IDENTIFYING BEARDED AND RINGED SEAL DIET– A COMPARISON OF STOMACH CONTENTS, STABLE ISOTOPES, FATTY ACIDS, AND FECAL DNA

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Abstract

Stomach contents, stable isotopes, fatty acids, and more recently fecal DNA are commonly used to infer the diet of marine mammals. However, how complementary or contradictory these methods are, especially when considering individual diet variability, remains poorly understood. This study assessed the differences in the dietary information resulting from stomach contents, stable isotopes, and fatty acids for adult bearded seals (*Erignathus barbatus*), and fishes identified from stomach contents and fecal DNA for bearded and ringed seals (*Pusa hispida*), harvested in Alaska for subsistence use. Stomach contents and fecal DNA provided information on recently consumed prey. In contrast, stable carbon and nitrogen isotopes of muscle and fatty acid profiles of blubber provided information on prey consumed and integrated over a longer time frame, but taxonomic resolution of prey was low. Overall, stomach contents provided the most dietary data, while fecal DNA delivered the least. Using denaturing gradient gel electrophoresis (DGGE) of 16S gene fragments, only 40% of the fecal samples (12 bearded and one ringed seal) produced detectable DNA suitable for reference gene amplification. Only three fish species could be positively identified in the diet of seals (Arctic cod, *Boreogadus saida*; shorthorn sculpin, *Myoxocephalus scorpius*; and an unknown snailfish species, Liparidae) when using fecal DNA. In a dietary comparison, and despite differences in dietary time frames, the relative occurrence (RO) of prey from stomach contents and the mean proportions of prey source groups from a Bayesian stable isotope mixing model (SIAR) were similar. The proportions of indicator fatty acids from full-thickness blubber, such as 16:4n-1, 20:5n-3, 20:4n-6, 20:1n-9, 22:1n-11, and the presence of non-methylene-interrupted fatty acids were similar to other fatty acid studies of bearded seals in Alaska, and suggest a benthic diet. Overall, the methods yielded different, but not necessarily contradictory results.

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Chapter 1: General Introduction

The arctic ecosystem is changing, and these changes may alter the diet of ice seal species living in the Arctic. Bearded (*Erignathus barbatus*) and ringed (*Pusa hispida*) seals are two of four species of ice-dependent seals in Alaska who use sea ice for pupping, molting, and as a resting platform (McLaren 1958, Burns 1981). Sea ice extent and thickness is decreasing in the Arctic (Beitler 2012, Stroeve et al. 2012), although these changes are not uniform across locations, and it is unknown exactly how this decrease in sea ice will affect the food web. Changes in the physical environment of the Arctic will have impacts on primary production, thus, marine mammals will likely see density and distribution shifts in their prey species as part of a bottom-up trophic cascade (Grebmeier et al. 2006, Bluhm and Gradinger 2008, Kovacs and Lydersen 2008). The Bering and Chukchi seas are benthic-dominated ecosystems, and, as summer sea ice decreases, a shift to a more pelagic-dominated ecosystem is anticipated (Grebmeier et al. 1988, 2006). Further, as sea ice retreats, increasing light irradiance could decrease the nutritional quality of prey by decreasing the proportion of polyunsaturated fatty acid (Leu et al. 2010, Wang et al. 2014) or changing the compostion (Kelly and Scheibling 2012) of primary producers. These changes could then propagate up the food chain with unknown consequences. The combined effects of habitat loss and changes in food web structure could cause ice-dependent species to be vulnerable to sea ice changes (Kovacs et al. 2011). In 2012, due to these predicted changes in the arctic environment, and concerns for the long-term survival of ice-associated pinnipeds, the Pacific population of bearded seals (U.S. Federal Register 2012*b*) and the Arctic Basin population of ringed seals (U.S. Federal Register 2012*a*) were listed as threatened under the Endangered Species Act.

Bearded and ringed seals are ice-associated phocid seals with a circumpolar distribution (McLaren 1958, Burns 1981). Bearded seals prefer shallow waters near moving sea ice that produces many openings (Burns 1981, Simpkins et al. 2003). In contrast, ringed seals prefer landfast ice or the edge of the ice pack (Frost and Lowry 1981, Simpkins et al. 2003). Pupping and molting for both species occurs in the spring, bearded seals give birth from mid-March through the first week of May (Burns 1981), and ringed seals pup in April (Frost and Lowry 1981). Both species also molt in the spring and early summer (Burns 1981, Frost and Lowry 1981). In Alaska, both of these species have extensive and large-scale migrations encompassing the Bering, Chukchi, and Beaufort seas (Frost et al. 2008, Cameron and Boveng 2009, Crawford et al. 2012). Bearded and ringed seals are also culturally and nutritionally important for indigenous peoples in the Arctic, and are an essential food source for polar bears (*Ursus maritimus*); thus, they are a critical link to high trophic levels in the arctic ecosystem.

Diet of ice seals is an important factor when monitoring response to change, such as climate warming, because a decrease in nutrition could lead to a decrease in the animal's health (Burek et al. 2008, Moore and Huntington 2008). Bearded seals are benthic generalists, known to eat a wide variety of invertebrates and fishes (Johnson et al. 1966; Lowry et al. 1980*a*; 1981*a*, *b*; Antonelis et al. 1994; Dehn et al. 2007; Quakenbush et al. 2011*a*), and the diet of ringed seals includes both pelagic and benthic prey (Johnson et al. 1966; Lowry et al. 1981*a*, *b*; Dehn et al. 2007; Quakenbush et al. 2011*b*). A shift in diet could lead to low prey availability, food with decreased nutritional value (Trites and Donnelly 2003, Leu et al. 2010, Wang et al. 2014), or increased energy expenditure associated with capture and digestion of novel prey (Rosen et al. 2007, Barboza et al. 2009). This could then result in less fat/blubber storage, decreased insulation, and consequently more energy needed for thermoregulation and locomotion (Rosen et

al. 2007). An overall shift in diet could also reduce availability of energy and resources for reproduction and disease resistance, ultimately resulting in population declines (Burek et al. 2008). However, a shift in diet does not necessarily imply a negative effect and decreased nutritional value for the predator. Quakenbush et al. (2011*a*, *b*) found that both bearded and ringed seals were consuming more fishes in the 2000s compared with the 1960s or 1970s without a noticeable decrease in body condition or fecundity. Consequently, investigations enhancing knowledge of ice seal diets, mainly due to the uncertainties about bottom-up food web effects, and adaptive responses of seals in the rapidly changing arctic ecosystem, remain an important issue.

Seal diet can be challenging to identify because feeding can often not be directly observed, and the removal of individuals from prey populations usually cannot be measured (Pierce and Boyle 1991). Therefore, indirect methods for estimating diet, such as identifying prey remains from stomachs or feces, chemical analysis of tissues (including fatty acids and stable isotopes), and DNA identification of prey remains are used (Pierce and Boyle 1991, Deagle et al. 2005, Budge et al. 2006, Newsome et al. 2010, Tollit et al. 2010). Each of these methods provide important dietary information, but each approach has limitations and biases (Bowen and Iverson 2013), and none of the techniques provide a complete description of diet.

Stomach content and fecal hard part analyses involve sorting and identifying prey remains found in the stomach or feces of the predator (Pierce and Boyle 1991). The collection of feces from haulouts has no (or minimal) effects on the predator (Tollit et al. 2010), whereas the collection of stomach contents is at a minimum invasive (e.g., lavage) and generally requires dead animals. If stomachs are collected from sick or stranded animals, they may not contain prey representative of an individual as part of a normal, healthy population (Pierce and Boyle

1991). Both stomach contents and fecal hard parts are limited by variable digestion and retention of prey (Bowen and Iverson 2013). For example, soft bodied prey (such as marine worms; Sheffield et al. 2001) and small fragile otoliths (e.g., salmon, *Oncorhynchus* spp.) digest quickly (Bowen 2000); in contrast, large otoliths (e.g., saffron cod, *Eleginus glacialis*) and cephalopod beaks can accumulate over several feedings and can be overrepresented (Pierce and Boyle 1991, Bowen and Iverson 2013). Stomach content and fecal hard part analyses render no dietary information if prey lack hard parts (e.g., tunicates and marine worms) or are digested completely (Pierce and Boyle 1991, Bowen 2000). Also, taxa that are secondarily ingested from prey (i.e., prey of prey) can cause false positives (Bowen and Iverson 2013). Nonetheless, stomach content (and to some extent fecal hard part) analyses are widely used and remain the gold standard for diet estimations of free-ranging populations.

Stable isotope analysis involves analyzing the ratio of heavy and light stable isotopes (e.g., carbon, $^{13}C/^{12}C$ and nitrogen, $^{15}N/^{14}N$) in tissues of the animal (Tollit et al. 2010) and comparing the ratios among groups of animals or food webs (e.g., Hobson and Welch 1992, Iken et al. 2010). Stable carbon isotope ratios can identify the carbon source and habitat where the animal was foraging (e.g., benthic vs. pelagic, onshore vs. offshore), whereas stable nitrogen isotope ratios represent the average trophic level at which the animal was foraging (Peterson and Fry 1987, Kelly 2000, Newsome et al. 2010). Other stable isotopes, such as oxygen ($^{18}O/^{16}O$) and sulfur ($^{34}S/^{32}S$) can also be useful to identify foraging areas. For example, the oxygen isotope ratio reflects water mass characteristics (e.g., fresh vs. saline) and is often used to interpret paleoclimates (Woodruff et al. 1981, Clementz and Koch 2001, Newsome et al. 2010), and sulfur can be used to identify the importance of marine vs. freshwater ecosystems (Peterson

and Fry 1987, Hoekstra et al. 2002). However, metabolic processes of the predator can complicate the interpretation of stable isotopes.

Tissues within an animal have different metabolic turnover rates and will therefore indicate dietary information for different time frames (Newsome et al. 2010). For example, stable isotope analysis of liver reveals more recent dietary information than stable isotope analysis of muscle (Newsome et al. 2010). Some metabolically inert tissues (e.g., claws, teeth, and vibrissae) only reflect diet during the time of growth, but these tissues continuously grow over time, thereby generating a time series of diet information for individual animals (Newsome et al. 2009, Hindell et al. 2012, Carroll et al. 2013). Stable isotope analysis is advantageous because tissues can be collected with minimally invasive techniques (e.g., biopsy or hair collection) from free-ranging animals (Tollit et al. 2010) and provide information on absorbed not just ingested prey (Kelly 2000). On the other hand, taxonomic resolution of prey items is low (Tollit et al. 2010). Stable isotope mixing models can estimate the proportional contributions of prey (Phillips 2012), but exact prey proportions cannot be generated when the number of prey sources is greater than the number of isotopes used plus one (Phillips and Gregg 2003). This makes the application of stable isotope mixing models challenging for generalists predators.

Fatty acid analysis uses the proportion of different fatty acids (i.e., fatty acid signature) in adipose tissue (blubber is often utilized for marine mammals) of a predator to make inferences about its diet or foraging ecology (Budge et al. 2006, Iverson 2009). Fatty acids have been used both qualitatively (e.g., Budge et al. 2007, Meynier et al. 2008, Cooper et al. 2009) and quantitatively (e.g., Iverson et al. 2004, Rosen and Tolllit 2012) to describe the diets of many pinniped species. Fatty acids are advantageous because they can be collected with minimally

invasive techniques (e.g., biopsy) from free-ranging animals (Iverson 2009). Fatty acids provide information on absorbed not just ingested prey (Budge et al. 2006), and the taxonomic resolution is higher than for stable isotopes (Bowen and Iverson 2013). Recently, fatty acid analysis has been combined with stable isotope analysis, (i.e., compound specific fatty acid analysis; Budge et al. 2008, Wang et al. 2014). This approach can be powerful to track sources of indicator fatty acids (e.g., primary production from pelagic and sympagic sources) to estimate and track their contribution through the food web to top level consumers (Budge et al. 2008).

Without quantitative analysis, fatty acids and stable isotopes can only indicate general differences in diet among animals (Budge et al. 2006, Martínez del Rio et al. 2009), but by using quantitative models, these two methods can estimate proportions of prey in the diet of a predator (Bowen and Iverson 2013). However, these models require knowledge of the fatty acid or stable isotope values of the prey (Iverson et al. 2004, Phillips 2012), prior knowledge of prey in the diet of a predator (usually obtained via stomach contents; Bowen and Iverson 2013), and metabolic information about the predator (Iverson et al. 2004, Parnell et al. 2010). Quantitative models for both fatty acids and stable isotopes are sensitive to the metabolic correction factors (Bond and Diamond 2011, Rosen and Tollit 2012). Predator metabolism alters both the fatty acid signature and stable isotope ratios of prey before incorporating them into the predator's tissues (Tieszen et al. 1983, Hobson et al. 1996, Iverson et al. 2004). Therefore, metabolic correction factors have been developed (i.e., stable isotope trophic enrichment factors and fatty acid calibration coefficients) and are used in quantitative models to account for the metabolic differences between the predator and prey (Iverson et al. 2004, Parnell et al. 2010). Unfortunately, controlled feeding studies to establish metabolic correction factors for diet estimates are sparse

and do not exist for most marine mammals (Newsome et al. 2010, Bond and Diamond 2011, Rosen and Tollit 2012), in particular in the Arctic, making these models problematic.

Most recently, prey DNA molecules found in the digestive tract and in feces of predators have been used to identify prey items (Deagle et al. 2005, 2013; Vestheim and Jarman 2008; Tollit et al. 2009). The main advantage of using DNA is that prey can be identified to the species level, even after digestion has destroyed diagnostic parts (Jarman et al. 2004, King et al. 2008), with no (or minimal) impact to the animal (Tollit et al. 2010). However, false positives or false negatives are possible (King et al. 2008, Bowen and Iverson 2013), and these biases are difficult to recognize in samples collected from wild populations (Deagle et al. 2005). Newer high-throughput DNA sequencing techniques have been successful in obtaining DNA from feces (Deagle et al. 2009). Quantitative estimates of diet using DNA still have many problems (Pompanon et al. 2012), and wide confidence limits should be associated with these estimates (Deagle et al. 2010). Yet, the high taxonomic resolution, the relative ease of fecal collection, as well as the rapid advancements in genetics and its application make this method promising for diet estimates of free ranging populations.

A direct comparison of stomach contents, stable isotopes, fatty acids, and fecal DNA performed on the same individuals is needed to determine which method, or combination of methods, provides the best representation of diet for bearded and ringed seals in Alaska. With the exception of prey DNA analysis, the dietary methods described above have been used previously to determine the diet of both bearded and ringed seals in Alaska (e.g., Lowry 1980*a*, *b*; Dehn et al. 2007; Cooper et al. 2009; Quakenbush et al. 2011 *a, b*). However, a direct comparison of these dietary techniques using the same individual ice seals has never been conducted. Therefore, the goal of this thesis is to use combinations of dietary methods (stomach

contents, stable isotopes, fatty acids, and fecal DNA) to compare and contrast the resulting dietary information. Although there are numerous published studies of stomach contents, stable isotopes, fatty acids, or fecal DNA of pinnipeds (e.g., Lowry et al. 1980*a*, *b*; Budge et al. 2007; Dehn et al. 2007; Cooper et al. 2009; Bowles and Trites 2013; Carroll et al. 2013), it is not well understood how the dietary information relates among the methods. Also, no ice seal diet studies have been completed using fecal DNA. Therefore, understanding how the dietary information gained from fecal DNA relates to other diet methods is useful. Chapter two of this thesis ("Identifying bearded seal diet – a comparison of individual seals using stomach contents, stable isotopes, and fatty acids") will compare the dietary information gained from stomach contents, stable isotopes, and fatty acids for bearded seals. Chapter two also includes a direct comparison between the proportions of prey source groups identified in stomach contents and a stable isotope mixing model. Chapter three ("Fish prey in bearded and ringed seal diet – a comparison of stomach contents and fecal DNA") will examine and compare fish taxa identified using stomach contents and fecal DNA of bearded and ringed seals. Understanding how the dietary information from these different methods relate will aid in the detection and interpretation of potential prey changes in bearded and ringed seals as arctic habitats change.

Chapter 2: Identifying bearded seal diet – a comparison of individual seals using stomach contents, stable isotopes, and fatty acids[1]

2.1 Abstract

Stomach contents, stable isotopes, and fatty acids are commonly used to infer the diet of marine mammals. However, how complimentary or contradictory these methods are, especially when considering individual diet variability, remains poorly understood. This study assesses the differences in the dietary information resulting from each of these methods for 76 adult bearded seals (*Erignathus barbatus*) harvested in Alaska for subsistence use. Stomach contents provided information on prey items recently consumed and can generally be interpreted without accounting for complex physiological and biochemical factors and interactions. Bearded seals are generalists, and we identified at least 60 prey taxa in their stomachs; benthic prey predominated with sculpins (Cottidae) occurring most often at 66% frequency of occurrence (FO), followed by shrimp at 64% FO, crab at 63% FO, and cod (Gadidae) at 55% FO. Stable carbon and nitrogen isotope analysis of muscle and fatty acid analysis of blubber provided information on prey consumed and integrated over a longer time frame, but taxonomic resolution of prey was low. We applied a stable isotope mixing model (SIAR) and compared the mean proportion of prey source groups with the relative occurrence (RO) of prey from stomach contents. Despite differences in dietary time frames, the RO of prey from stomach contents and the mean proportions of prey source groups from the stable isotope mixing model were similar. This did not hold true for octopus which occurred at a higher proportion using estimates from stable isotopes (13%) compared with the RO of stomach contents (3%). The proportions of indicator fatty acids, from full-thickness blubber, such as 16:4n-1, 20:5n-3, 20:4n-6, 20:1n-9,

[1]Bryan A. L., Quakenbush L., and Horstmann-Dehn L. 2014. Identifying bearded seal diet ─ a comparison of individual seals using stomach contents, stable isotopes, and fatty acids. Prepared for submission to Ecological Applications.

22:1n-11, and the presence of non-methylene-interrupted fatty acids were similar to other fatty acid signature studies of bearded seals in Alaska, and suggest a benthic diet. Overall, the three methods yielded different, but not necessarily contradictory results. However, fatty acid prey libraries for the Alaskan Arctic do not exist, precluding any specific prey identification using this method. Until a fatty acid prey library is available or more specific stable isotope mixing models are developed, analysis of stomach contents provides the most detailed description of bearded seal diet in Alaska.

2.2 Introduction

Arctic marine habitats are changing, and the extent and thickness of sea ice is decreasing in the Arctic (Beitler 2012, Stroeve et al. 2012), although these changes are not uniform for every location. As sea ice changes occur, there is increased concern for the marine species living in the Arctic (Kovacs et al. 2011). Bearded seals (*Erignathus barbatus*) are one species of ice-associated seal who use sea ice for pupping, molting, and as a resting platform (Burns 1981). Changes in sea ice coverage affect primary production (Bluhm and Gradinger 2008), thus, seals will likely experience prey density and prey distribution shifts as part of a bottom-up trophic cascade (Grebmeier et al. 2006, Bluhm and Gradinger 2008, Kovacs and Lydersen 2008). Further, as sea ice retreats, increasing light irradiance could decrease the nutritional quality of prey by decreasing the proportion of polyunsaturated fatty acids (e.g., n-3 and n-6 essential fatty acids) of primary producers (Leu et al. 2010, Wang et al. 2014), or the compostion of primary producers could change (Kelly and Scheibling 2012). These changes could then propagate up the food chain. The observed changes in sea ice habitat have prompted concerns for the long-term survival of bearded seals, and consequently, the Pacific population of bearded seals has

been listed as threatened under the Endangered Species Act in 2012 (U.S. Federal Register 2012).

Diet studies are important for understanding population health, reproduction, and response to ecosystem change. Yet, diet is not always easy to quantify, especially for most marine mammal populations, because foraging cannot usually be observed (Pierce and Boyle 1991). Therefore, dietary studies often rely on indirect measures, such as analysis of stomach contents, stable isotopes, and fatty acids, all of which have been used to estimate bearded seal diet in Alaska (e.g., Johnson et al. 1966; Lowry et al. 1980; 1981*a*, *b*; Dehn et al. 2007; Cooper et al. 2009; Quakenbush et al. 2011). Although each of these methods provides important dietary information, each has strengths and limitations and none of the methods provide an accurate representation of diet.

Stomach content analysis involves sorting and identifying prey remains found in the stomach of the predator. A limitation of stomach contents is the variable digestion and differing retention times of prey (Pierce and Boyle 1991, Bowen and Iverson 2013). For example, soft bodied prey (e.g., mollusks and marine worms) (Sheffield et al. 2001) and fishes with fragile otoliths (e.g., salmon, *Oncorhynchus* spp.) can be underrepresented in stomachs (or go undetected), whereas cephalopods (due to presence of digestion resistant beaks) and fishes with large otoliths (e.g., saffron cod, *Eleginus glacialis*) may be overrepresented (Pierce and Boyle 1991, Bowen 2000). Additionally, stomach content analysis renders no dietary information if prey are digested completely, lack hard parts (e.g., tunicates and marine worms), or the stomach is empty (Pierce and Boyle 1991, Bowen 2000). Lastly, collection of stomach contents is at a minimum invasive, and is generally performed *post mortem*.

Stable isotope methods involve analyzing the ratio of heavy to light stable isotopes (e.g., carbon, $^{13}C/^{12}C$ and nitrogen, $^{15}N/^{14}N$) in tissues of the animal (Tollit et al. 2010). Stable isotopes do not provide the taxonomic resolution achieved with stomach contents, but give information on nutrients assimilated from the diet, not just ingested prey (Post 2002, Bowen and Iverson 2013). Stable isotopes also integrate dietary information over longer time periods, representing feeding over days to months or even years depending on the turnover rate of the tissue analyzed (DeNiro and Epstein 1978, Tieszen et al. 1983, Tollit et al. 2010). Stable carbon isotope ratios can identify carbon source and habitat where the animal was foraging (e.g., benthic vs. pelagic, onshore vs. offshore), whereas stable nitrogen isotope ratios represent the average trophic level at which the animal was foraging (Peterson and Fry 1987, Hobson and Welch 1992, Kelly 2000, Newsome et al. 2010).

An understanding of regional isoscapes (Hobson 1999), and a pre-existing knowledge of commonly consumed prey items is generally needed to derive predator diet information from stable isotope data (Post 2002). Stable isotope mixing models have been developed to estimate proportions of prey items in the diet (Phillips and Gregg 2003, Moore and Semmens 2008, Parnell et al. 2010). These models combine the stable isotope values of the predator and prey sources, while accounting for trophic enrichment (i.e., difference in stable isotope ratios between predator and prey), to give proportional information about the prey consumed (Phillips 2012). However, due to model constraints, application of mixing models is limited by the number of prey categories that can be considered (Phillips and Gregg 2003, Phillips et al. 2005), and they are therefore less useful when approximating the diet of generalists known to consume a wide variety of prey (Bowen and Iverson 2013).

Fatty acid analysis uses the proportion of different fatty acids (i.e., fatty acid signature) in the tissue (blubber is often used for marine mammals) of a predator to make inferences about its diet or foraging ecology (Budge et al. 2006, Iverson 2009). Long chain fatty acids are generally taken up directly from the diet without modification, and are stored in fat deposits, allowing them to be traced back to dietary sources (Iverson 2009). Some unique fatty acid biomarkers of specific prey types remain unaltered and can be tracked through the food chain; for example, non-methylene-interrupted (NMI) fatty acids are created through *de novo* synthesis by mollusks (Budge et al. 2007, Thiemann et al. 2007), and 16:4n-1 is synthesized by diatoms (Budge et al. 2008). Although fatty acids that are accumulated in the blubber of pinnipeds represent diet assimilated over weeks to months (Budge et al. 2006), some fatty acids may be mobilized from blubber stores during fasting or lactation (Wheatley et al. 2007, 2008, Iverson 2009) altering the fatty acid proportions in blubber.

Fatty acids are not uniformly distributed throughout the blubber layer; therefore, it is important to sample the full thickness of blubber (Budge et al. 2006, Strandberg et al. 2008), which can be difficult to do with minimal invasive methods. Sampling location of blubber does not appear to influence estimates of fatty acid composition in pinnipeds, as long as the blubber is sampled from the main trunk (Lambert et al. 2013). Fatty acids can be used qualitatively to distinguish spatial or temporal variations in the diet among species or groups of animals (e.g., Budge et al. 2007, Cooper et al. 2009) or quantitatively (i.e., quantitative fatty acid analysis, QFASA) using a mixing model to estimate the proportional contribution of prey to the diet of the predator (e.g., Iverson et al. 2004, Meynier et al. 2010). The QFASA model requires the fatty acid profile of the predator, the fatty acid profiles of common prey items (generally from pre-existing knowledge of stomach contents), and a calibration coefficient that accounts for the

predator-specific metabolism (Iverson et al. 2004). At this time, neither a calibration coefficient nor a fatty acid prey library exists for bearded seals in Alaska.

Bearded seals are benthic generalists, known to eat a variety of invertebrates and fishes (Lowry et al. 1980, Antonelis et al. 1994, Dehn et al. 2007, Quakenbush et al. 2011). Recent studies using stable isotope ratios in claws (Carroll et al. 2013) and whiskers (Hindell et al. 2012) of bearded seals indicate that there is also high individual variability in foraging patterns. As many as 113 common (> 1% frequency of occurrence) prey taxa have been identified from stomachs of bearded seals harvested in Alaska (Quakenbush et al. 2011). These authors also found changes in bearded seal diet over time, with more fishes being consumed in the 2000s compared with the 1960s or 1970s. Quakenbush et al. (2011) specifically noted that in the 2000s, bearded seals had increased consumption of sculpins and consumed fewer crustaceans. Consequently, changes in bearded seal diet, and the ability to compare data from various studies applying different methods of dietary analysis remain important issues.

The complex nature of analyzing and interpreting bearded seal feeding ecology raises many questions, in particular, how the dietary information gained from numerous published studies of stomach contents, stable isotopes, and fatty acids relate to each other. The objective of this study was to apply all three of these methods to a group of individual adult bearded seals harvested in Alaska and compare the resulting dietary information.

2.3 Methods

2.3.1 Field Collections

Tissues from 76 (27 males and 49 females) adult bearded seals, harvested during legal subsistence hunts near Little Diomede Island, Point Hope, and Barrow, Alaska were collected

from 2004 to 2010 (Table 2.1). All seals were collected in the late spring/early summer (May-July). Samples were obtained as part of the ice seal biomonitoring program by the Alaska Department of Fish and Game (ADF&G) and the North Slope Borough Department of Wildlife Management. Out of all available seals, animals were selected if the teeth, stomach, muscle, and blubber were available. Tissues were removed from the seal, placed in plastic bags within 24 hours of death, and stored frozen at -20ºC until processed. Stomachs were removed without tying the ends and placed directly into plastic bags. Muscle and full-thickness blubber samples were collected by ADF&G personnel or the hunters from unknown locations on the trunk of the body. Seals were considered to be adults if they had an estimated tooth age of at least 5 years (Cameron et al. 2010, Quakenbush et al. 2011). Ages were estimated by counting cementum growth layers of sectioned teeth at Matson's Laboratory, Montana; one growth layer group was assumed per year (Mansfield and Fisher 1960, Benjaminsen 1973, Stewart et al. 1996).

2.3.2 Stomach Content Analysis

Stomachs were thawed in the laboratory at ADF&G, and the contents were gently rinsed in freshwater through 1.0 mm and 0.5 mm sieves. Prey items were sorted and identified to the lowest possible taxonomic level using standard reference keys (see Dehn et al. 2007, Quakenbush et al. 2011). Fish otoliths and cephalopod beaks were identified by W. Walker, Vashon, Washington. The remaining invertebrates were identified by C. Stark at the School of Fisheries and Ocean Sciences, University of Alaska Fairbanks (UAF), and by ADF&G staff. Stomach contents from 42 of the seals used in this study were also included in Quakenbush et al. (2011).

2.3.3 Stable Isotope Analysis

Approximately 1–2 g of seal muscle was freeze-dried (VirTis Sentry) for a minimum of 48 hrs and homogenized into a fine powder at the UAF Marine Mammal Laboratory using mortar and pestle. A subsample of 0.2 – 0.4 mg (dry weight) of ground muscle was weighed into a tin capsule using a mico-balance (Sartorius Model MP2) (Dehn et al. 2007). The stable isotope values were determined using a Thermo Scientific Delta V Plus Isotope Ratio Mass Spectrometer (IRMS) coupled to a Costech Elemental Analyzer (ESC 4010) at the Alaska Stable Isotope Facility at UAF. The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios are expressed as delta (δ) notation in parts per thousand (‰).

$$\delta R‰ = (R_{sample}/R_{standard})\text{-}1) \times 1{,}000$$

where δR represents the difference between stable isotope ratios of the sample and the standard. Standards were Vienna Pee Dee Belemnite (VPDB) and atmospheric N_2 for carbon and nitrogen, respectively. Peptone (n = 32) was used as an internal laboratory standard; the laboratory precision error (standard deviation) was 0.1 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. Most of the muscle stable isotope data were generated as part of this study, but non-lipid-extracted $\delta^{13}C$ and $\delta^{15}N$ values for 35 seals were provided by Carroll (2012).

Although bearded seal lumbar muscle is generally lean (Hoekstra et al. 2002), several of our samples had high C:N ratios (>3.5) indicating that lipids were present (Post et al. 2007). Lipids can alter stable carbon isotope ratios making the tissue appear depleted in ^{13}C (DeNiro and Epstein 1978, Post et al. 2007). On the other hand, lipid-extraction can affect the $\delta^{15}N$ value of muscle samples (Søreide et al. 2006). Therefore, lipid-extracted $\delta^{13}C$ and non-lipid-extracted $\delta^{15}N$ values were used in all analyses. Lipids were extracted from muscle using a modified Bligh and Dyer (1959) approach. Dried, homogenized samples were immersed in a 2:1

chloroform/methanol mixture and tissues were agitated for five minutes followed by a five-minute centrifugation. The supernatant was then discarded. This process was repeated (approximately 3 to 5 times) until the supernatant was visually colorless. Then lipid-extracted samples were freeze-dried again for a minimum of 12 hrs.

Stable isotope values for prey were mostly compiled from the literature (Dehn et al. 2007, Iken et al. 2010, Carroll 2012). Thus, stable isotope values were not available for all prey items consumed by bearded seals, but major taxonomic groups were represented. All prey used in this study were collected in the Bering, Chukchi, and Beaufort seas between 2000 and 2009 (Table 2.2). Prey for which stable isotope values were selected had a frequency of occurrence (FO) greater than 10% as determined from the stomach contents of seals in this study. Although sponges (Porifera) occur with a FO greater than 10%, we did not consider them to be a diet item because it is unknown if they are ingested incidentally when foraging. In addition, Porifera have a high ash content and low caloric density (Wacasey and Atkinson 1987) making them an unlikely choice as a valuable food item. Stable carbon and nitrogen isotope ratios of 16 prey taxa were used; echiurid (Echiuridae), bivalve (*Serripes groenlandicus*), unknown octopus spp. (Octopoda), Arctic (*Boreogadus saida)* and saffron (*Eleginus glacialis*) cod, Pacific sand lance (*Ammodytes hexapterus*), polychaete (Polynoidae), crab (*Telmessus cheiragonus, Hyas coarctatus, and Chionoecetes opilio),* gastropod (*Buccinum* spp.), shrimp (*Argis lar*), slender eelblenny (*Lumpenus fabricii*), sculpin (*Gymnocanthus tricuspis* and *Myoxocephalus scorpius*), and yellowfin sole (*Limanda aspera*) (Table 2.2). Lipid and non-lipid-extracted stable isotope values from two prey taxa, yellowfin sole (n = 2) and unknown octopus spp. (n = 3), were generated as part of this study. Muscle was subsampled from yellowfin sole and tentacles from octopus, the rest of the analytical procedures were the same as described above for seals.

2.3.4 Fatty Acid Analysis

A 500-mg section of full-thickness blubber was sub-sampled on a glass-covered surface, and any outer blubber surface was removed. Lipids were extracted from all samples using 2:1 chloroform/methanol (Folch et al. 1957, Parrish 1999). Lipids were then transesterified to fatty acid methyl esters (FAME) with Hilditch reagent (Budge et al. 2006). FAMEs were quantified using temperature-programmed gas chromatography (GC) on a Perkin Elmer Autosystem II Capillary FID /GC fitted with a 30 m x 0.25 mm internal diameter column coated with 50% cyanopropyl- methylpolysiloxane (DB-23) and linked to a computerized integration system (Varian Star software). Conditions for GC analysis of predator tissues were adapted from Budge et al. (2006). Routinely, 74 FAME were identified using authentic standards from NuCheck Prep and by GC-mass spectrometry. FAME extraction and analysis were completed by S. Budge at Dalhousie University. Shorthand nomenclature of A:Bn-X was used to describe each FAME, where A represents the number of carbon atoms, B characterizes the number of double bonds, n signifies the terminal methyl group, and X is the position of the double bond closest to the terminal methyl group. Fatty acid data from 62 of the 76 seals used in this study are also reported by Wang (2014). With the exception of NMI, fatty acids that occurred with <0.1% total proportion were not included in the analysis. NMI fatty acids were retained in the analysis because they originate directly from mollusks (Budge et al. 2008, Barnathan 2009), which are an important prey for bearded seals (Lowry et al. 1980, Dehn et al. 2007, Quakenbush et al. 2011).

2.3.5 Statistical Analysis

Percent frequency of occurrence (% FO) was calculated for stomach contents as the number of stomachs containing a particular fish or invertebrate taxon divided by the total number of stomachs that contained prey (x100). All statistical tests were run in R (version 2.14.2, R Development Core Team 2012), with $\alpha < 0.05$ considered significant. The stable isotope data were normally distributed and no transformations were necessary. Relative proportions of selected fatty acids were standardized to 100% and log-transformed using a log(1+X) transformation (Wang et al. 2014). We tested how stable isotope ratios and fatty acid proportions differed by sex, harvest location, harvest month, and time-period. Time-period was included to determine if there was a shift in diet after the 2007 September Arctic sea ice minimum (Beitler 2012). Because our samples were collected in spring/summer, they were separated into pre- and post-2008 (after the 2007 sea ice minimum) categories. Differences in stable carbon and nitrogen isotope ratios were each analyzed using one-way ANOVAs. Differences in fatty acid signatures were explored using the PERMANOVA function ADONIS in the R package vegan (Oksanen et al. 2013) on Bray-Curtis dissimilarity matrices.

Several confounding factors related to sampling complicated the analysis of our fatty acid and stable isotope data sets. In particular, sex ratios of samples were not equivalent across time periods, and different harvest locations were sampled at different times, making it difficult to tease apart time, location and sex effects. For example, more of the samples collected post-2008 were female, all samples collected in May were from Little Diomede Island, and almost all samples collected in July were from Barrow. The ADONIS tests for the fatty acid signatures did not significantly differ by sex, time period, or month, but harvest location was significant (R^2=0.08, $p < 0.05$). $\delta^{15}N$ values differed between males and females, and between time periods. For $\delta^{13}C$, significant differences were found for harvest location and month. However, we do

not believe that any differences by sex or harvest location are biologically meaningful. Bearded seals undergo extensive and large-scale migrations encompassing the Bering, Chukchi, and Beaufort seas (Frost et al. 2008, Cameron and Boveng 2009), and have muscle turnover rates of up to 2 years (Seymour et al. 2014); location of harvest is thus not likely to reflect location of foraging. Moreover, earlier studies using stomach content, stable isotope, or fatty acid analyses have found no differences in diet between male and female bearded seals (Budge et al. 2007, Dehn et al. 2007, Cooper et al. 2009, Carroll 2012). Thus, we pooled our stable isotope data and evaluated variability among individuals across the entire sample set. The lipid-extracted $\delta^{13}C$ and the non-lipid-extracted $\delta^{15}N$ values were used to generate seal cluster groups that occupied similar stable isotopic space, using k-means clustering. Fatty acid data were then visualized according to these stable isotope-based cluster groups using a non-metric multidimensional scaling (MDS) plot. A Mahalanobis distance test was performed on the first and second MDS dimensions to detect outliers (Filzmoser and Gschwandtner 2014).

2.3.6 Stable Isotope Mixing Model

Proportions of prey in seal diet were estimated using a stable isotope mixing model, stable isotope analysis in R (SIAR) version 4.2 (Parnell et al. 2010). SIAR is a Bayesian mixing model that incorporates the stable isotope values of both predator and prey, as well as a tissue-specific trophic enrichment factor. This generates probabilities for the prey input to the diet of the predator. The SIAR model allows for a greater number of prey sources compared with older models (Parnell et al. 2010), which were restricted to one more source than the number of stable isotopes used (Phillips and Gregg 2003). However, stable isotope mixing models have difficulties assigning proportions in the diet when prey items overlap in stable isotopic space

(Phillips et al. 2005), such that the predicative power of the model decreases as the number of sources increases (Parnell et al. 2010). Therefore, instead of using stable carbon and nitrogen isotope ratios for individual prey, prey were clustered into source groups using k-means clustering. All fishes were lipid-extracted, but most invertebrates were not. However, Iken et al. (2010) found that lipid-extraction had little or no effect on stable isotope ratios of benthic invertebrates in this region. Clustering resulted in seven prey groups that explained 95.9% of the variability. Echiurid, bivalve, octopus, and flounder fell out into groups by themselves (i.e., were isolated in stable isotopic space from other prey; Figure 2.1). All three crab species (*Telmessus cheiragonus, Hyas coarctatus, and Chionoecetes opilio)* clustered together into a group labeled "crab"; Table 2.2). The remainder of the prey species separated into two groups; "semidemersal" (Arctic and saffron cod, Pacific sand lance, and polychaete), and "epibenthic" (gastropod, shrimp, slender eelblenny, and both sculpin species; Table 2.2). Mean $\delta^{13}C$ and $\delta^{15}N$ values for the prey cluster groups and their standard deviations were used in the stable isotope mixing model. Standard deviations were generated for the prey source groups using a pooled variance (Ward et al. 2011).

We used trophic enrichment factors from harp seal (*Pagophilus groenlandicus*) muscle, $1.3 \pm 0.4‰$ for $\delta^{13}C$ and $2.4 \pm 0.4‰$ for $\delta^{15}N$ (Hobson et al. 1996, Carroll 2012) as the closest available relative to bearded seals. Lipid-extracted $\delta^{13}C$ and non-lipid-extracted $\delta^{15}N$ values were used in the model for all seals and prey when available. We did not include priors or concentration dependences in our model. The model was run for 500,000 iterations with a burn-in of 50,000. The model was run once with all bearded seals pooled into one group, and again with the seals separated into the stable isotope-based seal cluster groups (described above) to

identify more specific individual foraging patterns. Proportions of prey source groups in seal diet are reported as means with 95% credibility intervals.

2.3.7 Method Comparisons

To compare prey identified from the stomach contents of bearded seals with prey source groups used in the stable isotope mixing model, taxa identified in the stomachs were combined into the same prey source groups, and the percent relative occurrence (RO) of prey source groups was calculated. Percent RO was calculated as the number of stomachs that contain a prey source group divided by the cumulative number of prey source groups identified in all stomachs (x100 ± SE). To be consistent with stable isotope mixing model parameters, prey that occurred at < 10% FO in stomachs were excluded from the comparison (as well as Porifera, for reasons described above). Mean ROs were also calculated for each stable isotope-based seal cluster group. The percent RO for each prey source group in stomachs was compared to the mean proportion of that prey source group indicated by stable isotope mixing model. Lastly, we evaluated whether fatty acid composition of blubber differed among seal cluster groups, using the PERMANOVA function ADONIS in the R package vegan.

2.4 Results

2.4.1 Stomach Content Analysis

Nine bearded seal stomachs (12%) were empty and did not contain prey. The remaining 67 (88%) stomachs contained a minimum of 60 taxa (24 fish and 36 invertebrate taxa); the number of taxa per stomach ranged from 1 to 28. Sculpins occurred most often with a 66% FO, followed by shrimp at 64% FO, crabs at 63% FO, gadids at 55% FO, echiurids at 46% FO,

flounder at 45% FO, bivalves at 39% FO, and Pacific sand lance and eelblenny both at 25% FO (Table 2.3 and 2.4). Other prey with a FO of at least 10% included sponges (19%), gastropods (15%), cephalopods at 12% (octopus was the only cephalopod identified at 10% FO), and polychaetes at 10%. When stomach contents were restricted to the same prey source groups used in the stable isotope mixing model, the RO of prey source groups for all seals combined were as follows (arranged by increasing trophic level): echiurids at 14%, bivalves 11%, semidemersal 19%, octopus 3%, crab 18%, epibenthic 22%, and flounder at 13% (Figure 2.2).

2.4.2 Stable Isotope Analysis

The mean stable carbon isotope value of bearded seal muscle was -17.63 ± 0.90 (SD), and the mean stable nitrogen isotope value was 16.41 ± 0.92 (Table 2.5 and Figure 2.2). Using the k-means clustering, bearded seals were clustered into seven groups which accounted for 81.4% of the variability. All groups included samples from both pre- and post-2008, of both sexes, and seals from at least two harvest locations and collection months (Table 2.5 and Figure 2.1). For these groups, the $\delta^{13}C$ values ranged from -18.93 ± 0.17 to -15.38 ± 0.49, and the $\delta^{15}N$ ranged from 15.46 ± 0.67 to 18.00 ± 0.80.

The stable isotope mixing model (SIAR) indicated the overall mean proportions of prey source groups (with 95% credibility intervals) were as follows: echiurids 10% (0 to 22%), bivalves 11% (0 to 21%), semidemersal 15% (0 to 30%), octopus 13% (2 to 24%), crab 17% (0 to 32%), epibenthic 17% (1 to 31%), and flounder 18% (1 to 33%; Figure 2.2). Seals cluster groups 1 and 4 fell outside the polygon of stable isotope space represented by these prey source groups, indicating that either these prey are not consumed by these seals, or that the prey were collected in a region outside the seal foraging area in a location with a different stable isotope

signature. In addition, fecal DNA data for one of the seals in seal cluster group 1 indicated that it was a ringed seal (*Pusa hispida*), not a bearded seal (A. Bryan, unpublished data). This suggests that some tissues for this animal may have been mixed up post-collection. Fecal DNA from one other seal also indicated that it was a ringed seal, this seal was in cluster group 2 with a number of other seals, and therefore, it is unlikely that the muscle sample from this second seal was mixed up. The proportion of echiurids was highest for seal cluster group 5 at 20% (4 to 34%). The proportion of bivalves was highest in seal cluster group 3 at 34% (18 to 49%). The proportion of the "semidemersal" prey source group was highest in seal cluster groups 1, 2, 4, and 7, all at 14%. The proportion of octopus was the highest in seal cluster group 5 at 46% (34 to 58%). The proportion of crab was highest in seal cluster groups 4 and 7, both at 17%. The proportion of epibenthic prey source group was highest in seal cluster group 6 at 35% (15 to 57%). Finally, the proportion of flounder was highest in seal cluster group 2 at 20% (2% to 37% 2.2).

The proportion of the prey source groups differed when all seals were combined into a single model. When all seals were combined into one group, the stable isotope mixing model generated proportions of prey items for an "average" bearded seal, but some of the individual variability was lost. For example, the mean proportional contribution of bivalves to all seals was 11% (0 to 21%), but the seal cluster groups indicated that some seals were eating as much as 34% (18 to 49%) bivalves, while others were only eating 4% (0 to 10%) bivalves (Figure 2.2).

The RO of prey source groups in stomach contents were mostly similar to the proportions predicted by the stable isotope mixing model (Figure 2.2). Octopus was a notable exception; stable isotope data suggested that octopus makes up about 13% of the average seal's diet (2 to 24%), with some seals eating as much as 46% (34 to 58%) octopus. On the other hand, stomach

contents indicated that the highest RO for octopus occurred in seal cluster group 1 with 10%. For this same seal cluster group, the stable isotope mixing model indicated that 20% (0 to 37%) octopus was consumed.

2.4.3 Fatty Acid Analysis

Forty-seven fatty acids were identified in proportions greater than 0.1%. Six NMI fatty acids (proportions 0.04 – 0.43%) were also included in the analysis (as described above) for a total of 53 fatty acids used. The relative proportion of fatty acids for all seals combined are summarized in Appendix A. Fatty acid signatures of the seal cluster groups were significantly different ($R^2 = 0.04$, $p = 0.01$); specifically, seal cluster group 1 was different than seal cluster groups 6 and 7, and seal cluster group 2 was different than seal cluster group 7. However, visual inspection of the MDS plot indicated that there were some outliers (Figure 2.3). A Mahalanobis distance test confirmed that there were six outliers. Once these outliers were dropped, the seal cluster groups were no longer significantly different ($R^2 = 0.01$, $p = 0.44$). In addition, fecal DNA data for two of the six outliers indicate they were ringed seals, not bearded seals (A. Bryan, unpublished data), suggesting that some tissues may have been mixed up post-collection. Nothing unusual was detected in the stomachs of these six seals, meaning they contained prey commonly described for bearded seals. However, one of these outlier seal stomachs was empty. The outlier seals fell into three different seal cluster groups based on stable isotopes. Two of the seals were in seal cluster group 1, which was outside the prey polygon. The two seals identified as ringed seals from fecal DNA were in different seal cluster groups, indicating that at least muscle tissue from these seals was not mixed up. Some of the tissues from these two animals may have been mislabeled in the field or lab post-collection.

2.5 Discussion

We compared stomach contents, stable isotopes, and fatty acids for a group of adult bearded seals. All methods provided dietary information, but the extent and overlap varied. Stomach contents provided dietary information of about 60 prey taxa consumed, and the frequency with which these prey occurred. Stable isotope mixing models provided information on proportions of prey source groups to bearded seal diet over a longer time period; however, the taxonomic detail was lower compared with stomach contents, because the prey were grouped into seven groups of isotopically similar sources. Stable isotope data was useful for assessing individual variability of bearded seal diet; seals were clustered into groups that appeared to differ in their foraging behavior, with each group differing from the "average" bearded seal. Using fatty acids, we were not able to calculate prey proportions, because a fatty acid prey library does not exist for the Bering, Chukchi, and Beaufort seas. However, we did find proportions of fatty acids in the blubber of bearded seals that are similar to those reported in previous studies; they appear to be bearded seal-specific and clearly differentiate this species from other ice-associated pinnipeds in Alaska (Budge et al. 2007, Cooper et al. 2009).

The time frame captured by different dietary analyses (i.e., stomach contents, stable isotopes, fatty acids) varies substantially making direct comparisons difficult. Stomach contents represent relatively recent feeding events, but how much of the ingested diet is assimilated into the body tissue can vary by prey type (Lawson et al. 1997, Trumble et al. 2003). Moreover, stomach contents do not always represent recent meals; hard parts, such as otoliths and cephalopod beaks, can accumulate in the stomach over several feeding events (Pitcher 1980, Jobling and Breiby 1986), whereas soft-bodied prey, such as marine worms, are quickly digested

(one to five hours; Sheffield et al. 2001). In contrast, muscle stable isotopes and blubber fatty acids reflect prey that has been integrated into the body tissues over weeks to months (Bowen and Iverson 2013), although the exact tissue turnover times are unknown for bearded seals. Young and Ferguson (2013) estimated that it takes about one month to show a noticeable change in the stable carbon and nitrogen isotopes values in muscle of fasting ringed seals. Fasting is a metabolically taxing time when stored resources are being reallocated, so this change in isotope values might be much easier and quicker to detect than more subtle changes in isotopically similar prey. Seymour et al. (2014) used a mathematical equation based on published turnover rates in muscle of terrestrial mammals, and the relationship among body mass, metabolic rate, and tissue turnover rate to estimate the turnover of Pacific walrus (*Odobenus rosmarus divergens*) muscle at approximately two years. Fatty acids in blubber of pinnipeds are thought to represent diet integrated into the body over weeks to months (Budge et al. 2006). For harbor seal (*Phoca vitulina*) pups, Nordstrom et al. (2008) found that blubber fatty acid turnover occurred in about 1.5 to 3 months, but the turnover may not be linear and different prey fatty acids may turnover at different rates, making prey changes difficult to detect over short periods of time (i.e., months).

Stomach content analysis provided detailed information about prey species consumed by bearded seals and the relative proportion of those species in their diet. The prey consumed by these seals is consistent with prey found in other, larger studies of bearded seal stomach contents in Alaska (Lowry et al. 1980, Dehn et al. 2007, Quakenbush et al. 2011). Although overall stomach contents provided more dietary information compared with stable isotopes and fatty acids, 12% of the stomachs were empty and provided no dietary information. All seals in this study were apparently healthy when they were harvested for subsistence purposes. They can

therefore be presumed to have been foraging "normally" as compared to stranded marine mammals. Despite known biases, stomach content analysis is valuable, because it provides taxonomic resolution for prey items, often to the species level, which is rarely possible when using stable isotopes or fatty acids (Pierce and Boyle 1991, Bowen 2000, Bowen and Iverson 2013). Stomach content analysis is also advantageous because it provides baseline dietary information used when interpreting stable isotope or fatty acid data. Without this baseline dietary information, stable isotopes, for example, will provide only relative trophic information.

The stable carbon and nitrogen isotope ratios of bearded seal muscle encompassed a wide range of values (-19.31 to -14.98 $\delta^{13}C$ and 14.02 to 18.76 $\delta^{15}N$), similar to other studies of bearded seals in Alaska (Dehn et al. 2007, Carroll 2012, Carroll et al. 2013), including large individual variations in stable isotope ratios (Carroll et al. 2013). This range is the result of bearded seals feeding on both benthic and pelagic prey (Dehn et al. 2007), as confirmed by stomach contents data in this study. In addition, bearded seals move seasonally among the Bering, Chukchi, and Beaufort seas (Frost et al. 2008, Cameron and Boveng 2009), and each location has distinct stable isotope signatures (Schell et al. 1998, Dehn et al. 2007). The wide span in $\delta^{15}N$ is due to feeding at multiple trophic levels, as confirmed by a minimum of 60 prey taxa identified in bearded seal stomachs in this study, ranging from deposit and filter-feeding mollusks to fishes (Table 2.3 and 2.4).

Adding to the observed variability in bearded seal stable isotope ratios, there are also isotopic differences starting at the base of the food chain throughout Alaskan waters (Iken et al. 2010). Particulate organic matter from the Alaska Coastal Water (highly influenced by terrestrial fresh water) is more depleted in ^{13}C compared with Anadyr and Bering Shelf waters, and these differences propagate to higher trophic levels (Iken et al. 2010). Additionally, predators

consuming prey within the influence of Alaska Costal Water had higher stable nitrogen isotope values (average 2.5‰) than predators consuming prey within Anadyr Water (Iken et al. 2010). Stable carbon isotope values become more depleted in ^{13}C when moving northward into the Beaufort Sea (Schell et al. 1998), and they can also change seasonally depending on the contribution of ^{13}C enrichment from sea ice algae (Wang et al. 2014). Further, there may be isotopic differences for specific taxa within the water column. For example, it has been suggested that Arctic cod of similar size may forage on different prey in pelagic and benthic habitats (Rand et al. 2013). Stomach contents of Arctic cod caught in demersal trawls were dominated by sculpins, whereas cod caught in pelagic trawls were feeding mainly on euphausiids (Rand et al. 2013). These differences in prey composition would result in Arctic cod with different stable carbon and nitrogen isotope values. All of these factors make interpretation of bearded seal stable isotope ratios difficult for this region.

Stable isotope analysis indicated that individual bearded seals clustered into groups with different proportions of prey in the diet, suggesting variations in the longer-term foraging patterns of bearded seals. For all seals combined into the "average" seal group, the mean proportion of each of the seven prey source groups in seal diet ranged from 10 to 18% (Figure 2.2). However, when separated into seal cluster groups, it becomes apparent that some seals in the population are eating proportionally more of certain prey. For example, the mean proportion of bivalves for seal cluster group 3 was 34%, but only 11% for the "average" seal. This individual variability in foraging patterns, and potential preference and specialization for certain prey, has also been described in other stable isotope studies of bearded seals (Dehn et al. 2007, Hindell et al. 2012, Carroll et al. 2013).

As for many biological models, stable isotope mixing models are only as accurate as the parameter estimates provided, and the model will assign proportions to all prey groups selected, even if they were not present in the diet; in addition, missing prey sources will not be identified and apportioned (Parnell et al. 2010). Seal cluster groups 1 and 4 fell outside the polygon created by our prey source groups (Figure 2.1) indicating that the prey used in this model did not fully represent the prey consumed by the seals during the time period represented by the muscle tissue. Although we used commonly consumed prey taxa, the stable isotope ratios of the prey were mostly representative of the Chukchi Sea, not the Beaufort Sea or offshore in the Bering Sea where bearded seals may also be foraging. Variability in prey stable isotopes is high in Alaskan waters (Schell et al. 1998, Iken et al. 2010), thus, region-specific, and possibly seasonal prey libraries are needed. Incorporation of this regional variability in prey stable isotopes would likely better represent the diet of bearded seals using stable isotope mixing models. For example, using stable isotope mixing models and prey libraries from several different regions, Pomerleau et al. (2012) was able to identify the proportional contribution of prey in different regions of the Eastern Canadian Arctic to groups of bowhead whales (*Balaena mysticetus*).

Stable carbon and nitrogen isotope values also vary over time. The prey used in our model do not represent all of the years that seal muscle was collected. This could have an effect on the model fit (Witteveen et al. 2012). Mixing models are also sensitive to the metabolic correction factor (i.e., TEF) used (Bond and Diamond 2011), and species-specific correction factors do not exist for most marine mammals. We used trophic correction factors developed for harp seals (Hobson 1999), which were the most closely related taxon available. But harp seals have a somewhat smaller mass (Innes et al. 1981) than bearded seals, which affects their metabolic and tissue turnover rates (Newsome et al. 2010). If the trophic enrichment of harp seal

muscle is substantially different than bearded seal muscle, it could also have affected our mixing model results.

Fatty acid analysis provided the least detailed dietary information when compared with stomach contents and stable isotopes of bearded seals. The lack of a fatty acid prey reference library and calibration coefficients for bearded seals prevent application of mixing models, such as QFASA, to estimate proportions of prey in bearded seal diet. In controlled feeding studies, fatty acid calibration coefficients varied by phylogeny (i.e., phocids vs. otariids) as well as within a family leading to substantially erroneous diet estimates (Rosen and Tollit 2012). Without a prey reference library, fatty acid diet analysis is limited to distinguishing individuals or groups with similar diet, and actual prey items cannot be identified. Previous studies have used fatty acid data to qualitatively describe differences and resource partitioning among the five species of ice-associated pinnipeds in Alaskan waters, bearded, ringed, ribbon (*Histriophoca fasciata*), and spotted seals (*Phoca largha*) (Cooper et al. 2009), and walruses (Budge et al. 2007). Fatty acid signatures for some of these seal species varied over time (Wang 2014). Unfortunately, all of the seals in our study were adults harvested in the spring/summer; therefore, there are limitations to the qualitative comparisons that can be made. The presence of NMI fatty acids indicates that mollusks were part of the bearded seal diet (Budge et al. 2007), which is confirmed by stomach contents data in this study (Table 2.4). Monounsaturated fatty acids were found in high proportions (58.05%) of the total fatty acids, which is also consistent with other studies of bearded seals in Alaska (Budge et al. 2007, 2008). Monounsaturated fatty acids are most abundant in the outer, less metabolically active blubber layer, and are important for thermoregulation and membrane fluidity (Strandberg et al. 2008). Proportions of n-7 fatty acids and the fatty acid 20:4n-6 are common in benthic organisms (Iverson et al. 2002, Cooper et al.

2009) and were also similar to past studies (Cooper et al. 2009). Proportions of 20:1n-9 and 22:1n-11, which are generally high in pelagic fishes (Cooper et al. 2009), were also comparable to bearded seals analyzed by Cooper et al. (2009). The proportions of these indicator fatty acids in bearded seal blubber reflect comparable diet estimates indicative of a benthic generalist as described with stomach contents and stable isotopes, despite the lack of calibration coefficients and prey parameter input to run a QFASA model. However, for future studies, we recommend the development of a fatty acid prey library in the Alaskan Arctic to explore more specific information about diet from fatty acids, as well as newly emerging techniques, such as compound-specific stable isotope analysis (e.g., Budge et al. 2008).

Surprisingly similar proportions of prey source groups were detected in bearded seal diet using RO of stomach contents and stable isotope mixing models, given the substantial dietary time frame differences (hrs vs. months). This was not the case, however, for octopus, which was detected in lower proportions from stomach contents than inferred by stable isotopes. A previous study using stomach contents detected that more octopus were consumed in the Bering Sea than the Chukchi Sea (Quakenbush et al. 2011). Most of our samples (56 out of 76) were collected in the Chukchi and Beaufort seas, which could be a factor in the lower detection of octopus in the stomach contents in this study. Stable isotopes, on the other hand, represent a longer term diet, so our data may indicate higher consumption of octopus during the winter in the Bering Sea. It should be noted that the octopus used in the stable isotope mixing model was the only prey collected in the Beaufort Sea. The stable isotope mixing model may have proportioned more of the diet to octopus, because this diet item reflected the depleted carbon signature of the Beaufort Sea (Schell et al. 1998). Seal cluster groups identified with stable carbon and nitrogen isotopes did not significantly differ in fatty acid composition. This suggests variability in prey

preferences among individuals that was captured using stable isotopes, but was not detectable using fatty acid signatures. Nonetheless, the proportions of indicator fatty acids suggest a similar benthic generalist diet to that identified using the other methods.

A methods comparison study for snow crab (*Chionoecetes opilio*) in the Bering Sea compared diet information derived from stomach contents, stable isotopes, fatty acids, and prey abundance, and found that stomach contents and the stable isotope mixing model did not produce similar results, but that estimates improved when prior stomach content information was added to the model (Kolts et al. 2013). Using separation distance in principle component analysis, these authors found quantitative prey estimates for fatty acids that were similar to both stomach contents and prey abundance in the area of study. Similar to our study, Kolts et al. (2013) concluded that stomach contents provided the most detailed dietary information for a generalist consumer.

2.6 Conclusions

Stomach content analysis provided more specific information about prey taxa consumed by bearded seals than did stable isotope or fatty acid analyses. The proportions of prey source groups from the stable isotope mixing model were similar to the RO of prey source groups found in stomach contents. Without a fatty acid prey reference library, the fatty acid data were the most limited in providing dietary information for bearded seals sampled in this study. All three methods provided different but not necessarily contradictory dietary information. The usefulness of each of these methods depends on the dietary research question. If information on prey taxa is needed, then the analysis is limited to stomach contents or mixing models using stable isotope or fatty acid data. However, prior prey information from stomach contents is needed to implement

these models. Without regular access to animals via subsistence harvests, by-catch, or stranding events, stable isotope and fatty acid data are good, minimally invasive options for assessing the general trophic position and key foraging patterns and habitat of free-ranging marine mammals. Both methods are able to detect general foraging patterns and potential temporal changes; however, neither method is able to determine exactly what has changed. For future diet studies of bearded seals, we recommend the continued use of all methods.

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2.8 References

Antonelis, G. A., S. R. Melin, and Y. A. Bukhtiyarov. 1994. Early spring feeding habits of bearded seals (*Erignathus barbatus*) in the Central Bering Sea, 1981. Arctic 47:74–79.

Barnathan, G. 2009. Non-methylene-interrupted fatty acids from marine invertebrates: Occurrence, characterization and biological properties. Biochimie 91:671-678.

Beitler, J. 2012. Arctic sea ice extent settles at record seasonal minimum, National Snow and Ice Data Center. http://nsidc.org/arcticseaicenews/2012/09/arctic-sea-ice-extent-settles-at-record-seasonal-minimum/.

Benjaminsen, T. 1973. Age determination and the growth and age distribution from cementum growth layers of bearded seals at Svalbard. Fiskeridirektoratets skrifter. Ser Havunders 16:159–170.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911–917.

Bluhm, B., and R. Gradinger. 2008. Regional variability in food availability for Arctic marine mammals. Ecological Applications 18:S77–S96.

Bond, A. L., and A. W. Diamond. 2011. Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. Ecological Applications 21:1017–1023.

Bowen, W. D. 2000. Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. Canadian Journal of Fisheries and Aquatic Sciences 57:898–905.

Bowen, W. D. and S. J. Iverson. 2013. Methods of estimating marine mammal diets: A review of validation experiments and sources of bias and uncertainty. Marine Mammal Science 29: 719–754.

Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Marine Mammal Science 22:759–801.

Budge, S. M., A. M. Springer, S. J. Iverson, and G. Sheffield. 2007. Fatty acid biomarkers reveal niche separation in an Arctic benthic food web. Marine Ecology Progress Series 336:305–309.

Budge, S. M., M. J. Wooller, A. M. Springer, S. J. Iverson, C. P. McRoy, and G. J. Divoky. 2008. Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. Oecologia 157:117–129.

Burns, J. J. 1981. Bearded seal (*Erignathus barbatus*). Pages 145–170 *in* S. H. Ridgway and R. J. Harrison, editors. Handbook of marine mammals. Volume 2: Seals. Academic Press, New York, New York, USA.

Cameron, M. F., J. L. Bengtson, P. L. Boveng, J. K. Jansen, B. P. Kelly, S. P. Dahle, E. A. Logerwell, J. E. Overland, C. L. Sabine, G. T. Waring, and J. M. Wilder. 2010. Status review of the bearded seal (*Erignathus barbatus*). U.S. Dep. Commer., NOAA Tech. Memo. NMFS-AFSC-211, 246 pp.

Cameron, M. F., and P. L. Boveng. 2009. Habitat use and seasonal movements of adult and sub-adult bearded seals. Alaska Fisheries Science Center Quarterly Report, October-November-December 2009, 1–4 pp.

Carroll, S. S. 2012. Insight into the diet history of ice seals using isotopic signatures of muscle tissue and claws. M.S. thesis, University of Alaska Fairbanks, Fairbanks, AK.

Carroll, S. S., L. Horstmann-Dehn, and B. L. Norcross. 2013. Diet history of ice seals using stable isotope ratios in claw growth bands. Canadian Journal of Zoology 91:191–202.

Cooper, M. H., S. M. Budge, A. M. Springer, and G. Sheffield. 2009. Resource partitioning by sympatric pagophilic seals in Alaska: monitoring effects of climate variation with fatty acids. Polar Biology 32:1137–1145.

Dehn, L.-A., G. G. Sheffield, E. H. Follmann, L. K. Duffy, D. L. Thomas, and T. M. O'Hara. 2007. Feeding ecology of phocid seals and some walrus in the Alaskan and Canadian Arctic as determined by stomach contents and stable isotope analysis. Polar Biology 30:167–181.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506.

Filzmoser, P., and M. Gschwandtner. 2014. Multivariate outlier detection based on robust methods. R package version 2.0.4. http://CRAN.R-project.org/package=mvoutlier.

Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. The Journal of Biological Chemistry 226:497–509.

Frost, K. J., A. Whiting, M. F. Cameron, and M. A. Simpkins. 2008. Habitat use, seasonal movements and stock structure of bearded seals in Kotzebue Sound, Alaska. Tribal Wildlife Grants Program, Fish and Wildlife Service, Tribal Wildlife Grants Study U-4-IT. Final report from the Native Village of Kotzebue, Kotzebue, AK, for U.S. Fish and Wildlife Service, Anchorage, AK.16 pp.

Grebmeier, J. M., J. E. Overland, S. E. Moore, E. V. Farley, E. C. Carmack, L. W. Cooper, K. E. Frey, J. H. Helle, F. A. McLaughlin, and S. L. McNutt. 2006. A major ecosystem shift in the northern Bering Sea. Science 311:1461–1464.

Hindell, M. A., C. Lydersen, H. Hop, and K. M. Kovacs. 2012. Pre-partum diet of adult female bearded seals in years of contrasting ice conditions. PLoS ONE 7:e38307.

Hobson, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314–326.

Hobson, K. A., D. M. Schell, D. Renouf, and E. Noseworthy. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Canadian Journal of Fisheries and Aquatic Sciences 53:528–533.

Hobson, K. A., and H. E. Welch. 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Marine Ecology Progress Series 84:9–18.

Hoekstra, P. F., L. A. Dehn, J. C. George, K. R. Solomon, D. C. G. Muir, and T. M. O'Hara. 2002. Trophic ecology of bowhead whales (*Balaena mysticetus*) compared with that of other Arctic marine biota as interpreted from carbon-,nitrogen-, and sulfur-isotope signatures. Canadian Journal of Zoology 80:223–231.

Iken, K., B. Bluhm, and K. Dunton. 2010. Benthic food-web structure under differing water mass properties in the southern Chukchi Sea. Deep-Sea Research II 57:71–85.

Innes, S., Stewart, R. E. A. and Lavigne, D. M.1981. Growth in northwest Atlantic harp seals *Phoca groenlandica*. Journal of Zoology 194: 11–24.

Iverson, S. J. 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. Pages 281–308 *in* M. T. Arts, M. T. Brett, and M. Kainz, editors. Lipids in aquatic ecosystems. Springer, New York, New York, USA.

Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: A new method of estimating predator diets. Ecological Monographs 74:211–235.

Iverson, S. J., K. J. Frost, and S. L. C. Lang. 2002. Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability. Marine Ecology Progress Series 241:161–181.

Jobling, M., and A. Breiby. 1986. The use and abuse of fish otoliths in studies of feeding habits of marine piscivores. Sarsia 71:265–274.

Johnson, M. L., C. H. Fiscus, B. T. Ostenson, and M. L. Barbour. 1966. Marine mammals. Pages 877–924 *in*: N.J. Wilimovsky and J. N. Wolfe, editors. Environment of the Cape Thompson region, Alaska. U.S. Atomic Energy Commission, Oak Ridge, Tennessee, USA.

Kelly, J. F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78:1–27.

Kelly, J. R., and R. E. Scheibling. 2012. Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series 446:1–22.

Kolts, J. M., J. R. Lovvorn, C. A. North, J. M. Grebmeier, and L. W. Cooper. 2013. Relative value of stomach contents, stable isotopes, and fatty acids as diet indicators for a

dominant invertebrate predator (*Chionoecetes opilio*) in the northern Bering Sea. Journal of Experimental Marine Biology and Ecology 449:274–283.

Kovacs, K. M., and C. Lydersen. 2008. Climate change impacts on seals and whales in the North Atlantic Arctic and adjacent shelf seas. Science Progress 91:117–150.

Kovacs, K. M., C. Lydersen, J. E. Overland, and S. E. Moore. 2011. Impacts of changing sea-ice conditions on Arctic marine mammals. Marine Biodiversity 41:181–194.

Lambert, A., L. Meynier, L. C. Donaldson, W. D. Roe, and P. C. H. Morel. 2013. Body regional distribution and stratification of fatty acids in the blubber of New Zealand sea lions: implications for diet prediction. Journal of Comparative Physiology B 183:145–156.

Lawson, J. W., E. H. Miller, and E. Noseworthy. 1997. Variation in assimilation efficiency and digestive efficiency of captive harp seals (*Phoca groenlandica*) on different diets. Canadian Journal of Zoology 75:1285–1291.

Leu, E., J. Wiktor, J. Søreide, J. Berge, and S. Falk-Petersen. 2010. Increased irradiance reduces food quality of sea ice algae. Marine Ecology Progress Series 411:49–60.

Lowry, L. F., K. J. Frost, and J. J. Burns. 1980. Feeding of bearded seals in the Bering and Chukchi seas and trophic interaction with Pacific walruses. Arctic 33:330–342.

Lowry, L. F., K. J. Frost, and J. J. Burns. 1981*a*. Trophic relationships among ice-inhabiting phocid seals and functionally related marine mammals in the Bering Sea. U.S. Department of Commerce, NOAA, OCSEAP Environmental Assessment of the Alaskan Continental Shelf, Final Reports of Principal Investigators, Biological Studies 11:97–173.

Lowry, L. F., K. J. Frost, and J. J. Burns. 1981*b*. Trophic relationships among ice-inhabiting phocid seals and functionally related marine mammals in the Chukchi Sea. U.S. Department of Commerce, NOAA, OCSEAP Environmental Assessment of the Alaskan Continental Shelf, Final Reports of Principal Investigators, Biological Studies 11:37–95.

Mansfield, A. W., and H. D. Fisher. 1960. Age determination in the harbour seal, *Phoca vitulina* L. Nature 186:92–93.

Meynier, L., P. C. H. Morel, B. L. Chilvers, D. D. S. Mackenzie, and P. J. Duignan. 2010. Quantitative fatty acid signature analysis on New Zealand sea lions: model sensitivity and diet estimates. Journal of Mammalogy 91:1484–1495.

Moore, J. W., and B. X. Semmens. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecology Letters 11:470–480.

Newsome, S. D., M. T. Clementz, and P. L. Koch. 2010. Using stable isotope biogeochemistry to study marine mammal ecology. Marine Mammal Science 26:509–572.

Nordstrom, C. A., L. J. Wilson, S. J. Iverson, and D. J. Tollit. 2008. Evaluating quantitative fatty acid signature analysis (QFASA) using harbour seals *Phoca vitulina richardsi* in captive feeding studies. Marine Ecology Progress Series 360:245–263.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2013. vegan: Community Ecology Package. R package version 2.0-7. http://CRAN.R-project.org/package=vegan.

Parnell, A. C., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Source partitioning using stable isotopes: coping with too much variation. PLoS ONE 5:e9672.

Parrish C. C. 1999. Determination of total lipid, lipid classes and fatty acids in aquatic samples. Pages 4–12 *in* M. T. Arts, and B. C. Wainman, editors. Lipids in freshwater ecosystems. Springer, New York, New York, USA.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293–320.

Phillips, D. L. 2012. Converting isotope values to diet composition: the use of mixing models. Journal of Mammalogy 93:342–352.

Phillips, D. L., and J. W. Gregg. 2003. Source partitioning using stable isotopes: coping with too many sources. Ecosystem Ecology 136:261–269.

Phillips, D. L., S. D. Newsome, and J. W. Gregg. 2005. Combining sources in stable isotope mixing models: alternative methods. Oceologia 144:520–527.

Pierce, G. J., and P. R. Boyle. 1991. A review of methods for diet analysis in piscivorous marine mammals. Oceanography and Marine Biology 29:409–486.

Pitcher, K. W. 1980. Stomach contents and feces as indicators of harbor seal, *Phoca vitulina*, foods in the Gulf of Alaska. Fishery Bulletin 78:797–798.

Pomerleau, C., V. Lesage, S. H. Ferguson, G. Winkler, S. D. Petersen, and J. W. Higdon. 2012. Prey assemblage isotopic variability as a tool for assessing diet and the spatial distribution of bowhead whale *Balaena mysticetus* foraging in the Canadian eastern Arctic. Marine Ecology Progress Series 469:161–174.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718.

Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, and C. G. Montana. 2007. Getting to the fat of the matter: models, methods, and assumptions for dealing with lipids in stable isotope analysis. Oecologia 152:179–189.

Quakenbush, L., J. Citta, and J. Crawford. 2011. Biology of the bearded seal (*Erignathus barbatus*) in Alaska, 1961–2009. Final Report to National Marine Fisheries Service from the Alaska Department of Fish and Game. 71pp.

R Development Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org/.

Rand, K. M., A. Whitehouse, E. A. Logerwell, E. Ahgeak, R. Hibpshman, and S. Parker-Stetter. 2013. The diets of polar cod (*Boreogadus saida*) from August 2008 in the US Beaufort Sea. Polar Biology 36:907–912.

Rosen, D. A. S., and D. J. Tollit. 2012. Effects of phylogeny and prey type on fatty acid calibration coefficients in three pinniped species: implications for the QFASA dietary quantification technique. Marine Ecology Progress Series 467:263–276.

Schell, D. M., B. A. Barnett, and K. A. Vinette. 1998. Carbon and nitrogen isotope ratios in zooplankton of the Bering, Chukchi, and Beaufort seas. Marine Ecology Progress Series 162:11–13.

Seymour, J., L. Horstmann-Dehn, and M. Wooller. 2014. Inter-annual variability in the proportional contribution of higher trophic levels to the diet of Pacific walruses. Polar Biology 37:597–609.

Sheffield, G. G., F. H. Fay, M. A. Fedak, and B. P. Kelly. 2001. Laboratory digestion of prey and interpretation of walrus stomach contents. Marine Mammal Science 17:310–330.

Søreide, J. E., T. Tamelander, H. Hop, K. A. Hobson, and I. Johansen. 2006. Sample preparation effects on stable C and N isotope values: a comparison of methods in Arctic marine food web studies. Marine Ecology Progress Series 328:17–28.

Stewart, R. E. A., B. E. Stewart, I. Stirling, and E. Street. 1996. Counts of growth layer groups in cementum and dentine in ringed seals (*Phoca hispida*). Marine Mammal Science 12:383–401.

Strandberg, U., A. Kakela, C. Lydersen, K. M. Kovacs, O. Grahl-Nielsen, H. Hyvarinen, and R. Kakela. 2008. Stratification, composition, and function of marine mammal blubber: The ecology of fatty acids in marine mammals. Physiological and Biochemical Zoology 81:473–485.

Stroeve, J. C., M. C. Serreze, M. M. Holland, J. E. Kay, J. Malanik, and A. P. Barrett. 2012. The Arctic's rapidly shrinking sea ice cover: a research synthesis. Climate Change 110:1005–1027.

Thiemann, G. W., S. M. Budge, S. J. Iverson, and I. Stirling. 2007. Unusual fatty acid biomarkers reveal age-and sex-specific foraging in polar bears (*Ursus maritimus*). Canadian Journal of Zoology 85:505–517.

Tieszen, L. L., T. W. Boutton, K. G. Tesdahl, and N. A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37.

Tollit, D. J., G. J. Pierce, K. A. Hobson, W. D. Bowen, and S. Iverson. 2010. Diet. Pages 165–190 *in* I. L. Boyd, W. D. Bowen, and S. Iverson, editors. Marine mammal ecology and conservation: A handbook of techniques. Oxford University Press, Oxford, UK.

Trumble, S. J., P. S. Barboza, and M. A. Castellini. 2003. Digestive constraints on an aquatic carnivore: effects of feeding frequency and prey composition on harbor seals. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 173:501–509.

U.S. Federal Register. 2012. Endangered and threatened species; threatened status for the Beringia and Okhotsk distinct population segments of the *Erignathus barbatus nauticus* subspecies of bearded seal. Pages 76739–76768 *in* N. U.S. Department of Commerce, National Marine Fisheries Service, editor.

Wacasey, J. W., and E. G. Atkinson. 1987. Energy values of marine benthic invertebrates from the Canadian Arctic. Marine Ecology Progress Series 39:243–250.

Wang, S. W. 2014. Tracking carbon sources through an Arctic marine food web: insights from fatty acids and their carbon stable isotopes. Ph.D. dissertation, University of Alaska Fairbanks.

Wang, S. W., S. Budge, R. R. Gradinger, K. Iken, and M. Wooller. 2014. Fatty acid and stable isotope characteristics of sea ice and pelagic particulate organic matter in the Bering sea: tools for estimating sea ice algal contribution to Arctic food web production. Oecologia 174:699–712.

Ward, E. J., B. X. Semmens, D. L. Phillips, J. W. Moore, and N. Bouwes. 2011. A quantitative approach to combine sources in stable isotope mixing models. Ecosphere 44: 4645–4650.

Wheatley, K. E., P. D. Nichols, M. A. Hindell, R. G. Harcourt, and C. J. A. Bradshaw. 2007. Temporal variation in the vertical stratification of blubber fatty acids alters diet predictions for lactating Weddell seals. Journal of Experimental Marine Biology and Ecology 352:103–113.

Wheatley, K. E., P. D. Nichols, M. A. Hindell, R. G. Harcourt, and C. J. A. Bradshaw. 2008. Differential mobilization of blubber fatty acids in lactating weddell seals: evidence for selective use. Physiological and Biochemical Zoology 81:651–662.

Witteveen, B. H., G. A. J. Worthy, R. J. Foy, and K. M. Wynne. 2012. Modeling the diet of humpback whales: An approach using stable carbon and nitrogen isotopes in a Bayesian mixing model. Marine Mammal Science 28:E233–E250.

Young, B. G., and S. H. Ferguson. 2013. Seasons of the ringed seal: pelagic open-water hyperphagy, benthic feeding over winter and spring fasting during molt. Wildlife Research 40:52–60.

2.9 Tables

Table 2.1 Bearded seal samples collected for dietary analyses

Bearded seal samples collected for stomach content, stable isotope, and fatty acid analyses. Sample sizes by collection year, month, and sex of adult bearded seals harvested for subsistence purposes near Barrow, Point Hope, and Little Diomede Island, Alaska. Stomach, muscle, and blubber were collected for each individual and used for diet analyses.

			Month			Sex	
Location	Year	*n*	May	June	July	Female	Male
Little Diomede	2004	2	2			1	1
	2005	11	5	6		3	8
	2007	1		1		1	
	2009	4	4			2	2
	2010	2	1	1		1	1
	Subtotal	**20**	**12**	**8**		**8**	**12**
Point Hope	2005	7		7		5	2
	2006	5			5	3	2
	2007	2		2		2	
	2008	14		14		8	6
	2009	3		3		3	
	2010	14		14		12	2
	Subtotal	**45**		**40**	**5**	**33**	**12**
Barrow	2006	1			1	1	
	2010	10		9	1	7	3
	Subtotal	**11**		**9**	**2**	**8**	**3**
	Total	**76**	**12**	**57**	**7**	**49**	**27**

Table 2.2 Stable isotopes of prey included in the stable isotope mixing model

Stable carbon and nitrogen isotope values of prey included in the stable isotope mixing model (SIAR). Prey were clustered into seven prey source groups due to model constraints.

Species	Prey source group	Water mass	Year collected	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Source
Echiurid (Echiuridae spp.)	Echiurid	Chukchi Sea	2000	1	-19.70 ± 0.40*	9.60 ± 0.40*	Dehn et al. 2007
Greenland cockle *(Serripes groenlandicus*)	Bivalve	Bering shelf	2004	3	-18.33 ± 0.58	10.15 ± 0.59	Iken et al. 2010
Polychaete (Polynoidae spp.)	Semidemersal	Alaska Coastal Water	2004	5	-19.08 ± 0.68	14.19 ± 0.67	Iken et al. 2010
Pacific sand lance (*Ammodytes hexapterus*)	Semidemersal	Chukchi Sea	2009	10	-19.53 ± 0.40**	13.05 ± 1.02	Carroll 2012
Arctic cod (*Boreogadus saida*)	Semidemersal	Chukchi Sea	2009	10	-19.50 ± 0.32**	14.24 ± 0.69	Carroll 2012
Saffron cod (*Eleginus glacialis*)	Semidemersal	Chukchi Sea	2009	10	-19.70 ± 1.08**	13.42 ± 0.52	Carroll 2012
Octopus (Octopoda spp.)	Octopus	Beaufort Sea	2012	3	-21.41 ± 0.36**	14.07 ± 0.42	This study
Crab (*Telmessus cheiragonus*)	Crab	Alaska Coastal	2004	3	-18.84 ± 0.42	15.50 ± 0.54	Iken et al. 2010
Crab (*Hyas coarctatus*)	Crab	Alaska Coastal	2004	8	-18.48 ± 0.32	15.22 ± 0.82	Iken et al. 2010
Crab (*Chionoecetes opilio*)	Crab	Alaska Coastal	2004	9	-18.30 ± 0.46	14.75 ± 0.62	Iken et al. 2010
Gastropod (*Buccinum* spp.)	Epibenthic	Alaska Coastal	2004	3	-16.94 ± 0.27	15.58 ± 0.39	Iken et al. 2010
Shrimp (*Argis lar*)	Epibenthic	Alaska Coastal	2004	6	-17.15 ± 0.34	14.63 ± 0.70	Iken et al. 2010
Arctic staghorn sculpin (*Gymnocanthus tricuspis*)	Epibenthic	Chukchi Sea	2009	10	-17.88 ± 0.55**	15.53 ± 0.49	Carroll 2012
Shorthorn sculpin (*Myoxocephalus scorpius*)	Epibenthic	Chukchi Sea	2009	10	-17.75 ± 0.78**	14.96 ± 0.78	Carroll 2012
Slender eelblenny (*Lumpenus fabricii*)	Epibenthic	Chukchi Sea	2009	10	-17.64 ± 0.57**	15.09 ± 0.78	Carroll 2012
Yellowfin sole *(Limanda aspera)*	Flounder	Chukchi Sea	2009	2	-18.41 ± 0.13**	16.55 ± 0.60	This study

** Single sample, thus, two times the instrument error of Dehn et al. (2007) was used as a standard deviation in the mixing model.*

*** Indicates that stable carbon isotope values were lipid-extracted.*

Table 2.3 Percent frequency of occurrence of fishes in bearded seal stomachs

Percent frequency of occurrence (%FO) of fishes identified from stomach contents of 69 bearded seals harvested 2004 – 2010 in Alaska. %FO is the number of stomachs containing a fish taxon divided by the total number of stomachs containing prey (x100).

Fishes	%FO
All Osmeridae, Capelin, *Mallotus villosus*	1
All Gadidae	55
Arctic cod, *Boreogadus saida*	42
Saffron cod, *Eleginus gracilis*	36
Walleye pollock, *Gadus chalcogrammus or Theragra chalcogramma*	1
All Cottidae	66
Arctic staghorn sculpin, *Gymnocanthus tricuspis*	34
Sculpin species, *Gymnocanthus* spp.	4
Sculpin species, *Hemilepidotus* spp.	6
Sculpin species, *Icelus* spp.	3
Brightbelly sculpin, *Microcottus sellaris*	6
Arctic sculpin, *Myoxocephalus scorpioides*	3
Shorthorn sculpin, *Myoxocephalus scorpius*	27
Sculpin species, *Myoxocephalus* spp.	36
Ribbed sculpin, *Triglops pingelii*	1
Sculpin species, *Triglops* spp.	9
All Agonidae, poachers	1
All Liparidae	9
Snailfish species, *Careproctus* spp.	1
Variegated snailfish, *Liparis gibbus*	4
Snailfish species, *Liparis* spp.	1
All Zoarcidae	9
Wattled eelpout, *Lycodes palearis*	1
Polar eelpout, *Lycodes polaris*	1
Eelpout species, *Lycodes* spp.	6
All Stichaeidae	25
Blackline prickleback, *Acantholumpenus mackayi*	1
Daubed shanny, *Leptoclinus maculatus*	9
Slender eelblenny, *Lumpenus fabricii*	16
Slender eelblenny or snake prickleback, *Lumpenus* spp.	4
All Ammodytidae, Pacific sand lance, *Ammodytes hexapterus*	25

Table 2.3 Continued.

Fishes	Percent Frequency
All Pleuronectidae	45
Bering flounder, *Hippoglossoides robustus*	7
Yellowfin sole, *Limanda aspera*	9
Longhead dab, *Limanda proboscidea*	37
Righteye flounder species, *Limanda* spp.	3
Arctic flounder, *Pleuronectes glacialis*	1
All unidentified fish	64

Table 2.4 Percent frequency of occurrence of invertebrates in bearded seal stomachs

Percent frequency of occurrence (%FO) of invertebrates identified from stomach contents of 69 adult bearded seals harvested 2004–2010 in Alaska. %FO is the number of stomachs containing an invertebrate taxon divided by the total number of stomachs containing prey (x100).

Invertebrate taxon	%FO
All Porifera	19
All Polychaeta	10
Polynoidae	4
All Bivalvia	39
Nuculana spp.	1
Cyclocardia crebricostata	1
Serripes spp.	1
Macoma spp.	1
Mya spp.	1
Hiatella arctica	3
Liocyma spp.	1
All Cephalopoda	12
Octopus	10
Benthoctopus leioderma	9
Benthoctopus spp.	3
All Gastropoda	15
Velutina spp.	6
Euspira spp.	1
Natica spp.	1
Buccinum spp.	3
All Cirripedia	4
All Mysidae	1
Neomysis rayii	1
All Isopoda	3
Saduria entomon	1
All Amphipoda	4
Photis spp.	1
Gammarus spp.	1
Acanthostepheia spp.	1
All shrimp (Caridea)	64
Hippolytidae	3
Eualus gaimardii	1
Crangonidae	63
Argis lar	36
Argis spp.	12

Table 2.4 Continued.

Invertebrate taxon	Percent frequency
Crangon alaskensis or *septemspinosa*	27
Crangon dalli	12
Crangon spp.	13
Sclerocrangon boreas	24
Pandalidae	9
Pandalus goniurus	6
Pandalus spp.	1
All crab	63
Anomura	1
Paguridae	6
Pagurus spp.	3
Telmessus cheiragonus	27
Majidae	15
Chionoecetes opilio	4
Chionoecetes spp.	27
Hyas coarctatus	10
Hyas spp.	12
All Echiuridae	46
All Bryozoa	3
All Echinodermata	3

Table 2.5 Bearded seal cluster groups and mean stable isotopes of muscle

Bearded seal cluster groups and mean stable carbon and nitrogen isotope values ± 1 SD of muscle tissue. Seals were clustered using k-means clustering of their lipid-extracted stable carbon and non-lipid-extracted stable nitrogen isotope values. The communities where seal tissues were collected are abbreviated as, B-Barrow, P-Point Hope, and D-Little Diomede Island.

Group	Location	Year	*n*	Sex (M, F)	Month	$\delta^{13}C$ non-lipid-extracted	$\delta^{15}N$ non-lipid-extracted	$\delta^{13}C$ lipid-extracted	$\delta^{15}N$ lipid-extracted
All seals	B, P, D	2004 – 2010	76	27, 49	5, 6, 7	-18.14 ± 1.46	16.41 ± 0.92	-17.63 ± 0.90	16.56 ± 0.92
1	B, P, D	04, 06, 10	4	3, 1	5, 6, 7	-19.84 ± 1.80	18.17 ± 0.49	-18.67 ± 0.82	18.00 ± 0.8
2	B, P, D	05 ,06, 08, 10	18	8, 10	6, 7	-18.92 ± 1.25	16.87 ± 0.35	-18.25 ± 0.32	16.91 ± 0.51
3	B, P, D	07, 08, 09, 10	10	2, 8	5, 6	-17.53 ± 1.64	15.24 ± 0.50	-16.95 ± 0.26	15.46 ± 0.67
4	P, D	05, 09	3	1, 2	5, 6	-17.61 ± 1.92	17.52 ± 0.88	-15.38 ± 0.49	17.81 ± 0.87
5	P, D	05, 07, 08, 09, 10	8	2, 6	5, 6	-19.47 ± 1.57	15.4 ± 0.70	-18.93 ± 0.17	15.88 ± 0.92
6	B, P, D	04, 05, 06, 08, 09, 10	18	9, 9	5, 6, 7	-17.13 ± 0.53	16.89 ± 0.38	-17.01 ± 0.40	17.02 ± 0.50
7	B, P, D	05, 07, 08, 09, 10	15	2, 13	6, 7	-17.77 ± 0.75	15.92 ± 0.32	-17.58 ± 0.33	16.06 ± 0.49

2.10 Figures

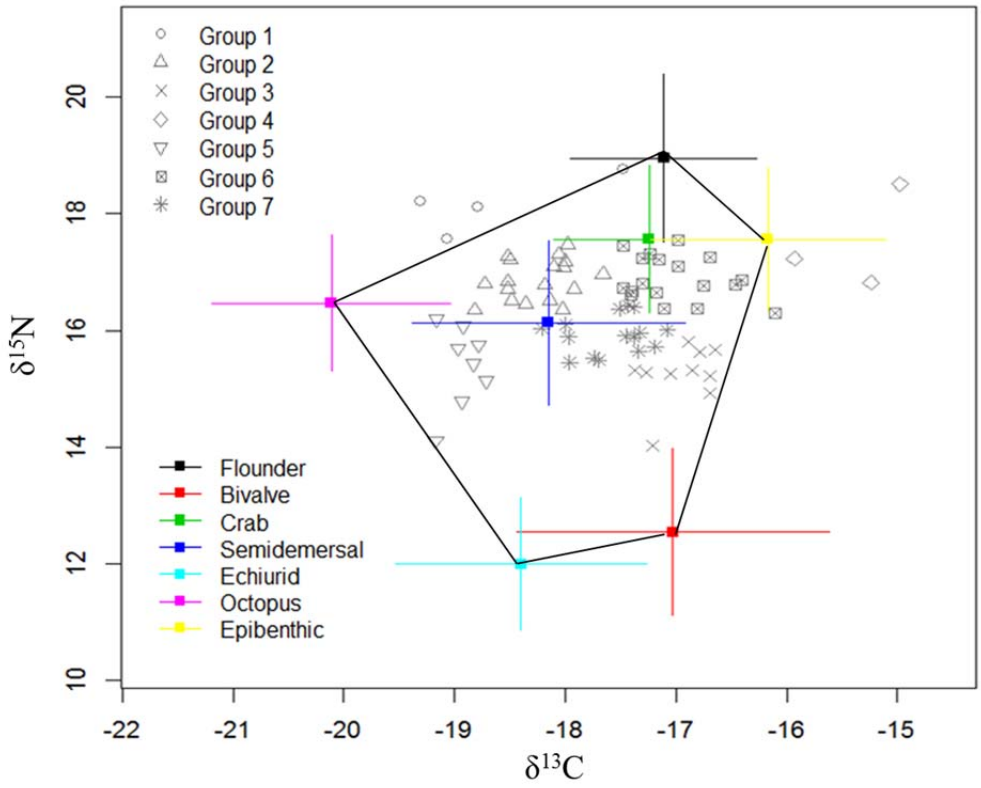

Figure 2.1 Stable isotopes of bearded seal muscle and prey source groups

Stable carbon ($\delta^{13}C$) and nitrogen (δ^{15} N) isotope values for adult bearded seal muscle and prey source groups. Points indicate values for individual seals coded by cluster group, as identified via k-means clustering (see methods for details). The polygon represents the stable isotope space that the stable isotope mixing model (SIAR) will be able to fit with the prey sources used in the model. Colored points are the mean values for each prey source group (±1 standard deviation). Prey source groups have been corrected for tissue enrichment (Parnell et al. 2010) using 1.3‰ for $\delta^{13}C$ and 2.4‰ for $\delta^{15}N$ (Hobson et al. 1996).

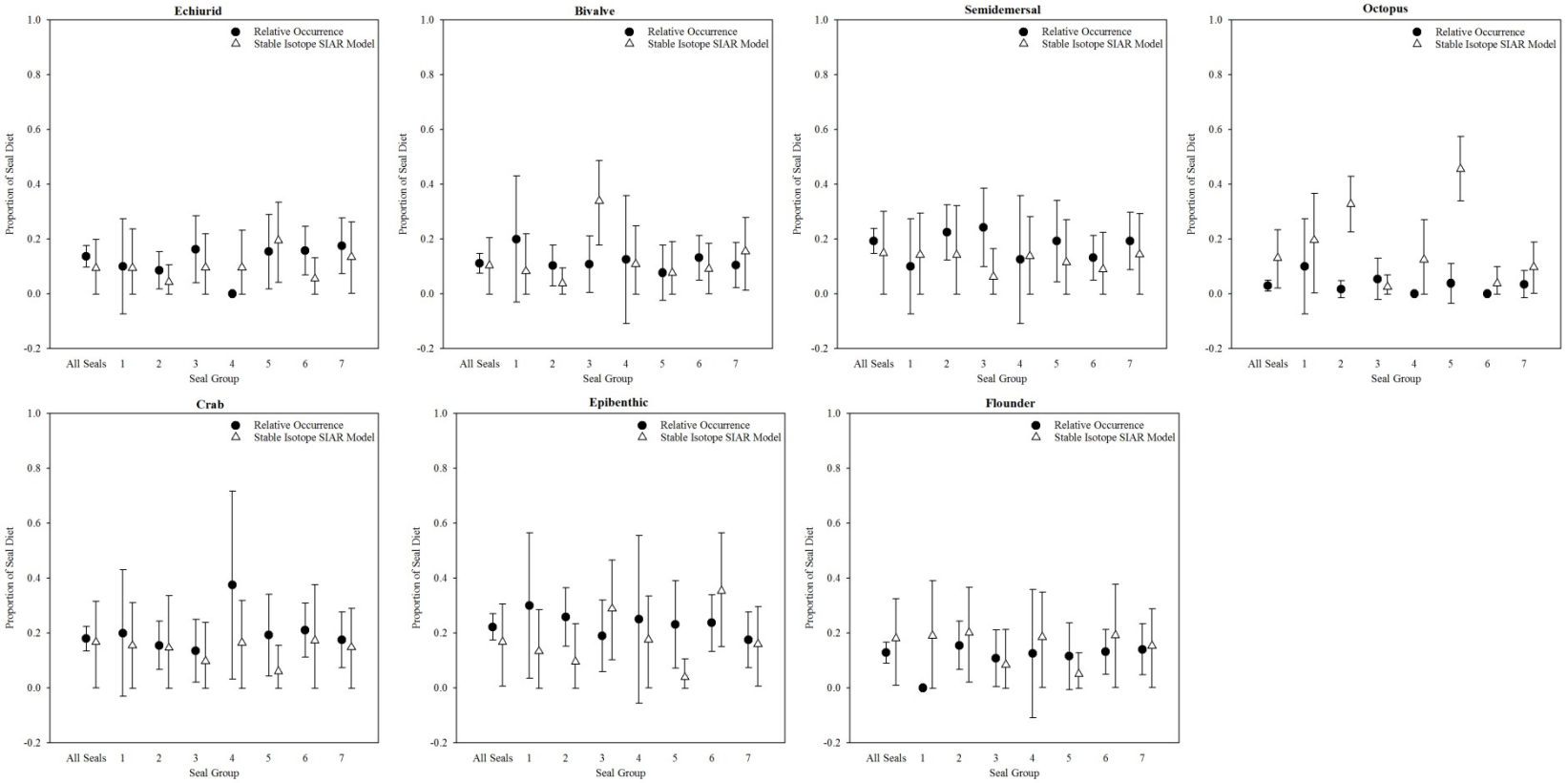

Figure 2.2 Proportion of prey source groups in bearded seal diet from stomach contents and stable isotopes

Comparison of the proportions of prey source groups in adult bearded seal diet obtained from stomach contents and stable isotope analyses. Stomach contents data are shown as the relative occurrence ± 1 SE. Mean prey proportions and 95% credibility intervals are illustrated as determined by the stable isotope mixing model (SIAR). All prey proportions were calculated for all seals combined, and seals separated into the seven seal cluster groups (see methods for details).

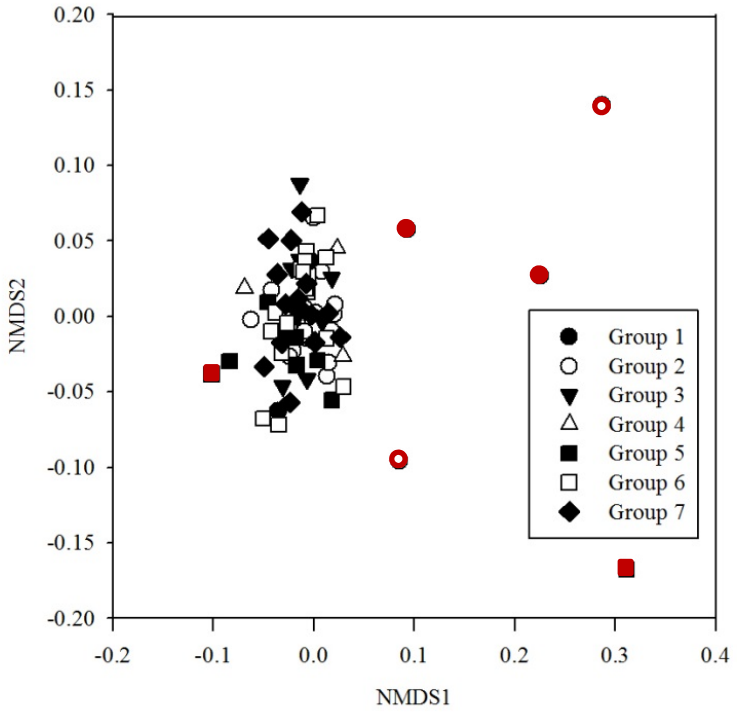

Figure 2.3 Multidimensional Scaling (MDS) plot of fatty acids from bearded seal blubber

Multidimensional Scaling (MDS) plot of 53 fatty acids in full-thickness blubber of 76 adult bearded seals harvested 2004–2010 in Alaska, MDS stress = 0.13. The groups represent the seal cluster groups identified using stable isotope analysis, these groups are represented by different symbols. Seals highlighted in red were found to be outliers using the Mahalanobis distance and were removed from subsequent fatty acid analysis.

2.11 Appendix

Appendix 2.11A. Proportions of fatty acids in bearded seal blubber

Proportions of fatty acids that occurred at >0.1% and NMI fatty acids (mean ± SD) in full-thickness blubber of adult bearded seals. Proportions are expressed as mass percent of total fatty acids.

Fatty acid	All seals *n* = 76	Outliers removed *n* = 70
Saturated		
14:0	2.44 ± 0.44	2.41 ± 0.40
15:0	0.36 ± 0.06	0.37 ± 0.05
i-16:0	0.14 ± 0.02	0.15 ± 0.02
16:0	7.31 ± 1.48	7.38 ± 1.37
i-17:0	0.34 ± 0.05	0.35 ± 0.04
ai-17:0	0.28 ± 0.06	0.29 ± 0.04
17:0	0.26 ± 0.07	0.27 ± 0.06
18:0	1.45 ± 0.46	1.48 ± 0.44
Subtotal	**12.59 ± 2.33**	**12.7 ± 2.17**
Monounsaturated		
20:1n-11	1.73 ± 0.88	1.66 ± 0.45
20:1n-9	2.36 ± 1.00	2.21 ± 0.64
20:1n-7	2.56 ± 0.82	2.66 ± 0.69
22:1n-11	0.37 ± 0.49	0.3 ± 0.17
22:1n-9	0.23 ± 0.14	0.22 ± 0.09
14:1n-9	0.11 ± 0.05	0.10 ± 0.05
14:1n-5	0.74 ± 0.28	0.70 ± 0.22
16:1n-11	0.35 ± 0.07	0.35 ± 0.07
16:1n-9	0.34 ± 0.05	0.33 ± 0.03
16:1n-7	20.53 ± 3.41	20.59 ± 3.00
16:1n-5	0.29 ± 0.03	0.29 ± 0.03
17:1(b)	0.22 ± 0.06	0.22 ± 0.05
17:1	0.60 ± 0.11	0.61 ± 0.10
18:1n-13	0.46 ± 0.12	0.48 ± 0.10
18:1n-11	0.55 ± 0.29	0.50 ± 0.16
18:1n-9	16.2 ± 2.95	16.11 ± 2.42
18:1n-7	9.84 ± 1.80	10.18 ± 1.38
18:1n-5	0.55 ± 0.09	0.55 ± 0.09
Subtotal	**58.02 ± 4.83**	**58.05 ± 4.58**
Polyunsaturated		
16:2n-4	0.43 ± 0.08	0.42 ± 0.06
16:3n-4	0.21 ± 0.06	0.21 ± 0.06
16:4n-1	0.32 ± 0.12	0.32 ± 0.12

Appendix 2.11A. Continued.

Fatty Acid	All seals $n = 76$	Outliers removed $n = 70$
18:2n-6	0.92 ± 0.14	0.91 ± 0.13
18:2n-4	0.25 ± 0.04	0.26 ± 0.03
18:3n-6	0.12 ± 0.02	0.12 ± 0.02
18:3n-4	0.27 ± 0.05	0.28 ± 0.04
18:3n-3	0.29 ± 0.09	0.28 ± 0.07
18:4n-3	0.64 ± 0.22	0.62 ± 0.21
18:4n-1	0.27 ± 0.07	0.27 ± 0.06
20:2n-9	0.15 ± 0.05	0.15 ± 0.03
20:2n-6	0.35 ± 0.06	0.36 ± 0.06
20:3n-6	0.14 ± 0.02	0.14 ± 0.02
20:4n-6	0.98 ± 0.22	1.02 ± 0.17
20:4n-3	0.45 ± 0.08	0.44 ± 0.08
20:5n-3	7.83 ± 1.75	7.87 ± 1.52
21:5n-3	0.55 ± 0.10	0.56 ± 0.10
22:4n-6	0.28 ± 0.08	0.29 ± 0.06
22:5n-6	0.27 ± 0.06	0.27 ± 0.06
22:5n-3	5.05 ± 0.99	5.00 ± 0.75
22:6n-3	7.57 ± 1.92	7.34 ± 1.42
Subtotal	**27.32 ± 3.78**	**27.13 ± 2.86**
Non-methylene interrupted		
20:2Δ5,11	0.04 ± 0.03	0.04 ± 0.03
20:2Δ5,13	0.09 ± 0.04	0.09 ± 0.04
20:3Δ5,11,14	0.04 ± 0.02	0.04 ± 0.02
22:2NMID	0.06 ± 0.03	0.06 ± 0.02
22:2Δ7,13	0.11 ± 0.06	0.11 ± 0.06
22:3Δ7,15	0.43 ± 0.15	0.45 ± 0.13
Subtotal	**0.75 ± 0.21**	**0.78 ± 0.18**
Total	98.67 ± 0.2	98.67 ± 0.32

Chapter 3: Fish prey in bearded and ringed seal diet – a comparison of stomach contents and fecal DNA[1]

3.1 Abstract

Current changes to sea ice habitat makes understanding diet of bearded (*Erignathus barbatus*) and ringed (*Pusa hispida*) seals important to managers. Through examination of stomach contents and prey DNA extracted from feces, we determined which method provided more dietary information about fish species consumed. From stomach contents of 21 bearded seals, we identified 1,810 individual fish, representing at least 20 species. We also identified 44 fish, representing at least six species, from four ringed seals stomachs. We used denaturing gradient gel electrophoresis (DGGE) of 16S gene fragments to identify fish prey species. Only 40% of the fecal samples (12 bearded and one ringed seal) produced detectable DNA suitable for reference gene amplification, and we were only able to positively identify three fish species in the diet of seals (Arctic cod, *Boreogadus saida;* shorthorn sculpin, *Myoxocephalus scorpius*; and an unknown snailfish species, family Liparidae) with the DGGE method. Shorthorn sculpin were found in six of the 12 bearded seal samples, and snailfish was found once. A single ringed seal fecal sample contained DNA from both Arctic cod and shorthorn sculpin. A number of additional unidentified amplicons were also found, which can potentially be identified with direct sequencing. Overall, more fish species were identifiable using the stomach contents of these seals than using the DGGE of reference gene amplifications from fecal DNA. However, a larger prey DNA reference library, a combination of both methods, or the implementation of next

[1]Bryan A. L., Horstmann-Dehn L., Hundertmark K. J., Quakenbush L., López, J. A. 2014. Fish prey in bearded and ringed seal diet – Comparison of stomach contents and fecal DNA. Prepared for submission to Polar Biology.

generation sequencing approaches could increase overall numbers of fish species identifiable in ice seal diets.

3.2 Introduction

Bearded (*Erignathus barbatus*) and ringed (*Pusa hispida*) seals are arctic pinnipeds that use sea ice for pupping, molting, and as a resting platform (Burns 1981; McLaren 1958). Arctic sea ice extent is decreasing (Beitler 2012; Stroeve et al. 2012), and this decrease in ice extent will likely have consequences for the marine food web (Bluhm and Gradinger 2008; Wang et al. 2014). The combined effects of habitat loss and changes in food web biomass could cause ice-dependent species to be vulnerable to sea ice changes (Kovacs et al. 2011). Due to sea ice habitat changes, concerns for the long-term survival of these species have led to the listing of the Pacific population of bearded seals (U.S. Federal Register 2012a) and the Arctic Basin population of ringed seals (U.S. Federal Register 2012b) as threatened under the Endangered Species Act.

Changes in sea ice coverage will have impacts on primary production; thus, seals will likely experience density and distribution shifts in their prey species as part of a bottom-up trophic cascade (Bluhm and Gradinger 2008; Kovacs and Lydersen 2008). Bearded seals are benthic generalists, known to eat a variety of invertebrates and fishes (Lowry et al. 1980a; Antonelis et al. 1994; Dehn et al. 2007; Quakenbush et al. 2011a), and ringed seals have a diet that includes both pelagic and benthic prey (Lowry et al. 1980b; Dehn et al. 2007; Quakenbush et al. 2011b). Changes in prey assemblages could force animals to target prey with decreased nutritional value (Leu et al. 2010; Trites and Donnelly 2003; Wang et al. 2014), but a shift in diet does not necessarily imply decreased nutritional value for the predator (Quakenbush et al. 2011a,

b). Consequently, investigations enhancing knowledge of ice seal diets, mainly due to the uncertainty about bottom-up food web effects, and adaptive responses of seals in the rapidly changing arctic ecosystem remain an important issue.

Diet studies are important for understanding population health and response to ecosystem change. Evaluating pinniped diet is challenging, because feeding cannot be directly observed (Pierce and Boyle 1991). Therefore, diet studies rely on indirect measures (e.g., stable isotopes, fatty acids, stomach contents, and prey DNA) to identify prey (Cooper et al. 2009; Dehn et al. 2007; Quakenbush et al. 2011a, b; Tollit et al. 2009), each with strengths and limitations. For example, stable carbon and nitrogen isotopes can indicate the carbon source and trophic level of prey consumed and assimilated (Post 2002), but have low taxonomic resolution. Stable isotope mixing models estimate the proportion of different prey types in diet, but perform poorly when approximating the diet of generalists known to consume a wide variety of prey (Bowen and Iverson 2013). Fatty acids can be used qualitatively to infer spatial or temporal differences in diet (Budge et al. 2006; Iverson 2009), or quantitatively to identify the relative proportions of prey taxa consumed (Iverson et al. 2004). Mixing models for both stable isotopes and fatty acids require prey reference libraries and predator-specific metabolic information (i.e., marker turnover times), but these data are not always available for free-ranging wildlife (Bond and Diamond 2011; Iverson 2009; Rosen and Tollit 2012).

Stomach content analysis allows prey taxa to be identified, often to the species level, but digestion or passage rates, size of prey, and size of hard parts (e.g., size of otoliths or cephalopod beaks) can cause some prey to be over- or underrepresented relative to their true proportion in the diet (Bowen 2000; Pierce and Boyle 1991). Conversely, fully digested prey, prey without hard parts, and empty stomachs do not provide any dietary information (Bowen 2000; Pierce and

Boyle 1991; Sheffield et al. 2001). Recently, prey DNA molecules found in the digestive tract and feces have been used to identify prey items (e.g., Bowles and Trites 2013; Deagle et al. 2013; Deagle et al. 2005b; Tollit et al. 2009). Using polymerase chain reactions (PCR), products can be yielded from single molecules of starting template DNA. The main advantage of using DNA is that prey can be identified to the species level, even after digestion has destroyed diagnostic parts (Jarman et al. 2004; King et al. 2008). However, false positives or false negatives are possible (Bowen and Iverson 2013; King et al. 2008), and these biases are difficult to recognize in samples collected from wild populations (Deagle et al. 2005b). PCR inhibitors can also be co-isolated with prey DNA during the template preparation process, preventing the DNA from replicating (King et al. 2008). Despite the limitations and biases of stomach content and DNA analyses, both allow a wide range of prey to be identified with high taxonomic resolution (Bowen and Iverson 2013). Species-level identification of prey is important to establish baseline dietary information (e.g., prey assemblages, prey size, and quantity of food consumed) that can then be used to create prey reference libraries for other dietary analyses, such as stable isotopes and fatty acids.

Given the strengths and weaknesses of different diet analysis methods, it is beneficial to employ a broad set of tools with complementary strengths to properly characterize ice seal feeding ecology. Here, we focus on the role that stomach content and DNA analysis can play to increase the knowledge of prey taxa consumed by free-ranging ice seals. Specifically, the objective of this study was to determine whether fecal DNA provided more dietary information than stomach contents about fish species consumed by bearded and ringed seals.

3.3 Methods

3.3.1 Sample Collections

Stomachs and lower colons were collected in 2008–2011 from 32 adult bearded and ringed seals (> 5yrs old) harvested during legal subsistence hunts near Point Hope and Barrow, Alaska (Table 3.1). The samples were collected as part of the ice seal biomonitoring programs conducted by the Alaska Department of Fish and Game (ADF&G) and the North Slope Borough Department of Wildlife Management. Twenty-two bearded seals (five males and 17 females) were collected in 2008–2010, and 10 ringed seals (three males and seven females) were collected in 2010 and 2011. As part of a larger study comparing diet methods, seals were selected if the stomach, lower colon, muscle, and blubber were available. Stomachs and intestines were removed from the seal, without tying the ends, placed in plastic bags within 24 hours of death, and stored frozen at –20ºC until processed. Ages were determined by counting cementum growth layers of sectioned teeth, one growth layer group was assumed per year (Benjaminsen 1973; Mansfield and Fisher 1960; Stewart et al. 1996). Previous diet studies showed no sex-related differences in Alaska-harvested bearded and ringed seals (Quakenbush et al. 2011a, b), so males and females of each species were pooled for this study.

As reference DNA samples, 12 forage fish species as well as muscle from both of the seal species were collected. The fish were collected during the 2009 Russian-American Long-term Census of the Arctic (RUSALCA) expedition in the Chukchi Sea using a plumb-staff beam trawl and surface trawl. Selected species were: rainbow smelt (*Osmerus mordax*); Arctic cod (*Boreogadus saida*); saffron cod (*Eleginus gracilis*); walleye pollock (*Gadus chalcogrammus or Theragra chalcogramma*); Arctic staghorn sculpin (*Gymnocanthus tricuspis*); shorthorn sculpin (*Myoxocephalus scorpius*); polar eelpout (*Lycodes polaris*); slender eelblenny (*Lumpenus fabricii*); Pacific sand lance (*Ammodytes hexapterus*); yellowfin sole (*Limanda aspera*);

longhead dab (*Limanda proboscidea*); and an unknown snailfish species (family Liparidae). These fish were chosen because they have high frequency of occurrence (> 10%) in either bearded or ringed seal stomach contents (Quakenbush et al. 2011a, b) and were available for analysis. The seal muscle was collected as part of ADF&G's biomonitoring program. Fish and seal muscle were stored frozen at -20ºC until DNA extraction.

3.3.2 Stomach Content Analysis

Stomachs were thawed in the laboratory, and the contents were rinsed in freshwater through 1.0 mm and 0.5 mm sieves. Prey items were sorted into broad categories and identified to the lowest possible taxonomic level (see Dehn et al. 2007; Quakenbush et al. 2011a; b for details). Invertebrates were common in the stomachs of bearded and ringed seals, but were not examined for the purposes of this study. Fish otoliths were identified by W. Walker, Vashon, Washington. Fish were counted, first, by adding any whole fish, and then by adding the maximum number of right or left otoliths plus half of the count of otoliths that could not be identified as right or left. No attempt was made to identify non-otolith fish tissue; if fish bones were present without otoliths, one unidentified fish was recorded as a minimum representation of the consumed fish. Percent frequency of occurrence (% FO) was calculated as the number of stomachs containing a particular fish taxon divided by the total number of stomachs that contained fish (x100).

3.3.3 DNA Extractions

Colons of bearded and ringed seals were partially thawed, cut on a bleached glass cutting board with a clean razor blade, and a 180–220 mg subsample of feces was taken and placed in a

DNA/RNA-free snap-cap vial. Total DNA was isolated using the reagents and protocols in the QIAamp DNA Stool Kit (QIAGEN 2010). The quality and quantity of isolated DNA was checked visually by ethidium bromide-stained agarose gel (0.8% w/v) electrophoresis.

Reference DNA was extracted from fish tissue and seal muscle using reagents and protocols from the Gentra Puregene Tissue Kit (QIAGEN 2011), with minor modifications. Instead of grinding the tissue with liquid nitrogen, placing the ground tissue in 300 µl of Cell Lysis solution, and incubating at 65ºC for one minute, a 5–10 mg piece of fish muscle was placed directly in a DNA/RNA free snap-cap vial with 250 µl of Cell Lysis solution and incubated at 65ºC for 15 minutes. Then, the remaining 50 µl of Cell Lysis solution was added along with the Puregene Proteinase K, and the sample was incubated overnight. After incubation, the samples were placed directly into an ice bath; no RNase A solution was added. The remaining steps were done without modification except that all centrifuge times were increased to five minutes. Fish DNA samples were viewed on a 1% agarose gel using the same conditions as described above for fecal DNA elutes.

3.3.4 PCR Amplifications

For fecal samples, we followed the protocol described by Tollit et al. (2009) to identify 16S ribosomal RNA gene fragments from prey species in the fecal DNA isolates. Briefly, the protocol employs a semi-nested PCR strategy to amplify a section of the 16S ribosomal RNA gene, a highly conserved mitochondrial gene that includes species-specific sequences in variable regions. The initial PCR reaction used universal primers 16SF1 (5'-GGACGAGAAGACCCT-3') and 16SallR (5'-CGCTGTTATCCCTAGGGTAACT-3'). This primer set generates a 290–308 base pair (bp) fragment from most vertebrate lineages. The follow-up, semi-nested PCR

used the fish-specific forward primer 16SfishF (5'-AGACCCTATGGAGCTTTAGAC-3') and reverse primer 16SallR to amplify a 282–300 bp fragment from the products of the initial PCR. This semi-nested strategy first generates a broad pool of amplicons from all prey present, followed by preferential amplifications of those amplicons derived from fish (Jarman et al. 2004; Tollit et al. 2009). In addition, the 16SallR primer in the second reaction carries a 39-bp GC-rich tail (Deagle et al. 2005a; Tollit et al. 2009) to improve band separation on the DGGE runs (Sheffield et al. 1989). To minimize the incidence of contamination from DNA in the environment, the PCR reactions were assembled at the University of Alaska Museum of the North, a PCR-free facility at the University of Alaska Fairbanks.

Primary PCR reactions were performed using 2.0 µl of fecal DNA isolate in 25 µl volumes with the following reagent concentrations; 1.33x Hot Star *Taq* Plus PCR Buffer, 2mM $MgCl_2$, 0.2 mM of dNTP's, 1x bovine serum albumin (BSA), 20 mM of each primer (16SF1 and 16SallR), and 0.025 U/µl of Hot Star *Taq* Plus. Primary PCR cycling conditions from Tollit et al. (2009) did not produce PCR products, so we used the following modified conditions: pre-incubation at 94ºC for 2:30 minutes, denaturing at 94ºC for 30 seconds, annealing at 54ºC for 30 seconds, extension at 72ºC for 30 seconds, and post-incubation at 72ºC for 6 minutes. Denaturing, annealing, and extensions were repeated for 40 cycles.

Semi-nested PCR reactions were carried out using the same reagent conditions, with 2.0 µl primary PCR products as template DNA. Semi-nested PCR cycling conditions followed the protocol in Tollit et al. (2009): pre-incubation at 95ºC for 15 minutes, denaturing at 94ºC for 30 seconds, annealing at 55ºC for 30 seconds, extension at 72ºC for 45 seconds, and post-incubation at 72ºC for 10 minutes. Denaturing, annealing, and extensions were repeated for 35 cycles.

After the first 25 µl of semi-nested products were exhausted, subsequent reactions used 1.0 µl of primary PCR products with the same final volume.

Negative and positive controls were included with every PCR reaction to check for contamination and reaction success. Primary and nested PCR products were viewed on a 1.8% agarose gel. The gels were run for 30 minutes at 100 volts. Gels were then stained for 15 minutes in 10% ethidium bromide solution, rinsed in fresh water for 4 minutes, and viewed on a Labnet, DyNA Light UV Transilluminator.

PCR reactions can be obstructed by inhibitors (e.g., bile salts and complex polysaccharides) present in fecal DNA that can decrease sensitivity or prevent successful PCR amplification, causing false negatives (Kreader 1996; Monteiro et al. 1997; Schrader et al. 2012). To assess the potential impact of PCR inhibitors on our ability to amplify fecal DNA fragments, we tested the effect of template DNA dilution (10, 100 and 1000-fold) with two isolates from bearded seal feces that did not yield products when undiluted. Semi-nested reactions were also run for one of these fecal samples using the original, 10-fold, and 1,000-fold dilutions. Products from these secondary PCRs were examined by DGGE at 56ºC to determine if multiple bands were present and to determine if the products generated were consistent across different dilutions of the starting template.

Fish and seal PCRs followed the same strategy as the one used with fecal DNA isolates with the following exceptions of constituents used in the primary reaction; 5x Green GoTaq Flexi buffer (replacing the Hot Star *Taq* Plus PCR Buffer), 2 mM $MgCl_2$, 0.2 mM dNTP's, 0.4 uM primers, and GoTaq (replacing Hot Star *Taq* Plus). No BSA was added to the primary reaction because the template DNA was of good quality. The thermal cycling conditions for both the primary and semi-nested reactions were the same as described previously for fecal DNA PCRs.

Primary and nested products were viewed on a 1.8% agarose gel using the same conditions as described previously.

3.3.5 Denaturing Gradient Gel Electrophoresis

DGGE separates DNA fragments based on size and/or nucleotide sequence. DNA fragments differing by as little as a single nucleotide site substitution can be separated (Tollit et al. 2009) by electrophoresis along a urea-formamide denaturation gradient in a polyacrylamide matrix at a controlled and fixed temperature (Deagle et al. 2005a; Tollit et al. 2009). The DGGE runs were performed on a DGGE-2401 apparatus (C.B.S Scientific Company, Inc). Gels were 7.5% acrylamide with a 35 to 60% urea-formamide gradient. The denaturing gradient was created using a GM-40 gradient maker (C.B.S Scientific Company, Inc) and a peristaltic variable flow mini-pump to gradually and progressively mix the following two acrylamide solutions while casting the gel: 1) 2.45 M urea, 14% formamide (w/v) in 1x Tris, acetic acid, EDTA (TAE) buffer, and 2) 4.20 M urea, 24% formamide in 1x TAE.

When pouring gels, 11.5 µl of each stock solution was placed in a glass beaker with 80 µl of ammonium persulfate solution and 5 µl of tetramethylethylenediamine (TEMED); 60 µl bromothymol blue was added to the 60% denaturant to make the gradient readily visible. Gels were then poured using a GM-40 gradient maker (C.B.S Scientific Company, Inc) and a mini-pump following the vertical gradient protocol from the DGGE-2401 manual. The 35% and 60% solutions were each poured into a separate side of the gradient maker which was placed on a magnetic stirrer. A magnet was placed into the 60% solution to mix the solutions as the gel was poured. The gradient maker was connected with tubing to a 21-gauge needle placed between two glass casting plates. The 60% solution dispensed from the gradient maker first, and the

solutions mixed as the gel was poured such that the top of the gel contained 35% denaturants. Once poured, a gel comb was added to the gel, and the gel was covered by plastic wrap to avoid evaporation and shrinkage as the gel polymerized.

Reference ladders of prey DNA bands were created by mixing PCR products from the fish samples into the following two sets: 1) rainbow smelt, Arctic cod, saffron cod, walleye pollock, shorthorn sculpin, and Pacific sand lance; and 2) Arctic staghorn sculpin, polar eelpout, slender eelblenny, yellowfin sole, longhead dab, and the snailfish. These reference ladders were used to identify amplicons generated from fecal DNA isolates by matching migration distance on DGGE runs; fish were split into two ladders to aid in identification. Replicate DGGE assays were performed at two different temperatures to improve confidence in species identification (Tollit et al. 2009). Gels were run at 60V, for 15 hrs, at both 56ºC and 60ºC (Tollit et al. 2009). Upon completion, the gels were stained for 25 minutes in 10% ethidium bromide solution, rinsed in fresh water for 5 minutes, and viewed using the same protocol as the PCR products on agarose gels as described previously.

For each sample, bands on the DGGE gels were classified as strong, well-defined bands, or weak, more diffuse bands. We did not identify thick smudges, dark coloration that did not fill the full width of a lane, light discoloration, or smudges that continued past the edges of a lane. Bands from fecal DNA PCR products that lined up with bands in the reference ladder at both temperatures (56ºC and 60ºC) were considered confirmed matches (Tollit et al. 2009). Percent FO was calculated as the number of fecal samples that contained a fish taxon divided by the total number of fecal samples that produced semi-nested PCR products (x100).

As a validation step, we sequenced primary PCR products generated from five bearded seal fecal samples that showed a single product type on DGGE runs to determine whether bands

represented seal DNA. The species provenance of the sequenced PCR fragments was determined by performing Basic Local Alignment Search Tool (BLAST) queries on the nucleotide databases maintained by the National Center for Biotechnology (Altschul et al. 1997).

3.4 Results

3.4.1 Stomach Content Analysis

All bearded seal stomachs (n = 22) contained prey, but one only contained invertebrates without fish. The remaining 21 stomachs (95%) contained a minimum of 1,810 individual fish of at least 20 species from seven families (Table 3.2); the number of individual fish identified per stomach ranged from 1 – 415. Arctic cod occurred most often with a 76% FO, followed by unidentified *Myoxocephalus* spp. at 57% FO, longhead dab at 52% FO, slender eelblenny at 48% FO, saffron cod and Arctic staghorn sculpin at 36% FO, and Pacific sand lance with a 33% FO (Table 3.2). Otoliths from 458 fishes (25%) were only identifiable to genera. They included sculpin (*Gymnocanthus* spp., *Hemilepidotus* spp., *Icelus* spp., *Myoxocephalus* spp., and *Triglops* spp.), snailfish (*Careproctus* spp. and *Liparis* spp.), eelblenny (*Lumpenus* spp.), and flounder (*Limanda* spp.). Otoliths from 73 fish were unidentifiable due to erosion by digestive processes.

Five of the ringed seal stomachs were empty (50%), one contained only invertebrates, and four contained fish (40%). A minimum of 44 fish were found in the four stomachs that contained fish, comprising at least six species from three families (Table 3.3); the number of fish identified per stomach ranged from 4 – 28. Arctic cod had the highest FO (75%), followed by saffron cod (25% FO), Arctic staghorn sculpin (25% FO), and Pacific sand lance (25% FO) (Table 3.3). In addition, two sculpin genera were observed (each at 25% FO), but species level identification was not possible (*Hemilepidotus* spp. and *Icelus* spp.).

3.4.2 DNA-Based Diet Analysis

Of the 32 samples examined (22 bearded, 10 ringed), 27 (22 bearded, 5 ringed) yielded DNA in quantities visually detectable on agarose gels. Twenty two of these (14 bearded, 8 ringed) yielded visually detectable primary PCR products, but only 13 (12 bearded, 1 ringed) produced semi-nested PCR products. We were unable to amplify any 16S fragments in ten of the fecal samples (8 bearded, 2 ringed).

Reference fish samples were run on DGGE gels, individually and in sets, to verify consistent migration rates and determine the relative placement of products from known fish species under our DGGE conditions. At both temperatures, the DGGE assays clearly separate the 16S PCR products from most of the reference fish species. However, two pairs of species co-migrate at 56ºC: shorthorn sculpin with Pacific sand lance, and longhead dab with yellowfin sole (Figure 3.1).

Using DGGE, we identified between two and seven different amplicons in 12 PCR products from bearded seal fecal DNA isolates, including six occurrences of shorthorn sculpin and one of snailfish (Table 3.4). Twenty additional amplicons did not match any of the fish in the reference ladder, and were noted as "unidentified fish". Not all DNA bands appeared on the gels with the same clarity and intensity. We also detected bearded seal DNA in all 12 fecal samples, including two different haplotypes (Table 3.4 and Figure 3.2). A faint band of contamination from ringed seal DNA was also detected in one bearded seal sample. Fecal DNA from a ringed seal yielded evidence of Arctic cod and shorthorn sculpin, as well as ringed seal.

Primary PCR products generated from five bearded seal fecal samples showed a single band, which did not migrate with the prey, on DGGE runs. BLAST searches of these sequences

yielded matches with 98% or 99% identity to known, previously reported bearded seal 16S sequences. The next closest matches were to other phocids (i.e., hooded seal, *Crystophora cristata* and crabeater seal, *Lobodon carcinophaga*) with 87% or 88% identity. Of these five bearded seal fecal samples, three successfully produced semi-nested products (samples 2, 10, and 11 in Table 3.4), the other two failed.

Results of dilution trials for fecal samples suggest that inhibitors were having an effect on our samples. All diluted DNA isolates (10-fold, 100-fold, and 1,000-fold) from both seal species produced primary PCR products, but the original concentrations did not. In one sample, semi-nested PCR products were also produced for both the 10-fold and 1,000-fold dilutions. When run on the DGGE, the 10-fold dilution resulted in seven bands, and the 1,000-fold had three, indicating that more prey DNA was amplified in the 10-fold dilution than the 1,000-fold. Bands were not matched to reference fish species because the gels were only run at one temperature.

For the twelve bearded seals that produced semi-nested PCR products, nine of the 12 fishes in the DNA reference ladder were also found in the stomach contents, but only two of the fish species were identified in the fecal DNA on the DGGE. The FO of shorthorn sculpin was higher in the fecal samples (50%) than in the stomach contents (33%), and snailfish was found more often in the stomach contents (25% FO) than in the fecal samples (8% FO; Table 3.5). Unknown fish were found using both methods. Using stomach contents, unidentified otoliths were detected with a 42% FO (Table 3.5), these cannot be further identified due to their advanced digestion. Unknown DNA bands, assumed to be fish, were found in all 12 fecal samples on the DGGE (100% FO; Table 3.5). The ringed seal had Arctic cod, saffron cod, and Pacific sand lance in its stomach, whereas the DNA results indicated that Arctic cod and shorthorn sculpin were consumed. For this one ringed seal, a combination of these methods

identified prey species not accounted for with each method independently, thus increasing dietary resolution.

3.5 Discussion

We examined the effectiveness of DGGE-based species identification for characterizing diet components when compared with visual identification of stomach contents. Previous studies have demonstrated that DNA and stomach contents can be combined to increase prey detection for generalist predators (Braley et al. 2010; Dunshea et al. 2013; Tollit et al. 2009). Although the number of fish species we identified using stomach contents was greater compared with DNA, when combined, we were able to increase the frequency of occurrence of shorthorn sculpin for both seal species. In our efforts to implement DGGE-based species detection in the study of ice seal diet, we encountered numerous limitations and issues with the DNA method, such as primer design, PCR amplification, effectiveness of DGGE at determining diet; similar problems been discussed in previous studies (Deagle et al. 2005b; King et al. 2008). These limitations could be mitigated if primers and protocols were optimized for these specific pinniped species (King et al. 2008).

3.5.1 Seal Diet

Using stomach contents we were able to identify 20 fish species and 1,855 individual fish in 22 bearded seal stomachs, and six fish species and 44 individual fish in four ringed seal stomachs. The DGGE method on the other hand did not generate as much usable dietary information. Of the 12 fishes used in the DNA fish reference library, only three were identified in the feces of ringed and bearded seals in this study (i.e., Arctic cod, shorthorn sculpin, and

snailfish). However, otoliths from all but one of the 12 reference fishes (walleye pollock) were found in the stomachs. Although the dietary information we gained from DNA was limited, the technique shows that it is possible to advance dietary information by combining data from both methods.

PCR inhibition was likely a factor for our low detection success using fecal DNA. Active control of inhibitors would be crucial in future work and may improve success. In addition, a number of DNA bands were generated from fecal samples that did not match fish from the reference library. However, these unknown taxa could be identified by excising the bands from the gel, and extracting and sequencing the DNA (Tollit et al. 2009). An abundance of seal DNA was also found in the fecal samples; all 13 samples that produced nested PCR products also yielded seal DNA even though the primer should have been specific for fish DNA.

The DGGE method has been applied successfully elsewhere. Using a combination of hard part analysis from scats and fecal DNA, Tollit et al. (2009) was able to increase detectability of Steller sea lion (*Eumetopias jubatus*) prey species by 22% with an average of about two prey occurrences per scat using DNA. However, this technique is difficult to standardize across laboratories and species, even within pinnipeds. We attempted to duplicate conditions used by Tollit et al. (2009) to detect prey DNA, but we had difficulty acquiring the same outcome for our samples as reported by these authors. PCR cycling conditions, for example, do not always generate repeatable results between laboratories. In addition, many of the complications we encountered generally go unreported, giving the impression of an easily implemented technique applicable to multiple species. As a routine diet monitoring tool, DGGE is time-intensive and cumbersome to use.

Hard part (i.e., stomach content and fecal) diet analysis has limitations, but when large numbers of samples are collected, it is possible to monitor changes over time (e.g., Labansen et al. 2011; Lance et al. 2012). Using stomach contents, Quakenbush et al. (2011a, b) found that in Alaska waters both bearded and ringed seals consumed more fishes in the 2000s compared with the 1960s and 70s. A number of otoliths in our study and the Quakenbush et al. (2011a, b) studies were only identifiable to the genus level due to the physical condition of the otolith (i.e., degree of digestion, breakage, or the age class of the fish being identified). Otoliths from juvenile fish of closely related species can be hard to distinguish (Frost 1981; Jobling and Breiby 1986; Morrow 1979). Unlike the unidentified otoliths, unknown DNA bands could be further identified using DNA sequencing. The ability to further identify prey clearly highlights an important advantage of DNA-based techniques over stomach contents.

Dietary information from both stomach contents and fecal DNA represent recently ingested prey items, but the digestive passage rates are not the same. Hard parts (e.g., otoliths) can accumulate in the stomach over several feeding events (Jobling and Breiby 1986). During a captive feeding trial, Murie and Lavigne (1986) found that otoliths were recoverable in stomach contents of grey (*Halichoerus grypus*), harp (*Pagophilus groenlandica*), and ringed seals between 0 and 12.9 hours after ingestion. In contrast, a captive feeding study with Steller sea lions found that prey DNA in feces was detectable within 48 hours of ingestion (Deagle et al. 2005b). Other factors can also affect the rate of digestion, such as increased food intake which can decrease the retention time in the digestive system (Trumble and Castellini 2005), and the nutritional content of prey (amount of lipid vs. protein) which can alter the uptake of nutrients (Trumble et al. 2003). Also, otolith digestion is species and size specific; this can lead to an underrepresentation of species with fragile otoliths or an overrepresentation of species with hard,

robust otoliths (Bowen 2000). Although it is not the only reason for our prey detection differences, digestion and passage rates likely contributed to our low number of fish matches between methods.

3.5.2 Methods Considerations

The taxonomic selectivity of the PCR primers determines the ultimate effectiveness of the DGGE technique in recovering a representative sample of the species present in a sample. The ideal primers would broadly, and with minimal bias, amplify target gene fragments from species in a target group (e.g., ray-finned fishes) and not those of the predator. For this reason, primer design is important in minimizing replication of predator DNA, and is most effective when prey species and the predator represent evolutionarily distant lineages (e.g. Deagle et al. 2005b; King et al. 2008). The primers and PCR conditions used in this study were designed for Steller sea lion diet studies (Deagle et al. 2005b; Tollit et al. 2009) to target a wide range of fish DNA, while eliminating Steller sea lion DNA. The 16SF primer used in the primary reaction is known to amplify both fish and pinniped DNA (Deagle et al. 2005b). We confirmed this cross-amplification in this study, which may have limited the effectiveness of the semi-nested PCR to generate a representative pool of amplicons from prey species initially present in the fecal sample. The 16SfishF primer should have targeted fishes, and eliminated the non-target DNA (including seal DNA) (Deagle et al. 2005b; Tollit et al. 2009), yet a substantial amount of predator amplicons appear in our semi-nested PCR products (Table 3.4 and Figure 3.2). We did not establish whether they represent carryover from the primary PCR or products of the semi-nested or secondary PCR. Development and incorporation of predator-specific blocking primers in the amplification strategy could help circumvent this limitation (Vestheim and Jarman 2008).

Our experiments to test for possible presence and effect of PCR inhibitors indicate widespread inhibition of PCR. Only 39% of the fecal samples in this study produced semi-nested PCR products. Reagents in the fecal DNA extraction kit are designed to eliminate or significantly reduce the concentration of common PCR inhibitors (QIAGEN 2010), however, some inhibitors will remain (Schrader et al. 2012). In addition, BSA was added to the PCR reactions to decrease the effect of inhibitors (King et al. 2008) by interacting with the inhibitory substances allowing successful amplifications (Nagai et al. 1998). While dilution can overcome the effect of inhibitors, it may decrease detection rates of rare targets because the quantity of prey DNA in feces is likely to be low (King et al. 2008). Amplification products from different dilutions of the same starting DNA isolates yielded different results on the DGGE. This suggests that there is a range or threshold where dilution of samples is a useful option for overcoming PCR inhibitors in fecal samples without compromising detection of prey DNA. Using Pacific walrus (*Odobenus rosmarus divergens*) feces, Bowles and Trites (2013) had similar amplification problems, which they attributed to non-specific binding primers, competition for primers, small amounts of predator DNA, or degraded DNA.

Our fecal sampling method could have affected the success of our prey detection. Deagle et al. (2005b), found that prey DNA detection was lower for samples taken from small distinct parts of individual scats than when larger samples were homogenized and then subsampled. We may have lowered our success rate, because we took a single small subsample of fecal content from the lower colon. However, another study, using the same single subsample method, and a similar group of ice seals (including many of these same individuals) produced ample prey DNA using high-throughput sequencing (Hundertmark & Horstmann-Dehn, unpublished data).

DGGE presents a number of practical challenges for diet analysis (King et al. 2008; Martin et al. 2006). Inconsistencies when pouring gels can alter the denaturing gradient, thus making band identification difficult (King et al. 2008). The DGGE can also distinguish DNA sequences with as little as one base pair difference; therefore, species with multiple alleles can confound the interpretation of bands (Deagle et al. 2005a). For example, we detected two putative haplotypes among bearded seals examined (Table 3.4 and Figure 3.2). These two haplotypes could be differentiated on the DGGE assays at 56ºC, but at 60ºC they showed identical migration characteristics.

If present, unidentifiable bands in the gel need to be excised and sequenced for proper species identification. To accurately identify all bands separated on the DGGE, a prey reference library would need to be constructed including all known prey species consumed. We chose a prey library that included many common fish prey species (identified in past studies to occur with >10% frequency in stomachs), yet we had more unknown than known DNA bands in our fecal samples. High-throughput sequencing techniques may prove more effective in identifying DNA of large numbers of prey taxa in fecal samples (Deagle et al. 2013; Pompanon et al. 2012), although this still requires a library of prey sequences. Given all the limitations outlined above, it is apparent that DGGE has very limited range of application as an efficient or effective method for diet analysis. Next generation sequencing technology has greater potential to produce representative lists of species composition in the diet. However, like DGGE, this approach requires development and optimization of primer sets that capture a diverse target group with minimal biases. Hybridization-capture approaches or the use of a large number of primer sets with complementary biases may prove useful refinements of DNA-based diet studies.

3.6 Conclusions

Despite the known biases and limitations, stomach contents generated a greater wealth of dietary information for bearded and ringed seals. Molecular DNA techniques have worked successfully for other pinnipeds and should have been equally successful for these ice-associated seals. We identified many possible caveats when extracting prey DNA from fecal samples that may help future studies refine their methods and lead to higher success. Although DGGE is quickly becoming an outdated technique, it can still be valuable and cost effective to answer dietary questions regarding a few prey species, specifically prey species that are generally difficult to identify in stomach contents, e.g., fish prey with fragile otoliths. Overall, stomach contents and molecular DNA techniques are useful and can be done in conjunction for a more complete description of diet.

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3.8 References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res 27:3389–3402

Antonelis GA, Melin SR, Bukhtiyarov YA (1994) Early spring feeding habits of bearded seals (*Erignathus barbatus*) in the Central Bering Sea, 1981. Arctic 47:74–79

Beitler, J (2012) Arctic sea ice extent settles at record seasonal minimum, National Snow and Ice Data Center. http://nsidc.org/arcticseaicenews/2012/09/arctic-sea-ice-extent-settles-at-record-seasonal-minimum/

Benjaminsen T (1973) Age determination and the growth and age distribution from cementum growth layers of bearded seals at Svalbard. Fiskeridir Skr Ser HavUnders 16:159–170

Bluhm B, Gradinger R (2008) Regional variability in food availability for Arctic marine mammals. Ecol Appl 18:S77–96

Bond AL, Diamond AW (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. Ecol Appl 21:1017–1023

Bowen WD (2000) Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. Can J Fish Aquat Sci 57:898–905

Bowen, WD, Iverson SJ (2013) Methods of estimating marine mammal diets: A review of validation experiments and sources of bias and uncertainty. Mar Mamm Sci 29:719–754

Bowles E, Trites AW (2013) Faecal DNA amplification in Pacific walruses (*Odobenus rosmarus divergens*). Polar Biol 36:755–759

Braley M, Goldsworthy SD, Page B, Steer M, Austin JJ (2010) Assessing morphological and DNA-based diet analysis techniques in a generalist predator, the arrow squid *Nototodarus gouldi*. Mol Ecol Resour 10:466–474

Budge SM, Iverson SJ, Koopman HN (2006) Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Mar Mamm Sci 22:759–801

Burns JJ (1981) Bearded seal (*Erignathus barbatus*). In: Ridgway SH, Harrison RJ (eds) Handbook of marine mammals, vol 2: Seals. Academic Press, New York, New York, USA, pp 145–170

Cooper MH, Budge SM, Springer AM, Sheffield G (2009) Resource partitioning by sympatric pagophilic seals in Alaska: monitoring effects of climate variation with fatty acids. 32:1137–1145

Deagle BE, Jarman SN, Pemberton D, Gales NJ (2005a) Genetic screening for prey in the gut contents from a giant squid (*Architeuthis* sp.). J Hered 96:417–423

Deagle BE, Thomas AC, Shaffer AK, Trites AW, Jarman SN (2013) Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: which counts count? Mol Ecol Resour 13:620–633

Deagle BE, Tollit DJ, Jarman SN, Hindell MA, Trites AW, Gales NJ (2005b) Molecular scatology as a tool to study diet: analysis of prey DNA in scats from captive Steller sea lions. Mol Ecol 14:1831–1842

Dehn L-A, Sheffield GG, Follmann EH, Duffy LK, Thomas DL, O'Hara TM (2007) Feeding ecology of phocid seals and some walrus in the Alaskan and Canadian Arctic as determined by stomach contents and stable isotope analysis. Polar Biol 30:167–181

Dunshea G, Barros NB, Berens McCabe EJ, Gales NJ, Hindell MA, Jarman SN, Wells RS (2013) Stranded dolphin stomach contents represent the free-ranging population's diet. Biol Letters 9: 1–5

Frost KJ (1981) Descriptive key to the otoliths of gadid fishes of the Bering, Chukchi, and Beaufort Seas. Arctic 34:55–59

Iverson SJ (2009) Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In: Arts MT, Brett MT, Kainz M (eds) Lipids in aquatic ecosystems. Springer, New York, New York, USA, pp 281–308

Iverson SJ, Field C, Bowen WD, Blanchard W (2004) Quantitative fatty acid signature analysis: A new method of estimating predator diets. Ecol Monogr 74:211–235

Jarman SN, Deagle BE, Gales NJ (2004) Group-specific polymerase chain reaction for DNA-based analysis of species diversity and identity in dietary samples. Mol Ecol 13:1313–1322

Jobling M, Breiby A (1986) The use and abuse of fish otoliths in studies of feeding habits of marine piscivores. Sarsia 71:265–274

King RA, Read DS, Traugott M, Symondson WOC (2008) Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol Ecol 17:947–963

Kovacs KM, Lydersen C (2008) Climate change impacts on seals and whales in the North Atlantic Arctic and adjacent shelf seas. Sci Progr 92:117–150

Kovacs KM, Lydersen C, Overland JE, Moore SE (2011) Impacts of changing sea-ice conditions on Arctic marine mammals. Mar Biodivers 41:181–194

Kreader CA (1996) Relief of amplification inhibition in PCR with bivine serum albumin or T4 gene 32 protein. Appl Environ Microbiol 62:1102–1106

Labansen AL, Lydersen C, Levermann N, Haug T, Kovacs KM (2011) Diet of ringed seals (*Pusa hispida*) from Northeast Greenland. Polar Biol 34:227–234

Lance MM, Chang W-Y, Jeffries SJ, Pearson SF, Acevedo-Gutiérrez A (2012) Harbor seal diet in northern Puget Sound: implications for the recovery of depressed fish stocks. Mar Ecol Prog Ser 464:257–271

Leu E, Wiktor J, Søreide J, Berge J, Falk-Petersen S (2010) Increased irradiance reduces food quality of sea ice algae. Mar Ecol Prog Ser 411:49–60

Lowry, LF, Frost KJ, and Burns JJ (1980*a*). Feeding of bearded seals in the Bering and Chukchi seas and trophic interaction with Pacific walruses. Arctic 33:330–342

Lowry, LF, Frost KJ, and Burns JJ (1980*b*). Variability in the diet of ringed seals, *Phoca hispida*, in Alaska. Can J Fish Aquat Sci 37:2254–2261

Mansfield AW, Fisher HD (1960) Age determination in the harbour seal, *Phoca vitulina* L. Nature 186:92–93

Martin DL, Ross RM, Quetin LB, Murray AE (2006) Molecular approach (PCR-DGGE) to diet analysis in young Antarctic krill *Euphausia superba*. Mar Ecol Prog Ser 319:155–165

McLaren IA (1958) The biology of the ringed seal (*Phoca hispida* Schreber) in the eastern Canadian Arctic. Bull Fish Res Board of Can No. 118:1–97

Monteiro L, Bonnemaison D, Vekris A, Petry KG, Bonnet J, Vidal R, Cabrita J, Megraud F (1997) Complex polysaccharides as PCR inhibitors in feces: *Helicobacter pylori* model. J Clin Microbiol 35:995–998

Morrow JE (1979) Preliminary keys to otoliths of some adult fishes of the Gulf of Alaska, Bering Sea, and Beaufort Sea. Department of Commerce, NOAA Technical Report, NMFS Circular 420. 32pp

Murie DJ, Lavigne DM (1986) Interpretation of otoliths in stomach content analyses of phocid seals: quantifying fish consumption. Can J Zool 64:1152–1157

Nagai M, Yoshida A, Sato N (1998) Additive effects of bovine serum albumin, dithiothreitol, and glycerol on PCR. Biochem and Mol Biol Int 44:157–163

Pierce GJ, Boyle PR (1991) A review of methods for diet analysis in piscivorous marine mammals. Oceanogr Mar Biol Annu Rev 29:409–486

Pompanon F, Deagle BE, Symondson WOC, Brown DS, Jarman SN, Taberlet P (2012) Who is eating what: diet assessment using next generation sequencing. Mol Ecol 21:1931–1950

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions Ecology 83:703–718

QIAGEN (2010) QIAamp® DNA Stool Handbook for DNA purification from stool samples. April 2010. Second Ed. 43pp.

QIAGEN (2011) Gentra® Puregene® Handbook. June 2011. Third Ed. 72pp.

Quakenbush L, Citta J, Crawford J (2011a) Biology of the bearded seal (*Erignathus barbatus*) in Alaska, 1961–2009. Final Report to National Marine Fisheries Service from the Alaska Department of Fish and Game. 71pp.

Quakenbush L, Citta J, Crawford J (2011b) Biology of the ringed seal (*Phoca hispida*) in Alaska, 1960–2010. Final Report to National Marine Fisheries Service from the Alaska Department of Fish and Game. 72pp.

Rosen DAS, Tollit DJ (2012) Effects of phylogeny and prey type on fatty acid calibration coefficients in three pinniped species: implications for the QFASA dietary quantification technique. Mar Ecol Prog Ser 467:263–276

Schrader C, A. S, Ellerbroek L, Johne R (2012) PCR inhibitors – occurrence, properties and removal. J Appl Microbiol 113:1014–1026

Sheffield GG, Fay FH, Fedak MA, Kelly BP (2001) Laboratory digestion of prey and interpretation of walrus stomach contents. Mar Mamm Sci 17:310–330

Sheffield VC, Cox DR, Lerman LS, Myers RM (1989) Attachment of a 40-base-pair G+C-rich sequence (GC-clamp) to genomic DNA fragments by the polymerase chain-reaction results in imporved detection of single-base changes. Proc Natl Acad Sci USA 86:232–236

Stewart REA, Stewart BE, Stirling I, Street E (1996) Counts of growth layer groups in cementum and dentine in ringed seals (*Phoca hispida*). Mar Mamm Sci 12:383–401

Stroeve, J C, Serreze MC, Holland MM, Kay JE, Malanik J, and Barrett AP (2012) The Arctic's rapidly shrinking sea ice cover: a research synthesis. Clim Change 110:1005–1027

Tollit DJ, Schulze AD, Trites AW, Olesiuk PF, Crockford SJ, Gelatt TS, Ream RR, Miller KM (2009) Development and application of DNA techniques for validating and improving pinniped diet estimates. Ecol Appl 19:889–905

Trites AW, Donnelly CP (2003) The decline of Steller sea lions *Eumetopias jubatus* in Alaska: a review of the nutritional stress hypothesis. Mamm Rev 33:3–28

Trumble SJ, Barboza PS, Castellini MA (2003) Digestive constraints on an aquatic carnivore: effects of feeding frequency and prey composition on harbor seals. J Comp Physiol B 173:501–509

Trumble SJ, Castellini MA (2005) Diet mixing in an aquatic carnivore, the harbour seal. Can J Zool 83:851–859

U.S. Federal Register (2012a) Endangered and threatened species; threatened status for the Beringia and Okhotsk distinct population segments of the *Erignathus barbatus nauticus* subspecies of bearded seal. FR 77(249):76739–76768. U.S. Department of Commerce N, National Marine Fisheries Service

U.S. Federal Register (2012b) Endangered and threatened species; threatened status for the Arctic, Okhotsk, and Baltic subspecies of the ringed seal and endangered status for the Ladoga subspecies of the ringed seal. FR 77(249):76705–76738. U.S. Department of Commerce N, National Marine Fisheries Service

Vestheim H, Jarman SN (2008) Blocking primers to enhance PCR amplification of rare sequences in mixed samples – a case study on prey DNA in Antarctic krill stomachs. Front in Zool 5:12

Wang SW, Budge S, Gradinger RR, Iken K, Wooller M (2014) Fatty acid and stable isotope characteristics of sea ice and pelagic particulate organic matter in the Bering sea: tools for estimating sea ice algal contribution to Arctic food web production. Oecologia. 174:699–712

3.9 Tables

Table 3.1 Seals collected for stomach content and DNA diet analyses

Sample sizes by collection year and sex of bearded and ringed seals harvested for subsistence purposes at Barrow and Point Hope, Alaska. Stomachs and lower colons were collected and used for diet analysis.

Species	Year	n	Location	Sex (M, F)	Month
Bearded seal	2008	1	Point Hope	(1, 0)	June
	2010	13	Point Hope	(2, 11)	June
	2010	8	Barrow	(2, 6)	June–July
Ringed seal	2010	1	Point Hope	(1, 0)	June
	2010	3	Barrow	(0, 3)	June–July
	2011	6	Barrow	(2, 4)	June–July

Table 3.2 Percent frequency of occurrence of fishes in bearded seal stomachs

Percent frequency of occurrence (%FO) of fishes identified from stomach contents of bearded seals collected during subsistence harvests in Alaska, 2008 and 2010 (n=21, 1 stomach contained only invertebrates). %FO is the number of stomachs containing a fish taxon divided by the total number of stomachs containing fish (x100).

Fishes	Percent Frequency
All Gadidae	81
Arctic cod, *Boreogadus saida*	76
Saffron cod, *Eleginus gracilis*	38
Walleye pollock, *Gadus chalcogrammus* or *Theragra chalcogramma*	5
All Cottidae	81
Arctic staghorn sculpin, *Gymnocanthus tricuspis*	38
Sculpin species, *Gymnocanthus* spp.	10
Sculpin species, *Hemilepidotus* spp.	14
Sculpin species, *Icelus* spp.	10
Brightbelly sculpin, *Microcottus sellaris*	14
Arctic sculpin, *Myoxocephalus scorpioides*	10
Shorthorn sculpin, *Myoxocephalus scorpius*	29
Sculpin species, *Myoxocephalus* spp.	57
Ribbed sculpin, *Triglops pingelii*	5
Sculpin species, *Triglops* spp.	10
All Liparidae	19
Snailfish species, *Careproctus* spp.	5
Variegated snailfish, *Liparis gibbus*	5
Snailfish species, *Liparis* spp.	5
All Zoarcidae	24
Wattled eelpout, *Lycodes palearis*	5
Polar eelpout, *Lycodes polaris*	5
Eelpout species, *Lycodes* spp.	14
All Stichaeidae	57
Daubed shanny, *Leptoclinus maculatus*	19
Slender eelblenny, *Lumpenus fabricii*	48
Slender eelblenny or snake prickleback, *Lumpenus* spp.	5
All Ammodytidae	33
Pacific sand lance, *Ammodytes hexapterus*	33

Table 3.2 Continued.

All Pleuronectidae	52
Yellowfin sole flounder, *Limanda aspera*	19
Longhead dab, *Limanda proboscidea*	52
Righteye flounder species, *Limanda* spp.	10
Arctic flounder, *Pleuronectes glacialis*	5
All Unidentified fish	29
Minimum no. of fish species eaten	20
Minimum no. of fish eaten	1,810

Table 3.3 Percent frequency of occurrence of fishes in ringed seal stomachs

Percent frequency of occurrence (%FO) of fishes identified from stomach contents of ringed seals collected during subsistence harvests in Alaska, 2010 and 2011 (n=4, 5 stomachs were empty and one contained only invertebrates). %FO is the number of stomachs containing a fish taxon divided by the total number of stomachs containing fish (x100).

Fishes	Percent Frequency
All Gadidae	75
Arctic cod, *Boreogadus saida*	75
Saffron cod, *Eleginus gracilis*	25
All Cottidae	50
Arctic staghorn sculpin, *Gymnocanthus tricuspis*	25
Sculpin species, *Hemilepidotus* spp.	25
Sculpin species, *Icelus* spp.	25
All Ammodytidae	50
Pacific sand lance, *Ammodytes hexapterus*	50
Minimum no. of fish species eaten	6
Minimum no. of fish eaten	44

Table 3.4 DNA bands identified from bearded and ringed seal feces

Variable DNA bands (and their interpretation) identified from feces using Denaturing Gradient Gel Electrophoresis (DGGE). Twelve bearded and one ringed seal produced DNA products.

Seal	Species	Arctic cod, *Boreogadus saida*	Shorthorn sculpin, *Myoxocephalus scorpius*	Snailfish species, Liparidae	Unidentified fish	Bearded seal, *Erignathus barbatus*	Ringed seal, *Pusa hispida*
1	Bearded		●	○	●(1), ○(1)	●†	
2	Bearded				○(1)	●	○
3	Bearded				○(1)	●	
4	Bearded		●		○(4)	●†	
5	Bearded		○		○(1)	●†	
6	Bearded				○(1)	●	
7	Bearded		●		○(1)	●	
8	Bearded				○(1)	●†	
9	Bearded				○(1)	●	
10	Bearded				○(1)	●	
11	Bearded		●		○(5)	●	
12	Bearded		○		○(1)	●	
13	Ringed	●	●				●

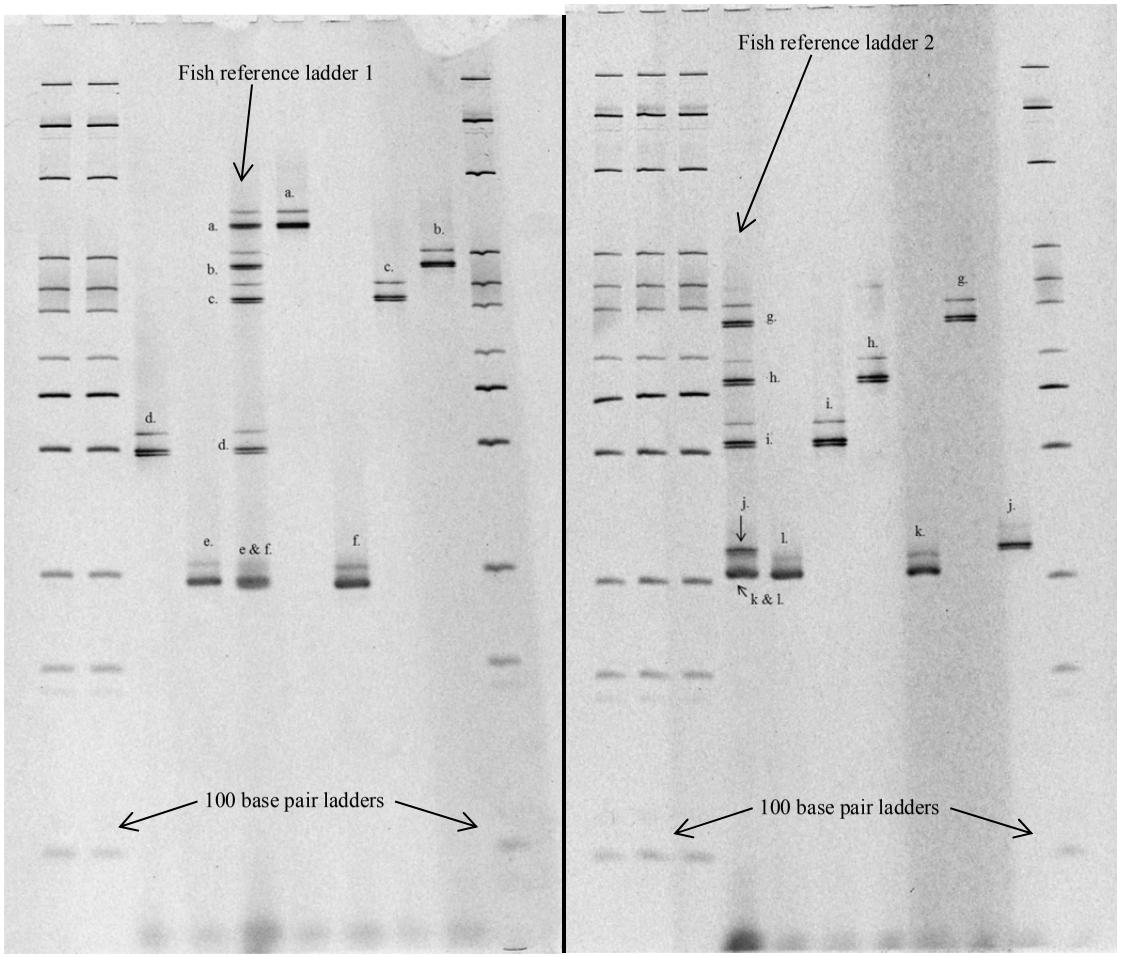
● Strong band
○ Weak band

† These bands traveled slightly further at 56°C than the reference bearded seal DNA, but in line with the reference bearded seal DNA at 60°C. We hypothesize that this is a second bearded seal allele.

Table 3.5 Percent frequency of occurrence of fishes in both stomach contents and DNA

Percent frequency of occurrence (%FO) of the 12 fish species included in the DNA reference ladder identified from stomach contents and fecal DNA from 12 bearded seals. %FO is the number of stomachs or fecal samples containing a fish taxon divided by the total number of stomachs or fecal samples containing prey (x100).

	Stomach contents	DNA
Fishes		
Rainbow smelt, *Osmerus mordax*	-	-
Arctic cod, *Boreogadus saida*	67	-
Saffron cod, *Eleginus gracilis*	50	-
Walleye pollock, *Gadus chalcogrammus* or *Theragra chalcogramma*	-	-
Arctic staghorn sculpin, *Gymnocanthus tricuspis*	50	-
Shorthorn sculpin, *Myoxocephalus scorpius*	33	50
Snailfish species, Liparidae	25	8
Polar eelpout, *Lycodes polaris*	-	-
Slender eelblenny, *Lumpenus fabricii*	67	-
Pacific sand lance, *Ammodytes hexapterus*	50	-
Yellowfin sole flounder, *Limanda aspera*	33	-
Longhead dab, *Limanda proboscidea*	67	-
All unidentified fish	42	100



a. Walleye Pollock (*Gadus chalcogrammus or Theragra chalcogramma*)
b. Saffron cod (*Eleginus gracilis*)
c. Arctic cod (*Boreogadus saida*)
d. Rainbow smelt (*Osmerus mordax*)
e. Shorthorn sculpin (*Myoxocephalus scorpius*)
f. Pacific sand lance (*Ammodytes hexapterus*)
g. Snailfish species (Liparidae)
h. Polar eelpout (*Lycodes polaris*)
i. Slender eelblenny (*Lumpenus fabricii*)
j. Arctic staghorn sculpin (*Gymnocanthus tricuspis*)
k. Longhead dab (*Limanda proboscidea*)
l. Yellowfin sole (*Limanda aspera*)

Figure 3.1 DNA gel image of reference fishes

DNA from 12 reference fish species separated using Denaturing Gradient Gel Electrophoresis (DGGE) at 56˚C. Bands from known fishes were used to identify fish bands from seal fecal DNA.

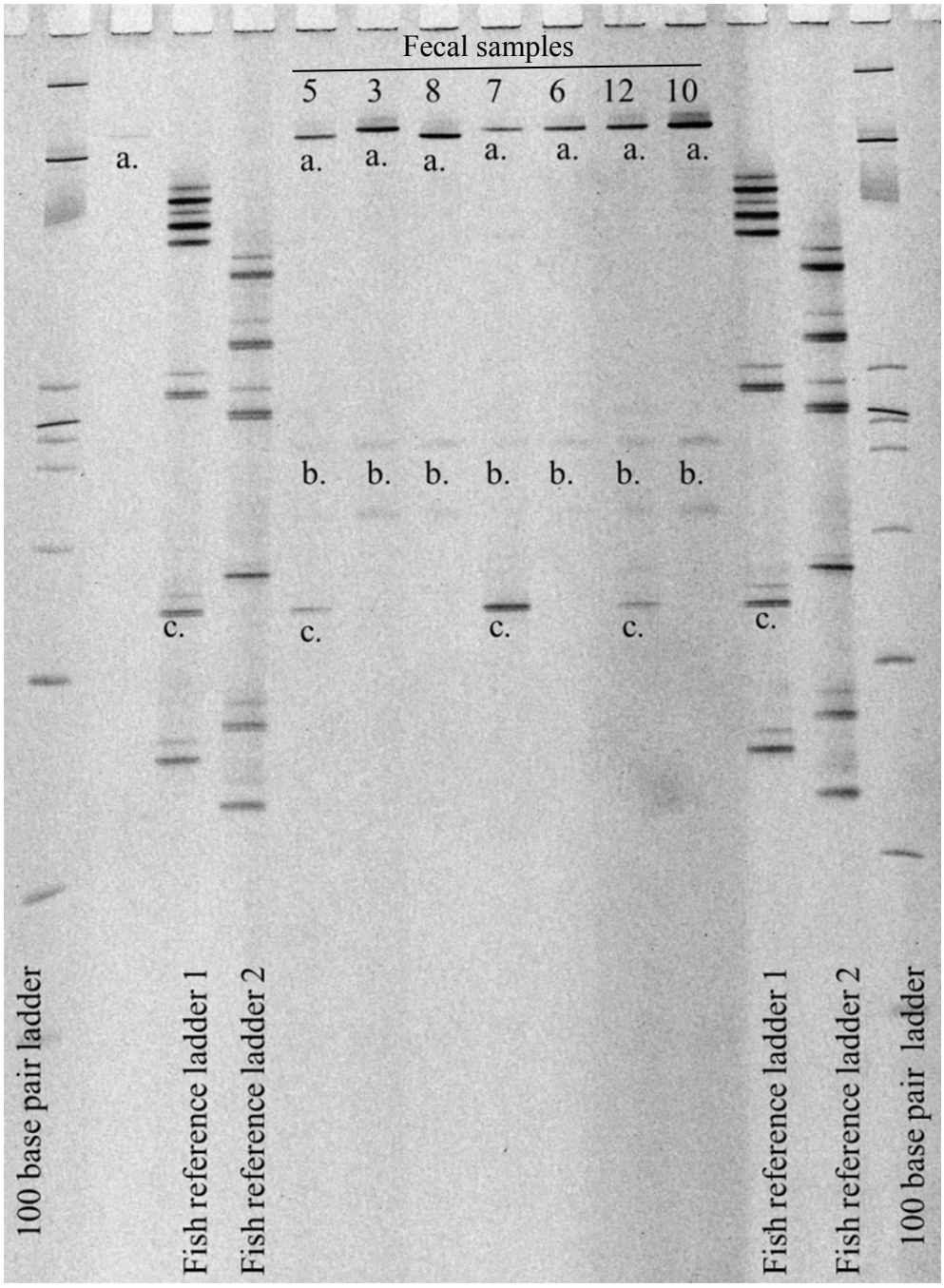


Figure 3.2 DNA gel image of seal fecal DNA and reference fishes

Fecal DNA from seven bearded seals separated using Denaturing Gradient Gel Electrophoresis (DGGE) at 60˚C. Bands in the fecal samples were identified by comparing against known fish prey and bearded seal DNA. Bands were only counted as matches if they lined up with a reference band at both 56˚C and 60˚C. Bands identified in these fecal samples include a. bearded seal DNA, b. unidentified fish, and c. shorthorn sculpin. Fecal sample ID numbers correspond to the seal sample numbers in Table 3.4.

3.11 Appendix

Appendix 3.11A. Co-author approval to include DNA chapter in thesis

Bryan, Anna L (DFG)

From: andresl.fish@gmail.com on behalf of Andres Lopez <jalopez2@alaska.edu>
Sent: Thursday, June 26, 2014 11:10 AM
To: Bryan, Anna L (DFG); UAF-Grad-School@alaska.edu
Subject: Re: I need your permission to use the DNA chapter in my thesis

Hi Anna,

I approve the inclusion of your DNA-based diet study in your thesis. I have copied the Graduate School on this message.

Best wishes for Wednesday!
Andres Lopez

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Chapter 4: General Conclusion

The arctic marine ecosystem is changing due to sea ice loss, and the changes will have an effect on the marine food web (Grebmeier et al. 2006), but the extent and exact impacts are unknown (Bluhm and Gradinger 2008). Additionally, these changes are not predicted to be uniform throughout the Arctic (Kovacs and Lydersen 2008). Changing sea ice dynamics could alter the pelagic-benthic coupling, leading to a shift from a benthic-dominated ecosystem to a more pelagic ecosystem (Grebmeier et al. 2006, Bluhm and Gradinger 2008). Alterations in primary production could also alter the nutritional quality of prey due to changes in fatty acid profiles of primary producers that then propagate up the food chain (Leu et al. 2010, Wang et al. 2014). Polyunsaturated fatty acids (PUFAs), such as n-3 and n-6 fatty acids are important for a variety of reasons including the development of eyes and brain, and membrane fluidity (Barboza et al. 2009), and PUFAs require less oxygen to fully metabolize (Gurr et al. 2002). Essential fatty acids (i.e., n-3 and n-6) can only be acquired from diet (Barboza et al. 2009). An alteration in nutritional quality of prey could lead to less fat storage, more energy needed for thermoregulation (Rosen et al. 2007), and a reduction in available energy and resources for reproduction and disease resistance, leading to decreased health, and ultimately resulting in population declines (Burek et al. 2008). Consequently, investigations like the present study that enhance our knowledge of ice seal diets in the rapidly changing arctic ecosystem remain important. Over the past decades, a wealth of dietary information has been generated for arctic predators using a range of techniques (e.g., Johnson et al. 1966; Lowry et al. 1980*a*, *b*; Seaman et al. 1982; Hoekstra et al. 2002; Dehn et al. 2007; Budge et al. 2008; Cooper et al. 2009; Loseto et al 2009; Sheffield and Grebmeier 2009; Quakenbush et al. 2011*b*; Bowles and Trites 2013;

Carroll et al. 2013; Seymour et al. 2014*a, b*). Yet, little is known about how the dietary information obtained using these different methods is inter-related, thus making direct or even temporal comparisons challenging.

This study used a combination of stomach contents, stable isotopes, and fatty acids to compare the dietary information generated by each method for a group of bearded seals (*Erignathus barbatus*), and stomach contents and fecal DNA to identify fishes in the diet of bearded and ringed seals (*Pusa hispida*). All these methods used in both chapters 2 ("Identifying bearded seal diet – a comparison of individual seals using stomach contents, stable isotopes, and fatty acids") and 3 ("Fish prey in bearded and ringed seal diet – a comparison of stomach contents and fecal DNA") provided dietary information, but the extent and overlap differed. The time frame captured by different dietary methods also varied substantially, making direct comparisons difficult. Stomach content and DNA analyses provided taxonomic information on the species consumed during recent feeding (Pitcher 1980, Jobling and Breiby 1986, Sheffield et al. 2001, Deagle et al. 2005). On the other hand, stable carbon and nitrogen isotopes and fatty acids provided more general information on diet integrated into the tissues of seals over time. Blubber fatty acids represent diet integrated over weeks to months (Bowen and Iverson 2013), and muscle stable carbon and nitrogen isotopes represent diet integrated over months to years (Young and Ferguson 2013, Seymour et al. 2014*a*). The exact dietary turnover time is unknown and varies with body mass, physiological state, and metabolic rate of the predator (Newsome et al. 2010, Seymour et al. 2014*a*). In addition, turnover rates of muscle and blubber may be different for different compounds (e.g., differential turnover or mobilization of saturated fatty acids and n-3 fatty acids, e.g., Wheatley et al. 2008).

In chapter 2, a direct comparison of stomach content, stable isotope, and fatty acid analyses was used to identify bearded seal diet. Stomach contents provided the most taxonomic information with over 60 fish and invertebrate prey taxa identified in bearded seals. The proportions of prey source groups detected using a stable isotope mixing model were similar to the relative occurrence (RO) of source groups from stomach contents. However, due to model restrictions, prey included in the model were limited to frequently consumed taxa and had to be pooled into prey source groups due to overlapping isotopic space. Fatty acid analysis of bearded seal blubber was limited to qualitative differences among individual bearded seals, because fatty acid data are lacking for prey items from the Bering, Chukchi, and Beaufort seas. The proportions of indicator fatty acids, from full-thickness blubber, such as 20:4n-6, 20:1n-9, and 22:1n-11, and the presence of non-methylene-interrupted fatty acids were similar to other fatty acid signature studies of bearded seals in Alaska that suggest a benthic diet (Budge et al. 2007, Cooper et al. 2009), in accordance with our stomach contents and stable isotope data.

In chapter 3, fishes identified with stomach content and DNA analyses were compared for bearded and ringed seals. Stomach contents generated a greater wealth of dietary information for both species. Using stomach contents, we were able to identify 20 fish species and 1,855 individual fish in bearded seals, and six fish species and 44 individual fish in ringed seals. On the other hand, with denaturing gradient gel electrophoresis (DGGE), only three of twelve reference fishes were identified in the feces of ringed and bearded seals (Arctic cod, *Boreogadus saida*; shorthorn sculpin, *Myoxocephalus scorpius*; and an unknown snailfish species, family Liparidae). However, otoliths from all but one of the 12 reference fishes (walleye pollock, *Gadus chalcogrammus* or *Theragra chalcogramma*) were found in the stomachs. In our efforts to implement DGGE-based DNA analysis, we encountered numerous limitations and problems

with the DNA method, including primer design, PCR amplification, and effectiveness of DGGE at determining diet. Similar problems have been described in previous studies (Deagle et al. 2005; King et al. 2008). These limitations could be mitigated in the future if primers and protocols were optimized for specific seal species (King et al. 2008).

The application of stomach contents, stable isotopes, fatty acids, and fecal DNA to determine diet also depends on the research question. For general questions or comparisons (e.g., differences in diet over time or among groups), stable isotopes and fatty acid signatures may be useful (Tollit et al. 2010). However, if the desired information is to expand upon what prey taxa are being consumed by a predator, then the analysis would currently be limited to stomach contents, prey DNA identification, or quantitative mixing models using stable isotopes or fatty acids if the predator consumes a small number of distinct prey (e.g., Bentzen et al. 2007, Tollit et al. 2010, Bowen and Iverson 2013, Seymour et al. 2014*b*). Feeding ecology approaches that rely on chemical methods become less useful if the number of prey species consumed by a predator is large and chemical signatures of prey overlap. In addition, taxonomic information provided by stomach contents is essential for developing prey libraries used in stable isotope mixing models, quantitative fatty acid analysis, and prey DNA comparisons making stomach contents the "gold standard" for diet analyses. However, stable isotope and fatty acid analyses have advantages because they can be conducted on archived (Newsome et al. 2009, Lind et al. 2012) or archeological (Misarti et al. 2009, Gregg et al. 2010, Newsome et al. 2010) specimens to gain insight into past conditions and diet. Additionally, stable isotope analysis of metabolically inert tissues, such as whiskers, claws, and teeth, are also useful for studying long-term variability in the diet of individual animals (Newsome et al. 2010, Carroll et al. 2013).

The impact on the animal being sampled is not the same for stomach contents, stable isotopes, fatty acids, and fecal DNA. Collection of stomach contents are invasive and generally require dead animals (Pierce and Boyle 1991), whereas tissues for stable isotope and fatty acid analysis can be collected with minimal to moderate invasive sampling from live animals (Tollit et al. 2010). Also, when available, feces used for DNA analysis can be easily collected from live animals at haulout sites or in the water without any physical disturbance to the animal (Tollit et al. 2010). Without regular access to animals via subsistence harvest, by-catch, or stranding, stable isotopes, fatty acids, and fecal DNA are good, minimally invasive, options for assessing the diet of free-ranging marine mammals.

Using a combination of stomach contents, stable isotopes, fatty acids, and fecal DNA in conjunction with each other will increase the amount of dietary information provided. For example, by using both stomach contents and stable isotope mixing model data, we were able to identify differences in the proportion of octopus in the diet of bearded seals during both recent foraging (stomach contents) and long-term diet (mixing model). Additionally, although the number of fish species we identified from stomach contents was greater than with fecal DNA, when combined, we were able to increase the frequency of occurrence for shorthorn sculpin for both bearded and ringed seals. Previous studies have also demonstrated that DNA and stomach contents can be combined to increase prey detection for generalist predators (Tollit et al. 2009, Braley et al. 2010, Dunshea et al. 2013). Newer high-throughput DNA sequencing techniques have been successfully used to identify 62 prey taxa in Australian fur seal (*Arctocephalus pusillus doriferus*) feces (Deagle et al. 2009). Although high-throughput sequencing can generate large amounts of DNA data, it still requires a library of prey sequences to interpret the

results. Thus, DNA approaches may compliment stomach contents, but are lacking without preexisting information provided by prey identification from stomachs.

For future diet studies of bearded and ringed seals in Alaska, the continued use of stomach content, stable isotope, fatty acid, and DNA analyses is recommend. Using combinations of these methods can help determine whether prey taxa are changing or whether the prey taxa are stable, but the nutritional content is altered. This is especially true for dietary methods that combine techniques and information, such as compound-specific stable isotope analysis, which can help identify some molecular changes in the nutritional make-up of prey (Budge et al. 2008, Wang et al. 2014). Analyzing the stable isotopes of whiskers or claws can provide individual variability in foraging over time (Carroll et al. 2013, Seymour et al. 2014*a*), and the use of archived or archeological samples can indicate how much variability existed in the diet of bearded and ringed seals before recent climate changes started to occur, i.e., before baselines have started to shift. Finally, the combination of diet analysis and physiological parameters, such as stable isotopes and cortisol levels (Bryan et al. 2013), could be a valuable tool for relating diet to physiological health of an animal.

The comparison of stomach contents, stable isotopes, fatty acids, and fecal DNA, as part of this research shed light on several factors that could be improved upon to increase prey detection when utilizing these methods to estimate ice seal diet. First, the development of a fatty acid prey library for arctic pinnipeds and other marine mammals in Alaska is crucial, so that fatty acids can be used quantitatively to identify potential changes to their diet. Second, due to stable isotopic variation among the Bering, Chukchi, and Beaufort seas, and ice seals migrating throughout this vast region (Frost et al. 2008, Cameron and Boveng 2009, Crawford et al. 2012), we recommend that separate stable isotope prey libraries be created for these Alaska waters.

Multiple prey libraries would allow researchers to compare the prey proportions of pinniped diet for each of these waters and potentially show if there is a greater reliance on one habitat over the other. Information on key foraging areas could aid in identifying important foraging habitat; this information is extremely valuable as the Pacific population of bearded seals and the Arctic Basin ringed seals were listed as threatened under the Endangered Species Act, and federal managers are required to designate critical habitat for these species (U.S. Federal Register. 2012*a*, *b*). Lastly, controlled feeding studies on ice-associated pinnipeds are recommended to determine the tissue turnover rate of both blubber fatty acids and muscle stable carbon and nitrogen isotopes.

In this study, stomach contents provided more dietary information on important prey taxa than stable isotope, fatty acid, and fecal DNA analyses for bearded seals, and more taxonomic information than fecal DNA for ringed seals. Although our implementation of the DGGE DNA method was fairly unsuccessful, molecular DNA techniques have worked effectively for other pinnipeds (Deagle et al. 2005, Tollit et al. 2009) and should have been equally successful for these ice-associated seals. High-throughput sequencing techniques may prove more operational in identifying DNA of large numbers of prey taxa in fecal samples (Pompanon et al. 2012, Deagle et al. 2013). Overall, for bearded seals, stomach contents, stable isotopes, and fatty acids all yielded different, but not necessarily contradictory results. All methods indicated that these seals are continuing to forage on a wide variety of benthic and pelagic prey taxa. The generalist strategy of bearded seals, and the use of both benthic and pelagic prey, may make them less vulnerable to climate induced changes to benthic ecosystem productivity (Bluhm and Gradinger 2008). The continued use of all these dietary methods, as well as applications of state-of-the-art techniques (such as compound-specific stable isotope analysis), will help to detect and interpret changes in the diet of ice-associated marine mammals as habitats change in the Arctic.

4.1 General References

Antonelis, G. A., S. R. Melin, and Y. A. Bukhtiyarov. 1994. Early spring feeding habits of bearded seals (*Erignathus barbatus*) in the Central Bering Sea, 1981. Arctic 47:74–79.

Barboza P.S., K. L. Parker, and I. D. Hume. 2009. Integrative wildlife nutrition. Springer. Verlag Berlin Heidelberg, Germany.

Beitler, J. 2012. Arctic sea ice extent settles at record seasonal minimum, National Snow and Ice Data Center. http://nsidc.org/arcticseaicenews/2012/09/arctic-sea-ice-extent-settles-at-record-seasonal-minimum/.

Bentzen, T. W., E. H. Follmann, S. C. Amstrup, G. S. York, M. J. Wooller, and T. M. O'Hara. 2007. Variation in winter diet of southern Beaufort Sea polar bears inferred from stable isotope analysis. Canadian Journal of Zoology 85:596–608.

Bluhm, B., and R. Gradinger. 2008. Regional variability in food availability for Arctic marine mammals. Ecological Applications 18:S77–S96.

Bond, A. L., and A. W. Diamond. 2011. Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. Ecological Applications 21:1017–1023.

Bowen, W. D. 2000. Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. Canadian Journal of Fisheries and Aquatic Sciences 57:898–905.

Bowen, W. D. and S. J. Iverson. 2013. Methods of estimating marine mammal diets: A review of validation experiments and sources of bias and uncertainty. Marine Mammal Science 29: 719–754.

Bowles E., and A. W. Trites. 2013. Faecal DNA amplification in Pacific walruses (*Odobenus rosmarus divergens*). Polar Biology 36:755–759.

Braley, M., S. D. Goldsworthy, B. Page, M. Steer, and J. J. Austin. 2010. Assessing morphological and DNA-based diet analysis techniques in a generalist predator, the arrow squid *Nototodarus gouldi*. Molecular Ecology Resources 10:466–474.

Bryan, H. M., C. T. Darimont, P. C. Paquet, K. E. Wynne-Edwards, and J. E. G. Smits. 2013. Stress and reproductive hormones in grizzly bears reflect nutritional benefits and social consequences of a salmon foraging niche. PLoS ONE 8:e80537.

Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Marine Mammal Science 22:759–801.

Budge, S. M., A. M. Springer, S. J. Iverson, and G. Sheffield. 2007. Fatty acid biomarkers reveal niche separation in an Arctic benthic food web. Marine Ecology Progress Series 336:305–309.

Budge, S. M., M. J. Wooller, A. M. Springer, S. J. Iverson, C. P. McRoy, and G. J. Divoky. 2008. Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. Oecologia 157:117–129.

Burek, K. A., F. M. D. Gulland, and T. M. O'Hara. 2008. Effects of climate change on Arctic marine mammal health. Ecological Applications 18:S126–S134.

Burns, J. J. 1981. Bearded seal (*Erignathus barbatus*). Pages 145–170 *in* S. H. Ridgway and R. J. Harrison, editors. Handbook of marine mammals. Volume 2: Seals. Academic Press, New York, New York, USA.

Cameron, M. F., and P. L. Boveng. 2009. Habitat use and seasonal movements of adult and sub-adult bearded seals. Alaska Fisheries Science Center Quarterly Report, October-November-December 2009, 1–4 pp.

Carroll, S. S., L. Horstmann-Dehn, and B. L. Norcross. 2013. Diet history of ice seals using stable isotope ratios in claw growth bands. Canadian Journal of Zoology 91:191–202.

Clementz, M. T., and P. L. Koch. 2001. Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. Oecologia 129:461–472.

Cooper, M. H., S. M. Budge, A. M. Springer, and G. Sheffield. 2009. Resource partitioning by sympatric pagophilic seals in Alaska: monitoring effects of climate variation with fatty acids. Polar Biology 32:1137–1145.

Crawford, J. A., K. J. Frost, L. T. Quakenbush, and A. Whiting. 2012. Different habitat use strategies by subadult and adult ringed seals (*Phoca hispida*) in the Bering and Chukchi seas. Polar Biology 35: 241–255.

Deagle, B., A. Chiaradia, J. McInnes, and S. N. Jarman. 2010. Pyrosequencing faecal DNA to determine diet of little penguins: is what goes in what comes out? Conservation Genetics 11:2039–2048.

Deagle, B. E., R. Kirkwood, and S. N. Jarman. 2009. Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. Molecular Ecology 18:2022–2038.

Deagle, B. E., A. C. Thomas, A. K. Shaffer, A. W. Trites, and S. N. Jarman. 2013. Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: which counts count? Molecular Ecology Resources 13:620–633.

Deagle, B. E., D. J. Tollit, S. N. Jarman, M. A. Hindell, A. W. Trites, and N. J. Gales. 2005. Molecular scatology as a tool to study diet: analysis of prey DNA in scats from captive Steller sea lions. Molecular Ecology 14:1831–1842.

Dunshea, G., N. B. Barros, E. J. Berens McCabe, N. J. Gales, M. A. Hindell, S. N. Jarman, and R. S. Wells. 2013. Stranded dolphin stomach contents represent the free-ranging population's diet. Biology Letters 9:1–5.

Dehn, L.-A., G. G. Sheffield, E. H. Follmann, L. K. Duffy, D. L. Thomas, and T. M. O'Hara. 2007. Feeding ecology of phocid seals and some walrus in the Alaskan and Canadian Arctic as determined by stomach contents and stable isotope analysis. Polar Biology 30:167–181.

Frost, K. J., and L. F. Lowry. 1981. Ringed, Baikal and Caspian seals—*Phoca hispida*, *Phoca sibirica*, and *Phoca caspica*. Pages 29–53 *in* S. H. Ridgway and R. J. Harrison, editors. Handbook of Marine Mammals. Volume 2. Seals: Academic Press, New York, New York, USA.

Frost, K. J., A. Whiting, M. F. Cameron, and M. A. Simpkins. 2008. Habitat use, seasonal movements and stock structure of bearded seals in Kotzebue Sound, Alaska. Tribal Wildlife Grants Program, Fish and Wildlife Service, Tribal Wildlife Grants Study U-4-IT. Final report from the Native Village of Kotzebue, Kotzebue, AK, for U.S. Fish and Wildlife Service, Anchorage, AK.16 pp.

Grebmeier, J. M., C. P. McRoy, and H. M. Feder. 1988. Pelagic-benthic coupling on the shelf of the northern Bering and Chukchi Seas. I. Food supply source and benthic biomass. Marine Ecology Progress Series 48:57–67.

Grebmeier, J. M., J. E. Overland, S. E. Moore, E. V. Farley, E. C. Carmack, L. W. Cooper, K. E. Frey, J. H. Helle, F. A. McLaughlin, and S. L. McNutt. 2006. A major ecosystem shift in the northern Bering Sea. Science 311:1461–1464.

Gregg, M. W., and G. F. Slater. 2010. A new method for extraction, isolation and transesterification of free fatty acids from archaeological pottery. Archaeometry 52:833–854.

Gurr, M. I., J. L. Harwood, and K. N. Frayn. 2002. Lipid Biochemistry. Oxford: Blackwell Science.

Hindell, M. A., C. Lydersen, H. Hop, and K. M. Kovacs. 2012. Pre-Partum diet of adult female bearded seals in years of contrasting ice conditions. PLoS ONE 7:e38307.

Hobson, K. A., D. M. Schell, D. Renouf, and E. Noseworthy. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Canadian Journal of Fisheries and Aquatic Sciences 53:528–533.

Hobson, K. A., and H. E. Welch. 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Marine Ecology Progress Series 84:9–18.

Hoekstra, P. F., L. A. Dehn, J. C. George, K. R. Solomon, D. C. G. Muir, and T. M. O'Hara. 2002. Trophic ecology of bowhead whales (*Balaena mysticetus*) compared with that of other Arctic marine biota as interpreted from carbon-,nitrogen-, and sulfur-isotope signatures. Canadian Journal of Zoology 80:223–231.

Iken, K., B. Bluhm, and K. Dunton. 2010. Benthic food-web structure under differing water mass properties in the southern Chukchi Sea. Deep-Sea Research II 57:71–85.

Iverson, S. J. 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. Pages 281–308 *in* M. T. Arts, M. T. Brett, and M. Kainz, editors. Lipids in Aquatic Ecosystems. Springer, New York, New York, USA.

Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: A new method of estimating predator diets. Ecological Monographs 74:211–235.

Jarman, S. N., B. E. Deagle, and N. J. Gales. 2004. Group-specific polymerase chain reaction for DNA-based analysis of species diversity and identity in dietary samples. Molecular Ecology 13:1313–1322.

Jobling, M., and A. Breiby. 1986. The use and abuse of fish otoliths in studies of feeding habits of marine piscivores. Sarsia 71:265–274.

Johnson, M. L., C. H. Fiscus, B. T. Ostenson, and M. L. Barbour. 1966. Marine mammals. Pages 877–924 *in* N.J. Wilimovsky and J. N. Wolfe, editors. Environment of the Cape Thompson region, Alaska. U.S. Atomic Energy Commission, Oak Ridge, Tennessee, USA.

Kelly, J. F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78:1–27.

Kelly, J. R., and R. E. Scheibling. 2012. Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series 446:1–22.

King, R. A., D. S. Read, M. Traugott, and W. O. C. Symondson. 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Molecular Ecology 17:947–963.

Kovacs, K. M., and C. Lydersen. 2008. Climate change impacts on seals and whales in the North Atlantic Arctic and adjacent shelf seas. Science Progress 91:117–150.

Kovacs, K. M., C. Lydersen, J. E. Overland, and S. E. Moore. 2011. Impacts of changing sea-ice conditions on Arctic marine mammals. Marine Biodiversity 41:181–194.

Leu, E., J. Wiktor, J. Søreide, J. Berge, and S. Falk-Petersen. 2010. Increased irradiance reduces food quality of sea ice algae. Marine Ecology Progress Series 411:49–60.

Lind, Y., B.-M. Bäcklin, K. Lundström, S. M. Budge, M. Walton, and O. Karlsson. 2012. Stability of fatty acid composition in seal blubber during long-term storage. Marine Ecology Progress Series 461:283–291.

Loseto, L. L., G. A. Stern, T. L. Connelly, D. Deibel, B. Gemmill, A. Prokopowicz, L. Fortier, and S. H. Ferguson. 2009. Summer diet of beluga whales inferred by fatty acid analysis of the eastern Beaufort Sea food web. Journal of Experimental Marine Biology and Ecology 374:12–18.

Lowry, L. F., K. J. Frost, and J. J. Burns. 1980*a*. Feeding of bearded seals in the Bering and Chukchi seas and trophic interaction with Pacific walruses. Arctic 33:330–342.

Lowry, L. F., K. J. Frost, and J. J. Burns. 1980*b*. Variability in the diet of ringed seals, *Phoca hispida*, in Alaska. Canadian Journal of Fisheries and Aquatic Sciences 37:2254–2261.

Lowry, L. F., K. J. Frost, and J. J. Burns. 1981*a*. Trophic relationships among ice-inhabiting phocid seals and functionally related marine mammals in the Bering Sea. U.S. Department of Commerce, NOAA, OCSEAP Environmental Assessment of the Alaskan Continental Shelf, Final Reports of Principal Investigators, Biological Studies 11:97–173.

Lowry, L. F., K. J. Frost, and J. J. Burns. 1981*b*. Trophic relationships among ice-inhabiting phocid seals and functionally related marine mammals in the Chukchi Sea. U.S. Department of Commerce, NOAA, OCSEAP Environmental Assessment of the Alaskan Continental Shelf, Final Reports of Principal Investigators, Biological Studies 11:37–95.

Martínez del Rio, C., N. Wolf, S. A. Carleton, and L. Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biological Reviews 84:91–111.

McLaren, I. A. 1958. The biology of the ringed seal (*Phoca hispida* Schreber) in the eastern Canadian Arctic. Bulletin of the Fisheries Research Board of Canada 118:1–97.

Meynier, L., P. C. H. Morel, B. L. Chilvers, D. D. S. Mackenzie, A. MacGibbon, and P. J. Duignan. 2008. Temporal and sex differences in the blubber fatty acid profiles of the New Zealand sea lion *Phocarctos hookeri*. Marine Ecology Progress Series 366:271–279.

Misarti N., B. P. Finney, H. Maschner, and M. Wooller. 2009. Changes in Northeast Pacific marine ecosystems over 4,500 years: Evidence from stable isotope analysis of bone collagen from archaeological middens. The Holocene 19:1139–1151.

Moore, S. E., and H. P. Huntington. 2008. Arctic marine mammals and climate change: impacts and resilience. Ecological Applications 18:S157–S165.

Newsome, S. D., M. T. Clementz, and P. L. Koch. 2010. Using stable isotope biogeochemistry to study marine mammal ecology. Marine Mammal Science 26:509–572.

Newsome, S. D., M. A. Etnier, D. H. Monson, and M. L. Fogel. 2009. Retrospective characterization of ontogenetic shifts in killer whale diets via $\delta^{13}C$ and $\delta^{15}N$ analysis of teeth. Marine Ecology Progress Series 374:229–242.

Parnell, A. C., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Source partitioning using stable isotopes: coping with too much variation. PLoS ONE 5:e9672.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293–320.

Phillips, D. L. 2012. Converting isotope values to diet composition: the use of mixing models. Journal of Mammalogy 93:342–352.

Phillips, D. L., and J. W. Gregg. 2003. Source partitioning using stable isotopes: coping with too many sources. Ecosystem Ecology 136:261–269.

Pierce, G. J., and P. R. Boyle. 1991. A review of methods for diet analysis in piscivorous marine mammals. Oceanography and Marine Biology 29:409–486.

Pitcher, K. W. 1980. Stomach contents and feces as indicators of harbor seal, *Phoca vitulina*, foods in the Gulf of Alaska. Fishery Bulletin 78:797–798.

Pompanon, F., B. E. Deagle, W. O. C. Symondson, D. S. Brown, S. N. Jarman, and P. Taberlet. 2012. Who is eating what: diet assessment using next generation sequencing. Molecular Ecology 21:1931–1950.

Quakenbush, L., J. Citta, and J. Crawford. 2011*a*. Biology of the bearded seal (*Erignathus barbatus*) in Alaska, 1961–2009. Final Report to National Marine Fisheries Service from the Alaska Department of Fish and Game.

Quakenbush, L., J. Citta, and J. Crawford. 2011*b*. Biology of the ringed seal (*Phoca hispida*) in Alaska, 1960–2010. Final Report to National Marine Fisheries Service from the Alaska Department of Fish and Game.

Rosen, D. A. S., and D. J. Tollit. 2012. Effects of phylogeny and prey type on fatty acid calibration coefficients in three pinniped species: implications for the QFASA dietary quantification technique. Marine Ecology Progress Series 467:263–276.

Rosen, D. A. S., A. J. Winship, and L. A. Hoopes. 2007. Thermal and digestive constraints to foraging behavior in marine mammals. Philosophical Transactions of the Royal Society B 362:2151–2168.

Seaman, G. A., L. F. Lowry, and K. J. Frost. 1982. Foods of belukha whales (*Delphinapterus leucas*) in western Alaska. Cetology 44:1–19.

Seymour, J., L. Horstmann-Dehn, and M. Wooller. 2014*a*. Inter-annual variability in the proportional contribution of higher trophic levels to the diet of Pacific walruses. Polar Biology 37:597–609.

Seymour, J., L. Horstmann-Dehn, and M. Wooller. 2014*b*. Proportion of higher trophic-level prey in the diet of Pacific walruses (*Odobenus rosmarus divergens*). Polar Biology 37:941–952.

Sheffield, G. G., F. H. Fay, M. A. Fedak, and B. P. Kelly. 2001. Laboratory digestion of prey and interpretation of walrus stomach contents. Marine Mammal Science 17:310–330.

Sheffield, G. G., and J. M. Grebmeier. 2009. Pacific walrus (*Odobenus rosmarus*): Differential prey digestion and diet. Marine Mammal Science 25:761–777.

Simpkins, M. A., Hiruki-Raring, G. Sheffield, J. M. Grebmeier, and J. L. Bengtson. 2003. Habitat selection by ice-associated pinnipeds near St. Lawrence Island, Alaska in March 2001. Polar Biology 26:577–586.

Stroeve, J. C., M. C. Serreze, M. M. Holland, J. E. Kay, J. Malanik, and A. P. Barrett. 2012. The Arctic's rapidly shrinking sea ice cover: a research synthesis. Climate Change 110:1005–1027.

Tieszen, L. L., T. W. Boutton, K. G. Tesdahl, and N. A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37.

Tollit, D. J., G. J. Pierce, K. A. Hobson, W. D. Bowen, and S. Iverson. 2010. Diet. Pages 165–190 *in* I. L. Boyd, W. D. Bowen, and S. Iverson, editors. Marine mammal ecology and conservation: A handbook of techniques. Oxford University Press, Oxford, U.K.

Tollit, D. J., A. D. Schulze, A. W. Trites, P. F. Olesiuk, S. J. Crockford, T. S. Gelatt, R. R. Ream, and K. M. Miller. 2009. Development and application of DNA techniques for validating and improving pinniped diet estimates. Ecological Applications 9:889–905.

Trites, A. W., and C. P. Donnelly. 2003. The decline of Steller sea lions *Eumetopias jubatus* in Alaska: a review of the nutritional stress hypothesis. Mammal Review 33:3–28.

U.S. Federal Register. 2012*a*. Endangered and threatened species; threatened status for the Arctic, Okhotsk, and Baltic subspecies of the ringed seal and endangered status for the Ladoga subspecies of the ringed seal. Pages 76705–76738 *in* N. U.S. Department of Commerce, National Marine Fisheries Service, editor.

U.S. Federal Register. 2012*b*. Endangered and threatened species; threatened status for the Beringia and Okhotsk distinct population segments of the *Erignathus barbatus nauticus* subspecies of bearded seal. Pages 76739–76768 *in* N. U.S. Department of Commerce, National Marine Fisheries Service, editor.

Vestheim, H., and S. N. Jarman. 2008. Blocking primers to enhance PCR amplification of rare sequences in mixed samples – a case study on prey DNA in Antarctic krill stomachs. Frontiers in Zoology 5:12.

Wang, S. W., S. Budge, R. R. Gradinger, K. Iken, and M. Wooller. 2014. Fatty acid and stable isotope characteristics of sea ice and pelagic particulate organic matter in the Bering sea: tools for estimating sea ice algal contribution to Arctic food web production. Oecologia 174:699–712.

Wheatley, K. E., P. D. Nichols, M. A. Hindell, R. G. Harcourt, and C. J. A. Bradshaw. 2008. Differential mobilization of blubber fatty acids in lactating weddell seals: evidence for selective use. Physiological and Biochemical Zoology 81:651–662.

Woodruff, F., S. M. Savin, and R. G. Douglas. 1981. Miocene stable isotope record: A detailed deep Pacific Ocean study and its paleoclimatic implications. Science 212:665–668.

Young, B. G., and S. H. Ferguson. 2013. Seasons of the ringed seal: pelagic open-water hyperphagy, benthic feeding over winter and spring fasting during molt. Wildlife Research 40:52–60.