absolute element concentrations.

The PCA of element pattern (Figure 2.3B) revealed two clearly separated groups with less variability between the species than the PCA of element concentrations (Figure 2.3A), as the four Pacific sleeper sharks observed to separate out in the PCA of element concentrations were not apparent in the analysis of pattern. PC1 and PC2 explained 95.5 % of the total variability in element concentrations among samples on the PCA of pattern, and results were similar to the results of GLM, as Greenland sharks separated out due to higher Cd, Ag, Hg, As (non-essential elements) and Cu (essential element) concentrations, and Pacific sleeper sharks separated out due to higher concentrations of Zn, Co, Mn (essential elements) and Rb (non-essential element).

The mean Se:Hg molar ratio in the Pacific sleeper shark (12.36 ± 5.46) was higher than that in the Greenland shark (3.26 ± 1.45).

DISCUSSION

Univariate and multivariate analysis of elements

Due to the increased risk of type II error (failure to reject a false null hypothesis) that occurs when individual elements are analyzed separately using repeated univariate analyses (Sokal and Rohlf 1981), this study included both univariate (GLM) and multivariate (PCA) analyses. Univariate and multivariate analyses of element data in Greenland sharks and Pacific sleeper sharks yielded

generally similar results, but the PCA of element concentrations did identify some differences in elements among four individual Pacific sleeper sharks. The variability in these individuals could potentially be explained by variable feeding events of the sharks, such as occasional cetacean/pinniped predation or scavenging (Smith and Baco 2003; Schaufler et al. 2005). However, further research is needed to determine the relationship between shark element concentrations and feeding ecology before this conclusion can be made. It should also be noted that interpreting feeding behavior and bioaccumulation trends of elements based solely on hepatic element concentrations should be done with caution due to the high turnover and metabolic activity of liver tissue. The PCA of element pattern is useful because it normalizes the data and minimizes the effect of individual element concentrations, and relative to the concentrations PCA, the pattern PCA more clearly separates the samples into two groups based on either species identity or location. Thus the results of this study demonstrate that multivariate statistics can be a useful tool in element studies, often identifying underlying variability that is not obvious from univariate analysis, but also providing a means to use all element data in analyses when there is insufficient samples sizes for univariate statistics. Similar results and suggestions were made for statistical analysis of elements in seabirds (Borgå et al. 2006).

Element/ length relationships within the sleeper shark species

Linear regression analysis did not reveal any significant relationships between hepatic element concentrations and shark length, although

element/length relationships have been previously demonstrated in other fishes (Riget et al. 2000; Adams and Onorato 2004; Muir et al. 2005). Certain non-essential elements, especially Hg (Campbell et al. 2005a) and to a lesser degree Rb (Campbell et al. 2005b), are known to increase with increasing trophic level and with increasing length in fishes (Adams and Onorato 2004; Muir et al. 2005) and one shark species (Storelli and Marcotrigiano 2002). This is likely because fish generally increase in trophic level as they grow larger and are able to feed on larger prey. The lack of a mercury/length relationship (Figure 2.2A, B) in the Greenland and Pacific sleeper sharks could be a result of the limited size range of sharks represented in this study. However, the lack of a mercury/length relationship could also indicate that these sharks are not necessarily increasing in trophic level as they grow larger (at least not within the size ranges sampled in this study), which is supported by the known scavenging behavior of Greenland sharks and Pacific sleeper sharks. For example, the Greenland shark that had the highest Hg concentration (1.16 ug/g, ww), and is therefore assumed to be feeding at the highest trophic level, had a fork length of 248 cm, which is at the low end of the range and below the mean of Greenland shark fork lengths sampled in this study. The scattered Hg concentrations along the length axis could also be reflective of the known omnivory of the Greenland and Pacific sleeper sharks, and could indicate variable marine mammal scavenging events. For example, it has been suggested, based on organic contaminants levels, that the frequency of marine mammal predation varies between individual Greenland sharks (Fisk et al. 2002), which would further explain the lack of a linear increase

in Hg with shark length. The lack of a mercury/ length relationship could also indicate that Hg does not bioaccumulate in the tissues of Greenland and Pacific sleeper sharks, although this explanation is highly unlikely because Hg has repeatedly been demonstrated to biomagnify. The impact of trophic position on Hg concentrations within the Greenland sharks and Pacific sleeper sharks is probably isolated from contributions of environmental Hg concentrations because both species were collected entirely in the same respective areas (i.e., all Greenland sharks were collected in Cumberland Sound and all Pacific sleeper sharks were collected in Prince William Sound), such that the environmental exposure is assumed to be the same for all individual representatives of each species.

The lack of a relationship between concentrations of essential elements and fork lengths in either Greenland sharks or Pacific sleeper sharks suggests that the physiological requirements of essential elements do not vary with size, and therefore likely age, over the size ranges of sharks sampled in this study. Although the relationship between element requirements and physiology in fish is poorly understood, a negative relationship between Zn concentration and fish size has been previously observed, suggesting a decrease in Zn requirement with size/age (Halden et al. 2000; Zhang and Wang 2005). As the documented symptoms of Zn deficiency in fish include depressed growth (Lovell 1998), Zn could potentially be required in higher concentrations in smaller, rapidly growing fishes. In the Greenland and Pacific sleeper sharks, this trend either does not occur, or occurs in sharks smaller than the ones samples in this study.

Differences in non-essential elements between the sleeper sharks

Based on similar results between the PCA and GLM, the concentrations of the non-essential elements Ag, As, Cd, Hg, and Rb differed between Greenland sharks (Cumberland Sound) and Pacific sleeper sharks (Prince William Sound), and all but Rb were higher in the Greenland sharks. Because non-essential elements are likely not regulated to a specific level, or regulated less efficiently than essential elements (Zhang and Wang 2005), we suggest that variances in the non-essential element concentrations are indicative of environmental exposure differences between the locations. The higher concentrations of the biomagnifying element Hg in the Greenland shark could also be attributed to the potentially higher trophic level of the Greenland shark relative to the Pacific sleeper shark (Fisk et al. 2002; Schaufler et al. 2005). Cadmium, which has also been shown to exhibit trophic transfer in some species (Muir et al. 1992), was significantly higher in Greenland sharks from Cumberland sound, which further suggests that the Greenland shark is feeding at a higher trophic level than the Pacific sleeper sharks. However, higher concentrations of Hg and Cd in the Greenland sharks may also reflect greater concentrations of these elements in the environment where they were collected (i.e., Cumberland Sound) relative to where the Pacific sleeper sharks were collected (i.e., Prince William Sound). Cadmium concentrations increase from the western to the eastern North American arctic in marine mammals (Dietz et al. 1998), which is consistent with the trends seen in the Greenland and Pacific sleeper sharks. Spatial trends in Hg in the North American arctic are more variable (Dietz et al. 1998), and thus it is

hard to assess differences in Hg levels in the sharks' ecosystems. Thus, a combination of a potentially higher trophic level and higher regional contaminants could explain higher Cd and Hg concentrations in the Greenland sharks.

Higher concentrations of As and Ag were also found in the Greenland sharks, which is likely due to regional differences in exposure since these compounds have not been observed to biomagnify in food webs (Campbell et al. 2005a). Although Rb biomagnified in an arctic marine food web, but to a lesser degree than Hg (Campbell et al. 2005a, b), it was the only non-essential element that was higher in the smaller and potentially lower trophic level Pacific sleeper sharks than in the Greenland sharks. Rubidium therefore contradicts the trend observed in Hg, but this could be due to variation in Rb levels between locations, with higher levels in Prince William Sound than in Cumberland Sound. Unfortunately, information on Rb concentrations in the environment is scarce and conclusions about regional differences are not possible.

Differences in essential element concentrations between the sleeper sharks

The Greenland shark and Pacific sleeper shark were assumed to have similar physiologies and similar concentrations of essential elements in individuals representing both species were expected. It was shown in several arctic seabird species that the essential element concentrations in individuals belonging to the same species remained relatively constant across wide geographical distances (Borgå et al. 2006). In the Greenland and Pacific sleeper sharks, however, which belong to the same Genus but not the same species, only two essential element concentrations (Mo, Se) did not differ while the other

four essential elements (Co, Cu, Mn, Zn) did differ between species. This suggests that either the metabolic regulation of essential elements is not highly efficient in cold-blooded fishes, and that environmental levels determined the observed concentrations in the sharks, or that regulation is efficient, and the Greenland shark and Pacific sleeper shark have different physiological requirements. However, the lack of information and data for comparison, and the complicated behavior and fate of elements in the environment requires caution be taken when comparing element concentrations between species/locations.

Elements in sharks collected in arctic waters vs. sharks collected in more temperate waters

Of the few studies that have analyzed element concentrations in sharks, several report data for muscle tissue only (Domi et al. 2005; Turoczy et al. 2000) and cannot be used for comparisons to the hepatic element concentrations reported in this study because muscle and liver have been shown to vary in their element concentrations (Storelli et al. 2003; Campbell et al. 2005a; Borgå et al. 2006). Past studies that have analyzed hepatic tissue from sharks sampled about the coast of Argentina (Marcovecchio et al. 1991) and the Mediterranean Sea (Storelli, et al. 2003; Storelli and Marcotrigiano 2002), have generally reported higher mean concentrations of As, Cd, Cu, Hg, Pb, Se and Zn than those observed in the Greenland and Pacific sleeper sharks. For example, the Greenland sharks from Cumberland Sound and the Pacific sleeper sharks from Prince William Sound had lower mean hepatic Cd concentrations (3.9 ± 1.3 µg/g, ww and 2.6 ± 1.3 µg/g, ww, respectively) than three species of sharks

sampled about the Argentinian coast: *Mustelus schmitti*, 5.92 ± 1.65; *Notorynchus* sp., 8.41 ± 0.32; *Schroederichthys bivius*, 7.93 ± 1.78 µg/g, ww (note that *Schroederichthys bivius* is the current valid name for *Halaeulurus bivius*) (Marcovecchio et al., 1991). A hammerhead shark species *Sphyrna zygaena,* from about the Mediterranean Sea, had an approximately five times higher hepatic Cd concentration (19.77 ± 1.29 µg/g, ww) than the sleeper sharks (Storelli et al. 2003). Similar to Cd results, the Greenland shark and Pacific sleeper shark had lower hepatic Hg concentrations than previously reported for sharks collected about Argentina, (*Mustelus schmitti*: 0.79 ± 0.39; *Schroederichthys bivius*: 2.26 ± 0.56; *Notorynchu* sp.:2.11 ± 0.33 µg/g, ww) (Marcovecchio et al., 1991) and in *Sphyrna zygaena,* from the Mediterranean Sea (35.89 ± 3.58 µg/g, ww) (Storelli et al. 2003). The variance in Hg concentrations among the shark species collected about Argentina reported in Marcovecchio et al. (1991) could be a result of differences in feeding ecology or closer proximity to emission sources relative to the Greenland and Pacific sleeper sharks. For example, *S. bivius* and *Notorynchus* sp. feed on a higher percentage of fish and have higher Hg concentrations relative to *M. schmitti*, but because specific trophic positions were not reported, comparisons between the feeding ecology of the sleeper sharks to that of the sharks collected about Argentina are difficult. Size differences among shark species could also contribute to variance in element concentrations, but *S. zygaena* were of similar size to the sharks in this study and Marcovecchio et al. (1991) only report lengths

for *M. schmitti*, which were much smaller than the Greenland and Pacific sleeper sharks.

Concerning essential elements, several studies have reported shark hepatic Zn concentrations (Marcovecchio et al. 1991; Storelli et al. 2003). Zn is of interest because it has been suggested to biomagnify (Campbell et al. 2005), although it appears to be efficiently regulated by fish (Kraemer et al. 2005; Zhang and Wang 2005). The variance in Zn concentrations observed in the food web could be attributed to different physiological requirements between invertebrates and vertebrates (i.e., higher Zn requirements in higher trophic level vertebrates), instead of biomagnification. Hepatic Zn concentrations in the Greenland shark and Pacific sleeper shark were compared with previously published hepatic concentrations of Zn in four different species of shark (Marcovecchio et al. 1991; Storelli et al. 2003) and in two mammal species (Campbell et al. 2005a) (Figure 2.4). This comparison reveals a casual observation that the different species of sharks, and the sharks and mammals, have different tissue concentrations, and likely different physiological requirements of Zn. The Zn concentrations also appear to be similar within, but different among, orders of sharks (Squaliformes, Carcharhiniformes, Hexanchiformes). Although this is preliminary data and no conclusions can be drawn, the comparison is nonetheless interesting.

Hg-Se relationships in sleeper sharks

The mean Se:Hg molar ratios in the Pacific sleeper shark (12.36 ± 5.46) and Greenland shark (3.26 ± 1.45) reflect the similar Se concentrations between the two species (0.54 ± 0.03, 0.52 ± 0.03 µg/g, ww, respectively) and the higher

Hg concentrations in the Greenland shark. A 1:1 molar ratio of Se:Hg has been suggested to mitigate toxicity of Hg, but is most often observed only in marine mammals with very high Hg concentrations (above approximately 2.0 µg/g, ww) (Braune et al. 1991; Dietz et al. 2000). Organisms with lower Hg concentrations relative to marine mammals, such as seabirds and fish, generally have excess concentrations of Se relative to Hg, resulting in Se:Hg ratios larger than 1 (Dietz et al. 2000; Campbell et al. 2005a; Borgå et al. 2006). For example, previously reported mean hepatic Se:Hg ratios include: 16.3 ± 15.2 for a species of cat shark (*Galeus melastomus*) (Storelli and Marcotrigiano 2002), 26.3 ± 44.1 for several species of seabird, and 198 ± 296 for teleost fishes (Dietz et al. 2000). However, a mean Se:Hg ratio of 1.14 ± 0.272 was reported for polar bears (Dietz et al. 2000), suggesting that high Hg concentrations (such as those observed in polar bears) induces a Se detoxification action, and that the Se:Hg ratio might tend towards 1:1 as Hg concentrations increase toward some species-specific threshold level (Storelli and Marcotrigiano 2002). Although the Hg concentrations in the Greenland shark were substantially lower than the Hg concentrations previously reported for marine mammals and scavenging seabirds, the mean molar Se:Hg ratio in the Greenland shark is more similar to reported values for the ringed seal (*Phoca hispida*) (2.2 ± 2.8) and the glaucous gull (*Larus hyperboreus*) (3.7 ± 1.4) (Campbell et al. 2005a) than to values reported for other fish (Dietz et al., 2000) and one shark species (Storelli and Marcotrigiano 2002). This relatively low Se:Hg ratio could indicate a low Hg threshold (i.e., a Se detoxification response that is potentially activated at low Hg

concentrations) for the Greenland shark, even though its Hg concentration seems low relative to other mammals and southerly fishes and sharks. However, it is difficult to draw conclusions about shark physiology, as both the natural levels and "normal" concentrations of Se and Hg in the sleeper sharks are not known.

Comparison of Hg in sharks collected in arctic waters to other arctic species

Due to the potential toxicity of Hg and its ability to biomagnify, Hg concentrations have been analyzed in several arctic organisms. Mean Hg concentrations were higher in the Greenland shark (0.49 ± 0.06 µg/g ww) than in arctic cod, *Boreogadus saida*, liver samples collected about Greenland (0.015 ± 0.002 µg/g, ww) (Campbell et al. 2005a), and in other arctic areas summarized by Muir et al., 1992 (0.01 ± 0.01 – 0.04 ± 0.02 µg/g, ww), which is likely due to the higher trophic level of the sharks. However, the mean Hg concentrations in the Greenland and Pacific sleeper sharks were much lower than previously reported hepatic Hg concentrations for the ringed seal, *Phoca hispida*, (22.51 ± 16.87 µg/g, ww) and the scavenging glaucous gull, *Larus hyperboreus*, (2.92 ± 1.06 µg/g, ww) collected about Greenland (Campbell et al. 2005a), despite the fact that these species probably all feed at similar trophic levels (Fisk et al. 2002). Lower concentrations of Hg in Greenland sharks compared to marine mammals and seabirds is a contradiction to what has been observed for biomagnifying organic contaminants such as poly-chlorinated biphenyls (PCBs) and dichloro-diphenyl-trichloroethane (DDT), the levels of which are much higher in Greenland sharks compared to ringed seals (Fisk et al. 2002). This discrepancy is most

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likely due to varying half lives between Hg and organic contaminants and provides insights on the feeding ecology of the shark species. Organic contaminants, such as PCBs, would have very long half lives, potentially decades, in large cold water fish species like Greenland sharks and Pacific sleeper sharks (Fisk et al. 2002), which are likely much longer than for Hg. Thus the occasional predation or scavenging of a marine mammal would result in a significantly higher exposure and accumulation of Hg and organic contaminants than from feeding on invertebrates or fish, and the increased concentrations of the organic contaminants would remain in the sharks much longer than the Hg. Thus, lower Hg concentrations in the shark compared to the seals and seabirds suggest that on average Greenland sharks feed at a lower trophic level, but higher organic contaminants levels suggest that at times the sharks feed at a higher trophic level. Concentrations of mercury in the liver of flathead sole, *Hippoglossoides elassodon*, (0.32 ± 0.1 µg/g, dw) (Meador et al. 2005) collected about Prince William Sound are similar to Hg concentrations in Pacific sleeper shark livers (0.12 ± 0.01 µg/g, ww), if the liver of the flathead sole is assumed to be approximately 50% water and most of the Hg in fish is assumed to be methylmercury. Concentrations of Hg in livers of harbor seals, *Phoca vitulina*, (range = 0.4-72 µg/g, ww, n = 23) from the Gulf of Alaska/Prince William Sound area (Miles et al. 1992) are much higher than those of Pacific Sleeper sharks. These results suggest that the Pacific sleeper sharks feed at lower trophic level than seals, even though Pacific sleeper sharks occasionally consume marine mammals (Bright 1959). However, evidence from fatty acids suggests that the

marine mammals consumed by Pacific sleeper sharks are baleen whales, which are likely at a lower trophic level than piscivorous seals (Schaufler et al. 2005).

CONCLUSION

This study presents the first element data reported for the only two shark species known to regularly inhabit arctic waters. The within-species comparison revealed that no non-essential element concentration was related to length in either the Greenland shark or the Pacific sleeper shark, which could suggest a lack of trophic level increase in sharks within the size range sampled in this study. No changes in physiological requirements with size were apparent based on the lack of significant essential element vs. length relationships in either species. Univariate and multivariate statistics suggested that the Greenland sharks had higher levels of certain non-essential elements relative to the Pacific sleeper shark, likely due to exposure variances (environmental and dietary). The variance in essential elements between the species could suggest that the Greenland and Pacific sleeper sharks have different physiological requirements. The Greenland and Pacific sleeper sharks had lower non-essential and essential element concentrations than those previously reported for sharks inhabiting lower latitudes. However, the mean molar Se:Hg ratio in the Greenland shark was lower than that observed in more southerly fishes, which could indicate a lower Hg threshold for the Greenland shark relative to other fishes. Baseline element data is important because the Greenland shark and Pacific sleeper shark are likely ecologically significant in the arctic marine ecosystem, and could be

sentinel species based on their feeding behavior. Further, very little element data exists for sharks and there is a need to better understand the trophic transfer, assimilation, and elimination of both non-essential and essential elements, which could be useful in not only addressing concerns over toxicity, but could also help in answering basic questions about shark life history and physiology.

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Table 2.1. Means (standard errors) for selected elements (µg/g, ww) in liver tissue of Pacific sleeper sharks collected about Prince William Sound and Greenland sharks collected about Cumberland Sound. Elements marked with an asterisk (*) are significantly different between species at $p<0.05$.

| Species | n | ESSENTIAL ELEMENTS | | | | | |
		Co*	Cu*	Mn*	Mo	Se	Zn*
Pacific sleeper shark	14	0.027	0.833	0.427	0.039	0.542	10.39
		(0.004)	(0.071)	(0.023)	(0.003)	(0.026)	(0.708)
Greenland shark	24	0.018	1.754	0.179	0.033	0.516	6.898
		(0.001)	(0.202)	(0.009)	(0.002)	(0.029)	(0.338)

| Species | n | NON-ESSENTIAL ELEMENTS | | | | | | |
		Ag*	As*	Cd*	Ga	Rb*	Sr	Hg*
Pacific sleeper shark	14	0.048	5.364	2.638	0.005	0.454	0.241	0.122
		(0.014)	(0.149)	(0.354)	(2E-04)	(0.026)	(0.011)	(0.010)
Greenland shark	24	0.198	9.815	3.913	0.005	0.383	0.206	0.492
		(0.022)	(0.702)	(0.436)	(2E-04)	(0.016)	(0.200)	(0.058)

Table 2.2. Means and standard errors (SE) for elements (µg/g, ww) in liver tissue of Pacific sleeper sharks collected about Prince William Sound and Greenland sharks collected about Cumberland Sound that were excluded from statistical analyses. The detection limits (DL) (µg/g) and the number of samples above the DL, which were included in the mean calculation, are provided.

Element	DL	PACIFIC SLEEPER SHARKS $n = 14$			GREENLAND SHARKS $n = 24$		
		n above DL	Mean	SE	*n* above DL	mean	SE
Ba	0.005	11	0.015	0.003	13	0.011	0.002
Cr	0.050	0	-	-	0	-	-
La	0.001	0	-	-	11	0.001	0
Li	0.005	6	0.006	0	7	0.005	0
Ni	0.010	2	0.020 – 0.150	-	7	0.043	0.016
Pb	0.002	2	0.002 – 0.150	-	5	0.017	0.009
Pt	0.001	7	0.001	0	21	0.001	0
Sb	0.001	4	0.001	0	4	0.001	0
Tl	0.001	0	-	-	0	-	-
U	0.001	0	-	-	13	0.001	0
V	0.002	5	0.010	0.002	17	0.010	0.001

Figure 2.1. Map showing sampling sites. Greenland shark samples (n= 24, 14 males and 10 females) were collected in Cumberland Sound and ranged in size from 234 cm to 322 cm (fork length). All Pacific sleeper shark samples (n=14, 7 males and 7 females) were collected in Prince William and ranged in size from 139 cm to 244 cm (fork length).
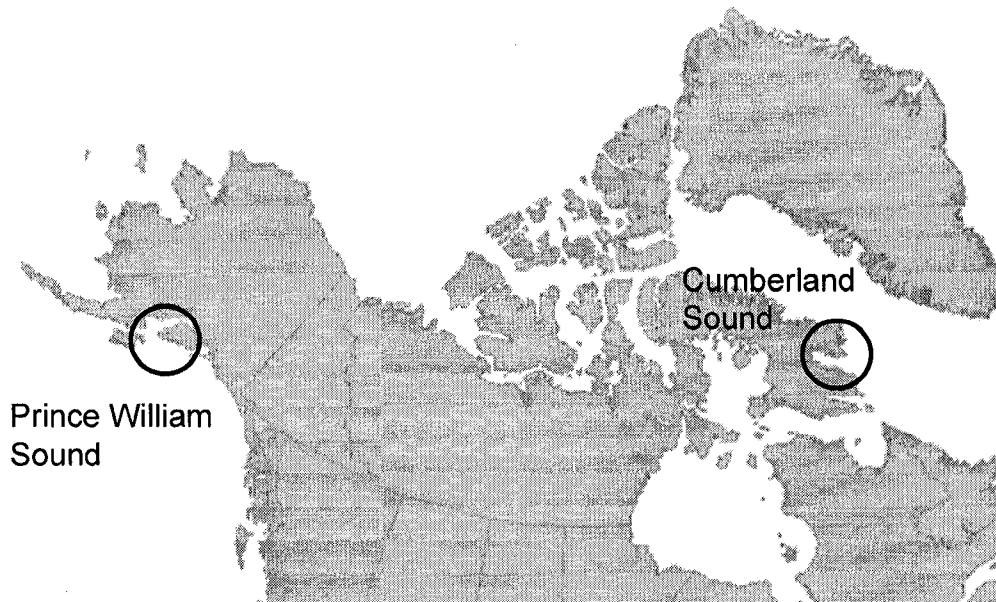
Figure 2.2. Hg vs. fork lengths of individual A) Pacific sleeper sharks (black circles) from Prince William Sound and 2) Greenland sharks (white circles) from Cumberland Sound.
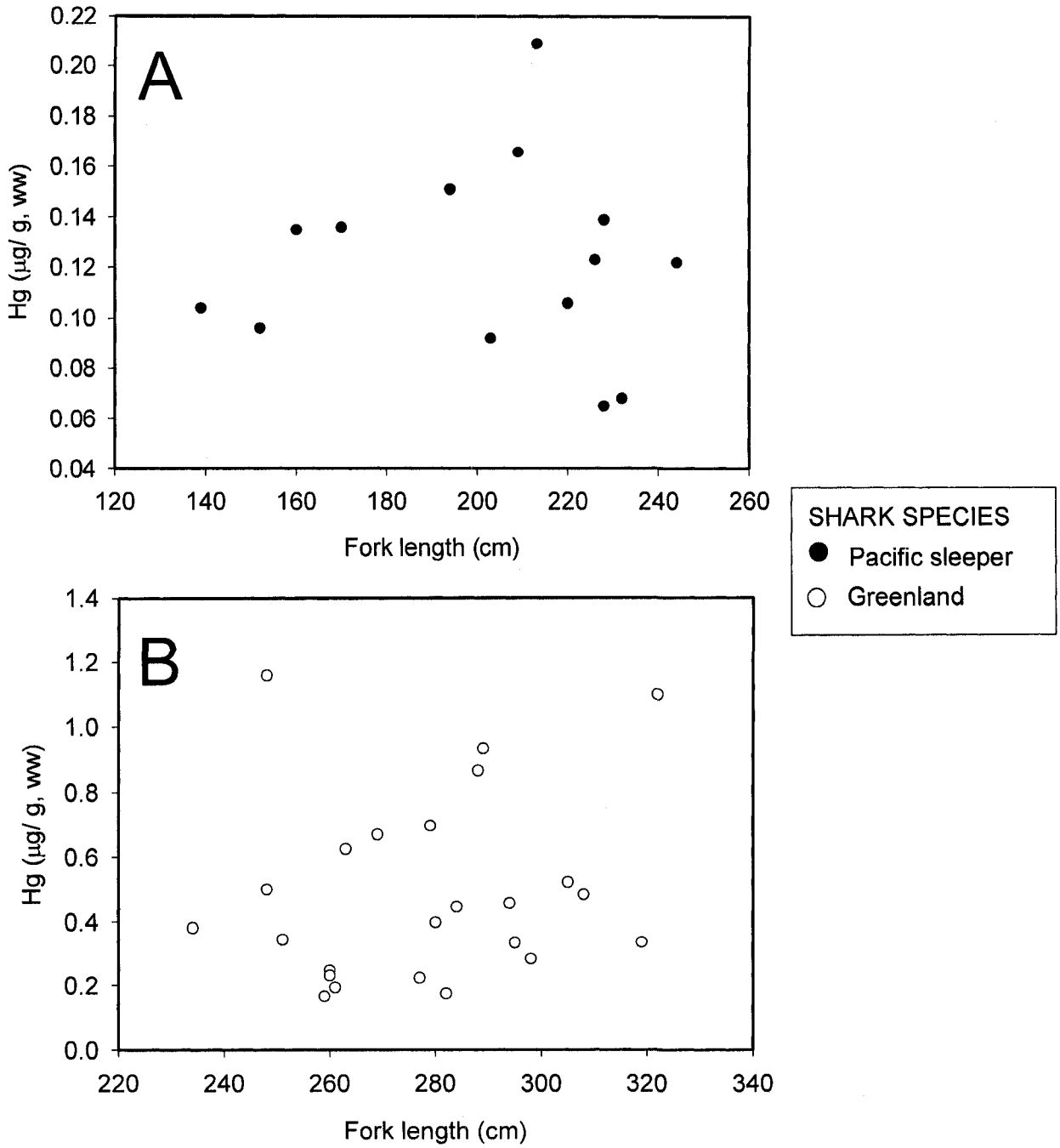
Figure 2.3. Biplot of Prince William Sound Pacific sleeper sharks' (black circles) and Cumberland Sound Greenland sharks' (white circles) scores on the principal components (PC) extracted by principal component analyses (PCA), and the elements' loadings on the PCs. A) Absolute element concentrations (log mg/kg) and B) standardized element concentrations (pattern) in liver tissue. Symbols are individual sample's scores, and arrows are the respective element pointing in the direction of increasing value. Only elements that correlated more than 20% are shown in the ordination plot, as the others do not contribute much to sample separation in the ordination diagram.
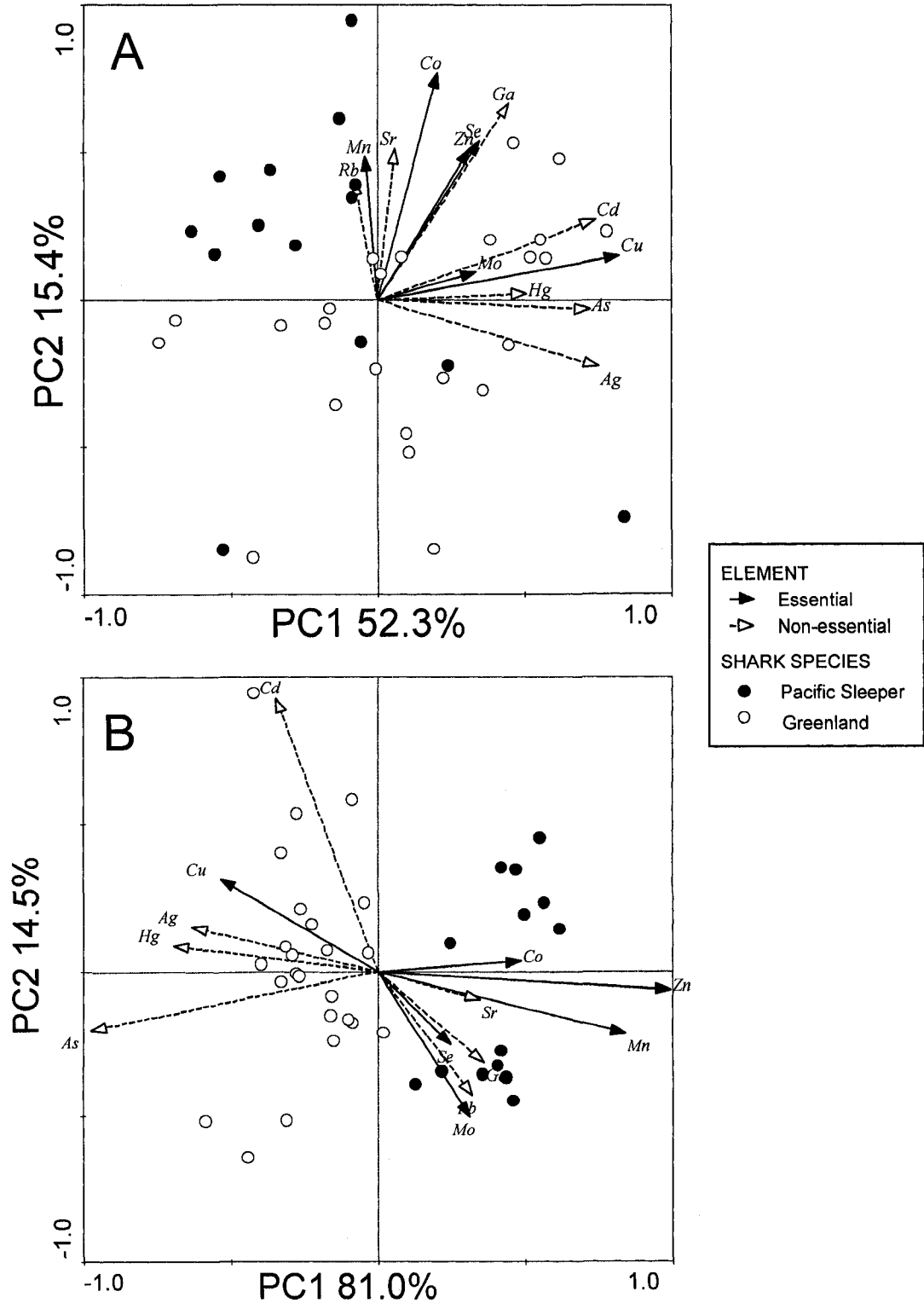
Figure 2.4. A comparison of hepatic Zn concentrations (mean ± SD) among several shark species, including: Pacific sleeper shark (*Somniosus pacificus*), Greenland shark (*Somniosus microcephalus*), smooth hammerhead shark (*Sphyrna zygaena*) (Storelli et al., 2003), narrownose smooth-hound (*Mustelus schmitti*) (Marcovecchio et al., 1991), narrowmouthed catshark (*Schroederichthys bivius*) (Marcovecchio et al., 1991), sevengill shark (*Notorynchus* sp.) (Marcovecchio et al., 1991); and two mammal species: ring seal (*Phoca hispida*) and glaucous gull (*Larus hyperboreus*) (Campbell et al., 2005a). The shark species belong to order Squaliformes (black bars), order Carchirhiniformes (grey bars), or order Hexanchiformes (white bar), and the ring seal and glaucous gull belong to the class Mammalia (hatched bars).
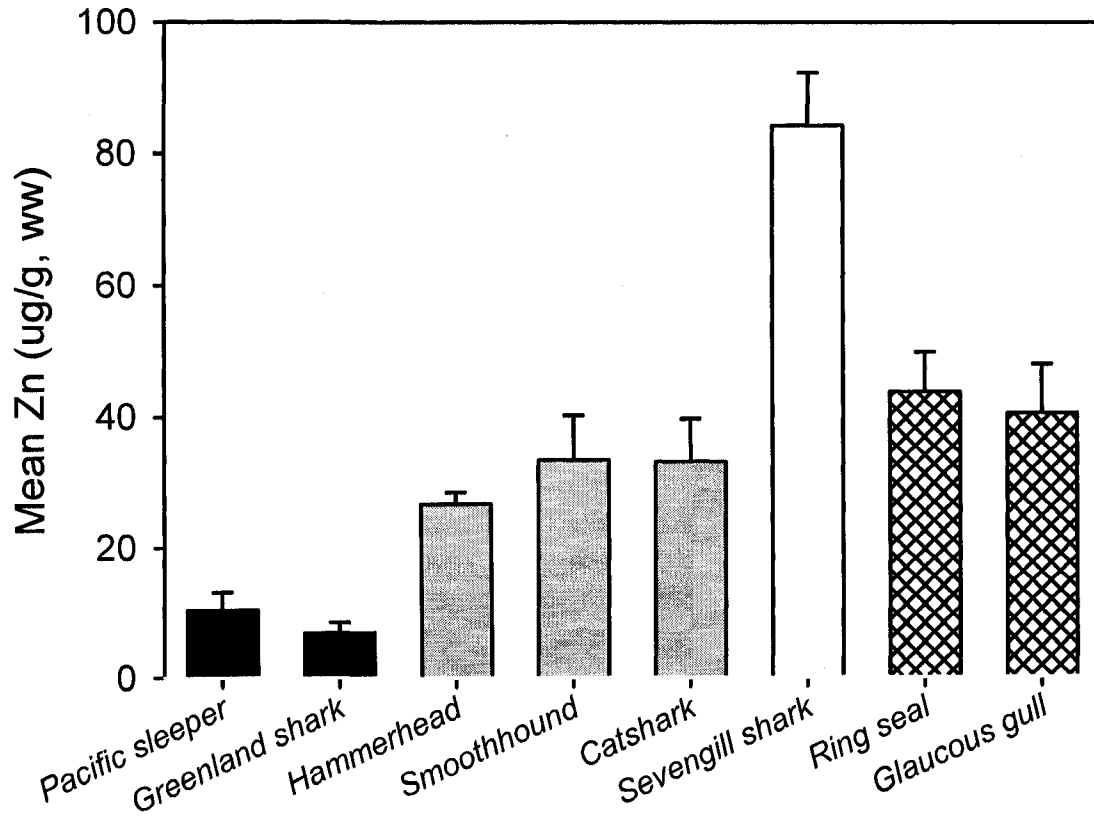
CHAPTER 3

ESTABLISHING TROPHIC LINKS IN A MARINE ECOSYSTEM THROUGH
INTEGRATION OF ESTABLISHED AND NOVEL TRACERS

INTRODUCTION

Investigating the structure of food webs provides information concerning feeding interactions and the movement of energy and contaminants through ecosystems, which is important knowledge for understanding many aspects of ecology (e.g., population dynamics, species diversity) (Cohen et al. 1993; Martinez 1995). In aquatic systems, understanding relative trophic positions and carbon sources in a food web is crucial for fisheries management and conservation purposes. Unfortunately, aquatic food webs are difficult to quantify due to the presence of multiple and complex feeding interactions (Layman et al. 2005), migratory and omnivorous species (Vander Zanden and Rasmussen 1996), the difficultly in obtaining a representative sample of species interacting in a food web (Layman et al. 2005) and/or collecting a large enough sample sizes to accurately estimate diet, especially when based on stomach content analysis (Arrington and Winemiller 2002).

Stomach content analysis is the most obvious way to investigate feeding interactions in a food web. However, stomachs often contain unidentifiable soft tissues (e.g., gelatinous invertebrates) or partially digested prey, provide only a snapshot of feeding behavior and require a large sample size to account for the occurrence of omnivory, variable feeding events and empty stomachs (Sherwood and Rose 2005; Layman et al. 2005; Arrington and Winemiller 2002). Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are commonly used in ecological studies to identify carbon sources and assign trophic positions in aquatic food webs, respectively (Vander Zanden et al. 1999; Hobson et al. 2002).

Stable isotope analysis is based on the premise that an organism's tissues become "enriched" in the heavier isotopes (i.e., ^{13}C and ^{15}N) relative to its food source (Peterson and Fry 1987). The difference in relative stable carbon and nitrogen isotope ratios between an organism and its food (e.g., $\delta^{15}N_{predator}$ - $\delta^{15}N_{prey}$) can be expressed as $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$, respectively. Values of $\Delta\delta^{15}N$ between an organism and its diet are commonly reported between 3-4‰, such that $\delta^{15}N$ is used to calculate trophic positions based on the $\delta^{15}N$ of a baseline organism (Peterson and Fry 1987; DeNiro and Epstein 1981; Minagawa and Wada 1984; Post 2002). Carbon stable isotopes differ to a lesser degree, by ~1‰ between an organism and its diet, but are higher in inshore/benthic organisms relative to offshore/pelagic organisms, and $\delta^{13}C$ is therefore utilized to identify carbon sources in aquatic food webs (Hobson and Welch 1992; France 1995). Stable isotopes are useful because unlike stomach content analysis, they provide an integrated view of an organism's diet (Hobson et al. 1995), and relative trophic positions and carbon sources can be determined for representative members of a food web by only sampling a few individuals of each species (e.g., 5-10 individuals) and without accounting for every organism within the food web.

Several sources of uncertainty exist surrounding the interpretation of stable isotope data, however, such as the assumption of consistent differences of $\delta^{15}N$ and $\delta^{13}C$ between predators and prey (Vanderklift and Ponsard 2003; Jardine et al. 2006). As well, stable isotope studies often rely on the $\delta^{13}C$ vs. $\delta^{15}N$ bi-plot to make inferences about feeding interactions among potentially

dozens of species within an aquatic ecosystem, which is of concern because stable isotopes are often not differentiated among carbon sources and trophic positions (Post 2002; Jardine et al. 2006; Gannes et al. 1997). Utilizing only two tracers to structure food webs in spatially large, complex ecosystems, such as in the marine environment, has potential for additional problems. For example, nitrogen isotope ratios have been shown to vary spatially within marine environments, potentially due to different nitrogen availability and utilization by organisms at the base of the food web (Iken et al. 2001; Wu et al. 1997). As marine ecosystems can have multiple habitats (i.e., benthic, pelagic, offshore, inshore, deep water) and independent food webs, there is potential for species to be incorrectly included within a food web (e.g., offshore species included in an inshore food web), leading to misidentified trophic positions (see Sherwood and Rose 2005).

To more accurately structure complex food webs, there is a need for additional tracers. One such tracer could be mercury (Hg). Mercury has been shown to biomagnifiy (i.e., increase in concentration with each trophic step (Gobas and Morrison 2000)) in fish, and relationships between log [Hg] and $\delta^{15}N$ have been shown to be very constant across food webs from many different types of ecosystems, including a tropical freshwater lake (Kidd et al. 2003), several lakes in northern Canada (Kidd et al. 1995; Power et al. 2002) and two arctic marine food webs (Atwell et al. 1998; Campbell et al. 2005). Mercury closely tracks protein due to its association with sulfur-containing amino acids (Harris et al. 2003), and $\delta^{15}N$ represents nitrogen in dietary proteins, which likely

explains consistency in the log [Hg]-δ^{15}N relationships. However, unlike δ^{15}N, mercury does not vary significantly within local marine environments or with ocean depth (Fitzgerald et al. 1998; Gill and Fitzgerald 1988) (although mercury does vary on large spatial scales, such as areas in the Atlantic vs. Pacific oceans, see Fitzgerald and Gill 1988; McMeans et al. 2007). Therefore, mercury could be useful for further resolving feeding interactions in marine systems and for identifying species that potentially belong to other food webs.

The marine environment surrounding Iceland supports several species of commercially important fishes that represent several different taxa and feeding strategies, as well as an omnivorous top predator, the Greenland shark, *Somniosus microcephalus*. Greenland sharks are megafaunal vertebrates in the Iceland marine environment, however no data exist concerning how the Greenland shark's trophic position and sources of carbon relate to other members of this food web (i.e., how Greenland sharks "fit" into the Iceland marine food web). Greenland sharks inhabit both deep and shallow water (Skomal and Benz 2004) and based on stomach contents (Bigelow and Schroeder 1948; Bright 1959; Yang and Page 1999) and contaminants and stable isotopes (Fisk et al. 2002), are known to consume fishes, invertebrates and marine mammals. The Iceland marine food web has never been assessed using stable isotopes, and because previous marine food web studies utilizing stable isotopes (Hobson and Welch 1992; Hobson et al. 1995; Hobson et al. 2002) and a combination of stable isotopes and mercury (Atwell et al. 1998; Campbell et al. 2005) have focused on warm-blooded top predators (i.e.,

seabirds and/or marine mammals), there is a need to investigate the ability of relatively established (i.e., stable isotopes) and novel (i.e., mercury) tracers to structure a marine food web with a cold-blooded top predator.

The overall goal of this research was to identify the trophic position of and sources of carbon to a marine species, the Greenland shark that is both omnivore and top predator, using stable isotopes of carbon and nitrogen and concentrations of mercury. The Greenland shark's spatially variable feeding behavior (i.e., feeding among different habitats) and species-diverse diet provide a unique opportunity to determine if the addition of mercury as a tracer results in higher resolution of feeding interactions and the identification of species that might not belong in the food web.

To address these goals, Greenland sharks and representative species were sampled about the coast of Iceland. Specifically, this research tested the following hypotheses: 1) that among-species there is a relationship between $\delta^{15}N$ and $\delta^{13}C$ that structures the food web with regards to trophic position and carbon source, 2) that the relationship between log [Hg] concentrations and $\delta^{15}N$ will reflect the trend previously observed in other aquatic food webs and 3) that including mercury as a tracer will provide more resolution on trophic links and their spatial relationships in the marine environment.

METHODS

Sampling of Greenland sharks

Greenland sharks (n = 20, 4 males, 16 females) were collected during November 2001-August 2005 as bycatch in the Iceland fishery via either long line or trawl net in the waters surrounding Iceland (Figure 3.1). All sharks were measured for total length and ranged in size from 340 cm to 480 cm with a mean ± 95% confidence interval (CI) total length of 415.6 ± 0.3 cm. Approximately 6 grams of muscle tissue (dorsal surface) and liver tissue (left lobe) were sampled from each shark. All tissue was stored in plastic bags and immediately frozen until analysis. Stomach contents for 15 individual sharks were identified as close to species level as possible, counted and weighed (Table 3.1).

Sampling of the Iceland marine food web

All samples for representative species of the Iceland marine food web, except for the porpoise were collected during August 2005-March 2007 and the sampling locations for each species are shown in Figure 3.1. All fish were measured for total length and those that were not gutted prior to sampling were weighed. The sample size and tissue analyzed are provided in Table 3.2. Approximately 4 grams of muscle tissue (left flank) and liver tissue (left lobe) were sampled from the following species (scientific name and total length given as mean ± 95% CI in parenthesis): pollock (*Pollachius virens*, 42.5 ± 3.0 cm), redfish (*Sebastes marinus*, collected inshore 36.3 ± 2.1 cm and collected offshore 35.5 ± 4.5 cm), argentine (*Argentina silus*, 37.3 ± 4.4 cm), tusk (*Brosme brosme*, 38.4 ± 13.2 cm), ling (*Molva molva*, 87.2 ± 11.4 cm) and blue ling (*Molva dypteryia*, 80.7 ± 28.6 cm). For the following species, which were gutted prior to sampling, 4 grams of muscle tissue (left flank) was collected: cod (*Gadus*

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morhua, 62.4 ± 7.1 cm), haddock (*Melanogrammus aeglefinus*, 59.5 ± 5.2 cm), wolffish (*Anarhichas lupus*, 51.3 ± 2.1 cm), plaice (*Pleuronectes platessa*, 42.8 ± 5.3 cm), lemon sole (*Microstomus kitt*, 32.0 ± 2.8 cm) and *Lycodes* (*Lycodes frigidus*, 40.8 ± 7.0 cm). Capelin (*Mallotus villosus*, 14.4 ± 1.0 cm) were analyzed whole. In addition, lesser black-backed gulls (*Laurus fuscus*) were collected in August 2005 and harbor porpoises (*Phocoena phocoena*) were sampled in April 1992. Approximately 4 grams of muscle tissue were sampled from the left breast muscle of the gulls and from the dorsal surface of the porpoises. Tissue from the left lobe of the liver was also collected from gulls. All specimens were immediately placed in whirl pack bags and frozen until analysis.

The following invertebrate species were collected: mixed zooplankton, snail (*Neptunea despecta*), Norway lobster (*Nephrops norvegicus*), hermit crab (*Pagurus bernhardus*), crab (*Lithodes maja*) and starfish (*Asterias rubens*). Zooplankton, which were collected via a vertical tow using a Hansen net, were not identified to species and due to their small size, several individuals were pooled into each "zooplankton" sample. The shells were removed from snails, lobsters, hermit crabs, crabs and starfish prior to analysis and the remaining soft tissues were analyzed. Shells were removed due to the potential of calcium carbonate to bias $\delta^{13}C$ results.

Stable isotope analysis

Capelin, zooplankton and invertebrates were analyzed whole and the remaining fish, shark, gull and porpoise samples were subsampled into approximately 2 gram batches before freeze drying for 48 hours. Tissue was then

pulverized using a ball mill grinder (SPEX CertiPrep 8000-D ball milling unit, SPEX CertiPrep; Metuchen, NJ, USA) and lipids were removed to decrease the influence of different lipid contents among samples (Post et al. 2007). To remove lipids, approximately 5 ml of 2:1 chloroform: methanol was added to homogenized samples and vortexed for approximately 30 seconds. Samples were allowed to sit for 24 hours, centrifuged and the solution was then decanted into pre-weighed aluminum trays for determination of lipids gravimetrically. The lipid extraction process was repeated a second time to more thoroughly remove lipids and achieve accurate lipid determination. Solvents were removed from the tissue by drying for 24 hours in a fumehood. To determine the effectiveness of the lipid extraction method, the entire process was repeated on three samples (wolffish muscle, Greenland shark muscle, Greenland shark liver) that had been previously lipid extracted using the above method. This second extraction produced only 3-10% more lipid, and thus it is assumed that >90% of lipid is removed by one extraction. Approximately 1 μg of tissue was weighed into tin capsules and stable carbon and nitrogen isotope ratios were determined on a continuous flow isotope ratio mass spectrometer (IRMS; Finnigan MAT Delta^{plus} (Thermo Finnigan, San Jose, CA, USA). The precision of the isotopic analyses was determined to be ±0.15‰ based on internal reference samples. Stable isotopes are expressed as a delta (δ) value using the equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000 \qquad (1)$$

where X is ^{15}N or ^{13}C and R is the ratio $^{15}N/$ ^{14}N or $^{13}C/$ ^{12}C. The standard reference material for carbon was Pee Dee Belemnite and for nitrogen atmospheric N_2.

Mercury analysis

Total mercury (Hg) in muscle, liver and whole capelin and invertebrate samples was analyzed by Atomic Absorption Spectrometry Vapor Generation (AAS-VG) at the Great Lakes Institute for Environmental Research (GLIER) (GLIER method # 01-002), which is accredited by the Canadian Association of Environmental Analytical Laboratories (CAEAL). Twenty percent of samples run were blanks, replicates and National Research Council Canada (NRC-CNRC) standards (Dorm-2, Dolt-3) to ensure accuracy. The instrumental detection limit was 0.1 µg/g, dry. As tissue was analyzed dry, all mercury concentrations are reported in µg/ g, dry weight (dw).

Data analysis

Unless otherwise stated, all statistical analyses were performed using SYSTAT® Version 11.0 (Systat Software Inc., San Jose, California, USA) and relationships were considered significant if $p < 0.05$. Values of $\delta^{15}N$ and $\delta^{13}C$ among species and between tissues (muscle vs. liver) were considered significantly different if their 95% confidence intervals (CI) did not overlap. Unless otherwise stated, all means are reported as mean ± 95% CI. Original values of $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ were calculated for Greenland sharks based on equations derived from Sherwoood and Rose (2005), which take into account the proportion of prey species found in the stomachs of the sharks and the

corresponding stable isotopes of the prey. The change in $\delta^{15}N$ and $\delta^{13}C$ between Greenland sharks and their diet (i.e., $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$, respectively) were calculated as follows, using $\delta^{15}N$ as an example:

$$\Delta\delta^{15}N = \delta^{15}N_{shark} - \Sigma\ (P_i \times \delta^{15}N_i) \qquad (2)$$

where $\delta^{15}N_{shark}$ and $\delta^{15}N_i$ are mean $\delta^{15}N$ signatures of sharks and individual prey species, respectively. P_i is the proportion of the ith prey item in the stomachs of the sharks and is equal to the ratio of total weight (kg) of prey i consumed by all sharks (W_i) to the total weight (kg) of all prey consumed by all sharks (W_{tot}).

Trophic positions were calculated for all fishes using both muscle and liver tissue mean $\delta^{15}N$. The mean $\delta^{15}N$ of argentine was used as a base for trophic position calculations as follows:

$$TP_i = 3.2 + (\delta^{15}N_{consumer} - \delta^{15}N_{argentine})/\ 3.4 \qquad (3)$$

where 3.2 is the estimated trophic level of argentine, $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ of the consumer of interest, and 3.4 is the increase of $\delta^{15}N$ with each trophic level (Vander Zanden and Rasmussen 2001; Post 2002), which is assumed to be constant for all organisms across the food web. Trophic positions were calculated using consumer $\delta^{15}N$ values of muscle and liver using $\delta^{15}N_{argentine}$ values for muscle tissue (10.75‰) and liver tissue (10.11‰), respectively. Trophic position calculations should be based on an organism that temporally integrates isotopes in a similar manner to the consumer of interest and spatially reflects the important sources of nutrients to the consumer (Post 2002). Trophic positions were not calculated for invertebrates because an appropriate base organism was not sampled from the Iceland food web. Argentine were used as a base for fish

because they are mobile and assumed to integrate isotopes spatially (at least from the pelagic environment). The trophic position of Greenland sharks (based on $\delta^{15}N$ of muscle tissue) was also determined using a model derived from Layman et al. (2005), which calculates trophic position for consumers that acquire nitrogen from more than one food web, as follows:

$$\text{Trophic position} = 3.2 + (\delta^{15}N_{shark} - [\delta^{15}N_{B1} \times \alpha + \delta^{15}N_{B2} \times (1-\alpha)]/\ 3.4 \qquad (4)$$

where B1 and B2 are the two baseline taxa representing the different sources of nutrients to the sharks, 3.2 is the trophic position of B1 and B2, α is the proportion of nitrogen that the consumer derived from the food web that B1 is a part of and 3.4 is the increase in $\delta^{15}N$ with each trophic level. The equation for estimating α is:

$$\alpha = (\delta^{13}C_{shark} - \delta^{13}C_{B2})/\ (\delta^{13}C_{B1} - \delta^{13}C_{B2}) \qquad (5)$$

where B1 and B2 are the baseline taxa. For the Iceland marine food web there appeared to be two sources of isotopically distinct carbon and nitrogen to the sharks, inshore organisms and offshore organisms. Argentine (B1) was used as the baseline for the offshore food web and plaice (2) were chosen as a baseline for the inshore organisms. Argentine and plaice were chosen as baseline taxa because they feed primarily on planktonic (Scott and Scott 1988) and benthic (Gibson and Robb 1996) invertebrates, respectively and should therefore incorporate isotopes to spatially represent the offshore and inshore organisms. The trophic position of argentine and plaice was estimated at 3.2 based on their reliance on invertebrates but occasional consumption of fish (Scott and Scott 1988; Gibson and Robb 1996). Two community-wide metrics were calculated for

the Iceland marine food web as derived from Layman et al. (2007): 1) $\delta^{15}N$ range, which is abbreviated NR and equals the difference between the maximum and minimum mean $\delta^{15}N$ and 2) $\delta^{13}C$ range, abbreviated CR and equals the difference in maximum and minimum mean $\delta^{13}C$.

Mercury data was log transformed to reduce skewness prior to analysis. To test whether mercury concentrations increased with trophic positions across the food web, a reduced major axis (RMA) regression was performed (RMA: Software for Reduced Major Axis regression, v. 1.17, A.J. Bohonak, 2004) on $\delta^{15}N$ vs. log [Hg] for whole invertebrates and muscle tissue of fishes (except for capelin as whole individuals were analyzed) and the seabird and mammal species. RMA is a model II regression and takes into account variation on both the x and y axes and is more appropriate than simple linear regressions (which only take into account variation on the y axis) when the x variable is measured with error (Sokal and Rohlf 1981). However, previous studies assessing the increase of mercury with $\delta^{15}N$ have been based almost entirely on simple linear regressions (see Riget et al. 2007). To asses the difference in slopes calculated using RMA and simple linear regressions, an ordinary least squares (OLS) regression was also performed on $\delta^{15}N$ and log [Hg] data of the same samples as analyzed by RMA.

As the feeding behavior of Greenland sharks was of interest, the relationship between Greenland shark length and $\delta^{15}N$ (muscle tissue) and length and log [Hg] (muscle tissue) was investigated using a general linear model (GLM), as mercury has been previously shown to increase with increasing fish

length (Somers and Jackson 1993; Trudel and Rasmussen 2006). The effects of sex on stable isotope and mercury values could not be determined due to the low number of males sharks sampled and because most remaining fish were gutted prior to sampling. Although there is the potential for seasonal effects on the feeding behavior of Greenland sharks, because they were collected during different times of the year, the effect of season on $\delta^{13}C$, $\delta^{15}N$ and/or log [Hg] values in Greenland sharks could not be determined due to the low number of sharks and therefore low power of statistical tests.

In addition, Isosource mixing models (Philips and Gregg 2003) were run using combinations of $\delta^{15}N$, $\delta^{13}C$ and log [Hg] as tracers in an attempt to estimate the contribution of potential prey in the Greenland shark's diet. This analysis used data from the muscle tissue of the Greenland shark and several potential prey species (the sources). Six of the prey species (redfish (inshore and offshore), cod, ling, argentine and porpoise) were included in the model based on their high prevalence in the stomachs of the Greenland sharks. *Lycodes*, which is a deep-sea (>2000m, this study), cold-water (<0°C, this study) species, were included to determine the degree Greenland sharks utilize deep-sea resources, as unidentifiable fragments of deepwater fishes have been observed in the stomachs of Greenland sharks about Iceland (Jörundur Svavarsson, personal communication). Three Isosource models were run and the source increment was set at 1% and the tolerance error was set at 0.1‰. Isosource outputs are reported as a range of minimum to maximum feasible solutions, and the 1-99 percentile range of solutions are reported here (see Philips and Gregg 2003;

Benstead et al. 2006). To account for the enrichment of ^{15}N and ^{13}C by Greenland sharks, each prey species was corrected by 3.4‰ and 1‰, respectively, the $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ values provided for fish by Post (2002) and Minagawa and Wada (1984). The linear $\delta^{15}N$ vs. log [Hg] relationship was used to calculate the increase of log [Hg] with each trophic level, which was represented by a 3.4‰ increase in $\delta^{15}N$. Therefore, the difference was taken between log [Hg] when $\delta^{15}N$ was set equal to 4.4‰ and 1‰. Each prey species' log [Hg] value was then corrected by the trophic increase of log [Hg] before being input into the model.

RESULTS

Stomach contents

All prey species found in stomachs of Greenland sharks are shown in Table 3.1. Redfish had the highest occurrence (60% of sharks), highest numbers (240 individuals) and contributed the most to the Greenland sharks diet by weight (46% of total weight) (table 3.1). Fragments of only five individual marine mammals were found in the shark's stomachs, but by weight, the combined marine mammal tissue contributed 11% to the diet of the Greenland sharks sampled.

Stable isotopes

All of the important fish species and one of the important marine mammals found in the stomach of the sharks were assessed for stable isotopes. Among all species and tissues analyzed from the Iceland marine food web, Greenland

shark liver tissue had the highest mean $\delta^{15}N$ (16.26 ± 0.43‰) and starfish the lowest (9.40 ± 0.53‰; whole body soft tissue) (Table 3.2, Figure 3.2). The $\delta^{15}N$ range (NR, see Layman et al. 2007) was therefore 6.86‰. *Lycodes* muscle $\delta^{15}N$ (15.13 ± 0.45‰) was significantly higher than all other fishes, except for the Greenland shark, which it did not statistically differ from based on CIs. Differences between maximum and minimum $\delta^{15}N$ among fishes sampled from the Iceland marine food web (this study), Newfoundland continental shelf (Sherwood and Rose 2005), North-water polyna (Hobson et al. 2002), Australian southeastern waters (Davenport and Bax 2002), Celtic Sea (Pinnegar et al. 2002) Georges Bank (Fry 1988) and Porcupine abyssal plain, northeastern Atlantic (Iken et al. 2001) are provided in Table 3.3. Intertissue comparisons revealed that liver tissue values of $\delta^{15}N$ were significantly higher than that of muscle tissue in Greenland sharks, pollock and ling by 0.93‰, 1.02‰ and 0.83‰, respectively (Table 3.2). Redfish collected offshore had significantly lower $\delta^{15}N$ values in liver tissue relative to muscle tissue by 0.91‰, and muscle $\delta^{15}N$ did not statistically differ from liver in the remaining species (redfish collected inshore, argentine, blue ling, tusk or gull).

The $\delta^{13}C$ range (abbreviated CR, see Layman et al. 2007) was 8.36‰ (from zooplankton (-22.34 ± 0.42‰) to snail (-13.98 ± 0.30‰); Table 3.2, Figure 3.2). Inshore/benthic organisms were generally ^{13}C enriched relative to offshore/pelagic organisms in the Iceland marine food web (Figure 3.2). The invertebrates and fishes increased on a gradient of $\delta^{13}C$ from offshore pelagic organisms (zooplankton, capelin, offshore redfish, argentine) to a mixture of

offshore benthic/ benthic pelagic organisms (*Lycodes*, Greenland shark, blue ling, ling, tusk, inshore redfish, pollock, cod, haddock, starfish, lobster) to inshore benthic organisms (lemon sole, plaice, wolffish, hermit crab, crab, snail), which generally agreed with previous knowledge concerning the habitats of these species and with their collection locations about Iceland in this study (Figure 3.1 and 2). The Greenland sharks displayed a relatively wide range of $\delta^{13}C$ values (-17.76 ± 0.56‰) which fall generally around the middle range of $\delta^{13}C$ values. Regarding the warm-blooded animals, the mean muscle $\delta^{13}C$ of porpoise was -18.46 ± 0.16‰ and gull was -17.54 ± 0.18‰, grouping them with the offshore pelagic fishes.

$\Delta\delta^{15}N$, $\Delta\delta^{13}C$ and trophic positions

The original $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ calculated for Greenland sharks, which incorporated the proportions of prey species found in the sharks' stomachs was 4.15‰ and 0.35‰, respectively (Table 3.1). Trophic positions calculated for representative Iceland fishes, seabird and marine mammal are shown in Table 3.2. The trophic position calculated for Greenland sharks (based on muscle tissue $\delta^{15}N$) using equation (4) listed in the methods section, which incorporated multiple sources of nitrogen and carbon, was 4.6 (Table 3.2). The calculated trophic position of sharks using equation (3), which was used for all other fishes, was 4.5.

Mercury and $\delta^{15}N$

Greenland sharks had the highest mean mercury concentrations (5.9 ± 1.2 µg/ g, dw) in muscle tissue and pollock and plaice had the lowest (0.1 ± 0.1 µg/ g,

dw), although a number of species (zooplankton, hermit crab, crab, starfish, capelin) were below detection limits (DL = 0.1 µg/g, dw) (Table 3.2). The RMA regression of log [Hg] and $\delta^{15}N$ (Figure 3.3) revealed a positive relationship among all species of the Iceland marine food web (log [Hg] = 0.308 * $\delta^{15}N$ + (-3.995), r^2=0.611) with a slope of 0.308. Due to concerns over a potential temporal bias in porpoise mercury concentration, the RMA regression of $\delta^{15}N$ vs. log [Hg] was also run excluding the porpoise, but the relationship remained positive (r^2=0.660) and the equation was altered only slightly (log[Hg] = 0.306 * $\delta^{15}N$ + (-4.020)). The porpoise were therefore left in the food web and log [Hg] vs. $\delta^{15}N$ regression, and although the potential for a temporal change in mercury concentration cannot be excluded, the similarity between regressions does suggest that the mercury levels in the porpoise have not changed substantially from the early 1990s, when they were collected, and 2005, when most of the food web was collected. Results of the simple linear regression (OLS) of $\delta^{15}N$ vs. log [Hg] (Figure 3.3) among all species of the Iceland marine food web revealed a significant positive relationship (log [Hg] = 0.239 * $\delta^{15}N$ + (-3.140), $p<0.0001$, r^2=0.612).

Based on GLM, no significant relationships were found between Greenland shark length and $\delta^{15}N$ ($F_{1,\ 15}$ = 0.06, p = 0.81) or length and log [Hg] ($F_{1,\ 14}$ = 0.32, p = 0.58). The length vs. mercury relationship in both muscle and liver tissue is represented graphically in Figure 3.4, which agrees with results from McMeans et al. (2007).

Isosource

Inclusion of all three tracers ($\delta^{13}C/ \delta^{15}N/$ log [Hg]) in the Isosource model provided better resolution of source (prey) contribution to the Greenland shark than δ13C/ $\delta^{15}N$ or $\delta^{15}N/$ log [Hg] alone (Table 3.4). The first two tracer combinations (i.e., $\delta^{13}C/ \delta^{15}N/$ log [Hg] and $\delta^{13}C/ \delta^{15}N$) revealed high resolution of offshore redfish and *Lycodes* percent contributions to the shark's diet, which is the most informative data provided by Isosource in this study because very low minima and very high maxima and/or large ranges between minima and maxima do not provide clear information about actual source contributions (see Benstead et al. 2006). Data from models run with $\delta^{13}C/ \delta^{15}N/$ log [Hg] and with $\delta^{13}C/ \delta^{15}N$ agreed with Greenland shark stomach content data that redfish are the most significant contribution to the Greenland shark's diet (Table 3.1), and further, suggested that the offshore redfish are a more significant source than the inshore redfish. None of the models predicted a high contribution of cod to the Greenland shark's diet, although cod are often found in stomachs of the sharks (Table 3.1). The $\delta^{13}C/ \delta^{15}N$ tracers indicated a higher contribution of argentine than the other two tracer combinations. The $\delta^{15}N/$ log [Hg] tracers suggested the highest potential contribution of ling and porpoise to the Greenland shark.

DISCUSSION

Feeding behaviors and sources of nutrients

Relative values of $\delta^{15}N$ in the species sampled from the Iceland marine food web were generally consistent with what is known about their feeding ecology. Increasing $\delta^{15}N$ among fishes reflected the increase in trophic positions

from invertebrate-feeding flat fish (i.e., plaice, lemon sole) to predominantly zooplanktivorous fishes (i.e., capelin, argentine), to piscivores (e.g., cod, ling, *Lycodes*) (Scott and Scott 1988; Gibson and Robb 1996; Bjelland et al. 2000). As well, previous studies reported similar ranges (maximum to minimum mean δ^{15}) of $\delta^{15}N$ and feeding behaviors (e.g., planktonivores, generalists, piscivores) based on $\delta^{15}N$ among fishes collected in marine ecosystems to that of the Iceland marine food web (Table 3.3; Iken et al. 2001; Sherwood and Rose 2005; Hobson et al. 2002; Fry 1988; Pinnegar et al. 2002; Davenport and Bax 2002). These results suggested that $\delta^{15}N$ is indicative of trophic position of fish from temperate to sub-arctic marine food webs.

The Greenland shark had $\delta^{15}N$ values that were greater than all species sampled in the Iceland marine food web except for *Lycodes*, which is consistent with previous conclusions that the Greenland sharks feeds, at least at times as an apex predator (Fisk et al. 2002). The $\delta^{15}N$ of *Lycodes* was higher than expected based on its putative diet of bottom crustaceans and predominantly fish (Bjelland et al. 2000). However, *Lycodes* is a deepwater organism, and although there are very little stable isotope data for the deep sea (but see Iken et al. 2001), different mechanism of nitrogen cycling and different forms of nitrogen utilization in deep-sea organisms could contribute to spatial variation in observed $\delta^{15}N$ (Sherwood and Rose 2005; Wu et al. 1997). Thus, determination of *Lycodes'* trophic position based on comparison of its $\delta^{15}N$ to values in pelagic and inshore benthic food webs may be inaccurate.

Values of $\delta^{13}C$ suggested that the Iceland marine food web sampled in this study actually represented two separate food webs; an inshore food web and an offshore food web, but with most of the species sampled feeding between both food webs. Species were separated first on their feeding inshore vs. offshore (i.e., more depleted ^{13}C vs. enriched ^{13}C) and further on their reliance of benthic (depleted) vs. pelagic (enriched) prey. The range of maximum to minimum $\delta^{13}C$ values observed among fishes from the Iceland marine food web generally agreed with: a) those reported in other marine food web studies (Table 3.3) (Iken et al. 2001; Sherwood and Rose 2005; Hobson et al. 2002; Fry 1988; Davenport and Bax 2002), b) previous knowledge concerning the habitats and feeding behavior of the Iceland fishes (Scott and Scott 1988; Gibson and Robb 1996; Bjelland et al. 2000) and c) where the samples were collected. For example, of the Iceland fishes, wolffish were the most ^{13}C enriched (i.e., most positive $\delta^{13}C$) and were collected inshore (Figure 3.1) and are known to feed on benthic crustaceans (Coad and Reist 2004). Argentine, on the other hand, were the most ^{13}C depleted based on fish muscle tissue (i.e., most negative $\delta^{13}C$), were collected offshore (Figure 3.1) and have been reported to feed on planktonic invertebrates (Scott and Scott 1988). Values of $\delta^{13}C$ in redfish collected from both offshore and inshore areas further support that $\delta^{13}C$ was tracking carbon sources in the Iceland marine food web, as the offshore and inshore redfish were relatively depleted and enriched in ^{13}C, respectively.

Many of the species from the Iceland marine food web appeared to fall somewhere in between the gradient of inshore vs. offshore and/or benthic vs.

pelagic, including the *Lycodes*, ling, blue ling, tusk, porpoise, inshore redfish, cod, haddock, pollock and Greenland shark. The predominantly offshore/ pelagic species appeared to be zooplankton and small pelagic fishes (represented by capelin and argentine) and the predominantly inshore/ benthic species appeared to be flat fishes, wolffish and invertebrates (except for zooplankton). Sherwood and Rose (2005) used $\delta^{13}C$ to calculate the percent reliance on benthic prey of several fishes about Newfoundland, then compared the result to a priori designations of feeding behavior (i.e., pelagic or benthic), and determined that many species previously considered either predominantly pelagic, like redfish, or predominantly benthic, like *Lycodes* species, were actually utilizing several carbon sources and could be assigned as "mixed" feeders. The variable $\delta^{13}C$ values within Greenland sharks are an indicator of variable feeding and of the sharks' known omnivory (Sweeting et al. 2005) and further suggest that the Greenland shark was integrating carbon from multiple sources within the Iceland marine food web. The large geographical range of top marine predators like sharks suggest that they act to couple local food webs and to increase food web stability (Brose et al. 2004), thus Greenland sharks could play a significant ecological role in the Iceland marine ecosystems by integrating discreet food webs. Further, the influence of the Greenland shark on ecosystems could also be unique, although difficult to define, because they combine a high trophic position with omnivorous feeding behavior.

Original $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ calculated for Greenland sharks based on proportion of prey species found in the sharks' stomachs was slightly higher for

δ^{15}N than the commonly used increment of 3.4‰ and slightly lower for δ^{13}C than the commonly reported value of 1‰ (Post 2002; Minagawa and Wada 1984). However, both $\Delta\delta^{15}$N and $\Delta\delta^{13}$C were within the published ranges for aquatic organisms (compiled by Vander Zanden and Rasmussen 2001), and all the major contributors to the stomach contents were analyzed for stable isotopes, suggesting that although the sample size of analyzed shark stomachs was low (*n* = 15) (especially considering that Sherwood and Rose (2005) quantified the stomachs of 1742 individual cod) the calculated $\Delta\delta^{15}$N and $\Delta\delta^{13}$C are likely a valid estimate of the trophic enrichment of nitrogen and carbon stable isotopes of Greenland sharks from their diet.

The significance of mercury as a tracer

Both RMA and OLS regressions revealed an increase in log [Hg] with δ^{15}N, suggesting that mercury increased with trophic position in the Iceland food web. The OLS regression of δ^{15}N vs. log [Hg] among members of the Iceland marine food web produced a similar slope to that observed in other aquatic ecosystems, which were also predominantly assessed using simple linear regressions (see Riget et al. 2007). RMA and OLS typically produce similar p and r^2 values but different slopes because RMA takes variability in both x and y variables into account. Therefore, RMA is a more appropriate statistic when the slope of δ^{15}N vs. log [Hg] is being considered, and in the Iceland marine food web, RMA resulted in a higher slope than OLS. It is unknown whether consistent slopes of log [Hg] vs. δ^{15}N would still be observed across various aquatic systems if the relationship was assessed using RMA regressions. Further, more

data is needed to identify other environmental or physical factors that could affect the observed slope across a food web. Although the slopes from both regressions differed, the same overall trend of increasing log [Hg] with δ^{15}N was observed in the Iceland marine food web.

Based on the log [Hg] and δ^{15}N relationship in the Iceland food web, tusk, ling, blue ling, offshore redfish and porpoise are likely the most significant contributors of mercury to Greenland sharks (Table 3.2) because these species had the highest mercury concentrations among the known dietary constituents of the Greenland sharks sampled in the Iceland marine food web. *Lycodes* also had high mercury concentrations, and although they have never been found in the stomachs of Greenland sharks, the sharks have been caught in Nordic seas where the *Lycodes* were sampled (i.e., to the north of Iceland, Jörundur Svavarsson, personal communication) (Figure 3.1) and are known to occur at depths below 2000 m (Benz et al. 2007), such that it is possible that Greenland sharks consume *Lycodes* and other deepwater fauna.

Although mercury generally agreed with stable nitrogen isotopes, the difference in mercury concentrations between Greenland sharks and *Lycodes* was unexpected due to their similar δ^{15}N. Based solely on stable isotope data, the Greenland shark and *Lycodes* feed at a similar trophic position and *Lycodes* appeared to be a top trophic level fish in the ecosystems sampled. However log [Hg] suggested that *Lycodes* were feeding at a significantly lower trophic position than the sharks, and around that of other fishes such as tusk and blue ling. Considering the accepted life history characteristics of *Lycodes*, that they inhabit

deep water and feed on benthic fishes and crustaceans, it is possible that they are feeding in a different food web that has higher baseline $\delta^{15}N$ than the other fishes sampled about Iceland. However, with only $\delta^{13}C$ and $\delta^{15}N$ data, there was no legitimate reason to suggest that other processes than diet were responsible for the observed stable isotope signature in *Lycodes* but not for other species. Therefore, the addition of mercury data provided a legitimate, objective reason to suggest that the high $\delta^{15}N$ in *Lycodes* might not be a result of feeding behavior, but was likely a result of higher baseline $\delta^{15}N$ in their food web.

The lack of a relationship between mercury and length within the Greenland sharks supports the idea that Greenland sharks are omnivorous, opportunistic feeders that do not increase in trophic position within the size range sampled in this study (see McMeans et al. 2007). The "shotgun" pattern of mercury concentrations with shark length could suggest recent consumption of marine mammal by sharks with noticeably high mercury concentrations, which supports Greenland shark stomach content data, because marine mammal was found in the stomachs of only a few individuals. The results from the Iceland marine food web suggest that mercury is useful as a tracer not only of large scale feeding interactions within entire food webs but is also useful for investigating the feeding behavior within individual species.

Furthering the scope of biochemical tracers

As stable isotopes, and potentially mercury, become more frequently used in ecological studies, new techniques are emerging for interpreting biochemical data, with the result of knowledge gained in a broader scope of ecological

disciplines. For example, estimating prey contribution to a predator's diet (e.g., Isosource, Philips and Gregg 2003) and calculating trophic positions (Sherwood and Rose 2005) on the level of populations, determining species and trophic diversity on the level of communities (Layman et al. 2007) and quantifying the movement of energy and contaminants utilizing mathematical modeling on the scale of ecosystems (Trudel and Rasumssen 2006), are all extending the uses of biochemical tracers. Several of these techniques were applied to the Iceland marine food web in an attempt to better understand the structure of the food web as a whole and to determine the usefulness and applicability of these techniques in food web studies.

Beyond redfish and *Lycodes*, Isosource could not provide clearly resolved prey contributions to the Greenland shark's diet, which likely reflects the complex nature of marine food webs, similarity in stable isotope values across species, and the omnivorous feeding behavior of the Greenland shark. Differences between Isosource results and shark stomach content data (e.g., the high prevalence of cod and argentine in the stomach contents but low contribution to the Greenland shark as suggested by Isosource) indicates either that the Isosource models do not work for this food web (due to inability to resolve prey contributions), that stomach contents do not reflect long term feeding behavior and/ or represent important sources of nutrients and contaminants, that important components of the food web are lacking for this study, or that calculated $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ are inaccurate.

The use of log [Hg] in the Isosource models provided new perspectives on trophic relationships within the Iceland food web, and to a great sense, the definition of trophic position. The difference between Isosource results when mercury was included and not included as a tracer suggested that certain prey species are more important than others in their contribution of nutrients versus contaminants to a predator. For example, based on the increase of the potential contribution of argentine to the diet of the Greenland shark when carbon was included as a tracer and the decrease in argentine's potential contribution when log [Hg] was used as a tracer indicates that although argentine are consumed by the sharks (because they are found in the stomachs), they could be more important to the carbon signature of the shark (based on $\delta^{13}C$), and less important to the shark's observed concentration of mercury, and potentially other elements (some of which are essential). On the other hand, removing $\delta^{13}C$ as a tracer (i.e., the model run using $\delta^{15}N$/ log [Hg] as tracers) increased the potential contribution of ling and porpoise to the Greenland shark, suggesting that these two species are significant contributors to the mercury concentration, and potentially protein, in the shark but not to the carbon. In other words, ling and porpoise are likely not consumed in high quantities relative to other fish (i.e., cod, argentitne), but because they are relatively high in mercury, could represent the most significant source of mercury to the sharks. Although the range between the minima and maxima was high and results must be interpreted with caution, including multiple tracers in the Isosource model and comparing results between

different combinations of tracers provided insight into the significant sources of nutrients and contaminants to predators.

The disconnect between $\delta^{15}N$ and log [Hg] in *Lycodes* reveled through linear regressions and through Isosource raises questions about the accuracy and applicability of calculated trophic positions. The idea of trophic positions, although arbitrary, are useful for food web studies as a way to describe where an organism obtains its energy and/ or contaminants, which is important for studying species interactions, population dynamics and for fisheries management. However, concerns about calculating trophic positions based on the assumption of a constant $\Delta\delta^{15}N$ among members of a food web (i.e., that $\Delta \delta^{15}N = 3\text{-}4‰$) is well stated in stable isotope research (Vanderklift and Ponsard 2003; Post 2002; Gannes et al. 1997; Jardine et al. 2006). Our results raise further concern, because depending on whether the focus, or question, is where an organism obtains its nitrogen or where the same organism obtains mercury, trophic positions appear to differ for the same individuals (based on $\delta^{15}N$ and mercury concentrations, respectively). Regardless of the accuracy of calculated trophic positions based on $\delta^{15}N$, calculating a number to represent where an organism is feeding in its environment (e.g., trophic position 3.3 or 4.2) is potentially a) different depending on whether the interest is where the consumer is getting its nitrogen or its contaminants (based on our results from this study) and b) unhelpful especially for omnivorous species that obtain nutrients from many different sources that cannot be expressed via a number. For example, the trophic position calculated for Greenland sharks (based on shark muscle tissue),

which took into account the sharks' reliance on multiple sources of nitrogen and carbon was equal to 4.6. However, 4.6 in itself does not actually reveal anything about the Greenland shark or its feeding behavior until it is interpreted in relation to the other members of its food web. As far as relating the Greenland shark to the rest of the food web, results from this study suggest that visually investigating and reporting relationships between $\delta^{13}C$, $\delta^{15}N$ and log [Hg] and comparing these data with stomach contents and existing knowledge concerning the biology of organisms provides much more insight and understanding of food web structure, feeding behavior and species interactions than by reporting trophic positions.

On the scale of communities, it has recently been suggested that community-wide metrics like NR and CR can be used in stable isotope studies to provide measures of trophic diversity and multiple basal resources within food webs, respectively (Layman et al. 2007). Comparing these metrics among systems would not be warranted unless similar sampling efforts had been made to accurately represent species from all trophic levels and carbon sources, however monitoring temporal trends in NR and CR within a system could be used to monitor potential alterations to food web structure. The Iceland marine food web has never before been assessed using stable isotopes, but measuring NR and CR values in future studies could help identify changes in the structure of the Iceland marine food web, potentially due to perturbations such as the removal of species, climate change and/or species invasions.

As new techniques continue to emerge for analyzing and interpreting biochemical data, our understanding of food web structure will surely advance. However, just as the validation of new techniques is of concern, so is the question of their applicability and usefulness.

CONCLUSION

The Greenland shark fits into its food web as a top predator (based on $\delta^{15}N$ and mercury) and as an integrator of carbon from multiple sources and potentially from multiple food webs (based on $\delta^{13}C$). The results of this study suggest that stable isotopes have utility in investigating complex marine food webs with multiple sources of carbon and the presence of omnivorous species. The collection of conspecifics from different locations (i.e., inshore and offshore redfish) was helpful for validating the ability of $\delta^{13}C$ to identify different carbon sources. The analysis of stomach contents from Greenland sharks was also useful for interpreting stable isotope data, and our results support the suggestion by Vander Zanden and Rasmussen (1996), Layman et al. (2005) and others that the combination of stable isotopes and stomach contents provides the most robust estimate of diet. Isosource models could not clearly resolve percent contributions of prey species to the Greenland shark, but were useful for identifying potentially important sources of carbon and mercury. The trend of mercury within the Iceland marine food web was similar to that previously observed in other systems, and although mercury generally supported stable isotope data, also suggested that *Lycodes* might be part of a different food web.

As stable isotope use in ecology continues to increase, our results indicate that the inclusion of additional tracers like mercury is crucial for correctly interpreting stable isotope data, especially when diverse species are sampled without a priori knowledge of food web structure and/ or baseline organisms for individual food webs.

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Table 3.1. Stomach contents of 15 individual Greenland sharks. Percent (%) in sharks is the number of stomachs in which each prey species was found divided by total number of stomachs (15). Total # is the number of individuals of each species found in all stomachs. W_i equals the total weight (kg) of all individuals of each species from all stomachs. W_{tot} is equal to the total weight (kg) of all prey from all stomachs. Diet proportion (P_i) is equal to W_i/W_{tot} (reported here as a % value). Stable isotopes are mean $\delta^{15}N$ and $\delta^{13}C$ of representative samples of each prey species and Greenland sharks from about Iceland. P_i was recalculated for trophic fractionations including only the species with stable isotope data.

Prey Species found in shark stomachs	% in sharks	total #	W_i (kg)	P_i (%)	Greenland shark trophic fractionation		
					P_i (%)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Unidentified gelatinous material	6.7	1	10.0	1.74			
Etmopterus princes (great laternshark)	6.7	1	0.2	0.03			
Raja sp. (unidentified ray)	6.7	2	3.0	0.52			
Teleostei (unidentified teleosts)	46.7	19	11.0	1.92			
Clupea harengus (Atlantic herring)	6.7	9	4.5	0.78			
Argentina silus (argentine)	26.7	14	8.9	1.55	1.92	10.75	-18.58
Gadus morhua (cod)	33.3	60	116.0	20.23	24.99	11.81	-16.60
Melanogrammus aeglefinus (haddock)	13.3	2	1.5	0.26	0.32	11.88	-16.02
Pollachius virens (pollock)	33.3	12	29.5	5.15	6.36	11.00	-16.68
Brosme brosme (tusk)	6.7	1	2.0	0.35	0.43	12.71	-16.78
Molva molva (ling)	6.7	2	4.0	0.70	0.86	12.00	-17.11
Gadiformes (unidentified ling, blue ling, tusk)	20.0	3	2.0	0.35			
Anarhichas lupus (wolffish)	26.7	6	10.2	1.78	2.20	10.14	-15.05
Anarhichas minor (spotted wolf fish)	6.7	1	1.0	0.17			
Pleuronectid (unidentified flatfish)	13.3	4	0.8	0.14			
Pleuronectes platessa (plaice)	6.7	10	6.0	1.05	1.29	10.21	-15.20
Limanda limanda (dab)	13.3	4	2.7	0.47			
Reinhardtius hippoglossoides (halibut)	6.7	1	4.0	0.70			
Cyclopterus lumpus (lumpsucker)	13.3	5	2.0	0.35			
Sebastes sp. (redfish)	60.0	240	265.0	46.22	57.10	10.94	-19.19
Mammalia (Unidentified mammal tissue)	13.3	2	64.0	11.16			
Pinniped	13.3	2	21.0	3.66	4.52	11.64	-17.54
Ursus maritimus (polar bear)	6.7	1	4.0	0.70		15.33	-17.76
					G. shark signature		
TOTALS		402	573.3 (W_{tot})		trophic fractionation	4.15	0.35

See equation (2) methods section for trophic fractionation calculations.

Table 3.2. Representative species of the Iceland marine food web: tissue analyzed and mean ± 95% CI $\delta^{15}N$, $\delta^{13}C$ and total mercury (Hg) (µg/ g, dw). Trophic levels (TL) are calculated using equation (3) (see methods).

Species	n	Tissue	$\delta^{15}N$ (‰)	TL	$\delta^{13}C$ (‰)	Hg (µg/ g, dw
Invertebrates						
Zooplankton	6	whole	9.70 ± 0.32		-22.34 ± 0.42	< DL
snail	6	whole	11.04 ± 1.25		-13.98 ± 0.30	0.38 ± 0.28
Norwegian lobster	2	whole	10.52		-16.16	0.19
hermit crab	3	whole	10.98 ± 0.77		-15.41 ± 0.26	< DL
crab	10	whole	10.56 ± 0.33		-14.41 ± 0.23	< DL
starfish	3	whole	9.40 ± 0.53		-16.14 ± 0.97	< DL
Chondricthyes						
Greenland shark	19	muscle	15.33 ± 0.34	4.6*	-17.76 ± 0.56	5.93 ± 1.17
	20	liver	16.26 ± 0.43	5.0	-18.98 ± 0.64	5.32 ± 2.73
Teleostei						
argentine	6	muscle	10.75 ± 0.64	3.2	-18.58 ± 0.42	0.20 ± 0.09
	5	liver	10.11 ± 0.33	3.2	-18.44 ± 0.27	0.41 ± 0.27
capelin	10	whole	9.95 ± 0.45	3.0	-19.58 ± 0.18	< DL
Atlantic cod	5	muscle	11.81 ± 0.45	3.5	-16.60 ± 0.21	0.18 ± 0.07
haddock	8	muscle	11.88 ± 0.45	3.5	-16.02 ± 0.36	0.16 ± 0.07
pollock	11	muscle	11.00 ± 0.46	3.3	-16.68 ± 0.25	0.12 ± 0.03
	5	liver	12.02 ± 0.20	3.8	-19.71 ± 0.68	0.09
tusk	6	muscle	12.71 ± 0.84	3.8	-16.78 ± 0.25	1.70 ± 0.18
	6	liver	11.52 ± 1.09	3.6	-18.36 ± 0.95	0.34 ± 0.19
ling	6	muscle	12.00 ± 0.42	3.6	-17.11 ± 0.14	0.89 ± 018
		liver	12.83 ± 0.15	4.0	-20.89 ± 0.50	0.18 ± 0.04
blue ling	6	muscle	12.96 ± 0.19	3.8	-17.81 ± 0.21	0.97 ± 0.30
	6	liver	13.65 ± 0.56	4.2	-20.24 ± 0.54	0.10 ± 0.02
wolffish	6	muscle	10.14 ± 0.98	3.0	-15.05 ± 0.39	0.39 ± 0.09
plaice	6	muscle	10.21 ± 0.71	3.0	-15.20 ± 0.37	0.12 ± 0.03
lemon sole	6	muscle	11.17 ± 0.95	3.3	-15.34 ± 0.28	0.25 ± 0.16
redfish (inshore)	6	muscle	11.52 ± 0.53	3.4	-16.74 ± 0.51	0.37 ± 0.14
	6	liver	11.56 ± 0.54	3.6	-18.66 ± 0.38	0.05
redfish (offshore)	6	muscle	10.94 ± 0.32	3.3	-19.19 ± 0.32	0.72 ± 0.33
	6	liver	10.03 ± 0.17	3.2	-19.54 ± 0.47	0.28 ± 0.04
Lycodes	9	muscle	15.13 ± 0.45	4.5	-18.77 ± 0.56	1.21 ± 0.26
Seabirds						
lesser black backed gull	3	muscle	10.40 ± 0.21	3.1	-18.46 ± 0.16	0.43 ± 0.19
	3	liver	10.47 ± 1.44	3.3	-18.36 ± 0.42	1.78 ± 0.21
Marine mammals						
Harbor porpoise	7	muscle	11.64 ± 0.49	3.5	-17.54 ± 0.18	1.29 ± 0.21

*See equation (3) and (4) for Greenland shark muscle TL calculation in methods section.

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Table 3.3. Maximum (max), minimum (min) and differences between maximum and minimum mean $\delta^{15}N$ (NR) and $\delta^{13}C$ (CR) values from the Iceland marine food web and six previously published marine food web studies.

Marine system	$\delta^{15}N$ (‰)			$\delta^{13}C$ (‰)			Reference
	NR	min	max	CR	min	max	
Iceland marine food web	5.19	10.14	15.33	4.14	-19.19	-15.05	this study
Newfoundland continental shelf	5.40	10.30	15.70	3.90	-21.40	-17.50	Sherwood and Rose (2005)
North Water polyna	3.40	10.60	14.00	2.20	-20.10	-17.90	Hobson et al. (2002)
Southeastern Australia	5.10	9.60	14.70	5.40	-20.60	-15.20	Davenport and Bax (2002)
Celtic Sea	7.00	10.20	17.20	n/a	n/a	n/a	Pinnegar et al. (2002)
Georges Bank	5.00	10.20	15.20	6.30	-22.10	-15.80	Fry (1988)
Porcupine abyssal plain (NE Atlantic)	5.24	10.28	15.52	2.35	-18.62	-16.27	Iken et al. (2001)

Table 3.4. The distribution of feasible contributions (1-99 percentile ranges) of each prey species to the Greenland shark, as provide by IsoSource using three different combinations of $\delta^{13}C$, $\delta^{15}N$ and log Hg as tracers.

Prey species	Tracers		
	$\delta^{13}C$, $\delta^{15}N$, log Hg	$\delta^{13}C$, $\delta^{15}N$	$\delta^{15}N$, log Hg
redfish (inshore)	0 – 11%	0 – 12%	0 – 31%
redfish (offshore)	47 – 67%	24 – 66%	0 – 54%
cod	0 – 8%	0 – 11%	0 – 20%
argentine	0 – 14%	0 – 45%	0 – 21%
ling	0 – 16%	0 – 14%	0 – 74%
porpoise	0 – 20%	0 – 18%	1 – 66%
Lycodes	18 – 24%	18 – 26%	1 – 20%

Figure 3.1. Sampling locations for representative invertebrate, fish, seabird and marine mammal species. The rectangle indicates sampling area for the following species: crab, lobster, hermit crab, starfish, snail, zooplankton, wolffish, lemon sole, pollock, haddock, plaice, cod, redfish (inshore), gull and porpoise.
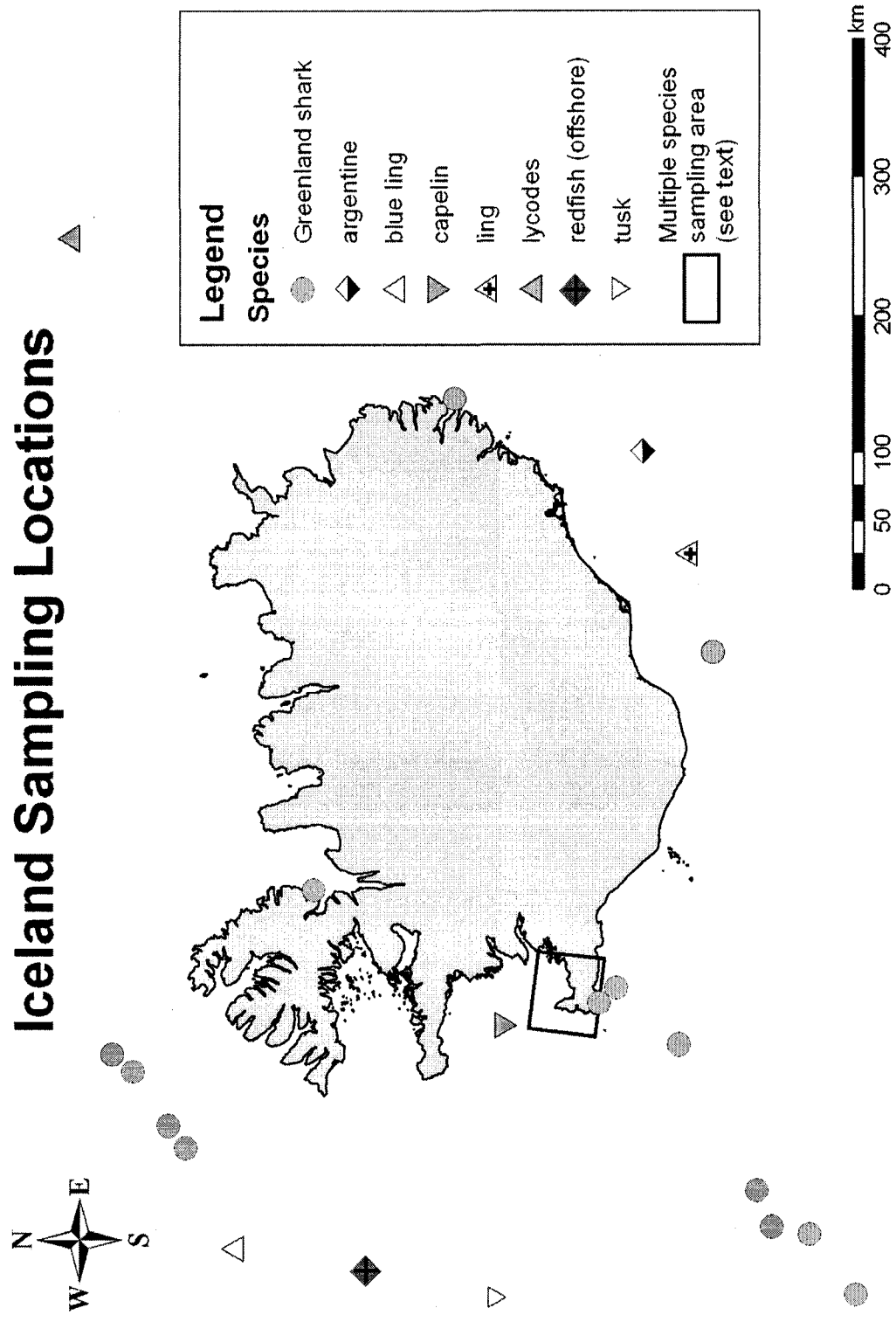

Iceland Sampling Locations

Legend
Species

Greenland shark
argentine
blue ling
capelin
ling
lycodes
redfish (offshore)
tusk

Multiple species
sampling area
(see text)

km
0   50   100   200   300   400

Figure 3.2. Mean ± 1SE of $\delta^{15}N$ and $\delta^{13}C$ for representative species of the Iceland marine food web.

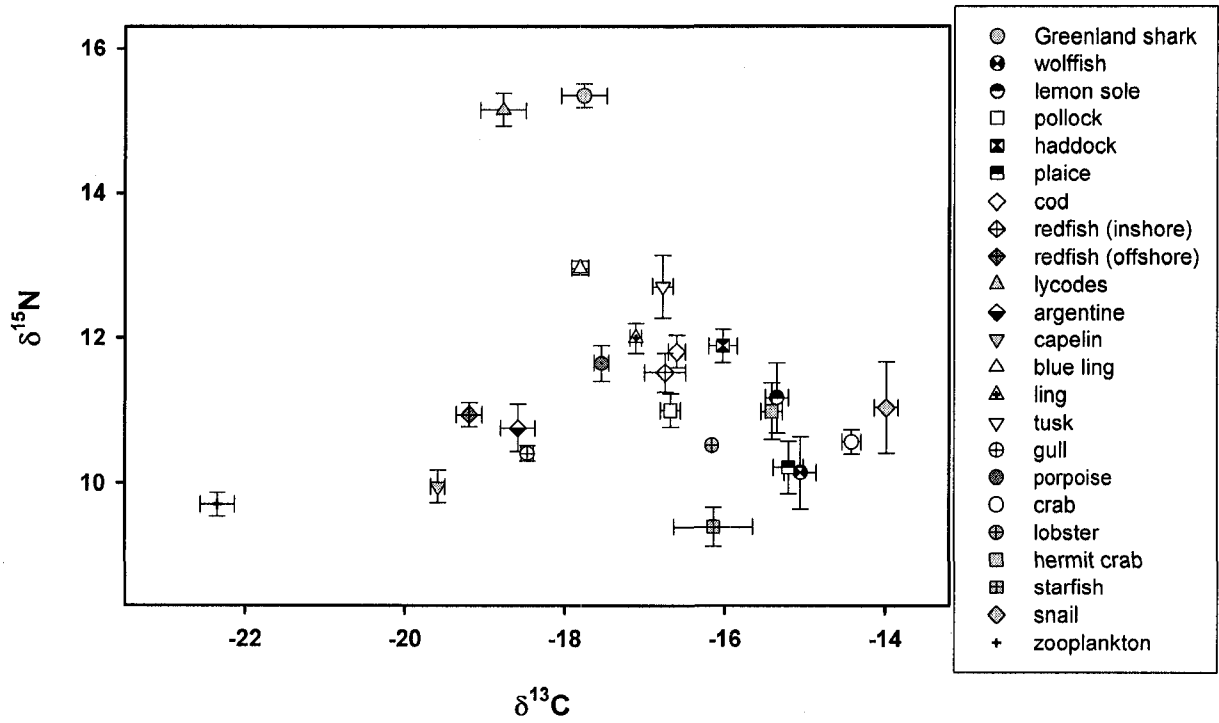|	
⊙	Greenland shark
⊖	wolffish
⊖	lemon sole
□	pollock
⊠	haddock
▣	plaice
◇	cod
⊕	redfish (inshore)
◆	redfish (offshore)
△	lycodes
◆	argentine
▽	capelin
△	blue ling
▲	ling
▽	tusk
⊕	gull
⊛	porpoise
○	crab
⊕	lobster
▣	hermit crab
⊞	starfish
◈	snail
+	zooplankton

Figure 3.3. Mean ± 1SE of $\delta^{15}N$ and log [Hg] for representative species of the Iceland marine food web.
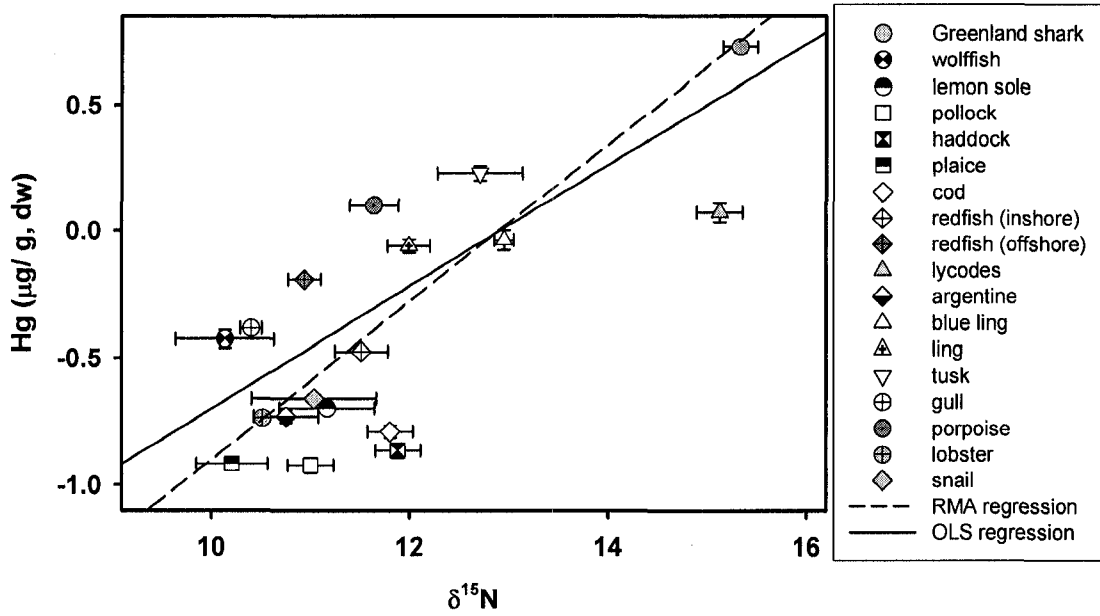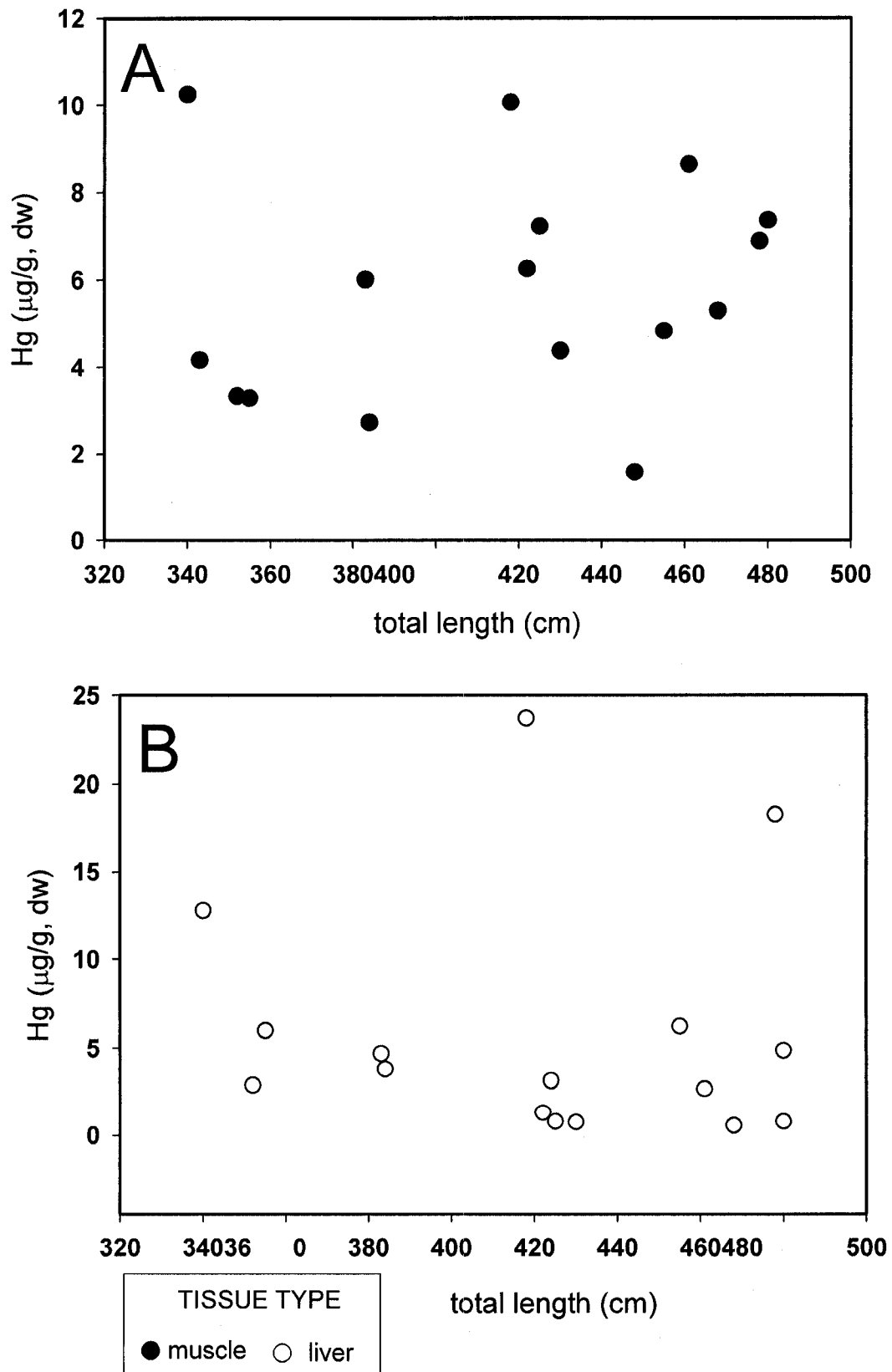
Figure 3.4. Total lengths (cm) and mercury (Hg) (µg/ g, dw) concentrations for individual Greenland shark (A) muscle tissue and (B) liver tissue.

CONCLUSION

The overall goal of this work was to use chemical tracers to investigate different aspects of sleeper shark biological and ecological processes, and in doing so, to answer questions about the ability of elements to act as tracers of these processes.

Chapter two focused on identifying the element profiles of two sleeper sharks. By comparing concentrations between the two species, several conclusions were reached. First, significantly different concentrations of non-essential elements were observed in Greenland sharks and Pacific sleeper sharks, which could indicate different environmental or dietary exposures between these species. Second, the differences in essential elements, which were generally higher in Pacific sleeper sharks, are thought to signify physiological differences between the species. These results suggest that elements, both essential and non-essential, have potential as tracers of environmental exposure and physiological processes in marine organisms.

Chapter three integrated data from the biomagnifying element mercury, stable nitrogen and carbon isotopes and stomach contents to examine trophic links within the Greenland shark's food web about Iceland with the goal of more specifically understand the trophic role of the Greenland shark. This study also aimed to test the ability of mercury, a novel tracer, and stable isotopes, established tracers, to structure a marine food web, and in doing so, to determine if mercury was useful for providing more information about feeding interactions in complex systems. Findings of this work supported previous data concerning the

Greenland shark's feeding behavior but also provided new insights and conclusions. First, Greenland sharks collected about Iceland feed at the highest trophic position and integrate carbon from multiple food webs. Second, the stable nitrogen isotope data were generally supported by mercury data, suggesting that both stable isotopes and mercury are useful for structuring marine food webs. Third, the integration of mercury and stable isotopes was able to identify that certain prey species, like redfish and argentine, are more important sources of carbon to the Greenland shark, whereas marine mammal (i.e. porpoise) are more significant contributors of contaminants. Finally, the addition of mercury data provided a legitimate reason to suggest that processes other than diet were responsible for $Lycodes$' observed $\delta^{15}N$. Chapter three also raised questions about the accuracy and applicability of calculated trophic positions, as our results suggested that trophic positions of Greenland sharks could vary depending on whether the consideration is the shark's major source of carbon or contaminants.

The overall conclusions of this work are that elements are useful as ecological tracers of exposure, potentially physiological processes and of food web structure and that mercury could be considered necessary for correctly interpreting stable isotope data for diverse arrays of species that could represent multiple food webs.

VITA AUCTORIS

Bailey McMeans was born in 1982 in Knoxville, Tennessee, USA. She graduated from Centennial High School in 2000 and went on to obtain a B.Sc. in Biology in 2004. She is currently a candidate for the Master's degree in Environmental Science at the Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario, Canada.