Trophic Links: Forage Fish, Their Prey, and Ice Seals in the Northeast Chukchi Sea

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Chapter 1: General Introduction

The goal of this research was to document trophic structure in the northern Chukchi Sea by examining feeding ecology of Arctic fishes and ice seals to provide a baseline for assessing how trophic structure may vary among years with different ice habitat conditions. To achieve this goal, we examined the diet and short and longer-term records of trophic levels in a selection of fishes and ice seals. The short and longer-term diets of eleven Arctic fish species were examined via, respectively, stomach contents and muscle stable isotope analyses. Interannual variability of short and longer-term diets of five of these fish species were examined across three years, 2008–2010. Stable isotope ratios within ice seal muscle tissue were used to compare their diets during 2002–2003 and 2007–2010. Finally, seasonal and potential interannual differences in ice seal diet were described using stable isotope ratios in ice seal claws.

The northeastern Chukchi Sea from Point Hope to Barrow is experiencing increased oil and gas resource exploration and development pressure, along with ever-increasing rates of global climate change. There have been reductions in the extent and thickness of perennial ice in the Chukchi and Beaufort Seas since 1979 (Moline et al. 2008). Minimum sea ice extent occurs in September, and 2007 was a particularly low-ice year relative to the average from satellite records during 1979–2000 (NSIDC 2011a). Since 2007, less of the thicker multi-year ice has persisted leading to a sea ice decline in 2012 surpassing that of 2007 (Arctic Research Consortium of the United States [ARCUS] 2012; NSIDC 2012). It will not be possible to distinguish between future anthropogenic or natural effects on the trophic structure in the Chukchi Sea without a basis of comparison.

Sea ice reduction in the Arctic may lead to modifications in productivity and/or food-web structure in the Arctic Ocean during 2007 (Arrigo et al. 2008). The high biomass of phytoplankton may have been immediately consumed by pelagic crustaceans that develop earlier and more rapidly in warmer waters (Bluhm and Gradinger 2008; Forest et al. 2011). Arctic fishes and seals may take advantage of the prey source presented by more abundant, pelagic crustaceans in years of high annual primary productivity (Bluhm and Gradinger 2008). A pelagic-dominated food web would reduce the input of more refractory carbon to the seafloor (Bluhm and Gradinger 2008), potentially impacting benthic biomass over time (Dunton et al. 2005). Species composition is likely changing in the Chukchi Sea as faunal ranges shift northward (Grebmeier 2012) and as the ecosystem shifts from a benthic to a pelagic-dominated system (Bluhm and Gradinger 2008). Assessment of trophic structure during recent sea ice minima is needed to understand potential effects of climate change.

Ice seals may be directly impacted by climate change as the sea ice platform they use for resting, pupping, and molting diminishes. In response to evident sea-ice habitat loss and predicted reduced snow cover, the Arctic Basin population of ringed seals (*Pusa hispida*) and the Okhotsk population of bearded seals (*Erignathus barbatus*) have been listed as threatened under the Endangered Species Act (ESA) by the National Oceanic and Atmospheric Administration

([NOAA] 2012a). Because sea ice in the Bering Sea is expected to persist in winter and is not present in the summer, the Bering Sea population of spotted seals (*Phoca largha*) and the entire species of ribbon seals (*Histriophoca fasciata*) are expected to remain unaffected by the summer sea ice minima of the Arctic Ocean, and have not been listed under the ESA (NOAA 2008, 2009). Ice seals may be indirectly affected by changes to prey resources, such as increased competition and changes in prey distribution and abundance (Grebmeier 2012).

Fishes are a key component in the Arctic food web. The most abundant demersal fishes in the Chukchi Sea are Gadidae (cods), Cottidae (sculpins), and Stichaeidae (pricklebacks) (Norcross et al. 2010). Additional families examined in this study include Clupeidae (herrings), Osmeridae (smelts), Zoarcidae (eelpouts), Ammodytidae (sand lances), and Pleuronectidae (righteye flounders). Research on these Arctic fish species is limited for various reasons including short ice-free periods, logistical difficulties, and political boundaries (Mecklenburg et al. 2008). Changes in the Arctic ecosystem will likely lead to changes in fish diets. Past and future information about fish diets could be compared with information about recent diets to enhance general knowledge of energy flow in this dynamic ecosystem.

Stable nitrogen and carbon isotope ratios have been used to study food webs and identify likely dietary sources for many fauna, including Arctic species (Hobson and Welch 1992; Dehn et al. 2006; Bentzen et al. 2007). Nitrogen exists in two forms; as a lighter, more common stable isotope (^{14}N) and as a heavier, less abundant stable isotope (^{15}N). Organisms preferentially use the lighter isotope for metabolic processes resulting in proportionally more of the heavier isotope being integrated into their tissues (Peterson and Fry 1987; Newsome et al. 2010). Stable nitrogen isotope ratios ($^{15}N/^{14}N$, expressed as $\delta^{15}N$ values) describe a species' trophic level in the trophic pyramid. The tissues of a consumer will be enriched in ^{15}N by about 3‰ compared to its prey (Peterson and Fry 1987; Kelly 2000) and thus will have higher $\delta^{15}N$ values (Figure 1-1). Stable carbon isotope ratios ($^{13}C/^{12}C$, expressed as $\delta^{13}C$ values) have been used to illustrate carbon sources and habitat use (Figure 1-1; Schell et al. 1989; Kline et al. 1997; Dehn et al. 2007). For example, ice algae trapped in brine channels exhaust the available lighter isotope (^{12}C) and transition to using the heavier isotope (^{13}C) resulting in ice algae being more enriched in ^{13}C compared to phytoplankton (Kennedy et al. 2002; Gradinger 2008). Additionally, benthic organisms consume recycled material, making their tissues more enriched in the heavier carbon isotope compared to pelagic organisms foraging on fresh phytoplankton (Iken et al. 2005). Predators foraging on sea-ice-associated prey or benthic organisms can have tissues more enriched in ^{13}C relative to pelagic foragers. Coupling stable nitrogen and carbon isotope ratios creates an isotope signature for a predator that will vary based on the proportions of different prey items consumed.

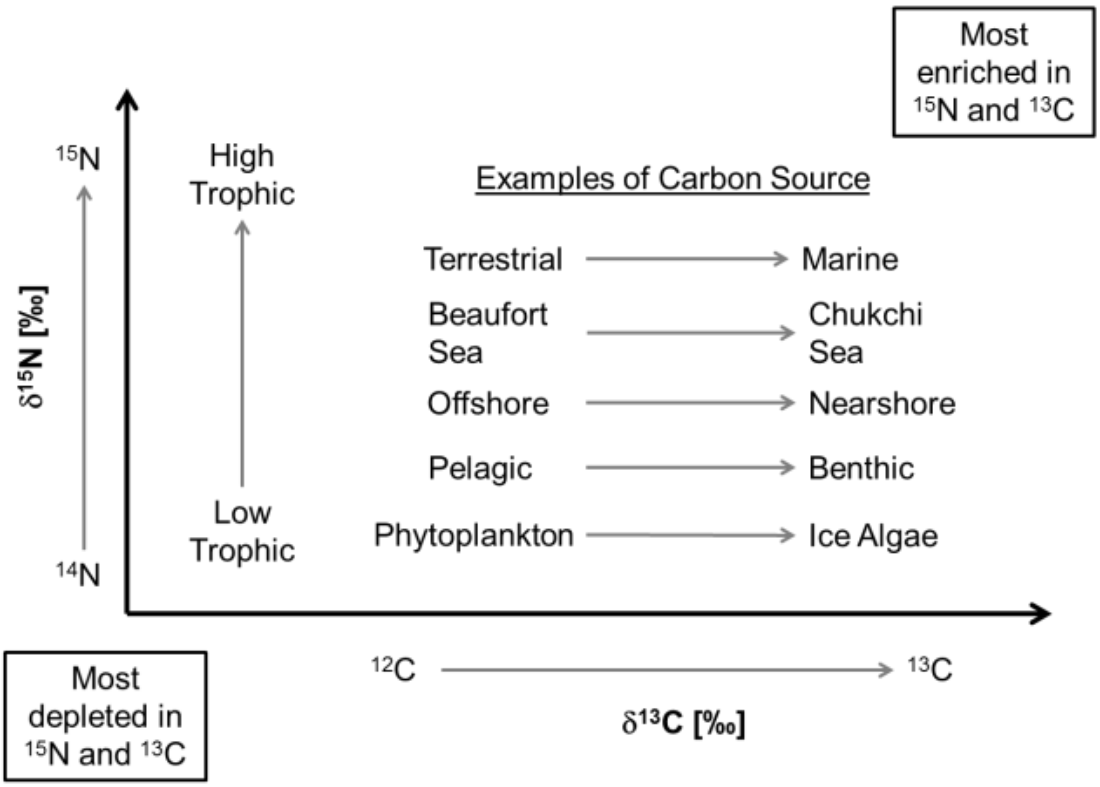

Figure 1-1. **Nitrogen and Carbon Explanation.** Illustration shows the various factors that influence stable nitrogen and carbon isotope ratios. More positive $\delta^{15}N$ values (y-axis) means the organism is feeding at a higher trophic level (Kelly 2000). More positive $\delta^{13}C$ values (x-axis) may indicate the organism is consuming marine instead of terrestrial organisms (Clementz et al. 2003), foraging in the Chukchi Sea vs. the Beaufort Sea (Schell et al. 1989; Dunton et al. 2006), foraging nearshore vs. offshore (Burton and Koch 1999), foraging more in the benthic vs. pelagic zone (Hobson et al. 2002; Iken et al. 2005), or consuming more ice algae vs. phytoplankton (Kennedy et al. 2002; Gradinger 2008).

Isotopic mixing models have become powerful tools to evaluate predator diets and describe the proportional consumption of prey (Phillips and Gregg 2001, 2003; Bentzen et al. 2007; Moore and Semmens 2008; Parnell et al. 2010). In order to make inferences about prey contribution to the diet of a predator, a comparison must be made between the isotopic signatures of the predator and its prey. Three factors are inserted into mixing models: stable carbon and nitrogen isotope ratios of the predator and its prey, and a trophic enrichment factor. The trophic enrichment factor is the incremental change in stable isotope ratios from prey to predator tissues and is used to evaluate all food-web components in the same isotopic space (Peterson and Fry 1987). Moreover, knowledge of isotopic turnover rates for the tissue of the predator is crucial to accurately interpret the diet timeframe described by stable isotopes (Newsome et al. 2012).

A key component to the successful application of mixing models is the distinctive isotopic signatures of prey items (Gannes et al. 1998). If prey signatures overlap, the model confounds the proportional contribution of each source (Phillips and Gregg 2003). The diet of a predator can be described using a single consumption percentage for each prey source if there are only two or three isotopically distinct prey items (# isotopes + 1; Phillips and Gregg 2003). Many predators,

however, have a varied diet leading to a range of possible solutions for proportional contributions of prey items to the diet. As the number of food sources increases, the uncertainty to the contribution of each source increases as well (Phillips and Gregg 2003). Bayesian isotope mixing models allow for the incorporation of more than three dietary sources (prey) and produce probable dietary solutions for each (Parnell et al. 2010). In addition, these models account for biological variability in stable carbon and nitrogen isotope ratios of predator and prey and include measurement error (Parnell et al. 2010).

This report is organized as a series of chapters addressing different project components:

In **Chapter 2**, feeding ecology was documented for eleven fish species using stomach content and stable isotope analyses. Fish stomach content data provide information about important prey taxa in diets from the previous day(s), while isotope ratios of fish tissue describe diets averaged over the previous year (Buchheister and Latour 2010). Fish prey taxa collected during stomach content analysis were analyzed for stable nitrogen and carbon isotope ratios. Total body homogenate of fishes was processed for stable isotope analysis as we were examining seal diets for this overall project as well. Additionally, we compared stable isotope ratios for both non-lipid-extracted and lipid-extracted samples of both fish muscle and total body homogenate; those results are provided as an appendix in this chapter.

In **Chapter 3**, interannual diets were investigated for five of the fish species from the previous chapter. The particular species were selected because a larger sample size was available across multiple, consecutive years from 2008 to 2010. As stable isotope ratios of fish tissue describe diets averaged over the previous year, fish diets from 2007 to 2010 were explained using isotope mixing models to show the proportional contribution of prey groups. Additionally, mixing model performance was assessed with a larger predator sample size and as a separate investigation with stable isotope ratios of prey from a single year compared with a multi-year average.

In **Chapter 4**, stable isotope ratios within the muscle tissue of three ice seal species were used to compare diets during 2002−2003 and 2007−2010. Isotope mixing models were used to describe the proportional contribution of prey guilds to the diets of ringed, bearded, and spotted seals. To our knowledge, tissue turnover rates have not been examined for marine mammals. However, experimental studies have documented tissue turnover rates of terrestrial mammals, i.e., mice, gerbils, alpacas, and steers (Tieszen et al. 1983; MacAvoy et al. 2005; Sponheimer et al. 2006; Bahar et al. 2009), and those rates were used to extrapolate tissue turnover rates of ice seals.

In **Chapter 5**, potential interannual differences in diets of ice seals were described using the dietary record deposited in claws. In a previous study, a chronological record of diet was documented using stable isotope ratios of ringed seal claws (Ferreira et al. 2011). In this chapter, stable isotope ratios in claws were examined for ringed seals and an additional three species, bearded, spotted, and ribbon seals. Up to ten years of dietary information is presented in an individual claw, and, therefore, compiled information from claws analyzed in this study included

diet information from 1998 to 2010. Additionally, we assessed potential age-related differences in diet and examined fetal, natal, weaning, and post-weaning stable isotope signatures in claws. Results for ringed and bearded seal claws were published February 2013 in the Canadian Journal of Zoology (doi:10.1139/cjz-2012-0137).

In **Chapter 6**, major conclusions from each component of this research were compiled to identify potential interannual feeding trends of Arctic predators during the recent sea ice minima. Making connections as to interannual variations between fish diets and seal diets may demonstrate their adaptation potential to changes in food web structure. As sea ice extent continues to decrease in the Arctic, this study provides baseline dietary information for Arctic fishes and ice seals during initial reduced sea ice years that may be beneficial for future research.

Chapter 2: Comparison of Short-term and Long-term Diets of Eleven Arctic Fish Species

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2.1 Introduction

The Chukchi Sea has a high biomass of benthic organisms for an Arctic area (Grebmeier and Dunton 2000; Hunt et al. 2013). However, as the Arctic climate changes so will the distribution of prey resources (Grebmeier et al. 2006, Grebmeier 2012). The Chukchi Sea is similar to the northern Bering Sea, an ecosystem that recently has become more dominated by pelagic species (Grebmeier et al. 2006, Grebmeier 2012). Sea ice extent has been at historic lows since 2007 (NSIDC 2013). During 2007, there was a high abundance of pelagic crustacean grazers and consumers, particularly more Pacific species, in the Chukchi Sea (Eisner et al. 2012). A pelagic-dominated food web would reduce the input of refractory material to the seafloor (Bluhm and Gradinger 2008), potentially impacting benthic biomass over time (Dunton et al. 2005; Wassmann and Reigstad 2011) and foraging success of benthic predators (Grebmeier et al. 2006).

Fishes are a key component in the Arctic food web. Research of Arctic fish species is limited for various reasons, e.g., short ice-free periods, remoteness and logistical difficulties, political boundaries (Mecklenburg et al. 2008). Most fish species in the Chukchi Sea are benthic or benthopelagic predators while few are strictly pelagic (Mecklenburg et al. 2008). The most abundant demersal fishes in the Chukchi Sea are Gadidae (cods), Cottidae (sculpins), and Stichaeidae (pricklebacks) (Norcross et al. 2010). Additional families examined in this study include Clupeidae (herrings), Osmeridae (smelts), Zoarcidae (eelpouts), Ammodytidae (sand lances), and Pleuronectidae (righteye flounders). Species from these families are commonly identified in stomach contents of ice seals (Quakenbush et al. 2009, 2010a, b). Changes in the composition of fish prey as a result of diminished sea ice extent can quickly propagate to apex predators (Grebmeier 2012). Therefore, understanding the variability in diets of Arctic fishes is helpful in the assessment of current and potential abiotic effects on food web structure in this dynamic ecosystem.

Feeding ecology studies of Arctic fishes using stomach contents can be enhanced by stable isotope analysis. Stomach content analysis provides high taxonomic resolution of prey on a short time scale, but can underestimate the dietary contribution of soft-bodied prey (Brush et al. 2012), such as fish tissue and polychaete worms, and overestimate hard-bodied prey (Sheffield et al. 2001), such as clams and snails. It is also a labor-intensive process that requires familiarity with the specialized task of taxonomic identification. Alternatively, information from stable isotope analysis is of relatively poor taxonomic resolution (Carrasco et al. 2012), yet provides estimates of assimilated diet integrated into the tissues of a consumer over a period of time (Buchheister and Latour 2010). Stable nitrogen isotope ratios indicate the trophic position at which a fish feeds; tissues of predators are enriched in the heavier isotope (^{15}N) by about 3‰ compared to prey (Peterson and Fry 1987). Stable carbon isotope ratios illustrate carbon source and habitat

use (Kline et al. 1998). For example, benthic organisms consume recycled material, making their tissues more enriched in the heavier carbon isotope (^{13}C) compared to pelagic organisms foraging on fresh phytoplankton (Iken et al. 2005). Assessing short-term (stomach contents) and long-term diet (stable isotope ratios) of Arctic fishes is essential to understand trophic relationships between primary consumers and apex predators.

The goal of this research was to document trophic structure in the Chukchi Sea by examining the feeding ecologies of Arctic fish species. This was part of a larger project investigating trophic links from the prey of fish through fish to ice seals. Stomach content and stable isotope analyses were performed on eleven Arctic fish species collected from the Chukchi Sea during 2007–2010. Fish stomach content data provided information about important prey taxa in diets from the previous day(s) while stable isotope ratios of fish tissue described diets averaged over the previous year (Buchheister and Latour 2010). Adding to the baseline knowledge of feeding ecology of Arctic fishes may enhance general understanding of their adaptation potential to changes in food-web structure.

2.2 Materials and Methods

2.2.1 Fish Collections

Fishes used in this study were collected offshore in the Chukchi Sea across a four-year period during research cruises in August–September 2007, July 2008, July–October 2009, and August–September 2010 (Figure 2-1). Eleven species were selected for diet and stable isotope analysis (Table 2-1): Pacific Herring (*Clupea pallasii*), Capelin (*Mallotus villosus*), Arctic Cod (*Boreogadus saida*), Saffron Cod (*Eleginus gracilis*), Arctic Staghorn Sculpin (*Gymnocanthus tricuspis*), Shorthorn Sculpin (*Myoxocephalus scorpius*), Canadian Eelpout (*Lycodes polaris*), Stout Eelblenny (*Anisarchus medius*), Slender Eelblenny (*Myoxocephalus scorpius*), Pacific Sand Lance (*Ammodytes hexapterus*), and Bering Flounder (*Hippoglossoides robustus*). Capelin and Pacific Sand Lance were collected by midwater trawl, and the other species were collected by bottom trawl. These species are from major taxonomic families found in the Chukchi Sea and are commonly observed as the prey of ice seals (Quakenbush et al. 2009, 2010a, b).

Fishes were frozen at sea and transported to the University of Alaska Fairbanks (UAF) Fisheries Oceanography Laboratory where detailed processing was conducted. In the laboratory, each fish was thawed, and total length was measured to the nearest mm. For this study, two length classes of fishes were analyzed: small (≤70 mm) and large (>70 mm). Two length classes of fish were analyzed because small fish typically have different feeding strategies than larger fish (Schael et al. 1991). The length that an ontogenetic shift of feeding occurs for each of the 11 species is unknown. Thus, only one length demarcation was chosen to represent seal prey because fish larger than 70 mm are known to be the main prey for ringed seals (Lowry et al. 1980a). Where available, stomach contents were examined from at least 20 fish within each category (species x length class) and fish tissue was examined by stable isotope analysis from at least five

individuals within each category. The small length class of Pacific Herring and Capelin were not available for stomach content or stable isotope analyses, and small Saffron Cod were not available for stomach content analysis.

2.2.2 Stomach Content Analysis

Whole fishes were thawed, and stomachs were excised, covered in water, and frozen until processing. Thawed stomachs were blotted on lens paper, and wet weight of the stomach was measured to the nearest 0.0001 g using an Orion series HR200 precision balance. Prey taxa were removed from the stomach and the empty weight and approximate percent fullness (0–100%) of the stomach were recorded. Prey taxa were sorted into class- or family-level taxonomic groupings. Each prey item, determined by the presence of a head, was counted. All prey of the same taxonomic group were combined, blotted on lens paper, and weighed to the nearest 0.0001 g. Fragments of organisms were included when they could be definitively identified to a taxonomic group. Prey fragments were assigned a count of one only where no heads were observed. This process was repeated for each taxonomic group of prey in every stomach. Prey were aggregated into broad taxonomic groupings for analysis, i.e., phylum, class, or order.

A total of 1,365 stomachs were examined across eleven species of fish and two length classes (Table 2-1). For diet analysis, 1,253 stomachs were examined, and 112 stomachs were excluded because they were empty. In order to analyze diets of fishes, an index of relative importance (%IRI) was calculated for each prey taxon in each species and length group as follows:

$$IRI = (\%N+\%W) / \%O$$

where %N is the percentage by count of a certain prey taxon, %W is the percentage of the weight of the prey taxon, and %O is the percentage of occurrence of prey taxa over all taxa present for that species and length class (Pinkas et al. 1971). Using three measures of fish prey dietary importance makes IRI a useful tool for stomach content. Solely using %N is biased toward prey that are numerous and small (e.g., copepods) while %W is biased toward prey that are relatively rare and large (e.g., fish tissue; Hyslop 1980; Liao et al. 2001). The IRI is reported for each prey taxa as a percentage of total IRI for each category, i.e., %IRI. We used the count of prey taxa, at approximately the class level of taxonomy, as an indicator of diet diversity; we considered prey that contributed more than 5% to be particularly important to diet.

2.2.3 Sample Preparation for Stable Isotope Analysis

Samples for stable nitrogen and carbon isotope analysis were prepared for 222 fishes and 444 fish prey (Table 2-2, Table 2-3). Each sample of a prey taxon was pooled over multiple fish stomachs, regardless of fish species, at a station, and where necessary, multiple stations were pooled to increase prey sample mass to yield sufficient tissue for stable isotope analysis, i.e., > 0.2 mg freeze dried. Prey were aggregated into broad taxonomic groups for stable isotope analysis, with the lowest taxonomic classification being order. Barnacle cyprids and isopods were not analyzed for stable carbon isotope ratios because the sample volume was too small for lipid

extraction. Whole fish and prey were frozen at -20°C and freeze-dried for approximately 48 hours using a VirTis BT 6K ES freeze dryer. We analyzed the stable isotope ratios of individual whole fish because a primary goal of this research was to assess the trophic contribution that the whole fish provides to the apex predator, i.e., ice seals. Because it is typical of stable isotope research on fishes to examine muscle tissue, and methods in published literature do not consistently use lipid extract, we compared the stable isotope ratios of whole fish and fish muscle tissue, both with and without lipid extraction (see Chapter 2 Appendix).

Whole fish without stomachs were processed for non-lipid-extracted $^{15}N/^{14}N$ ratios and lipid-extracted $^{13}C/^{12}C$ ratios. Extracting lipids from samples can alter the stable nitrogen isotope signature (Pinnegar and Polunin 1999; Sweeting et al. 2006) but removes the stable carbon isotope signature of fats (DeNiro and Epstein 1977), leaving only the stable carbon isotope signature of the tissue. Whole freeze-dried fish were homogenized using a mortar and pestle. Lipids were extracted from one-half of the sample using a modified version of Bligh and Dyer (1959). Samples were immersed in a 2:1 chloroform/methanol mixture with a solvent volume about three times the sample volume (Logan et al. 2008). Each sample was agitated for five minutes followed by five minutes of centrifugation at 605 g (3000 rpm) using a VWR Clinical 50 centrifuge. The supernatant containing lipids was discarded. Lipid extraction was repeated approximately three to five times until the supernatant was colorless after centrifugation (Logan et al. 2008). Lipid-extracted samples were dried overnight in a fume hood, re-freeze dried for approximately two hours the following day and re-homogenized.

All prey samples were processed for non-lipid-extracted $^{15}N/^{14}N$ ratios and lipid-extracted $^{13}C/^{12}C$ ratios while prey with exoskeletons were also acid fumed. Exoskeleton carbonates of invertebrates can impact stable carbon isotope results (Søreide et al. 2007); therefore, samples of prey having exoskeletons were processed to assess non-treated $^{15}N/^{14}N$ ratios and acid fumed/lipid-extracted $\delta^{13}C/^{12}C$ ratios. Freeze-dried prey tissues were fumed with saturated HCl vapors for four hours in a vacuum chamber. Samples then were soaked in a 2:1 chloroform/methanol mixture for approximately four hours; the solvent was removed, and fresh chemicals were added. Lipid extraction was repeated three times and the samples were freeze-dried for an additional two hours before analyzing stable nitrogen ($^{15}N/^{14}N$) and carbon ($\delta^{13}C/^{12}C$) isotope ratios.

Whole fish and fish prey were analyzed for $\delta^{15}N$ and $\delta^{13}C$ values at the Alaska Stable Isotope Facility at UAF. A sub-sample of ground fish tissue or fish prey, i.e., 0.2−0.4 mg dry weight, was weighed into tin capsules using a microbalance (Sartorius Model M2P). Stable isotope analysis was performed using a Finnigan MAT Delta^{Plus}XP Isotope Ratio Mass Spectrometer (IRMS) directly coupled to a Costech Elemental Analyzer (ECS 4010). The $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios are expressed in conventional delta (δ) notation, relative to atmospheric N_2 (atm.) and Vienna Pee Dee Belemnite (VPDB), respectively. Peptone was used as a laboratory standard. The precision

of analyses, expressed as one standard deviation from multiple analyses of peptone (n = 90) conducted during runs of samples for fish, was 0.2‰ for $\delta^{15}N$ and 0.1‰ for $\delta^{13}C$.

For each replicate of prey type collected from fish stomachs, trophic level was calculated using the equation:

$$TL_{prey} = (\delta^{15}N_{consumer} - \delta^{15}N_{POM})/3.4 + 1$$

where TL_{prey} is the trophic level of prey aggregated from multiple fish stomachs, consumer is fish prey, and POM is particulate organic matter. Water mass differences in POM have been found across the Chukchi Sea (Iken et al. 2010). As our samples were collected in the eastern Chukchi, the baseline value of $\delta^{15}N$ for POM we used was 5.2, the average of Bering Sea Water (5.63) and the more depleted Alaska Coastal Water (4.56; Iken et al. 2010). For each individual fish for which $\delta^{15}N$ was analyzed, trophic level was calculated using the equation:

$$TL_{fish} = (\delta^{15}N_{consumer} - \delta^{15}N_{primary\ consumer})/3.4 + 2$$

where TL_{fish} is the trophic level of fish predator, consumer is fish predator, primary consumer is copepod, and $\delta^{15}N$ values were from samples that had not been acid-fumed or lipid-extracted. The baseline value of $\delta^{15}N$ for the primary consumer was the average for copepods from fish stomachs in this study (8.77**)**. This value of $\delta^{15}N$ for copepods is within the spring and summer variation of *Calanus glacialis* (9.09 ± 0.66 to 12. 41 ± 0.59) in Amundsen Gulf in the eastern Beaufort Sea (Forest et al. 2011). The mean trophic ^{15}N enrichment of *C. glacialis* was 2.8–4.7‰. The increase of $\delta^{15}N$ in marine food webs is usually 3–4‰ per trophic level (Michener and Schell 1994). The average trophic nitrogen fractionation for aquatic consumers, 3.4 (Vander Zanden and Rasmussen 2001, Post 2002), is the enrichment of $\delta^{15}N$ between trophic levels that we used in these equations and other recent trophic analyses for the Chukchi Sea (Iken et al. 2010, Tu et al. 2015).

2.2.4 Statistical Analysis

Analysis of variance (ANOVA) was used to test the difference in mean stable isotope values within and among fish species, with a significance level of 5%. If normality and equal variance assumptions were met, one-way ANOVA was used to determine whether the mean isotope ratios differed among fish species as well as between small and large fish. If differences were found, a pairwise multiple comparison procedure using the Holm-Sidak Method (t) was used to determine which species differed. If normality and equal variance assumptions were not met, a nonparametric Kruskal-Wallis one-way ANOVA was used followed by a pairwise multiple comparison procedure using Dunn's Method (Q) to determine which species differed. The same statistical method was used to examine the difference in mean isotope values among prey taxa. All statistical tests were conducted in SigmaPlot Version 12.0 (Systat Software, Inc. 2011).

2.3 Results

2.3.1 Stable Isotope Ratios of Fish Prey

There were differences in stable isotope ratios among fish prey though the standard deviations for each prey taxon were understandably large (Figure 2-2). The primary difference was that nematodes were significantly more enriched in ^{15}N than lower trophic prey: cumaceans (Q = 8.124), copepods (Q = 7.511), amphipods (Q = 6.746), euphausiids (Q = 5.357), crabs (Q = 4.977), and barnacles (Q = 3.970). Barnacles had the lowest and nematodes had the highest $\delta^{15}N$ values (Table 2-4; Figure 2-3). Range in $\delta^{15}N$ values was largest for amphipods (9.8‰), and ranges for the other prey taxa in decreasing order were: tanaids > polychaetes > copepods > cumaceans > shrimps > euphausiids > ostracods > mysids > barnacles > crabs > mollusks > nematodes > isopods > fishes. Trophic level (TL) for prey of fish was well represented by $\delta^{15}N$ values, which is expected as TL was calculated from $\delta^{15}N$ values. TL of nematodes was 3.6 and only 1.0 for barnacles (Table 2-4). There were no significant differences in $\delta^{13}C$ values among prey taxa ($p > 0.05$). Amphipods had the lowest and mysids had the highest $\delta^{13}C$values (Figure 2-4). Range in $\delta^{13}C$ values was largest for amphipods (7.9‰), and ranges for the other prey taxa in decreasing order were: mysids > euphausiids > fishes > cumaceans > copepods > polychaetes > crabs > shrimps > nematodes > tanaids > mollusks > ostracods. Barnacles, crabs, and shrimps identified in stomach contents were pelagic early life stages.

2.3.2 Short- and Long-term Diets for Fish Length Class

Within species, fish of the small length class consumed low-trophic crustaceans (Tables 2-5, 2-6) and typically had lower $\delta^{15}N$ and $\delta^{13}C$ values than the large length class (Figures 2-5, 2-6). Copepods were more important in the short-term diets of small Arctic Cod, Canadian Eelpout, Stout Eelblenny, Slender Eelblenny, Pacific Sand Lance, and Bering Flounder (Tables 2-5, 2-6) than they were in the diets of larger fish. Amphipods were important in the diets of both Cottidae species, with %IRI being higher for the small length class (Table 2-6). Small fish were significantly more depleted in ^{15}N than large fishes, with the exception of Canadian Eelpout and Stout Eelblenny (Table 2-7). Small fish were significantly more depleted in ^{13}C than the large length class, with the exception of Saffron Cod, Shorthorn Sculpin, Canadian Eelpout, and Bering Flounder (Table 2-7). Among species, stable isotope signatures of small fishes created five significantly distinct fish predator groups (Table 2-8, Figure 2-5). Conversely, stable isotope signatures of large fishes indicated two groups without overlapping standard deviations of carbon (Table 2-9, Figure 2-6); a lower trophic, more benthopelagic feeding group (Pacific Herring, Capelin, Arctic Cod, Saffron Cod, Pacific Sand Lance), and a higher trophic, more benthic feeding group (Arctic Staghorn Sculpin, Shorthorn Sculpin, Canadian Eelpout, Stout Eelblenny, Slender Eelblenny, Bering Flounder).

Trophic Levels of fish, as with prey, were proportional to $\delta^{15}N$ values. TL values ranged from 2.5 for small Pacific Sand Lance to 4.1 for large Canadian Eelpout and Stout Eelblenny (Table 2-4).

For all fish species examined, TL was lower for fish ≤70 mm than >70 mm. Pelagic-feeding fishes had TL values ≤3.0 for small fishes and ≤3.6 for large fishes. Benthic-feeding fishes had TL values ≤3.3 for small fishes and ≤3.6 for large fishes. Both lower TL limits were associated with Slender Eelblenny while, in the same family, Stout Eelblenny and Canadian Eelpout had the highest TL value for large fishes (4.1), and the second highest for small fishes (3.9), respectively. There was a broader range of TL values for fish prey than for fishes (Figure 2-7). The mean TL of small Sand Lance was lower than the TL for six prey categories. Nematodes had a larger mean TL than large Pacific Sand Lance, Rainbow Smelt, and Pacific Herring, and all small fishes examined except Stout Eelblenny and Canadian Eelpout.

2.3.3 Short- and Long-term Diets Within Fish Families

Both short- and long-term diets for Gadidae demonstrated that Saffron Cod consumed more high-trophic prey than Arctic Cod. Polychaetes and amphipods were important in Saffron Cod short-term diets while copepods had the greater importance to Arctic Cod short-term diets (Table 2-5). The diversity of prey consumed was similar for the large length class of both species (Table 2-5). Stable isotope signatures were not significantly different between Arctic Cod and Saffron Cod (Tables 2-8, 2-9), but large Saffron Cod had a higher standard deviation for $\delta^{15}N$ values (Figure 2-6).

Short-term diets showed amphipods were the prey of highest importance to both species of Cottidae and both length classes, and long-term diets indicated similar trophic levels between species. Polychaetes were important in the short-term diet for both length classes of Arctic Staghorn Sculpin but less important in Shorthorn Sculpin diets (Table 2-6). The number of prey with greater than 5% IRI was higher for large Shorthorn Sculpin than for either length classes of Arctic Staghorn Sculpin (Table 2-6). Small Shorthorn Sculpin had higher $\delta^{13}C$ values than small Arctic Staghorn Sculpin (Table 2-8, Figure 2-5), and stable isotope signatures were not significantly different between large-sized Cottidae (Table 2-9).

Both short- and long-term diets for Stichaeids showed Stout Eelblenny consumed more high-trophic prey than Slender Eelblenny. Crabs and nematodes were more important in short-term diets of Stout Eelblenny than in short-term diets of Slender Eelblenny (Table 2-6). For each length class, Stout Eelblenny had more diverse short-term diets than Slender Eelblenny (Table 2-6). $\delta^{13}C$ values were not significantly different for both eelblenny species (Tables 2-8, 2-9), but Stout Eelblenny had a higher standard deviation for $\delta^{15}N$ values (Figures 2-5, 2-6).

2.3.4 Short- and Long-term Diets Among Fish Species

Among the five benthopelagic feeding fishes, Pacific Herring, Capelin, and Pacific Sand Lance had the least diverse short-term diets (Table 2-5), while Pacific Herring, Arctic Cod, and Saffron Cod had comparable long-term diets (Table 2-9, Figure 2-6). Copepods were the most important prey for Capelin and Pacific Sand Lance compared with other prey taxa. Euphausiids dominated %IRI in short-term diets of Pacific Herring. Fishes were prey for Pacific Herring but were absent

from stomach contents of Capelin and Pacific Sand Lance. Arctic Cod and Saffron Cod consumed a wider variety of prey taxa compared to the other three benthopelagic feeding fishes, yet low-trophic crustaceans had the highest %IRI in cod diets compared to the other prey taxa. There were no significant differences in either $\delta^{15}N$ or $\delta^{13}C$ values among benthopelagic feeding fishes (Table 2-9); however, the large length class of Pacific Herring, Arctic Cod, and Saffron Cod had higher standard deviations for $\delta^{15}N$ values than the large length class of Capelin and Pacific Sand Lance (Figure 2-6). Small Pacific Sand Lance had the lowest $\delta^{15}N$ values, and large Saffron Cod had the highest $\delta^{15}N$ values (Figure 2-3). Small and large Arctic Cod had the lowest and highest $\delta^{13}C$ values, respectively (Figure 2-4).

Among the six benthic feeding fishes, low-trophic crustaceans were most important in short-term diets, while long-term diets showed both length classes of Canadian Eelpout and Stout Eelblenny consumed more high-trophic prey than the other benthic feeding fishes. Amphipods had the highest %IRI in diets of all benthic feeding fishes, with the exception of both length classes of Stout Eelblenny and small Slender Eelblenny (Table 2-6). Only a few other prey taxa had >5% IRI in diets of benthic feeding fishes, i.e., mysids and shrimps for Bering Flounder, crabs and shrimps for Shorthorn Sculpin, and crabs and nematodes for Stout Eelblenny. The small length class of Canadian Eelpout and Stout Eelblenny had similar $\delta^{15}N$ values that were significantly higher than the other small benthic feeding fishes (Table 2-8, Figure 2-5). Small Shorthorn Sculpin had significantly higher $\delta^{13}C$ values compared with the other small benthic feeding fishes (Table 2-8, Figure 2-5). There were no significant differences in either $\delta^{15}N$ or $\delta^{13}C$ values among large benthic feeding fishes (Table 2-9, Figure 2-6). Small Bering Flounder had the lowest $\delta^{15}N$ values, and large Stout Eelblenny had the highest $\delta^{15}N$ values (Figure 2-3). Small Bering Flounder had the lowest $\delta^{13}C$ values, and large Slender Eelblenny had the highest $\delta^{13}C$ values (Figure 2-4).

2.4 Discussion

We used stomach contents of a large quantity of fishes to quantify short-term information about fish diets, and stable isotope ratios to quantify long-term diet information. These two types of information are complementary as prey taxa must be identified in order to explain observed stable isotope ratios. Stomach content analysis provides only a glimpse into recent diet history and potentially misses large portions of the predator's feeding ecology. Prey assimilated and integrated into tissues during the past year demonstrated that these fish predators fed at a higher trophic level than was described by simply investigating stomach contents. Intra-family diet comparisons revealed that fishes may have recently consumed similar prey taxa yet have dissimilar long-term diets, particularly for the small length class. For the large length class, long-term diets were similar among fish species likely because they have diverse short-term diets of isotopically similar prey taxa. Ultimately, the proportions of prey consumed and assimilated provide the key to understanding feeding ecology. A combination of stomach content and stable isotope analyses added to the general knowledge of the feeding ecologies of eleven fish species in the Arctic, an area where diet information is especially limited (Mecklenburg et al. 2008).

Prey taxa consumed likely reflected their abundance in the environment. Three prey taxa categories (amphipods, copepods, and euphausiids) contributed 92 – 100% of the index of relative importance (IRI) of diets of both size classes of the five benthopelagic fish species: Pacific herring, capelin, Arctic cod, saffron cod and Pacific sand lance. Though euphausiids were not important in the diets of benthic feeding fish, amphipods and copepods made up 60 – 97 % of the IRI. The dominance of three prey taxa for the IRI values for all 11 fish species examined indicated that Chukchi Sea fish select prey based on availability more than preferential foraging for particular prey. Therefore, major changes in the availability of these three taxa can directly impact fish and in turn potentially impact their ice seal and other upper trophic predators.

The stable isotope signatures of fish prey observed in the present study had large standard deviations, which was likely because the taxonomic groupings were sufficiently general that they encompassed both benthic and pelagic animals. Benthic organisms typically have higher $\delta^{15}N$ values as they consume more recycled material than pelagic fauna (Iken et al. 2005). High trophic (mainly benthic) prey taxa in this study included mysids, polychaetes, mollusks, tanaids, isopods, juvenile shrimps, mysids, fishes, and nematodes; lower trophic prey taxa included barnacle cyprids, cumaceans, copepods, juvenile crabs, euphausiids, amphipods, and ostracods. These lower trophic crustaceans can include benthic and pelagic animals. There are gammarid amphipods in both benthic and pelagic domains while hyperiid amphipods are purely pelagic (Vinogradov et al. 1996). The presence of pelagic prey in stomach contents did not guarantee that a fish was not feeding demersally as said prey can be found throughout the water column, including at the bottom (Arctic Ocean Diversity [ArcOD] 2008). Moreover, the short-term diet should not be extrapolated to apply to other seasons. Stomach contents and stable isotope ratios do not document foraging strategy but instead illustrate feeding ecology.

Low-trophic crustaceans can have higher $\delta^{15}N$ and $\delta^{13}C$ values than observed in this study, which could be due to one or many causes. One likely case is that crustaceans examined in this study, of which some were taken from stomach contents of small fishes, may have been considerably smaller than were analyzed in other studies. Amphipods from this study had lower mean $\delta^{15}N$ values than those collected in the Chukchi Sea prior to 2004 (Iken et al. 2010; Feder et al. 2011). Amphipods from this study may have consumed more phytoplankton or copepods in 2007–2010 and more detritus in previous studies. In Arctic seas, amphipods, copepods, and euphausiids can be found throughout the water column feeding on phytoplankton, small zooplankton, and detritus (ArcOD 2008). Copepods and euphausiids from this study had lower mean $\delta^{15}N$ and higher mean $\delta^{13}C$ values than these collected from the northern Chukchi Sea (Schell et al. 1998). Differences in mean $\delta^{15}N$ values between the two studies may relate to a greater consumption of detritus in 1984–1994 compared to copepods and euphausiids from this study. Differences in $\delta^{13}C$ values between this and earlier studies may relate to variable lipid content, as lipids were not extracted in Schell et al. (1998). Crabs can have higher $\delta^{15}N$ and $\delta^{13}C$ values than observed in this study (Iken et al. 2010; Feder et al. 2011) as they are scavengers. However, crabs analyzed for this study were juveniles, i.e., *Chionoecetes* sp. and Paguridae zoea. These early life stages are

benthic or planktonic carnivores (ArcOD 2011) and can have lower δ^{15}Nvalues than older individuals. Cumaceans are benthic foragers consuming microorganisms and organic matter (Watling 2005). This prey taxon had higher mean δ^{15}N values and similar mean δ^{13}C values in this study compared to cumaceans collected from the Beaufort Sea in 2002 (Iken et al. 2005). Differences in mean δ^{15}N values may be a result of cumaceans receiving more recycled material from surface waters in this study than those collected in 2002. Lower δ^{13}C values are typically documented for organisms in the Beaufort Sea relative to the Chukchi Sea (Dunton et al. 1989); however, this was not the case for cumaceans in this study. Other processes may have impacted δ^{13}C values, such as proximity to shore during collection. Besides regional or interannual differences in stable isotope signatures for low-trophic crustaceans, the species analyzed may have led to differences in stable isotope ratios between this study and other studies. Lower trophic feeding by amphipods, copepods, and euphausiids in this study compared to studies prior to 2007 may relate to high productivity during reduced sea ice cover in the Arctic (Arrigo et al. 2008; NSIDC 2012), resulting in greater availability of phytoplankton and small zooplankton (Eisner et al. 2012).

High-trophic prey from this study had similar stable isotope signatures compared to other studies, with a few exceptions. Ostracods have a variety of feeding strategies (ArcOD 2008, 2011), and this was illustrated by their large range in δ^{15}N values. When comparing stable isotope signatures of ostracods between studies, δ^{15}N values were similar and δ^{13}C values were higher in this study compared to ostracods collected from the Beaufort Sea (Iken et al. 2005). Ostracods from the Beaufort Sea had lower δ^{13}C values likely because ostracods analyzed from the Beaufort Sea were mostly pelagic inhabitants (Iken et al. 2005). Tanaids are bottom-dwelling filter feeders and predators (ArcOD 2011). Tanaids from the Chukchi Sea had similar δ^{15}N and δ^{13}C values compared to those from the Beaufort Sea (Iken et al. 2005). Mollusks processed for stable isotope ratios were primarily pieces of bivalves. Bivalves from this study had less variable stable isotope ratios than species collected from the Chukchi during 2004 (Iken et al. 2010). Only seven bivalve samples were processed for stable isotope ratios from this study, while nine species of bivalves, along with multiple replicates, were analyzed by Iken et al. (2010), thus they were able to capture a larger range of variability. Fishes as prey had lower mean δ^{15}N values and typically lower mean δ^{13}C values compared to fish species collected from the Chukchi Sea during 2004 (Iken et al. 2010). Fishes as prey were likely younger individuals or pelagic species, both of which feed lower trophically and more pelagically, thus having lower δ^{15}N and δ^{13}C values. Shrimps in this study had lower mean δ^{15}N and δ^{13}C values than shrimps from studies prior to 2004 (Iken et al. 2010; Feder et al. 2011), and this matches the juvenile feeding behavior, i.e., low-trophic, pelagic prey, of shrimps from this study. Mysids are generally considered benthic or epibenthic, with few species being truly pelagic (ArcOD 2011). Mysids are omnivorous (ArcOD 2011) and had higher mean δ^{15}N and δ^{13}C values in this study than mysids collected from the eastern Beaufort Sea (Dunton et al. 2012). That study analyzed mysids from nearshore lagoons along the eastern Beaufort Sea that were likely part of a shorter food chain, thus producing lower stable isotope signatures compared to mysids from this study. Polychaetes in this study had similar stable

isotope signatures to those collected from the Chukchi in 2004 (Iken et al. 2010), and their high $\delta^{15}N$ values match their feeding behaviors as filter feeders or predators (ArcOD 2011). Species of nematodes have various trophic roles and can be deposit feeders, epibenthic feeders, predators, scavengers, or parasites (Heip et al. 1985; Jensen 1987). Based on the high $\delta^{15}N$ values observed in this study, it is likely these nematodes were either parasitic or predators consuming such prey as polychaetes.

As fish get bigger in size, they consume larger prey (Lowry and Frost 1981; Morrow 1980) and forage deeper (Huse and Toresen 1996; Cui et al. 2012), leading to higher trophic and more benthic isotope signatures. Where both small and large length classes were available for comparison in the present study, copepods had a greater importance in short-term diets of small compared to large fish. More precise analysis of Arctic Cod diet at 10 mm increments of body length across the northeastern Chukchi and U.S. Beaufort Seas indicated that in both seas, increases in fish size were accompanied by a marked decrease in consumption of copepods (Gray 2015). Consuming a greater proportion of copepods likely resulted in small fish from this study having lower $\delta^{15}N$ and $\delta^{13}C$ values than large fish, although there were a few exceptions. Canadian Eelpout was the only species in this study that fed on the same trophic level and carbon source regardless of length class. This may mean that small Canadian Eelpout are able to consume large prey and forage as deep as large Canadian Eelpout; this premise is supported by fieldwork in the Beaufort Sea, which in 2012 collected Canadian Eelpout of 46−210 mm in length at a depth of 350 m in the Beaufort Sea (Norcross et al. unpub. data). The size of fish available for analysis and the specified length classes in this study may have contributed to the lack of significant differences in stable isotope ratios between length classes for other fish species. For example, both length classes of Bering Flounder had similar $\delta^{13}C$ values; however, sampling more individuals smaller than 50 mm would likely show more indication of pelagic feeding (Edenfield et al. 2011). We recommend that future studies specifically examine ontogenetic shifts in fish diets. Among fish species, standard deviations of stable isotope signatures for small fish overlapped less than they did for large fishes likely due to the less varied diet for small fish. For example, compared to large Arctic Cod, small Arctic Cod are more specialized feeders occupying a narrower feeding niche (Cui et al. 2012). A mixture of many prey taxa of different trophic levels and carbon sources likely led to a dilution of stable isotope signatures making long-term diets of this study more similar among the larger fish from all species.

Gadidae fed on isotopically similar prey taxa; yet, the proportional contribution of high-trophic prey was greater for Saffron Cod. Morrow (1980) found that Arctic Cod primarily feed on plankton, which matches with results of this study where copepods, amphipods in particular, were the most important prey. Arctic Cod also consume mysids (Lowry and Frost 1981; Craig et al. 1982; Fechhelm et al. 1984; Coyle et al. 1997), shrimps (Lowry and Frost 1981; Fechhelm et al. 1984; Coyle et al. 1997), and fishes (Craig et al. 1982; Fechhelm et al. 1984; Coyle et al. 1997; Cui et al. 2012). These prey taxa are also common in stomach contents of Saffron Cod

(Craig and Haldorson 1981; Fechhelm et al. 1984; Morrow 1980). Although mysids, shrimps, and fishes were minor contributors to stomach contents of both cod species in this study, these prey taxa likely have greater importance in fish diets later in the year. For instance during the winter, mysids can have a greater contribution to diets of Arctic Cod collected nearshore while fishes as prey occur in greater proportions in Arctic Cod collected offshore (Craig et al. 1982). The proportional contribution of these isotopically similar prey taxa can vary between these species of Gadidae. In another Chukchi Sea study, Arctic Cod ate more mysids and fishes, while saffron consumed more shrimps (Coyle et al. 1997). Although the proportions are different, these prey taxa create similar isotope signatures in both species of Gadidae. Saffron Cod had a higher standard deviation for $\delta^{15}N$ values than Arctic Cod, likely a result of consuming more high-trophic prey, such as polychaetes. Similarly to findings for large Gadids in this study, Saffron Cod collected from the eastern Chukchi during 2004 had larger standard deviations for $\delta^{15}N$ and $\delta^{13}C$ values and had higher mean $\delta^{15}N$ values than Arctic Cod (Iken et al. 2010). In the southeast Chukchi Sea, Saffron Cod also had higher mean $\delta^{15}N$ values than Arctic Cod (Feder et al. 2011). A greater contribution of polychaetes likely led to higher standard deviations for $\delta^{15}N$ values for Saffron Cod as this prey taxon is common in Saffron Cod stomachs (Table 2-5; Fechhelm et al. 1984; Morrow 1980) but contributes minimally to Arctic Cod diets (Table 2-5; Lowry and Frost 1981; Cui et al. 2012). Comparisons of stable isotope ratios for fishes are not made directly between this study and others because different techniques were applied. For example, Iken et al. (2010) and Feder et al. (2011) examined fish muscle instead of total body homogenate, which we found to have different $\delta^{15}N$ values (Chapter 2 Appendix). Moreover, previous studies did not lipid extract tissues, and this can result in lower $\delta^{13}C$ values (Chapter 2 Appendix). The high diversity of prey taxa in Arctic Cod diets from this study supports the claims that Arctic Cod take advantage of a variety of food sources and trophic niches (Lowry and Frost 1981; Fechhelm et al. 1984), and the same appears true for Saffron Cod even when fewer Saffron Cod stomachs were examined.

For Cottidae, the importance of polychaetes was greater in diets of Arctic Staghorn Sculpin than in diets of Shorthorn Sculpin, yet both species fed at a similar trophic level. Amphipods are major prey for both species (Table 2-6; Atkinson and Percy 1991; Cui et al. 2012). Crabs, shrimps, and fishes were less important in short-term diets of large Arctic Staghorn Sculpin than in short-term diets of large Shorthorn Sculpin from this study. Even though fewer Shorthorn Sculpin stomachs were analyzed in this study, possibly resulting in an overestimation of the importance of crabs, shrimps, and fishes, the difference in the importance of these taxa to diets between the two species matches with other diet studies. Crabs, shrimps, and fishes make a small contribution and are not common. For example, Coyle et al. 1997 found these taxa were < 11% IRI and occurred at a single site in the Chukchi Sea, and Atkinson and Percy (1991) found no presence of these taxa in stomach contents of Arctic Staghorn Sculpin. Crabs are less important in Shorthorn Sculpin diets from the Chukchi Sea (Table 2-6) but have approximately 40% IRI in stomach contents of Shorthorn Sculpin collected from the Bering Sea (Cui et al. 2012). Shrimps and fishes each had approximately 20% IRI in stomach contents of Shorthorn Sculpin (Atkinson

and Percy 1991). Contrary to other diet studies, polychaetes were more important in short-term diets of Arctic Staghorn Sculpin than in short-term diets of Shorthorn Sculpin from this study. For instance, polychaetes are primary prey for Arctic Staghorn Sculpin and Shorthorn Sculpin (Atkinson and Percy 1991; Cui et al. 2012). Long-term diets of small Shorthorn Sculpin had a more benthic signature than small Arctic Staghorn Sculpin, and this may be a result of differences in fish lengths analyzed. If fish smaller than 60 mm were analyzed for stable isotope ratios, small Shorthorn Sculpin might have had a more pelagic signature similar to small Arctic Staghorn Sculpin. Both species of the large length class had similar $\delta^{15}N$ values in this study. Conversely, Shorthorn Sculpin collected from the eastern Chukchi Sea during 2004 had higher mean $\delta^{15}N$ values than Arctic Staghorn Sculpin (Iken et al. 2010). These long-term comparisons indicate these predators have relatively diverse diets that are likely to vary interannually depending on the availability of prey.

Both species of Stichaeidae had diverse diets and are high-trophic predators. While copepods and amphipods were major prey items for Slender Eelblenny in this study, these low-trophic crustaceans are less important in short-term diets compared to benthic polychaetes (Atkinson and Percy 1991). Other common prey taxa in stomach contents of Slender Eelblenny included benthic species such as mollusks, cumaceans, isopods, tanaids, and nematodes (Aktinson and Percy 1991). Aktinson and Percy (1991) reported that Slender Eelblenny have a particularly diverse diet. This study indicated a diverse diet for Slender Eelblenny and even more prey taxa in the Stout Eelblenny diet, although the latter may have been due to our examination of a larger number of that species. Short-term diet information is limited for Stout Eelblenny from the Arctic; however, general diet consists of polychaetes, bivalves, and crustaceans (Makushok 1986). In this study, Nematodes had the third highest %IRI in diets of both species and both length classes of Stichaeidae (Table 2-5), and they were also shown to be important in diets of Slender Eelblenny in the Canadian Arctic (Atkinson and Percy 1991). Nematodes and polychaetes, both high-trophic prey taxa, are likely major prey for species of Stichaeidae. In this study of fishes collected from the eastern Chukchi Sea, Stout Eelblenny had higher standard deviations for $\delta^{15}N$ values than Slender Eelblenny. Alternatively, Slender Eelblenny collected from the eastern Chukchi Sea during 2004 had higher mean $\delta^{15}N$ values compared to Stout Eelblenny (Iken et al. 2010). The proportional contribution of high-trophic prey to the diets of Stout and Slender Eelblenny likely varies interannually depending on prey availability.

Contrary to short-term diets of benthopelagic feeding fishes, long-term diets showed Pacific Herring, Arctic Cod, and Saffron Cod had more similar long-term diets that were at a higher trophic level than Capelin and Pacific Sand Lance. In this study, either copepods or euphausiids were the most important prey taxa in the stomach contents of Pacific Herring, Capelin, and Pacific Sand Lance, and these prey taxa may have consisted of both pelagic and benthic prey types. Capelin are considered to be epipelagic (Jarvela and Thorsteinson 1999), yet stomach contents contain harpacticoid copepods, cumaceans, and mysids (Fechhelm et al. 1984). Pacific Sand Lance (*Ammodytes hexapterus*) is a pelagic schooling feeder (Ciannelli 1997; Robards et al.

1999) but is also considered semi-demersal as it buries in the substrate (Robards et al. 1999; Pirtle and Mueter 2011). Similarly to Capelin, stomach contents of Pacific Sand Lance contain harpacticoid copepods and mysids (Fechhelm et al. 1984; Field 1988), but diets of Pacific Sand Lance additionally consist of polychaetes and gammarid amphipods (Field 1988). Pacific Sand Lance forage on epibenthic invertebrates during fall and winter (Rogers et al. 1979). Benthic invertebrates, such as polychaetes, likely contribute more to diets of Pacific Sand Lance than diets of Capelin as Pacific Sand Lance had a larger range in $\delta^{15}N$ values in this study. Capelin and Pacific Sand Lance can have similar stable isotope signatures, with Capelin having a more pelagic signature in certain years. Larval fishes are prey for Capelin and Pacific Sand Lance (Fechhelm et al. 1984; Sturdevant et al. 2001) while post-larval fishes are frequent and substantial prey items in stomach contents of Pacific Herring (Table 2-5; Fechhelm et al. 1984). The contribution of post-larval fish to Pacific Herring diets likely led to the higher standard deviations for $\delta^{15}N$ values compared to Capelin and Pacific Sand Lance. This likely also occurs in the Bering Sea where Pacific Herring have higher mean $\delta^{15}N$ values than Capelin (Kurle and Worthy 2001). Pacific Herring, Capelin, and Pacific Sand Lance had less diverse diets than Arctic Cod and Saffron Cod in this study. This supports the claim that Capelin are more selective feeders than Arctic Cod and Saffron Cod (Fechhelm et al. 1984). However, the similarity in prey diversity for Pacific Herring and Capelin in this study does not support Capelin being more selective than Pacific Herring (Fechhelm et al. 1984). Diets for Pacific Herring can be more diverse than detected in this study, possibly due to our small sample size (n=10). For example, Fechhelm et al. (1984) observed cumaceans, mysids, isopods, and polychaetes in an earlier study of Pacific Herring stomach contents. Besides feeding on benthic prey items, benthopelagic feeding fishes can have a more benthic carbon signature by consuming pelagic prey taxa feeding under the ice (Kennedy et al. 2002). Collections for the present study occurred during the open water season and thus did not observe short-term diets from underneath the sea ice.

The proportional contribution of prey taxa consumed could vary depending on prey availability, potentially altering the trophic structure of benthic feeding fishes. For benthic feeding fishes, long-term diets of Canadian Eelpout were more similar to species of Stichaeidae, while long-term diets of Bering Flounder were more similar to species of Cottidae. Relative to other fish species in this study, the higher %IRI of nematodes in diets of two species of Stichaeidae may mean that both are actively feeding on nematodes. Nematodes are not important in stomach contents of Canadian Eelpout compared to Stichaeidae, but another high-trophic prey taxon, polychaetes, are major prey in short-term diets (Atkinson and Percy 1991). This prey taxon likely contributes more to diets of Canadian Eelpout based on the higher $\delta^{15}N$ values for this fish compared to the other fish species examined in this study. Similarly to this study, *Lycodes* spp. have higher $\delta^{15}N$ values than Slender Eelblenny (Dunton et al. 2012). Bering Flounder could have higher $\delta^{15}N$ values than observed in this study. Fish prey made up more than 30% IRI in stomach contents of Bering Flounder collected at four sites in the Chukchi Sea during the 1990s (Coyle et al. 1997) while fish had less than 2% IRI in the short-term diets of Bering Flounder in this study. Sampled Bering Flounder had a larger mean length in the 1990s (Coyle et al. 1997)

compared to large fish from this study. A larger fish could more easily consume large prey, e.g., fish (Gibson and Ezzi 1987), which could explain some diet differences between the two studies. Fish species from this study had a different trophic structure compared to fish species collected from the eastern Chukchi Sea during 2004 (Iken et al. 2010). For fishes collected in 2004, Shorthorn Sculpin had higher mean $\delta^{15}N$ values than Arctic Cod, Saffron Cod, Arctic Staghorn Sculpin, Stout Eelblenny, Slender Eelblenny, and Bering Flounder.

Low-trophic crustaceans appeared to be more common in the stomach content analysis from this study than other short-term diet studies (e.g., Craig and Haldorson 1981; Craig et al. 1982; Fechhelm et al. 1984; Field 1988; Atkinson and Percy 1991; Coyle et al. 1997). Short-term diet analysis can be affected by regional, seasonal, and annual differences in prey availability. For example, stomach contents of Arctic Cod collected from the northeastern Chukchi Sea primarily consist of pelagic/planktonic organisms, and Arctic Cod collected from the northern Bering Sea mainly consume benthic prey (Lowry and Frost 1981). Pelagic crustaceans are calorically valuable prey during the autumn season as they build up lipid stores to prepare for winter diapause (Falk-Petersen et al. 2009). This study and other fish diet studies in the Chukchi Sea (Craig and Haldorson 1981; Lowry and Frost 1981; Fechhelm et al. 1984; Atkinson and Percy 1991; Coyle et al. 1997) sampled during the summer/fall when fishes are logistically easier to collect and when pelagic crustaceans are abundant. We recommend that future feeding ecology studies incorporate more winter sampling to allow for recognition of seasonal changes in diets of Arctic fish species. Earlier stomach content studies (Craig and Haldorson 1981; Craig et al. 1982; Fechhelm et al. 1984; Field 1988; Atkinson and Percy 1991; Coyle et al. 1997) documented a greater consumption of higher trophic prey compared to this study that collected fishes during 2007–2010, indicating interannual differences in prey availability. During 2007–2012, sea ice extent was at record lows (NSIDC 2012). Expansion of ice-free area and longer duration of the open water season led to higher annual primary productivity during 2007 (Arrigo et al. 2008) and supported more pelagic crustacean grazers and consumers (Eisner et al. 2012). Arctic fishes may have taken advantage of this higher abundance of low-trophic, pelagic prey during recent low ice years. Higher trophic prey are likely important prey taxa seasonally (e.g., winter, Craig et al. 1982), regionally (e.g., offshore, Craig et al. 1982; the Bering Sea, Lowry and Frost 1981), and interannually.

The combination of summer diets and isotopic signatures provides evidence useful in inferring year round diet and foraging realm. Summer diets in this study—as well as most of the published literature on diet—should not be considered a reliable indicator of year-round diet for that species. For example, Saffron Cod had almost exclusively amphipods in their guts, whereas Arctic Cod had mostly copepods. Yet Saffron Cod and Arctic Cod could not be differentiated isotopically. Perhaps the slightly wider confidence range of the trophic level of Saffron Cod indicates a more diverse year-round diet. Representations of diet based on stomach contents need to be more narrowly interpreted as summer diet only.

This study documents the low number of major fish prey taxa (~ 4) and indicates that only 8 of 20 identified prey taxa constitute >5% of the IRI of any fish species and size class. Such data suggest there may be less redundancy and lower resilience in the low diversity and short food chain in the Arctic food web in the Chukchi Sea than in the food webs of more southerly, temperate seas.

With regard to assessing potential impacts of OCS oil and gas or other human development impacts in the Chukchi Sea, it is critical to understand which fish are most likely to be impacted in the pelagic water column and which are most likely to be impacted in the benthic realm. These may differ in open water season (for which stomach contents provide diet information) and the rest of the year (for which isotopes provide diet information).

Analyzing stomach contents from the same region across multiple years may help to narrow dietary shifts to seasonal or interannual changes in prey availability. Further research is needed to understand the dietary shifts that may be related to regional, seasonal, or interannual prey availability. Interannual differences were described by long-term diets of fish collected during years of reduced sea ice extent, 2007–2010 (Chapter 3; NSIDC 2012). This study provides additional baseline information regarding the feeding ecologies of eleven species across eight different fish families in the northeast Chukchi Sea.

The fish species included here were chosen because they are important ice seal prey. Nevertheless, they also represent the most common fish species in the Chukchi Sea. Other less common marine fish species in the Chukchi Sea food web include Yellowfin Sole, Starry Flounder, Walleye Pollock, Arctic Shanny and Bering Cisco. Future comparisons of stomach contents (summer diet) and isotope contents (longer term, approximately annual diet) of these species can provide additional ecological insights into the functioning of the Chukchi Sea ecosystem.

2.5 Acknowledgments

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2.6 Tables

Table 2-1. **Inventory of Fishes for Stomach Content Analysis**. Number of stomachs analyzed from fishes collected from the Chukchi Sea. (*n*) = number of fish with empty stomachs. Ranges and mean lengths exclude fish that had empty stomachs.

Fishes Family, scientific and common names	**Length Class**	***n***	**min length (mm)**	**max length (mm)**	**mean length (mm)**
CLUPEIDAE (HERRINGS)					
Clupea pallasii	small (≤ 70 mm)	-	-	-	-
Pacific herring	large (> 70 mm)	10 (1)	198	230	210
OSMERIDAE (SMELTS)					
Mallotus villosus	small (≤ 70 mm)	-	-	-	-
Capelin	large (> 70 mm)	21	105	129	117
GADIDAE (CODS)					
Boreogadus saida	small (≤ 70 mm)	208 (13)	16	70	52
Arctic cod	large (> 70 mm)	194 (13)	71	252	103
Eleginus gracilis	small (≤ 70 mm)	-	-	-	-
Saffron cod	large (> 70 mm)	20	83	268	142
COTTIDAE (SCULPINS)					
Gymnocanthus tricuspis	small (≤ 70 mm)	107 (8)	31	70	49
Arctic staghorn sculpin	large (> 70 mm)	55 (1)	71	134	84
Myoxocephalus scorpius	small (≤ 70 mm)	48 (3)	30	68	57
Shorthorn sculpin	large (> 70 mm)	21	71	188	113
ZOARCIDAE (EELPOUTS)					
Lycodes polaris	small (≤ 70 mm)	69 (20)	31	70	49
Canadian eelpout	large (> 70 mm)	116 (13)	71	200	107
STICHAEIDAE (PRICKLEBACKS)					
Anisarchus medius	small (≤ 70 mm)	20	43	70	61
Stout eelblenny	large (> 70 mm)	206 (7)	71	158	108
Lumpenus fabricii	small (≤ 70 mm)	22 (11)	43	70	63
Slender eelblenny	large (> 70 mm)	21 (4)	71	219	159
AMMODYTIDAE (SAND LANCES)					
Ammodytes hexapterus	small (≤ 70 mm)	31 (9)	29	70	53
Pacific sand lance	large (> 70 mm)	20	71	188	93
PLEURONECTIDAE (FLATFISHES)					
Hippoglossoides robustus	small (≤ 70 mm)	114 (4)	48	68	56
Bering flounder	large (> 70 mm)	62 (5)	73	115	93
Total Count		1365 (112)			

Table 2-2. **Inventory of Fishes for Stable Isotope Analysis**. Sample sizes and lengths (min, max, and mean) of Arctic fishes analyzed for stable nitrogen and carbon isotope ratios.

Fishes Family, scientific and common names	**Length Class**	***n***	**min length (mm)**	**max length (mm)**	**mean length (mm)**
CLUPEIDAE (HERRINGS)					
Clupea pallasii	small (≤ 70 mm)	-	-	-	-
Pacific herring	large (> 70 mm)	10	198	230	209
OSMERIDAE (SMELTS)					
Mallotus villosus	small (≤ 70 mm)	-	-	-	-
Capelin	large (> 70 mm)	15	87	125	108
GADIDAE (CODS)					
Boreogadus saida	small (≤ 70 mm)	10	36	58	46
Arctic cod	large (> 70 mm)	15	71	132	104
Eleginus gracilis	small (≤ 70 mm)	5	61	70	66
Saffron cod	large (> 70 mm)	10	87	262	137
COTTIDAE (SCULPINS)					
Gymnocanthus tricuspis	small (≤ 70 mm)	15	35	67	49
Arctic staghorn sculpin	large (> 70 mm)	14	75	113	88
Myoxocephalus scorpius	small (≤ 70 mm)	5	60	68	64
Shorthorn sculpin	large (> 70 mm)	15	71	188	95
ZOARCIDAE (EELPOUTS)					
Lycodes polaris	small (≤ 70 mm)	10	39	61	44
Canadian eelpout	large (> 70 mm)	10	71	176	113
STICHAEIDAE (PRICKLEBACKS)					
Anisarchus medius	small (≤ 70 mm)	8	59	67	63
Stout eelblenny	large (> 70 mm)	15	77	148	111
Lumpenus fabricii	small (≤ 70 mm)	5	43	68	62
Slender eelblenny	large (> 70 mm)	10	90	212	143
AMMODYTIDAE (SAND LANCES)					
Ammodytes hexapterus	small (≤ 70 mm)	5	52	67	61
Pacific sand lance	large (> 70 mm)	15	92	154	124
PLEURONECTIDAE (FLATFISHES)					
Hippoglossoides robustus	small (≤ 70 mm)	15	41	67	57
Bering flounder	large (> 70 mm)	15	75	188	102
Total Count		222			

Table 2-3. **Inventory of Prey for Stable Isotope Analysis**. Count of fish prey samples analyzed for stable nitrogen and carbon isotope ratios. Prey were gathered from the stomachs of more than one fish and pooled before analysis of stable isotope ratios; therefore, the maximum number of fish stomachs that contributed to the sample is listed. Prey that were consumed as early life stages are indicated with an asterisk (*).

Phylum	**Subphylum**	**Prey Taxa**	**$\delta^{15}N$ *(n)***	**$\delta^{13}C$ *(n)***	**Max # of Fish Stomachs**
Annelida		Polychaetes	46	29	181
Mollusca		Mollusks	7	4	22
Arthropoda	Crustacea	Amphipods	153	116	822
		Barnacles *	2	-	6
		Copepods	60	41	498
		Crabs *	9	4	14
		Cumaceans	50	25	138
		Euphausiids	24	20	93
		Isopods	3	-	7
		Mysids	19	13	71
		Ostracods	5	1	9
		Shrimps *	14	11	31
		Tanaids	16	2	60
Nematode		Nematodes	18	6	92
Chordata	Vertebrata	Fishes	18	14	25
		Total Count	444	286	2069

Table 2-4. **Trophic Levels and Stable Isotopes for Fishes and Their Prey**. Average and standard deviation of values; n = number of fish or prey (pooled) contributing to those values. Fishes and prey are listed in phylogenetic order.

			δ^{15}N and Trophic Level				δ^{13}C	
Taxon	Length Class	*n*	Trophic Level Mean ± StDev		δ15N Mean ± StDev		*n*	mean ± StDev
FISHES								
CLUPEIDAE (HERRINGS)								
Clupea pallasii	≤70 mm	0	-	-	-	-	0	- -
Pacific Herring	>70 mm	10	3.6 ±	0.2	14.07 ±	0.66	10	-20.72 ± 0.59
OSMERIDAE (SMELTS)								
Mallotus villosus	≤70 mm	0	-	-	-	-	0	- -
Capelin	>70 mm	15	3.1 ±	0.2	12.38 ±	0.66	15	-19.14 ± 0.47
Osmerus dentex	≤70 mm	0	-	-	-	-	0	- -
Rainbow Smelt	>70 mm	10	3.4 ±	0.1	13.53 ±	0.45	10	-23.09 ± 0.35
GADIDAE (CODS)								
Boreogadus saida	≤70 mm	14	3.0 ±	0.2	12.04 ±	0.69	14	-20.45 ± 0.67
Arctic Cod	>70 mm	20	3.6 ±	0.2	14.10 ±	0.78	20	-19.48 ± 0.60
Eleginus gracilis	≤70 mm	5	3.0 ±	0.1	12.03 ±	0.43	5	-21.09 ± 0.25
Saffron Cod	>70 mm	15	3.6 ±	0.4	14.16 ±	1.20	15	-19.74 ± 1.07
COTTIDAE (SCULPINS)								
Gymnocanthus tricuspis	≤70 mm	15	3.4 ±	0.3	13.56 ±	1.04	15	-18.80 ± 0.61
Arctic Staghorn Sculpin	>70 mm	14	3.8 ±	0.3	15.06 ±	1.05	19	-18.02 ± 0.64
Myoxocephalus scorpius	≤70 mm	5	3.4 ±	0.1	13.44 ±	0.18	5	-17.17 ± 0.16
Shorthorn Sculpin	>70 mm	15	3.7 ±	0.3	14.47 ±	1.04	15	-17.59 ± 0.72
ZOARCIDAE (EELPOUTS)								
Lycodes polaris	≤70 mm	10	4.0 ±	0.1	15.70 ±	0.48	10	-18.42 ± 0.71
Canadian Eelpout	>70 mm	10	4.1 ±	0.2	15.87 ±	0.61	15	-17.93 ± 0.67
STICHAEIDAE (PRICKLEBACKS)								
Anisarchus medius	≤70 mm	8	3.9 ±	0.3	15.38 ±	1.02	8	-18.65 ± 0.45
Stout Eelblenny	>70 mm	15	4.1 ±	0.3	15.81 ±	0.90	20	-17.63 ± 0.62
Lumpenus fabricii	≤70 mm	5	3.3 ±	0.3	13.07 ±	1.18	5	-18.82 ± 0.38
Slender Eelblenny	>70 mm	10	3.6 ±	0.3	14.36 ±	0.98	15	-17.49 ± 0.69
AMMODYTIDAE (SAND LANCES)								
Ammodytes hexapterus	≤70 mm	5	2.5 ±	0.1	10.32 ±	0.47	5	-20.05 ± 0.34
Pacific Sand Lance	>70 mm	15	3.2 ±	0.4	12.81 ±	1.31	15	-19.43 ± 0.62
PLEURONECTIDAE (FLATFISHES)								
Hippoglossoides robustus	≤70 mm	15	3.5 ±	0.3	13.79 ±	0.87	15	-18.59 ± 0.86
Bering Flounder	>70 mm	15	3.8 ±	0.3	14.79 ±	0.87	20	-18.42 ± 0.33
Total Count of Fishes		246					271	
FISHES' PREY								
PHYLUM ANNELIDA								
Polychaete		46	3.2 ±	0.5	12.53 ±	1.79	29	-19.27 ± 1.04
PHYLUM MOLLUCSA								
Mollusc		7	2.5 ±	0.4	10.25 ±	1.32	4	-18.96 ± 0.41
PHYLUM ARTHROPODA, SUBPHYLUM CRUSTACEA								
Amphipod		153	2.3 ±	0.6	9.62 ±	2.19	116	-20.10 ± 1.25
Barnacle		2	1.0 ±	0.9	5.17 ±	3.13	0	-
Copepod		60	2.1 ±	0.6	8.77 ±	2.02	41	-20.70 ± 1.07
Crab		9	2.1 ±	0.5	8.91 ±	1.58	4	-17.84 ± 1.56
Cumacean		50	1.9 ±	0.5	8.42 ±	1.67	25	-18.51 ± 1.22
Euphausiid		24	2.3 ±	0.7	9.52 ±	2.22	20	-20.66 ± 1.36
Isopod		3	2.6 ±	0.4	10.65 ±	1.52		
Mysid		19	2.9 ±	0.4	11.72 ±	1.49	13	-19.98 ± 1.90
Ostracod		5	2.4 ±	0.8	9.91 ±	2.86	1	-17.16
Shrimp		14	2.7 ±	0.6	10.89 ±	2.16	11	-19.94 ± 0.97
Tanaid		16	2.5 ±	0.7	10.35 ±	2.42	2	-19.50 ± 1.25
PHYLUM NEMATODA								
Nematode		18	3.6 ±	0.3	13.92 ±	1.12	6	-19.26 ± 0.95
PHYLUM CHORDATA, SUBPHYLUM VERTEBRATA								
Fish		18	2.8 ±	0.2	11.48 ±	0.75	14	-20.02 ± 1.42
Total Count of Prey		444					286	

Table 2-5. **Diet Composition of Benthopelagic Feeding Fishes from the Chukchi Sea**. Numbers represent % IRI (index of relative importance). Prey taxa organized from highest to lowest % IRI among benthopelagic feeding fishes. Prey that were consumed as early life stages are indicated with an asterisk (*). Prey taxa that contributed more than 5% IRI to diet are in bold font. *n* is count of stomachs analyzed for each species, excluding the number of empty stomachs.

	Clupeidae	**Osmeridae**	**Gadidae**		**Gadidae**	**Ammodytidae**	
	Pacific herring	**Capelin**	**Arctic cod**		**Saffron cod**	**Pacific sand lance**	
	n = 9	*n* = 21	*n* = 195	*n* = 181	*n* = 20	*n* = 22	*n* = 20
Prey Taxa	**large** > 70 mm	**large** > 70 mm	**small** ≤ 70 mm	**large** > 70 mm	**large** > 70 mm	**small** ≤ 70 mm	**large** > 70 mm
Copepods	< 0.1	**66.4**	**100.0**	**65.9**	< 0.1	**99.8**	**80.2**
Euphausiids	**97.4**	**16.1**	< 0.1	**6.9**	0.1	-	**19.8**
Amphipods	2.2	**17.5**	< 0.1	**24.8**	**91.7**	< 0.1	-
Polychaetes	-	-	-	< 0.1	**6.5**	-	-
Fishes	< 0.1	-	< 0.1	1.9	-	-	-
Cumaceans	-	-	-	0.4	0.7	-	-
Shrimps *	-	-	-	0.2	0.4	-	-
Barnacles *	0.3	-	< 0.1	< 0.1	-	0.2	-
Nematodes	-	-	-	-	0.3	-	-
Ostracods	-	-	-	-	0.2	-	-
Crustaceans, unidentified	-	-	< 0.1	< 0.1	< 0.1	-	< 0.1
Tanaids	-	-	-	< 0.1	< 0.1	-	-
Mollusks	-	-	< 0.1	-	< 0.1	-	-
Crabs *	-	-	-	< 0.1	-	-	-
Mysids	-	-	-	< 0.1	< 0.1	-	-
Isopods	-	-	-	< 0.1	-	-	-
Animals, unidentified	-	-	-	< 0.1	-	-	-
Sponges	-	-	-	-	-	-	-
Echinoderms	-	-	-	-	-	-	-
Plants, unidentified	-	-	-	-	-	-	-
Diversity (count prey taxa)	5	3	7	14	12	3	3
(count prey taxa > 5%)	1	3	1	3	2	1	2

Table 2-6. **Diet Composition of Benthic Feeding Fishes from the Chukchi Sea**. Numbers represent % IRI (index of relative importance). Prey taxa organized from highest to lowest % IRI among benthic feeding fishes. Prey that were consumed as early life stages are indicated with an asterisk (*). Prey taxa that contributed more than 5% IRI to diet are in bold font. *n* is count of stomachs analyzed for each species, excluding the number of empty stomachs.

	Cottidae		**Cottidae**		**Pleuronectidae**	
	Arctic staghorn sculpin		**Shorthorn sculpin**		**Bering flounder**	
	n = 99	*n* = 54	*n* = 45	*n* = 21	*n* = 110	*n* = 57
Prey Taxa	**small** ≤ 70 mm	**large** > 70 mm	**small** ≤ 70 mm	**large** > 70 mm	**small** ≤ 70 mm	**large** > 70 mm
Amphipods	**90.1**	**80.1**	**96.2**	**70.2**	**68.3**	**55.8**
Copepods	1.3	-	0.2	-	**12.9**	< 0.1
Mysids	-	< 0.1	0.1	< 0.1	**16.9**	**25.9**
Polychaetes	**6.3**	**18.0**	0.8	1.3	1.0	2.8
Crabs *	< 0.1	-	0.7	**5.5**	-	-
Crustaceans, unidentified	< 0.1	0.5	< 0.1	-	0.2	< 0.1
Shrimps *	-	< 0.1	-	**9.5**	0.1	**11.2**
Nematodes	< 0.1	-	-	-	-	< 0.1
Barnacles *	0.9	-	1.5	3.8	< 0.1	< 0.1
Fishes	< 0.1	0.3	< 0.1	**9.7**	0.1	1.8
Mollusks	0.1	0.3	< 0.1	-	< 0.1	0.3
Cumaceans	1.3	0.1	0.1	< 0.1	< 0.1	0.2
Euphausiids	< 0.1	< 0.1	0.2	-	0.1	1.8
Ostracods	< 0.1	-	< 0.1	< 0.1	< 0.1	< 0.1
Tanaids	-	-	-	-	-	-
Sponges	< 0.1	0.5	-	< 0.1	-	-
Plants, unidentified	-	-	-	-	-	< 0.1
Isopods	-	-	-	-	0.2	0.1
Animals, unidentified	-	-	-	-	< 0.1	< 0.1
Echinoderms	< 0.1	-	-	< 0.1	-	0.1
Diversity (count prey taxa)	14	10	12	11	14	17
(count prey taxa > 5%)	2	2	1	4	3	3

Table 2-6 continued**. Diet Composition of Benthic Feeding Fishes from the Chukchi Sea**. Numbers represent % IRI (index of relative importance). Prey taxa organized from highest to lowest % IRI among benthic feeding fishes. Prey that were consumed as early life stages are indicated with an asterisk (*). Prey taxa that contributed more than 5% IRI to diet are in bold font. *n* is count of stomachs analyzed for each species, including the number of empty stomachs.

	Stichaeidae		**Stichaeidae**		**Zoarcidae**	
	Stout eelblenny		**Slender eelblenny**		**Canadian eelpout**	
	n = 20	*n* = 199	*n* = 11	*n* = 17	*n* = 49	*n* = 103
Prey Taxa	**small** ≤ 70 mm	**large** > 70 mm	**small** ≤ 70 mm	**large** > 70 mm	**small** ≤ 70 mm	**large** > 70 mm
Amphipods	**21.9**	-	-	**54.3**	**53.0**	**96.8**
Copepods	**68.3**	**59.8**	**71.3**	**35.5**	**41.1**	0.3
Mysids	-	< 0.1	-	-	-	< 0.1
Polychaetes	0.2	3.2	-	1.6	4.9	2.1
Crabs *	-	**20.9**	0.4	-	-	-
Crustaceans, unidentified	-	< 0.1	**25.2**	0.4	< 0.1	-
Shrimps *	-	-	-	-	-	0.3
Nematodes	**5.6**	**7.9**	2.4	3.4	-	-
Barnacles *	2.6	1.8	-	2.8	0.7	< 0.1
Fishes	-	< 0.1	-	-	-	< 0.1
Mollusks	0.1	3.2	-	1.5	0.1	0.1
Cumaceans	0.3	1.2	-	0.3	0.2	0.3
Euphausiids	-	< 0.1	-	< 0.1	-	-
Ostracods	< 0.1	0.9	0.7	0.2	-	< 0.1
Tanaids	1.0	0.7	-	< 0.1	-	-
Sponges	-	< 0.1	-	-	-	< 0.1
Plants, unidentified	-	0.3	-	-	-	-
Isopods	< 0.1	< 0.1	-	-	-	< 0.1
Animals, unidentified	-	< 0.1	-	-	-	< 0.1
Echinoderms	-	-	-	-	-	-
Diversity (count prey taxa)	10	17	5	11	7	13
(count prey taxa > 5%)	3	3	2	2	2	1

Table 2-7. **ANOVA for Stable Isotope Ratios between Fish Length Classes**. Analysis of variance results for stable nitrogen and carbon isotope ratios between small and large fish. Either Holm-Sidak Method (*t*) or Dunn's Method (Q) were used for the pairwise multiple comparison. Bold font indicates significant differences.

	One-Way Anova Results for Isotopes Between Small (≤ 70 mm) and Large (> 70 mm) Fish			
	$\delta^{15}N$		$\delta^{13}C$	
Arctic cod	**t = 6.334**	**p < 0.001**	**Q = 3.384**	**p < 0.05**
Saffron cod	**Q = 3.062**	**p < 0.05**		$p > 0.05$
Arctic staghorn sculpin	**t = 3.861**	**p < 0.001**	**t = 3.734**	**p < 0.001**
Shorthorn sculpin	**Q = 1.964**	**p < 0.05**		$p > 0.05$
Canadian eelpout		$p > 0.05$		$p > 0.05$
Stout eelblenny		$p > 0.05$	**t = 4.616**	**p < 0.001**
Slender eelblenny	**Q = 2.205**	**p < 0.05**	**t = 4.489**	**p < 0.001**
Pacific sand lance	**t = 4.084**	**p < 0.001**	**t = 2.112**	**p = 0.049**
Bering flounder	**t = 3.130**	**p = 0.004**		$p > 0.05$

Table 2-8. **ANOVA for Stable Isotope Ratios of the Small Length Class between Fish Species**. Analysis of variance results for stable nitrogen (top right corner) and carbon isotope ratios (bottom left corner) for the small length class among fish species. Significant differences were examined using Holm-Sidak Method (t). Bold font indicates significant differences.

Small Fishes (≤ 70 mm)		**One-Way Anova Results for $\delta^{15}N$ Values Among Species**								
		Arctic cod	**Saffron cod**	**Arctic staghorn sculpin**	**Shorthorn sculpin**	**Canadian eelpout**	**Stout eelblenny**	**Slender eelblenny**	**Pacific sand lance**	**Bering flounder**
One Way Anova Results for $\delta^{13}C$ Values Among Species	**Arctic cod**		$p > 0.05$	**$t = 3.763$, $p = 0.005$**	$p > 0.05$	**$t = 9.348$, $p < 0.001$**	**$t = 8.001$, $p < 0.001$**	$p > 0.05$	**$t = 4.508$, $p < 0.001$**	**$t = 4.477$, $p < 0.001$**
	Saffron cod	$p > 0.05$		**$t = 3.667$, $p = 0.007$**	$p > 0.05$	**$t = 8.285$, $p < 0.001$**	**$t = 7.284$, $p < 0.001$**	$p > 0.05$	**$t = 3.339$, $p = 0.018$**	**$t = 4.231$, $p = 0.001$**
	Arctic staghorn sculpin	**$t = 7.154$, $p < 0.001$**	**$t = 7.058$, $p < 0.001$**		$p > 0.05$	**$t = 6.477$, $p < 0.001$**	**$t = 5.159$, $p < 0.001$**	$p > 0.05$	**$t = 7.757$, $p < 0.001$**	$p > 0.05$
	Shorthorn sculpin	**$t = 10.093$, $p < 0.001$**	**$t = 9.886$, $p < 0.001$**	**$t = 5.049$, $p < 0.001$**		**$t = 5.092$, $p < 0.001$**	**$t = 4.216$, $p = 0.001$**	$p > 0.05$	**$t = 6.104$, $p < 0.001$**	$p > 0.05$
	Canadian eelpout	**$t = 7.921$, $p < 0.001$**	**$t = 7.790$, $p < 0.001$**	$p > 0.05$	**$t = 3.625$, $p = 0.008$**		$p > 0.05$	**$t = 5.929$, $p < 0.001$**	**$t = 12.141$, $p < 0.001$**	**$t = 5.763$, $p < 0.001$**
	Stout eelblenny	**$t = 6.664$, $p < 0.001$**	**$t = 6.815$, $p < 0.001$**	$p > 0.05$	**$t = 4.152$, $p = 0.002$**	$p > 0.05$		**$t = 5.020$, $p < 0.001$**	**$t = 10.988$, $p < 0.001$**	**$t = 4.494$, $p < 0.001$**
	Slender eelblenny	**$t = 5.302$, $p < 0.001$**	**$t = 5.737$, $p < 0.001$**	$p > 0.05$	**$t = 4.149$, $p = 0.002$**	$p > 0.05$	$p > 0.05$		**$t = 5.380$, $p < 0.001$**	$p > 0.05$
	Pacific sand lance	$p > 0.05$	$p > 0.05$	**$t = 3.843$, $p = 0.004$**	**$t = 7.260$, $p < 0.001$**	**$t = 4.758$, $p < 0.001$**	**$t = 3.902$, $p = 0.004$**	**$t = 3.111$, $p = 0.037$**		**$t = 8.321$, $p < 0.001$**
	Bering flounder	**$t = 7.977$, $p < 0.001$**	**$t = 7.709$, $p < 0.001$**	$p > 0.05$	**$t = 4.399$, $p < 0.001$**	$p > 0.05$	$p > 0.05$	$p > 0.05$	**$t = 4.493$, $p < 0.001$**	

Table 2-9. **ANOVA for Stable Isotope Ratios of the Large Length Class between Fish Species.** Analysis of variance results for stable nitrogen (top right corner) and carbon isotope ratios (bottom left corner) for the large length class among fish species. Significant differences were examined using Holm-Sidak Method (t) or Dunn's Method (Q). Bold font indicates significant differences.

	Large Fishes (> 70 mm)	**One-Way Anova Results for $\delta^{15}N$ Values Among Species**										
		Pacific herring	**Capelin**	**Arctic cod**	**Saffron cod**	**Arctic staghorn sculpin**	**Shorthorn sculpin**	**Canadian eelpout**	**Stout eelblenny**	**Slender eelblenny**	**Pacific sand lance**	**Bering flounder**
One Way Anova Results for $\delta^{13}C$ Values Among Species	**Pacific herring**		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
	Capelin	$p > 0.05$		$p > 0.05$	$p > 0.05$	**Q = 5.309, $p < 0.05$**	**Q = 4.106, $p < 0.05$**	**Q = 6.288, $p < 0.05$**	**Q = 6.775, $p < 0.05$**	**Q = 3.477, $p < 0.05$**	$p > 0.05$	**Q = 4.839, $p < 0.05$**
	Arctic cod	$p > 0.05$	$p > 0.05$		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
	Saffron cod	$p > 0.05$	$p > 0.05$	$p > 0.05$		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
	Arctic staghorn sculpin	**Q = 4.919, $p < 0.05$**	$p > 0.05$	**Q = 3.751, $p < 0.05$**	**Q = 3.938, $p < 0.05$**		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	**Q = 4.090, $p < 0.05$**	$p > 0.05$
	Shorthorn sculpin	**Q = 5.543, $p < 0.05$**	**Q = 3.830, $p < 0.05$**	**Q = 4.437, $p < 0.05$**	**Q = 4.549, $p < 0.05$**	$p > 0.05$		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
	Canadian eelpout	**Q = 3.921, $p < 0.05$**	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$		$p > 0.05$	$p > 0.05$	**Q = 5.179, $p < 0.05$**	$p > 0.05$
	Stout eelblenny	**Q = 5.803, $p < 0.05$**	**Q = 4.120, $p < 0.05$**	**Q = 4.728, $p < 0.05$**	**Q = 4.809, $p < 0.05$**	$p > 0.05$	$p > 0.05$	$p > 0.05$		$p > 0.05$	**Q = 5.535, $p < 0.05$**	$p > 0.05$
	Slender eelblenny	**Q = 5.590, $p < 0.05$**	**Q = 4.006, $p < 0.05$**	**Q = 4.549, $p < 0.05$**	**Q = 4.683, $p < 0.05$**	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$		$p > 0.05$	$p > 0.05$
	Pacific sand lance	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	**Q = 3.755, $p < 0.05$**	**Q = 4.442, $p < 0.05$**	$p > 0.05$	**Q = 4.732, $p < 0.05$**	**Q = 4.553, $p < 0.05$**		**Q = 3.598, $p < 0.05$**
	Bering flounder	**Q = 3.762, $p < 0.05$**	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	

2.7 Figures

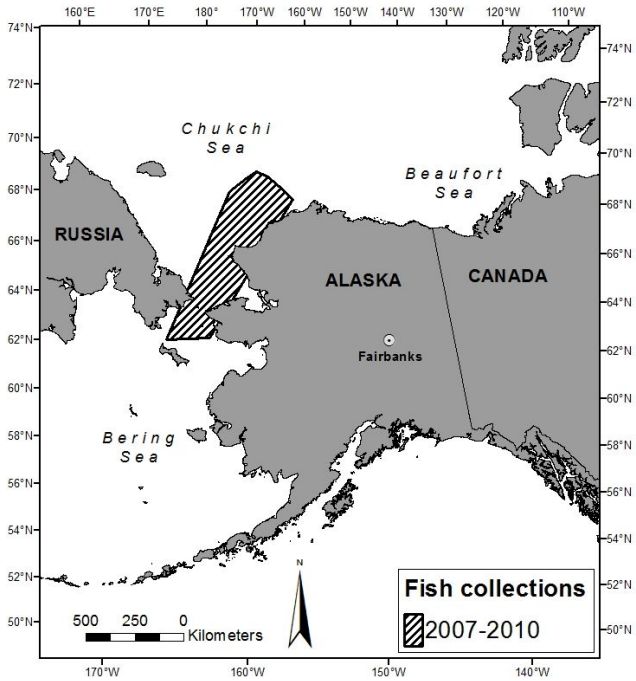

Figure 2-1. **Sample Collection Map**. Map showing area of fish collections in the Chukchi and northern Bering Seas from 2007 to 2010.

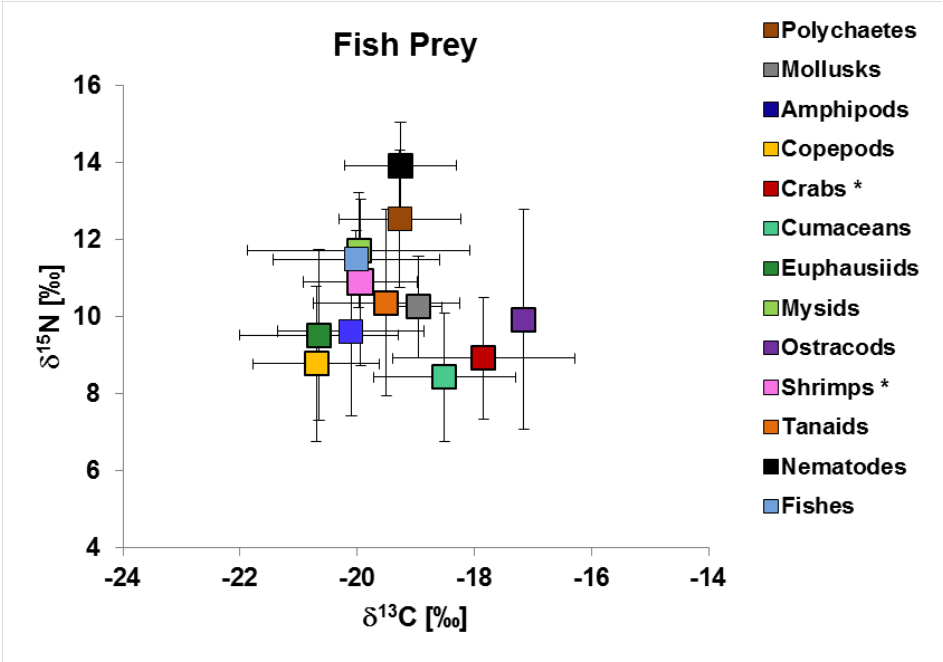

Figure 2-2. **Stable Isotope Signatures of Fish Prey**. Stable nitrogen and carbon isotope ratios of prey collected from the stomach contents of Arctic fishes. Early life stages of prey are indicated with an asterisk (*). Symbols represent values averaged across multiple years, 2007–2010. Standard deviations are illustrated by the bars.

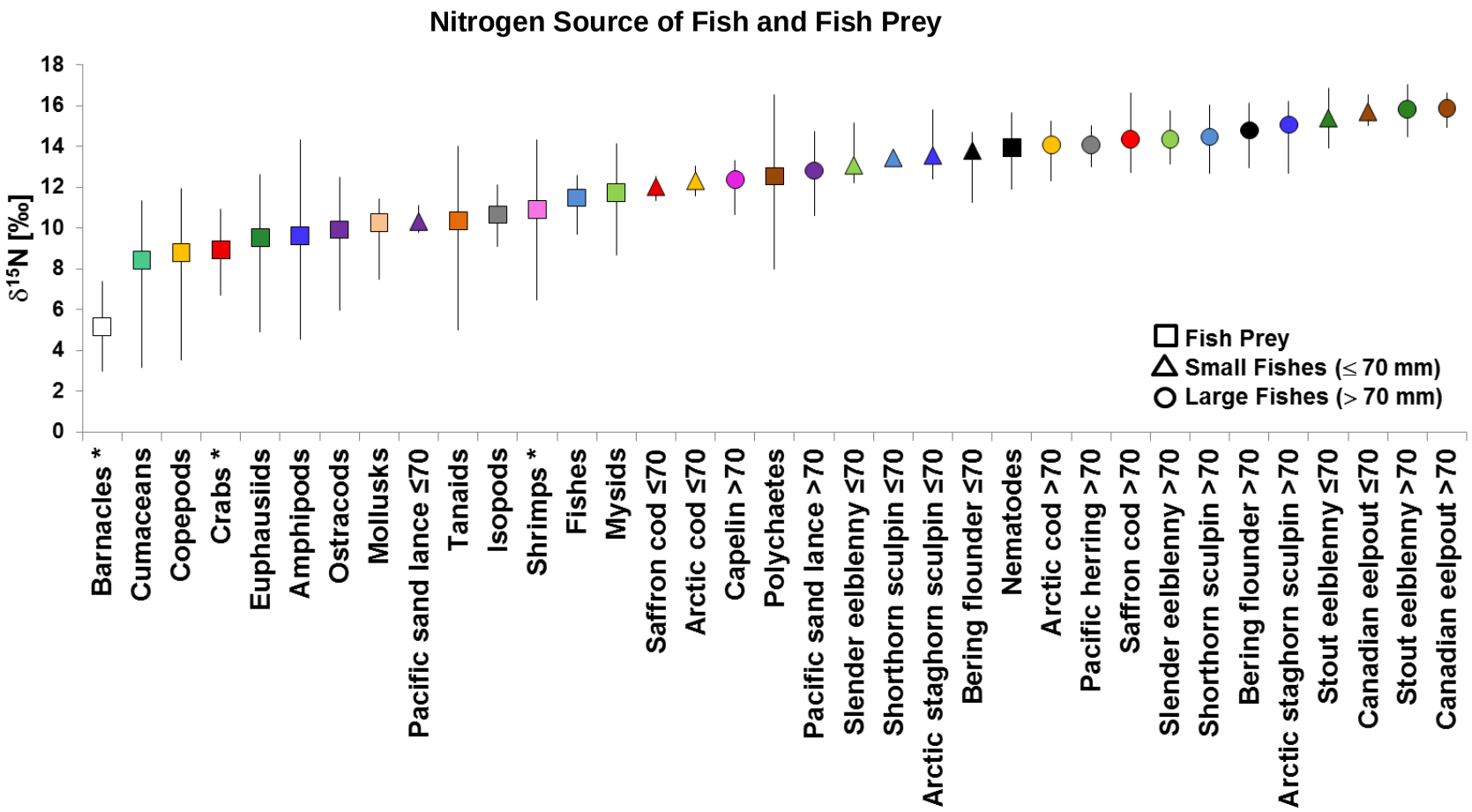

Figure 2-3. **Stable Nitrogen Isotope Ratios of Fish Prey (squares), Small Fishes (triangles), and Large Fishes (circles).** Early life stages of prey are indicated with an asterisk (*). Symbols represent an averaged value across multiple years, 2007–2010. Bars illustrate the range of values (minimum and maximum $\delta^{15}N$).

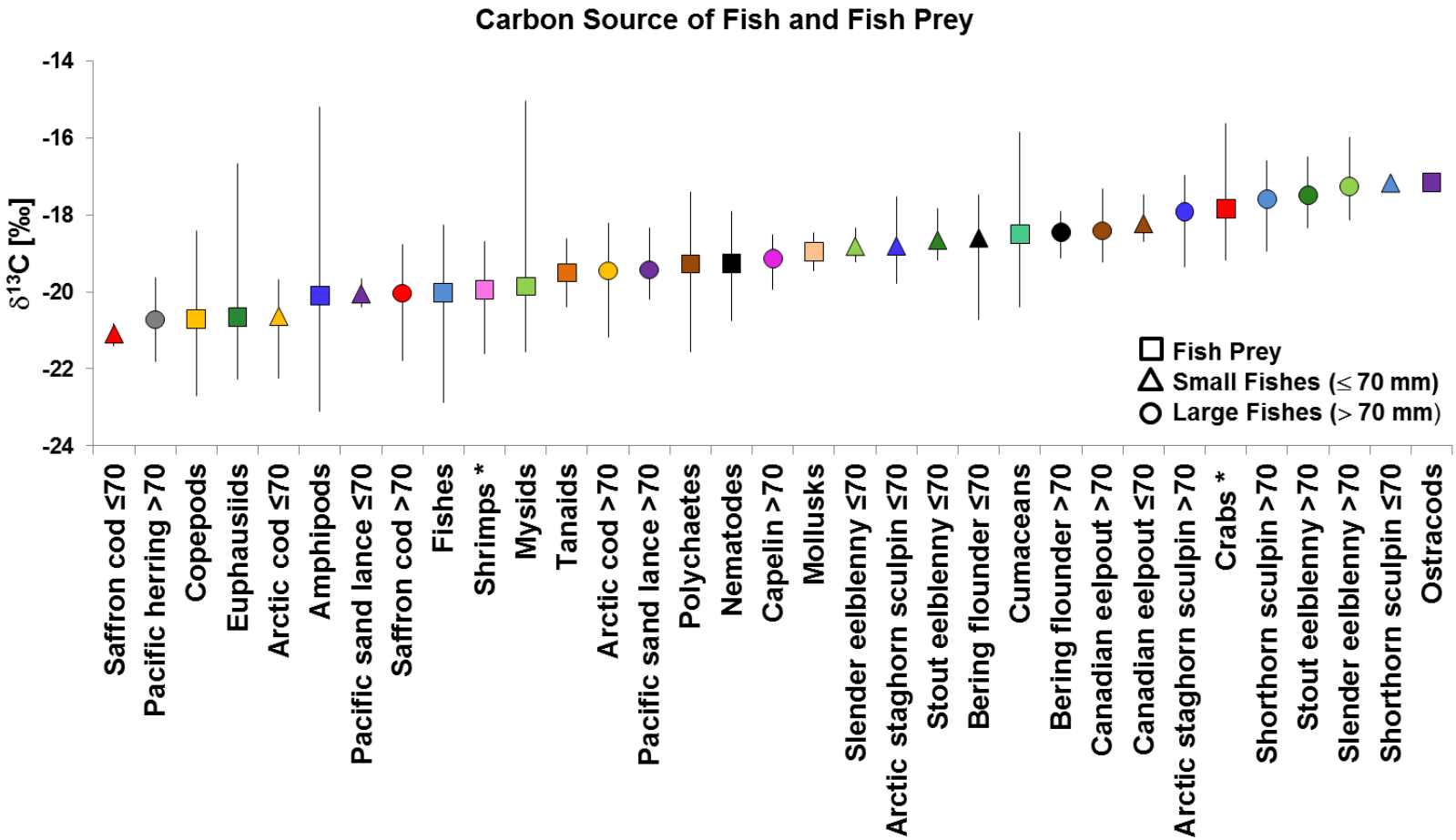

Figure 2-4. **Stable Carbon Isotope Ratios of Fish Prey (squares), Small Fishes (triangles), and Large Fishes (circles).** Early life stages of prey are indicated with an asterisk (*). Symbols represent an averaged value across multiple years, 2007–2010. Bars illustrate the range of values (minimum and maximum $\delta^{13}C$).

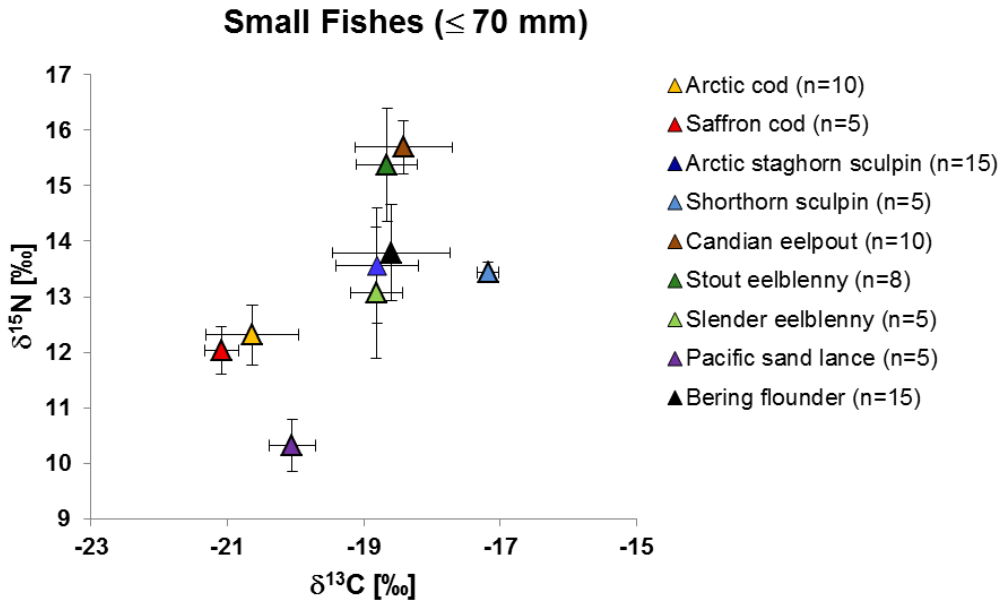

Figure 2-5. **Stable Isotope Signatures of Small Fishes**. Stable nitrogen and carbon isotope ratios of small fishes (≤ 70 mm). Symbols represent values averaged across multiple years. Standard deviations are illustrated by the bars.

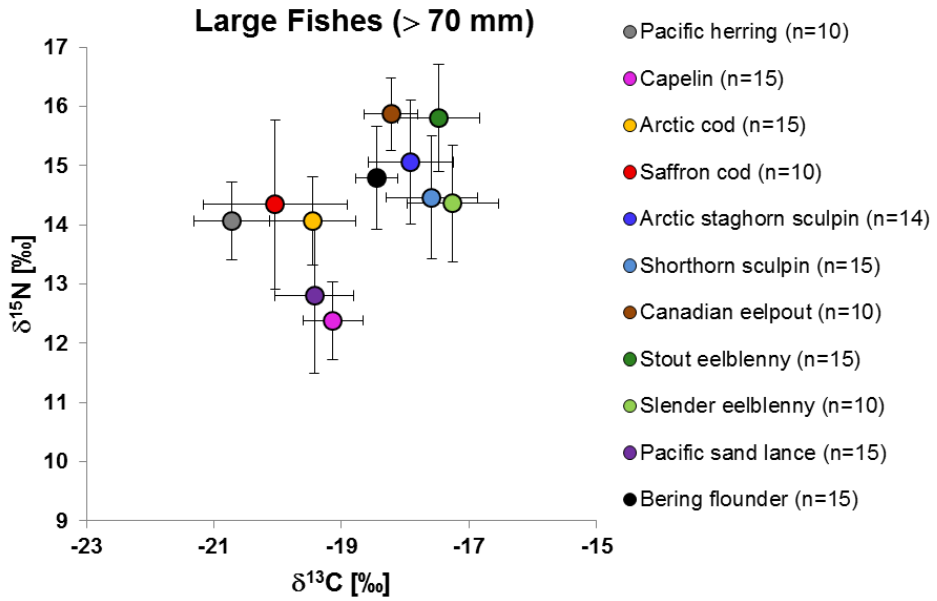

Figure 2-6. **Stable Isotope Signatures of Large Fishes.** Stable nitrogen and carbon isotope ratios of large fishes (> 70 mm). Symbols represent values averaged across multiple years. Standard deviations are illustrated by the bars.

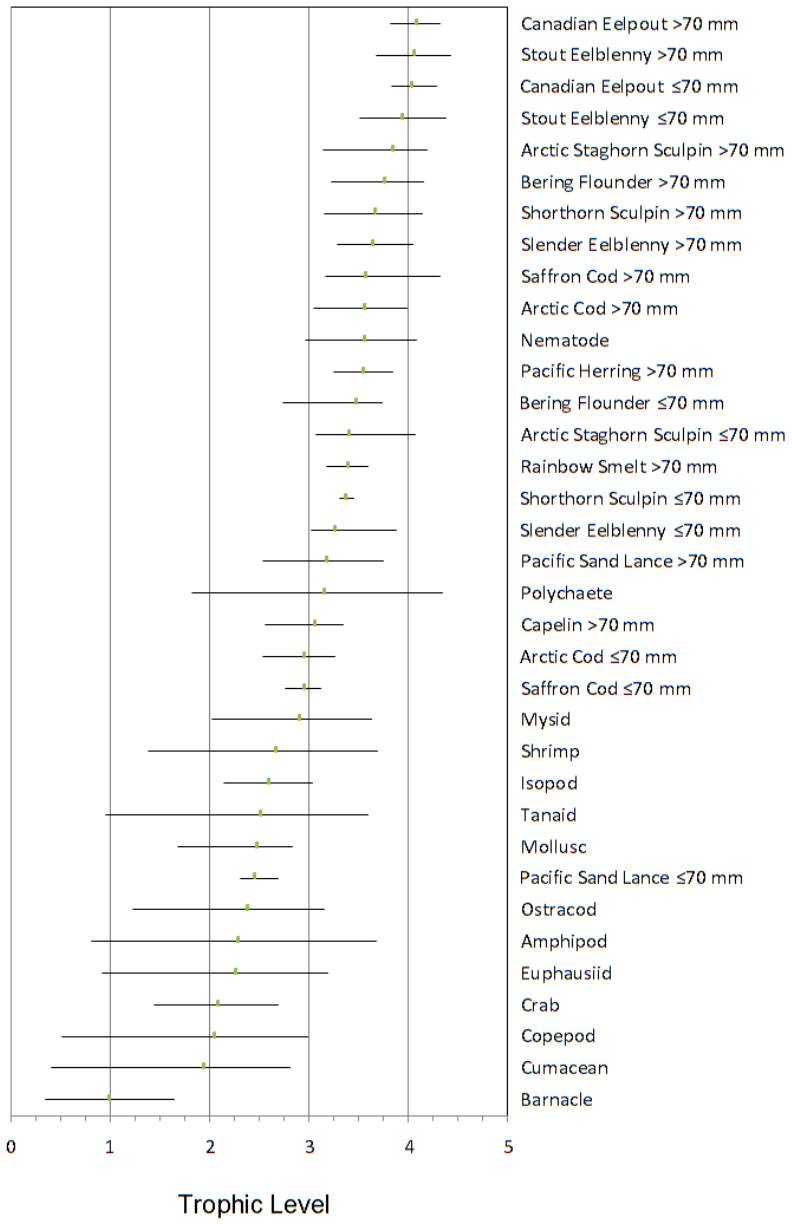

Figure 2-7. **Trophic Level of Fishes and Prey**. Minimum, maximum and average (dot) values of trophic level for fishes and their prey. Included is Rainbow Smelt for which we analyzed TL for seal prey (Chapter 4), though it is not included in this chapter.

Appendix 2-1a. **Stable Isotope Ratios of Muscle Tissue and Whole Fishes.** Stable nitrogen and carbon isotope ratios for muscle tissue and total body homogenate (whole fish excluding stomachs) of 11 fish species. *n* is count of fish that muscle tissue and total body homogenate was analyzed from the same individual. LE is an abbreviation for lipid-extracted. Significant differences were examined using a paired t-test. If data did not pass the Shapiro-Wilk normality test, a Wilcoxon Sign Rank test was applied.

		Non-LE Fish Muscle		LE Fish Muscle		Non-LE Whole Fish		LE Whole Fish	
Species	*n*	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Pacific herring *(Clupea pallasii)*	5	14.10 ± 0.35	-22.52 ± 0.77	15.27 ± 1.46	-20.82 ± 0.65	13.56 ± 0.44	-23.13 ± 0.42	14.49 ± 0.42	-21.07 ± 0.47
Capelin *(Mallotus villosus)*	5	12.72 ± 0.39	-20.01 ± 0.38	14.54 ± 0.57	-19.09 ± 0.19	12.21 ± 0.48	-22.52 ± 1.12	13.08 ± 0.46	-19.24 ± 0.18
Arctic cod *(Boreogadus saida)*	10	13.67 ± 1.39	-20.41 ± 0.64	14.08 ± 1.65	-19.78 ± 0.66	12.84 ± 1.56	-21.95 ± 0.75	13.41 ± 1.92	-19.79 ± 0.56
Saffron cod *(Eleginus gracilis)*	5	12.89 ± 0.33	-21.81 ± 0.19	13.76 ± 0.38	-21.50 ± 0.17	12.03 ± 0.43	-22.14 ± 0.32	12.92 ± 0.27	-21.09 ± 0.25
Arctic staghorn sculpin *(Gymnocanthus tricuspis)*	13	15.59 ± 0.97	-18.83 ± 0.72	15.58 ± 1.00	-18.44 ± 0.57	15.05 ± 0.98	-19.51 ± 0.74	14.79 ± 1.12	-18.21 ± 0.78
Shorthorn sculpin *(Myoxocephalus scorpius)*	5	14.42 ± 0.65	-18.44 ± 0.29	15.26 ± 0.91	-18.45 ± 0.32	14.31 ± 0.43	-19.06 ± 0.42	13.75 ± 0.68	-17.14 ± 0.42
Canadian eelpout *(Lycodes polaris)*	12	16.59 ± 0.56	-18.56 ± 0.53	16.54 ± 0.51	-18.07 ± 0.60	15.90 ± 0.60	-18.94 ± 0.60	16.03 ± 0.86	-18.30 ± 0.44
Stout eelblenny *(Anisarchus medius)*	16	16.18 ± 0.65	-18.84 ± 0.51	16.48 ± 0.84	-18.19 ± 0.47	16.11 ± 0.72	-19.42 ± 0.52	15.97 ± 0.86	-17.96 ± 0.74
Slender eelblenny *(Lumpenus fabricii)*	5	15.27 ± 0.80	-18.36 ± 0.21	15.61 ± 0.84	-18.19 ± 0.13	15.09 ± 0.78	-19.44 ± 0.40	14.58 ± 0.97	-17.30 ± 0.62
Pacific sand lance *(Ammodytes hexapterus)*	5	13.96 ± 0.39	-20.35 ± 0.43	15.34 ± 0.71	-19.72 ± 0.41	13.39 ± 0.46	-21.39 ± 0.21	13.91 ± 0.37	-19.66 ± 0.27
Bering flounder *(Hippoglossoides robustus)*	9	15.00 ± 0.88	-18.81 ± 0.30	15.25 ± 0.88	-18.42 ± 0.21	14.58 ± 1.01	-19.52 ± 0.81	14.78 ± 1.18	-18.07 ± 0.47

Appendix 2-1b. **Statistical Comparison of Stable Isotope Ratios.** Applied to Muscle Tissue and Whole Fishes data reported in Appendix 2-1a.

Species	*n*	Non-LE Whole Fish Vs. Non-LE Fish Muscle		Non-LE Fish Muscle Vs. LE Fish Muscle		Non-LE Whole Fish Vs. LE Whole Fish	
		$\delta^{15}N$ *t, p*	$\delta^{13}C$ *t, p*	$\delta^{15}N$ *t, p*	$\delta^{13}C$ *t, p*	$\delta^{15}N$ *t, p*	$\delta^{13}C$ *t, p*
Pacific herring *(Clupea pallasii)*	5	$p > 0.05$	$t = 2.815$ $p = 0.048$	$p > 0.05$	**$t = -5.229$ $p = 0.006$**	**$t = -5.095$ $p = 0.007$**	$p > 0.05$
Capelin *(Mallotus villosus)*	5	**$t = 3.738$ $p = 0.020$**	**$t = 7.348$ $p = 0.002$**	**$t = -10.781$ $p < 0.001$**	**$t = -8.319$ $p = 0.001$**	**$t = -7.092$ $p = 0.002$**	**$t = -7.464$ $p = 0.002$**
Arctic cod *(Boreogadus saida)*	10	**$t = 4.671$ $p = 0.001$**	**$t = 7.341$ $p < 0.001$**	**$Z = 2.090$ $p = 0.037$**	**$t = -8.353$ $p < 0.001$**	**$t = -2.715$ $p = 0.024$**	**$t = -12.459$ $p < 0.001$**
Saffron cod *(Eleginus gracilis)*	5	**$t = 7.413$ $p = 0.002$**	**$t = 5.248$ $p = 0.006$**	$p > 0.05$	**$t = -3.373$ $p = 0.028$**	**$t = -5.357$ $p = 0.006$**	**$t = -13.307$ $p < 0.001$**
Arctic staghorn sculpin *(Gymnocanthus tricuspis)*	13	**$t = 7.746$ $p < 0.001$**	**$t = 5.417$ $p < 0.001$**	$p > 0.05$	**$t = -4.873$ $p < 0.001$**	$p > 0.05$	**$Z = 3.180$ $p < 0.001$**
Shorthorn sculpin *(Myoxocephalus scorpius)*	5	$p > 0.05$	**$t = 3.134$ $p = 0.035$**	**$t = -3.203$ $p = 0.033$**	$p > 0.05$	**$t = 4.301$ $p = 0.013$**	**$t = -44.871$ $p < 0.001$**
Canadian eelpout *(Lycodes polaris)*	12	**$t = 4.328$ $p = 0.001$**	**$t = 3.210$ $p = 0.008$**	$p > 0.05$	**$t = -3.395$ $p = 0.006$**	$p > 0.05$	**$t = -6.282$ $p < 0.001$**
Stout eelblenny *(Anisarchus medius)*	16	$p > 0.05$	**$t = 5.248$ $p < 0.001$**	**$Z = 2.482$ $p = 0.011$**	**$t = -5.141$ $p < 0.001$**	$p > 0.05$	**$t = -9.084$ $p < 0.001$**
Slender eelblenny *(Lumpenus fabricii)*	5	$p > 0.05$	$p > 0.05$	**$t = -3.542$ $p = 0.024$**	**$t = -3.484$ $p = 0.025$**	$p > 0.05$	**$t = -8.223$ $p = 0.001$**
Pacific sand lance *(Ammodytes hexapterus)*	5	**$t = 9.306$ $p < 0.001$**	**$t = 4.688$, $p = 0.009$**	**$t = -6.363$ $p = 0.003$**	$p > 0.05$	**$t = -3.223$ $p = 0.032$**	**$t = -17.447$ $p < 0.001$**
Bering flounder *(Hippoglossoides robustus)*	9	**$t = 2.838$ $p = 0.022$**	$p > 0.05$	**$t = -2.786$ $p = 0.024$**	**$t = -3.504$ $p = 0.008$**	$p > 0.05$	**$t = -5.230$ $p < 0.001$**

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Chapter 3: Interannual Diet Variability for Five Arctic Fish Species in the Chukchi Sea

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3.1 Introduction

Recent abiotic changes to the Arctic ecosystem may alter biotic factors, more specifically food web structure, on an interannual scale. Advection of warmer waters into the Arctic Ocean and continued loss of thicker multi-year ice resulted in the period of 2007–2012 having the lowest September ice extents in satellite records since recording began in 1979 (NSIDC 2012). The expansion and longer duration of the open water season led to a relatively high annual primary productivity in the Arctic Ocean during 2007 (Arrigo et al. 2008). The high biomass of phytoplankton may have been immediately consumed by pelagic grazers that develop earlier and rapidly in warmer waters (Bluhm and Gradinger 2008; Forest et al. 2011). Arctic fishes may take advantage of the prey source presented by more abundant, pelagic grazers in years of high annual primary productivity (Bluhm and Gradinger 2008). A pelagic-dominated food web would reduce the input of more refractory carbon to the seafloor (Bluhm and Gradinger 2008), potentially impacting benthic biomass over time (Dunton et al. 2005; Grebmeier 2012).

Identification of interannual changes in the diets of marine fishes in the Arctic requires basic knowledge of the feeding ecology of benthopelagic and demersal fishes. Information about diets of these fishes comes from four decades of collections spanning the U.S. and Canadian Arctic waters. Arctic Cod (*Boreogadus saida*) primarily consume copepods (Lowry and Frost 1981; Coyle et al. 1997; Cui et al. 2012). Epibenthic prey such as amphipods, mysids, and shrimps, also have been found in the stomachs of Arctic Cod (Lowry and Frost 1981; Coyle et al. 1997; Walkusz et al. 2012). Arctic Staghorn Sculpin (*Gymnocanthus tricuspis*) mainly eat polychaetes, mollusks, and amphipods (Atkinson and Percy 1991; Coyle et al. 1997; Cui et al. 2012). The diet of Canadian Eelpout (*Lycodes polaris*) consists of polychaetes, mollusks, amphipods, and copepods (Atkinson and Percy 1991). Stout Eelblenny (*Anisarchus medius*) have diverse diets of polychaetes, mollusks, amphipods, shrimps, nematodes, fishes, and other crustaceans (Atkinson and Percy 1991; Edenfield et al. 2011). Bering Flounder (*Hippoglossoides robustus*) eat infaunal amphipods, crabs, shrimps, and fishes (Coyle et al. 1997). These observations of stomach contents are generalized and provide no insight as to interannual variability of prey consumption.

Dietary information from stomach contents and stable isotope studies are complementary methods for assessing feeding ecology over short- and long-term time frames (Giraldo et al. 2011; Polito et al. 2011). Stomach content analyses provide high taxonomic resolution of prey on a short time scale, but can underestimate the amount of soft-bodied prey (Brush et al. 2012) such as fish tissue and polychaete worms. Alternatively, information from stable isotope analysis is of relatively poor taxonomic resolution (Carrasco et al. 2012), yet provides estimates of assimilated diet over an integrated period of time (Buchheister and Latour 2010). As stable isotopes reflect diet over a longer timeframe than stomach content information (Brush et al. 2012), they can illustrate the interannual variation in fish diets that cannot be observed by stomach content

analyses alone (Wainright et al. 1993). Stable nitrogen isotope ratios describe trophic position as the tissues of a consumer are enriched in the heavier isotope (^{15}N) by about 3‰ compared to the prey source (Peterson and Fry 1987). Stable carbon isotope ratios illustrate carbon source and habitat use (Kline et al. 1997). For example, benthic organisms consume recycled material, making their tissues more enriched in the heavier carbon isotope (^{13}C) compared to pelagic organisms that forage on fresh phytoplankton (Iken et al. 2005). Ice algae trapped in brine channels can be more enriched in ^{13}C compared to phytoplankton (Hobson et al. 1995). Coupling stable nitrogen and carbon isotope ratios can then create an isotope signature for a predator that will vary based on the proportions of different prey items consumed. Coupling stable nitrogen and carbon isotope ratios can then create an isotope signature for a predator that will vary based on the proportions of different prey items consumed.

Isotope mixing models are used to describe the proportional contribution of prey to a predator's diet (Phillips and Gregg 2001, 2003; Moore and Semmens 2008; Parnell et al. 2010). They can show prey of annual ecological importance and illustrate changes in the proportions of prey across years (Polito et al. 2011). In order to make inferences about the proportional contribution of prey, it is necessary to know the stable nitrogen and carbon isotope ratios of the predator and the prey as well as a trophic enrichment factor. The trophic enrichment factors (i.e., fractionation factors) are the tissue-specific incremental change in the stable isotope ratios as they are incorporated from prey into the tissues of the consumer (Peterson and Fry 1987). Including these values into the isotope mixing model allows for evaluating all food-web components in the same isotopic space. Knowledge of isotopic turnover rates for the tissue of the predator is also crucial to accurately identify the diet timeframe described by stable isotopes (Newsome et al. 2012).

The goal of this study was to identify interannual trends in diets of five Arctic fish species by examining stomach contents, stable isotope ratios of whole fish and prey, and isotope mixing models. This study was part of a larger project investigating trophic links from fish prey to fish to ice seals. This paper builds upon a portion of this project that examined the diet of 11 fish species (Chapter 2). In this paper, we concentrate on five of those fish species in greater detail across three consecutive years. Sea ice extent was lower in 2007 compared to 2008–2010 (NSIDC 2012). During low ice years, the Arctic food web may become more pelagically dominated (Bluhm and Gradinger 2008), and we hypothesize that Arctic fishes may have increased foraging efforts on low-trophic, pelagic prey during 2007. As stable isotopes describe assimilated fish diet from the previous year (Buchheister and Latour 2010), isotope mixing models for fish collected during 2008–2010 will show feeding ecology from 2007–2009.

3.2 Materials and Methods

3.2.1 Fish Collections

Fishes used in this study were collected offshore in the Chukchi Sea during research cruises in 2008, 2009, and 2010 (Figure 3-1). The collection times were July 2008, July–October 2009, and

September–October 2010. Five fish species were selected for diet and stable isotope analysis, because samples were available from more than one year and they represented major taxonomic families found in the Chukchi Sea: Arctic Cod (Gadidae, cods), Arctic Staghorn Sculpin (Cottidae, sculpins), Canadian Eelpout (Zoarcidae, eelpouts), Stout Eelblenny (Stichaeidae, pricklebacks), and Bering Flounder (Pleuronectidae, righteye flounders).

Fishes were frozen at sea and transported to the University of Alaska Fairbanks (UAF) Fisheries Oceanography Laboratory where detailed processing was conducted. In the laboratory, each fish was thawed, and total length was measured to the nearest mm. Prey size and prey items typically increase as the fish gets larger (Morrow 1980). For this study, two length classes of fishes were selected and analyzed: small (≤70 mm) and large (>70 mm). Two length classes of fish were analyzed because small fish typically have different feeding strategies than larger fish (Schael et al. 1991). Fish larger than 70 mm are main prey for ringed seals (Lowry et al. 1980a). Where available, stomach contents were examined from at least 20 fish per length class per year, and total fish body homogenates were analyzed for stable nitrogen and carbon isotope ratios from five individuals per species per length class per year.

3.2.2 Stomach Content Analysis

Whole fishes were thawed, and stomachs were excised, covered in water, and frozen until processing. Thawed stomachs were blotted on lens paper, and wet weight of the stomach was measured to the nearest 0.0001 g using an Orion series HR200 precision balance. Prey were removed from the stomach and the empty weight and approximate percent fullness (0–100%) of the stomach was recorded. Prey were sorted into class- or family-level taxonomic groupings. Each prey item, determined by the presence of a head, was counted. All prey of the same taxonomic group were combined, blotted on lens paper, and weighed to the nearest 0.0001 g. Fragments of organisms were included when they could be definitively identified to a taxonomic group. Prey fragments were assigned a count of one only when no heads were observed. This process was repeated for each taxonomic group of prey in every stomach. Prey were reported as broad taxonomic groupings, i.e., phylum, class, or order; however, diet analysis was performed at a higher taxonomic resolution when possible.

For two length classes of five fish species over three years, 1,151 stomachs were examined (Table 3-1). For diet analysis, 1,067 stomachs were analyzed, and 84 stomachs were excluded because they were empty. In order to analyze diets of fishes, an index of relative importance (%IRI) was calculated for each prey taxa for each category, i.e., species per length class per year. The IRI was calculated for each prey taxon in each species and length group as follows:

$$IRI = (\%N+\%W) / \%O$$

where %N is the percentage by count of a prey taxon, %W is the percentage of the weight of the prey taxon, and %O is the percentage of occurrence of prey taxa over all taxa present for that species and length class (Pinkas et al. 1971). Using three measures of fish prey dietary

importance makes IRI a useful tool for stomach content analysis. Using solely %N is biased toward prey that are numerous and small (e.g., copepods) while %W is biased toward prey that are relatively rare and large (e.g., fish; Hyslop 1980; Liao et al. 2001). The IRI in this paper is reported for each prey taxa as a percentage of total IRI for each category (%IRI).

3.2.3 Sample Preparation for Stable Isotope Analysis

Samples for stable nitrogen and carbon isotope analysis were prepared for 267 fish prey and 127 fishes from 2008 to 2010. Each sample of a prey taxon was pooled over multiple fish stomachs, regardless of fish species, at a station, and where necessary, multiple stations were pooled to increase prey sample mass to yield sufficient tissue for stable isotope analysis, i.e., > 0.2 mg freeze-dried. Prey were aggregated into broad taxonomic groups for stable isotope analysis, with the lowest taxonomic classification being order. Whole fish and prey were frozen at -20 °C and then freeze-dried for approximately 48 hours using a VirTis BT 6K ES freeze-dryer.

Whole fish without stomachs were processed for non-lipid-extracted $^{15}N/^{14}N$ ratios and lipid-extracted $^{13}C/^{12}C$ ratios. Extracting lipids from samples removes the stable carbon isotope signature of fats (DeNiro and Epstein 1977), leaving only the stable carbon isotope signature of the tissue, but the extraction procedure can alter the stable nitrogen isotope signature (Pinnegar and Polunin 1999; Sweeting et al. 2006). Freeze-dried whole fish were homogenized using a mortar and pestle. Lipids were extracted from one-half of the sample of ground whole fish using a modified version of Bligh and Dyer (1959). Dried, homogenized samples were immersed in a 2:1 chloroform/methanol mixture with a solvent volume about three times the sample volume (Logan et al. 2008). Each sample was agitated for five minutes followed by five minutes of centrifugation at 605 g (3000 rpm) using a VWR Clinical 50 centrifuge. The supernatant containing lipids was discarded. This process was repeated until the supernatant was colorless after centrifugation (Logan et al. 2008), approximately three to five times depending on the lipid content of the fish. Lipid-extracted samples were dried overnight in a fume hood, re-freeze dried for approximately two hours the following day and re-homogenized.

All prey samples were processed for non-lipid-extracted $^{15}N/^{14}N$ ratios and lipid-extracted $^{13}C/^{12}C$ ratios while prey having exoskeletons were also acid fumed. Exoskeleton carbonates of invertebrates can impact stable carbon isotope ratios (Søreide et al. 2007); therefore, samples of prey having exoskeletons were processed to assess non-treated $^{15}N/^{14}N$ ratios and acid-fumed/lipid-extracted $^{13}C/^{12}C$ ratios. Freeze-dried prey tissues were fumed with saturated HCl vapors for four hours in a vacuum chamber. Samples were then soaked in a 2:1 chloroform/methanol mixture for approximately four hours; the solvent was removed, and fresh chemicals were added. Lipid extraction was repeated three times and the samples were freeze-dried for an additional two hours before analyzing stable nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) isotope ratios.

Whole fish and fish prey were analyzed for $\delta^{15}N$ and $\delta^{13}C$ values at the Alaska Stable Isotope Facility at UAF. A sub-sample of ground fish tissue and fish prey, 0.2–0.4 mg dry weight, was weighed into tin capsules using a microbalance (Sartorius Model M2P). Stable isotope analysis was performed using a Finnigan MAT Delta^{Plus}XP Isotope Ratio Mass Spectrometer (IRMS) directly coupled to a Costech Elemental Analyzer (ECS 4010). The $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios are expressed in conventional delta (δ) notation, relative to atmospheric N_2 (atm) and Vienna Pee Dee Belemnite (VPDB), respectively. Peptone was used as a laboratory standard. The precision of analyses, expressed as one standard deviation from multiple analyses of peptone (n = 90) conducted during runs of samples for fish, was 0.2‰ for $\delta^{15}N$ and 0.1‰ for $\delta^{13}C$.

3.2.4 Stable Isotope Mixing Model

The Bayesian isotopic mixing model, SIAR (Stable Isotope Analysis in R, Version 4.1.1, Parnell and Jackson 2011), was used to determine the relative proportions of prey in fish diets. $\delta^{15}N$ and $\delta^{13}C$ values of whole fish were grouped by year and length class to determine dietary proportions of the corresponding sampled population. The target sample size of predators was five fish (per species, per year, per length class); however, this size of sample was not available in some cases (Table 3-2), e.g., specimens of Canadian Eelpout from the research cruise in 2008 were not available for analysis.

Muscle turnover rates and associated stable isotope signatures were used as a proxy for whole fish turnover in this study. Isotopic turnover for fish muscle is approximately one year for nitrogen and 10 months for carbon (Buchheister and Latour 2010). Therefore, stable isotope ratios of whole fish collected in summer 2008 would represent the averaged diet consumed from summer 2007 to summer 2008. Mixing model results described integrated fish diets from the previous year.

Isotope mixing model performance decreases when too many prey groups are included (Parnell et al. 2010). Thus, non-metric multidimensional scaling (MDS) ordination of stable nitrogen and carbon isotopes of fish prey was used to visually portray the correlation of prey taxa (PRIMER v. 6.1). MDS ordination plots have no interpretable axes and are based on simple matching coefficients calculated between pairs of prey taxa samples. For MDS analysis, absolute values of stable isotopes were normalized, and a Euclidean distance matrix was calculated. Taxa represented by points that are closer together in an MDS plot have similar isotope signatures; taxa that are farther apart are less similar and correspond to different values (Clarke et al. 2008). A stress of <0.1 is considered to be a good fit while a stress of <0.2 is a reasonable fit (Clarke and Warwick 2001).

The mean and standard deviation for $\delta^{15}N$ and $\delta^{13}C$ of each prey group were averaged across years and inserted into the isotope mixing models. Averaged $\delta^{15}N$ and $\delta^{13}C$ values of prey groups were adjusted to account for trophic enrichment factors of fish tissue, i.e., 3.8‰ for $\delta^{15}N$ (Hobson and Welch 1992) and 2.0‰ for $\delta^{13}C$ (Bosley et al. 2002; Barnes et al. 2007; Sweeting et

al. 2007). In order to create the standard deviations for the trophic enrichment factor, instrument error was doubled, i.e., ± 0.4‰ for $\delta^{15}N$ and ± 0.2‰ for $\delta^{13}C$. Mixing model estimates are presented as 50%, 75%, and 95% credibility intervals (Bayesian confidence intervals) and as a mean percent contribution to diet for each prey group.

For each individual fish for which $\delta^{15}N$ was analyzed, trophic level was calculated using the equation:

$$TL_{fish} = (\delta^{15}N_{consumer} - \delta^{15}N_{primary\ consumer})/3.4 + 2$$

where TL_{fish} is trophic level of fish predator, consumer is the fish predator, primary consumer is copepod, and $\delta^{15}N$ values were from samples that had not been acid-fumed or lipid-extracted. The baseline value of $\delta^{15}N$ for the primary consumer was the average for copepods from fish stomachs in this study (8.77**)**. This value of $\delta^{15}N$ for copepods is within the spring and summer variation of *Calanus glacialis* (9.09 ± 0.66 to 12. 41 ± 0.59) in Amundsen Gulf in the eastern Beaufort Sea (Forest et al. 2011). The mean $\delta^{15}N$ enrichment of *C. glacialis* was 2.8–4.7‰. The increase of $\delta^{15}N$ in marine food webs is usually 3–4‰ per trophic level (Michener and Schell 1994). The average trophic nitrogen fractionation for aquatic consumers, 3.4 (Vander Zanden and Rasmussen 2001, Post 2002), is the enrichment of $\delta^{15}N$ between trophic levels that we used in these equations and other recent trophic analyses for the Chukchi Sea (Iken et al. 2010, Tu et al. 2015).

Mixing models were examined using different sample sizes of predator tissues. A larger quantity of samples was available for fish muscle compared to the five samples of whole fish analyzed for this study. Mixing models for Stout Eelblenny collected in 2009 were compared using sample sizes of 5, 10, 15, and 20 fish. Stable isotope samples were randomly chosen to be included in the mixing model. Increasing the predator sample size narrowed the credibility intervals such that confidence in largest sample size was highest because of the small credibility intervals (Chapter 3 Appendix 3.1). Thus, while our results provided sufficient information to detect interannual differences, we recommend a sample size of at least 10 predators for future studies.

Stable isotope ratios of fish prey groups from 2008 to 2010 were averaged prior to insertion into mixing models. As stable isotope ratios document integrated fish diets from the previous year (Buchheister and Latour 2010), it was appropriate to include stable isotope ratios of prey from the previous year into mixing models. Additionally, fish stomachs from 2009 contained some prey taxa that were not present in year 2008 fish stomach contents. Interannual differences in prey stable isotope ratios may impact mixing model results documenting interannual changes in fish diets. Mixing models for three fish species were compared when using stable isotope ratios of prey averaged across multiple years (2008−2010) versus using stable isotope ratios of prey from the previous year that fish were collected. The results were similar as the 95% credibility intervals and the means did not vary more than 4% between the mixing models (Chapter 3 Appendix 3-2). Therefore, averaging prey stable isotope ratios across years should suffice in capturing general changes in fish diets over time.

3.2.5 Statistical Analysis

Statistical tests were conducted in SigmaPlot Version 12.0 (Systat Software, Inc. 2011). Analysis of variance (ANOVA) was used to test the difference in mean stable isotope ratios among years for fish, with a significance level of 5%. If normality and equal variance assumptions were met, one-way ANOVA was used to determine if stable isotope ratios of fish differed among years. If differences were found, a pairwise multiple comparison procedure using the Holm-Sidak Method (t) was used to determine which years differed. If normality and equal variance assumptions were not met, a Kruskal-Wallis one-way ANOVA was used; a posthoc test was not necessary as no significant differences were found among years.

3.3 Results

3.3.1 Fish Prey Groups

Based on the MDS plots, stable nitrogen and carbon isotope ratios of fish prey (Figure 3-2) appeared to cluster into three groups: a low-trophic, pelagic group consisting of amphipods and copepods (Figures 3-3, 3-4); a low-trophic, benthic group consisting of mollusks, crabs, and cumaceans, and; a high-trophic group consisting of mysids, shrimps, tanaids, polychaetes, nematodes, and fishes. Euphausiids seemed to be intermediate. Barnacles, isopods, and ostracods, while present in stomach contents of fishes, were excluded from prey groupings, because the sample volume was too small for stable isotope analyses.

3.3.2 Short-term Diets Based on Stomach Content Analysis

There were no interannual differences in prey importance for small (<70 mm) Arctic Cod; however, diet of large-sized (>70 mm) Arctic Cod was dominated by copepods during 2010. The only major prey taxon for small Arctic Cod in 2009 and 2010 was copepods (Table 3-3). Copepods were more important to large-sized Arctic Cod diets in 2010 compared to 2008 or 2009. Euphausiids were more important to large Arctic Cod collected in 2009 compared to 2008.

There were no interannual differences in prey consumed by small Arctic Staghorn Sculpin based on stomach contents; conversely, large Arctic Staghorn Sculpin fed on relatively more benthic prey taxa during 2010. Primarily amphipods, and to a lesser extent polychaetes and cumaceans, were the most important diet items for small Arctic Staghorn Sculpin (Table 3-4). Main prey for large Arctic Staghorn Sculpin in 2008 and 2009 were similar to those of small Arctic Staghorn Sculpin in all years, although the importance of polychaetes was higher and amphipods lower in large Arctic Staghorn Sculpin diets. In 2010, fishes and mollusks were major taxa in the large Arctic Staghorn Sculpin diet, while the importance of polychaetes doubled and that of amphipods simultaneously decreased by about 50%.

The short-term diet of small-sized Canadian Eelpout was dominated by copepods during 2010; however, interannual variations in prey importance were not observed for large Canadian

Eelpout. In 2009, the major prey taxon for small Canadian Eelpout was amphipods. Amphipods were superseded by copepods as the most important prey taxa in 2010 (Table 3-5). Polychaetes were important diet items for small Canadian Eelpout in 2010 compared to 2009. Amphipods were a dominant prey for large Canadian Eelpout in both 2009 and 2010. Canadian Eelpout of either length class were unavailable for diet analysis in 2008.

Both small and large length classes of Stout Eelblenny showed interannual differences in stomach contents, with a relatively greater importance of nematodes in 2009 and 2010 compared to 2008 (Table 3-6). Copepods were a major prey taxon in all years for small Stout Eelblenny. Polychaetes and barnacles were also valuable food items in 2008. In 2010, amphipods and nematodes became leading diet items for small Stout Eelblenny, while copepods were less dominant. Main prey taxa for large Stout Eelblenny included copepods, amphipods, polychaetes, and nematodes. However, the relative importance of these prey taxa was not consistent throughout the years. The contribution of copepods to large Stout Eelblenny diet declined in 2009, and the dietary contribution of polychaetes and nematodes increased.

Stomach contents of small-sized Bering Flounder showed interannual differences in the contribution of mysids while only the main prey taxa varied for large-size Bering Flounder interannually. Amphipods were an important prey taxon for small Bering Flounder across all years, although their importance in stomach contents generally declined over the years. The importance of mysids increased from 2008 to 2010. Polychaetes, copepods, and euphausiids were also important to small Bering Flounder in some years, though without a consistent pattern (Table 3-7). As with small Bering Flounder, amphipods were major diet items for large Bering Flounder in all three years. Polychaetes were main prey items only in 2008, and the importance of mysids was highest in 2009. Shrimps were found to be major diet items for large Bering Flounder in 2009 and 2010.

3.3.3 Long-term Diets Using Stable Isotope Ratios and Mixing Models

Interannual trends in long-term diets were not documented for either length class of Arctic Cod. $\delta^{15}N$ or $\delta^{13}C$ values (Table 3.8) were not significantly different among years for either small (p = 0.103 and 0.100, respectively) or large Arctic Cod (p = 0.272 and 0.750, respectively; Figure 3-5). There was a slightly higher mean proportional contribution (~8%) of high-trophic prey to large Arctic Cod diets during 2008−2010 (Figure 3-6). The mean trophic level (TL) of small Arctic Cod was always less than that of large Arctic Cod and for both sizes was less in 2008 than in later years (Table 3-8).

Interannual trends were observed in the long-term diet of small and large Arctic Staghorn Sculpin. $\delta^{15}N$ or $\delta^{13}C$ values (Table 3-8) were not significantly different among years for small Arctic Staghorn Sculpin (p = 0.134 and 0.280, respectively; Figure 3-5). As with Arctic Cod, smaller fish had lower TL than larger fish and higher TL in 2010 than in 2009 (Table 3-8). Mixing models illustrated that high-trophic prey had the highest mean proportional contribution

to long-term diets of small Arctic Staghorn Sculpin during 2009/2010 compared to 2007−2009 (Figure 3-7). Large Arctic Staghorn Sculpin from 2008 (t = 2.744, p = 0.038) and 2009 (t = 3.716, p = 0.010) had significantly higher $\delta^{13}C$ values than fish from 2010 (Figure 3-5). Likewise, mixing models showed large Arctic Staghorn Sculpin consumed more low-trophic, benthic prey during 2007−2009 compared to 2009/2010 (Figure 3-7). During 2009/2010, high- trophic prey had a higher mean proportional contribution to long-term diets of large Arctic Staghorn Sculpin compared to diets during 2007−2008, i.e., 50% vs. 36%, respectively. However, 95% credibility intervals overlapped among years for these prey groupings and potential interannual differences were not considered significant (Payton et al. 2003; Julious 2004).

Interannual trends were not apparent in the long-term diets of both length classes of Canadian Eelpout. Small Canadian Eelpout from 2009 had significantly higher $\delta^{13}C$ values (Table 3-8) than fish from 2010 (t = 2.743, p = 0.025; Figure 3-5). However, small Canadian Eelpout did not always have smaller TLs than larger eelpouts, and there was not an interannual pattern (Table 3-8). Mixing models showed a slightly greater mean contribution of low-trophic, benthic prey to diets of small Canadian Eelpout in 2008/2009 than in 2009/2010 (6%; Figure 3-8). $\delta^{15}N$ or $\delta^{13}C$ values were not significantly different among years for large Canadian Eelpout (p = 0.056 and 0.818, respectively), and this matches mixing model results where the mean proportional contribution did not vary by more than 3% (Figure 3-8). Diet during 2007/2008 could not be assessed for Canadian Eelpout because no fish of either length class were available from 2008 for stable isotope analysis.

Interannual trends in the long-term diet of Stout Eelblenny were similar between both length classes. Small Stout Eelblenny from 2009 had significantly lower $\delta^{15}N$ values (Table 3-8) than fish from 2010 (t = 4.205, p = 0.006; Figure 3-5), and TL in 2009 was less than in 2010. Likewise, small Stout Eelblenny consumed proportionally more low-trophic, benthic prey during 2008/2009, while small fish fed on more high-trophic prey during 2009/2010 (Figure 3-9). Small Stout Eelblenny from 2008 were not available for stable isotope analysis to address feeding ecology during 2007/2008. Large Stout Eelblenny from 2008 had significantly lower $\delta^{15}N$ values (Table 3-8) than fish from 2009 (t = 4.439, p = 0.002) and 2010 (t = 6.770, p < 0.001) (Figure 3-5), and the TLs increased each year. Additionally, large Stout Eelblenny from 2009 had significantly lower $\delta^{15}N$ values than fish from 2010 (t = 2.331, p = 0.038). From 2007 to 2010, there was a decrease in the mean proportional contribution of low-trophic, benthic prey. The mean proportional contribution of high-trophic prey increased from 40% in 2007/2008 to 50% in 2008/2009 and stayed high in 2009/2010 (Figure 3-9). Although, for both length classes of Stout Eelblenny, 95% credibility intervals overlapped among years for prey groupings and potential differences were therefore not considered significant (Payton et al. 2003; Julious 2004).

Interannual trends in long-term diet were observed for both length classes of Bering Flounder. $\delta^{15}N$ or $\delta^{13}C$ values (Table 3-8) were not significantly different among years for small Bering Flounder (p = 0.496 and 0.072, respectively; Figure 3-5); yet, TLs increased each year, and mixing models showed small Bering Flounder fed on more low-trophic, pelagic prey during

2007/2008 compared to 2008−2010 (Figure 3-10). Large Bering Flounder from 2008 had significantly lower $\delta^{15}N$ values (Table 3-8) than fish from 2009 (t = 3.648, p = 0.010) and 2010 (t = 3.636, p = 0.007) (Figure 3-5). TL was lower in 2008 than in 2009 and 2010. Likewise, mixing models for large Bering Flounder demonstrated more high-trophic feeding during 2008−2010 compared to 2007/2008 (Figure 3-10). For both length classes of Bering Flounder, potential differences in mixing model results were not considered significant due to overlapping credibility intervals (Payton et al. 2003; Julious 2004).

3.4 Discussion

Fishes in the Arctic do not consume the same kinds of food each year. We showed that in some years, high-trophic prey were consumed in larger proportion, whereas the reverse might be true for the same species in other years. However, such changes are difficult to prove for extended periods of time (e.g., between years) using the customary method of analyzing stomach contents. We were able to identify and verify interannual trends in fish diets in the Arctic by combining traditional stomach content analysis with stable isotope ratios and stable isotope mixing models. Stable isotope analysis and mixing models are used increasingly in ecological studies to examine prey contributions and prey shifts (Bentzen et al. 2007, Yeakel et al. 2009; Bond and Diamond 2011, Dalerum et al. 2012). However, mixing models are based on a variety of assumptions and are only useful when combined with direct observations that identify prey sources; consequently, examination of contents of fish stomachs cannot be entirely replaced.

Stomach contents are not the best indicator of interannual changes to diet. Inspection of fish stomach contents provides high resolution of prey taxa in the gut, but only offers short-term diet information, yet fish diets can vary seasonally thus biasing interpretations (Hop et al. 1992; Eloranta et al. 2010). Stomach content analysis of Arctic fishes may be biased towards low-trophic, pelagic prey because many dietary studies of Arctic fishes in the Chukchi Sea take place during the summer/fall (Lowry and Frost 1981; Fechhelm et al. 1984; Coyle et al. 1997; Cui et al. 2012) when these prey taxa are more abundant. Unfortunately, Arctic Cod (Fechhelm et al. 1984) is the only one of the five species we examined for which winter gut contents have been examined. Mysids and fishes make up a greater proportion of stomach contents of Arctic Cod during the winter than summer (Craig et al. 1982), substantiating expected seasonal bias. Within-year differences in prey consumption emphasize the need for a tool that measures more than a snapshot of fish diet.

Other inherent biases in gut analysis reiterate the need for measurements that are less, or differently, biased. Portraying stomach contents as percent weight (Craig et al. 1982) may over represent prey taxa that are large (Hyslop 1980; Liao et al. 2001), e.g., shrimps versus mysids. Conversely, copepods are repeatedly found in stomachs of Arctic fishes in high numbers (Fechhelm et al. 1984), over-representing frequently occurring and numerous prey (Hyslop 1980; Liao et al. 2001). Furthermore, the importance of prey taxa in diets may be overestimated due to the small sample size of stomachs available for analysis, which is further confounded with

interannual disparities in the availability of some fish species in this study. In addition to temporal biases, regional differences in high-trophic prey availability may influence the interpretation of stomach content analysis of Arctic fishes. For instance, shrimps were more than 10% important in the short-term diet of Arctic Staghorn Sculpin collected from one site in the Chukchi Sea (Coyle et al. 1997).

The underestimation of soft-bodied prey that are readily digested, e.g., polychaetes and nematodes (Brush et al. 2012) is a prominent bias in stomach content analysis but it can be circumvented through stable isotope analysis. High-trophic polychaetes appear to contribute minimally to diets of Canadian Eelpout compared to low-trophic, hard-shell mollusks (Atkinson and Percy 1991). Low-trophic amphipods was the only major prey item identified in stomach contents of Canadian Eelpout in all years we studied, which is the opposite of our stable isotope results. Based on the high $\delta^{15}N$ values, Canadian Eelpout are high-trophic predators and likely consume polychaetes that cannot be seen in stomach contents because of digestion or off-season consumption. Therefore, high-trophic prey appear to be more important to diets of Arctic fishes than explained by stomach content analysis alone.

In some species-year combinations, there was agreement in trophic analysis with short- and long-term diets. Arctic Staghorn Sculpin gut contents and isotopes showed that high-trophic prey contributed more in 2010 than in the two earlier years. Their stomach contents in 1990 – 1991 also were dominated by polychaetes and other high-trophic prey (Coyle et al. 1997), validating our observations in 2010. Whereas both short- and long-term diets of Stout Eelblenny also were in agreement but displayed different interannual results: high-trophic prey made up more of food consumed during 2009 and 2010 than in 2008. Previous stomach content information is not available for Stout Eelblenny from the Arctic, but it is likely that soft-bodied polychaetes are primary prey for Stout Eelblenny as for Slender Eelblenny, a closely related species (Atkinson and Percy 1991).

Small Arctic fishes likely took advantage of the favorable pelagic foraging period of 2007. Mixing models showed that small Bering Flounder were feeding on proportionally more low-trophic, pelagic prey during 2007/2008 compared to 2008−2010. In 2007, there was an increase in the annual primary production in the Chukchi Sea compared to average values from 1998 to 2002 (Arrigo et al. 2008). Elevated primary production, warmer waters, and a longer open water season supported a higher abundance of pelagic crustacean grazers and consumers, e.g., copepods (Eisner et al. 2012). Copepod development was faster in 2007 compared to 2008, likely due to warmer waters (Matsuno et al. 2011). Moreover, the higher flow of warm Pacific Water into the Chukchi Sea may have led to the advection of copepods into the Chukchi Sea (Ashjian et al. 2010), adding to the high zooplankton biomass and diversity observed in 2007 (Matsuno et al. 2011). Feeding more extensively on these abundant pelagic crustaceans likely resulted in the lower $\delta^{15}N$ values of small Bering Flounder, thus leading to the observed mixing model results showing an increase in low-trophic, pelagic feeding. For three of the five fish species examined

in this study, small fish were not available from 2008 to add support to the mixing model results of low-trophic, pelagic feeding during 2007/2008.

An increase in the high-trophic feeding of Arctic fishes in years after 2007 may relate to the amount of seasonal production reaching the benthos. During 2008 and 2009, the density and diversity of macrofaunal animals, e.g., polychaetes, were greater than in 2010 (Blanchard et al. 2013). The greater density of these benthic organisms in 2008 and 2009 were supported by a greater proportion of zooplankton reaching the benthos (Blanchard et al. 2013). For instance, Pacific copepods advected into the Chukchi Sea in 2007 probably did not survive the Arctic winter (Grebmeier 2012), adding to the input of material to the benthic ecosystem. Arctic Staghorn Sculpin, Stout Eelblenny, and Bering Flounder likely took advantage of this heightened benthic productivity during 2009. From 2008 to 2010, there was an increase in the abundance and biomass of zooplankton in the Chukchi Sea (Questel et al. 2013). Increased grazing by these abundant planktonic consumers would decrease the amount of seasonal production to the seafloor, causing an increase in overwintering mortality of macrofauna and hence the decline in benthic density during 2010 (Blanchard et al. 2013). A decrease in benthic feeding during 2010 was not documented in the mixing models of Arctic fishes. High-trophic foraging of Arctic fishes during 2010 may be a result of consuming benthic prey that were isotopically enriched. Less input of seasonal production to the seafloor would mean benthic organisms would need to consume more recycled material, thus leading to higher δ^{15}N values of benthic consumers (Iken et al. 2010). In turn, fishes consuming these benthic organisms will have higher δ^{15}Nvalues. If benthic biomass continues to decrease, demersal Arctic fishes may switch to foraging more pelagically.

Mixing models provide insight into general changes in the feeding ecology of predators because taxonomic resolution of stable isotopes is limited. For instance, prey taxa had to be assigned to mixing model groups to create signatures that are more distinct in isotopic space than if all prey taxa were included in the model. Moreover, these groups are not going to consist of the same prey taxa in all instances because stable isotope ratios may vary by region (Dunton et al. 2006; Iken et al. 2010) or season (Forest et al. 2011). Regional differences in stable isotope ratios of prey taxa in the Arctic could lead to multiple interpretations. For instance, prey species collected in the Beaufort Sea (Dunton et al. 2012) had lower δ^{13}C values than prey collected from the Chukchi during this study. When examining fish that mixing models indicated were foraging more pelagically, the fish may have actually been foraging in the Beaufort Sea as both factors lead to lower δ^{13}C values (Figure 1-1). Care should be taken when interpreting mixing model estimates. Isotope mixing models can be a more useful tool when they are applied to studies encompassing a smaller regional scale, thus eliminating some of the many factors that influence stable carbon isotopes (Figure 1-1). We showed that mixing models are a useful tool when assessing interannual changes in trophic feeding,

Turnover rate of fish tissues may be longer than the rate assumed in this study. The estimated one-year isotope turnover rate used in this study is based on experimental studies on summer

flounder (*Paralichthys dentatus*) in water temperatures of 20°C (Buchheister and Latour 2010). Turnover rates are slower for juvenile winter flounder (*Pseudopleuronectes americanus*) in 13°C compared to 18 °C water temperatures (Bosley et al. 2002). The isotopic turnover rate may be much slower for fish living in water temperatures less than 5°C experienced in the Chukchi Sea (Weingartner et al. 2013). Additionally, turnover rates may be slower for adult versus juvenile fish due to reduced growth and metabolism of older fish (Weidel et al. 2011). Likewise, physiological changes associated with maturity or age may affect the time frame reflected by stable isotope ratios in Arctic fishes.

Understanding the feeding ecology of Arctic fishes during low ice years could be enhanced by employing additional techniques to complement stomach content and stable isotope analysis. Because stomach contents can underestimate soft-bodied prey (Brush et al. 2012), DNA analysis of predators' feces could increase detection of these elusive prey taxa (Casper et al. 2007; Tollit et al. 2009); however, this is impractical for fish predators in the wild and still only provides a snapshot view of diet. Alternatively, examining trophic structure using fatty acid analysis of fish and their prey does not have a short-term bias. Similar to stable isotopes, fatty acid signatures present an integrated long-term view of a predator's diet, but with higher taxonomic resolution (Bowen and Iverson 2012). In addition, fatty acid analysis can give better insight into interannual differences in the consumption and contribution of ice algae versus phytoplankton (Scott et al. 1999) and these differences could be traced through the food web (Budge et al. 2008), thus establishing a more direct connection to interannual changes in trophic structure during low ice years.

In conclusion, our results show variation in the diet of Arctic fishes during years of reduced ice cover, likely due to the higher abundance of pelagic crustaceans. Sea ice extent in 2012 was the lowest on record, even superseding the previous minimum ice extent of September 2007 (NSIDC 2012). Continued low sea ice extent (NSIDC 2012) may influence the diet of Arctic fishes, and consequently their health and abundance. Warm climate conditions in the Arctic have been shown to favor larger, lipid-rich copepods over the smaller species (Questel et al. 2013); in turn, this could positively impact fish populations (Coyle et al. 2011). Fishes are important players in the short Arctic food chain leading to high-trophic level predators such as ice seals and polar bears (*Ursus maritimus*). Thus our detection of short- and long-term variability in fish diets and consequent change in the trophic level of the fish itself may help explain changes at higher trophic levels.

3.5 Acknowledgments

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3.6 Tables

Table 3-1. **Inventory of Fishes for Stomach Content Analysis**. Number of stomachs analyzed from fishes collected from the Chukchi Sea. (*n*) = number of fish with empty stomachs. Minimum, maximum, and mean lengths exclude fish with empty stomachs.

		Length Comparison for Stomach Content Analysis												
		2008				2009				2010				
Species		*n*	min length [mm]	max length [mm]	mean length [mm]	*n*	min length [mm]	max length [mm]	mean length [mm]	*n*	min length [mm]	max length [mm]	mean length [mm]	**Total *n***
Arctic cod														
(Boreogadus saida)	small (≤70 mm)	0	-	-	-	43 (8)	16	70	41	165 (5)	27	70	53	208 (13)
	large (>70 mm)	20 (3)	78	252	112	84 (4)	71	215	107	90 (6)	71	159	89	194 (13)
Arctic staghorn sculpin														
(Gymnocanthus tricuspis)	small (≤70 mm)	21	46	67	53	46 (5)	31	70	53	40 (3)	31	64	41	107 (8)
	large (>70 mm)	18 (1)	74	134	95	31	71	120	81	6	71	107	85	55 (1)
Canadian eelpout														
(Lycodes polaris)	small (≤70 mm)	0	-	-	-	40 (18)	37	70	57	29 (2)	31	70	47	69 (20)
	large (>70 mm)	0	-	-	-	61 (4)	71	200	104	55 (9)	71	200	111	116 (13)
Stout eelblenny														
(Anisarchus medius)	small (≤70 mm)	1	70	70	70	13	43	64	55	6	60	70	65	20
	large (>70 mm)	20	73	158	127	90 (4)	71	139	103	96 (3)	76	131	105	206 (7)
Bering flounder														
(Hippoglossoides robustus)	small (≤70 mm)	28 (2)	39	70	57	72 (2)	29	70	51	14	40	63	48	114 (4)
	large (>70 mm)	18 (1)	73	168	114	30 (2)	71	179	92	14 (2)	73	188	93	62 (5)

Table 3-2. **Inventory of Fishes for Stable Isotope Analysis**. Sample sizes and lengths (min, max, and mean) of Arctic fishes analyzed for stable nitrogen and carbon isotope ratios.

		Length Comparison for Stable Isotope Analysis												
		2008				2009				2010				
Species		*n*	min length [mm]	max length [mm]	mean length [mm]	*n*	min length [mm]	max length [mm]	mean length [mm]	*n*	min length [mm]	max length [mm]	mean length [mm]	**Total *n***
Arctic cod														
(Boreogadus saida)	small (≤70 mm)	0	-	-	-	5	36	43	40	5	46	58	53	10
	large (>70 mm)	5	80	123	97	5	104	132	122	5	71	109	91	15
Arctic staghorn sculpin														
(Gymnocanthus tricuspis)	small (≤70 mm)	5	49	67	53	5	37	43	40	5	35	64	54	15
	large (>70 mm)	5	80	90	85	5	76	113	88	4	75	107	90	14
Canadian eelpout														
(Lycodes polaris)	small (≤70 mm)	0	-	-	-	5	40	61	45	5	39	44	42	10
	large (>70 mm)	0	-	-	-	5	71	176	121	5	78	139	104	10
Stout eelblenny														
(Anisarchus medius)	small (≤70 mm)	0	-	-	-	3	59	64	62	5	60	67	63	8
	large (>70 mm)	5	77	148	120	5	84	132	105	5	98	131	108	15
Bering flounder														
(Hippoglossoides robustus)	small (≤70 mm)	5	44	65	59	5	49	67	60	5	41	63	53	15
	large (>70 mm)	5	75	101	82	5	76	179	112	5	79	188	112	15

Table 3-3. **Diet Composition of Arctic Cod**. Numbers represent % IRI (index of relative importance). Prey taxa contributing more than 5% to diet in any given year are in bold. Prey that were consumed as early life stages are indicated with an asterisk (*). Prey taxa organized from highest to lowest % IRI. *n* is count of stomachs analyzed for each species, excluding the number of empty stomachs.

Arctic cod *(Boreogadus saida)*	**Small (≤ 70 mm)**			**Large (> 70 mm)**		
	n = 0	*n* = 35	*n* = 160	*n* = 17	*n* = 80	*n* = 84
Prey	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**
Copepods	-	**99.8**	**100.0**	**66.3**	**48.6**	**91.6**
Amphipods	-	-	< 0.1	**31.2**	**33.9**	**6.6**
Euphausiids	-	< 0.1	< 0.1	1.2	**12.6**	1.2
Fishes	-	< 0.1	-	0.4	4.0	< 0.1
Cumaceans	-	-	-	0.1	0.5	0.4
Barnacles *	-	0.2	< 0.1	0.4	< 0.1	< 0.1
Shrimps *	-	-	-	-	0.4	0.1
Other Crustaceans	-	-	< 0.1	0.4	< 0.1	< 0.1
Crabs *	-	-	-	-	0.1	-
Mysids	-	-	-	-	< 0.1	< 0.1
Mollusks	-	< 0.1	-	-	-	-
Polychaetes	-	-	-	-	< 0.1	< 0.1
Isopods	-	-	-	-	< 0.1	< 0.1
Animals, unidentified	-	-	-	-	< 0.1	-
Tanaids	-	-	-	-	-	< 0.1

Table 3-4. **Diet Composition of Arctic Staghorn Sculpin**. Numbers represent % IRI (index of relative importance). Prey taxa contributing more than 5% to diet in any given year are in bold. Prey that were consumed as early life stages are indicated with an asterisk (*). Prey taxa organized from highest to lowest % IRI. *n* is count of stomachs analyzed for each species, excluding the number of empty stomachs.

Arctic staghorn sculpin *(Gymnocanthus tricuspis)*	**Small (≤ 70 mm)**			**Large (> 70 mm)**		
	n = 21	*n* = 41	*n* = 37	*n* = 17	*n* = 31	*n* = 6
Prey	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**
Amphipods	**90.4**	**86.7**	**89.4**	**79.8**	**82.9**	**29.3**
Polychaetes	**5.0**	**9.0**	**3.1**	**15.1**	**15.4**	**38.7**
Mollusks	-	0.4	< 0.1	-	< 0.1	**18.0**
Fishes	-	< 0.1	-	-	< 0.1	**12.4**
Cumaceans	3.9	0.1	4.3	0.5	0.1	-
Crustaceans, unidentified	0.2	< 0.1	< 0.1	4.3	-	1.5
Copepods	0.4	1.3	2.4	-	-	-
Barnacles *	< 0.1	2.4	0.6	-	-	-
Sponges	-	< 0.1	0.2	-	1.4	-
Shrimps *	-	-	-	0.2	-	-
Euphausiids	-	-	< 0.1	-	0.1	-
Ostracods	-	0.1	-	-	-	-
Nematodes	< 0.1	-	-	-	-	-
Crabs *	-	< 0.1	-	-	-	-
Mysids	-	-	-	-	< 0.1	-
Echinoderms	-	< 0.1	-	-	-	-

Table 3-5. **Diet Composition of Canadian Eelpout**. Numbers represent % IRI (index of relative importance). Prey taxa contributing more than 5% to diet in any given year are in bold. Prey that were consumed as early life stages are indicated with an asterisk (*). Prey taxa organized from highest to lowest % IRI. *n* is count of stomachs analyzed for each species, excluding the number of empty stomachs.

Canadian eelpout *(Lycodes polaris)*	**Small (≤ 70 mm)**			**Large (> 70 mm)**		
	n = 0	*n* = 22	*n* = 27	*n* = 0	*n* = 57	*n* = 46
Prey	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**
Amphipods	**-**	**91.4**	**25.6**	**-**	**96.4**	**93.6**
Copepods	**-**	4.8	**66.9**	-	0.2	1.2
Polychaetes	**-**	0.3	**6.9**	-	3.1	1.2
Barnacles *	-	3.3	< 0.1	-	< 0.1	< 0.1
Shrimps *	-	-	-	-	-	1.9
Mollusks	-	-	0.2	-	< 0.1	1.5
Cumaceans	-	0.1	0.3	-	0.2	0.6
Mysids	-	-	-	-	< 0.1	0.1
Sponges	-	-	-	-	0.1	-
Fishes	-	-	-	-	0.1	-
Crustaceans, unidentified	-	-	< 0.1	-	-	-
Isopods	-	-	-	-	< 0.1	-
Ostracods	-	-	-	-	< 0.1	-
Animals, unidentified	-	-	-	-	-	< 0.1
Crabs *	-	-	-	-	-	-

Table 3-6. **Diet Composition of Stout Eelblenny**. Numbers represent % IRI (index of relative importance). Prey taxa contributing more than 5% to diet in any given year are in bold. Prey that were consumed as early life stages are indicated with an asterisk (*). Prey taxa organized from highest to lowest % IRI. *n* is count of stomachs analyzed for each species, excluding the number of empty stomachs.

Stout eelblenny *(Anisarchus medius)*	**Small (≤ 70 mm)**			**Large (> 70 mm)**		
	n = 1	*n* = 13	*n* = 6	*n* = 20	*n* = 86	*n* = 93
Prey	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**
Copepods	**83.2**	**86.4**	**28.3**	**65.6**	**43.9**	**67.8**
Amphipods	**-**	4.9	**39.8**	**24.0**	**26.6**	**14.7**
Nematodes	**-**	3.5	**25.3**	0.3	**11.8**	**8.0**
Barnacles *	**8.9**	2.5	2.5	**5.3**	1.2	0.6
Polychaetes	**7.8**	0.5	**-**	0.6	**8.1**	2.1
Mollusks	-	-	0.7	2.5	3.6	2.9
Cumaceans	-	1.4	0.1	0.6	3.1	0.6
Tanaids	-	0.5	3.2	< 0.1	1.1	0.8
Ostracods	-	-	0.1	1.1	0.5	1.1
Plants, unidentified	-	-	-	-	-	1.2
Isopods	-	0.1	-	-	< 0.1	< 0.1
Crustaceans, unidentified	-	-	-	-	< 0.1	0.1
Animals, unidentified	-	-	-	< 0.1	< 0.1	< 0.1
Mysids	-	-	-	-	< 0.1	< 0.1
Euphausiids	-	-	-	-	< 0.1	< 0.1
Fishes	-	-	-	-	< 0.1	< 0.1
Sponges	-	-	-	-	< 0.1	< 0.1

Table 3-7. **Diet Composition of Bering Flounder**. Numbers represent % IRI (index of relative importance). Prey taxa contributing more than 5% to diet in any given year are in bold. Prey that were consumed as early life stages are indicated with an asterisk (*). Prey taxa organized from highest to lowest % IRI. *n* is count of stomachs analyzed for each species, excluding the number of empty stomachs.

Bering flounder *(Hippoglossoides robustus)*	**Small (≤ 70 mm)**			**Large (> 70 mm)**		
	n = 26	*n* = 70	*n* = 14	n = 17	n = 28	n = 12
Prey	**2008**	**2009**	**2010**	**2008**	**2009**	**2010**
Amphipods	**82.2**	**65.4**	**51.3**	**61.0**	**47.3**	**56.8**
Mysids	2.3	**17.4**	**39.8**	2.4	**34.0**	**5.5**
Polychaetes	**8.4**	0.7	-	**30.2**	0.8	0.2
Euphausiids	0.2	< 0.1	**6.7**	-	0.6	**27.9**
Shrimps *	-	0.1	-	-	**14.4**	**9.2**
Copepods	2.8	**15.7**	1.7	-	< 0.1	-
Crustaceans, unidentified	3.7	0.1	0.1	2.2	-	-
Fishes	0.1	0.2	-	3.8	1.7	-
Mollusks	0.3	< 0.1	-	-	0.6	-
Isopods	-	0.2	0.3	-	0.1	-
Cumaceans	0.1	< 0.1	-	-	0.2	0.2
Barnacles *	-	< 0.1	-	0.2	-	0.2
Nematodes	-	-	-	0.2	< 0.1	-
Animals, unidentified	-	< 0.1	-	-	0.1	-
Echinoderms	-	-	-	-	0.1	-
Ostracods	-	< 0.1	-	-	0.1	-
Plants, unidentified	-	-	-	< 0.1	< 0.1	-

Table 3-8. **Trophic Level and Stable Isotope Values for Fishes.** Mean and standard deviation by length class and year.

Fishes Family, scientific and common names	Length Class	Year	δ^{15}N and Trophic Level *n*	Trophic Level Mean ± StDev	δ15N Mean ± StDev	δ^{13}C *n*	mean ± StDev
GADIDAE (CODS)							
Boreogadus saida	≤70 mm	2009	9	2.9 ± 0.2	11.73 ± 0.64	9	-20.54 ± 0.78
Arctic Cod	≤70 mm	2010	5	3.1 ± 0.1	12.60 ± 0.35	5	-20.28 ± 0.41
	≤70 mm Total		**14**	**3.0 ± 0.2**	**12.04 ± 0.69**	**14**	**-20.45 ± 0.67**
	>70 mm	2008	5	3.4 ± 0.3	13.61 ± 1.05	5	-19.29 ± 0.77
	>70 mm	2009	10	3.6 ± 0.2	14.24 ± 0.69	10	-19.50 ± 0.32
	>70 mm	2010	5	3.6 ± 0.2	14.32 ± 0.55	5	-19.63 ± 0.90
	>70 mm Total		**20**	**3.6 ± 0.2**	**14.10 ± 0.78**	**20**	**-19.48 ± 0.60**
COTTIDAE (SCULPINS)							
Gymnocanthus tricuspis	≤70 mm	2008	5	3.4 ± 0.4	13.60 ± 1.25	5	-18.48 ± 0.65
Arctic Staghorn Sculpin	≤70 mm	2009	5	3.2 ± 0.1	12.88 ± 0.30	5	-19.11 ± 0.24
	≤70 mm	2010	5	3.6 ± 0.3	14.19 ± 1.03	5	-18.82 ± 0.76
	≤70 mm Total		**15**	**3.4 ± 0.3**	**13.56 ± 1.04**	**15**	**-18.80 ± 0.61**
	>70 mm	2008	5	3.6 ± 0.4	14.16 ± 1.31	5	-17.77 ± 0.66
	>70 mm	2009	5	4.0 ± 0.1	15.53 ± 0.49	10	-17.88 ± 0.55
	>70 mm	2010	4	4.0 ± 0.1	15.59 ± 0.37	4	-18.66 ± 0.46
	>70 mm Total		**14**	**3.8 ± 0.3**	**15.06 ± 1.05**	**19**	**-18.02 ± 0.64**
ZOARCIDAE (EELPOUTS)							
Lycodes polaris	≤70 mm	2009	5	4.1 ± 0.2	15.83 ± 0.61	5	-17.95 ± 0.60
Canadian Eelpout	≤70 mm	2010	5	4.0 ± 0.1	15.56 ± 0.30	5	-18.89 ± 0.47
	≤70 mm Total		**10**	**4.0 ± 0.1**	**15.70 ± 0.48**	**10**	**-18.42 ± 0.71**
	>70 mm	2009	5	4.0 ± 0.1	15.44 ± 0.45	10	-17.77 ± 0.75
	>70 mm	2010	5	4.2 ± 0.1	16.30 ± 0.42	5	-18.26 ± 0.33
	>70 mm Total		**10**	**4.1 ± 0.2**	**15.87 ± 0.61**	**15**	**-17.93 ± 0.67**
STICHAEIDAE (PRICKLEBACKS)							
Anisarchus medius	≤70 mm	2009	3	3.6 ± 0.1	14.32 ± 0.40	3	-18.92 ± 0.29
Stout Eelblenny	≤70 mm	2010	5	4.1 ± 0.2	16.02 ± 0.62	5	-18.49 ± 0.48
	≤70 mm Total		**8**	**3.9 ± 0.3**	**15.38 ± 1.02**	**8**	**-18.65 ± 0.45**
	>70 mm	2008	5	3.8 ± 0.1	14.78 ± 0.35	5	-17.16 ± 0.33
	>70 mm	2009	5	4.1 ± 0.2	16.00 ± 0.52	10	-17.71 ± 0.69
	>70 mm	2010	5	4.3 ± 0.1	16.64 ± 0.41	5	-17.92 ± 0.54
	>70 mm Total		**15**	**4.1 ± 0.3**	**15.81 ± 0.90**	**20**	**-17.63 ± 0.62**
PLEURONECTIDAE (FLATFISHES)							
Hippoglossoides robustus	≤70 mm	2008	5	3.4 ± 0.4	13.46 ± 1.29	5	-18.63 ± 0.24
Bering Flounder	≤70 mm	2009	5	3.5 ± 0.2	13.78 ± 0.56	5	-17.97 ± 0.72
	≤70 mm	2010	5	3.6 ± 0.2	14.14 ± 0.62	5	-19.18 ± 1.05
	≤70 mm Total		**15**	**3.5 ± 0.3**	**13.79 ± 0.87**	**15**	**-18.59 ± 0.86**
	>70 mm	2008	5	3.5 ± 0.2	13.87 ± 0.55	5	-18.54 ± 0.50
	>70 mm	2009	5	3.9 ± 0.2	15.25 ± 0.68	10	-18.36 ± 0.30
	>70 mm	2010	5	3.9 ± 0.2	15.24 ± 0.55	5	-18.42 ± 0.18
	>70 mm Total		**15**	**3.8 ± 0.3**	**14.79 ± 0.87**	**20**	**-18.42 ± 0.33**
Total Count			246				

3.7 Figures

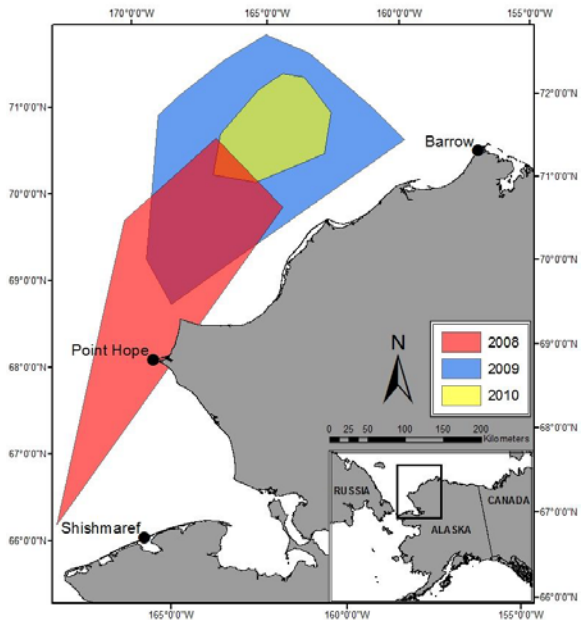


Figure 3-1. **Sample Collection Map**. Map showing areas of fish collections in the Chukchi Sea in 2008, 2009, and 2010.

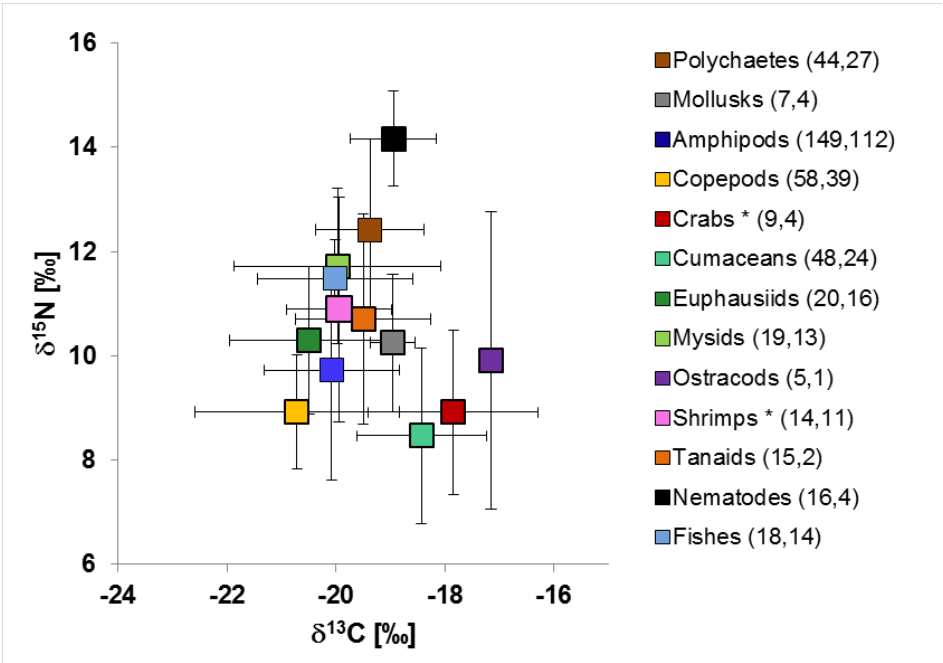


Figure 3-2. **Stable Isotope Signatures for Fish Prey**. Stable nitrogen and stable carbon isotope ratios for prey from the stomach contents of Arctic fishes. Stable isotope ratios of prey taxa were averaged across multiple years, 2008 – 2010. Numbers next to taxa represent count of samples analyzed for $\delta^{15}N$ and $\delta^{13}C$ values. Bars are standard deviations.

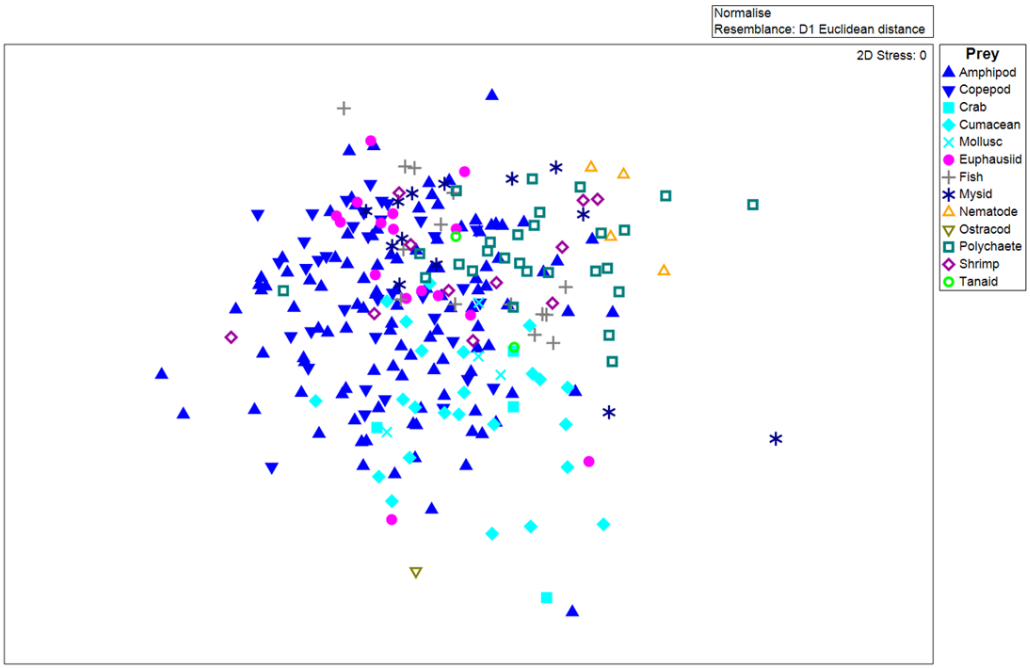


Figure 3-3. **MDS Plots of Fish Prey**. Non-metric multidimensional scaling of stable nitrogen and carbon isotope ratios in fish prey taxa pooled across multiple years, 2008–2010. Colors indicate groups of prey with similar values: amphipods – copepods, mollusks – juvenile crabs – cumaceans, and all other prey.

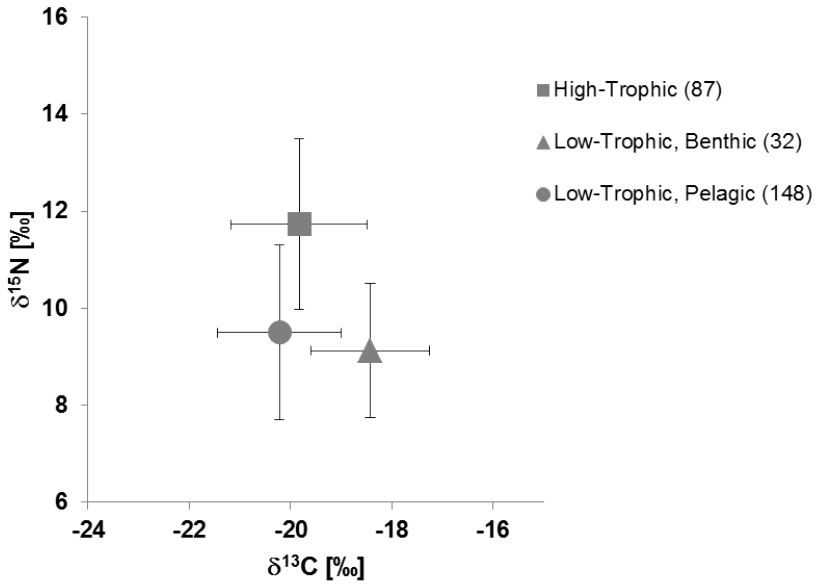


Figure 3-4. **Stable Isotope Signatures for Fish Prey Groups**. Stable nitrogen and carbon isotope ratios of fish prey groups used in mixing models. Prey groups were created based on MDS plots (See Figure 3-2). Fish prey groups consist of low-trophic, pelagic (amphipods, copepods), low-trophic, benthic (mollusks, juvenile crabs, cumaceans), and high-trophic prey taxa (polychaetes, euphausiids, mysids, juvenile shrimps, tanaids, nematodes, fishes). Numbers next to prey groups represent count of samples analyzed for $\delta^{15}N$ and $\delta^{13}C$ values. Standard deviations are illustrated by the bars.

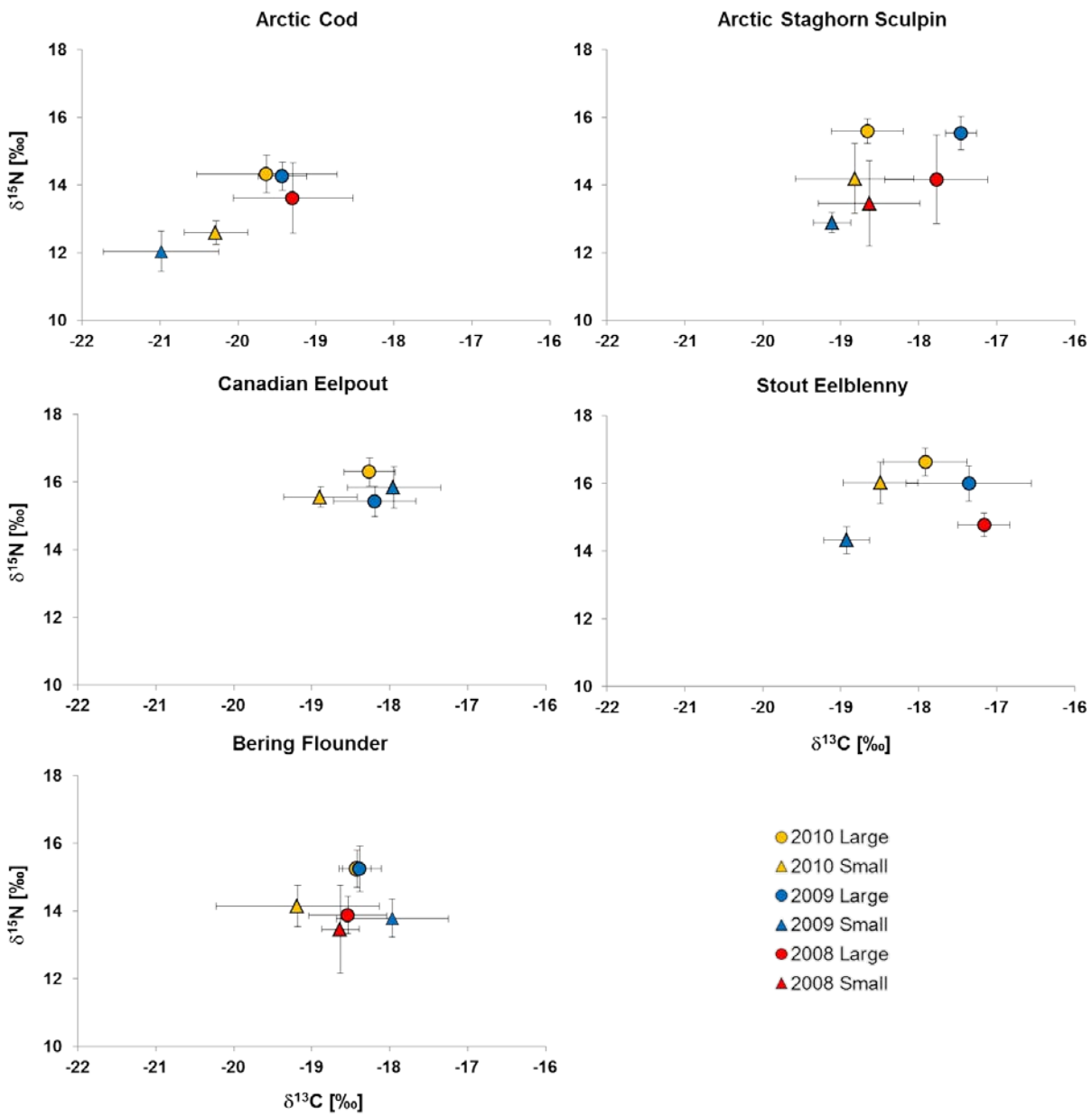


Figure 3-5. **Interannual Stable Isotope Signatures for Fishes**. Stable nitrogen and carbon isotope ratios of each fish species interannually and by length class, small (≤ 70 mm) and large fishes (> 70 mm). Symbols represent mean values; bars are standard deviations. For each *n*=5, except 2010 large Arctic Staghorn Sculpin (*n*=4) and 2009 small Stout Eelblenny (*n*=3).

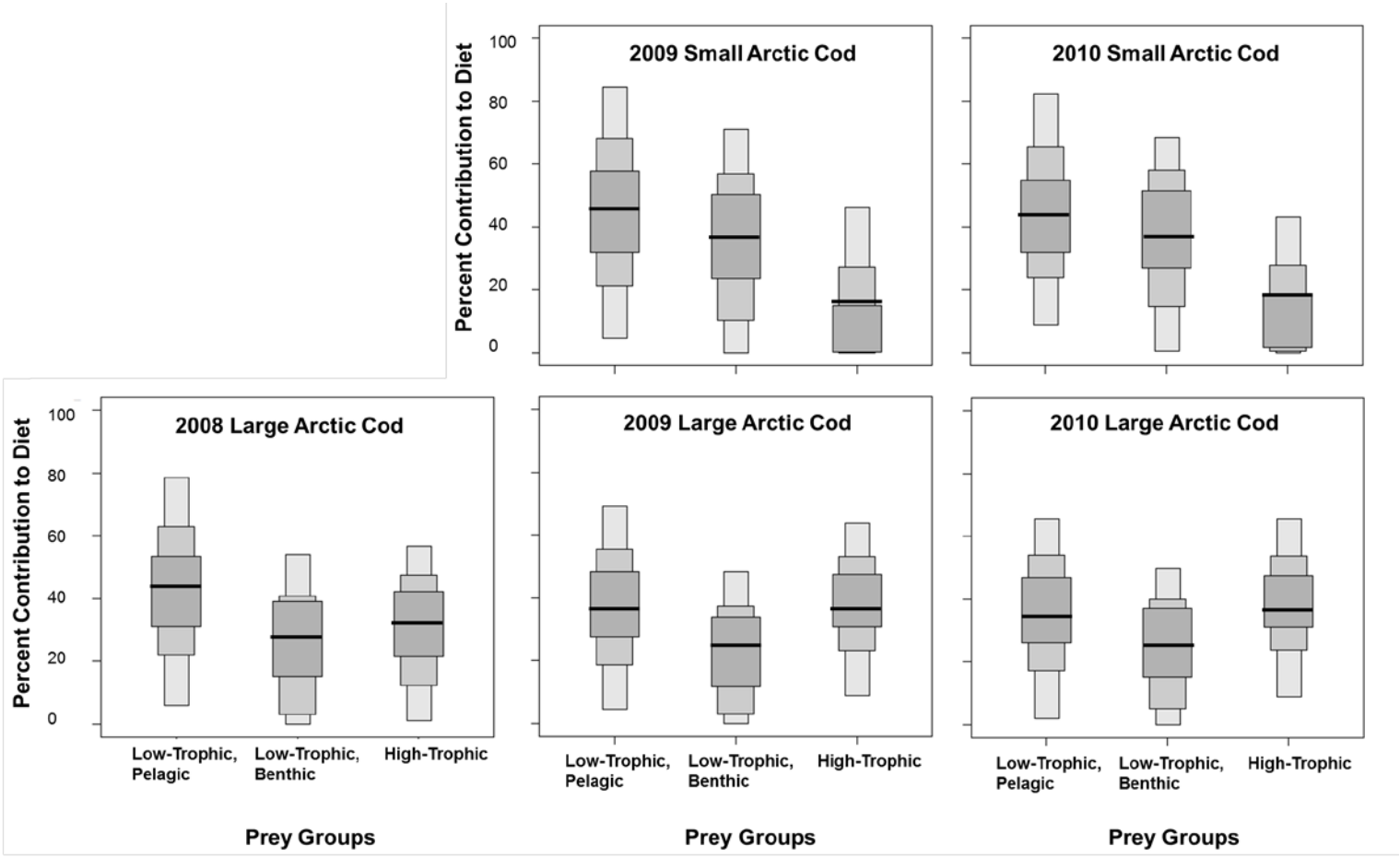


Figure 3-6. **Isotope Mixing Models for Arctic Cod**. Proportional contribution of low- and high-trophic prey to diets of small (≤ 70 mm) and large (> 70 mm) Arctic Cod as 50%, 75%, and 95% credibility intervals and mean values (vertical black line) based on the SIAR mixing model. Year indicates fish collection; mixing models show diet averaged from the past year, e.g., 2008 large Arctic Cod figure shows diet averaged across 2007–2008. See Figure 3-4 for fish prey groups.

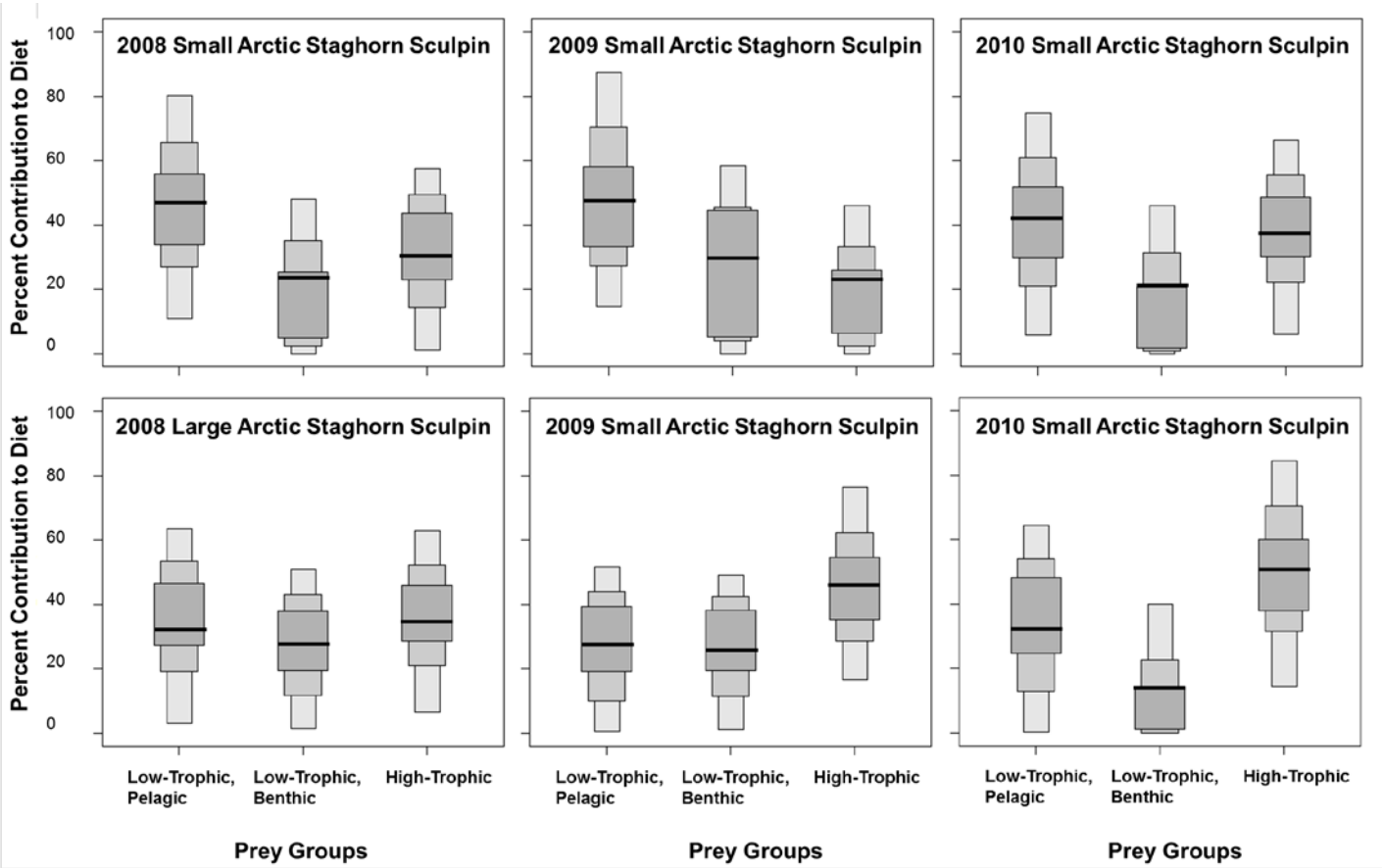


Figure 3-7. **Isotope Mixing Models for Arctic Staghorn Sculpin**. Proportional contribution of low- and high-trophic prey to diets of small (≤ 70 mm) and large (> 70 mm) Arctic Staghorn Sculpin as 50%, 75%, and 95% credibility intervals and mean values (vertical black line) based on the SIAR mixing model. Year indicates fish collection; mixing models show diet averaged from the past year, e.g., 2008 large Arctic Staghorn Sculpin figure shows diet averaged across 2007–2008. See Figure 3-4 for fish prey groups.

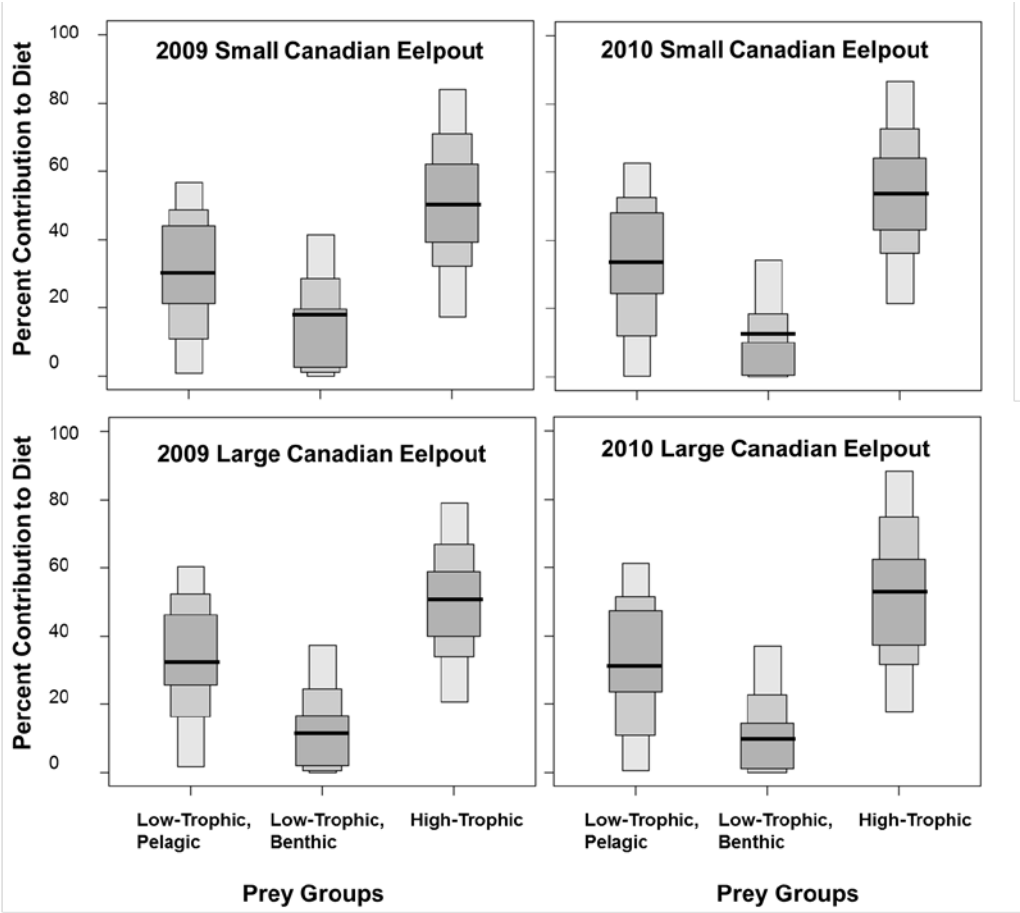


Figure 3-8. **Isotope Mixing Models for Canadian Eelpout**. Proportional contribution of low- and high-trophic prey to diets of small (≤ 70 mm) and large (> 70 mm) Canadian Eelpout as 50%, 75%, and 95% credibility intervals and mean values (vertical black line) based on the SIAR mixing model. Year indicates fish collection; mixing models show diet averaged from the past year, e.g., 2009 large Canadian Eelpout figure shows diet averaged across 2008–2009. See Figure 3-4 for fish prey groups.

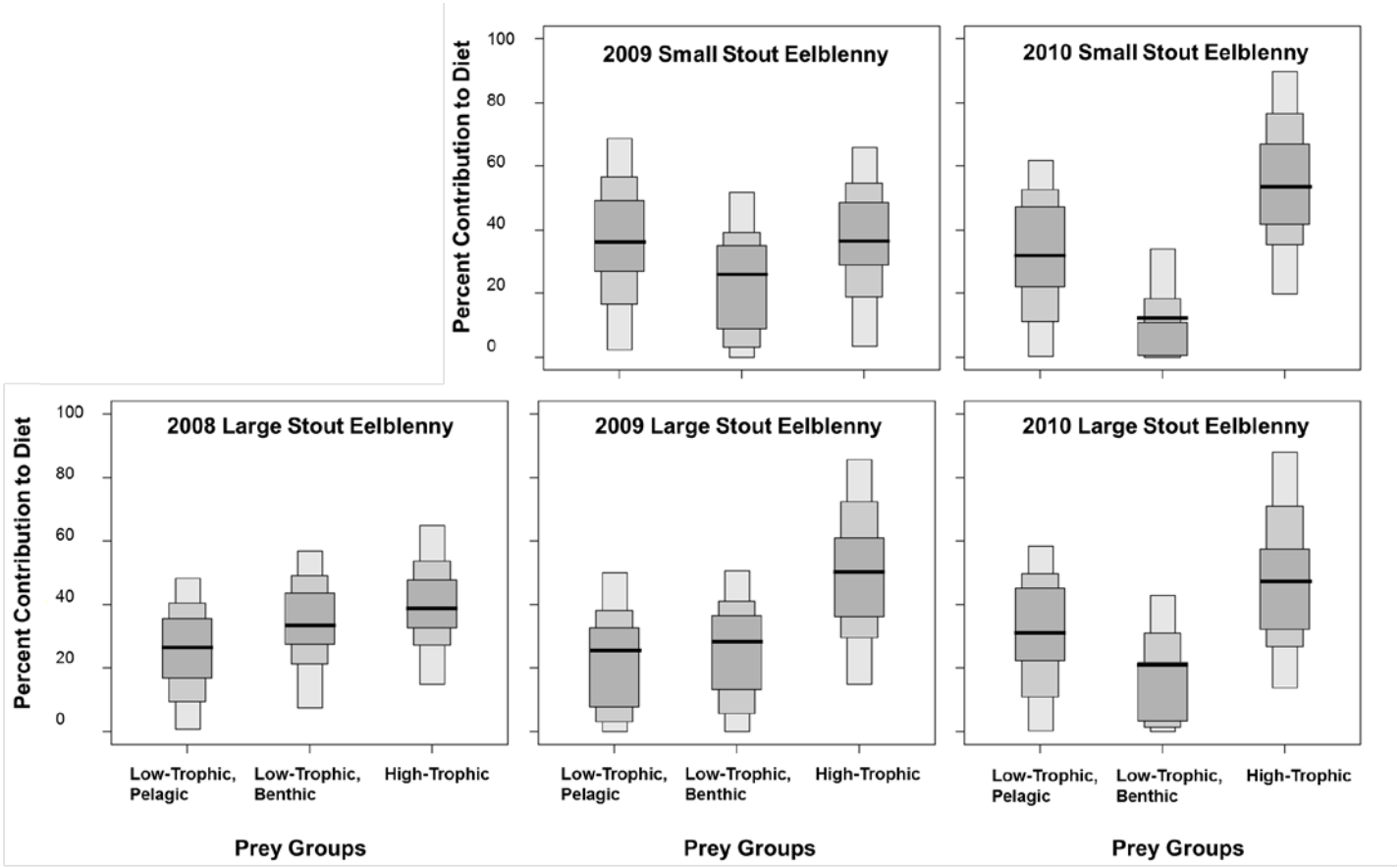


Figure 3-9. **Isotope Mixing Models for Stout Eelblenny**. Proportional contribution of low- and high-trophic prey to diets of small (≤70 mm) and large (> 70 mm) Stout Eelblenny as 50%, 75%, and 95% credibility intervals and mean values (vertical black line) based on the SIAR mixing model. Year indicates fish collection; mixing models show diet averaged from the past year, i.e., 2008 large Stout Eelblenny figure shows diet averaged across 2007–2008. See Figure 3-4 for fish prey groups.

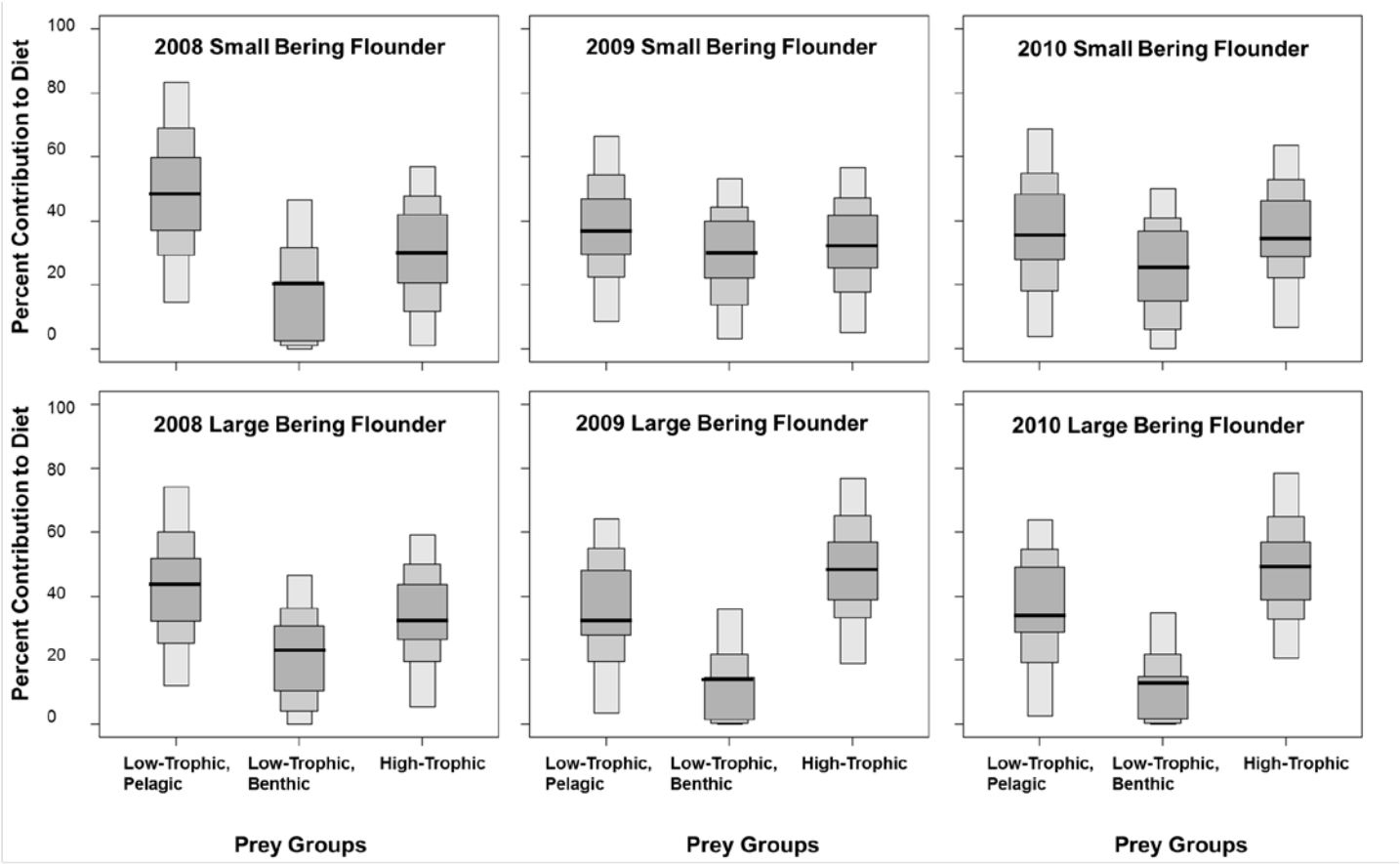
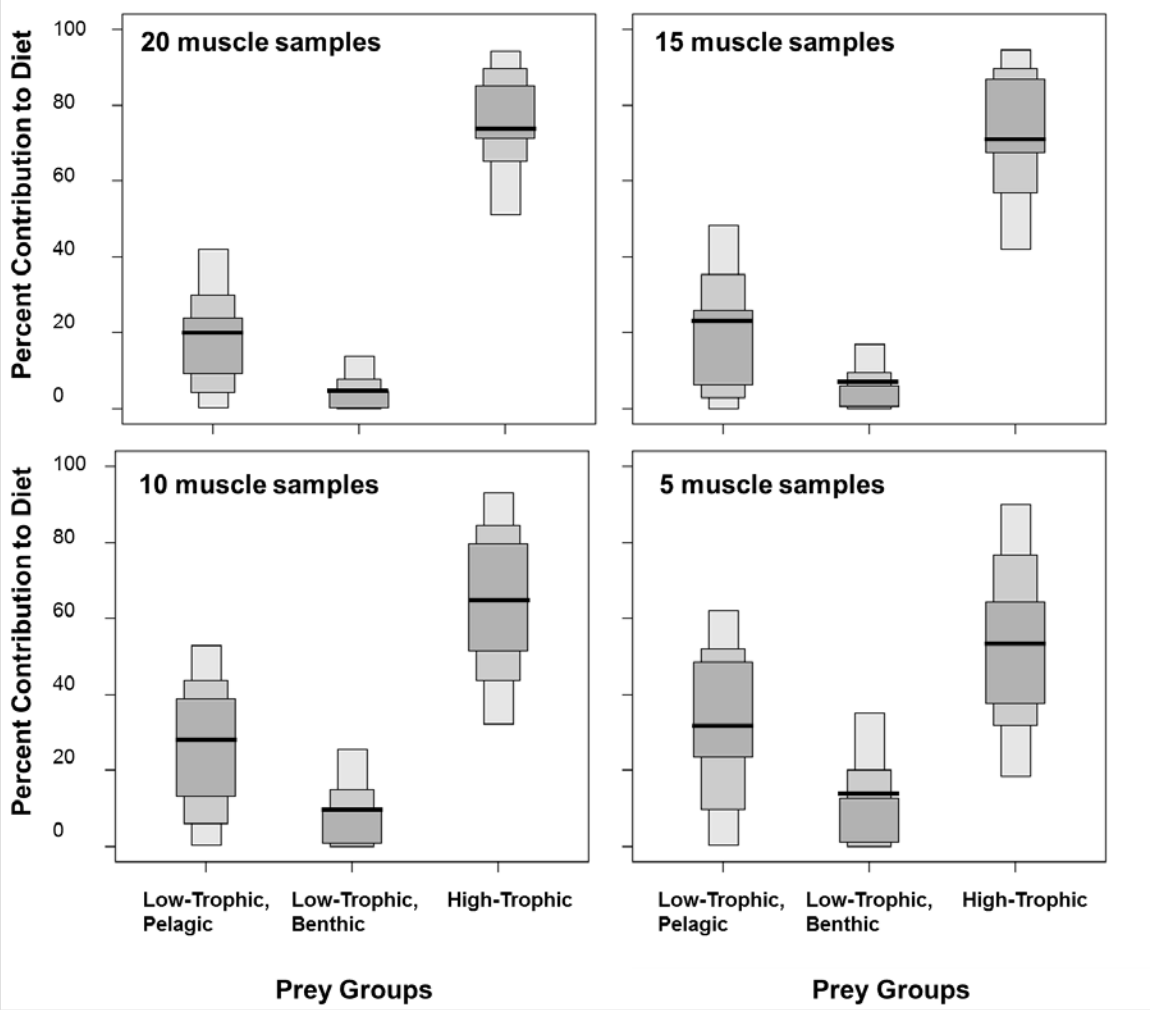


Figure 3-10. **Isotope Mixing Models for Bering Flounder**. Proportional contribution of low- and high-trophic prey to diets of small (≤70 mm) and large (> 70 mm) Bering Flounder as 50%, 75%, and 95% credibility intervals and mean values (vertical black line) based on the SIAR mixing model. Year indicates fish collection; mixing models show diet averaged from the past year, i.e., 2008 large Bering Flounder figure shows diet averaged across 2007–2008. See Figure 3-4 for fish prey groups.

3.8 Appendices



Appendix 3-1. **Isotope Mixing Model Comparison for Predator Sample Size**. Results of isotope mixing models for various sample sizes of the predator. Predator isotope ratios were from the muscle of large (> 70 mm in length) Stout Eelblenny collected during 2009. Fish prey groups were determined by MDS (See Figure 3-3).

Appendix 3-2. **Averaged Prey Versus Single Year Collection**. Prey stable isotope signatures averaged across multiple years (2008–2010) or from year prior to when fish were collected (2009). Mixing models vary by stable isotope signatures of fish prey included in the model. Mixing model estimates: 95% credibility intervals and mean values for the large length class (> 70 mm) of three Arctic fish species. Fish prey groups were determined by MDS (See Figure 3-3). For 2009 prey taxa, juvenile crabs were not available for stable isotope analysis.

		Isotope Mixing Model Results							
		Prey Groups - Averaged				Prey Groups - 2009			
2010 Large Fishes	Prey Grouping	$\delta^{15}N$ [‰]	$\delta^{13}C$ [‰]	95% CI	$\bar{x}$	$\delta^{15}N$ [‰]	$\delta^{13}C$ [‰]	95% CI	$\bar{x}$
Arctic cod									
(Boreogadus saida)	High Trophic	11.66 ± 1.86	-19.80 ± 1.37	9.1 - 66%	38.7	11.72 ± 1.57	-19.94 ± 1.44	8.4 - 66%	38.5
	Low Trophic, Benthic	9.13 ± 1.39	-18.43 ± 1.17	0.0 - 50%	25.3	9.27 ± 1.45	-18.76 ± 1.13	0.0 - 50%	26.1
	Low Trophic, Pelagic	9.50 ± 1.80	-20.22 ± 1.22	2.7 - 65%	35.9	9.78 ± 1.86	-20.33 ± 1.28	2.4 - 64%	35.4
Stout eelblenny									
(Anisarchus medius)	High Trophic			12 - 87%	48.1			11 - 86%	45.2
	Low Trophic, Benthic			0.0 - 44%	20.4			0.0 - 48%	24
	Low Trophic, Pelagic			0.1 - 59%	31.5			0.4 - 58%	30.8
Bering flounder									
(Hippoglossoides robustus)	High Trophic			20 - 78%	48.6			18 - 78%	47.4
	Low Trophic, Benthic			0.0 - 35%	14.7			0.0 - 39%	18.0
	Low Trophic, Pelagic			3.7 - 64%	36.7			2.6 - 61%	34.7

Chapter 4: Interannual Variations in the Diet of Ice Seals Assessed by Isotopic Mixing Models

Authors: Sara Carroll, Larissa Horstmann-Dehn, Brenda Norcross

4.1 Introduction

In the past few decades, average atmospheric temperatures in the Arctic have increased twice as fast as in the rest of the world (Arctic Climate Impact Assessment 2004). Rapid climate change is illustrated by the minima of summer ice extent occurring in 2007−2012 (National Sea Ice Data Center [NSIDC] 2012). The 2007 sea ice extent was at an unprecedented low until it was surpassed by the minimum sea ice extent of 2012 (NSIDC 2012). There have been reductions in the extent and thickness of perennial ice in the Chukchi and Beaufort Seas since 1979 (Moline et al. 2008). Determining organism responses to ecosystem changes will further the general understanding of adaptation potential and the possible consequences of a warming climate for Arctic marine mammal populations.

Ice seals are dependent on sea ice as a resting, feeding, and pupping platform. In response to continued sea-ice habitat loss and reduced snow cover, the National Oceanic and Atmospheric Administration (NOAA) Fisheries Service has listed the Arctic Basin population of ringed seals (*Pusa hispida*) and the Okhotsk population of bearded seals (*Erignathus barbatus*) as threatened under the Endangered Species Act (ESA) (NOAA 2012a). In 2009, NOAA decided not to list the Bering Sea population of spotted seals (*Phoca largha*) under the ESA; however, the smaller southern population (Yellow Sea and Sea of Japan) was listed as threatened (NOAA 2009).

Diminished sea ice thickness and extent may increase energetic costs for many Arctic seal species. As the Arctic marine ecosystem changes, so will the distribution and abundance of prey resources (Grebmeier et al. 2006). The lower nutritional quality of prey could then propagate up the food chain. This starts with essential fatty acids of ice algae being negatively correlated with increased irradiance due to reduced sea ice cover (Leu et al. 2010). Modifications to the food web, as sea ice diminishes, may lead to changes in seal diets, i.e., consumption of prey in reduced quantities or nutritional quality. Changes in prey quality, abundance, or distribution could lead to detrimental effects for ice seals such as decreased body condition, impaired immune response (Burek et al. 2008), reduced fecundity (Harwood et al. 2000), and ultimately population declines (Simmonds and Isaac 2007). Thus, it is important to examine the feeding ecology of ice seals on a temporal scale and document possible changes over time.

The objective of this study was to examine interannual changes in the trophodynamics of ice seal diets to assess their foraging plasticity. Stable nitrogen and carbon isotope ratios have been used to examine food webs and identify likely dietary sources for Arctic species (Hobson and Welch 1992; Dehn et al. 2006; Bentzen et al. 2007). Stable nitrogen isotope ratios are indicative of the trophic level at which an individual feeds. As an organism consumes nutrients, it preferentially uses the lighter nitrogen isotope (^{14}N) for metabolic processes and integrates the heavier isotope (^{15}N) into tissues, leading to a stepwise enrichment of ^{15}N in the food web (Kelly 2000). Stable

carbon isotope ratios typically provide information on carbon source and habitat usage such as benthic versus pelagic foraging (Dehn et al. 2007; Horstmann-Dehn et al. 2011). Benthic algae become enriched in the heavier carbon isotope (^{13}C) because they have minimal replenishment of the lighter isotope (^{12}C) through the benthic boundary layer (France 1995). In contrast, planktonic algae are likely to experience increased water turbulence and be depleted in ^{13}C (France 1995). Coincidentally, ice algae trapped in brine channels exhibit similar ^{13}C enrichment (Kennedy et al. 2002).

An advantage to using stable isotopes in feeding ecology studies is that they provide dietary information over an integrated time period by reflecting assimilated, not just ingested food (Peterson and Fry 1987). Tieszen et al. (1983) found that stable isotopes in muscle described integrated diet for gerbils (*Meriones unguiculatus*) consumed over a previous couple of months. However, mass-specific metabolic rates of gerbils are faster than for large mammals such as ice seals, so tissues of larger animals have substantially slower turnover times. For example, the half-life of muscle (and associated stable carbon isotope signature) in alpacas (*Lama pacos*) and bovines is roughly six times longer than that of gerbils (Sponheimer et al. 2006; Bahar et al. 2009). Half-life refers to the time required for half of the tissue to resemble a new diet (MacAvoy et al. 2006). Stable isotope ratios for muscle of large mammals thus describe long-term dietary averages of likely several months to a year (Sponheimer et al. 2006). Stable isotope turnover studies do not exist for marine mammal muscle.

Isotopic mixing models have become powerful tools to estimate predator diets and describe the proportional consumption of prey (Phillips and Gregg 2001, 2003; Bentzen et al. 2007; Moore and Semmens 2008; Parnell et al. 2010). In order to make inferences about prey contribution to the diet of a predator, a comparison must be made between the stable isotope signatures of the predator and its prey. Thus, three factors are inserted into mixing models: stable nitrogen and carbon isotope ratios of the predator and prey and a trophic enrichment factor. The trophic enrichment factor is the incremental change in stable isotope ratios from prey to predator tissues and is used to evaluate all food web components in the same isotopic space (Peterson and Fry 1987). The key to successful application of mixing models is distinct isotopic signatures of prey items (Gannes et al. 1998). If prey signatures overlap, the model confounds the proportional contribution of each source (Phillips and Gregg 2003). Diet of a predator can be described using a single consumption percentage for each prey source if only two or three isotopically distinct prey items are eaten. However, many predators have a varied diet leading to a range of possible solutions for proportional contributions of prey items to the diet. Thus, as the number of food sources increases, the uncertainty to the particular contribution of each source increases as well (Phillips and Gregg 2003). Bayesian isotopic mixing models allow for the incorporation of more than three dietary sources and produce probable dietary solutions for each (Parnell et al. 2010). In addition, these models account for biological variability in stable nitrogen and carbon isotope ratios of predator and prey and include measurement error (Parnell et al. 2010).

For this study, isotopic mixing models were used to describe the proportional contribution of prey sources to the diets of ice seals. In order to assess potential interannual changes in diet, stable isotope signatures in the muscle of ringed, bearded, and spotted seals were examined in 2003 and from 2008 to 2010. Sea ice extent was lower during 2007−2012 relative to previous years (NSIDC 2012), and this ecosystem change may result in differences of seal diets. As diminished sea ice, earlier ice melt, and warmer waters may favor a pelagic dominated food web (Bluhm and Gradinger 2008), we hypothesize that ice seals may then capitalize on more abundant pelagic prey sources rather than preferred prey during years of reduced ice cover in the Arctic Ocean. Overall, diet analysis on a temporal scale may help to assess the foraging plasticity of these Arctic pinniped species.

4.2 Materials and Methods

4.2.1 Seal Sampling

Muscle was collected from ringed (*Pusa hispida*), bearded (*Erignathus barbatus*), and spotted seals (*Phoca largha*) during Alaska Native subsistence harvests in Barrow, Point Hope, Shishmaref, Little Diomede, and Hooper Bay (Figure 4-1). Ice seals migrate long distances throughout marine waters in the Arctic and sub-Arctic (Kotzebue IRA and Arctic Web Publications 2010; Paulatuk, Holman, and Tuktoyaktuk Hunters and Trappers Committees 2011). Population structure of ice-associated seals is poorly understood, and subpopulations may exist (Kelly et al. 2010). However, for the purposes of this study, individuals of their respective species were considered part of the same population although they come from different, geographically spread-out communities along Alaska's coastline.

A total of 416 seal muscle samples were compared in this study (Table 4-1). Ringed and bearded seals were sampled in May and June of 2008−2010. Spotted seals were sampled in October and September of 2008 and 2009. Seal muscle was collected shortly after death (less than 12 hours), placed in Ziploc® or Whirlpak™ bags, and frozen at −20°C until processing for analysis of stable nitrogen and carbon isotope ratios. Ten muscle samples from 2008 were provided by the University of Alaska Museum of the North in Fairbanks (UAM, Loan # 2010.001.Mamm). To extend the interannual comparison, seal muscle samples collected during this study were compared to samples harvested in 2003 (Dehn et al. 2007). Seal teeth and front-flipper claws were used for age class classification. Jaws were soaked in hot water for approximately 15 min. Teeth were extracted, carefully cleaned of gum tissue, and sent to Matson's Laboratory LLC in Montana for sectioning, mounting, and staining (Giemsa blood stain, Wohlbach formula, Ricca Chemical Company, Arlington, Texas, USA). Seal age was estimated by counting growth layer groups in the cementum of canine and postcanine teeth (Stewart et al. 1996), or by counting growth layer groups of claws for a minimum age estimate (McLaren 1958). One light and one dark growth layer are assumed per year in seal teeth and claws (McLaren 1958; Benjaminsen 1973; Stewart et al. 1996). Seals were assigned to one of three age classes: young-of-the-year

(YOY, <1 yr.), subadult (1−4 yrs.), and adult (≥ 5 yrs.) (Boveng et al. 2009; Cameron et al. 2010; Kelly et al. 2010)

4.2.2 Fish Sampling

Fishes are common prey for ice seals, and selected species were processed for stable nitrogen and carbon isotope ratios. Fishes were collected during research cruises in the Chukchi Sea using bottom and surface trawls during 2007, 2009, and 2010 (Figure 4-1; Norcross et al. 2013). Fish species were chosen for stable nitrogen and carbon isotope analysis based on their frequency of occurrence in stomachs of ice seals (Quakenbush et al. 2009, 2010a, b) and their availability from research cruise collections. Twelve fish species were analyzed: Arctic Staghorn Sculpin (*Gymnocanthus tricuspis*), Bering Flounder (*Hippoglossoides robustus*), Canadian Eelpout (*Lycodes polaris*), Shorthorn Sculpin (*Myoxocephalus scorpius*), Slender Eelblenny (*Lumpenus fabricii*), Stout Eelblenny (*Anisarchus medius*), Arctic Cod (*Boreogadus saida*), Capelin (*Mallotus villosus*), Pacific Herring (*Clupea pallasii*), Pacific Sand Lance (*Ammodytes hexapterus*), rainbow smelt (*Osmerus mordax*), and Saffron Cod (*Eleginus gracilis*). For each fish species, stomachs were removed from ten individuals that were greater than 70 mm in total length for a separate study.

4.2.3 Sample Processing

The archived muscle samples from UAM were stored by the curators in 100% ethanol for two months prior to use. Ethanol preservation does not affect stable nitrogen and carbon isotope ratios of quail (*Coturnix japonica*) muscle (Hobson et al. 1997). For further validation, muscle from five ringed and five bearded seals was analyzed for stable nitrogen and carbon isotope ratios as both non-ethanol-preserved and as preserved in 100% ethanol for two months. Stable nitrogen and carbon isotope ratios were not significantly different between the two treatments in ringed and bearded seal (Chapter 4 Appendix 4-1). Thus, samples stored in ethanol were included in the analysis of this study (Table 4- 1).

Approximately 5 mg of muscle was freeze-dried (VirTis Sentry) for a minimum of 48 h and ground into a fine powder at the University of Alaska Fairbanks (UAF) Marine Mammal Laboratory. Lipids are depleted in ^{13}C, and their presence can influence the carbon isotope signature of tissues (DeNiro and Epstein 1977). Seal muscle is typically lean and does not require lipid extraction to normalize stable carbon isotope ratios (Hoekstra et al. 2002). For further validation, muscle from five individuals of each of the three seal species was analyzed for stable nitrogen and carbon isotope ratios as both non- lipid-extracted and lipid-extracted. Stable nitrogen isotope ratios were significantly different between the two treatments for ringed seals (Chapter 4 Appendix 4-2). Stable carbon isotope ratios were not significantly different between the two treatments for all three seal species (Chapter 4 Appendix 4-2). Muscle appeared to be mostly free of lipids that are depleted in ^{13}C (DeNiro and Epstein 1977). Thus, lipids were not

removed from seal muscle samples prior to stable isotope analysis to avoid skewing stable nitrogen isotope ratios.

Whole fishes without stomachs were freeze-dried for a minimum of 48 h and homogenized using a mortar and pestle. Extracting lipids from samples removes the stable carbon isotope signature of fats (DeNiro and Epstein 1977), leaving only the stable carbon isotope signature of the tissue, but the extraction procedure can alter the stable nitrogen isotope signature (Chapter 4 Appendix 4-3; Pinnegar and Polunin 1999; Sweeting et al. 2006). Therefore, fish total body homogenates from the same individual were processed to obtain non-lipid-extracted $^{15}N/^{14}N$ ratios and lipid-extracted $^{13}C/^{12}C$ ratios. Lipid was extracted from fish tissues using a modified technique described by Bligh and Dyer (1959). Samples were immersed in a 2:1 chloroform/methanol mixture with a solvent volume about three times the sample volume (Logan et al. 2008). Each sample was agitated for five minutes followed by five minutes of centrifugation at 605 g (3000 rpm) using a VWR Clinical 50 centrifuge. The supernatant containing lipids was discarded. Lipid extraction was repeated until the supernatant was colorless after centrifugation (Logan et al. 2008), approximately three to five times. Lipid-extracted samples were dried overnight in a fume hood; freeze-dried for approximately two hours the following day and re-homogenized.

4.2.4 Stable Isotope Analysis

Seal muscle and whole fish were analyzed for $\delta^{15}N$ and $\delta^{13}C$ values at the Alaska Stable Isotope Facility at UAF. A sub-sample of ground seal and fish tissue, 0.2−0.4 mg dry weight, was weighed into tin capsules using a microbalance (Sartorius Model M2P). Stable isotope analysis was performed using a Finnigan MAT Delta^{Plus}XP Isotope Ratio Mass Spectrometer (IRMS) directly coupled to a Costech Elemental Analyzer (ECS 4010). The $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios are expressed in conventional delta (δ) notation, relative to Vienna Pee Dee Belemnite (VPDB) and atmospheric N_2 (atm.), respectively. Peptone was used as a laboratory standard. The precision of analyses, expressed as one standard deviation from multiple analyses of peptone (n = 63) conducted during runs of samples, was 0.1‰ for both $\delta^{15}N$ and $\delta^{13}C$.

4.2.5 Statistical Analysis and Isotope Mixing Model

Non-metric multidimensional scaling (MDS) ordination of stable nitrogen and carbon isotopes of seal muscle was used to visually portray the correlations of sex and age class (PRIMER v. 6.1). Factors inserted into the program were sex, age class, and sex and age class combined. Additionally, $\delta^{15}N$ values were tested individually to investigate any confounding effects of YOY seals. YOY seals have elevated $\delta^{15}N$ values due to maternal nitrogen transfer via the placenta and nursing as well as increased metabolic and nitrogen demands during growth (Dehn et al. 2007). MDS ordination plots have no interpretable axes and are based on simple matching coefficients calculated between pairs of age classes or sex. For MDS analysis, absolute values of stable isotopes were used; no other transformation was applied. Bray-Curtis similarity matrices coefficients were ranked and reordered to group samples. Age classes or sexes represented by

points closer together in an MDS plot have similar stable isotope signatures; age classes or sexes that are farther apart are less similar (Clarke et al. 2008). A stress of <0.1 is considered to be a good fit (Clarke and Warwick 2001). For each seal species, stable nitrogen and carbon isotope ratios of muscle were similar for sex (Chapter 4 Appendix 4-4), age class (Chapter 4 Appendix 4-5), and sex/age class (Chapter 4 Appendix 4-6). Additionally, no difference was found in stable nitrogen isotope ratios of muscle for age class (Chapter 4 Appendix 4-7). Therefore, all sexes and age classes were pooled to increase sample size and statistical precision, and stable isotope data for each seal species was incorporated into stable isotope mixing models.

All statistical tests were conducted in SigmaPlot Version 12.0 (Systat Software, Inc. 2011). For all statistical analyses, an alpha less than 0.05 was considered significant. $\delta^{15}N$ and $\delta^{13}C$ values of muscle were compared among the three ice seal species. $\delta^{15}N$ values did not pass the equal variance test, and $\delta^{13}C$ values did not pass the normality test; therefore, $\delta^{15}N$ and $\delta^{13}C$ values were analyzed using a Kruskal-Wallis one-way ANOVA, followed by a pairwise multiple comparison procedure using Dunn's Method. For fish species, $\delta^{15}N$ (non-lipid-extracted) and $\delta^{13}C$ values (lipid-extracted) were analyzed using one-way ANOVA to determine appropriate groupings for mixing models. $\delta^{15}N$ values passed the normality and equal variance test. Thus a Holm-Sidak Method (t) was used as a posthoc procedure. $\delta^{13}C$ values did not pass the equal variance test. Thus, differences among species were examined with a pairwise multiple comparison procedure using Dunn's Method (Q).

Model performance decreases when more prey items are included (Parnell et al. 2010); therefore, prey items were grouped into trophic guilds due to mixing model constraints. Seal prey were combined into trophic guilds based on sharing similar isotopic space and their relative importance in seal diets (Phillips et al. 2005; Quakenbush et al. 2009, 2010a, b). The five different guilds consisted of: (a) high-trophic, benthic prey, (b) mid- trophic, benthopelagic prey, (c) mid-trophic, pelagic prey, (d) low-trophic, benthic prey, and (e) low-trophic, pelagic prey. Fish prey from Chukchi Sea collections were grouped as: (a) high-trophic, benthic guild, (b) mid-trophic, benthopelagic guild, and (c) mid-trophic, pelagic guild (Table 4-**2**). Based on stable isotope signatures, the demersal fishes Arctic Staghorn Sculpin, Bering Flounder, Canadian Eelpout, Shorthorn Sculpin, Slender Eelblenny, and Stout Eelblenny, were considered part of the high-trophic, benthic guild, while Arctic Cod, Capelin, Pacific Herring, Pacific Sand Lance, and Saffron Cod were part of the mid-trophic, benthopelagic guild (Table 4-2). Demersal fishes had higher $\delta^{15}N$ values than Capelin, Pacific Sand Lance, and Rainbow Smelt (Table 4-3). Mid-trophic, benthopelagic fishes had lower $\delta^{13}C$ values than the high-trophic, benthic fishes, with the exception of Bering Flounder (Table 4-3). Rainbow Smelt were considered to be mid- trophic, pelagic prey because they had lower $\delta^{13}C$ values than other fishes examined in this study, with the exception of Pacific Herring (Table 4-3). Other prey that could be considered mid-trophic, pelagic prey are younger fish because smaller fish consume more copepods (Chapter 2) and typically have lower $\delta^{13}C$ values than larger fish, e.g., small vs. large Arctic Staghorn Sculpin (Edenfield et al. 2011). Other prey taxa that could be considered high-trophic, benthic prey

include crabs, shrimps, and polychaetes which all share similar isotopic space with demersal fishes (Iken et al. 2010; Feder et al. 2011). Crabs and polychaetes are prevalent in bearded seal diets while shrimps are common in ringed and spotted seal diets (Quakenbush et al. 2009, 2010a, b). Isopods can have similar $\delta^{15}N$ and $\delta^{13}C$ values as the mid-trophic, benthopelagic fishes from this study (Dehn et al. 2007). Greenland cockle (*Serripes groenlandicus*, Iken et al. 2010) is commonly consumed by bearded seals (Quakenbush et al. 2010b) and was selected as a representative prey item for a low-trophic, benthic guild for bearded seals. The euphausiid *Thysanoessa raschii* (Iken unpub. data) was selected as a typical representative for the low-trophic, pelagic guild (guild e) for ringed and spotted seals. Pelagic amphipods, e.g., hyperiid amphipods, are also a planktonic crustacean prey for spotted and ringed seals and share similar isotopic space with *T. raschii* (Quakenbush et al. 2009, 2010a; Feder et al. 2011).

The Bayesian isotopic mixing model, SIAR (Stable Isotope Analysis in R, Version 4.1.1, Parnell and Jackson 2011) was used to determine the relative proportions of prey in seal diets. $\delta^{15}N$ and $\delta^{13}C$ values of seal muscle were grouped by year to document dietary proportions of the corresponding sampled population. Potential prey species of ice seals were grouped as previously described (Table 4-2) and their mean and standard deviation for $\delta^{15}N$ and $\delta^{13}C$ were inserted into SIAR. For the low-trophic, benthic guild, both non- lipid-extracted $\delta^{15}N$ and $\delta^{13}C$ were included in the mixing model (Iken et al. 2010). Stable carbon isotope ratios of benthic invertebrates are not affected by lipid extraction in contrast to pelagic invertebrates (Iken et al. 2010). For pelagic invertebrate prey, both lipid-extracted $\delta^{15}N$ and $\delta^{13}C$ values were used based on availability. $\delta^{15}N$ and $\delta^{13}C$ values of prey items were adjusted to account for trophic enrichment factors for harp seal (*Pagophilus groenlandicus*) muscle, i.e., 2.4 ± 0.4‰ for $\delta^{15}N$ and 1.3 ± 0.4‰ for ^{13}C (Hobson et al. 1996). Mixing model estimates are presented as 95% credibility intervals (Bayesian confidence intervals), along with a mean percent contribution to diet for each prey guild.

4.2.6 Mixing Model Performance

Prey $\delta^{15}N$ and $\delta^{13}C$ values inserted into the isotope mixing model were from a single year (2009), whereas the values for ice seals came from four different years (2003, 2008– 2010). Mixing models were assessed to determine whether interannual differences in prey stable isotope ratios impact mixing model results and interannual changes in seal diets. Mixing models for ringed and bearded seals were compared when using stable isotope ratios of prey from the previous year that seals were harvested (assuming approximately one year turnover of seal muscle; Figure 4-2) versus the use of stable isotope ratios of prey averaged across multiple years (2008, 2009). Demersal fishes were the only prey that were available from multiple years including years examined in this study. Demersal fishes collected during 2008 had significantly lower $\delta^{15}N$ values compared with demersal fishes collected during 2009 ($t = 6.019$, $p < 0.001$). Therefore, mixing models for both ringed and bearded seal muscle collected from 2009 and 2010 were assessed when including high-trophic, benthic prey from 2008 and 2009, respectively. Stable isotope ratios of high-trophic, benthic prey were then averaged for 2008/2009 and included in the

model. The results were similar for all prey guilds as the 95% credibility intervals and the means did not vary more than 10% among the mixing models (Figure 4-3). The collection year of prey available to this study suffices in capturing general changes in trophic level and feeding source of ice seals over time. The TL of seals is represented as follows:

$$TL_{seal} = (\delta^{15}N_{consumer} - \delta^{15}N_{primary\ consumer})/3.4 + 2$$

Where TL_{seal} is trophic level of seal predator, consumer is seal predator, primary consumer is copepod, and $\delta^{15}N$ values were from samples that had not been acid-fumed or lipid-extracted. The baseline value of $\delta^{15}N$ for the primary consumer was the average for copepods from fish stomachs in this study (8.77**)**. This value of $\delta^{15}N$ for copepods is within the spring and summer variation of *Calanus glacialis* (9.09 ± 0.66 to 12. 41 ± 0.59) from the Amundsen Gulf in the eastern Beaufort Sea (Forest et al. 2011). The mean trophic ^{15}N enrichment of *C. glacialis* was 2.8–4.7‰. The increase of $\delta^{15}N$ in marine food webs is usually 3–4‰ per trophic level (Michener and Schell 1994). The average trophic nitrogen fractionation for aquatic consumers, 3.4 (Vander Zanden and Rasmussen 2001, Post 2002), is the enrichment of $\delta^{15}N$ between trophic levels that we used in these equations and other recent trophic analyses for the Chukchi Sea (Iken et al. 2010, Tu et al. 2015).

4.2.7 Tissue Turnover Calculation

Turnover rates for ice seals are unknown, but as a first approximation we calculated turnover in ice seals based on body mass (body-mass specific metabolic rates) from other mammals. A larger animal has a lower metabolic rate per unit weight compared with a smaller animal (Kleiber 1947) resulting in a slower turnover of tissues in the animal with the greater body mass (MacAvoy et al. 2006). For example, muscle turnover rates (half- life of $\delta^{13}C$) for mice (*Mus musculus*), gerbils, alpacas, and steers (*Bos primigenius*) are 23.9 (MacAvoy et al. 2005), 27.6 (Tieszen et al. 1983), 178.7 (Sponheimer et al. 2006), and 151.0 days (Bahar et al. 2009), respectively. These experimental values were used to estimate muscle turnover rates for ice seals. The mass-specific metabolic rate is proportional to body size to the -0.25 power (West et al. 1997), a relationship that also applies to tissue turnover, i.e., half-life of ^{13}C = mass (in grams)$^{-0.25}$ (Carleton and Martínez del Rio 2005). Experimental values for the half-life of ^{13}C and mass$^{-0.25}$ for mice, gerbils, alpacas, and steers plotted on a logarithmic scale yielded a curvilinear regression (Figure 4-2) represented by:

$$y = 180.15 – 369.35\ (x^{-0.25})$$

where x is the total body mass of the mammal in grams and y represents the half-life of isotopic incorporation in days. This agrees with Kolokotrones et al. (2010) who showed mass and metabolic rate, i.e., tissue turnover, have a convex curvature on a logarithmic scale.

The regression equation from Figure 4-2 was used to estimate muscle turnover for an average-sized ringed, bearded, and spotted seal, i.e., and 45,000 g, 260,000 g, and 90,000 g, respectively (Fedoseev 2000). Blubber mass has been excluded, i.e., lean body mass, for each species, as

blubber is relatively metabolically inert (Schmidt-Nielsen 1984). Including blubber in mass estimates, especially for Arctic marine mammals with substantial blubber layers that add considerably more to body mass (Cameron et al. 2010), can lead to an overestimation of turnover rate. In order to estimate percent blubber mass, lean body mass was calculated using the LMD-index (length, mass, depth) (Ryg et al. 1990), and this percentage was subtracted from the average body mass. For each species, standard length and body mass (Fedoseev 2000) were used for the LMD-index. Instead of using dorsal blubber thickness for depth (Ryg et al. 1990), information was only available for sternal blubber thickness of these species (Quakenbush et al. 2009, 2010a, b). In addition, blubber thickness varies seasonally and reaches its maximum in winter. Thus, winter averages were used (Quakenbush et al. 2009, 2010a, b).

4.3 Results

Based on mean body mass, excluding blubber mass, the half-life of isotopic incorporation for ringed, bearded, and spotted seals was 150, 162, and 154 days, respectively (Figure 4-2). Doubled half-lives (to estimate complete muscle turnover rates) of isotopic incorporation for ringed, bearded, and spotted seals were approximately 10.0, 10.8, and 10.3 months, respectively. Based on these tissue turnover calculations, stable isotope ratios for seal muscle estimate integrated diet across approximately the previous 10-11 months. For example, stable isotope ratios/mixing models for ringed seals collected in May 2008 will show an integrated isotope signature that reflects feeding from July 2007 to May 2008.

Stable isotope signatures of muscle were variable among individuals within ice seal species (Figure 4-4); yet, stable isotope signatures of muscle were distinct among species. Muscle of spotted seals had significantly higher $\delta^{15}N$ values ($p < 0.0001$, $F = 48.35$, $df = 2$) compared to ringed and bearded seals (Table 4-1). As trophic levels were calculated from $\delta^{15}N$ values, the same patterns were seen in TL. Muscle of bearded seals was significantly enriched in ^{13}C ($p < 0.0001$, $F = 50.75$, $df = 2$) compared to ringed and spotted seals (Table 4-1).

Mixing model results illustrated high-trophic, benthic prey, mid-trophic, pelagic prey or both are key components in ringed and bearded seal diets, while low-trophic, pelagic prey are important in spotted seal diets. SIAR results are presented as upper and lower 95% credibility intervals, along with the mean value, for each prey source. Ringed seals consumed a higher mean proportional contribution of high-trophic, benthic prey and mid- trophic, pelagic prey, in 2002/2003 and 2009/2010, whereas low-trophic, pelagic prey were more prevalent in ringed seal diets in 2007−2009 (Table 4-4). For bearded seal diets, the mean proportional contribution of high-trophic, benthic prey was highest in 2002/2003 and decreased during 2007−2010, while the mean proportional contribution of mid-trophic, benthopelagic prey increased (Table 4-4). Spotted seal diets mainly consisted of low-trophic, pelagic prey (Table 4-4). Spotted seal muscle samples analyzed in this study were mostly from young seals, i.e., 70% (65 of 92 seals with age estimates, 13 seals had unknown age) were YOY to two years of age. The mean proportional contribution of mid-trophic, benthopelagic and mid-trophic, pelagic prey to spotted seal diets was highest

during 2007/2008 (Table 4-4). For all three seal species, credibility intervals overlapped for all proportional contributions of prey items and significant differences were not found for different prey groups across years. These finding are similar to results of previous studies by Payton et al. (2003) and Julious (2004).

4.4 Discussion

In general, the three ice seal species examined in this study fed on similar prey taxa; however, the proportions of prey consumed created distinct stable isotope signatures among the different seal species. $\delta^{15}N$ and $\delta^{13}C$ values of muscle indicated that ringed, bearded, and spotted seals fed at slightly different trophic levels; spotted seals had an average trophic level higher than bearded seals, which in turn had a higher trophic level than ringed seals. Ringed seal and bearded seal muscles had relatively depleted ^{15}N signatures compared with spotted seals, indicating a mixed diet of low-trophic prey (e.g., pelagic amphipods for ringed seals and bivalves for bearded seals) and high-trophic prey (e.g., fishes). Cods, euphausiids, amphipods, mysids, and shrimps are all important prey for ringed seals (Lowry et al. 1980a; Dehn et al. 2007). The relatively high $\delta^{15}N$ values of spotted seal muscle suggest a diet primarily comprised of fishes. Spotted seals consume both demersal and pelagic fishes that are also prey for ringed and bearded seals (Boveng et al. 2009). The relatively high $\delta^{13}C$ values of bearded seal muscle are consistent with benthic feeding (Dehn et al. 2007; Horstmann-Dehn et al. 2011). Bearded seals consume a variety of benthopelagic and demersal fishes (e.g., cods, eelpouts, flatfishes, pricklebacks, and sculpins) and invertebrates (e.g., bivalves, crabs, and shrimps) (Cameron et al. 2010).

4.4.1 Ringed Seals

Arctic Cod is a dominant prey item for ringed seals during ice-covered years while invertebrates become more important during the open-water season (Kelly et al. 2010). Mixing model results for ringed seals provide further evidence of a mixed fish and pelagic crustacean diet (Table 4-4). Shrimps have been documented as an important food item for ringed seals (Lowry et al. 1980a), and the isotopic guild of high-trophic, benthic prey could be indicative of shrimps (Iken et al. 2010).

Stable isotope mixing models can be used to detect interannual variations in diets of ice seals. While some interesting patterns emerged between years, overall prey proportions were not significantly different. Stable isotope ratios of muscle from ringed seals harvested in May provides dietary information averaged from about the previous 10 months (Figure 4-2), e.g., July 2007 to May 2008. Low-trophic, pelagic prey were more prominent in the diet of ringed seals harvested in 2008 and 2009 (Table 4-4) likely relating to a higher abundance of pelagic crustaceans during 2007−2009. Elevated annual primary production (Arrigo et al. 2008), early ice retreat coupled with warmer waters (NSIDC 2011a), and a longer open water season during 2007 supported a higher abundance of pelagic crustaceans, e.g., euphausiids (Forest et al. 2011; Matsuno et al. 2011; Eisner et al. 2012). Moreover, the higher flow of warm Pacific Water into

the Chukchi Sea may have led to the advection of euphausiids into the Chukchi Sea (Ashjian et al. 2010), adding to the high zooplankton biomass and diversity observed in 2007 (Matsuno et al. 2011). Feeding more extensively on these abundant pelagic crustaceans likely resulted in the observed mixing model results showing an increase in low-trophic, pelagic feeding for ringed seals during 2007–2009. From 2008 to 2010, there was an increase in the abundance and biomass of zooplankton in the Chukchi Sea (Questel et al. 2013). Fish abundance may also increase in response to an increase in the biomass of zooplankton (Overland and Stabeno 2004; Grebmeier et al. 2006). Consuming more pelagic fishes, e.g., young Arctic fishes, may have resulted in the mixing model results showing more mid-trophic, pelagic feeding during 2009/2010. Stable nitrogen and carbon isotope ratios in muscle provide evidence that ringed seals may be capitalizing on the more abundant prey sources (Dehn et al. 2007).

4.4.2 Bearded Seals

Mixing model results from this study showed that low-trophic, benthic prey, e.g., bivalves, were minor contributors to bearded seal diets, contrary to published accounts (Johnson et al. 1966; Lowry et al. 1980b). This may be a response to niche competition with other predators. Pacific walruses (*Odobenus rosmarus divergens*) largely depend on clams as a prey resource (Lowry et al. 1980b). Increased competition or low abundance of clams, possibly in accordance with reduced sea ice and walruses exhibiting a central foraging strategy from shore (Sheffield and Grebmeier 2009), could put pressure on bearded seals and effectively change their foraging efforts (Lowry et al. 1980b). However, the late spring and summer importance of bivalves as bearded seal prey (Lowry et al. 1980b) cannot be ruled out because, as mentioned before, stable isotope results from muscle are an integrated, long-term descriptor of diet. A greater contribution of high- trophic, benthic prey, e.g., crabs and shrimps, to bearded seal diets during the summer (Quakenbush et al. 2010b) and throughout the year may mask the seasonal importance of low-trophic, benthic prey, e.g., bivalves.

Interannually, isotope mixing model results indicate bearded seal diet consists of fewer high-trophic, benthic organisms, e.g., crabs, shrimps, demersal fishes, and more mid- trophic, benthopelagic prey, e.g., cod species, for 2002/2003 compared with 2007−2010 (Table 4-4). Muscle of bearded seals from 2003 and 2008−2010 represents averaged diet over approximately the previous 10.8 months (Figure 4-2). Bearded seals focus foraging efforts on the benthos (Cameron et al. 2010). Declining sea ice and earlier ice melt is thought to result in a pelagic-dominated food web as ice-free waters expand (Bluhm and Gradinger 2008). When ice retreats earlier in the year, low light levels and little stratification delay the phytoplankton bloom cycle. By the time the bloom develops, herbivorous zooplankton have become well established and consume much of the phytoplankton. This leads to decreased carbon flux to the benthos and reduced benthic productivity (Bluhm and Gradinger 2008). Mixing model results in this study provide evidence that bearded seals are able to adjust to less benthic biomass by foraging on benthopelagic prey sources as observed during ice minima years of 2007−2010 (NSIDC 2012). Fishes are considered of relatively minor importance to bearded seal diets (Lowry et al. 1980b).

Yet, the prevalence of fishes in bearded seal diets may vary interannually. For example, fishes were consumed more frequently by bearded seals during the 2000s than the 1970s (Quakenbush et al. 2010b).

4.4.3 Spotted Seals

Key resources for spotted seals appeared to be low-trophic, pelagic prey (Table 4-4). Low-trophic crustaceans, e.g., amphipods and euphausiids, are dominant prey for spotted seal pups (Gol'tsev 1971; Bukhtiyarov et al. 1984) and after the first year spotted seals consume more fish (Gol'tsev 1971). Over 50% (53 of 92 seals with estimate ages) of the spotted seal muscle samples analyzed in this study were collected from young spotted seals (YOY to 1 year). Mixing model data for mostly young spotted seals, therefore, result in the large contribution of low-trophic, pelagic prey observed in this study.

Interannual differences in the contribution of mid-trophic, pelagic, e.g., smelt, and mid- trophic, benthopelagic prey, e.g., Pacific Herring and Capelin, to spotted seal diets documented in this study may be a result of the amount of feeding in nearshore habitats. The distribution of spotted seals has been documented by satellite tracking (Lowry et al. 1998). Spotted seals are typically found on ice floes near the ice front in late winter/spring, and as the ice breaks up, they either move to nearshore haulouts in the Bering Sea or travel north into the Chukchi Sea from summer to early autumn (Lowry et al. 1998). During the open water season, spotted seals spend long time periods at sea and make infrequent trips to coastal haulouts (Lowry et al. 1998). Haulout locations for spotted seals in Alaska are located near spawning areas for fishes, e.g., Pacific Herring, Capelin, and smelt (Quakenbush 1988). The diet of spotted seals, as described by stable isotopes, is estimated to represent approximately the previous 10.3 months (Figure 4-2). The higher proportional contribution of mid-trophic, pelagic and mid-trophic, benthopelagic prey in the diet of spotted seals during 2007/2008 may be the result of spending more time foraging nearshore on fishes compared to seal diets during 2002/2003 and 2008/2009 foraging more offshore on low-trophic, pelagic prey (Table 4- 4).

4.4.4 Among Seal Species

A comparison of mixing model data among ice seal species is perplexing as mixing model results were somewhat contradictory. Ringed seals consumed a higher proportion of high-trophic, benthic prey during 2009/2010 while bearded seals (typically a benthic generalist) fed on a lower proportion of high-trophic, benthic prey during 2009/2010 compared to previous years (Table 4-4). In addition, ringed seals ate proportionally more low-trophic, pelagic prey during 2007−2009; whereas, spotted seals consumed proportionally more low-trophic, pelagic prey during 2008/2009 (Table 4-4). These contrasting results could relate to the distribution of each seal species and/or regional variations in food sources. For example, during 2007, euphausiids were more abundant in the Chukchi Sea, and copepods were more abundant in the Bering Sea (Eisner et al. 2012). Ringed seals may have been taking advantage of the greater abundance of

euphausiids in the Chukchi Sea while the availability of low-trophic, pelagic prey was lower for spotted seals foraging in the Bering Sea. Copepods are not prey items identified in stomach contents of spotted seals (reviewed in Boveng et al. 2009; Quakenbush et al. 2009). The high individual variability and opportunistic nature in feeding habits of ice seals was demonstrated in this study (Figure 4-4) and likely led to the wide credibility intervals for each prey grouping.

Ice seals are opportunistic predators and most likely will conserve energy and consume abundant prey sources regardless of moderate differences in caloric value. The energetic density of dietary lipids is over twice that of carbohydrates and proteins (Schmidt-Nielsen 1997). Anthony et al. (2000) and Ball et al. (2007) found lipid contents of rainbow smelt (8.7%) and flatfishes (7.9%) were almost twice that of Arctic Cod (4.5%) but considerably lower than Capelin (24.3%). Generally, pelagic fishes have higher caloric values (based on dry weight) compared with demersal fishes (Ball et al. 2007), e.g., 20.5 kJ g^{-1} for Capelin and 16.5 kJ g^{-1} for flatfishes (Anthony et al. 2000). More specifically, nearshore demersal fishes have intermediate gross energy densities while schooling pelagic fishes have either relatively high or low caloric value directly related to their lipid content (Anthony et al. 2000). Lipid content varies for fishes with regard to size, sex, reproductive status, month, year, and location (Anthony et al. 2000). Lipid-rich prey are good sources of energy if they can be digested properly. The higher lipid content of Capelin compared to Pacific Herring actually results in lower assimilation efficiency in seal guts (Lawson et al. 1997a; Trumble et al. 2003). Ultimately, ice seals demonstrate preferential feeding but will eat what is available if necessary (Lindstrøm et al. 1998). Energy spent traveling to foraging grounds may lead to depletion of blubber stores and thickness, resulting in a potential increase of energy needed to augment insulation losses and buoyancy control (Rosen et al. 2007). Instead of searching long distances for more energy dense food items, piscivorous predators may gain more energy consuming a plentiful resource of potentially lower quality. Ice seals preferentially feed on prey items that are numerous. For example, during spring 1981 bearded seal stomachs from the Bering Sea showed a high frequency of occurrence of Capelin due to the presence of dense schools around St. Matthew Island (Antonelis et al. 1994). Similarly, regional differences in the diet of spotted seals were correlated to abundance and seasonal distribution of their food source (Bukhtiyarov et al. 1984). Mixing model results from this study provide further evidence of interannual differences in the diets of ice seals that most likely are on account of abundant prey sources.

Mixing models should be used in a more general sense when assessing changes in diets of predators that consume diverse prey taxa. Stable isotope and mixing model analyses provide an integrated view of diet and highlights important annual prey sources and their potential changes. In this study, isotope mixing models had limitations due to sample availability. Because the prey samples only come from a single year (2009), we assumed that stable isotope signatures of prey did not vary interannually. Due to the timing of subsistence harvests and research cruises, the majority of ice seal samples and their prey did not come from the same season; therefore, seasonal/ within-year variation is not accounted for in the isotope mixing models. Regardless,

diet will still be captured in the muscle tissue, and the mixing models will illustrate general changes in diet, e.g., lower trophic feeding of ringed seals during 2007−2009. Ultimately, care should be taken when interpreting mixing model results, and isotope values of all potential prey items need to be scrutinized, especially when estimating diets of predators with varied prey sources and those spanning large geographic ranges. This initial examination using isotope mixing models is beneficial as a starting point to assess potential interannual changes in the diet of ice seals during years of reduced ice cover in the Arctic.

Additional limitations when assessing mixing model results were the estimated stable isotope turnover rates. Stable isotope turnover and integration into muscle tissue are poorly understood for mammals and have not been investigated for marine mammals. We extrapolated tissue turnover rates for marine mammals based on the tissue turnover experimental data for a range of body masses of terrestrial mammals (Tieszen et al. 1983; MacAvoy et al. 2005; Sponheimer et al. 2006; Bahar et al. 2009). Ice seals in the Arctic may have similar (reviewed in Lavigne et al. 1986; Dierauf and Gulland 2001) or faster (Castellini et al. 1991) tissue turnover rates than terrestrial mammals.

The composition of protein and lipids within a prey item can also influence stable nitrogen and carbon isotope ratios assimilated into predator tissues. Seals alter their assimilation routes to adjust for differing macronutrient composition of prey (Zhao et al. 2006a). For example, pinnipeds relying on a protein-rich diet need to consume more prey mass to match the caloric value contained in a lipid-rich diet. Elevated dietary protein intake can then result in enhanced rates of protein catabolism, excretion of ^{14}N, and enrichment of ^{15}N in tissues (Zhao et al. 2006b). The amount of nitrogen and carbon assimilated from each prey source was not incorporated into the mixing model. This information is not available for ice seals and is beyond the scope of this study. In addition, prey items were combined into trophic guilds and adding elemental concentration values is inappropriate as the digestive efficiencies and assimilation rates of seals may differ for prey items within the trophic guilds, e.g., poor digestion of chitinous exoskeleton of crustaceans resulting in lower assimilation compared to fish (Keiver et al. 1984; Lawson et al. 1997b; Trumble et al. 2003).

Further confounding factors complicating mixing model analyses are variable metabolic rates based on sex, age, season, and nutritional and physiological state of the animal. Breeding males, lactating females, and young seals are likely to have different metabolic rates and tissue turnover rates, e.g., younger animals have higher metabolic demands than older seals due to enhanced growth rates (Newsome et al. 2010). Moreover, starving animals have tissues enriched in ^{15}N due to break-down and re-assimilation of body-own proteins (Hobson et al. 1993). However, in contrast to terrestrial mammals, marine mammals will not catabolize protein during sometimes extensive fasting periods. Nonetheless, during times of starvation, marine mammals no longer refrain from protein sparing and begin to break down lean tissue mass (Castellini and Rea 1992). The excreted ^{14}N is not being restored by dietary protein, and the animal is essentially feeding on itself, thus leading to an enrichment of ^{15}N in tissues (Gannes et al. 1997). This could lead to

biased results and misinterpretations in the apportionment of prey to dietary proportions. As seals were harvested for subsistence use and hunters preferentially select fat and presumably healthy animals, it is unlikely that seals included in this study were in stage III starvation.

4.5 Acknowledgments

We thank the subsistence hunters in the communities of Barrow, Point Hope, Shishmaref, Little Diomede, Savoonga, and Hooper Bay for making ice seal samples available. The University of Alaska Coastal Marine Institute (BOEM Cooperative Agreement M09AC15432) provided funding for this project. Partial support for Horstmann- Dehn came from the National Science Foundation (ARC-0902177). We gratefully acknowledge the North Slope Borough Department of Wildlife Management, the Alaska Department of Fish and Game, Arctic Marine Mammal Program, Alaska Stable Isotope Facility, and the University of Alaska Museum of the North in Fairbanks for their support with this project. Specifically, we thank T. Hepa, C. Hanns, B. Adams, G. Krafsur, J. Herreman, L. Quakenbush, A. Bryan, J. Seymour, C. Gleason, M. Wooller, T. Howe, N. Haubenstock, A. Blanchard, B. Holladay, and L. Edenfield for their assistance during sample collections, sample processing, and statistical analysis. A special thank you to A. Ruby, N. Farnham and all of the fisheries lab technicians for their help with sample processing. We also thank M. Wooller and L. Quakenbush for their review of this manuscript and helpful comments. Marine mammal samples were collected under the authority of National Marine Fisheries Service Scientific Research Permit Numbers 358- 1585 and 358-1787 issued to the Alaska Department of Fish and Game, Arctic Marine Mammal Program, and DWM-814-1899 issued to the North Slope Borough, Department of Wildlife Management. Funding for sample collections was provided by the National Oceanic and Atmospheric Administration Fisheries Service. Fishes were collected and euthanized under UAF International Animal Care and Use Committee protocol 07-47.

4.6 Tables

Table 4-1. **Stable Isotope Signatures of Ice Seals.** Stable nitrogen and carbon isotope ratios for muscle of ice seals during each year. Number of samples analyzed in Bayesian isotopic mixing models (*n*) and parenthesis show the number of samples that were stored in ethanol.

Species	Year of Collection	*n*	$\delta^{15}N$ [‰] Non-Lipid-Extracted	$\delta^{13}C$ [‰] Non-Lipid-Extracted
Ringed Seal *(Pusa hispida)*	2003	31	16.88 ± 0.63	-19.63 ± 1.64
	2008	14 (5)	16.51 ± 0.64	-18.73 ± 0.77
	2009	14	16.46 ± 1.00	-18.88 ± 1.07
	2010	22	17.06 ± 0.79	-19.11 ± 0.53
Total *(n)*		**81**		
Bearded Seal *(Erignathus barbatus)*	2003	62	17.21 ± 0.79	-17.94 ± 0.96
	2008	51 (5)	16.39 ± 0.96	-17.58 ± 0.91
	2009	54	16.74 ± 0.94	-17.83 ± 1.27
	2010	63	16.28 ± 0.94	-17.88 ± 0.88
Total *(n)*		**230**		
Spotted Seal *(Phoca largha)*	2003	29	18.22 ± 1.14	-18.42 ± 0.64
	2008	20	17.43 ± 0.80	-18.65 ± 0.87
	2009	56	17.69 ± 0.77	-19.13 ± 0.84
Total *(n)*		**105**		
Total Sample Size *(n)*		**416**		

Table 4-2. **Stable Isotope Signatures of Ice Seal Prey.** Stable nitrogen and carbon isotope ratios for select fishes and invertebrate prey of ice seals. Number of samples analyzed in Bayesian isotopic mixing models (*n*). Shaded columns are values used in mixing models. (N/A, not available)

Species	Mixing Model Grouping	Examples of Other Prey Taxa in Grouping	Year of Collection	*n*	$\delta^{15}N$ [‰] Non-Lipid-Extracted	$\delta^{15}N$ [‰] Lipid-Extracted	$\delta^{13}C$ [‰] Non-Lipid-Extracted	$\delta^{13}C$ [‰] Lipid-Extracted	Source
Arctic Staghorn Sculpin *(Gymnocanthus tricuspis)*	High-Trophic, Benthic	Crabs, Shrimps, Polychaetes	2009	10	15.53 ± 0.49	15.50 ± 0.98	-19.28 ± 0.60	-17.88 ± 0.55	this study
Bering Flounder *(Hippoglossoides robustus)*	High-Trophic, Benthic	Crabs, Shrimps, Polychaetes	2009	10	15.25 ± 0.68	15.38 ± 0.72	-19.67 ± 0.99	-18.36 ± 0.30	this study
Canadian Eelpout *(Lycodes polaris)*	High-Trophic, Benthic	Crabs, Shrimps, Polychaetes	2009	10	15.44 ± 0.45	15.73 ± 0.70	-18.84 ± 0.56	-17.77 ± 0.75	this study
Shorthorn Sculpin *(Myoxocephalus scorpius)*	High-Trophic, Benthic	Crabs, Shrimps, Polychaetes	2009	10	14.96 ± 0.78	14.83 ± 1.26	-19.13 ± 0.42	-17.75 ± 0.78	this study
Slender Eelblenny *(Lumpenus fabricii)*	High-Trophic, Benthic	Crabs, Shrimps, Polychaetes	2009	10	15.09 ± 0.78	14.88 ± 1.05	-19.44 ± 0.40	-17.64 ± 0.57	this study
Stout Eelblenny *(Anisarchus medius)*	High-Trophic, Benthic	Crabs, Shrimps, Polychaetes	2009	10	16.00 ± 0.52	15.98 ± 0.79	-19.36 ± 0.76	-17.71 ± 0.69	this study
Arctic Cod *(Boreogadus saida)*	Mid-Trophic, Benthopelagic	Isopods	2009	10	14.24 ± 0.69	14.96 ± 0.66	-21.17 ± 0.63	-19.50 ± 0.32	this study
Capelin *(Mallotus villosus)*	Mid-Trophic, Benthopelagic	Isopods	2010	10	12.51 ± 0.51	13.43 ± 0.57	-22.47 ± 0.85	-19.41 ± 0.28	this study
Pacific Herring *(Clupea pallasii)*	Mid-Trophic, Benthopelagic	Isopods	2010	10	14.07 ± 0.66	14.71 ± 0.41	-22.85 ± 0.57	-20.72 ± 0.59	this study
Pacific Sand Lance *(Ammodytes hexapterus)*	Mid-Trophic, Benthopelagic	Isopods	2010	10	13.34 ± 0.76	14.12 ± 0.66	-22.05 ± 0.72	-19.71 ± 0.32	this study
Saffron Cod *(Eleginus gracilis)*	Mid-Trophic, Benthopelagic	Isopods	2009	10	13.42 ± 0.52	13.88 ± 0.27	-20.80 ± 0.84	-19.70 ± 1.08	this study
Rainbow Smelt *(Osmerus mordax)*	Mid-Trophic, Pelagic	Young Fishes	2007	10	13.53 ± 0.45	14.21 ± 0.51	-23.87 ± 0.27	-23.09 ± 0.35	this study
Greenland Cockle *(Serripes groenlandicus)*	Low-Trophic, Benthic	Bivalves	2004	6	8.07 ± 1.05	N/A	-17.74 ± 0.31	N/A	Iken et al. 2010
Arctic Krill *(Thysanoessa rashii)*	Low-Trophic, Pelagic	Amphipods	N/A	N/A	N/A	12.25 ± 1.16	N/A	-19.69 ± 0.96	Iken unpublished

Table 4-3. **ANOVA for Stable Isotope Ratios Among Fishes.** Either Holm-Sidak Method (t) or Dunn's Method (Q) were used for the pairwise multiple comparison. Bold font indicates significant differences.

		One-Way Anova Results for $\delta^{15}N$ Values Among Species											
		Arctic staghorn sculpin	**Bering flounder**	**Canadian eelpout**	**Shorthorn sculpin**	**Slender eelblenny**	**Stout eelblenny**	**Arctic cod**	**Capelin**	**Pacific herring**	**Pacific sand lance**	**Saffron cod**	**Rainbow smelt**
One Way Anova Results for $\delta^{13}C$ Values Among Species	**Arctic staghorn sculpin**		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	**$t = 3.757$, $p = 0.011$**	**$t = 8.777$, $p < 0.001$**	**$t = 4.246$, $p = 0.002$**	**$t = 6.353$, $p < 0.001$**	**$t = 6.128$, $p < 0.001$**	**$t = 5.793$, $p < 0.001$**
	Bering flounder	$p > 0.05$		$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	**$t = 7.960$, $p < 0.001$**	**$t = 3.429$, $p = 0.031$**	**$t = 5.536$, $p < 0.001$**	**$t = 5.312$, $p < 0.001$**	**$t = 4.976$, $p < 0.001$**
	Canadian eelpout	$p > 0.05$	$p > 0.05$		$p > 0.05$	$p > 0.05$	$p > 0.05$	**$t = 3.495$, $p = 0.026$**	**$t = 8.514$, $p < 0.001$**	**$t = 3.984$, $p = 0.005$**	**$t = 6.090$, $p < 0.001$**	**$t = 5.866$, $p < 0.001$**	**$t = 5.531$, $p < 0.001$**
	Shorthorn sculpin	$p > 0.05$	$p > 0.05$	$p > 0.05$		$p > 0.05$	$p > 0.05$	$p > 0.05$	**$t = 8.729$, $p < 0.001$**	$p > 0.05$	**$t = 5.760$, $p < 0.001$**	**$t = 5.486$, $p < 0.001$**	**$t = 5.075$, $p < 0.001$**
	Slender eelblenny	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$		$p > 0.05$	$p > 0.05$	**$t = 7.501$, $p < 0.001$**	$p > 0.05$	**$t = 5.077$, $p < 0.001$**	**$t = 4.852$, $p < 0.001$**	**$t = 4.517$, $p < 0.001$**
	Stout eelblenny	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$		**$t = 5.123$, $p < 0.001$**	**$t = 10.142$, $p < 0.001$**	**$t = 5.612$, $p < 0.001$**	**$t = 7.718$, $p < 0.001$**	**$t = 7.494$, $p < 0.001$**	**$t = 7.159$, $p < 0.001$**
	Arctic cod	**$Q = 3.920$, $p < 0.05$**	$p > 0.05$	**$Q = 4.091$, $p < 0.05$**	**$Q = 4.100$, $p < 0.05$**	**$Q = 4.408$, $p < 0.05$**	**$Q = 4.126$, $p < 0.05$**		**$t = 6.147$, $p < 0.001$**	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
	Capelin	**$Q = 3.732$, $p < 0.05$**	$p > 0.05$	**$Q = 3.903$, $p < 0.05$**	**$Q = 3.912$, $p < 0.05$**	**$Q = 4.220$, $p < 0.05$**	**$Q = 3.937$, $p < 0.05$**	$p > 0.05$		**$t = 5.549$, $p < 0.001$**	$p > 0.05$	$p > 0.05$	**$t = 3.654$, $p = 0.016$**
	Pacific herring	**$Q = 4.622$, $p < 0.05$**	**$Q = 3.613$, $p < 0.05$**	**$Q = 4.750$, $p < 0.05$**	**$Q = 4.757$, $p < 0.05$**	**$Q = 4.988$, $p < 0.05$**	**$Q = 4.776$, $p < 0.05$**	$p > 0.05$	**$Q = 3.271$, $p < 0.05$**		$p > 0.05$	$p > 0.05$	$p > 0.05$
	Pacific sand lance	**$Q = 3.581$, $p < 0.05$**	**$Q = 3.263$, $p < 0.05$**	**$Q = 3.709$, $p < 0.05$**	**$Q = 3.716$, $p < 0.05$**	**$Q = 3.947$, $p < 0.05$**	**$Q = 3.735$, $p < 0.05$**	$p > 0.05$	$p > 0.05$	$p > 0.05$		$p > 0.05$	$p > 0.05$
	Saffron cod	**$Q = 3.680$, $p < 0.05$**	$p > 0.05$	**$Q = 3.852$, $p < 0.05$**	**$Q = 3.860$, $p < 0.05$**	**$Q = 4.168$, $p < 0.05$**	**$Q = 3.886$, $p < 0.05$**	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$		$p > 0.05$
	Rainbow smelt	**$Q = 5.470$, $p < 0.05$**	**$Q = 4.461$, $p < 0.05$**	**$Q = 5.599$, $p < 0.05$**	**$Q = 5.605$, $p < 0.05$**	**$Q = 5.837$, $p < 0.05$**	**$Q = 5.625$, $p < 0.05$**	**$Q = 4.565$, $p < 0.05$**	**$Q = 4.961$, $p < 0.05$**	$p > 0.05$	**$Q = 3.764$, $p < 0.05$**	**$Q = 4.225$, $p < 0.05$**	

Table 4-4. **Isotope Mixing Model Data for Ice Seal Muscles.** Proportional contribution of prey guilds for ice seals by year. Data are provided as lower and upper 95% credibility intervals, along with the mean value, as determined by the SIAR mixing model. Examples of prey taxa within each guild include crabs, shrimps, and demersal fishes (High-Trophic, Benthic), isopods and cods (Mid-Trophic, Benthopelagic), young fishes (Mid-Trophic, Pelagic), bivalves (Low-Trophic, Benthic), and amphipods and euphausiids (Low-Trophic, Pelagic) (N/A, not applicable).

Species	Year Diet Represents	Prey Guild									
		High-Trophic, Benthic		Mid-Trophic, Benthopelagic		Mid-Trophic, Pelagic		Low-Trophic, Benthic		Low-Trophic, Pelagic	
		95 % CI	mean	95 % CI	mean	95 % CI	mean	95 % CI	mean	95 % CI	mean
Ringed seal											
(Pusa hispida)	2002/2003	34 - 55%	44.5	0.0 - 20%	6.8	33 - 58%	45.9	N/A		0.0 - 7.8%	2.9
	2007/2008	22 - 50%	36.0	0.0 - 47%	24.2	14 - 41%	27.7	N/A		0.0 - 27%	12.1
	2008/2009	14 - 48%	31.1	0.0 - 48%	24.1	12 - 44%	28.6	N/A		0.0 - 33%	16.2
	2009/2010	32 - 54%	44.2	0.0 - 26%	9.0	35 - 50%	43.2	N/A		0.0 - 11%	3.7
Bearded Seal											
(Erignathus barbatus)	2002/2003	61 - 76%	69.0	0.0 - 19%	7.0	16 - 29%	22.9	0.0 - 2.8%	1.1	N/A	
	2007/2008	37 - 65%	50.3	2.0 - 55%	31.4	0.0 - 19%	9.0	3.0 - 15%	9.2	N/A	
	2008/2009	39 - 68%	53.6	1.2 - 54%	28.8	1.3 - 26%	13.8	0.0 - 8.3%	3.8	N/A	
	2009/2010	28 - 60%	43.5	1.3 - 65%	34.3	0.5 - 26%	13.2	2.5 - 16%	9.0	N/A	
Spotted Seal											
(Phoca largha)	2002/2003	0.0 - 24%	7.5	0.0 - 40%	14.5	0.0 - 22%	6.9	N/A		37 - 97%	71.1
	2007/2008	0.0 - 39%	15.2	0.0 - 48%	22.4	0.0 - 34%	12.2	N/A		17 - 90%	50.2
	2008/2009	0.0 - 9.5%	3.2	0.0 - 19%	6.1	0.0 - 9.0%	3.1	N/A		72 - 100%	87.6

4.7 Figures

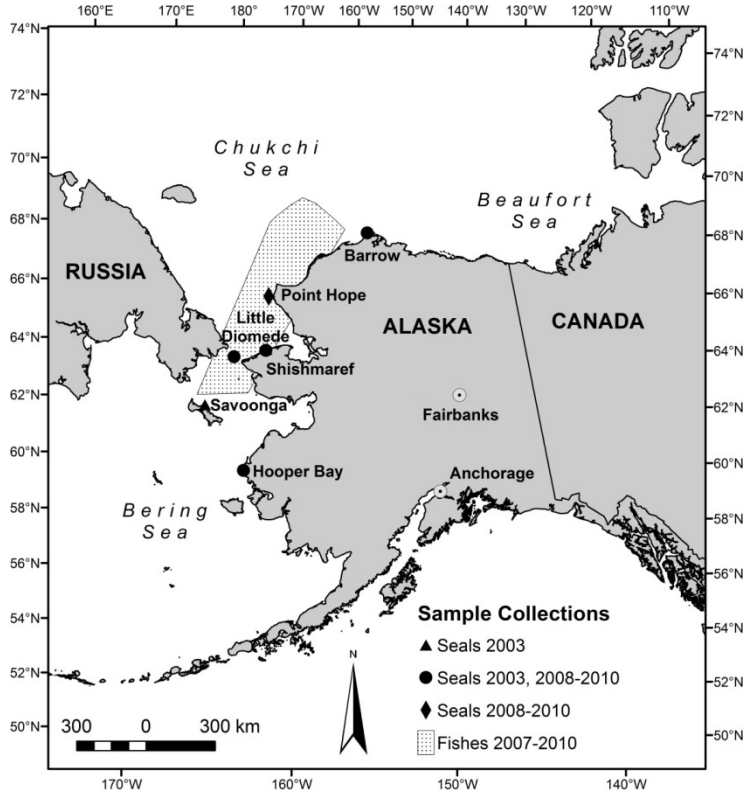


Figure 4-1. **Sample Collection Map.** Location map showing Alaska Native subsistence communities where ice seal samples were collected and the region (stippled) where fishes were caught.

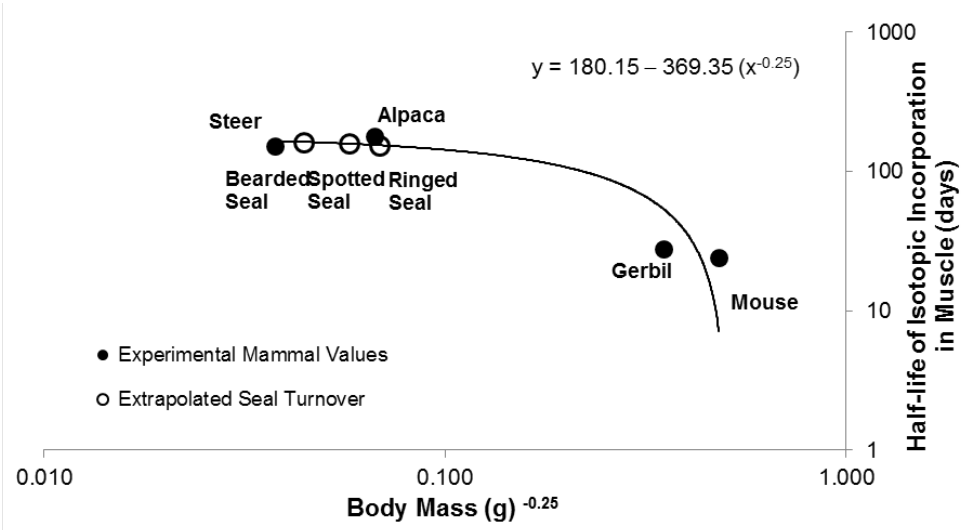


Figure 4-2. **Muscle Turnover Based on Body Mass.** Half-life of stable isotope integration into tissue (muscle turnover in days) based on a total body mass in grams, i.e., half-life of stable carbon isotope turnover = mass$^{-0.25}$ (Carleton and Martínez del Rio 2005). Experimental results for terrestrial mammals (solid circles, Tieszen et al. 1983; MacAvoy et al. 2005; Sponheimer et al. 2006; Bahar et al. 2009) were used to create a curvilinear regression; $y = 180.15 - 369.35\ (x^{-0.25})$. This regression equation was then used to estimate muscle turnover for ice seals (open circles) based on lean body mass.

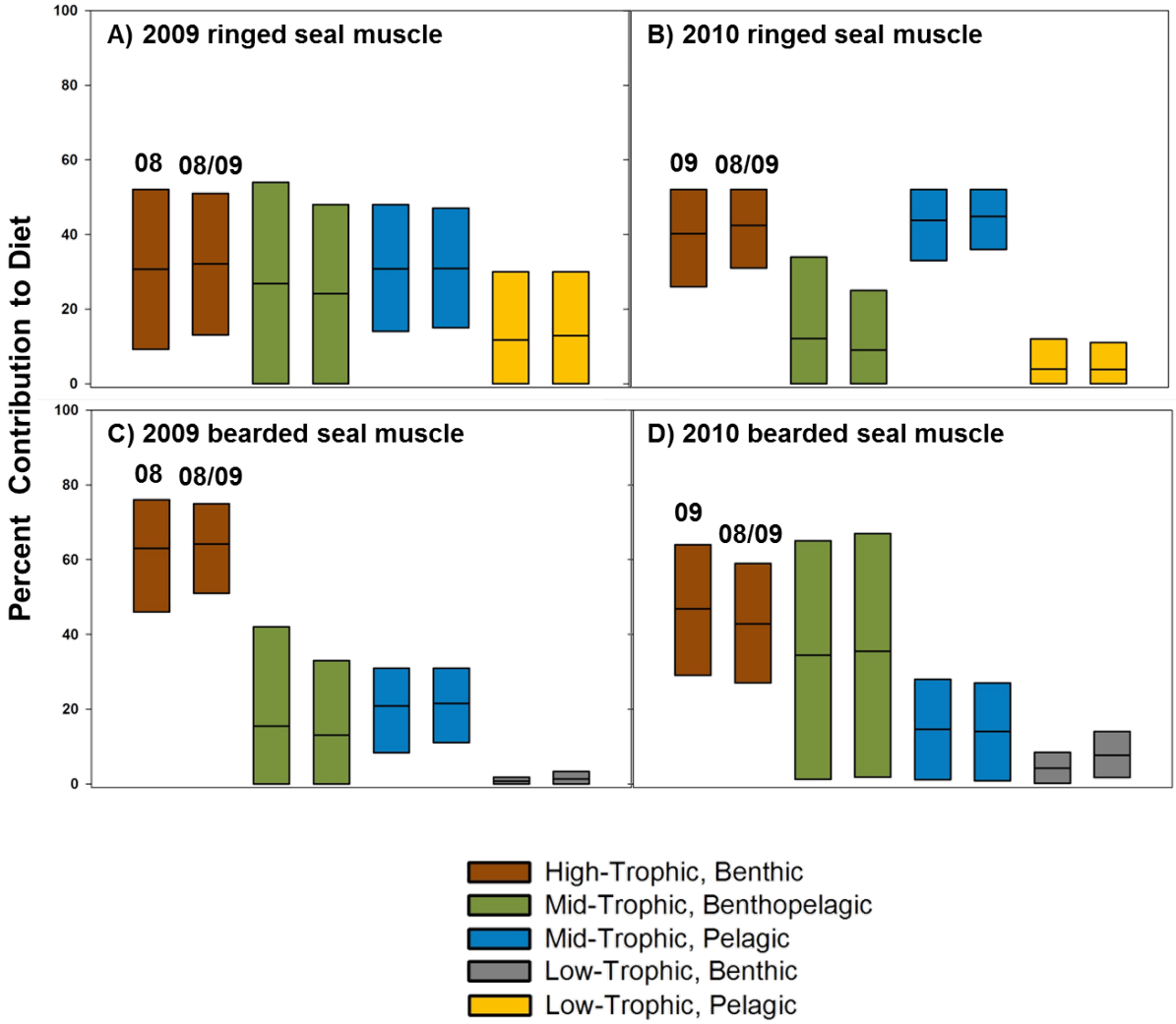


Figure 4-3. **Mixing Models with Prey Year Differences**. Mixing model results for ringed seal muscle from 2009 (a) and 2010 (b), and bearded seal muscle from 2009 (c) and 2010 (d). Results are presented as 95% credibility intervals and mean values (vertical black lines). The first column shows percent contribution of each prey group when stable isotope ratios for demersal fishes from the year prior to seal collections are included in the model. The second column shows percent contribution of each prey group when stable isotope ratios for demersal fishes (2008/2009) are averaged and included in the model.

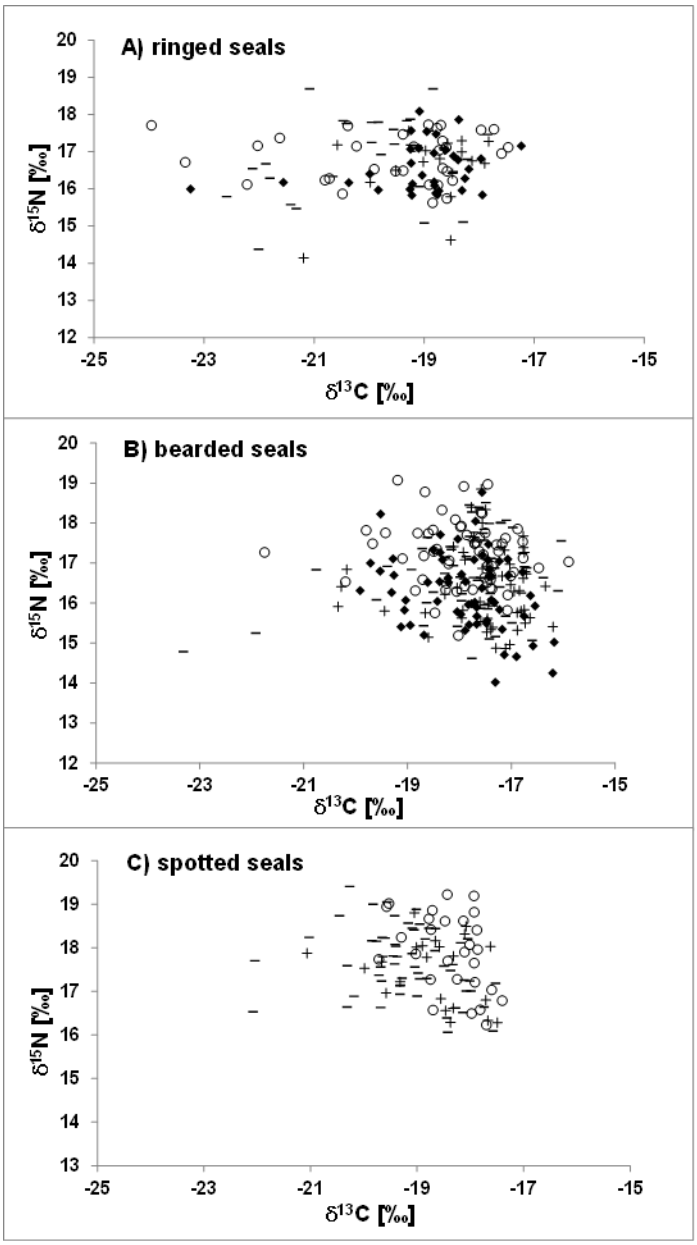


Figure 4-4. **Stable Isotope Signatures for the Sampled Population of Ice Seals.** Stable nitrogen versus carbon isotope ratios for muscle of A) ringed, B) bearded, and C) spotted seals. Symbols represent harvest year for individual seals, i.e., 2003 (open circle), 2008 (cross), 2009 (line), and 2010 (solid diamond).

4.8 Appendices

Appendix 4-1. **Ethanol Preservation Effects on Stable Isotope Ratios of Seal Muscle.** Stable nitrogen and carbon isotope ratios for non-preserved muscle samples and muscle samples preserved in 100% ethanol. A paired t-test was used to compare stable isotope ratios between treatments.

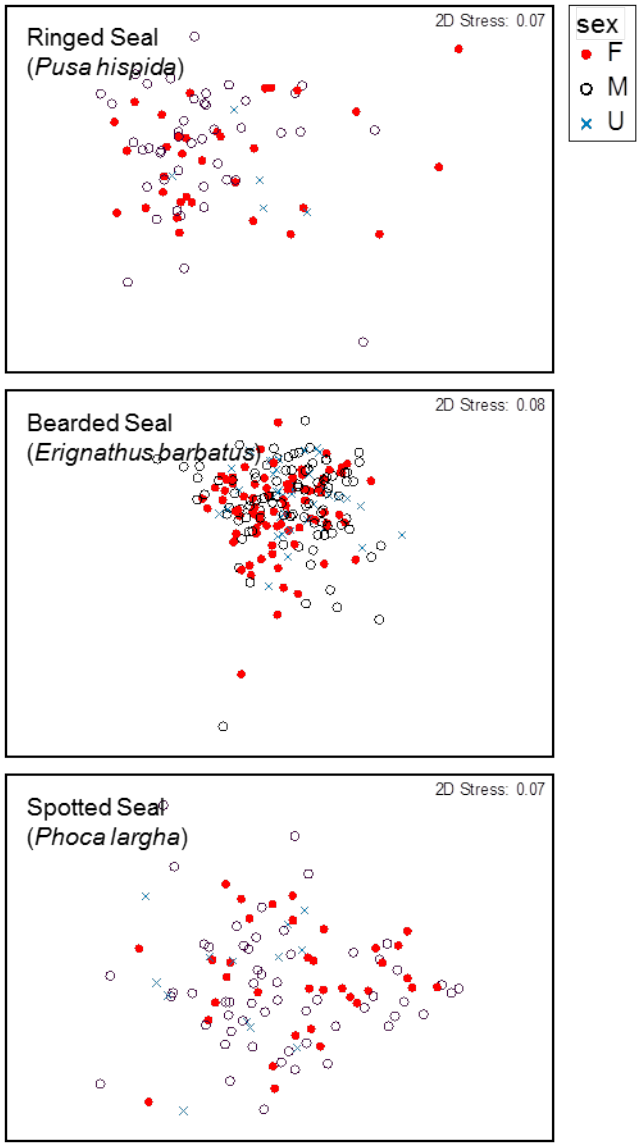
Species	*n*	$\delta^{15}N$ [‰] Non-Preserved	$\delta^{15}N$ [‰] Preserved in 100% Ethanol	$\delta^{15}N$ *t, p*	$\delta^{13}C$ [‰] Non-Preserved	$\delta^{13}C$ [‰] Preserved in 100% Ethanol	$\delta^{13}C$ *t, p*
Ringed Seal *(Pusa hispida)*	5	16.68 ± 1.07	16.90 ± 0.92	t = 1.397, p = 0.235	-18.19 ± 0.81	-18.01 ± 0.71	t = 1.189, p = 0.300
Bearded Seal *(Erignathus barbatus)*	5	16.42 ± 0.83	16.83 ± 0.25	t = 1.411, p = 0.231	-18.74 ± 0.24	-18.65 ± 0.31	t = 2.655, p = 0.057

Appendix 4-2. **Lipid Extraction Effects on Stable Isotope Ratios of Seal Muscle.** Stable nitrogen and carbon isotope ratios for non-lipid-extracted and lipid-extracted seal muscle. A paired t-test was used to compare stable isotope ratios between treatments. Bold font indicates significant differences.

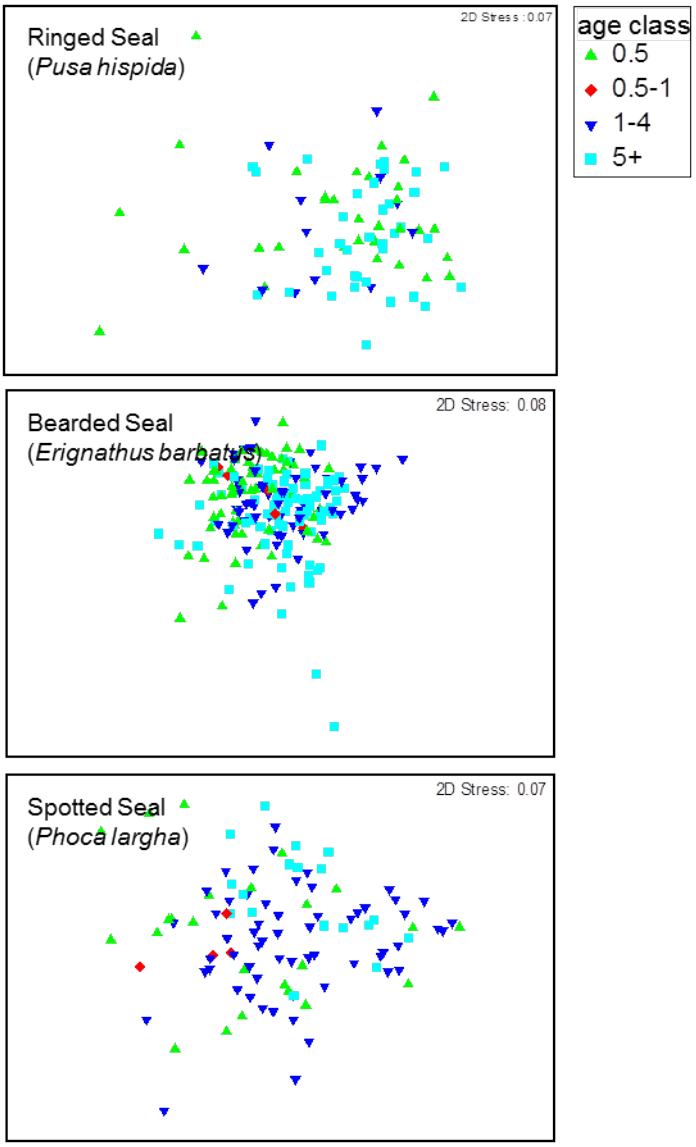
Species	*n*	$\delta^{15}N$ [‰] Non-Lipid-Extracted	$\delta^{15}N$ [‰] Lipid-Extracted	$\delta^{15}N$ *t, p*	$\delta^{13}C$ [‰] Non-Lipid-Extracted	$\delta^{13}C$ [‰] Lipid-Extracted	$\delta^{13}C$ *t, p*
Ringed Seal *(Pusa hispida)*	5	16.08 ± 0.20	16.69 ± 0.27	**t = -6.220, p = 0.003**	-18.83 ± 0.12	-19.00 ± 0.44	t = 0.787, p = 0.475
Bearded Seal *(Erignathus barbatus)*	5	16.34 ± 1.17	16.31 ± 0.73	t = 0.097, p = 0.927	-18.35 ± 0.91	-18.44 ± 0.79	t = 1.003, p = 0.373
Spotted Seal *(Phoca largha)*	5	17.01 ± 0.77	17.64 ± 0.79	t = -1.513, p = 0.205	-18.89 ± 0.82	-18.47 ± 0.71	t = -2.496, p = 0.067

Appendix 4-3. **Lipid Extraction Effects on Stable Isotope Ratios in Fish Tissue.** Stable nitrogen and carbon isotope ratios for non-lipid-extracted and lipid-extracted total body homogenate of fishes. A paired t-test was used to compare stable isotope ratios between treatments. Bold font indicates significant differences.

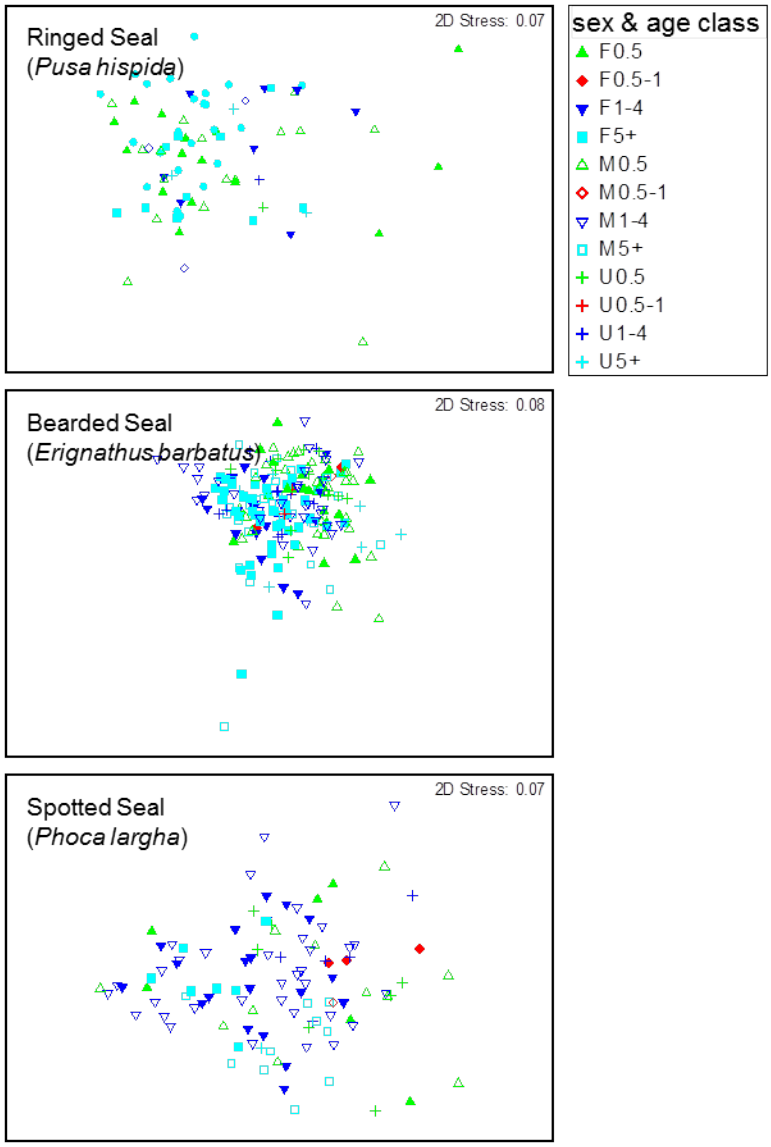
Species	*n*	$\delta^{15}N$ [‰] Non-Lipid-Extracted	$\delta^{15}N$ [‰] Lipid-Extracted	$\delta^{13}C$ [‰] Non-Lipid-Extracted	$\delta^{13}C$ [‰] Lipid-Extracted	$\delta^{15}N$ *t, p*	$\delta^{13}C$ *t, p*
Arctic Staghorn Sculpin *(Gymnocanthus tricuspis)*	10	15.53 ± 0.49	15.50 ± 0.98	-19.28 ± 0.60	-17.88 ± 0.55	p > 0.05	***t* = -6.317** ***p* = 0.003**
Bering Flounder *(Hippoglossoides robustus)*	10	15.25 ± 0.68	15.38 ± 0.72	-19.67 ± 0.99	-18.36 ± 0.30	p > 0.05	***t* = -3.188** ***p* = 0.033**
Canadian Eelpout *(Lycodes polaris)*	10	15.44 ± 0.45	15.73 ± 0.70	-18.84 ± 0.56	-17.77 ± 0.75	p > 0.05	***t* = -7.860** ***p* = 0.001**
Shorthorn Sculpin *(Myoxocephalus scorpius)*	10	14.96 ± 0.78	14.83 ± 1.26	-19.13 ± 0.42	-17.75 ± 0.78	p > 0.05	***t* = -7.148** ***p* < 0.001**
Slender Eelblenny *(Lumpenus fabricii)*	10	15.09 ± 0.78	14.88 ± 1.05	-19.44 ± 0.40	-17.64 ± 0.57	p > 0.05	***t* = -6.588** ***p* = 0.003**
Stout Eelblenny *(Anisarchus medius)*	10	16.00 ± 0.52	15.98 ± 0.79	-19.36 ± 0.76	-17.71 ± 0.69	p > 0.05	***t* = -8.223** ***p* = 0.001**
Arctic Cod *(Boreogadus saida)*	10	14.24 ± 0.69	14.96 ± 0.66	-21.17 ± 0.63	-19.50 ± 0.32	***t* = -4.865** ***p* < 0.001**	***t* = -8.691** ***p* < 0.001**
Capelin *(Mallotus villosus)*	10	12.51 ± 0.51	13.43 ± 0.57	-22.47 ± 0.85	-19.41 ± 0.28	***t* = -12.006** ***p* < 0.001**	***t* = -12.629** ***p* < 0.001**
Pacific Herring *(Clupea pallasii)*	10	14.07 ± 0.66	14.71 ± 0.41	-22.85 ± 0.57	-20.72 ± 0.59	***t* = -4.840** ***p* < 0.001**	***t* = -15.226** ***p* < 0.001**
Pacific Sand Lance *(Ammodytes hexapterus)*	10	13.34 ± 0.76	14.12 ± 0.66	-22.05 ± 0.72	-19.71 ± 0.32	***t* = -7.486** ***p* < 0.001**	***t* = -11.935** ***p* < 0.001**
Saffron Cod *(Eleginus gracilis)*	10	13.42 ± 0.52	13.88 ± 0.27	-20.80 ± 0.84	-19.70 ± 1.08	**t = -4.000** **p = 0.003**	**t = -5.197** **p < 0.001**
Rainbow Smelt *(Osmerus mordax)*	10	13.53 ± 0.45	14.21 ± 0.51	-23.87 ± 0.27	-23.09 ± 0.35	***t* = -10.236** ***p* < 0.001**	***t* = -7.989** ***p* < 0.001**



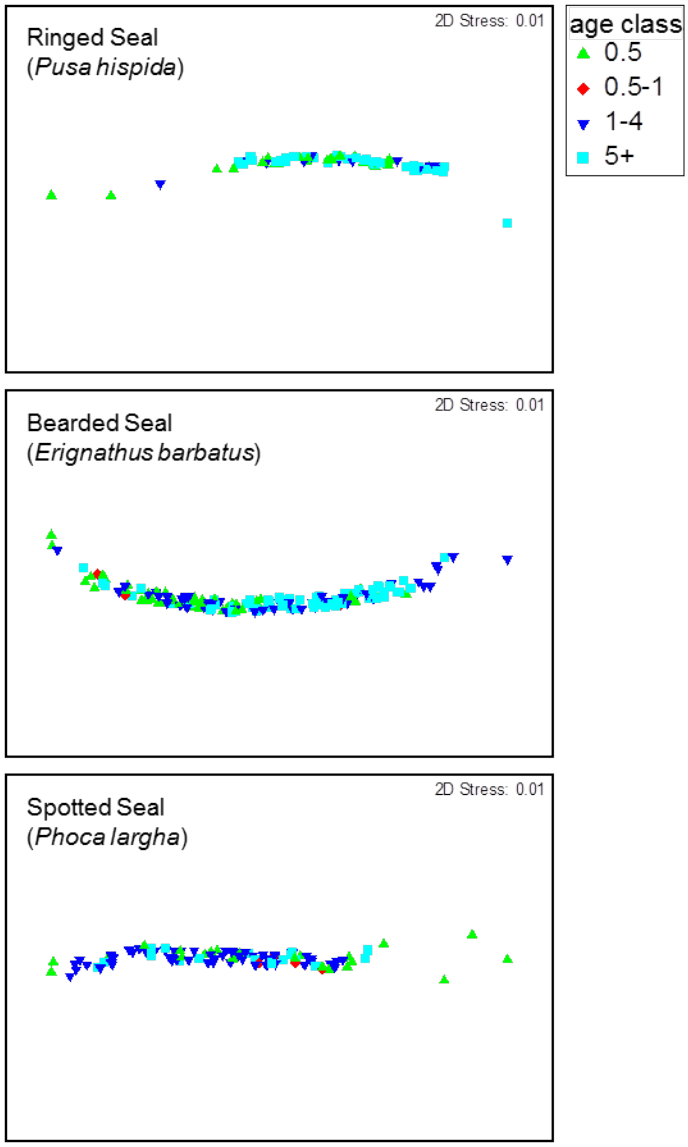
Appendix 4-4. **MDS Plots of $\delta^{15}N$ and $\delta^{13}C$ Values for Seal Muscle by Sex**. Non-metric multidimensional scaling of stable nitrogen and carbon isotope ratios in seal muscle to compare among sex for each seal species. Sex of the seal was either female (F), male (M), and unknown (U).



Appendix 4-5. **MDS Plots of $\delta^{15}N$ and $\delta^{13}C$ Values for Seal Muscle by Age Class**. Non-metric multidimensional scaling of stable nitrogen and carbon isotope ratios in seal muscle to compare among age classes for each seal species. Age classes consist of young-of-the-year (0.5 and 0.5-1 yr.), subadults (1-4 yrs.), and adults (+5 yrs.) (Quakenbush et al. 2009, 2010a).



Appendix 4-6. **MDS Plots of $\delta^{15}N$ and $\delta^{13}C$ Values for Seal Muscle by Sex and Age Class**. Non-metric multidimensional scaling of stable nitrogen and carbon isotope ratios in seal muscle to compare among sex and age class combined for each seal species. Sex of the seal was either female (F), male (M), or unknown (U). Age classes consist of young-of-the-year (0.5 and 0.5–1 yr.), subadults (1–4 yrs), and adults (+5 yrs) (Quakenbush et al. 2009, 2010a).



Appendix 4-7. **MDS Plots of δ^{15}N Values for Seal Muscle by Age Class**. Non-metric multidimensional scaling of stable nitrogen isotope ratios in seal muscle to compare among age classes. Age classes consist of young-of-the-year (0.5 and 0.5–1 yr.), subadults (1-4 yrs.), and adults (+5 yrs.) (Quakenbush et al. 2009, 2010a).

Chapter 5: Diet History of Ice Seals Using Stable Isotope Ratios in Claw Growth Bands[1]

Sara Carroll, Larissa Horstmann-Dehn, Brenda Norcross

5.1 Introduction

Ringed (*Pusa hispida*), bearded (*Erignathus barbatus*), spotted (*Phoca largha*), and ribbon seals (*Histriophoca fasciata*) use different habitats within the Arctic ecosystem (Simpkins et al. 2003) and have diverse feeding ecologies. Ringed seals prefer land-fast ice along the coast and have a diet predominantly comprised of ice-associated prey, i.e., gadids, euphausiids, amphipods, and mysids (McLaren 1958; Lowry et al. 1980a; Quakenbush et al. 2010a). In contrast, bearded seals are typically benthic foragers and are found in drifting pack ice over shallow coastal areas (Burns and Frost 1979; Lowry et al. 1980b; Dehn et al. 2007). Common prey items identified in the stomachs of bearded seals are demersal fishes, e.g., eelpouts, flatfishes, pricklebacks, and sculpins, as well as benthic invertebrates, e.g., bivalves, crabs, and shrimps (Johnson et al. 1966; Finley and Evans 1983; Quakenbush et al. 2010b). However, bearded seals also may consume pelagic fish species, such as Capelin[2] (*Mallotus villosus*) (Antonelis et al. 1994). Spotted seals are found in broken ice floes near the ice front during the winter/spring and during the open water season they make frequent visits to coastal haulouts (Lowry et al. 1998). Spotted seals feed on similar fish and invertebrate species as both ringed and bearded seals (Gol'tsev 1971; Bukhtiyarov et al. 1984; Quakenbush et al. 2009). Ribbon seals are associated with loose pack ice during the breeding season and then become pelagic when waters are ice free (Burns 1970). Less is known about the diet of ribbon seals compared to the other seal species, but prey items include demersal and pelagic fishes, amphipods, bivalves, cephalopods, crabs, euphausiids, and decapod shrimps (Shustov 1965; Frost and Lowry 1980). Alterations to diet strategies of these species may occur in response to changes in habitat structure.

In the last decade, Arctic sea ice has decreased in extent and thickness. Minimum sea ice extent occurs in September and was lowest in 2007 relative to the average from satellite records during 1979–2000 (NSIDC 2012). In the following summers since 2007, less of the thicker multi-year ice persisted leading to a sea ice decline in 2012 surpassing that of 2007 (ARCUS 2012). In response to sea-ice habitat loss and predicted reduced snow cover, the National Oceanic and Atmospheric Administration (NOAA) listed the Arctic Basin population of ringed seals and the Okhotsk population of bearded seals as threatened under the Endangered Species Act (ESA; NOAA 2012a). Because sea ice in the Bering Sea is expected to persist in winter and is not present in the summer, the Bering Sea population of spotted seals and the entire species of ribbon seals are expected to remain unaffected by the summer sea ice minima of the Arctic Ocean and have not been listed under the ESA (NOAA 2008, 2009). Ice seals may be directly impacted by climate change as the sea ice platform they use for resting, pupping, and molting diminishes,

[1]Portions of this chapter were published February 2013 in the Canadian Journal of Zoology (doi:10.1139/cjz-2012-0137)

[2]Fish species common and scientific names have been altered to match the remaining sections of this report and are as established by the American Fisheries Society (Page et al. 2013).

along with more subtle, indirect effects on prey resources. The food web may become more pelagically dominated during years with reduced ice cover in the Arctic Ocean as less-recycled material is exported to the seafloor (Bluhm and Gradinger 2008), potentially impacting the foraging success of benthic predators.

The nutritional changes exhibited by individual seals may ultimately hinder the growth of the population. As sea ice diminishes, a decrease in the availability of benthic prey for bearded seals (Grebmeier et al. 2006; Bluhm and Gradinger 2008; Grebmeier 2012) could result in increased competition for resources and decreased body condition. Lower body condition of female ringed seals has been correlated to lower ovulation rates in Canada, and years of low ice can cause additional negative impacts on fecundity (Harwood et al. 2000). Moreover, nutritional stress can hinder the immune response of marine mammals and increase susceptibility to disease (Burek et al. 2008). In December 2011, NOAA declared an unusual mortality event for ringed seals and other ice-associated pinnipeds in the Arctic characterized by delayed or unusual molt, skin lesions, internal organ lesions, and immune system changes (NOAA 2012b). The cause of the outbreak has not yet been determined. Overall, examining the feeding ecology of ice seals during ice minima years may give insight into how these species respond to interannual changes in the food web structure.

Stable nitrogen and carbon isotope ratios have been used extensively to study food-web structure in the Arctic (Hobson and Welch 1992; Bentzen et al. 2007; Dehn et al. 2007; Iken et al. 2010). Stable nitrogen isotope ratios describe the trophic level at which an individual feeds with stepwise enrichment of ^{15}N occurring from low to higher trophic levels (Peterson and Fry 1987; Kelly 2000). Carbon isotope ratios have been used to determine carbon source and illustrate habitat use (Schell et al. 1989; Dehn et al. 2007). For example, benthic organisms rely on organic material from surface waters that ultimately undergoes bacterial remineralization, leading to tissues enriched in ^{13}C compared to pelagic organisms that consume fresh phytoplankton (McConnaughey and McRoy 1979). Thus, predators consuming benthic prey items have tissues more enriched in ^{13}C than those feeding pelagically (Dehn et al. 2007). Compared to feeding ecology studies using stomach contents to document ingested prey, stable isotopes have the distinct advantage in that samples can be obtained minimally invasively, and they can elucidate dietary nitrogen and carbon that has been assimilated and integrated into tissues over a period of time depending on the tissue examined.

Long-term dietary records can be documented in continuously growing, metabolically inert keratin and dentine structures (Schell et al. 1989; Cherel et al. 2009; Newsome et al. 2009). Stable nitrogen and carbon isotope ratios of ringed seal claws have been analyzed to provide a long-term feeding record (Ferreira et al. 2011). An unbroken time-series of data is deposited in the keratinized and cornified claw sheath (Bragulla and Homberger 2009), more specifically the blade horn covering the lateral walls of the claw (Ethier et al. 2010). Along the sheath, i.e., horn, of a seal claw is a series of alternating light and dark keratin growth bands. The exact timing in the deposition of alternating light and dark horn bands is uncertain, but it has been documented

that the light band represents horn grown during summer, and the dark band is horn grown during winter (McLaren 1958; Benjaminsen 1973). Each set of light and dark horn bands represents a year of growth for the seal (Benjaminsen 1973). Up to 10 years of dietary information can be documented in the claw sheath as ringed and bearded seals use their claws to maintain breathing holes (Smith and Stirling 1975) resulting in wear at the tip of the claw, i.e., oldest horn growth. Stable nitrogen and carbon isotope ratios of growth layer groups in ringed seal claws can illustrate the interannual variation in diet among individuals (Ferreira et al. 2011). The overall goal of this study was to examine stable nitrogen and carbon isotope ratios in seasonal growth bands in claw sheaths of ice seals to infer seasonal and interannual changes in feeding ecology.

As diminished sea ice, earlier ice melt, and warmer waters may favor a pelagic dominated food web (Bluhm and Gradinger 2008), we hypothesize that ice seals may then capitalize on more abundant pelagic prey sources rather than preferred prey during years of reduced ice cover in the Arctic Ocean. To test this hypothesis we described general diet history for each species over the time period recorded in claw sheaths, and examined species-specific feeding history among the sampled population for interannual differences, particularly during the reduced sea ice extent of 2007. Additionally, we assessed potential age-related differences in diet and examined fetal, natal, weaning, and post-weaning stable isotope signatures in claws.

5.2 Materials and Methods

Claws of ringed, bearded, spotted, and ribbon seals were collected opportunistically during Alaska Native subsistence harvests in the coastal communities of Barrow, Point Hope, Shishmaref, Little Diomede, and Hooper Bay (Figure 5-1). Ringed seal sampling occurred during late winter through fall of 2008−2010. Bearded seals were collected during summer 2008−2010, during fall of 2009 for spotted seals, and summer 2007 and winter 2010 for ribbon seals. The whole front flipper or a single claw from the front flipper was collected shortly after death (less than 12 hours), stored in Ziploc® or Whirlpak™ bags, and frozen at −20 °C. Ice seal claws were obtained under the authority of National Marine Fisheries Service Scientific Research Permit Numbers 358-1585 and 358-1787 issued to the Alaska Department of Fish and Game, Arctic Marine Mammal Program (ADFG-AMMP) and DWM- 814-1899 issued to the North Slope Borough, Department of Wildlife Management.

Fifty-six claws from all species of various ages (Table 5-1) were prepared. The distal end of the digit was cut from digit I or II of the front flipper. All claws were trimmed of fur and tissue. Each claw was labeled with a unique identifier and placed in a glass vial filled with distilled water and situated in a water bath. Claws were soaked in an ultrasonic water bath (Branson 3510, frequency of 40 kHz) at 38°C for a minimum of 30 min or until the ungual crest at the base of the distal phalanx softened. The ungual crest and remaining cuticle skin of the cornified claw sheath were carefully shaved off with a scalpel blade. Each claw was placed back into a glass vial with a 2:1 chloroform/methanol solution completely covering the claw. Vials were returned to the water

bath and sonicated for 10 min at 38 °C. Even though keratin is mostly free of lipids (Newsome et al. 2010), the chloroform/methanol mixture was used to remove residual deposits or any lipid contamination as a result of handling from the claw surface. The solvent was replaced with distilled water and each claw was sonicated for an additional 10 min. After cleaning, claws were immersed in distilled water to enhance the visibility of the seasonal bands, and photographs were taken and sketches were produced. The lateral surface of each seasonal band along the blade horn of the claw sheath was drilled to a depth of less than 0.5 mm using a Dremel Stylus™Lithium-ion Cordless Rotary Tool (model: 1100-01) equipped with a size 105 engraving cutter to obtain horn powder for stable nitrogen and carbon isotope analysis. Drilling speed was set to 10 000−14 000 rpm. Bands were drilled starting from the base to the tip of the cornified claw sheath, i.e., most recently produced horn to older horn grown in successive seasons. Light seasonal bands were drilled first followed by the dark seasonal bands. Observations of horn band pigmentation during the month of collection, i.e., growth at base of the claw, showed that seasonal bands agreed with the findings by McLaren (1958), with light horn bands representing spring/summer growth, i.e., April–July, and dark bands displaying fall/winter growth, i.e., August–March (Table 5-1). For young seals (1−3 yrs.), additional sections were drilled to document potential pre-natal (formed during fetal development) and constriction region/natal notch (formed around the time of birth; as described by McLaren 1958) stable isotope signatures (Figure 5-2). The prenatal region was drilled about one centimeter from the tip of the claw sheath. Approximately 0.4 mg of dry horn powder from each band was collected using weigh paper and stored in 1 mL glass vials. The particular digit sampled from the front flipper was not always consistent because claws were collected opportunistically. Therefore in addition to the 56 claws, horn samples of all five claws from a front flipper of a single male ringed seal were drilled and analyzed to assess the variability of stable isotope ratios in seasonal growth bands of each cornified claw sheath (Table 5-1).

In addition to seal claws from the front flipper, teeth were used to estimate the ages of seals. Seasonal growth bands in seal claws provide minimum age estimates (McLaren 1958; Burns 1967; Benjaminsen 1973) as claw wear is removing horn bands at the distal end. Similarly, bearded seal teeth wear down at a linear rate. Thus, cementum growth layers are a better indicator of seal age compared to dentine (Benjaminsen 1973). In order to obtain age estimates from teeth, lower jaws were soaked in hot water for approximately 15 min; teeth were extracted, carefully cleaned of gum tissue, and sent to Matson's Laboratory LLC in Montana for sectioning, mounting, and staining (Giemsa blood stain, Wohlbach formula, Ricca Chemical Company). When possible seal ages were estimated by counting growth layer groups in the cementum of canine and postcanine teeth (Stewart et al. 1996), otherwise, seal claws provided minimum age estimates. Age estimates ranged from 4 to 37 years for ringed, 5 to 20+ years for bearded, 1 to 8 years for spotted, and 8 to 13 years for ribbon seals (Table 5-1).

Horn samples from seasonal bands of claws were analyzed for ^{15}N and $\delta^{13}C$ values at the Alaska Stable Isotope Facility at UAF. A subsample of horn powder, 0.2−0.4 mg, was weighed into tin capsules using a microbalance (Sartorius Model M2P). Stable isotope analysis was performed

using a Finnigan MAT Delta^{Plus}XP Isotope Ratio Mass Spectrometer (IRMS) directly coupled to a Costech Elemental Analyzer (ECS 4010, Italy). Stable isotope ratios are expressed in conventional delta (δ) notation:

$$\delta X (‰) = [(R_{sample}/ R_{standard}) - 1] \times 10^3$$

where X is ^{15}N or ^{13}C and represents the relative difference between isotope ratios in the horn sample (Rsample, $^{15}N/^{14}N$ or $^{13}C/^{12}C$) and in standard gases, i.e., atmospheric N_2 and Vienna Pee Dee Belemnite, respectively. Peptone was used as a laboratory standard and was run every 10 samples. The precision of analysis, expressed as one standard deviation from multiple analyses of peptone (n = 106) conducted during the runs of samples for $\delta^{15}N$ and $\delta^{13}C$, was 0.1‰ and < 0.1‰, respectively.

Prior to data analysis, all seal claw horn samples were corrected for the Suess Effect. Fossil fuels are depleted in ^{13}C (Keeling 1979). As the amount of anthropogenic CO2 released into the atmosphere increases, a higher amount of dissolved organic carbon depleted in ^{13}C is exchanged from the atmosphere to the ocean, i.e., Oceanic Suess Effect (Revelle and Suess 1957). The following Suess Effect exponential correction factor was used according to Misarti et al. (2010) for Arctic marine mammals:

$$\text{Suess Effect Correction Factor} = a^{(b*0.027)}$$

This correction factor was applied to the $\delta^{13}C$ value of each seasonal claw horn band in accordance with the year represented by the band. The variable a represents the maximum annual rate of $\delta^{13}C$ decrease in the North Pacific (i.e., −0.014, Quay et al. 1992), and variable b corresponds to the year of claw horn band deposition minus 1850, i.e., the start of the Industrial Revolution. An additional correction was proposed by Misarti et al. (2010) to account for the discrimination by primary producers for ^{13}C in response to elevated CO_{2aq} in the ocean as CO_{2atm} increases. However, this factor was not applied to data in this study because it is a small correction similar to instrument error (~0.1‰) and parameters needed for this calculation, i.e., cell growth rate, surface area of the cell, salinity, and sea surface temperatures, are variable across the ice seal geographic range (within and among species) and thus make it difficult to generalize.

In order to test the variability in stable isotope ratios of seasonal growth bands among claws from the same individual, statistical analysis was performed in SigmaPlot Version 12.0 (Systat Software, Inc. 2011). A repeated-measures ANOVA test for heterogeneity was used to compare both $\delta^{15}N$ and $\delta^{13}C$ values in seasonal growth bands among the five claw sheaths of the one individual, similar to the statistical approach used by Ferreira et al. (2011). Normal distribution assumptions were not met based on the Shapiro-Wilk test. Thus, stable isotope ratios among the five claw sheaths were analyzed using the Friedman repeated-measures ANOVA on ranks. In addition, a Spearman's rank-order correlation test was applied to test the correlation of both $\delta^{15}N$ and $\delta^{13}C$ values in seasonal growth bands among each cornified claw sheath. A p-value of less than 0.05 was considered significant.

In order to examine the general diet of ice seals, confidence intervals were created to illustrate "typical" stable isotope ratios displayed by the sampled population for each species. Studentized residuals were computed to depict extreme values or "anomalies." For spotted seal pups, a mean value and standard deviation was calculated for the change in isotope ratios during each developmental stage (e.g., fetal to natal claw horn growth stage). $\delta^{15}N$ and $\delta^{13}C$ values of claw sheaths corresponding to fetal/pup development were removed from the analysis of juvenile and adult seals; this included growth from prenatal to the first seasonal band located post-natal notch on the surface of the cornified claw sheath (Figure 5-2). Excluding stable isotope signatures likely relating to fetal/pup developmental phases from analysis removes the possible bias related to maternal nutrient transfer during nursing (Jenkins et al. 2001; Stegall et al. 2008). A linear mixed-effects model with temporal pseudoreplication (Crawley 2007) was run independently for each species in the computer software R (Version 2.11.1, R Development Core Team 2011) for both $\delta^{15}N$ and $\delta^{13}C$ values of ringed, bearded, and spotted seal claw sheaths. The fixed effects were the stable nitrogen and carbon isotope ratios, and the random effects were the seasonal growth bands for each seal. The standard deviation of $\delta^{15}N$ and $\delta^{13}C$ values across seal individuals within a species was extracted from the mixed-effects model and used to calculate a standard error for each season. Two standard errors from the species mean $\delta^{15}N$ and $\delta^{13}C$ values were used to create confidence intervals for both $\delta^{15}N$ and $\delta^{13}C$ values of each species. Additionally, studentized residuals were calculated in the computer software program R, with values greater than two considered extreme.

To identify possible trends for interannual variations over the recorded diet history of ringed, bearded, and spotted seals, residuals for both $\delta^{15}N$ and $\delta^{13}C$ values of seasonal bands were normalized among individuals for each seasonal growth band. Standard deviations of these averaged residuals were used to create upper and lower confidence intervals. Graphic representations of stable isotope data used to examine general diet and interannual trends were created using SigmaPlot Version 12.0 (Systat Software, Inc. 2011).

The percent contribution of variability from each source, i.e., among individual seals, season, and residual error, was assessed using their standard deviations. The standard deviations provided by the linear, mixed-effects model with temporal pseudoreplication were squared to show the variance for all three sources. Each variance was divided by the total variance to acquire the percent contribution of variability among individual seals, seasons, and residual error.

5.3 Results

5.3.1 Variation Among Digits

The $\delta^{15}N$ and $\delta^{13}C$ values among the five cornified claw sheaths from the same seal were not significantly different among the claws ($p = 0.319$). Moreover, $\delta^{15}N$ and $\delta^{13}C$ values of growth bands for each season across each claw (digit I−V) from the same individual were highly correlated (Table 5-2, Figure 5-3). When comparing growth bands from digit I–V, there was one

less seasonal band of the cornified claw sheath present at the tip of each consecutive claw, with approximately four seasonal growth bands not present at the distal end of digit V (Figure 5-3).

5.3.2 Stable Isotope History by Species

Ringed Seal

Isotopic signatures in the growth bands of ringed seal claws were highly variable both seasonally and interannually within individuals, and no consistent seasonal pattern was apparent (Figure 5-4a, Figure 5-4b, Figure 5-5a, Figure 5-5b). The range of $\delta^{15}N$ values in growth bands of ringed seal claw sheaths among individuals was 15.0 to 19.4‰, with maximum variations in seasonal bands within an individual seal claw sheath ranging from 0.4‰ to 2.4‰ (Figure 5-4a). Typical $\delta^{15}N$ values ranged from 16.8 to 17.4‰, based on confidence intervals over the time period of the largest sample size (i.e., fall/winter 2001 to spring/summer 2009; $n > 10$ seals). $\delta^{13}C$ values in seasonal bands of ringed seal claws among individuals ranged from -21.1 to -14.6 ‰, with maximum variations in seasonal bands within an individual seal claw sheath ranging from 0.5‰ to 1.5‰, excluding one individual having a variation of 5.4‰. Large seasonal fluctuations in $\delta^{13}C$ were observed for this one ringed seal with $\delta^{13}C$ values decreasing by more than 3.5‰ below the lower confidence interval during fall/winter 2001 and 2003 (Figure 5-5a). Typical $\delta^{13}C$ values ranged from −17.0 to −16.0‰, based on confidence intervals over the time period of the largest sample size (i.e., fall/winter 2001 to spring/summer 2009; $n > 10$ seals).

Bearded Seal

Isotopic signatures in the growth bands of claw horns among bearded seals were also highly variable (Figure 5-6a, Figure 5-6b, Figure 5-7a, Figure 5-7b). $\delta^{15}N$ values in seasonal growth bands of bearded seal claw sheaths among individuals ranged from 14.6 to 18.2‰, with maximum variations in seasonal bands within an individual seal claw sheath ranging from 0.5‰ to 1.2‰ (Figure 5-6a). Typical $\delta^{15}N$ values ranged from 16.3 to 16.9‰, based on confidence intervals over the time period of the largest sample size (i.e., fall/winter 2003 to spring/summer 2008; $n > 10$ seals). Variability of $\delta^{13}C$ values among individual bearded seals was also high; however, $\delta^{13}C$ values were relatively stable within each individual (Figure 5-7a). $\delta^{13}C$ values in seasonal growth bands of bearded seal claw sheaths among individuals ranged from −18.3 to −13.7‰, with maximum variations in seasonal bands within an individual seal claw sheath ranging from 0.2‰ to 1.7‰. Typical $\delta^{13}C$ values ranged from −16.3 to −15.4‰, based on confidence intervals over the time period of the largest sample size (i.e., fall/winter 2003 to spring/summer 2008; $n > 10$ seals).

Spotted Seal

Our sample of claw sheaths for spotted seals included 14 young animals (1−3 yrs.) and allowed us to examine the isotopic differences among fetal, natal, weaning, and post-weaning time periods. For spotted seal pups, $\delta^{15}N$ values increased, and $\delta^{13}C$ values generally decreased from

fetal to weaning claw horn growth (Figure 5-8a). $\delta^{15}N$ values increased by 1.2 ± 0.5‰ from fetal to the natal period (mean change in stable isotope ratios ± SD) in 79% of claw sheaths for all spotted seals. The remaining 21% of claw sheaths showed an increase in $\delta^{15}N$ values by < 0.5‰ from fetal to natal period. The pattern for $\delta^{15}N$ between natal and weaning claw horn growth was not consistent and $\delta^{15}N$ values for some claws increased while others decreased. $\delta^{13}C$ decreased by 0.9 ± 0.6‰ from fetal to weaning claw horn growth for 79% of pups while it increased by 0.6 ± 0.4‰ for the remaining 21% of pups.

For spotted seal juveniles, stable nitrogen and carbon isotopic signatures after weaning were generally constant with the exception of four seals. $\delta^{15}N$ values decreased by 1.3 ± 0.5‰ between weaning and first winter foraging (mean change in stable isotope ratios ± SD) in all spotted seal pups (Figure 5-8a). For four of 14 individuals, $\delta^{15}N$ values increased over the next four foraging seasons (Figure 5-8b). A general increase in $\delta^{15}N$ values, however, was not apparent for all juveniles after their first winter foraging as claws for these seals had higher $\delta^{15}N$ values compared to the four seals, i.e., values greater than 16‰ (Figure 5-8b). $\delta^{13}C$ values did not vary for the majority of juvenile seals during subsequent foraging seasons, and values ranged from −16.8 to −13.5‰ (Figure 5-8b).

Claw sheaths for adult spotted seals (> 4 years) displayed high variability for $\delta^{15}N$ values among individuals while $\delta^{13}C$ values were relatively constant (Figure 5-9a, Figure 5-9b, Figure 5-10a, Figure 5-10b). $\delta^{15}N$ values in seasonal bands of spotted seal claw sheaths among individuals ranged from 15.3 to 17.7‰, with maximum variations in seasonal bands within an individual seal claw sheath ranging from 0.5‰ to 1.3‰ (Figure 5-9a). Typical $\delta^{15}N$ values ranged from 16.3 to 17.3‰, based on confidence intervals over the time period of the largest sample size (i.e., fall/winter 2005 to spring/summer 2009; n > 10 seals). $\delta^{13}C$ values in seasonal bands of spotted seal claw sheaths among individuals ranged from −16.7 to −14.8‰, with maximum variations in seasonal bands within an individual seal claw sheath ranging from 0.3‰ to 1.1‰ (Figure 5-10a). Typical $\delta^{13}C$ values ranged from −15.7 to −15.2‰, based on confidence intervals over the time period of the largest sample size (i.e., fall/winter 2005 to spring/summer 2009; n > 10 seals).

Ribbon Seal

Ribbon seal claws showed a broad range of $\delta^{15}N$ values, but $\delta^{13}C$ values were similar across individuals and remained relatively constant over years (Figure 5-11a, Figure 5-11b). Among individuals, $\delta^{15}N$ variability was high with one seal claw sheath having lower values compared to the other two seal claw sheaths, i.e., minimum $\delta^{15}N$ value of 13.5‰ versus 17.8‰ for the other two seals (Figure 5-11a). Maximum variations in seasonal bands per individual claw sheath ranged from 0.7‰ to 1.7‰. Generally, $\delta^{15}N$ values increased with seal age. $\delta^{13}C$ ranges for ribbon seal claw growth bands were similar to those of adult spotted seals, i.e., −17.1 to −14.9‰. Maximum variations in seasonal bands per individual ranged from 0.4‰ and 1.1‰.

5.3.3 Interannual Comparison

Proportionally more ringed seal claw sheaths of the sampled population showed a decrease in $\delta^{15}N$ values during 2007 while across all individuals $\delta^{13}C$ values decreased over time (Figure 5-4b, Figure 5-5b). $\delta^{15}N$ values for 71% (12 of 17) of ringed seals decreased by 1.0 ± 0.3‰ from fall/winter 2006 to spring/summer 2008 (mean change in stable isotope ratios ± SD), with 58% (7 of 12) of the claw sheath isotope signatures falling below the lower confidence interval (Figure 5-4a). Furthermore, $\delta^{15}N$ values for 50% (5 of 10) of ringed seal claw sheaths increased by 1.2 ± 0.4‰ from spring/summer 2008 to spring/summer 2010, while two seals did not have data beyond 2008 as they were harvested in 2008. Standardized residuals displayed an overall decrease in $\delta^{15}N$ values during 2007 followed by a subsequent increase after 2008 (Figure 5-4b). An interannual trend for $\delta^{13}C$ values was not apparent when assessing isotope values in the claw horn (Figure 5-5a); however, standardized residuals revealed $\delta^{13}C$ values decreasing from 1998 to 2010 (Figure 5-5b).

During 2007, some bearded seal claw sheaths had lower $\delta^{15}N$ values compared to the previous years, whereas a decreasing trend in $\delta^{13}C$ values occurred after 2007. More specifically, $\delta^{15}N$ values for 56% (9 of 16) of bearded seal claw sheaths decreased by 1.0 ± 0.3‰ from fall/winter 2006 to spring/summer 2008 (Figure 5-6a). The remaining 44% (7 of 16) showed only minor deviations in $\delta^{15}N$ (< 0.5‰). Standardized residuals showed a decrease in $\delta^{15}N$ values from 1999 to 2000 and from 2006 to 2008 (Figure 5-6b); however, the sample size was small during the earlier years, i.e., n = 6 seals. An interannual trend for $\delta^{13}C$ values was not apparent when assessing isotope values in the claw horn (Figure 5-7a); however, standardized residuals displayed lower $\delta^{13}C$ values during spring/summer 2010 compared to previous years (Figure 5-7b). However, the sample size was small during spring/summer 2010 (Figure 5-7b).

During 2006, $\delta^{15}N$ values were lower for young-of-the-year and adult spotted seals. Spotted seal pups generally had $\delta^{15}N$ values ranging from 18.5 to 19.5‰; however, during 2006, 71% (5/7) of nursing pups had lower $\delta^{15}N$ values ranging from 16.1 to 17.2‰ (Figure 5-8a). During fall/winter 2006, claw sheaths for the two oldest seals, i.e., age 6+, showed a decrease in $\delta^{15}N$ values by about 0.8‰ (Figure 5-9a), dropping below the lower confidence interval. Standardized residuals displayed low $\delta^{15}N$ values during fall/winter 2006 for subadult/adult spotted seals; however, this was not lower compared to other years examined (Figure 5-9b). An interannual trend was not apparent for $\delta^{13}C$ values of subadult/adult spotted seal claw sheaths (Figure 5-10a) or when examining standardized residuals (Figure 5-10b). Overall, variations in $\delta^{15}N$ or $\delta^{13}C$ values were high among individuals for ringed, bearded, and spotted seals, while season contributed less to the total variance (Table 5-3).

5.4 Discussion

To our knowledge, the use of claw isotopes within individuals to provide a 10-year diet series represents the first successful application of this new technique to seal species. Similar isotopic

signatures were obtained from the same seasonal growth bands in one individual seal regardless of which digit the claw sheath came from (Table 5-2). However, fewer growth bands were retained on the shorter digits (digits III–V), possibly a result of increased wear of digits on the outer edge of the front flipper. Therefore, digit I or II should be collected and analyzed for the longest possible diet history. In addition to the low variation of isotope signatures among digits, the high correlation in stable isotope signatures of the same seasonal growth bands among digits in this study also confirms the consistency of our methods and our ability to obtain horn powder from specific growth bands. No significant differences in $\delta^{15}N$ and $\delta^{13}C$ values among ringed seal digits were also documented by Ferreira et al. (2011) and they also recommended digit I or II because they are larger in size and have distinct growth bands. Pigments, particularly melanin, of keratinized structures have been found to influence $\delta^{13}C$ values in bird feathers, with dark portions being more depleted in ^{13}C while $\delta^{15}N$ values were not affected (Michalik et al. 2010). A consistent pattern of dark bands (i.e., fall/winter growth) with depleted ^{13}C values compared to light bands was not documented in this study. It is unclear how pigmentation affected stable isotope ratios in growth bands of seal claw sheaths from this study, and this warrants further investigation.

5.4.1 Ringed Seal

The relatively depleted isotopic signatures of ringed seal claw sheaths compared to bearded seal claw sheaths are consistent with a primarily pelagic feeding strategy (Kovacs 2007), but may also indicate feeding in the Beaufort Sea, a region known to be depleted in ^{13}C (Saupe et al. 1989; Schell et al. 1989; Dunton et al. 2006). Ringed seals may consume a mixture of pelagic crustaceans and fishes, but prey proportions are highly variable by season and region (Lowry et al. 1980a). The range in $\delta^{15}N$ values observed for claw horn sheaths of ringed seals spans about two trophic levels, i.e., 3‰ per trophic level (Peterson and Fry 1987), but also varies widely within individuals. The range and variability of $\delta^{15}N$ values agree with a flexible and opportunistic diet comprised of lower and higher trophic level prey. From late fall to early spring, ringed seal diet mainly consists of gadids, but during summer invertebrates become more important (Johnson et al. 1966; Lowry et al. 1980a; Smith 1987). However, the results of our study indicate that a consistent seasonal switch in prey of different trophic levels, i.e., fishes versus krill, does not occur. On the other hand, epibenthic shrimps (e.g., *Sclerocrangon* spp.) can be important prey for ringed seals during spring and summer (Lowry et al. 1980a) and this prey taxon has $\delta^{15}N$ values similar to demersal fishes (Iken et al. 2010). Thus, differences between summer and winter prey may not be detected based on $\delta^{15}N$ values depending upon the prey eaten even though a seasonal prey switch may have occurred. On average, $\delta^{15}N$ values were higher for all growth bands analyzed in this study compared to growth bands from ringed seals analyzed by Ferreira et al. (2011), i.e., 17.1 ± 0.8‰ compared to 15.6 ± 1.5‰ (mean ± SD), respectively. Higher $\delta^{15}N$ values observed in this study may be a result of a greater consumption of demersal fishes, e.g., sculpins (Cottidae) or pricklebacks (Stichaeidae), and epibenthic shrimps by ringed seals in the Alaskan Arctic (Lowry et al. 1980a; Quakenbush et al. 2010a) than for the

ringed seals in the Hudson Bay analyzed by Ferreira et al. (2011). In addition, lower $\delta^{15}N$ values in muscle from ringed seals in the Hudson Bay region were attributed to regional differences in food-web structure, i.e., shorter food chain, compared to seal muscle from other Arctic regions (Young et al. 2010). Typical $\delta^{13}C$ values for the claw horn of ringed seals were lower compared to those in bearded seals, which may support pelagic foraging regardless of the season for ringed seals while bearded seals feed on benthic organisms more enriched in ^{13}C compared to pelagic organisms (Iken et al. 2010). The exceptionally depleted ^{13}C growth bands observed during fall/winter of 2001 and 2003, corresponding to the second and fourth fall/winter foraging for one ringed seal individual (Figure 5-5), could be the result of consuming fairly depleted ^{13}C prey sources during these seasons, e.g., smelt (*Osmerus mordax*) or Pacific Herring (*Clupea pallasii*). However, it is more likely that the depleted carbon signatures of this seal are due to foraging in the Beaufort Sea. Ice seal prey, i.e., cod, amphipod, and shrimp, from the Bering and Chukchi Seas are more enriched in ^{13}C compared to those from the eastern Beaufort Sea (Dunton et al. 1989; Saupe et al. 1989). Thus, the exceptionally low $\delta^{13}C$ values for fall/winter growth bands of this ringed seal would be consistent with a Beaufort Sea signature. Correspondingly, Dehn et al. (2007) found differences in carbon isotope signatures in muscle tissue of ringed seals harvested in Barrow (Chukchi/Beaufort Seas) versus those harvested in Ulukhaktok, Canada (Beaufort Sea). Changes in feeding location between the Beaufort and Chukchi Seas have also been illustrated as $\delta^{13}C$ oscillations in continuously growing baleen plates of bowhead whales (*Balaena mysticetus)* (Schell et al. 1989). Ringed seals in the Alaskan and Canadian Arctic are known to exhibit extensive movement ranges occupying the Bering, Chukchi, and Beaufort Seas (Crawford et al. 2011; Paulatuk Holman, and Tuktoyaktuk Hunters and Trappers Committees 2011). On average, all growth bands from this study were more enriched in ^{13}C (-16.6 ± 0.9‰) relative to growth bands from ringed seals analyzed in Ferreira et al. (2011), i.e., -18.6 ± 0.8‰, -18.4 ± 0.7‰, and -17.1 ± 0.6‰ for different areas within Hudson Bay. Similarly, muscle tissue from ringed seals harvested in the Hudson Bay was depleted in ^{13}C compared to ringed seals from other Arctic regions and it was suggested that the depleted ^{13}C signatures were a result of terrigenous input into the Hudson Bay (Young et al. 2010). Overall, high variability in $\delta^{15}N$ and $\delta^{13}C$ values among individuals from this study and Ferreira et al. (2011) illustrate the highly opportunistic nature of ringed seals.

5.4.2 Bearded Seal

Isotopic signatures of bearded seal claw horns are consistent with their benthic diet (Antonelis et al. 1994; Dehn et al. 2007; Quakenbush et al. 2010b), but there is high variability in diet among individuals. Bearded seals consume a variety of benthic and epibenthic invertebrates, along with demersal and pelagic fishes (Kosygin 1971; Dehn et al. 2007; Quakenbush et al. 2010b). Benthic prey are typically enriched in ^{15}N compared to pelagic food webs (Iken et al. 2005). However, typical $\delta^{15}N$ values for bearded seal claws were lower compared to ringed seals suggesting a high contribution of lower trophic level prey to the diets of bearded seals. Clams can be frequent prey for older bearded seals (Lowry et al. 1980b; Dehn et al. 2007; Quakenbush et al. 2010b), and this

prey taxon is relatively depleted in ^{15}N compared to benthic scavengers (Iken et al. 2010). Muscle tissue of bearded seals is enriched in ^{15}N compared to ringed seals (Young et al. 2010), or $\delta^{15}N$ is within similar ranges for both species (Dehn et al. 2007). Ultimately, $\delta^{15}N$ ranges can overlap between ringed and bearded seals as ringed seals may also feed on epibenthic shrimps and demersal fishes (Quakenbush et al. 2010a), and bearded seals have a diverse diet of lower and higher trophic invertebrates, along with demersal and pelagic fishes (Quakenbush et al. 2010b). High variability in $\delta^{13}C$ values among individual bearded seal claw sheaths in this study indicates diverse individual foraging strategies, which may be related to individual prey preference and/or different prey preferences among ages and between sexes. Smaller ranges in $\delta^{13}C$ have been documented for muscle tissue among bearded seals (Hobson et al. 2002; Dehn et al. 2007). This study documented minimal variation in $\delta^{13}C$ over time for an individual while simultaneously showing large variability among individuals. The highest $\delta^{13}C$ values likely belong to a more focused benthic consumer in the Bering Sea, i.e., feeding on a food source enriched in ^{13}C in a region enriched in ^{13}C (Dunton et al. 2006), and the lowest $\delta^{13}C$ values suggest a more pelagic forager. Low variability in $\delta^{13}C$ values for individual bearded seals over time could be related to a preference for certain prey guilds (Dehn et al. 2007), although the high variability in $\delta^{15}N$ values suggests this is not the case. Foraging in the same general region throughout the time period recorded in claw horn sheaths may be more likely. Bearded seals tend to migrate seasonally; during fall/winter they reside near the ice edge in the Bering Sea and as ice recedes they move to the southern edge of the Chukchi and Beaufort Seas pack ice for the duration of the summer (reviewed in Cameron et al. 2010; Boveng et al. 2012). While perpetual shifts in $\delta^{13}C$ values corresponding to the season were not observed for bearded seals, they may have been masked by changes within prey, such as seasonal changes in lipid content or regional differences in $\delta^{13}C$ values of prey. In fall/winter, shrimps and crabs are prevalent in bearded seal stomachs, while clams and fishes occur more frequently during spring/summer (Johnson et al. 1966; Lowry et al. 1980b). Bearded seals may have been feeding on benthic organisms relatively enriched in ^{13}C but in an area depleted in ^{13}C. For example, similar carbon signatures could occur if bearded seals fed on benthic prey in the Beaufort Sea and then switched to pelagic prey relatively depleted in ^{13}C but from an area enriched in ^{13}C such as in the Bering Sea (Dunton et al. 2006).

5.4.3 Spotted Seal

Stable isotope ratios in claw horns corresponding to prenatal and postnatal growth in spotted seals provide information on nutrient transfer from the mother and its incorporation by the pup. All claw sheaths that included prenatal growth bands showed an increase in $\delta^{15}N$ values from fetal to natal claw horn growth consistent with transfer and fractionation of maternal protein to fetal development. This isotopic enrichment has been reported in both terrestrial and marine mammals using a variety of soft tissues and keratinized structures (Hobson et al. 2000; Jenkins et al. 2001; Stegall et al. 2008). The growth band immediately following the natal notch represents an integrated stable isotope signature of the spring/summer diet of the pup, which includes the

nursing and weaning time periods. Spotted seal pups are born on ice floes between April and mid-May and begin foraging on their own in late May to early June (reviewed in Boveng et al. 2009). Thus, the observed increase in $\delta^{15}N$ values from natal to weaning claw horn growth corresponds to nursing and reliance on body reserves after weaning (Newsome et al. 2006). In contrast, the observed decrease in $\delta^{15}N$ values from natal to weaning claw horn growth likely reflects nursing combined with some feeding on lower trophic level prey. Spotted seal juveniles mainly consume crustaceans such as gammarid amphipods but consumption of shrimps and pelagic fishes such as Pacific Sand Lance (*Ammodytes hexapterus*), Arctic Cod (*Boreogadus saida)*, and Saffron Cod (*Eleginus gracilis*) increase with seal age (Gol'tsev 1971; Bukhtiyarov et al. 1984). Foraging on higher trophic level prey, e.g., teleosts, varies with individual juvenile seals (Burns 1999) and $\delta^{15}N$ values in claw growth bands of four seals from this study illustrated a gradual increase in trophic level feeding of seals, while the other ten seals fed at a higher trophic level immediately after their first foraging season. Although, seasonal bands enriched in ^{15}N could result from a difficult transition to feeding after weaning where pups must rely on their own protein catabolism (Castellini and Rea 1992); however, it is unlikely to occur for more than two seasons, and values were similar to nitrogen signatures of adult spotted seals. Phocid milk has a high-fat content of about 40% (Oftedal et al. 1988; Iverson et al. 1993) so that seal pups quickly develop a blubber layer during a short nursing period. Milk with high lipid content is depleted in ^{13}C (Newsome et al. 2010), and this is reflected in a continuous decrease in $\delta^{13}C$ values from fetal horn growth to the first claw growth band for seals in this study. In general, young-of-the-year pups have tissues enriched in ^{15}N and depleted in ^{13}C compared to their mothers (Polischuk et al. 2001) or other older individuals (Newsome et al. 2006; Dehn et al. 2007; Orr et al. 2011).

Spotted seal claw horns of subadults and adults showed high variability in trophic level feeding, but low variation in carbon source among individuals. Diets of spotted seals predominately consist of fishes, e.g., gadids, Pacific Herring, Walleye Pollock (*Gadus chalcogrammus*), Pacific Sand Lance, and smelts (Lowry et al. 1981; Bukhtiyarov et al. 1984; Dehn et al. 2007, Quakenbush et al. 2009). Other prey groups found in spotted seal stomachs include cephalopods, crustaceans, demersal fishes, and mollusks (Bukhtiyarov et al. 1984; Dehn et al. 2007; Quakenbush et al. 2009). A diet consisting mainly of fishes would have $\delta^{15}N$ values of about 17‰, i.e., Arctic Cod are about 14.9 ± 0.6‰ (Iken et al. 2010) plus an enrichment factor of 2.3‰ for claws (Hobson et al. 1996). Therefore, $\delta^{15}N$ values show a diet mainly comprised of prey enriched in ^{15}N such as pelagic or demersal fishes, or epibenthic shrimps. Growth bands depleted in ^{15}N occurred during the fall/winter season in spotted seals (Figure 5-9a), and this may be the result of consumption of ice-associated crustaceans, i.e., amphipods (Quakenbush et al. 2009). High variability in $\delta^{15}N$ values among individuals suggests a preference for certain prey types. For example, in this study a 4-year-old spotted seal fed at a higher trophic level than older seals in this study. This may be the result of the younger seal foraging on decapod shrimps (Gol'tsev 1971; Bukhtiyarov et al. 1984), which can have higher $\delta^{15}N$ values similar to demersal fishes (Iken et al. 2010). The values of $\delta^{15}N$ in claw sheaths were on average about 1‰ lower for

spotted seals than ringed seals (Figures 5-4a, 5-11a) in contrast to results from Dehn et al. (2007) where $\delta^{15}N$ values of muscle were 1‰ higher (reaching maximum values of about 19‰) for spotted seal than for ringed seals. Spotted seals in this study may have had a higher proportion of ice-associated crustaceans in their diet because younger seals dominated our sample (Table 5-1). Age-related changes in the diet of spotted seals have been described previously, with young seals relying more on ice-associated crustaceans and the relative importance of fish increases with age (Gol'tsev 1971). Similarly, the upper range of $\delta^{15}N$ values for spotted seal muscle documented by Dehn et al. (2007) could be due to the incorporation of walleye pollock in seal diets. Walleye pollock can be enriched in ^{15}N compared to Arctic Cod or Pacific Herring as adult pollock feed on juvenile fishes (Kurle and Worthy 2001). The confidence interval for $\delta^{13}C$ values was similar in spotted and bearded seal claw horns with growth bands for these two species being relatively enriched in ^{13}C compared to ringed seals, and this suggests a mixed diet of benthic and pelagic prey (even for juvenile and subadult spotted seals). However, pelagic fishes occur more frequently in the diet of spotted seals compared to demersal fishes or benthic invertebrates, with the exception of decapod shrimps (Quakenbush et al. 2009). A diet high in pelagic (planktivorous) fish was also documented for spotted seals using fatty acid analysis (Cooper et al. 2009). Thus, it is more likely that growth bands enriched in ^{13}C are characteristic of foraging nearshore and under the sea ice. Nearshore habitats are enriched in ^{13}C relative to offshore regions; higher nutrient levels from upwelling leads to faster growth of primary producers, thus causing an enrichment of ^{13}C in these organisms (reviewed in Newsome et al. 2010). During the open-water season, spotted seals aggregate at coastal haulouts (reviewed in Boveng et al. 2009) near spawning areas of Capelin and Pacific Herring (Quakenbush 1988), where they rest between feeding bouts. Consumption of pelagic fishes that are foraging on prey sources nearshore would produce seal tissues enriched in ^{13}C. Although spotted seals are found nearshore in summer (enriched in ^{13}C) and move offshore in winter (normally not enriched in ^{13}C) remaining close to the ice front in the Bering Sea (Burns 2002), the $\delta^{13}C$ values from their claw sheaths are similar between winter and summer growth bands. This suggests that spotted seals forage on ice-associated prey during the winter (enriched in ^{13}C). Spotted seals may be consuming Arctic Cod underneath the sea ice and this fish species has a diet primarily consisting of copepods (*Calanus* spp.) and amphipods (*Gammarus wilkitzkii, Apherusa glacialis, Onisimus nanseni,* and *Onisimus glacialis*) (Lowry and Frost 1981; Bradstreet and Cross 1982; Lønne and Gulliksen 1989). Copepods (*Calanus* spp.) overwinter in deeper waters during a period of diapause (Gradinger 1995). However, amphipods (*G. wilkitzkii, A. glacialis, O. nanseni,* and *O. glacialis*) live permanently associated with the ice and feed mainly on detritus and some ice algae during the winter (Poltermann 2001). Both sources of carbon (detritus and ice algae) would produce consumer tissues enriched in ^{13}C (McConnaughey and McRoy 1979; Kennedy et al. 2002) leading to seal tissues enriched in ^{13}C.

5.4.4 Ribbon Seal

Isotopic signatures of ribbon seal claws depict age-related differences in diet, high variation in trophic level feeding among individuals, and a stable carbon source. Juvenile and subadult ribbon seals have been found to consume lower trophic level crustaceans and then transition to foraging on fishes and cephalopods (Arsen'ev 1941; Fedoseev 2000; Dehn et al. 2007). In this study, a gradual increase in trophic level feeding was documented for two ribbon seals (Figure 5-11). One ribbon seal had the lowest $\delta^{15}N$ values of any ice seal in this study and may have been diving deep in the Bering Sea (reviewed in Boveng et al. 2008) to consume squid as cephalopods can be depleted in ^{15}N (Kurle et al. 2011) compared to demersal fishes and decapods (Iken et al. 2010). Alternatively, this seal may have foraged on amphipods, euphausiids, and/or clams (Shustov 1965; Frost and Lowry 1980; Bukhtiyarov 1990), which would have a similar signature as squid. Demersal and pelagic fishes are primary prey for ribbon seals in spring (Shustov 1965; Frost and Lowry 1980; Bukhtiyarov 1990) consistent with spring/summer growth bands being more enriched in ^{15}N. However, the lack of seasonality in $\delta^{15}N$ values suggests that ribbon seals may also prey on higher trophic level prey (e.g., teleosts) throughout the year. Ribbon seal $\delta^{13}C$ values were remarkably stable over the length of the claw with very little variation among individuals, season, and years. This indicates that ribbon seals feed in areas with similar carbon sources suggesting that movements into the Chukchi Sea are rare, and coastal areas are not where ribbon seals feed. Their geographic range and remote distribution in the pack ice and the Bering Sea shelf (Braham et al. 1984; Simpkins et al. 2003) are consistent with these findings. Ribbon seals reside in the Bering Sea during the ice-free months, and few are seen or harvested north of the Bering Strait in Alaska (reviewed in Boveng et al. 2008).

5.4.5 Interannual Variability

Interannual variability of stable isotope ratios in claws indicates ice seals are versatile predators that are able to adjust to changing food sources depending on availability, which supports our hypothesis. The extremely low minimum summer sea ice extent for five consecutive years occurred from 2007 through 2011, with 2007 having the record low sea ice extent in the Arctic aside from 2012 (NSIDC 2012). Standardized residuals of $\delta^{15}N$ values for ringed seal claws decreased during 2007, and this may be related to less sea ice in that year and its effects on food-web structure. Less sea ice and earlier ice melt are predicted to result in a more pelagic-dominated food web rather than the current more benthic- dominated food web (Bluhm and Gradinger 2008). The expansion and longer duration of the open-water period in the Arctic led to an increase in annual primary production in 2007 relative to 2006 (Arrigo et al. 2008). Ultimately, elevated primary production, early ice retreat coupled with warmer waters (NSIDC 2011a), and a longer open water season supported a higher abundance of pelagic crustacean grazers and consumers (Forest et al. 2011; Matsuno et al. 2011; Eisner et al. 2012) that could then be consumed by ice seals. Values of $\delta^{15}N$ indicated that ringed seals were feeding at a lower trophic level in 2007, which may be due to a greater availability of pelagic crustaceans. Ringed seals are known to consume dense swarms of euphausiids and amphipods during summer and

early autumn (Lowry et al. 1980a). Sea ice extent was relatively low from 2008 to 2010, compared to the median from 1979 through 2000 (NSIDC 2012); however, $\delta^{15}N$ values in ringed seal claw horns deposited after 2008 indicated they were feeding at a higher trophic level. Fish abundance may also increase in response to an increase in the biomass of zooplankton (Overland and Stabeno 2004; Grebmeier et al. 2006). A decrease in $\delta^{13}C$ values of ringed seal claws from 1998 to 2010 (found) may be a result of a decrease in sea ice extent in the Arctic. Between 1998 and 2007, there was a decrease in the 12−month running mean in sea ice extent with an increase after 2008 before decreasing again (NSIDC 2011b). A decrease in sea ice reduces the area available for ice algae to grow, which may result in less biomass of ice algae to support secondary consumers (Carroll and Carroll 2003). In turn, zooplankton may change from foraging under the sea ice to foraging more pelagically. Consequently, secondary consumers and higher trophic level predators have lower $\delta^{13}C$ values, i.e., phytoplankton are more deplete in ^{13}C relative to ice algae (Gradinger 2008). Overall, interannual trends in trophic level feeding suggest that ringed seals are flexible to climate change impacts on the food web.

For bearded seal claw horn sheaths, a decrease in $\delta^{15}N$ values during 2007 and lower $\delta^{13}C$ values after 2007 may provide support for more pelagic feeding in years of reduced sea ice cover. Feeding on lower trophic levels in 2007 may be a result of bearded seals consuming bivalves. Alternatively, similar to ringed seals, these seals may have been consuming a higher proportion of pelagic crustaceans. For example, euphausiids (*Thysanoessa* spp.) and amphipods (e.g., *Gammarus* spp.) are prey items commonly identified in both ringed and bearded seal stomachs (Johnson et al. 1966; Quakenbush et al. 2010a, b). However, these species may be found throughout the water column (euphausiids; ArcOD 2010) and are associated with the sea-ice and sea-bottom (e.g., *Gammarus wilkitzkii*). Zooplankton feeding under the ice or near the sea floor may have similar carbon isotope signatures because ice algae trapped in brine channels exhibit similar ^{13}C enrichment as algae in the benthos (Kennedy et al. 2002). Pelagic zooplankton are typically depleted in ^{13}C relative to benthic consumers or zooplankton grazing on ice algae (McConnaughey and McRoy 1979; Kennedy et al. 2002). Lower $\delta^{13}C$ values during spring/summer 2010 (Figure 5-7a) may suggest bearded seals were foraging more pelagically over time due to a decrease in benthic biomass. Benthic biomass is dependent on the quality and quantity of food reaching the benthos (Grebmeier et al. 1988). A decrease in benthic biomass may gradually occur over time, but it is likely on a scale of several years (Dunton et al. 2005). Thus, an overall response of bearded seals feeding more pelagically may not be as immediate. Inferences regarding an increase in pelagic foraging for bearded seals should be interpreted with caution as mean $\delta^{13}C$ values may be biased by the relatively small sample size of claws having stable isotope data during 2010. Ultimately, stable isotope signatures in claw horn samples may provide evidence of an increase in pelagic foraging for bearded seals; however, the use of stable isotopes to track these changes may not be ideal due to regional variations in carbon source as discussed above.

While an interannual trend was not apparent when assessing standardized residuals for $\delta^{15}N$ values of spotted seal claw horn samples, during 2006 spotted seals may have consumed lower trophic level prey. About 70% of the spotted seal pups born in 2006 had lower $\delta^{15}N$ values compared to seals born in 2007 and 2008 (Figure 5-8a). This indicates differences in maternal trophic level and nutrient transfer to the pup. In addition, two adult spotted seals also fed lower trophically in fall/winter 2006, suggesting a response to changes in the Arctic food web. Although, summer sea ice extent in the Arctic has been relatively low from 2007 through 2011, the winter sea ice extent in the Bering Sea was greater than average during winter 2012, i.e., second highest sea ice extent in January compared to averages from 1979 through 2000 (NSIDC 2012). Moreover, ocean temperatures in the Bering Sea were colder from winter 2006 to winter 2009 compared to 2000 through 2005 (Overland et al. 2009). Colder temperatures may have allowed for Arctic species to extend their range into sub-Arctic waters (Grebmeier et al. 2006); thus, more secondary consumers may have been available for consumption by spotted seals. However, the sample size of 2006 spotted seal claw horns was small, so it is difficult to generalize results. The apparent lower trophic level feeding during fall/winter 2006 may be a result of individual seal prey preference or local variation in food sources.

Anomalous stable isotope ratios in seal claw sheaths may be influenced by multiple factors and demonstrate the opportunistic feeding habits of seals. Extreme $\delta^{15}N$ values may be a result of foraging on a wide range of trophic levels. Extreme $\delta^{13}C$ values may be caused by changes in primary productivity or foraging in different geographic regions. The 60–90% contribution of variance described among seals (Table 5-3) is possibly a result of differences in physiology or diet of each individual, e.g., metabolic rates based on age or gender and nutritional quality of their diet (Newsome et al. 2010). The 10–40% contribution of variance related to residual error (Table 5-3) may be a result of changes in primary productivity or feeding in different locations. Overall, high variability of diets within the seal populations confounds the variation explained by temporal effects, i.e., season (Table 5-3). This further demonstrates the potential and ability of ice seal populations to adjust to changes in food-web structure in the Arctic.

Examining other tissues and additional investigations using stable isotope signatures of claw horn sheaths (i.e., using archived claws to reach further back in time, such as capturing the regime shift in the late 1970s; Hare and Mantua 2000) could aid in describing the diet strategies of ice seals and food chain effects. For processing of cornified growth bands, claws should be soaked until the bony ungual crest and perioplic horn (Ethier et al. 2010) can be removed easily. The two to three growth bands underneath the ungual crest, i.e., the youngest horn formed, are essential for minimum age estimates and stable isotope analysis. The seasonal resolution using stable isotope signatures in claw growth bands is low as each band represents an integrated isotopic signature over several months. Future studies may be able to provide better insight into seasonal changes in ice seal diets by using micro drills to process each growth band of the claw horn at 300 µm intervals (Newsome et al. 2010). Furthermore, a combination of stable isotope analysis of whisker and claw horn could improve the seasonal resolution (Cherel et al. 2009), as

whiskers have relatively faster growth rates (Zhao and Schell 2004) while simultaneously documenting interannual differences in diet over time. Understanding diet alterations for a predator with a variable diet and a wide geographic range complicates interpretations when using an integrated descriptor such as stable isotope ratios. Ultimately, stable isotopes are not a replacement for stomach content analysis but are a valuable supplemental tool to enhance our understanding of integrated seasonal or interannual feeding ecology of ice seals.

Differences in the seal muscle and seal claw portions of the study represent differences in the particular individuals and total numbers analyzed in each study as well as differences in the estimated period of diet integration in muscle and claw layers. Table 5-4 highlights some of the differences between this Chapter 5 study of seal claws and the Chapter 4 study of muscle tissue.

The variability among individuals and interannual variations in diet documented in this study exemplifies the opportunistic nature of ringed, bearded, spotted, and ribbon seals and their adaptation potential to changes in food-web structure in the Arctic. Claw horn samples provide a unique glimpse into the feeding history of ice seals. In addition to providing dietary information at the population level, similar to long-term studies examining stomach contents (Quakenbush et al. 2009, 2010a, 2010b), claw horn samples also describe the long-term diet of individuals. This technique allows us to demonstrate that ringed and bearded seals fed lower trophically during the 2007 ice minimum and may have fed more pelagically during years of reduced sea ice extent, which supports our hypothesis that ice seals may capitalize on more abundant pelagic prey sources during years of reduced ice cover in the Arctic Ocean.

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5.6 Tables

Table 5-1. **Inventory of Claws Collected from Ice Seals.** Ice seal claw sheaths analyzed for stable nitrogen and carbon isotope ratios. The asterisk marks the individual ringed seal where all five claws were analyzed from one front flipper. Some seal tooth age estimates are still to be determined (TBD), whereas others are not available (NA).

				Estimated age (years)		
Location	**Year**	**Month**	**Base band**	**Tooth**	**Claw**	**Sex**
Ringed Seals						
Point Hope	2010	February	Dark	10	8.5 +	Male
Hooper Bay	2010	February	Dark	13	11.5 +	Female
Hooper Bay	2010	February	Dark	20	11.5 +	Male
Barrow	2010	March	Dark	NA	8 +	Unknown
Barrow	2010	March	Dark	NA	11.5 +	Female
Barrow	2010	March	Dark	NA	11.5 +	Unknown
Point Hope	2009	June	Light	8	8	Male
Barrow	2008	July	Light	TBD	9 +	Female
Barrow	2009	July	Light	18	9 +	Male
Point Hope	2010	July	Light	21	8 +	Male
Point Hope	2010	July	Light	37	10 +	Male
Barrow	2010	July	Light	TBD	7	Male
Barrow	2010	July	Light	TBD	12 +	Female
Barrow	2008	August	Dark	TBD	7.5 +	Female
Barrow	2011	September	Dark	NA	9+	Male*
Shishmaref	2009	October	Dark	4	4	Unknown
Shishmaref	2009	October	Dark	8	6 +	Male
Shishmaref	2009	October	Dark	10	8.5 +	Male
Bearded Seals						
Little Diomede	2009	May	Light	5	5	Male
Point Hope	2009	June	Light	7	7	Female
Point Hope	2010	June	Light	11	10 +	Female
Barrow	2010	June	Light	TBD	10 +	Unknown
Point Hope	2010	June	Light	20	11 +	Unknown
Barrow	2010	June	Light	TBD	11 +	Male
Barrow	2010	June	Light	TBD	8 +	Male
Barrow	2008	July	Light	TBD	4	Male
Barrow	2008	July	Light	TBD	4	Male
Barrow	2008	July	Light	TBD	5	Unknown
Barrow	2008	July	Light	TBD	5	Unknown
Barrow	2008	July	Light	NA	5	Unknown
Barrow	2008	July	Light	TBD	7 +	Female
Barrow	2010	July	Light	TBD	8 +	Male
Barrow	2008	July	Light	TBD	9 +	Male
Barrow	2008	July	Light	NA	10 +	Male

Table 5-1 continued. **Inventory of Claws Collected from Ice Seals.** Ice seal claw sheaths analyzed for stable nitrogen and carbon isotope ratios. The asterisk marks the individual ringed seal where all five claws were analyzed from one front flipper. Some seal tooth age estimates are still to be determined (TBD), whereas others are not available (NA).

					Estimated age (years)		
	Location	**Year**	**Month**	**Base band**	**Tooth**	**Claw**	**Sex**
Spotted Seals							
	Barrow	2009	July	Light	8	7 +	Unknown
	Shishmaref	2009	September	Dark	3	3	Male
	Shishmaref	2009	September	Dark	NA	3	Male
	Shishmaref	2009	September	Dark	6	6	Male
	Shishmaref	2009	October	Dark	1	1	Female
	Shishmaref	2009	October	Dark	1	1	Female
	Shishmaref	2009	October	Dark	1	1	Male
	Shishmaref	2009	October	Dark	TBD	1	Female
	Shishmaref	2009	October	Dark	2	2	Male
	Shishmaref	2009	October	Dark	TBD	2	Male
	Shishmaref	2009	October	Dark	TBD	2	Unknown
	Shishmaref	2009	October	Dark	3	3	Female
	Shishmaref	2009	October	Dark	3	3	Male
	Shishmaref	2009	October	Dark	TBD	3	Female
	Shishmaref	2009	October	Dark	TBD	3	Female
	Shishmaref	2009	October	Dark	TBD	3	Female
	Shishmaref	2009	October	Dark	4	4	Male
	Shishmaref	2009	October	Dark	5	5	Male
	Shishmaref	2009	October	Dark	TBD	5	Unknown
	Shishmaref	2009	October	Dark	TBD	7.5 +	Female
Ribbon Seals							
	Hooper Bay	2010	February	Dark	8	8.5 +	Male
	Hooper Bay	2010	February	Dark	11	9.5 +	Female
	Point Hope	2007	June	Light	13	12 +	Female

Table 5-2. **Correlation of Stable Isotope Signatures Among Digits**. Spearman's rank-order correlation *p*-value describing correlation for stable nitrogen and carbon isotope ratios in the same seasonal claw growth bands among all five claws (Digit I–V) from the left, front flipper of one male ringed seal (*Pusa hispida*).

	Nitrogen				
	Digit I	Digit II	Digit III	Digit IV	Digit V
Digit I					
Digit II	< 0.001				
Digit III	< 0.001	< 0.001			
Digit IV	< 0.001	< 0.001	0.002		
Digit V	< 0.001	< 0.001	0.006	< 0.001	

	Carbon				
	Digit I	Digit II	Digit III	Digit IV	Digit V
Digit I					
Digit II	< 0.001				
Digit III	< 0.001	< 0.001			
Digit IV	< 0.001	< 0.001	< 0.001		
Digit V	< 0.001	0.003	0.003	< 0.001	

Table 5-3. **Variation of $\delta^{15}N$ and $\delta^{13}C$ in Claw Horns Among Seals and Season.** Total percent contribution of variance among individuals, seasons, and residual error for ringed (*Pusa hispida*), bearded, (*Erignathus barbatus*), and spotted seals (*Phoca largha*). Standard deviations for each source were extracted from linear, mixed effects model with temporal pseudoreplication and squared to create the variance. Variance for each source was then divided by the total variance to compute the percent contribution.

Source of Variance (%)	**Ringed Seal**		**Bearded Seal**		**Spotted Seal**	
	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Among Individuals	59.1	75.9	73.8	91.1	71.7	65.0
Season	0.1	0.1	0.4	0.1	0.4	0.4
Residual	40.8	23.9	25.8	8.8	27.9	34.7

Table 5-4. **Summary of Key Parameters for Four Ice Seal Species** (source: Carol Fairfield, BOEM).

Seal Species	Ringed (threatened)	Bearded (threatened)	Spotted (threatened)	Ribbon (species of concern)
Habitat	Land-fast Ice	Drifting pack ice – shallow coastal	Broken ice floes & open water/land haulouts	Loose pack ice & ice free pelagic
Prey species from literature	Cod, hake, euphausiids, amphipods, shrimp	Demersal & pelagic fish capelin, bivalves, crabs, shrimp	Fish & invertebrates like ringed & bearded seals	Demersal & pelagic fish, amphipods, bivalves, cephalopods, crabs, euphausiids, decapod shrimp
Prey species from Muscle Isotope	High trophic benthic crabs, shrimp, Demersal & young fish; mid trophic benthopelagic isopods, cod	High trophic benthic, Demersal fish, mid-trophic benthopelagic isopods, cod, capelin, young fish	Low trophic pelagic amphipods, euphausiids	N/A
δ^{15}N Muscle Isotope	16.46 to 17.06	16.28 to 17.21	17.43 to 18.22	N/A
δ^{15}N Claw Isotope*	15.0 to 19.4 (16.8 to 17.4) Decrease starting in summer 2007, lowest summer 2008, increased thru winter 2009, then decrease	14.6 to 18.2 (16.3 to 16.9) Began decreasing after summer 1999 thru winter 2000, increased thru summer 2006, then decreased thru summer 2008, increased thru winter 2009,then decrease	Juvenile: ~14.5 to ~18.4 Adult: 15.3 to 17.7 (16.3 – 17.3)	~13.5 to ~17.8
δ^{13}C Muscle Isotope	-19.63 to -18.73	-17.94 to -17.58	-19.13 to -18.42	N/A
δ^{13}C Claw Isotope*	-21.1 to -14.6 (-17.0 to -16.0) Decreased over time starting in winter 2005, lowest winter 2007,increased thru winter 2008,decreased thru summer 2010; general decrease from 1998 to 2010	-18.3 to -13.7 (-16.3 to -15.4) Decreased winter 1998 thru winter 2000, increase thru winter 2001, decrease summer 2002, increase thru summer 2003, steady/slight decline thru summer 2009, increase winter 2009, big decline thru summer 2010.	Juvenile: -16.8 to -13.5 Adult: -16.7 to -14.8 (-15.7 to -15.2)	-17.1 to -14.9
Differences	Muscle shows feeding on fewer euphausiids & amphipods, more crabs; Much larger δ^{13}C range in claws, but typical δ^{13}C less depleted than muscle.	Muscle shows feeding on more mid-trophic benthic & pelagic young fish, isopods, cod but fewer bivalves; δ^{13}C in muscle more depleted than typical range in claws, but within overall claw range, which is large.	Muscle shows feeding more on amphipods, euphausiids, low trophic pelagic; also shows DIFFERENT diet than ringed & bearded, unlike literature; δ^{15}N in muscle larger than in claws of both juvenile and adults, and δ^{13}C in muscle more depleted than in claws of juveniles and adults.	N/A
Years Covered muscle isotope**	July-Aug 2007-2009	July-Aug 2007-2009	Nov-Dec 2007-2008	N/A
Years covered claw isotope	winter 1998 - summer 2010	winter 1998- summer 2010	Juvenile: winter 2006 to summer 2009 Adults: winter 2002 to summer 2009	winter 1995 - winter 2009

*Numbers in parentheses indicate typical values; **Muscle Years Covered indicates when prey consumed, 10 months prior to collection

5.7 Figures

Figure 5-1. **Sample Collection Map.** Map of the location of Alaska Native subsistence communities where claws of ice seals were collected.

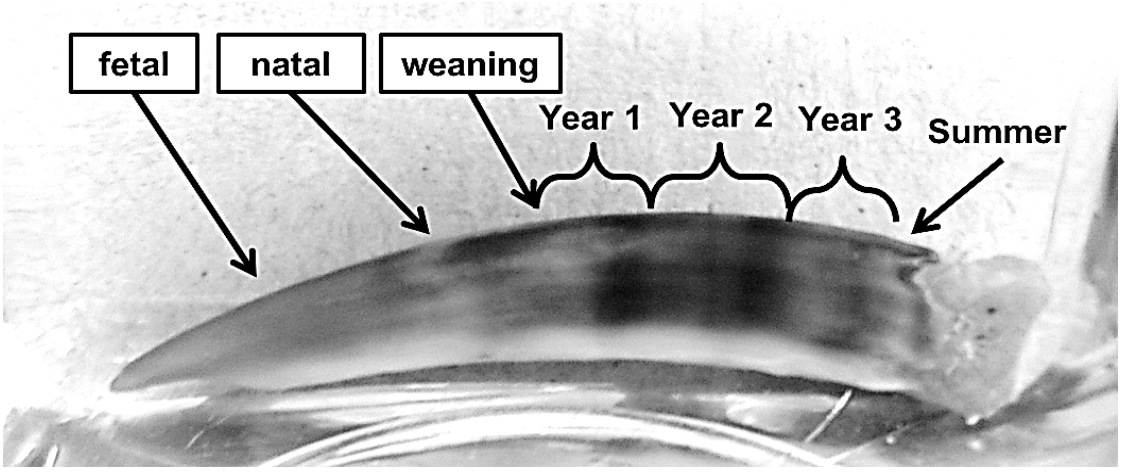

Figure 5-2. **Seal Claw Photo Description.** Lateral view of a claw from a 3-year-old spotted seal. The claw sheath consists of a series of light (spring/summer) and dark (fall/winter) seasonal bands. A pair of bands represents the horn formed in roughly one year. The horn at the base of the claw represents the most recent growth and the tip is the oldest horn growth. Labels indicate drilling locations to assess stable nitrogen and carbon isotope ratios for pre-natal growth (fetal), constriction region at time of birth (natal), and post-natal growth (weaning).

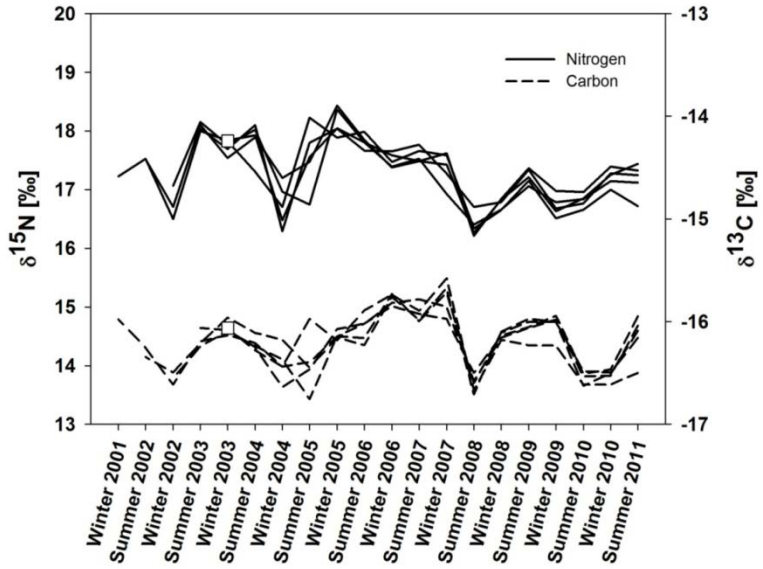

Figure 5-3. **Variation Among Digits.** Stable nitrogen (solid line) and carbon (dashed line) isotope ratios for seasonal growth bands of all five claws (digits I–V) from the left, front flipper of a male ringed seal (*Pusa hispida*). White square symbol marks the distal, i.e., oldest growth, band still present in the cornified claw sheath of the fifth digit.

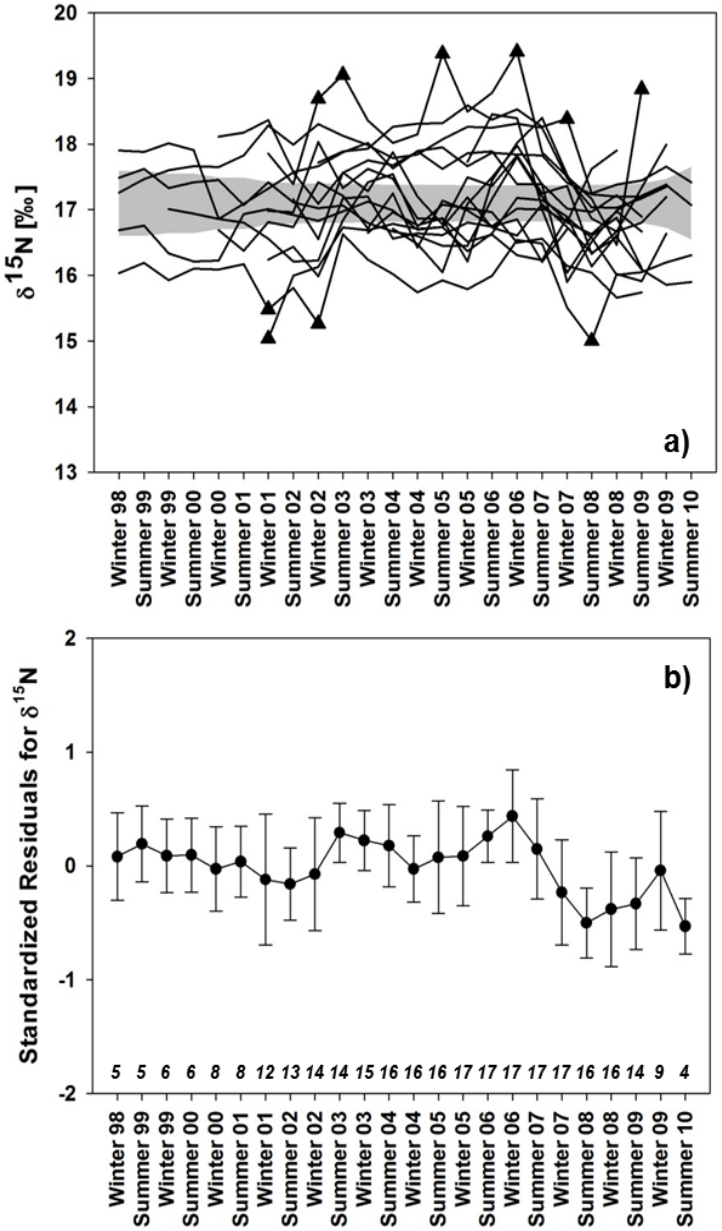

Figure 5-4. **δ^{15}N Values for Ringed Seal Claw Sheaths. (a)** Black lines show the δ^{15}N values for seasonal growth bands of each ringed seal (*Pusa hispida*) claw sheath, with lengths of lines varying based on the number of seasons present for each individual claw. The gray region shows 95% confidence limits created using a linear mixed-effects model with temporal pseudoreplication. Triangles are "anomalies", i.e., extreme values, based on studentized residuals greater than 2. **(b)** Dots are mean residuals of δ^{15}N values for each season among seals and the bar illustrates the standard deviations of these residuals. Numbers above the x-axis show the number of seals included in the analysis for that season. For seasonal bands (x-axis), winter corresponds to trophic level of seals during fall/winter, while summer describes trophic level during spring/summer.

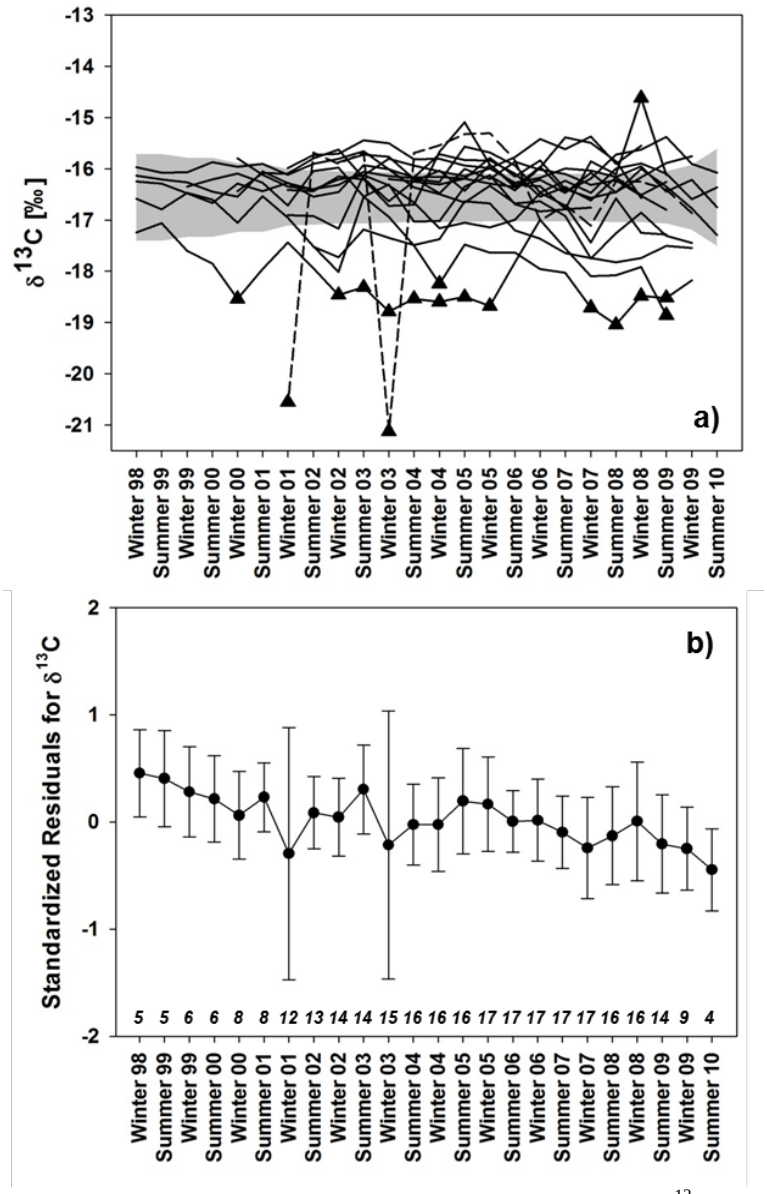

Figure 5-5. **δ^{13}C Values for Ringed Seal Claw Sheaths. (a)** Black lines show the δ^{13}C values for seasonal growth bands of each ringed seal (*Pusa hispida*) claw sheath, with lengths of lines varying based on the number of seasons present for each individual claw. The dotted black line shows an individual having seasonal growth bands especially depleted in ^{13}C. The gray region shows 95% confidence limits created using a linear mixed-effects model with temporal pseudoreplication. Triangles are "anomalies", i.e., extreme values, based on studentized residuals greater than 2. **(b)** Dots are mean residuals of δ^{15}N values for each season among seals and the bar illustrates the standard deviations of these residuals. Numbers above the x-axis show the number of seals included in the analysis for each season. For seasonal bands (x-axis), winter corresponds to trophic level of seals during fall/winter, while summer describes trophic level during spring/summer.

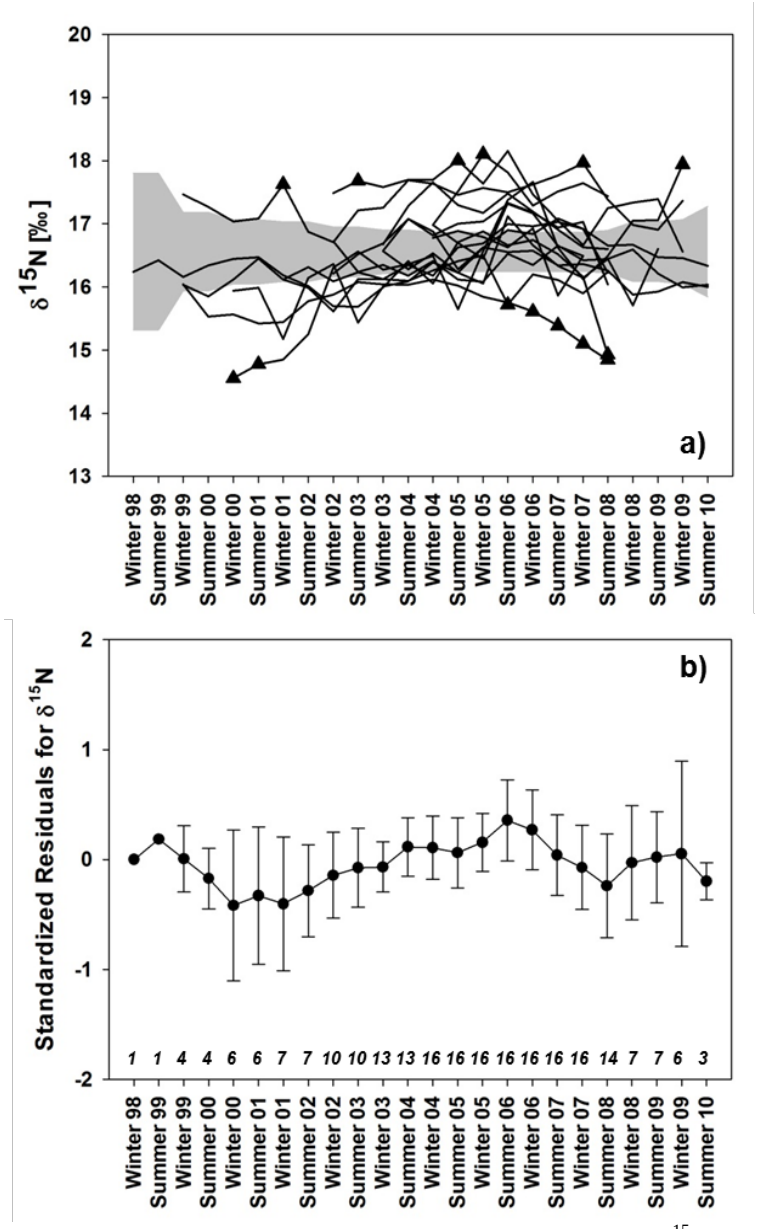

Figure 5-6. **δ^{15}N Values for Bearded Seal Claw Sheaths. (a)** Black lines show the δ^{15}N values for seasonal growth bands of each bearded seal (*Erignathus barbatus*) claw sheath, with lengths of lines varying based on the number of seasons present for each individual claw. The gray region shows 95% confidence limits created using a linear mixed-effects model with temporal pseudoreplication. Triangles are "anomalies", i.e., extreme values, based on studentized residuals greater than 2. **(b)** Dots are mean residuals of δ^{15}N values for each season among seals and the bar illustrates the standard deviations of these residuals. Numbers above the x-axis show the number of seals included in the analysis for each season. For seasonal bands (x-axis), winter corresponds to trophic level of seals during fall/winter, while summer describes trophic level during spring/summer.

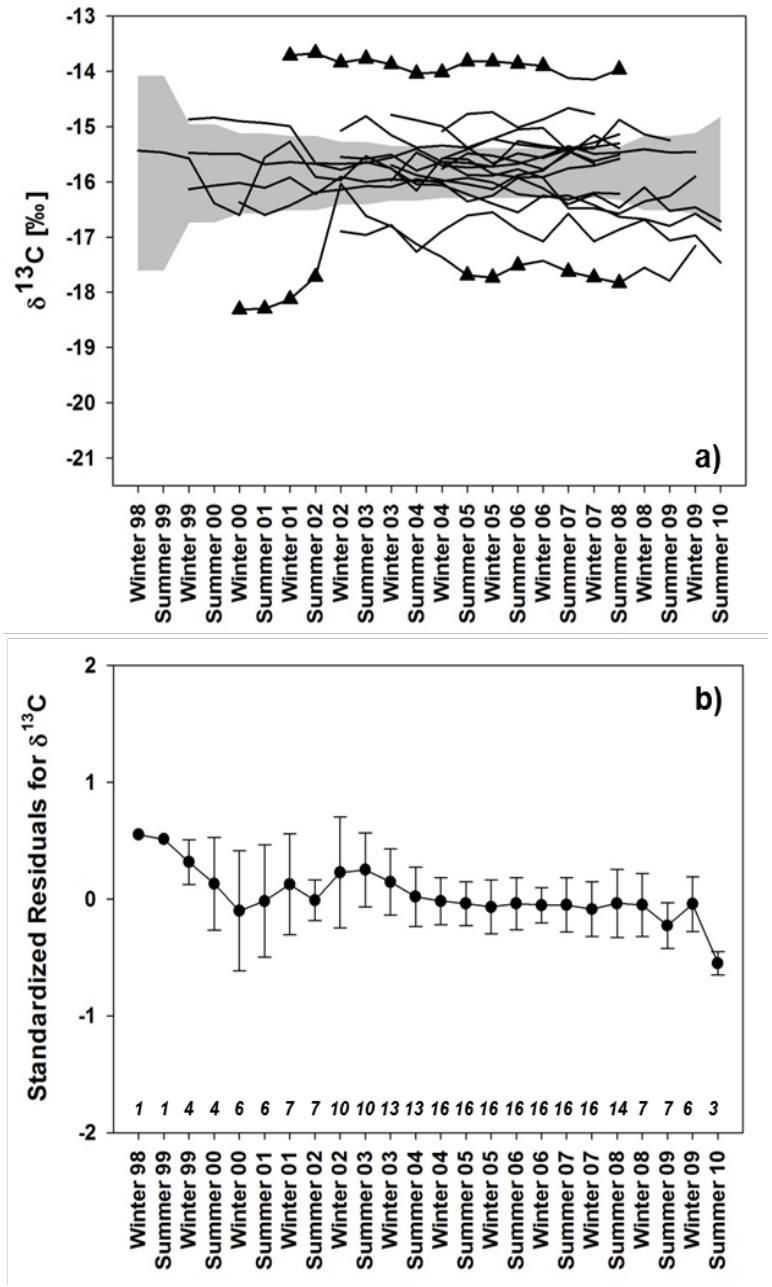

Figure 5-7. **δ^{13}C Values for Bearded Seal Claw Sheaths. (a)** Black lines show the δ^{13}C values for seasonal growth bands of each bearded seal (*Erignathus barbatus*) claw sheath, with lengths of lines varying based on the number of seasons present for each individual claw. The gray region shows 95% confidence limits created using a linear mixed-effects model with temporal pseudoreplication. Triangles are "anomalies", i.e., extreme values, based on studentized residuals greater than 2. **(b)** Dots are mean residuals of δ^{15}N values for each season among seals and the bar illustrates the standard deviations of these residuals. Numbers above the x-axis show the number of seals included in the analysis for each season. For seasonal bands (x-axis), winter corresponds to trophic level of seals during fall/winter, while summer describes trophic level during spring/summer.

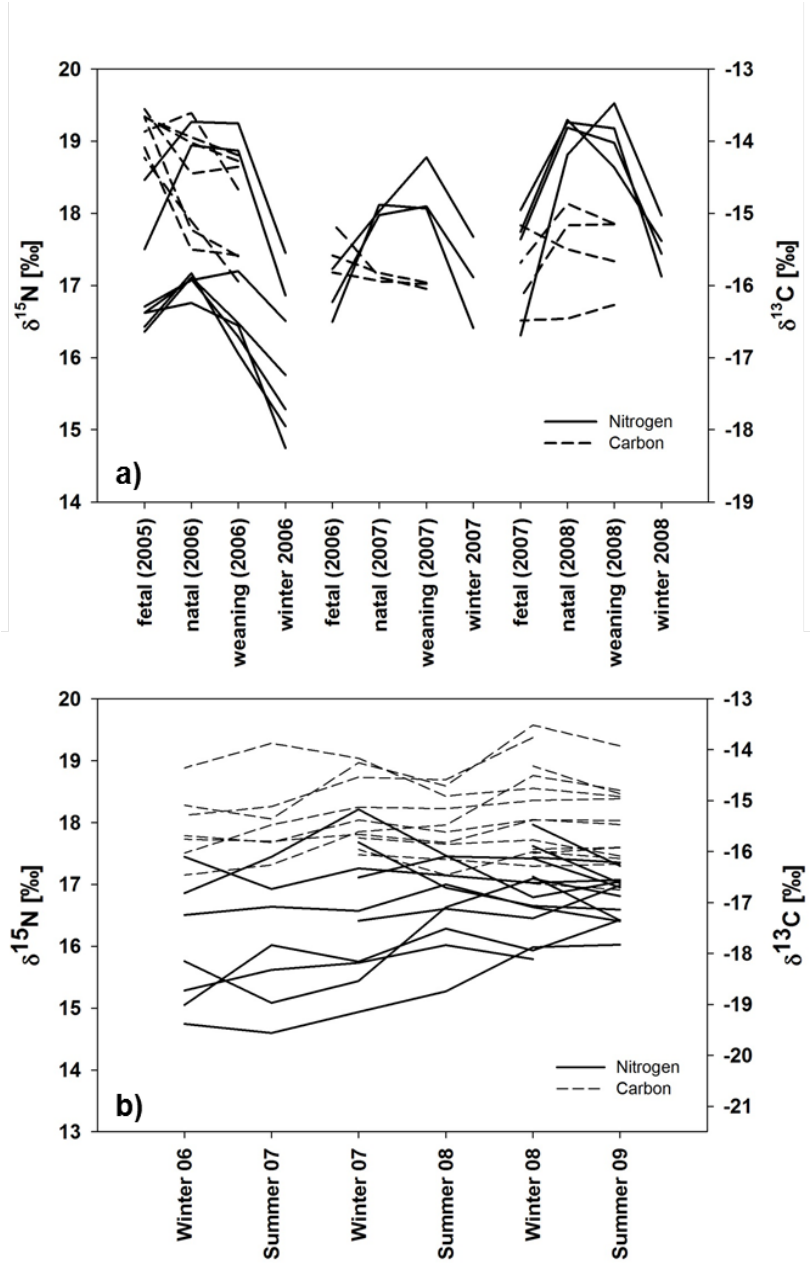

Figure 5-8. **Stable Isotope Signatures for Young Spotted Seal Claws.** Stable nitrogen (solid line) and carbon (dashed line) isotope ratios for seasonal growth bands of spotted seal claws (n = 14) ranging from one to three years of age. Isotope signatures recorded in claws during (**a**) fetal/pup developmental phases, i.e., fetal (tip of claw), natal (constriction region), weaning (first light band after natal notch), and fall/winter foraging claw horn growth (first dark band after natal notch). A continuation of stable nitrogen and carbon isotope signatures recorded in claws during (**b**) juvenile development from first winter foraging until harvest in summer 2009.

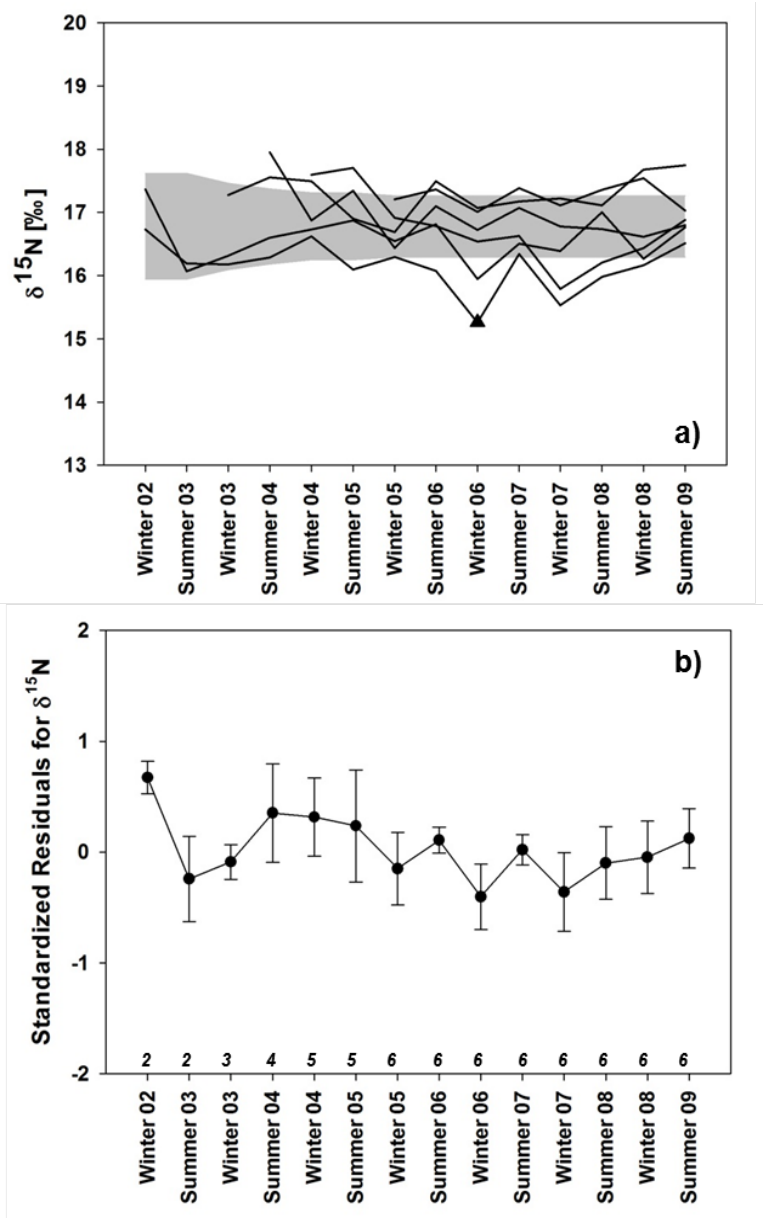

Figure 5-9. **δ^{15}N Values for Spotted Seal Claw Sheaths. (a)** Black lines show the δ^{15}N values for seasonal growth bands of each spotted (*Phoca largha*) claw sheath, with lengths of lines varying based on number of seasons present for each individual claw. The gray region shows 95% confidence limits created using a linear mixed-effects model with temporal pseudoreplication. Triangles are "anomalies", i.e., extreme values, based on studentized residuals greater than 2. **(b)** Dots are mean residuals of δ^{15}N values for each season among seals and the bar illustrates the standard deviations of these residuals. Numbers above the x-axis show the number of seals included in the analysis for each season. For seasonal bands (x-axis), winter corresponds to trophic level of seals during fall/winter, while summer describes trophic level during spring/summer.

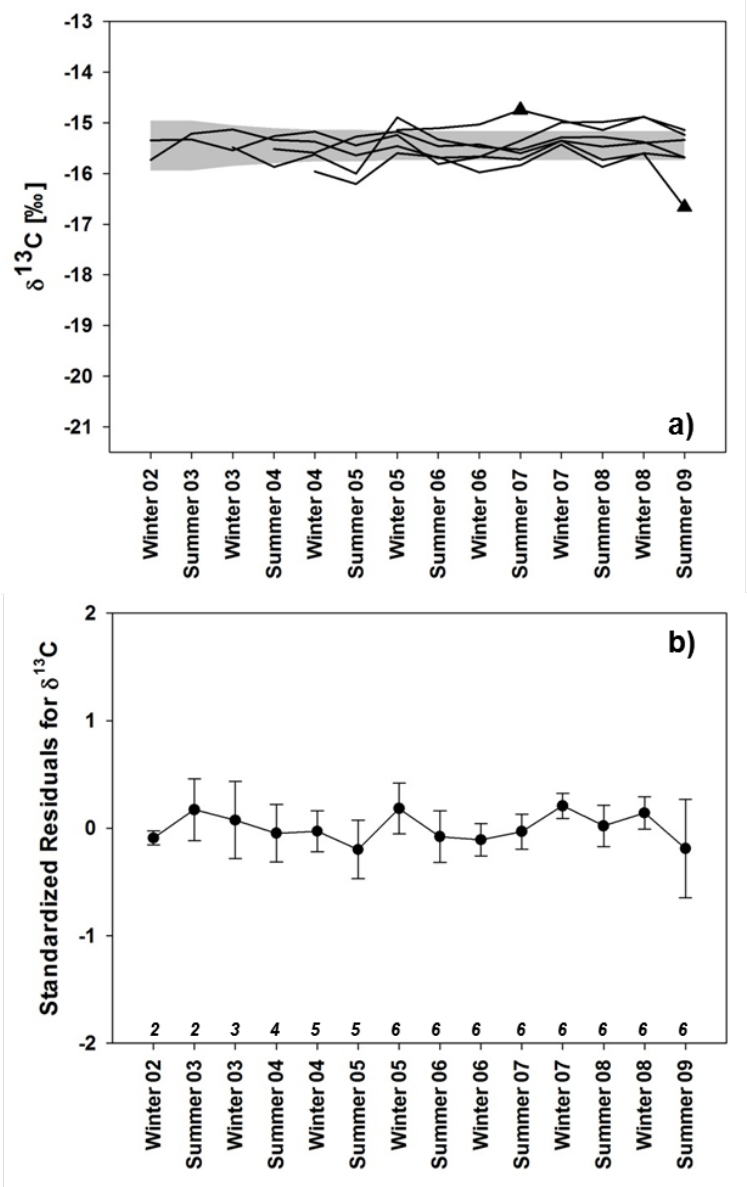

Figure 5-10. **δ^{13}C Values for Spotted Seal Claw Sheaths. (a)** Black lines show the δ^{13}C values for seasonal growth bands of each spotted seal (*Phoca largha*) claw sheath, with lengths of lines varying based on number of seasons present for each individual claw. The gray region shows 95% confidence limits created using a linear mixed-effects model with temporal pseudoreplication. Triangles are "anomalies", i.e., extreme values, based on studentized residuals greater than 2. (**b)** Dots are mean residuals of δ^{13}C values for each season among seals and the bar illustrates the standard deviations of these residuals. Numbers above the x-axis show the number of seals included in the analysis for each season. For seasonal bands (x-axis), winter corresponds to trophic level of seals during fall/winter, while summer describes trophic level during spring/summer.

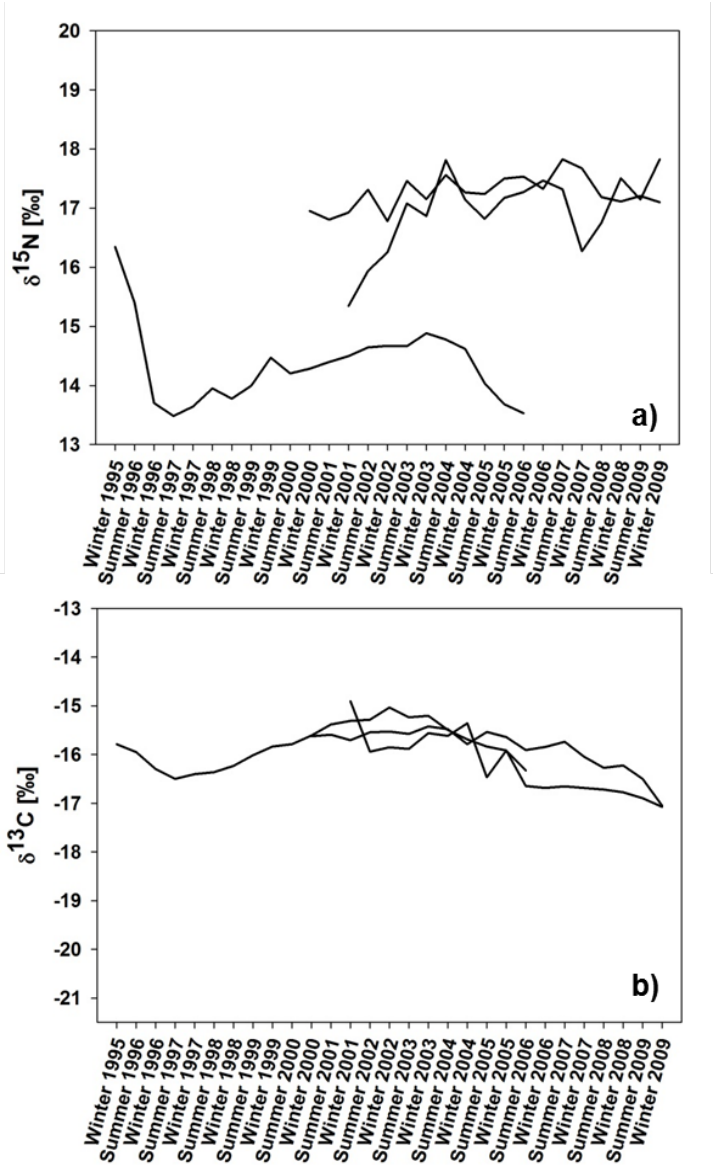

Figure 5-11. **Stable Isotope Signatures for Ribbon Seal Claws.** Stable nitrogen (**a**) and carbon (**b**) isotope ratios for seasonal growth bands of ribbon seal claw sheaths. For seasonal bands (x-axis), winter corresponds to trophic level of seals during fall/winter, while summer describes trophic level during spring/summer.

Chapter 6: Conclusions

This study filled a pressing need for baseline information on the feeding ecology of marine fishes and ice seals in the Arctic. Previous studies have been limited to a single year and have not captured ecosystem variability over time. This project addressed this variation by analyzing short-term and long-term diets of Arctic predators over consecutive years, particularly during sea ice minimum years. The combined effects of sea ice habitat loss and altered food web structure leave populations of ice seals and fishes extremely vulnerable to additional disturbances, e.g., increased ship traffic, noise, and sub-Arctic species range extensions. Accurate assessment of population health and development of effective management strategies is necessary for good stewardship by oil and gas exploration. Results from this project provide an enhanced understanding of energy flow and adaptive responses in the rapidly changing Arctic ecosystem. Feeding ecology of Arctic fishes and ice seals were described as four different components of this study (Chapters 2 – 5) as described below:

Comparison of short-term and long-term diets of eleven Arctic fish species (Chapter 2) describes general diets using stomach contents and stable isotope ratios for which there are little to no information. Stomach contents of Arctic fishes mainly consisted of pelagic crustaceans; however, based on stable isotope results, higher trophic prey likely make a greater contribution to diets over time. Short-term diets, as determined by stomach content analysis, and long-term integrated diets, as determined by stable isotope analysis, showed inconsistencies that indicate seasonal variability is confounded when using solely summer diet analysis. This highlights the importance of assessing feeding ecology using multiple tools.

Interannual diet variability for five Arctic fish species in the Chukchi Sea (Chapter 3) explores changes in fish diets over time using a Bayesian isotope mixing model approach. Low-trophic, pelagic prey contributed more to long-term diets of fish during 2007/2008, and the contribution of high-trophic prey increased from 2008 to 2010 (Figure 6-1). While trophic level (based on stable isotopes) of fish prey stayed constant during 2008-2010 (Figure 6-1), interannual variability in the feeding of Arctic fishes may correspond to an increase in abundance of pelagic crustaceans in the Chukchi Sea during 2007, a low-ice year. Higher trophic foraging by Arctic fishes in years following 2007 is consistent with the currently accepted hypothesis of benthic-pelagic uncoupling during minimum sea ice cover. This model approach is an effective tool to identify ecosystem variability over time.

Interannual variation in the diet of ice seals assessed by isotopic mixing models (Chapter 4) examines trophic variability in apex predators, i.e., Arctic pinnipeds. Diets of bearded seals had a lower contribution of benthic prey and consequently higher contribution of benthopelagic prey from 2007 to 2010 (Figure 6-1), again indicative of benthic-pelagic uncoupling. Pelagic ice seals, i.e., ringed and spotted seals, capitalized on abundant low-trophic, pelagic prey, particularly during 2007−2009 for ringed seals and 2008/2009 for spotted seals (Figure 6-1). The three species of ice seals that we examined are opportunistic predators. As such, ice seals may not be

vulnerable to changes in prey populations. If the climate shifts to a pelagic-dominated ecosystem, ice seals will likely take advantage of pelagic prey sources.

Diet history of ice seals using stable isotope ratios in claw growth bands (Chapter 5) investigates long-term diet patterns of ice-associated pinnipeds (Table 5-4). Examination of seal claws was unique in that it allowed documentation of time series up to ten years for individual seals. Stable isotope ratios within claw growth bands illustrated an increase in trophic foraging and likely more pelagic foraging by both ringed and bearded seals from 2007 to 2010 (Figure 6-1), again affirming patterns observed in previous components of this study.

Stomach content and stable isotope analyses are complementary techniques for assessing feeding ecology. Stomach content analysis provides the background information necessary for understanding stable isotope results. Prey items can be identified at a higher taxonomic resolution when examining stomach contents (Carrasco et al. 2012). Additionally, stomach content information can be used to calculate energetics (Dyck and Kebreab 2009). Stomach content analysis has been extensively used historically and is a basis for comparison among studies, making it the "gold standard" for feeding ecology. However, this technique does have biases. Stomach content analysis only provides dietary information from the previous days. Retention of prey hard parts and secondarily ingested prey can overestimate the importance of some species (Sheffield et al. 2001). The digestive state of prey can hinder identification and underestimate the importance of soft-bodied prey (Sheffield et al. 2001; Brush et al. 2012). Alternatively, stable isotope analysis is a more rapid technique and assesses diet over a longer time frame than stomach content analysis (Hesslein et al. 1993). Examining stable isotope ratios from different types of tissues can provide information that illustrate various time periods (Peterson and Fry 1987), with long-term dietary records even documented in keratinized structures (Schell et al. 1989; Cherel et al. 2009; Newsome et al. 2009). However, integrated diet can be difficult to interpret because differential turnover rates of tissues, diets consisting of a mixture of diverse prey taxa, and the physiological state of the predator (e.g., starvation and pregnancy) can influence stable isotope ratios (Newsome et al. 2010). The turnover rates of tissues are typically determined in captive settings (Hobson et al. 1996), and these rates are unknown for many species, especially Arctic predators. Comparing diet results from two techniques enhanced the range of our assessment making this study less myopic by adding more layers and depth.

The benefit of examining across multiple trophic levels and the ecosystem approach of this study provides insight into general food web effects instead of effects on just a single species. A change in environmental conditions, such as the low-ice year of 2007 and the resultant benthic-pelagic uncoupling, can be seen in both fish and seal diets. Many studies have identified ice seals as indicators of Arctic ecosystem health (Laidre et al. 2008; Moore and Huntington 2008; Cameron et al. 2010; Kelly et al. 2010). Results of our study show that fishes are key components of the food web that might also be indicators of Arctic ecosystem health. Seals seem to be resilient and adaptable to changes in food availability, and we do not have sufficient long-

term data for individual fish to make this conclusion. For example, fish vertebrate grow predictably (Fjelldal et al. 2013), i.e., can be aged, and may serve as a tool to monitor long-term changes in the diet of an individual.

Additional techniques, to complement stomach content and stable isotope analysis, could enhance the understanding of feeding ecology of Arctic fishes during low ice years. Diet determination and direct observation of fish and seal feeding behavior can be challenging, particularly in the Arctic, due to the remote distribution and seasonally restricted access to habitat. Many studies have therefore utilized indirect methods to describe diets, including stomach content analysis, identification of hard structures in feces, chemical feeding ecology (including stable isotopes and fatty acids), and more recently DNA-based approaches (Sheffield et al. 2001; Arim & Naya 2003; Budge et al. 2006; Dehn et al. 2007; Cooper et al. 2009; Tollit et al. 2009; Quakenbush et al. 2009, 2010a, b). Integrated approaches, such as stable isotope and fatty acid analysis, have become increasingly popular methods to assess marine mammal diets as these can be applied to minimally invasive biopsy samples (Bradshaw et al. 2003; Herman et al. 2005; Horstmann-Dehn et al. 2011). Primary producers synthesize a number of unique fatty acids that are passed to, but not synthesized by, higher trophic levels. Thus, the stable carbon isotope ratio of specific fatty acids (compound-specific stable isotope analysis) originating from different sources of marine primary production, e.g., open-water algae vs. ice-bound algae, can be used as biomarkers and can be traced into consumers throughout the food web (Budge et al. 2008). Reduced ice cover in the Arctic results in increased irradiation that decreases the quality of the fatty acids in ice algae, which in turn propagates through the food web (Leu et al. 2010). Use of fatty acids as an assessment tool expands understanding from dietary intake to include prey quality and health of the consumer. This study documents diet content but not diet quality. We did not examine body condition and health with regard to food intake; therefore, we cannot make conclusions about the effects of changes in trophic feeding to the health of Arctic fishes and ice seals. To date, a comprehensive assessment has not been done using this full range of available tools in combination with the “gold standard” stomach content analysis. Additionally, studies need to incorporate more seasonal sampling. A comparison of multiple dietary assessment techniques and multi-year sampling are necessary to develop a comprehensive understanding of energy flow in the Arctic.

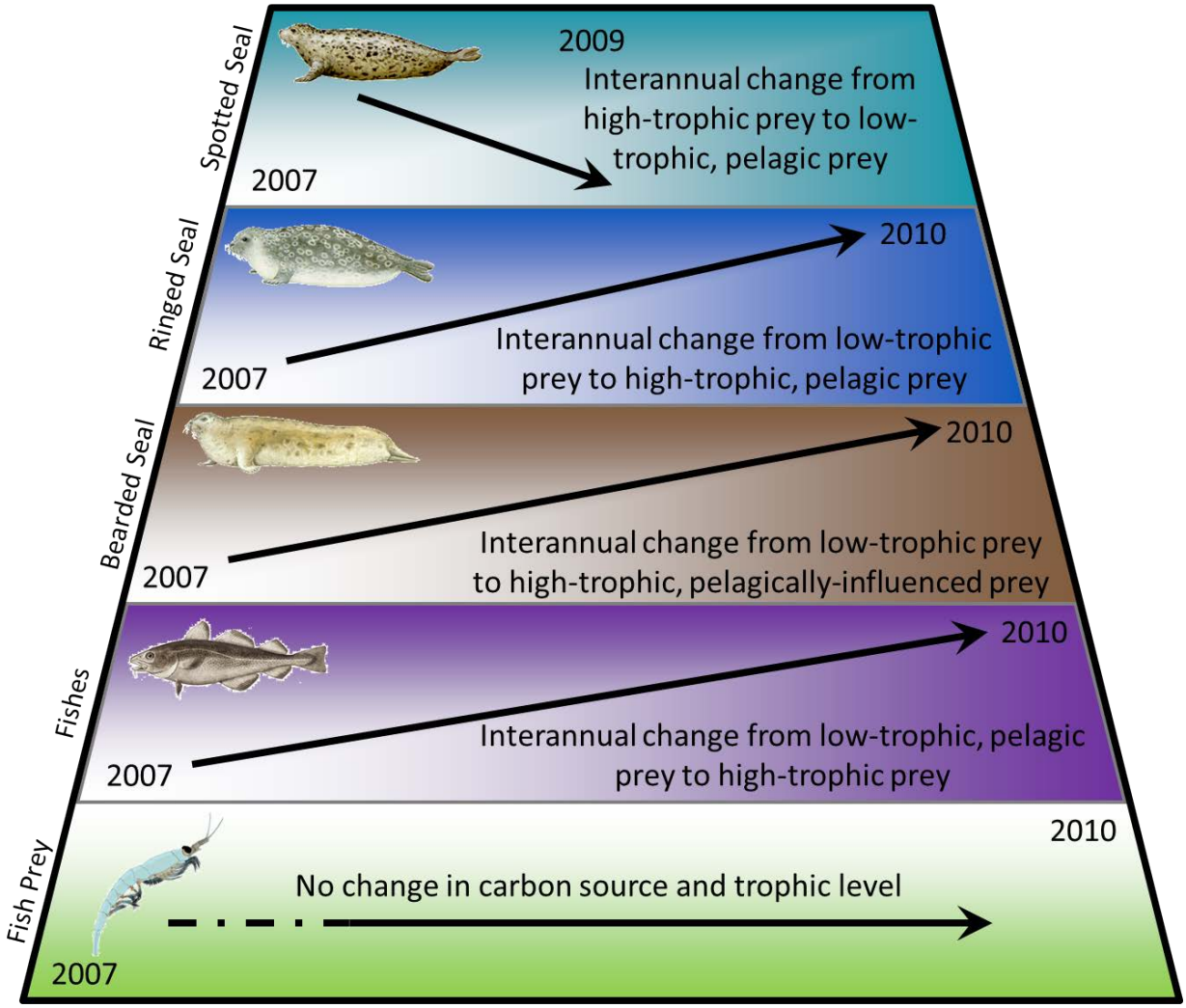

Figure 6-1. **Summary of Conclusions.** Interannual trends in the diet of Arctic fishes and ice seal species. Higher trophic feeding by Arctic fishes and ice seals in years following 2007 is consistent with the currently accepted hypothesis of benthic-pelagic uncoupling during years of reduced sea ice cover in the Arctic.

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The Department of the Interior Mission

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The Bureau of Ocean Energy Management (BOEM) works to manage the exploration and development of the nation's offshore resources in a way that appropriately balances economic development, energy independence, and environmental protection through oil and gas leases, renewable energy development and environmental reviews and studies.