THE COST OF ENERGY TRANSFORMATION AND DIGESTIBILITY OF MACRONUTRIENTS IN NORTHERN FUR SEALS (*CALLORHINUS URSINUS*)

by

MARIANA DIAZ GOMEZ

B.Sc. (Hons), Memorial University of Newfoundland, 2010

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Zoology)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

January 2016

Abstract

Bioenergetic studies can quantify the conversion of chemical energy contained in food to biologically useful energy to understand how changes in diet quality and quantity affect overall energy budgets and nutritional status. However, chemical energy is intrinsically linked to the macronutrients contained in food (i.e., lipid and protein) in terms of energetic density and digestive efficiency. For northern fur seals (*Callorhinus ursinus*) it is unknown how efficiently they transform dietary gross energy to net energy. I fed six trained adult female fur seals eight experimental diets composed of four prey species (capelin, walleye pollock, Pacific herring, and Magister squid), alone or combined. I measured the fur seals' digestive efficiency for energy and macronutrients across diets. I also investigated the effect of dietary intake on digestive efficiency, and tested the hypothesis that mixed-species diets provide a greater nutritional return than equivalent single-species diets. I quantified net energy uptake by measuring excreta energy loss and measuring heat increment of feeding. My results revealed significant differences between digestive parameters across diets. I found that digestible energy (95.9–96.7%) was negatively affected by both ingested mass and dietary crude protein. Furthermore, urinary energy loss (9.3–26.7%) increased significantly with increases in dietary crude protein. I also found that the heat increment of feeding (4.3–12.4%) increased with decreasing dietary lipid content. Overall, net energy gain (57.9–83.0%) was positively correlated with lipid content. I found that macronutrient digestibility differed across diets and that, overall, lipids were more digestible (96.0–98.4%) than crude proteins (95.7–96.7%). Also, dietary protein influenced the ability of fur seals to digest lipids and proteins. Overall, my results demonstrate that low lipid prey not only contain less gross energy, but result in proportionally lower net energy gain following digestion, partly due to decreasing digestibility of lipids in high protein diets. I also found that, counter to predictions, mixed-species diets do not provide fur seals with greater energetic or macronutrient gains than single-species diets. These findings contribute to understanding the nutritional ecology of northern fur seals and the impact that changes in diet can have on the fur seals' nutritional state.

Preface

I was the main designer of the experiments described in the present thesis, with suggestions by my supervisors David Rosen and Andrew Trites. All data collection was made possible with the help of veterinary, training, and technician staff from both the Vancouver Aquarium and the Marine Mammal Energetics and Nutrition Laboratory. All experiments were accomplished under the approval from the Animal Care Committees of the Vancouver Aquarium and the University of British Columbia (Permit #A10-0342).

I conducted all of the in-house sample analyses, which included proximate composition, energy density, and manganese concentration of fish and fecal samples. A representative set of fish and fecal samples was also analyzed for these parameters by SGS Canada Inc. (Burnaby, Canada) to corroborate the accuracy of my in-house results. I performed all of the data analysis, and was the primary writer of both the thesis and any related manuscripts. Chapters 2 and 3 were formatted as manuscripts for submission in scientific journals.

Table of contents

List of tables

List of figures

List of abbreviations

GEI Gross energy intake

DE% Digestibility energy

FEL% Fecal energy loss

UEL% Urinary energy loss

HIF% Heat increment of feeding

ME% Metabolizable energy

NE% Net energy

DMD% Dry-matter digestibility

RMR Resting metabolic rate

TC Thermoregulation cost

ADC Apparent digestibility coefficients

ADCcp Apparent digestibility coefficient of crude protein

ADC_{lipid} Apparent digestibility coefficient of lipid

$\dot{V}O_2$ Rate of oxygen consumption

$\dot{V}CO_2$ Rate of carbon dioxide production

Mn^{2+} Manganese

Acknowledgements

I would like to thank my co-supervisors Dr. David Rosen and Dr. Andrew Trites for all their invaluable guidance and support through every step of my thesis. In particular, thank you Andrew for teaching me how to communicate my research more effectively to others and for all the advice on how to write with better prose. I also thank you Dave for taking the time to explain things that were unclear to me while at the same time allowing me to figure things out on my own; these past years have been a delight under your supervision (most of it anyways!). Also, I would like to thank my committee members Dr. Jeffrey Richards and Dr. Ian Forster for providing me with encouragement, suggestions, and remarks that enhanced the quality of my thesis. I want to thank Andrew and Dave for the opportunity to undertake my research at the Marine Mammal Energetics and Nutrition Laboratory located in the Vancouver Aquarium. Additionally, thank you Pamela Rosenbaum for all the hard work you do behind the stage; I appreciate the support you provided when I needed it.

The completion of my thesis was a group effort, and I would like to thank the many people that worked hard to ensure the success of my project. From the Vancouver Aquarium I would like to thank all training staff, for being so dedicated in their caring for the animals as well as their efforts to make data collection possible; I will always remember all the great times we shared together. I would like to thank Billy Lasby, Nigel Walker, Danielle Hyson, Malgosia Kaczmarska, and Troy Neale. From the veterinary staff I would like to thank Dr. Martin Haulena, Chelsea DeColle, Sion Cahoon, Gwyneth Nordstrom, Dr. Chelsea Anderson, and Dr. Kelly Britt, for their great attitude that energized everyone. From the MMRU staff I would like to thank Rebecca Barrick, Brandon Russell, and Robert Marshall for their assistance with the experimental set up, data collection, and for all the great laughs.

I would also like to thank the laboratory staff at the Marine Ecosystems and Aquaculture Division (MEAD), Fisheries and Oceans Canada, for their precious teachings and guidance while caring for my safety at all times. Especially I would like to thank, Dr. Ian Forster, Mr. Mahmoud Rowshandeli.

Most of all I am thankful to have shared my time with my fellow members (past and present) from the Marine Mammal Research Unit. I thank you all for being attendant to my needs (particularly statistical needs), for providing great editorial corrections, and above all for

encouraging me right until the last minute. I could had never made it without you all and for that I will always be thankful. Particularly thanks to: Beth Volpov, you were an angel to me, even when you were busy you made time to help me, Carling Gerlinsky and Alex Dalton for their caring and infinite help, Elizabeth Goundie for always having the time to help me and for the great memories we built together, Brianna Wright for being a great example of strength and being always willing to revise my work. I would also like to thank Sarah Fortune, Hassen Allegue, Lindsay Aylesworth, Tiphaine Jeanniard du Dot, Erin Rechsteiner, Barbara Koot, Chad Nordstrom, Frances Robertson, Rachel Neuenhoff, Austen Thomas, Brian Battaile, Katie Haman, Aaron Purdy, and Benjamin Nelson. A special thanks goes to Morgan Davies for his help with sample processing and instructions on how to use the laboratory equipment.

I personally would like to thank my family for all their amazing support and encouragement that, even though miles of land separates us, were always there for me. I would like to thank my mother, Beatriz Gomez Cervantes, for being the inspiration of my life and for teaching me how to dream on a large scale. I would like thank my father, Rogelio Diaz Mejia, for his endless love and for teaching me to put life into a perspective to always find the joy in it. Finally, but by far not last, I want to thank my two beautiful sisters, Carolina Lorenz and Elisa Swan, for their precious advice and for always making time to listen to me when I needed them. Also, I want to thank my step mother Eunice Saavedra Salazar and my step brother Cesar Cervantes for cheering me on to give my best until the last day.

Chapter 1: Introduction

Consumption of prey provides animals access to environmental chemicals, in the form of nutrients that can be oxidized for energy. This chemical energy is liberated when macronutrients (i.e., lipids, proteins, and carbohydrates) are broken down (Moyes and Schulte 2007). Lipids provide the most chemical energy (37.7 kJ g^{-1}), followed by proteins (17.8 kJ g^{-1}) and carbohydrates (15.9 kJ g^{-1} for monosaccharides) (Blaxter 1989). Prey consumed by marine mammals vary seasonally in their abundances and distributions and also in their chemical compositions (although carbohydrates are negligible in most prey of marine mammals) (Sinclair et al. 1994; Vollenweider et al. 2011). In response to such differences, marine mammals have evolved diverse physiological and behavioural adaptations to capture prey and optimize the potential benefits from foraging on various taxa of cephalopods, crustaceans, and fish (Reynolds and Rommel 1999). These adaptations include being capable of altering their foraging strategies accordingly to changes in their ecosystem in order to satisfy energetic and nutritional demands. For example, northern fur seals (*Callorhinus ursinus*) have been shown to increase their foraging effort and switch between prey types to compensate for variation in seasonal prey distribution, composition, or abundance (Swartzman and Haar 1983; Gentry and Kooyman 1986; Sinclair et al. 1994). Knowing how changes in prey quality or quantity may affect the capacity of marine mammals to absorb the chemical energy and macronutrients contained in their prey is needed to better understand the impact that changes in prey may have on meeting the physiological demands of individual marine mammals, and ultimately, the consequences on their nutritional ecology and life history.

Optimal foraging theory proposes that animals should target prey that maximize their energy intake while minimizing acquisition costs (Stephens and Krebs 1986). However, the geometric framework theory of nutrition suggests that animals from various taxa actively select foods that balance macronutrient intake over energy maximization (Raubenheimer and Simpson 1999; Kohl et al. 2015). In evaluating these theories, it is important to recognize that energy is a byproduct of the macronutrient ratios contained within the prey, whereby the breakdown and assimilation of both lipids and proteins provide caloric gain. Thus, these two components of prey (energy and macronutrients) are intrinsically interconnected, particularly since animals have specific requirements for each of these independent of the energy they provide.

To obtain energy and macronutrients from prey, marine mammals must physically and chemically digest prey components to transform prey into biologically useful commodities. While past digestibility studies on pinnipeds have shown that energy and macronutrient digestibility vary among prey types, most studies only tested single-species diets (e.g., Keiver et al. 1984; Fisher et al. 1992). However, such homogeneous diets are not representative of free-ranging pinniped prey consumption. Studies that investigate how changes in prey composition impact a pinniped's efficiency for digesting energy and macronutrients should use diets that are comparable to that consumed by pinnipeds in the wild in order to better understand the nutritional impacts that changes in the biotic environment can have on pinnipeds.

The ultimate goal of my research was to determine the net energy gained by northern fur seals consuming eight different diets that varied in their chemical composition to understand the key factors that influence energy transformation. Specifically, I tested the following four hypotheses:

1. Net energy gained from specific diets (as a proportion of gross energy intake) declines disproportionally with decreases in quality (energy density) of the diet;
2. Macronutrient digestive efficiency varies across experimental diets;
3. Differences in macronutrient content are directly related to changes in overall digestive efficiency; and
4. There is no greater energetic or macronutrient benefit to consuming mixed-species diets over single-species diets.

Net energy gain: digestive processes are not always cheap

The chemical energy contained in prey is defined as gross energy (GE). Consumers must transform ingested gross energy into biologically useable energy (ATP) via several key digestive processes (Fig. 1.1; Lavigne et al. 1982). Each of these processes require metabolic activity and thus use chemical energy. In this context, the energy required to break down and digest nutrients could be considered as unavoidable energy "lost" to the system (i.e., is not available to the animal for other processes). Breakdown of food components (i.e., lipids, proteins, and carbohydrates that provide the gross energy) begins immediately after prey ingestion with physical breakdown of the food items, chemical and enzymatic degradation during digestion,

GROSS ENERGY (GE) of food (heat of combustion)

Faecal Energy (FE)

1. undigested food
2. enteric microbes and their products
3. secretions into the gastro-intestinal tract
4. cellular debris from gastro-intestinal tract

APPARENT DIGESTIBLE ENERGY (DE)

Urinary Energy (UE)
1. food origin
2. endogenous origin

Gaseous products of digestion (primarily methane)

METABOLIZABLE ENERGY (ME)[2]

Heat Increment[3] (HI)
(wasted heat unless animal is below lower critical temperature)
1. heat of nutrient metabolism
2. heat of fermentation

NET ENERGY (NE)

MAINTENANCE ENERGY (NE_m)
1. Basal metabolism[3]
2. Voluntary activity[3]
3. Energy to keep body warm if below thermal neutral environment, and when need for heat energy is above that supplied by heat increment
4. energy to keep body cool when above the thermal neutral environment

PRODUCTION ENERGY (NE_p)
1. energy storage foetus, semen, growth, fat, milk, hair, etc.
2. work (part of this is lost as heat)

Figure 1.1 Energy transformation and utilization by animals (taken from Lavigne et al. 1982). The pathway shows the conversion of gross energy contained in prey to net energy extracted by the consumer and its subsequent allocation to maintenance energy and production energy. All of the steps in the pathway are generally measurable to determine animal energetics, where apparent digestible energy (DE) is referred as apparent because excreta contain endogenous and undigested wastes, measured as fecal energy loss (FE) and urinary energy loss (UE). Although, heat increment of feeding (HI) occurs concurrently with digestion and absorption of digestive products, as well as with production of nitrogenous wastes and such, it appears in the pathway to be subtracted from metabolizable energy (ME). Subtraction of the energetic cost of HI from ME results in net energy gain (NE).

followed by intracellular absorption of the digestive products (i.e., fatty acids, glycerols, amino acids, nucleotides, and monosaccharides). Quantifying the energetic losses within the pathway

enables accurate estimates of the efficiencies of each step and, ultimately, the amount of energy derived by the consumer from a meal.

Energy that is not digested by the gastrointestinal system is directly lost through feces, and the remaining energy is known as digestible energy (DE) (i.e., the energy that was truly digested and absorbed). This is often referred to as apparent digestible energy, because it does not account for the endogenous metabolic waste products excreted in the feces that may also contribute energy density in the excreta. Fecal energy loss (FEL) can be measured by either collecting complete fecal output or by partial fecal sampling along with using a chemical marker to quantify how much prey is "represented" by an individual fecal sample. This may take the form of an added inert chemical marker such as chromium sesquioxide, or a naturally-occurring marker such as manganese. However, in pinniped digestive studies, chromium added to the food clusters together or is digested differently than the food, such that non-representative passage rates and inconsistent concentrations result in unreliable estimates of FEL (Fisher et al. 1992). Manganese, being a biologically natural component of food with minimal physiological absorption, does not have such problems, and has been used in several studies of pinniped digestion (Fadely et al. 1990). DE and FEL have been estimated for nine pinniped species, including northern fur seals (see Table 3 in Rosen and Trites 2000a; Stanberry 2003).

Additional energy is lost from the digestible energy via urinary products. Urinary energy loss (UEL) is mainly the result of protein deamination, where nitrogenous waste products are passed in urine as urea, creatine, etc. Of the energy lost through excreta, UEL is usually greater than FEL because the urinary waste products (unlike fecal products) contain molecular structures that have been assimilated and later excreted by the consumer. As a consequence, UEL is directly proportional to the metabolized nitrogen, such that the energy lost through urine is reported as a proportion of the digestible energy rather than the gross energy. The energy remaining after accounting for the energy lost through production and excretion of urine is known as metabolizable energy (ME) and is represented as a proportion of the gross energy intake (Fig. 1.1). ME is logistically difficult to quantify in pinnipeds because of the challenges with total urine collection. For this reason, only three studies have successfully estimated UEL in ringed seals (*Phoca hispida*), harp seals (*Phoca groenlandica*), and grey seal (*Halichoerus grypus*) (Parsons 1977; Keiver et al. 1984; Ronald et al. 1984).

The last stage in the energy transformation path is the heat increment of feeding (HIF), also known as the specific dynamic action (SDA). HIF is the metabolic response to the ingestion, physical breakdown, chemical digestion, absorption and assimilation of food that incurs an additional energetic cost to consumers. HIF is measured as an increase in postprandial metabolic rate above resting levels (Secor 2008). Gas respirometry is the most widely used method to measure HIF, where rates of oxygen consumption and carbon dioxide production can be quantified inside an experimental chamber (Rosen and Trites 1997). Following a baseline measurement of resting metabolic rate (RMR) on an inactive postabsorptive animal, the animal is fed a meal of known size and composition. Measurement of metabolic rate begins immediately following meal consumption, and continues as metabolism increases throughout the digestion of the meal, until metabolic rate returns to pre-feeding baseline (Secor 2008). Total oxygen consumption above "resting levels" is then converted to energy expended in meal digestion to estimate the energy used as a proportion of that consumed; therefore, HIF is reported as a percentage of GE (Secor 2008).

HIF has not been measured in northern fur seals, but has been measured in five other pinniped species (Parsons 1977; Ashwell-Erickson and Elsner 1981; Gallivan and Ronald 1981; Markussen and Øritsland 1991; Barbour 1993; Rosen and Trites 1997; Rosen and Trites 2003; Rosen et al. 2015). Subtraction of HIF from the metabolizable energy yields an estimate of the net energy (NE). NE is the energy that is available to animals from dietary intake that can be used by the consumer to fulfill its physiological requirements. NE can be expressed in either absolute amounts or as a proportion of the gross energy intake (NE%).

Net energy is used for the animal's maintenance or production requirements (Fig. 1.1). As such, a proportion of NE is used to sustain thermoregulatory metabolic needs in addition to RMR. Active thermoregulation takes place when homeotherms are outside of their thermal neutral zone, where maintaining core body temperatures requires additional energy expenditure through increases in metabolic rates (Gordon et al. 1972).

The efficiency with which chemical gross energy contained in prey is transformed into net energy varies depending on different factors, such as prey composition, the physical environment, enzymes present, and characteristics of the consumer's digestive system and developmental stage (Schneider and Flatt 1975; Reid et al. 1980). As a result, the NE that a

consumer gains from specific diets cannot be predicted solely from GE content. Therefore, bioenergetic studies with pinnipeds, such as northern fur seals, that investigate the NE gain from prey are essential to describing the ecological significance that changes in dietary intake have on the individual's energetic budgets. The majority of past pinniped bioenergetic studies have measured energy digestibility, fecal energy loss and urinary energy loss, while only a few studies have measured heat increment of feeding and metabolizable energy. However, net energy has only been estimated for two pinniped species: harbour seals (*Phoca vitulina*) and spotted seals (*Phoca largha*) (Ashwell-Erickson and Elsner 1981).

Many of these previous controlled feeding studies on pinnipeds have also measured dry-matter digestibility (DMD) (Rosen and Trites 2000b). DMD is a measure of the proportion of indigestible to digestible dry matter in food, such that foods high in indigestible matter (e.g., large bones and hair) have low DMD values. However, the link between dry matter assimilation and net energy gain is tenuous (Rosen and Trites 2000b).

While the previous feeding studies have provided important pieces of information regarding pinniped bioenergetics, the majority of them fed animals single-species diets, which are not representative of free-ranging pinnipeds dietary intakes. Pinnipeds are considered generalists predators known to consume over 100 different species of prey (Riedman 1990). Free-ranging pinnipeds continuously face the complex challenge of regulating their energetic and nutritional intake to balance prey selection with optimal intake requirements, while accounting for external (e.g., prey availability, environmental conditions, foraging costs) and internal (e.g., developmental stages, nutritional state) factors. As a consequence, pinniped prey consumption is a dynamic process that depends on different ecological and physiological factors. Information on how these factors interact to affect the energetic and nutritional budgets of pinnipeds is important for making accurate inferences about the impacts that changes in prey availability may have on individuals and ultimately populations.

The middle child of nutrition: macronutrient digestion

Many theories regarding animal nutrition—such as optimal foraging theory and predator-prey relationships—focus solely on levels of energy gain (Grodzinski 1975; Stephens and Krebs 1986; Barbosa and Castellanos 2005). Likewise, most individual or population food consumption models, particularly for marine mammals, are based on the energetic content of food (e.g.,

Winship et al. 2002; Fortune et al. 2013). However, modern nutritional theory proposes that carnivores target ingestion of specific ratios of macronutrients (i.e., lipid, protein, carbohydrates) rather than simply being energy-driven predators (Mayntz et al. 2009; Kohl et al. 2015). Thus, understanding the macronutrients that an animal obtains from different diets is important for several reasons. First, the overall energy digestibility (and hence energy gain) is a product of the digestibility of the individual macronutrients. Also, animals have specific macronutrient requirements that are distinct from those for energy, such that measurements of digestibility efficiency provides information of the animal's energetic and nutritional budget.

Break down of macronutrients provides animals with the building units they need for the maintenance and replacement of essential tissues, enzymes, hormones, etc. Specific macronutrient needs will not only differ between species, but will also vary during their life history due to changing requirements. For instance, in the early years of an animal's life, consumption of higher than the average protein intake is needed to support the higher demands for somatic growth versus maintenance and replacement of adult animal tissue (Robbins 1993). Protein demands are also higher during an animal's reproductive stage (Stevens and Hume 1995). Carnivorous mammals, such as pinnipeds, have the highest protein requirements, where an average mature mammal requires that 18-30% of their daily intake needs be met by dietary protein (Robbins 1993). Furthermore, marine mammals that use fur for insulation, particularly for those that undergo yearly molts, amino acids are also in high demand to maintain and recover vital pelage (Perrin et al. 2002). Northern fur seals rely heavily on their fur for thermal homeostasis, rather than blubber like most other pinnipeds (Reynolds and Rommel 1999). Thus, such marine mammals should consume prey that provide them with sufficient dietary protein to sustain their protein needs and prevent shortfalls that could lead to malnourishment.

Unlike for proteins, it is unclear whether animals have specific lipid intake requirements. There is no question that the majority of the marine mammals inhabiting cold geographical ranges (whether on land or deep underwater) depend on a thick layer of blubber tissue (subcutaneous lipid storage) to provide thermal insulation (Reynolds and Rommel 1999; Strandberg et al. 2008). It is also understood that lipid compounds are essential for energy storage in the form of adipose tissue in anticipation of fasting, extensive exercise, or periods of insufficient energy intake. However, most of these insights are based on analyzing the

functionality of animal fats and have not contributed to understanding whether marine mammals have specific fatty acid requirements to fulfill important biosynthetic pathways to sustain such essential fatty tissues.

Past studies of macronutrient digestion in pinnipeds relied on descriptive analysis which limited their ability to further examine the factors influencing the differences in macronutrient digestion (Goodman-Lowe et al. 1999). Similarly as with energy digestibility, these studies made use of single-species test diets, despite the fact free-ranging pinnipeds consume various prey that changes in macronutrient composition on an annual and seasonal basis (Riedman 1990; Van Pelt et al. 1997; Logerwell and Schaufler 2005; Vollenweider et al. 2011). Understating the total lipid and protein that pinnipeds gain when consuming diets that are more representative of pinnipeds in the wild is important given the potential physiological consequences that discrepancies between required and gained macronutrients could cause, such as nutritional stress (King and Murphy 1985). However, past pinniped studies have overlooked the role that micronutrient digestibility may play on an animal's nutritional state and also on their overall health and fitness (Elrod and Butler 1993; Boersma and Elser 2006; Solon-Biet et al. 2015a; Solon-Biet et al. 2015b).

Northern fur seals under nutritional stress: the unresolved concern

Northern fur seals inhabiting the Bering Sea are considered to be opportunistic predators (Gentry and Kooyman 1986; Riedman 1990) and are among the most pelagic of pinnipeds (spending all but 35-45 days at sea per year). Studies that have collected stomach contents and fecal samples have identified over 40 families of prey consumed by northern fur seals, but the majority of fur seal diets are dominated by just a few key species including juvenile walleye pollock (*Theragra chalcogrammus*), Northern smoothtongue (*Leuroglossus schmidti*), Atka mackerel (*Pleurogrammus monopterygius*), capelin (*Mallotus villosus*), Pacific herring *(Clupea pallasii*), as well as various squid species (e.g., Gonatidae) (Kajimura 1984; Sinclair et al. 1994; Call and Ream 2012). Notably, several of these key prey species are also commercially important in the Bering Sea (NMFS 1993).

A number of pinniped species inhabiting the North Pacific Ocean and Bering Sea have experienced recent population declines, including one northern fur seal population that breeds on the Pribilof Islands (St. George and St. Paul) (Alverson 1992; National Research Council 1996;

NMFS 2007). Research has revealed a potential relationship between various pinniped population declines and the reduction in quality (i.e., energy density) and diversity of prey available to them (Alverson 1992; Castellini 1993; Trites and Larkin 1996; Merrick et al. 1997; Rosen and Trites 2000a). Northern fur seals on the Pribilof Islands numbered approximately 2.1 million in the late 1940's and early 1950's (NMFS 1993). During 1956 and 1968 female fur seals at Pribilof Islands were heavily harvested with the justification that this selective harvest of females would increase the productivity of the herd (which did not occur; Fowler 1982; French and Reed 1990; Trites and Larkin 1996). Other unexplained downward population trends have occurred since the harvesting of females ceased in 1968 (Trites 1992) and, as of 2014, the population numbered about 550,000 with a declining pup production of about 3.7% per year since 1998 (Towell et al. 2014).

Like most other otariid seals, nursing northern fur seal pups undergo a period of fasting while their mothers make foraging trips away from the breeding grounds in order to pay for the cost of maternal care (Perrin et al. 2002). Bioenergetic studies on free-ranging reproductive female northern fur seals have suggested that energy transfer between fur seals and pups may be decreasing (Costa and Gentry 1986). This decrease in energy transfer may be due to several possible reasons. First, insufficient energy intake during maternal foraging trips may result in females being unable to produce sufficient quantities of nutrient-rich milk (Costa and Gentry 1986). Second, females may have to extend their foraging trips to ensure adequate energy intake. As a result, the pup's ability to sustain an adequate nutritional status while fasting between suckling periods may be insufficient, leading to emaciation (i.e., starvation coupled with disease and injury), which was identified in the past to be the main cause of death among pups (Keyes 1965). Clearly, energy allocation among females and pups is highly dependent on the mother's ability to acquire sufficient energy and nutrients to satisfy both of their needs.

Major fluctuations in the biodiversity and abundance of prey available in the Gulf of Alaska and eastern Bering Sea may put the reduced fur seal populations at greater risk. In the 1950's, herring biomass exceeded 3-5 metric tons and was considered the dominant fish species, while in the 1970s, herring numbers had dropped and the biomass of pollock had increased. By the late 1980's, pollock accounted for approximately 85% of the fish biomass in the area (NMFS 1993). Concurrent with the population decline of northern fur seals, the Bering Sea was heavily

fished, particularly for walleye pollock. As a result of the changes in fish abundance and fishing pressure, some suggested that pollock fisheries were competing with fur seals (Sergeant 1976; Fowler 1982; Trites 1992; NMFS 2007), and may have altered the quantity and quality of prey consumed by fur seals, either through reduction in the total abundance or through changes in age class of prey (Fowler 1982; Swartzman and Haar 1983; NMFS 2007). Other potential explanations for the fur seal population decline include entanglement in fishing gear and other debris, toxins in the environment, as well as the indirect effects of climate change on ocean biodiversity (Fowler 1982; Trites 1992; NMFS 1993; NMFS 2007).

Although the reason behind the current decline of northern fur seals and many other pinnipeds in the Bering Sea is still a topic of controversy, the nutritional stress hypothesis has emerged as a likely explanation for the overall decline in pinnipeds and seabirds in the Bering Sea. This hypothesis postulates that, even if there is sufficient fish for animals to consume, the quality of fish available do not provide the appropriate amounts of energy and nutrient return to meet their requirements (Rosen and Trites 2000a; Trites and Donnelly 2003; Rosen and Trites 2005). The long-term consequences of inappropriate nutritional gains result in negative physiological impacts, such as reduced birth rates, fertility, and body size as well as increased pup and juvenile mortality (Elrod and Butler 1993; Trites and Donnelly 2003). Ultimately, it is possible that all of these factors contribute synergistically to decrease individual lifespan and resultant population declines (Solon-Biet et al. 2015a; Solon-Biet et al. 2015b).

Even though the nutritional stress hypothesis has emphasized the importance of prey quality on the overall health and fitness of pinnipeds, the research to validate this hypothesis has been limited (Calkins et al. 1998; Rosen and Trites 2000a; Trites and Donnelly 2003; Rosen and Trites 2005; Rosen 2009; Calkins et al. 2013). Therefore, studies that expand the knowledge of pinniped bioenergetics should be of particular priority to provide an accurate understanding of the inter-relationships between top pinniped predators, fisheries, and prey, which would potentially inform and aid conservation management.

Thesis research goals

The overall aim of my thesis was to investigate which factors of dietary intake (e.g., prey composition, ingested food mass, etc.) contribute to potential differences in the digestive efficiency of energy and macronutrients in northern fur seals. Specifically, I quantified the net

energy gain of individual fur seals consuming diets of different compositions as a step towards understanding whether such differences could potentially influence the population dynamics of northern fur seals.

Previous dietary studies with northern fur seals concentrated on measuring differences in energy digestibility while animals consumed single-species diets and only a few investigated the energetic cost of the heat increment of feeding. Additionally, previous studies on northern fur seals did not quantify the complete energy transformation pathway. Therefore, my first goal was to quantify the fur seals' net energy gain as accurately as possible by feeding them experimental diets composed of combinations of key prey species consumed by wild fur seals (Chapter 2). This included measuring the fur seal's heat increment of feeding to calculate the energetic cost of digestion across the different experimental diets, which had never been examined before in fur seals. Also within Chapter 2, I tested whether changes in dietary intake would affect short-term metabolic activities, such as resting metabolic rate or thermoregulation capacity. My hope was that the findings from Chapter 2 would provide a greater understanding of how changes in quantity or quality of prey could be negatively impacting the energetic budget of fur seals in the Bering Sea, as well as other declining pinnipeds inhabiting the same region, and provide further evaluation of the physiological basis to the nutritional stress hypothesis.

Studies on pinniped nutrition have measured the digestive efficiency of macronutrients and, as with studies on energy digestibility, the feeding studies only used single-species diets. Macronutrient digestibility in northern fur seals has never been quantified. I therefore aimed to measure the potential differences in digestibility of macronutrients (i.e., lipid and protein digestibility) in northern fur seals, concurrent with measuring net energy gain (Chapter 3). Within Chapter 3, I further examined which constituents of diet composition affected the ability of fur seals to digest essential macronutrients. Additionally, I strived to investigate whether shifts in prey composition interacted with macronutrient digestibility in an effort to understand if these parameters impact the nutritional state of wild fur seals, and shed light on potential negative long-term health effects at the population level (Chapter 3).

My thesis consists of two chapters written as independent manuscripts for publication in peer-reviewed journals. The main research outcome of Chapter 2 focuses on net energy gain in northern fur seals and the importance of prey quality, while Chapter 3 concentrates on the

digestibility of vital macronutrients. The results from these two chapters are interconnected, as changes in fur seals' digestive efficiency due to changes in food components affect both energy and macronutrients because their assimilation occurs simultaneously.

Overall, I sought to increase knowledge of the energy transformation pathway for northern fur seals, as well as for other closely related pinniped species. I also sought to increase understanding of the mechanism by which changes in prey quantity and quality may negatively affect the fur seals' energetic and nutritional status and gain insight into potential consequences of dietary shifts on the long-term overall health and fitness state at the population level. Finally, I sought to provide scientific findings to ecosystem managers and policy makers, and help with conservation efforts to protect free-ranging fur seals and other pinniped species of the North Pacific Ocean and Bering Sea.

Chapter 2: Net energy gained by northern fur seals is impacted more by diet quality than by diet diversity

Summary

Understanding whether northern fur seals (*Callorhinus ursinus*) are negatively affected by changes in prey quality or diversity could provide insights into their on-going population decline in the central Bering Sea. We investigated how six captive female fur seals assimilated energy from eight different diets consisting of four prey species (walleye pollock, Pacific herring, capelin and Magister squid) fed alone or in combination. Net energy was quantified by measuring fecal energy loss, urinary energy loss, and heat increment of feeding. Digestible energy (95.9–96.7%) was high (reflecting low fecal energy loss), and was negatively affected by ingested mass and protein content of the diets. Urinary energy loss (9.3–26.7%) increased significantly for high-protein diets. Heat increment of feeding (4.3–12.4%) significantly changed with diet and was lower for high-lipid diets. Overall, net energy gain (57.9–83.0%) was affected by lipid content and varied significantly across diets. Dietary changes did not significantly affect resting metabolic rate or thermoregulation cost. Mixed-species diets also did not provide any energetic benefit over single-species diets. Our study demonstrates that net energy gain was higher from lipid-rich diets, and that diet quality was more important in terms of energy retention to fur seals than diet diversity. These findings suggest that fur seals consuming lower-quality prey in the Bering Sea would be challenged to obtain sufficient energy to satisfy energetic and metabolic demands, independent of high prey abundance.

Introduction

Energy is the currency of classical optimal foraging theory, which postulates that foragers should maximize their energy gain while minimizing the energetic costs of obtaining prey (Stephens and Krebs 1986). Ecological theories of predator-prey relationships also employ energy as a currency of profitability that should be maximized (Barbosa and Castellanos 2005). Similarly, many bioenergetic models assume that the gross energy contained in prey translates directly to the energy gained by predators (Grodzinski 1975). However, digestive processes can be energetically costly and can disproportionately distort the value of different prey items. Bioenergetics recognize that net energy gain, which is the energy remaining after metabolic and digestive processes have occurred, is the true measure of energy available to fuel the predator's

physiological demands (Lavigne et al. 1982). Energy transformation is a dynamic process that depends on many factors, such as prey composition, the presence of enzymes, and the characteristics of the consumer's digestive system (Schneider and Flatt 1975). Hence, the energy gained by a predator is a function of the predator's ability to search for and obtain food in a timely manner, as well as their physiological capability to absorb digestive products.

Animals are constantly faced with the complex challenge of regulating their energetic and nutritional intake in such a way that their prey selection meets their optimal intake requirements, while accounting for external (e.g., prey availability, environmental conditions, foraging costs) and internal (e.g., developmental stage, nutritional state) (Raubenheimer and Simpson 1999) factors. Pinnipeds are generalist predators, as reflected by the wide variety of prey species they typically consume (Riedman 1990). However, the diversity of prey consumed may not reflect prey abundance and occurrences alone, but may also reflect satisfying optimal intake requirements.

Diets composed of mixed prey species that are nutritionally complementary to each other have been hypothesized to enhance both the rate of breakdown and the post-digestive utilization of nutrients (Penry and Jumars 1987; Singer and Bernays 2003). For example, mixed-species diets reportedly provide phocid seals with significantly greater levels of energy intake than equivalent single-species diets (Goodman-Lowe et al. 1999; Trumble and Castellini 2005). Results from these and other studies that fed mixed-species diets (composed of multiple prey species) to a variety of predator species are consistent with the hypothesis that mixed-species diets provide the consumer with significantly higher returns than single-species diets.

Understanding how changes in diet composition affect the energetic and nutritional budgets of animals is essential for making accurate inferences about the impacts that changes in prey availability may have on populations. This is particularly true for declining populations of threatened or endangered species that may have difficulty obtaining sufficient energy and nutrients due to reductions in the quantity (biomass), quality (energy density), or diversity (numbers of species) of prey (Trites and Donnelly 2003; Rosen 2009). Nutritional stress, a failure to match required energetic or nutritional demands with the gains (King and Murphy 1985), has been suggested as a mechanism to explain the recent population declines of many species of seabirds and marine mammals inhabiting the central Bering Sea and the Gulf of

Alaska (Pitcher 1990; Trites and Larkin 1996; National Research Council 1996) as revealed by the relationships between rates of declines and reductions in the quality and diversity of prey available to them (Alverson 1992; Castellini 1993; Decker et al. 1995; Merrick et al. 1997; Rosen and Trites 2000a; Trites et al. 2007; Calkins et al. 2013).

The population of northern fur seals (*Callorhinus ursinus*) inhabiting the North Pacific Ocean and Bering Sea has declined dramatically in the Eastern Pacific from 2.1 million individuals in the late 1940's and early 1950's to ~550,000 in 2014. At present, pup production is declining ~3.7% per year on the Pribilof Islands in the central Bering Sea (Towell et al. 2014). Northern fur seals are known to change their seasonal foraging behaviours in response to their changing energetic needs, as well as the daily and seasonal movements of their prey (Gentry and Kooyman 1986; Gentry 2002). Their main prey species include juvenile walleye pollock (*Theragra chalcogrammus*), Atka mackerel (*Pleurogrammus monopterygius*), capelin (*Mallotus villosus*), Pacific herring (*Clupea pallasii*), and various squid species (e.g., *Gonatidae*) (Riedman 1990; Sinclair et al. 1994; Call and Ream 2012). Shifts in the quantity and age class of fish consumed by fur seals in the Bering Sea occurred in the late 1970's (Swartzman and Haar 1983) concurrent with changes in the abundance of fish stocks (NMFS 1993). This observed change in dietary intake has led to the hypothesis that the caloric intake of fur seals has declined. Reductions in net energy gains have obvious negative impacts on individual development, survival, reproductive fitness and, ultimately, population growth rates. However, the impact of the dietary changes on the net energy gained by individual northern fur seals is unknown.

Previous controlled feeding studies have investigated aspects of how northern fur seals digest different single-species diets. For instance, Miller (1978) and Fadely (1990) investigated the dry matter digestibility (the proportion of dry matter in food that is absorbed) of different single-species diets consumed by northern fur seals. These studies provided basic information on the digestive efficiency of fur seals, but did not examine critical pathways of energy transformation and assimilation. Additionally, they did not examine the digestibility of mixed-species diets, which are more representative of what wild fur seals consume.

The goal of our study was to investigate the efficiency of energy transformation and absorption by six captive, female northern fur seals fed eight different diets. A secondary goal was to test the hypothesis that mixed-species diets provide greater energetic gain than single-

species diets. Experimental diets were composed of four prey species of varying compositions (fed alone or in combination). The fur seals' complete digestive pathway was calculated in order to quantify net energy gain by measuring three pathways of digestive energy loss: fecal energy loss, urinary energy loss, and the heat increment of feeding. In addition, we also investigated potential short-term physiological changes in the fur seals' metabolism due to dietary shifts. Results from our study are an essential step in understanding the relationship between diets and energy budgets of northern fur seals, and aid in evaluating whether dietary shifts are negatively affecting the energetic status of northern fur seals in the North Pacific and Bering Sea.

Materials and methods

Animals

Experiments were conducted throughout November 2012–June 2013 on six female northern fur seals that were 4.5 years of age, with a body mass of 19.5–28.9 kg at the start of the study. The fur seals were captured as pups (approximately 4 months old) in October 2008 from St. Paul Island, Alaska, USA. Subsequently, the fur seals were housed at the University of British Columbia's Marine Mammal Energetics and Nutrition Laboratory, located at the Vancouver Aquarium (British Columbia, Canada). All experimental manipulations were in accordance with the guidelines of the University of British Columbia Animal Care Committee (#A10-0342) and the Canadian Council on Animal Care. The fur seals' standard diet consisted of thawed Pacific herring and market squid (*Loligo opalescens*), supplemented with vitamins, fed three times a day. The fur seals had access to continuous-flow seawater pools (with adjacent haulout space) that reflected local ocean temperatures during the experimental period (8.6–10.6˚ C). Fur seals were weighed daily on a platform scale (± 0.02 kg) prior to feeding.

Test diets and experimental design

The fur seals were subject to eight test diets that were hand-fed by trainers three times a day to ensure the schedule of food intake was consistent across trials. Experimental diets lasted three weeks and were composed of four key prey items that fur seals encounter in the wild: Pacific herring, walleye pollock, capelin, and Magister squid (*Berryteuthis magister*), fed alone or in combinations. Animals were previously exposed to herring and capelin, but not to pollock or Magister squid. The amount of fish consumed by the fur seals was recorded daily.

Table 2.1 Proximate composition (crude protein and lipid content), energy density, manganese (Mn^{2+}) concentration (dry-weight basis) and mean body size and weight (± SD) of a subsample of four species of prey (n=12 of each) experimentally fed to six female northern fur seals.

Experimental prey	Water (%)	Total lipid (%)	Crude protein (%)	Energy density (kJ g^{-1})	Mn^{2+} (ppm)	Fish length (cm)	Fish weight (g)
Pacific herring (*Clupea pallasii*)							
Batch A (main source)	68.5	41.6	51.4	24.3	5.1	19.9 (1.5)	93.0 (20.8)
Batch B (Magister diet only)	69.2	37.0	53.6	22.9	5.5	18.5 (0.6)	64.0 (6.7)
Walleye pollock (*Theragra chalcogramma*)	75.3	32.8	57.5	22.1	2.4	24.5 (2.2)	134.0 (33.2)
Capelin (*Mallotus villotus*)	82.6	4.0	81.6	15.2	2.9	15.0 (1.0)	24.0 (5.4)
Magister squid (*Berryteuthis magister*)	71.3	44.3	46.7	23.2	2.8	--	--

The different prey items were chosen to represent a range of proximate compositions and energy densities (Table 2.1). The aim was for the fur seals to be fed at a constant level of gross energy intake (GEI) that approximated maintenance levels, such that the fur seals were neither gaining nor losing body mass (Kleiber 1975). As maintenance energy requirements varied between fur seals, a separate target GEI was predetermined for each fur seal at the start of each feeding trial. These target GEI's were also adjusted with observed changes in body mass during the experiment to try to ensure body mass was held constant across all diets. The energy (GEI) required for maintenance was estimated to be between 11,500–12,500 kJ per day. This resulted in differences in the amounts of ingested mass by the individual fur seals per diet.

The eight test diets consisted of 1) herring only, 2) pollock only, 3) capelin only, 4) herring + pollock (50% by energy), 5) herring + capelin (50%), 6) pollock + capelin (50%), 7) herring (batch B) + Magister squid (50%), 8) herring + pollock + capelin (33%). Quantities of each prey type in the mixed-species diets were balanced to provide equal levels of gross energy (resulting in different amounts of ingested mass). All six of the fur seals were subject to all test diets, except for diet 7 that lasted two weeks and was only consumed by four of the fur seals due to a shortage of Magister squid. Additionally, all of the diets used herring from the same batch

(batch A) with the exception of the Magister squid diet that used a different batch of herring (batch B) (Table 2.1). The fur seals were divided into three treatment groups with two fur seals per group, and test diets were randomly assigned to each group to counter any potential effects due to seasonality. Each feeding trial was conducted over a three-week period and consisted of three phases: acclimation occurred during the first week of feeding, fecal samples were collected during the second week, and metabolic trials (detailed below) were conducted during the third week.

Fecal sample collections

During the second week of each trial, fecal samples were collected several times a day from the bottom of the holding pool or haulout area for subsequent digestibility analyses. Animals were held in pools according to diet groups, but fecal samples from individual fur seals were distinguished by using coloured micro-grit markers (Micro Tracers Inc., 1370 Van Dyke Avenue, San Francisco, USA). Approximately 5–6 g of micro-grits were fed via gel capsules inserted into the opercular cavity of the fish over the first two feedings of the day. Fecal samples were collected and date, time, mass, and fur seal identity was noted (by color) for each sample. Samples were frozen in sealed plastic bags at -20˚C until further analysis.

Metabolic rate measurements

The resting metabolic rate (RMR), added thermoregulation cost (TC), and heat increment of feeding (HIF%) for each fur seal on each diet were measured via open-circuit respirometry. During the measurements of RMR and TC the animals were in a fasted state (>16 hrs after their last meal) as well as during the initial metabolic rate baseline for HIF (further explained below). Also, for the entire duration of metabolic measurements, animal behaviour, ambient air temperature, and metabolic chamber temperature were noted every 5 min.

RMR is the total energy used by animals to perform vital bodily functions while in a relaxed and postabsorptive state (Kleiber 1975). RMR was measured for each fur seal on the last day of each feeding trial. The fur seals voluntarily entered a custom 340 L metabolic chamber (dimensions: 0.92 m x 0.61 m x 0.61 m) where rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured while the animal rested in ambient air. $\dot{V}O_2$ and $\dot{V}CO_2$ were measured by continuously drawing ambient air through the metabolic chamber at a set rate of 125 L min^{-1} using a Sable Systems Model 500H Mass Flow Controller which

continuously corrected the flow to standard temperature and pressure (Sable Systems, Las Vegas, USA). Subsamples of excurrent air were then desiccated using anhydrous $CaSO_4$ (Drierite; Hammond Drierite, Xenia, OH) and were analyzed afterwards to quantify $\dot{V}O_2$ and $\dot{V}CO_2$ in the chamber via Sable Systems FC-1B and CA-1B gas analyzers, respectively (Sable Systems, Las Vegas, USA). Gas concentrations were monitored and recorded to a portable computer every 0.5 sec using Sable Systems' Expedata software. Ambient air baselines at the start and end of each trial were used to account for any system drift. Changes in O_2 and CO_2 concentrations compared to ambient air baselines were converted into $\dot{V}O_2$ (Withers 1977). RMR was determined as the lowest continuous average $\dot{V}O_2$ maintained for 20 min during the last 30 min of the 45-min trial.

The potential additional cost of thermoregulation in cold water (TC) was measured immediately following the RMR measurement. After completion of the RMR measurement, data recording was paused and the chamber was filled roughly two-thirds full with continuously flowing 2˚C water. When the fur seal was partially submerged, the metabolic trial resumed and the animal's metabolic rate was measured while in water for an additional 30 min. Thermoregulation cost (TC)—the metabolic rate while in the water—was determined as the lowest continuous average $\dot{V}O_2$ maintained for ~10 minutes during the last 20 min of the 30-min trial. TC was calculated as the change in metabolic rate compared to the previously measured RMR in air.

The heat increment of feeding (HIF%) is the increase in metabolism resulting from the mechanical and chemical digestion of a recent meal. Measurements of HIF% were conducted during the last week of each diet trial. RMR was initially measured (as previously described) and after completion of the 30-min trial to obtain an RMR baseline, data collection was then temporarily paused while the fur seal was fed a meal of known energetic content (6294.7 ± 1202.2 kJ, approximately half of their daily GEI) while remaining inside the metabolic chamber. Data collection then resumed and $\dot{V}O_2$ was monitored for about 5–6 hrs afterwards to capture the entire postprandial rise in metabolism. $\dot{V}O_2$ was then converted into rates of energy utilization (1 L O_2 = 20.1 kJ; Blaxter 1989) to quantify the energetic cost of HIF%. Ultimately, HIF% was calculated as the total increase in energy utilized above RMR, expressed as a percentage of the GEI of the ingested meal.

Fish prey and fecal laboratory analysis

Fish and fecal samples were analyzed in-house (see below) and additional samples were sent to a commercial laboratory (SGS Canada Inc., Burnaby, Canada) for quality control (i.e., to provide a correction factor for in-house measurements). At least 10 samples of each of the prey items were analyzed by SGS Canada Inc. for proximate composition (moisture, lipid and protein), energy density, and manganese (Mn^{2+}) concentration (Table 2.1). An additional 10 samples of each of the fish items were similarly analyzed in-house for the same analyses (see below).

Three separate fecal samples per fur seal per diet were selected for proximate composition analysis; the majority of the samples were collected 24-hrs apart from each other. From these fecal samples, 16 representative samples were sent to SGS Canada Inc. to be analyzed for Mn^{2+} concentration, while 138 were analyzed in-house. Fecal and fish samples were thawed, ground (fish only) and two duplicate subsamples (~25 g each) were then placed in polycarbonate vials and weighed to the nearest milligram. Fecal and fish samples were refrozen, and subsequently freeze-dried for 36 hrs to a constant mass (Freeze dryer Freezone 6, LABCONCO, Kansas City, USA). Dried samples were reweighed to determine dry matter and water content.

Freeze-dried samples were used to measure proximate composition, energy density, and Mn^{2+} concentration. Throughout all laboratory analyses, sample variation of $\leq$5% was considered as acceptable between subsample replicates. Total energy density of fecal and fish samples was determined by combustion of duplicates of 1 g of dried sample using an oxygen bomb calorimeter (6400 Automatic isoperibol calorimeter, Parr Instrument Company, Moline, USA). Total crude protein of fish and fecal samples was determined by measuring Total Kjeldahl Nitrogen (TKN) of duplicates of approximately 0.2 g of dried sample with the addition of 2 Kjeltabs Cu catalyst tablets (FOSS, Eden Prairie, USA) using the Kjeldahl method (AOAC 1990) via spectrophotometric flow injection analyzer (FOSS FIAstar 5000 TKN analyzer unit, Eden Prairie, USA) measured at 590 nm. Nitrogen concentration was multiplied by a factor of 6.25 to determine total crude protein, based on the assumption that 100 g of crude protein contains 16 g of nitrogen (Robbins 1993). Lipid content from duplicate samples of approximately 2 g of feces and 1.5 g of fish were measured using a modified

chloroform/methanol extraction technique (Bligh and Dyer 1959). It is important to note that most of the fish and fecal samples used in our experiment were relatively high in lipid (>2%), which in some studies has led to underestimated lipid content (Iverson et al. 2001; Budge et al. 2006). However, our in-house lipid and protein laboratory analyses were corroborated against the SGS Laboratory results (which used different proximate composition analysis methods) to ensure the compatibility of our techniques. Both protein and lipid content of samples were expressed as a percentage of total dried samples.

Fish and fecal Mn^{2+} concentrations were determined through wet oxidation of duplicates of dried 0.4 g of fish and 0.2 g of fecal samples. Concentrations were determined by using an atomic absorption spectrophotometer (Perkin-Elmer 2380; 279.5 nm wavelength, slit width 0.2 nm, oxidizing air-acetylene flame; Perkin-Elmer, Montreal, Canada). Standard curves were generated with a Mn^{2+} standard stock solution of 1.0 ppm $MnNO_3$ by serial dilutions to approximate 0.02, 0.04, 0.06, 0.08, 0.10, 0.20, 0.40 Mn^{2+} concentrations (ppm).

Digestibility calculations

Laboratory results for the fish and fecal samples per animal per diet were averaged together to provide a single value for an entire diet trial for each individual fur seal. All calculations were done on a dry-matter basis.

Calculations of digestibility efficiency require a means of determining the amount of prey "represented" by a fecal sample. Naturally occurring manganese (Mn^{2+}) content in fish prey and feces has been widely utilized as an inert marker (given its low biological requirements) to quantify digestibility of fur seals and other pinnipeds in previous studies (Fadely et al. 1990; Lawson et al. 1997; Rosen and Trites 2000b). However, the low concentrations of Mn^{2+} in the prey samples led to unacceptable levels of variance in the in-house analyses, therefore, only the Mn^{2+} results obtained from SGS Laboratories were used.

GEI was calculated by multiplying ingested mass by the energy density of the prey items, in proportion to the amount fed of each experimental diet.

DMD% is the relative assimilation of dry materials, and was calculated as the change in concentration of Mn^{2+} concentration between diet and feces (Schneider and Flatt 1975):

$$\text{DMD } (\%) = \left(1 - \frac{C_i}{C_f}\right) \times 100$$

where *C* is the concentration of Mn^{2+} in diet (*i*) and feces (*f*).

Digestible energy (DE%)—the amount of energy assimilated—was calculated as (Mårtensson et al. 1994):

$$DE\ (\%) = \left(1 - \frac{C_i \times E_f}{C_f \times E_i}\right) \times 100$$

where *E* is the energy density of the ingested diet (*i*) and feces (*f*).

Fecal energy loss (FEL%)—the inverse of DE%—was calculated as:

$$FEL\ (\%) = 1 - DE\%$$

To calculate urinary energy loss (UEL) per day, apparent digestible nitrogen intake (ANI) was first calculated as:

$$ANI\ (g\ d^{-1}) = \frac{total\ crude\ protein\ consumed \times digestibility\ of\ crude\ protein}{6.25}$$

where digestibility of crude protein was calculated as (Mårtensson et al. 1994):

$$Protein\ Digestibility\ (\%) = \left(1 - \frac{C_i \times P_f}{C_f \times P_i}\right) \times 100$$

where *P* is the crude protein content of the ingested diet (*i*) and feces (*f*).

UEL was calculated with the following formula based on data from Keiver et al. (1984):

$$UEL\ (kJ\ d^{-1}) = (6.128 \times ANI + 14.737) * 4.186$$

The estimated energetic content in urine was then represented as a proportion of DE% (rather than GEI) since urinary losses are proportional to absorbed nitrogen, and independent of what is lost in the feces (Dierauf and Gulland 2001). While UEL% is most accurately reported as

a proportion of DE%, UEL% was also calculated as a proportion of GEI for data analysis only to keep statistical analysis consistent across all variables.

Metabolizable energy (ME%)—the energy that remains available after accounting for the energy lost through the excreta, expressed as a percentage of GEI—was calculated as:

$$ME\ (\%) = \left(\frac{GEI-(FEL+UEL)}{GEI}\right) \times 100$$

Net energy (NE) is the total energy gained by fur seals after accounting for the energy lost through excreta and through the HIF%. NE% is this value expressed as a percentage of GEI, and was calculated as:

$$NE\ (\%) = \left(\frac{GEI-(FEL+UEL+HIF)}{GEI}\right) \times 100$$

Testing effect of diet on digestibility and bioenergetics

Statistical differences in digestive and physiological parameters attributable to diet type were determined via linear mixed-effect (LME) models using R 3.1.2 statistical software (R Core Team 2014). Models were fitted using maximum likelihood estimates as required for LME model comparison using the package *nlme* (Pinheiro et al. 2015). Digestible data and dietary macronutrient intake data were arcsine and logit transformed in attempts to normalize the data. However, transformations did not resulted in normalized data. Furthermore, modeling with such transformed data did not change either the overall outcome or the patterns observed in the models that showed to be significant during preliminary analysis. We therefore used the untransformed proportional data given that the residuals of our models with untransformed data met the assumptions of the models used (see below) (Wilson et al. 2013). LME models were built in a step-wise fashion to assess the ability of the fixed factors to explain differences in the response variables, such that models containing fixed effect factors hierarchically nested within the null model (lacking a fixed effect factor) were compared against the null model and models with fewer fixed effects by likelihood ratio tests (LRT) and by comparison of Akaike information criterion (AIC) values. LME models accounted for repeated measures and variability within and among animals by treating Animal ID as a random effect, which also allowed

inferences from the sample population to be applied to their wild counterparts (Pinheiro and Bates 2000; Crawley 2007; Zuur et al. 2009; Galecki and Burzykowski 2013).

The first step in the data analysis was to determine the statistical influence of diet type on the following response variables: percent change in body mass, dry matter digestibility, digestible energy, fecal energy loss, urinary energy loss, heat increment of feeding, and net energy gain. Due to the relatedness between response variables, we examined each relationship independently, and diet was always the only fixed factor tested in this initial analysis. To investigate the nature of significant differences between response variables and diets, a *post-hoc* Tukey contrasts simultaneous test for general linear hypotheses was used after fitting the separate models.

When preliminary analysis demonstrated that diet type was a significant factor, subsequent analyses explored which components of the diet were at the root of the relationship by testing a number of relevant fixed effects, but excluding diet type. Fixed effects that were tested as potential model factors included: food mass intake (kg d^{-1}), gross energy intake (kJ d^{-1}), diet lipid intake (% per day dry-weight), diet protein intake (% per day dry-weight), and lipid to protein intake ratio. All models were compared against the null model using an LRT test. Models with the same response variable but different dependent variables in this analysis were compared by AIC values as described by Pinheiro and Bates (2000) and Crawley (2007), by selecting the lowest AIC and most parsimonious model (i.e., more complex models were tested against those with fewer fixed effects). The selected best-fit model contained the factor that best explained the trends observed in the response variables and thus fitted the data the most accurately while fulfilling the assumptions of the LME models (Pinheiro and Bates 2000; Crawley 2007; Zuur et al. 2009; Galecki and Burzykowski 2013).

To test whether mixed-species diets provided a greater than expected digestibility efficiency, expected DMD% and expected DE% of mixed-species diets were calculated (except for herring and Magister squid diet). The expected digestibility of energy for mixed-species diets (except for herring + Magister squid diet) was calculated as a weighted mean from the observed DE% of the single-species diet counterparts, proportional to the energy densities of each individual prey species in the diet according to (Forster 1999):

$$Expected\ DE\ (\%) = \frac{(Mass_{F1} \times Energy_{F1} \times DE\%_{F1}) + (Mass_{F2} \times Energy_{F2} \times DE\%_{F2})}{(Mass_{F1} \times Energy_{F1} + Mass_{F2} \times Energy_{F2})}$$

Similarly, expected DMD% for the mixed diets were calculated from the observed DMD% from the relevant single-species diet counterparts, weighed proportional to the ingested mass (dry-weight) of each component species. Statistical differences between the expected and observed DMD% and DE% were determined using a Welch two sample *t*-test.

Statistical differences in metabolic measurements—specifically, resting metabolic rate (RMR) and the added thermoregulation cost (TC)—attributable to changes in diet were determined via LME models in the same manner as previously explained. One sample *t*-tests were also used to determine whether the added cost of TC was significantly different from zero. Preliminary analysis resulted in data from one of the fur seals (ME08) being considered an outlier because it failed to fulfill the assumptions of the LME models when included in the analysis (e.g., residual normality and homogeneity) (Dalton et al. 2014). Excluding this animal from all RMR and TC metabolic data analysis resulted in all LME models meeting the assumptions of normality of the random effect and of the residual errors and homogeneity of the variance (Pinheiro and Bates 2000; Crawley 2007; Zuur et al. 2009; Galecki and Burzykowski 2013).

Significance was determined at the 5% rate of error for all tests, and results are presented as mean ± Standard Deviation (SD) where appropriate.

Results

Changes in body mass

The overall mean body mass of the fur seals at the start of each of the diet trials was 23.3 ± 0.4 kg (mean ± SD; Table 2.2). Despite minor changes in body mass while on the different diets (ranging from herring and pollock diet +1.3 ± 3.3%, to herring and Magister squid diet -2.3 ± 2.3%), there were no significant differences in percent body mass change due to diet (%) (F_{33}= 0.4, p= 0.9).

Table 2.2 Mean (± S.D.) body mass of six captive female northern fur seals at the start of feeding trial, mean ingested mass (wet) for the eight experimental diets with their respective proximate composition (crude protein and lipid content), energy density, and manganese (Mn^{2+}) concentration (dry-weight basis).

Diet	Mean body mass (kg)	Mean Ingested mass (kg)	Water (%)	Total lipid (%)	Crude protein (%)	Energy density (kJ g^{-1})	Mn^{2+} (ppm)
Herring	23.9	1.6	68.5	38.0	47.1	24.3	5.1
	(3.5)	(0.3)	(3.6)	(0.01)	(0.01)	(0.01)	(0.01)
Pollock	23.1	2.3	75.3	35.8	62.8	22.1	(2.4)
	(3.1)	(0.3)	(1.3)	(0.01)	(0.01)	(0.01)	(0.01)
Capelin	23.2	3.3	82.6	3.3	67.6	15.2	2.9
	(3.3)	(0.5)	(1.4)	(0.01)	(0.01)	(0.01)	(0.01)
Herring + Pollock	23.1	2.0	72.4	37.0	54.6	23.1	3.8
	(3.2)	(0.1)	(0.04)	(0.01)	(0.1)	(0.01)	(0.02)
Herring + Capelin	23.0	2.9	79.0	15.9	60.2	18.7	3.7
	(2.7)	(0.3)	(0.04)	(0.1)	(0.08)	(0.04)	(0.01)
Herring + Magister Squid	23.8	2.4	70.3	43.2	57.2	23.1	4.3
	(3.2)	(0.2)	(0.01)	(0.1)	(2.8)	(0.01)	(0.02)
Pollock + Capelin	22.9	3.0	79.9	15.9	65.8	18.3	2.7
	(2.8)	(0.5)	(0.5)	(2.2)	(0.3)	(0.5)	(0.03)
Herring + Pollock + Capelin	23.1	2.6	77.9	21.0	60.7	19.7	3.4
	(2.8)	(0.5)	(0.06)	(0.2)	(0.9)	(0.05)	(0.01)

Prey item and dietary characteristics

Proximate composition, energy density, and manganese concentration differed among the four experimental prey items (dry-weight basis; Table 2.1). Overall, Magister squid had the highest lipid content (44.3%), while capelin had the lowest (4.0%). Conversely, capelin had the highest protein content (81.6%), while Magister squid had the lowest (46.7%). Herring (batch A) had the highest energy density (24.3 kJ g^{-1}), and capelin had the lowest (15.2 kJ g^{-1}).

For the eight experimental diets, proximate compositions (dry-weight basis) and energy density also differed significantly (Table 2.2). Lipid content varied from 3.3 ± 0% (capelin diet) to 43.2 ± 0.1% (herring and Magister squid diet) (LRT= 265.3, $p<0.001$). Diet protein content also differed significantly, ranging from 47.1 ± 0% (herring diet) to 67.6 ± 0% (capelin diet) (LRT=366.9, $p<0.001$), while energy density ranged from 15.2 ± 0 kJ g^{-1} (capelin diet) to 24.3 ± 0 kJ g^{-1} (herring diet) (LRT=266.7, $p<0.001$). As the eight diets were balanced for GEI at maintenance levels, ingested mass also differed with diet (wet-weight) (LRT= 76.9, $p<0.001$). Mean ingested mass ranged from 1.6 ± 0.3 kg (herring only diet) to 3.3 ± 0.5 kg (capelin only diet) (Table 2.2).

Fecal energy density was significantly different across diets (Table 2.3), with the pollock diet having the lowest fecal energy density (7.7 ± 1.1 kJ g^{-1}) and the herring and Magister squid diet having the highest (12.9 ± 0.9 kJ g^{-1}) (LRT= 56.5, $p<0.001$). However, to calculate total daily FEL%, these data were combined with the Mn^{2+} data and the prey energy density data (*see* equation 3). The Mn^{2+} of the fecal samples ranged from 20.7 ± 3.3 ppm for the pollock diet to 60.9 ± 9.7 ppm for the herring diet (Table 2.3).

Despite the fact that GEI was targeted to be within a specific range of daily intake (i.e., 11,500–12,500 kJ d^{-1}) regardless of diet type, daily GEI differed significantly across diets (LRT= 61.1, $p<0.001$; Table 2.4) due to two anomalies. The first was that the animals refused to eat sufficient quantities of capelin (~3.3 kg), which resulted in GEI being significantly lower while consuming capelin than for other diets (8712.9 ± 1409.7 kJ d^{-1}). The second was that the herring and Magister squid diet had the highest GEI (15866.0 ± 1426.8 kJ d^{-1}), a byproduct of an attempt to maximize the use of the available Magister squid as the fur seals showed high enthusiasm to its consumption. Surprisingly, these differences in GEI (and related differences in net energy gain) did not result in statistically significant changes in body mass.

Changes in digestibility and bioenergetics due to changes in diet

There were significant differences in DMD% among the experimental diets (LRT= 38.0, $p<0.001$; Table 2.4). DMD% was significantly lower for pollock (88.2 ± 1.1%) than most other diets, and highest for the herring and Magister squid diet (92.3 ± 0.1%). DMD% also decreased significantly with increased protein content of diets (%) (LRT= 9.9, $p= 0.002$; Fig. 2.1).

Table 2.3 Mean (± SD) proximate composition (crude protein and lipid content), energy density and manganese (Mn^{2+}) concentration (dry-weight basis) of fecal samples from six captive female northern fur seals when consuming experimental diets.

Diet	Water (%)	Total lipid (%)	Crude protein (%)	Energy density (kJ g^{-1})	Mn^{2+} (ppm)
Herring	64.2	7.4	19.8	10.1	60.9
	(4.6)	(1.5)	(2.2)	(1.1)	(9.7)
Pollock	70.2	4.7	14.3	7.7	20.7
	(4.6)	(0.7)	(1.2)	(1.1)	(3.3)
Capelin	68.8	7.3	20.3	9.7	30.5
	(4.1)	(1.3)	(2.0)	(0.9)	(7.7)
Herring + Pollock	69.0	5.8	16.8	9.1	39.0
	(4.6)	(1.2)	(2.2)	(1.9)	(3.0)
Herring + Capelin	67.5	6.7	19.4	9.5	41.8
	(4.7)	(1.4)	(2.0)	(1.2)	(7.4)
Herring + Magister Squid	65.2	12.9	22.9	12.9	56.2
	(1.7)	(0.8)	(1.8)	(0.9)	(2.6)
Pollock + Capelin	69.2	5.7	17.4	8.7	24.9
	(4.8)	(0.7)	(1.5)	(1.3)	(6.5)
Herring + Pollock + Capelin	68.7	6.4	18.0	9.1	37.8
	(4.5)	(1.1)	(1.9)	(0.9)	(7.1)

FEL%, expressed as a percentage of GEI, ranged from 3.1 ± 0.3–4.1 ± 0.6% and was significantly different across diets (LRT= 19.6, p= 0.006; Table 2.4). The lowest FEL% was for the herring only diet and the highest FEL% was for the pollock and capelin diet. Both the protein content (%) and mean ingested mass significantly affected FEL%, such that increases in proportion of protein (LRT= 9.5, p= 0.002) and ingested mass (LRT= 9.4, p= 0.002) resulted in increased FEL%.

Similarly, DE%, which is the inverse of FEL%, was observed to be high overall and differed significantly by diet (LRT= 19.6, p= 0.006; Table 2.4). DE% ranged from 95.9 ± 0.7% for the pollock and capelin diet, to 96.9 ± 0.3% for the herring only diet. It was also inversely related to both mean ingested mass (LRT= 9.4, p= 0.002) and protein content of diets (%) (LRT= 9.5, p= 0.002), such that increased intake in either ingested mass or protein resulted in decreased DE% (Fig. 2.2).

Both DMD% and DE% reflect digestive efficiencies, but the former is a measure of dry-matter digestibility while the latter is determined on an energetic basis. While there was a significant positive relationship between DMD% and DE% across all eight experimental diets (Fig. 2.3; DE%= 0.21×DMD% + 77.8), the measures are not interchangeable as evident by the slope of 0.2 and weak correlations. While a standard r^2 cannot be calculated on mixed linear models, the model had both a r_m^2 and $r_c^2 = 0.38$ where r_m^2 stands for marginal r^2 and signifies the variance explained by fixed factors in the model, and r_c^2 stands for conditional r^2 and is understood as the variance explained by both fixed and random factors (LRT= 21.6, p<0.001).

UEL%, expressed as percentage of GEI, ranged from 8.9 ± 0.1–22.0 ± 0.2% and was significantly different across diets (LRT= 247.9, p<0.001; Table 2.4). The lowest UEL% was from the herring only diet and the highest UEL% was from the capelin diet. There was a significant interaction between the protein content (%) and lipid content (%) among experimental diets such that the interaction of both factors together affected UEL% significantly more than each factor separately. UEL% increased with increases in protein and decreased with increases in lipid content (LRT= 62.8, p<0.001).

ME% available to the fur seals was calculated by the subtraction of fecal and urinary energy losses from the GEI (Table 2.4). ME% differed significantly by diet (LRT= 197.5, p<0.001). The lowest amount of energy available was 70.3 ± 1.5% from the capelin only diet, while the highest was 87.2 ± 0.2% from the herring only diet. ME% increased significantly with increasing lipid content of diets (%) (LRT= 133.1, p<0.001).

HIF% differed significantly by experimental diet (LRT= 36.9, p<0.001; Table 2.4). HIF% was significantly greater while consuming the capelin only diet (12.4 ± 2.0%) and the least costly while consuming the herring only diet (4.3 ± 1.0%). Furthermore, HIF% varied

Table 2.4 Mean (± SD) dry matter digestibility (DMD%), gross energy intake (GEI), fecal energy loss (FEL%), digestible energy (DE%), apparent digestible nitrogen intake (ANI), urinary energy loss (UEL%), metabolizable energy (ME%), heat increment of feeding (HIF%), and net energy (NE%) of six captive female northern fur seals across the eight experimental diets.

Diet	DMD%	GEI ($kJ\ d^{-1}$)	FEL%	DE%	ANI ($g\ d^{-1}$)	UEL%	ME%	HIF%	NE%
Herring	91.5	12135.7	3.1	96.9	39.4	9.9	87.2	4.3	83.0
	(1.1)	(2412.5)	(0.3)	(0.3)	(7.9)	(0.1)	(0.2)	(1.0)	(1.0)
Pollock	88.2	12688.6	3.7	96.3	50.9	10.3	86.4	6.5	80.0
	(1.1)	(1570.4)	(0.5)	(0.5)	(6.1)	(0.1)	(0.5)	(3.8)	(3.5)
Capelin	90.2	8712.9	4.0	96.0	72.3	26.7	70.3	12.4	57.9
	(1.9)	(1409.7)	(0.8)	(0.8)	(11.6)	(1.9)	(1.5)	(2.0)	(2.6)
Herring + Pollock	90.1	12482.5	3.5	96.5	45.3	10.1	86.8	7.1	79.7
	(0.4)	(787.9)	(0.7)	(0.7)	(2.7)	(0.1)	(0.7)	(2.3)	(2.8)
Herring + Capelin	90.8	11301.8	3.5	96.5	65.3	18.6	78.5	7.9	70.6
	(1.2)	(1245.9)	(0.4)	(0.4)	(6.8)	(0.1)	(0.3)	(3.0)	(3.1)
Herring + Magister Squid	92.3	15866.0	3.9	96.1	55.1	9.3	87.1	6.0	81.1
	(0.1)	(1426.8)	(0.2)	(0.2)	(4.8)	(0.1)	(0.1)	(1.5)	(1.5)
Pollock + Capelin	88.6	11118.7	4.1	95.9	66.3	18.1	78.5	6.9	71.6
	(2.2)	(1613.2)	(0.6)	(0.7)	(10.9)	(1.2)	(1.1)	(2.0)	(1.2)
Herring + Pollock + Capelin	91.0	11472.6	3.3	96.7	59.5	15.8	81.4	5.2	76.2
	(1.0)	(2184.1)	(0.4)	(0.4)	(11.5)	(0.2)	(0.4)	(1.1)	(1.0)

Note: All digestibility measures are expressed as a proportion of GEI, except for UEL% which is expressed as a proportion of DE%.

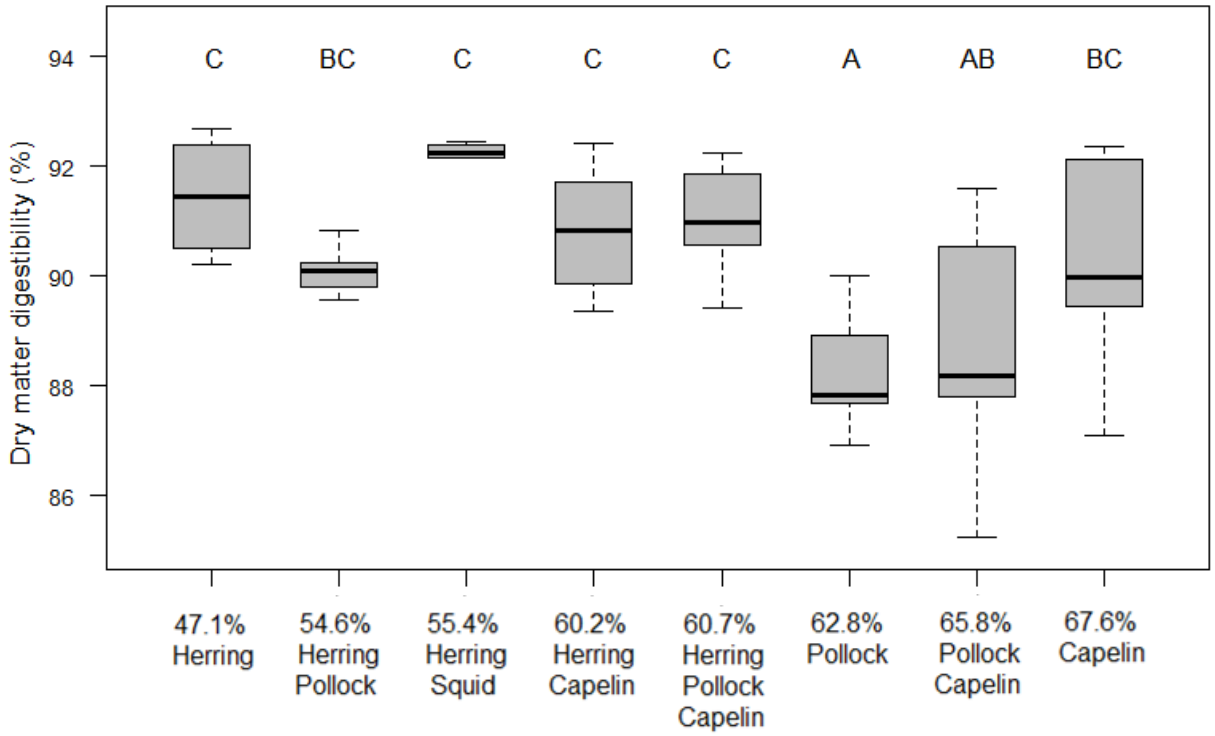

Figure 2.1 Dry matter digestibility (DMD%) of the eight experimental diets tested in six captive female northern fur seals. Diets are arranged accordingly from low to high protein content (%), as denoted above the diet labels. Each box represents the median (thick horizontal line), first and third quartile ("hinges") and 95% confidence interval of median ("notches"). Data for each diet trial are from the fur seals, with the exception of herring and squid diet which was only consumed by four of the animals. Letters above boxes indicate significant differences between diets ($p<0.05$).

significantly with the lipid content of the diets (%), where HIF% decreased as lipid content increased (LRT= 15.3, p= 0.001; Fig. 2.4)

Total net energy gain (kJ day^{-1}) increased significantly with increases in GEI (kJ day^{-1}) across experimental diets with a positive relationship (Fig. 2.5; NE= 1.04GEI – 3043.9, r_m^2= 0.927 and r_c^2=0.937). However, NE% as a proportion of GEI also differed by diet (LRT= 122.3, p<0.001; Table 2.4). NE% was lowest while consuming the capelin diet, where animals retained only 57.9 ± 2.6% of the ingested energy and highest when consuming the herring diet, where they retained 83.0 ± 1.0%. Lipid content in the diets (%) was a significant factor in determining

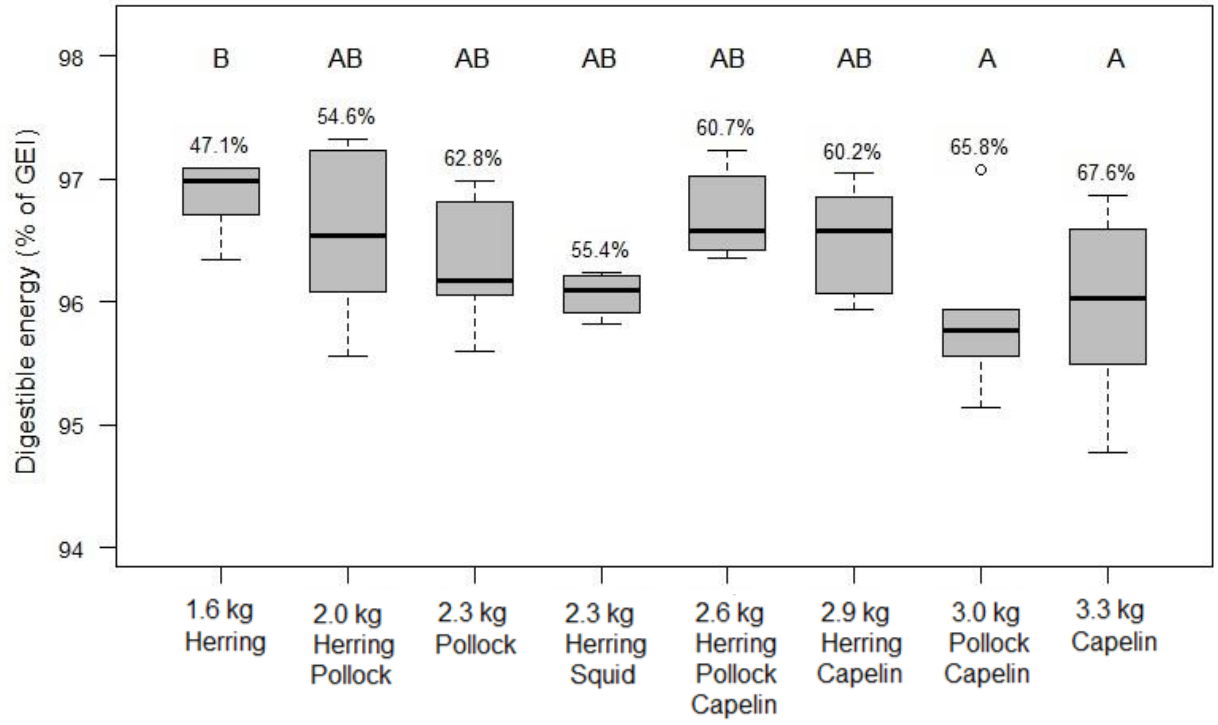

Figure 2.2 Digestible energy (DE%) of the eight experimental diets tested in six captive female northern fur seals. Diets are arranged accordingly from low to high mean ingested mass (wet-weight) during experimental trials, as denoted above the diet labels. Each box represents one diet trial for the fur seals, with the exception of the herring and squid diet which was only consumed by four of the animals. Numbers above individual boxes indicate the protein content (%) for each of the experimental diets. Letters above boxes indicate significant differences between diets ($p<0.05$).

NE%, such that fur seals retained the most NE% from fattier diets (LRT= 77.7, $p<0.001$; Fig. 2.6).

Changes on digestive efficiency due to diet mixing

Comparisons between observed DMD% and expected DMD% of mixed-species diets (based on calculations from observed DMD% of their single-species diet components) showed no significant changes in DMD% due to diet mixing for any of the diets ($p>0.05$). Similarly, expected DE% values were not significantly different for any of the mixed-species diets when

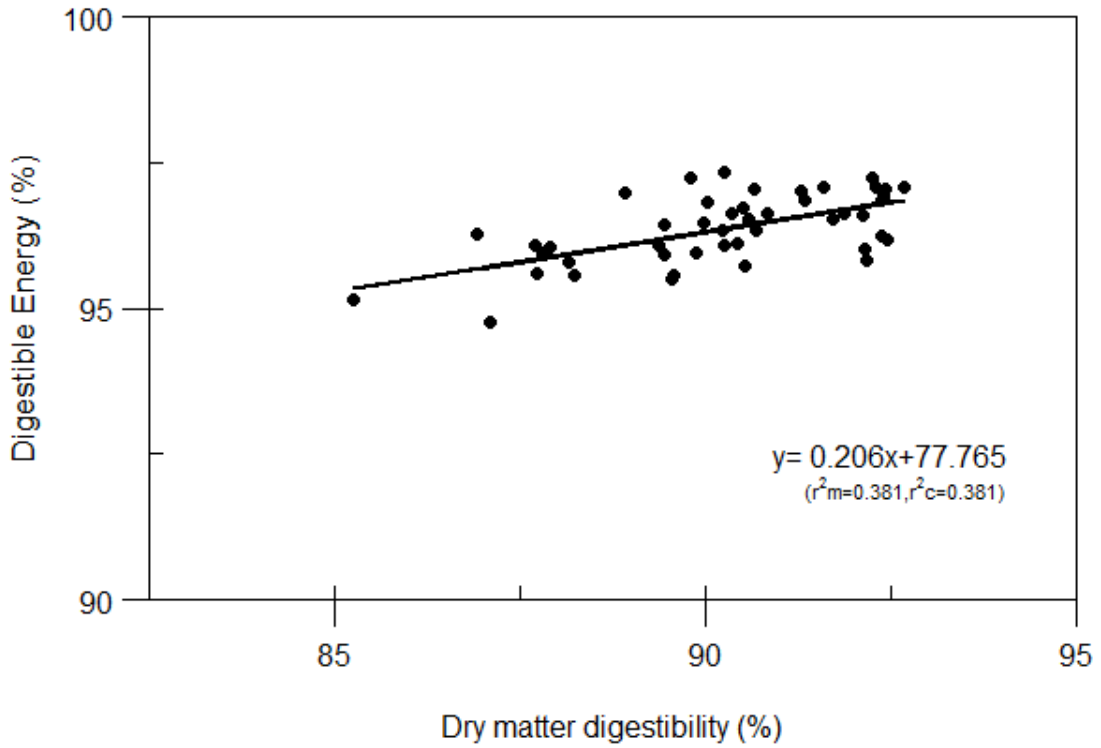

Figure 2.3 Relationship between dry matter digestibility (DMD%) and digestible energy (DE%) for six captive female northern fur seals when consuming eight experimental diets. Each point is the mean for one of the individual fur seals in each experimental diet, with the exception of herring and Magister squid diet which was only consumed by four of the animals.

compared to their respective observed DE% ($p>0.05$). Therefore, diet mixing did not provide a significant advantage to fur seals to better assimilate either dry matter or energy.

Effect of diet on metabolism

The average mass-specific resting metabolic rate (RMR) while the fur seals were resting in ambient air across all diets was 10.0 ± 3.4 mL O_2 kg^{-1} min^{-1}. While mean mass-specific RMR ranged from 8.2 ± 4.3 4 for the pollock diet to 11.9 ± 4.6 mL O_2 kg^{-1} min^{-1} for the herring and Magister squid diet (Table 2.5), it did not significantly differ among the experimental diets (F_{26}= 2.2, p= 0.07; Fig. 2.7). Mean mass-specific metabolic rate while the fur seals were partially submerged in 2˚C water was 17.1 ± 4.1 mL O^2 kg^{-1} min^{-1}, and this ranged from 13.8 ± 4.4 for the pollock diet to 18.9 ± 5.4 mL O_2 kg^{-1} min^{-1} for the herring and pollock diet (Table 2.5).

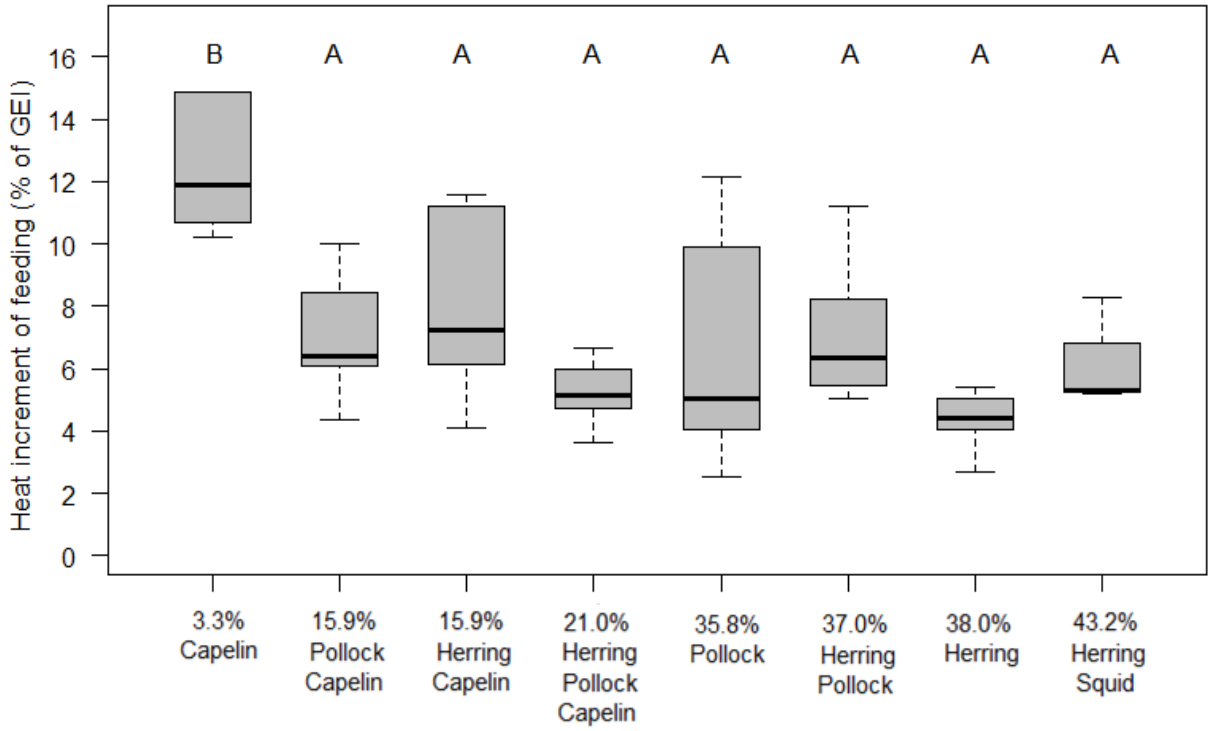

Figure 2.4 Heat increment of feeding (HIF%) of the eight experimental diets consumed by six captive female northern fur seals. Diets are arranged accordingly from low to high lipid content (%) in the experimental diets, as denoted above the diet labels. Each box represents one diet trial for the fur seals, with the exception of the herring and squid diet which was only consumed by four of the animals. Letters above individual boxes indicate significant differences between diets ($p<0.05$).

The added thermoregulation cost (TC) of being partially submerged in 2˚C water—calculated as the average mass-specific amount of oxygen consumed above RMR—was 6.9 ± 3.3 mL O^2 kg^{-1} min^{-1}. The added TC ranged from 5.6 ± 3.3 to 8.1 ± 3.1 mL O^2 kg^{-1} min^{-1}, where the lowest rate was for the pollock diet and the highest for capelin (Fig. 2.8). Also, TC was found to be significantly different from zero ($p<0.05$) for all diets, with the exception of the fur seals on the herring and Magister squid diet ($p= 0.05$). The latter exception is likely the result of the smaller sample size for the TC trial, since only data from three out of the four animals consuming the diet was collected. However, TC was not significantly different across experimental diets ($F_{26}= 1.2$, $p= 0.4$).

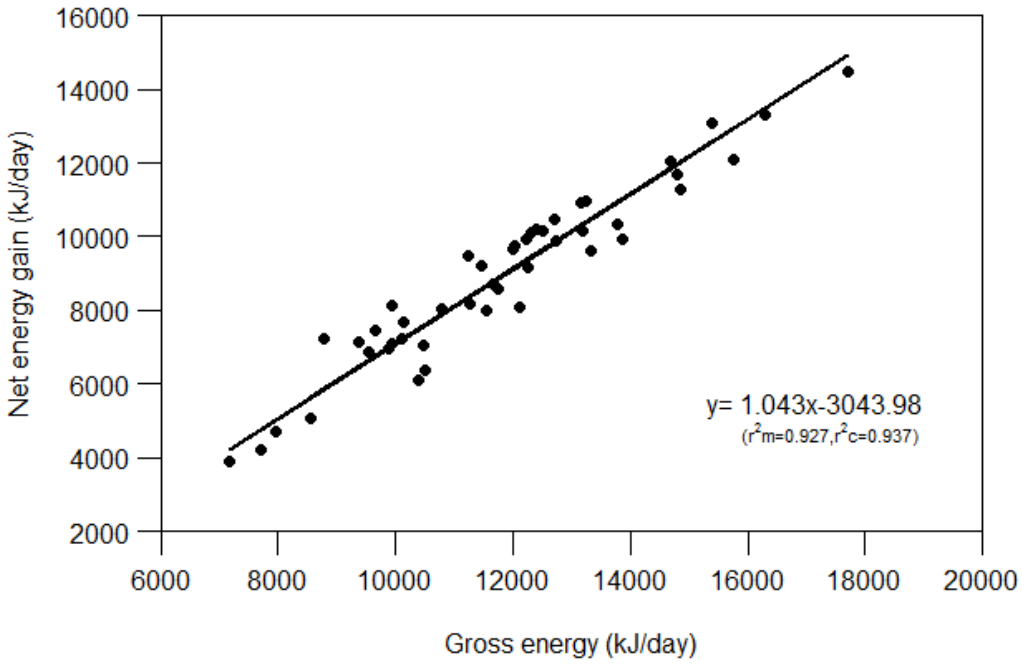

Figure 2.5 Relationship between net energy gain (NE%) and gross energy intake (GEI) in six captive female northern fur seals. Each point represents the mean value for an individual animal on one experimental diet.

Discussion

In most broad classifications, food with a high energy density is considered to be of "high quality", implying that it readily provides sufficient energy to its consumer. The chemical energy ingested via food is defined as a consumer's gross energy intake (GEI), and is derived from the breakdown of its individual components. For fish, this is a product of their lipid and protein content. It has been estimated that one gram of lipid contains ~37.7 kJ of energy while one gram of protein provides 17.8 kJ (Blaxter 1989). However, the net energy gain (NE%)—the biologically useful energy (ATP) available to the consumer after food has been broken down and assimilated—is different. While NE% is roughly proportional to GEI, it is affected by various factors such as composition of diet, the level of food intake, and nutritional status (Schneider and Flatt 1975).

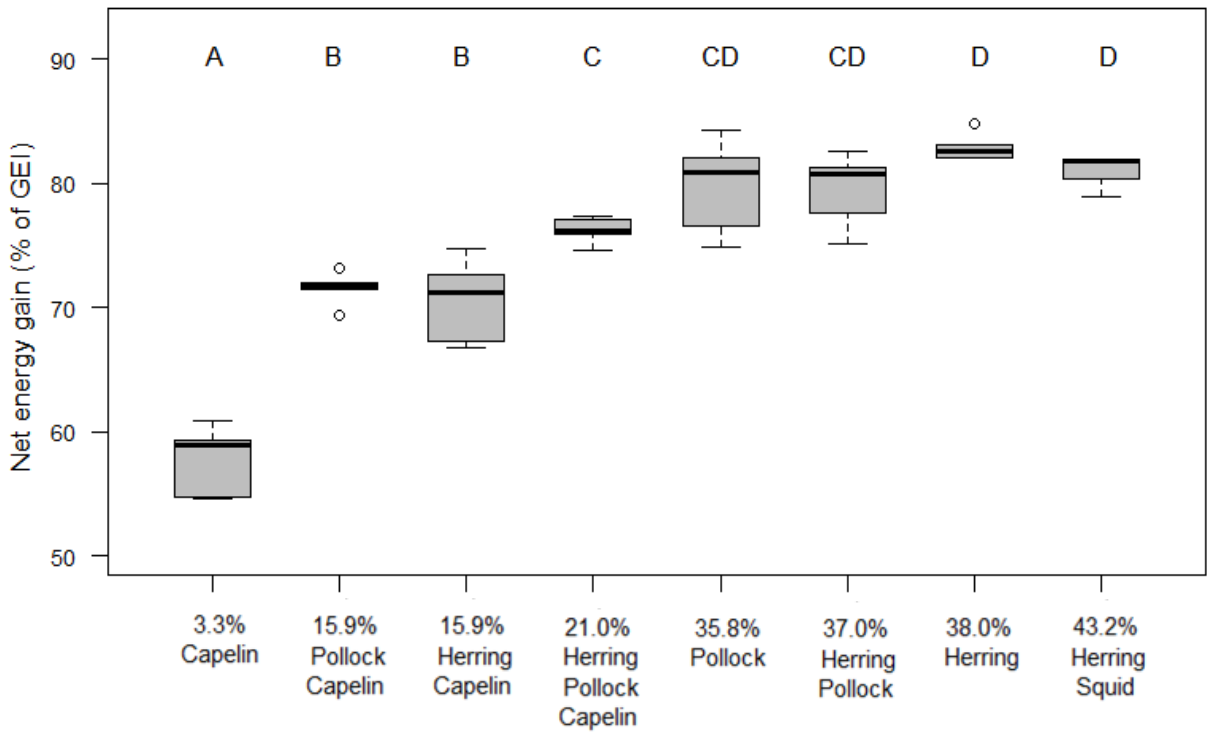

Figure 2.6 Net energy gain (NE% of gross energy intake GEI) from the eight experimental diets tested in six captive female northern fur seals. Diets are arranged accordingly from low to high lipid content (%) in the experimental diets, as denoted above the diet labels. Each box represents one diet trial for the fur seals, with the exception of herring and squid diet which was only consumed by four of the animals. Letters above individual boxes indicate significant differences between diets ($p<0.05$).

It is therefore necessary to empirically assess energy loss throughout the digestive process of an animal to determine and understand the NE% benefit of a particular diet.

Our study is the first to measure the complete pathway of energy transformation for an otariid, including digestible energy (DE%), the heat increment of feeding (HIF%), metabolizable energy (ME%), and net energy gained (NE%). Further, unlike previous studies with fur seals and most other pinnipeds, our study compared digestive efficiencies of mixed-species diets. Overall, our results showed that low energy density prey items—those normally classified as "low quality"—yielded significantly less NE% to the fur seals than would be predicted solely on the basis of GEI.

Table 2.5 Mean (± S.D.) mass-specific resting metabolic rate (RMR) while in ambient air, metabolic rate while partially submerged in 2˚C water and added thermoregulation cost (TC) (mL O^2 kg^{-1} min^{-1}) of six female northern fur seals (excluding ME08), consuming eight experimental diets. TC was calculated as the change in metabolic rate between RMR in ambient air and when the fur seals were partially submerged in 2˚C water.

Diet	RMR	Metabolic rate in water	TC
Herring	11.5 (3.5)	17.4 (4.7)	6.1 (3.3)
Pollock	8.2 (4.3)	13.8 (4.4)	5.6 (3.3)
Capelin	8.6 (3.8)	16.7 (5.1)	8.1 (3.1)
Herring + Pollock	11.3 (3.5)	18.9 (5.4)	7.6 (5.3)
Herring + Capelin	11.4 (4.1)	18.5 (4.9)	7.1 (3.0)
Pollock + Capelin	9.2 (4.3)	15.3 (4.5)	6.0 (2.5)
Herring + Magister squid	11.9 (4.6)	18.4 (4.7)	6.5 (3.0)
Herring + Pollock + Capelin	8.8 (4.7)	16.7 (5.6)	7.9 (3.3)
Mean (all diets)	10.1 (1.5)	17.0 (1.7)	6.9 (0.9)

Note: data for the herring and squid diet was only collected from three out of the four animals that consumed the diet.

This was largely due to the lower digestibility of protein vs. lipid, compounded by the negative effect of required increased prey mass intake. Furthermore, contrary to the mixed diet hypothesis, there appeared to be no benefit in terms of energy digestibility associated with consuming mixed-species diets over equivalent single-species diets.

Changes in digestibility and bioenergetics due to changes in diet

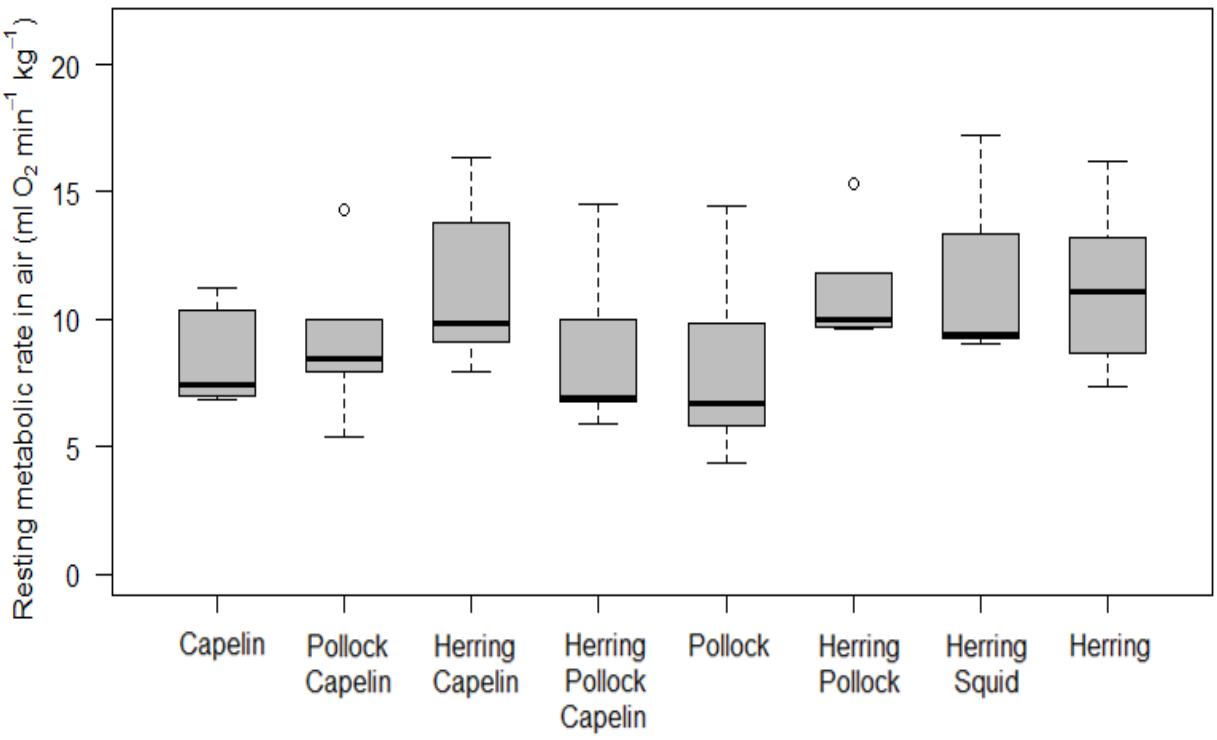

Figure 2.7 Mean mass-specific resting metabolic rate in ambient air of six captive female northern fur seals across eight experimental diets. Boxes represent the mean of each diet trial consumed by fur seals (excluding animal ME08), with the exception of herring and squid diet for which only data from three out of the four animals that consumed the diet was collected ($p<0.05$).

In the past, many studies have quantified the dry matter digestibility (DMD%)—a measure of the proportion of indigestible to digestible dry matter in food—as a proxy for energetic digestibility in pinnipeds. The fur seal's DMD% in our study was high and varied significantly across diets (Table 2.4). DMD% was lowest for walleye pollock, (Fig. 2.1), due to a combination of its high protein content and the fact that pollock has large bony structures compared to the other prey consumed, making it more challenging to digest.

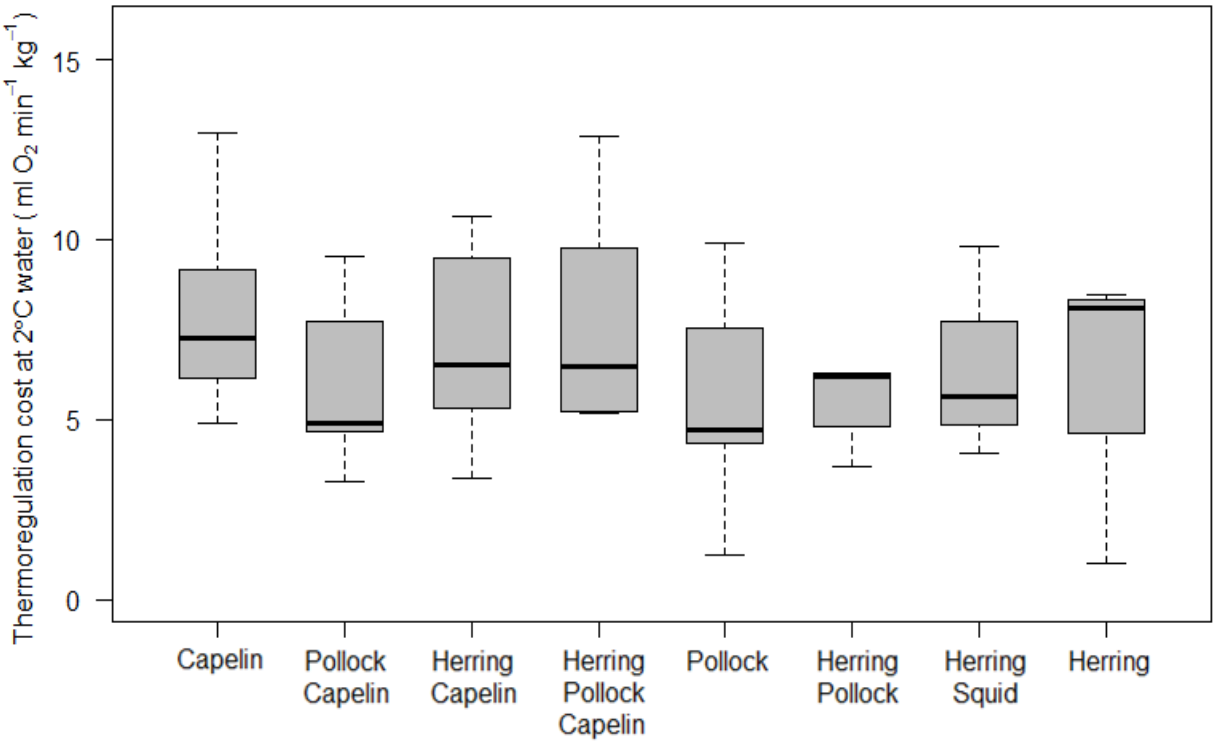

Figure 2.8 Mean mass-specific thermoregulation cost above the resting metabolic rate in ambient air when six female northern fur seals were partially submerged in 2˚C water across all eight experimental diets. Boxes represent the mean of each diet trial consumed by fur seals (excluding animal ME08), with the exception of herring and squid diet for which only data from three out of the four animals that consumed the diet was collected ($p<0.05$).

Dry matter digestibility values in our study were consistent with previous pinniped studies (Rosen and Trites 2000b). For example, our DMD values for the pollock diet (88.2±1.1%) where similar to those of Miller (1978), who reported that the DMD% for northern fur seals on a pollock diet (86.6–90%) were lower than for diets of herring capelin, or squid. While the highest DMD% in our study was for the herring and Magister squid diet (92.3±0.1%), the DMD of our herring only diet (91.5±1.1%) was comparable to previous fur seal studies by both Miller (1978) and Fadely (1990). Even though DMD% values are often reported, they are not informative with respect to the energy absorbed from the various diets (Rosen and Trites 2000b). Previous studies on northern fur seals did not extend their research beyond the

measurement of DMD%, which has meant that the energy transformation pathway for fur seals has been unclear.

Our values of digestible energy (DE%) were generally high across diets (Table 2.4), and were comparable to previous pinniped studies (Rosen and Trites 2000b), as well as other carnivorous terrestrial mammals consuming either meat or fish (Barbiers et al. 1982; Best 1985; Pritchard and Robbins 1990). The similarly high DE% among these carnivorous species was expected because they are all characterized by relatively simple stomachs and short intestinal tracts (Stevens and Hume 1995).

However, digestible energy (and its inverse, fecal energy loss) was not constant across diets for the fur seals—and was negatively affected by increases in both protein content of the diet and ingested mass (Fig. 2). The significant decrease in DE% with increasing protein content of the diet may be explained by the fact that, among all of the components in food, the breakdown and assimilation of proteins to obtain energy takes the most time and effort (Blaxter 1989). Protein molecules are long chains of amino acids with strong peptide bonds, which require great mechanical and chemical effort to break down, and require more time to digest (Blaxter 1989; Stevens and Hume 1995). This means that diets higher in protein content would have higher digestive costs and would provide less DE%. This decrease in DE% values with increasing nitrogen intake has been consistently observed in other pinniped species (Keiver et al. 1984; Ronald et al. 1984). DE% was also significantly affected by increases in ingested mass. This decrease in the efficiency of the digestive process with increases in food consumption levels has been confirmed in other species (Schneider and Flatt 1975), and is due in part to a decrease in chemical and mechanical efficiency (because of the higher food bolus), as well as the increased energy required to produce more fecal waste (both of which contribute to decreases in DE% gain).

The protein content in our experimental diets also affected the fur seal's urinary energy loss (UEL%), whereby the diets with the greatest protein content had the greatest UEL% (Tables 2.2 and 2.4). Lipid content of the diet also had a significant interaction along with protein content, which affected UEL%. This may be partly attributable to the complementary relationship between lipid and protein in food, such that lipid-rich diets are low in protein content and *vice versa*. It is suspected that protein was the primary driver of the relationship, as the

breakdown of protein produces more wastes than the breakdown of other dietary components. The primary nitrogenous waste product is ammonia, which is a toxic byproduct that must be transformed to urea to be eliminated. The UEL% values of the fur seals were comparable to those of previous studies with pinnipeds (Parsons 1977; Ashwell-Erickson and Elsner 1981; Keiver et al. 1984; Ronald et al. 1984; Goodman-Lowe et al. 1999). However, it is interesting to note that the UEL% from diets containing capelin (including the capelin only diet) was unexpectedly high.

Urinary energy loss is notoriously difficult to directly measure from complete urine collection in large mammals. In our study, UEL% was estimated from the apparent digestible nitrogen intake of each diet, using equations generated from previous studies with phocid seals (Keiver et al. 1984; Goodman-Lowe et al. 1999). These phocid studies found that the measured energy density of urine was higher (leading to higher UEL% values) than if calculated solely from energetic values per gram of nitrogen as urea (Keiver et al. 1984). This suggests that an unidentified component that was not of nitrogenous origin within the urine contributed to the energetic content of the samples (Keiver et al. 1984). As a result, our estimated UEL% were approximately 1.5 times higher than estimates based solely on nitrogen content.

While the values for urinary energy loss in our study may seem higher than previous estimates, it is worth noting that most previous UEL% studies in pinnipeds have been undertaken using herring diets that had a relatively low protein content (lipid-rich). An exception was a study where harbour seals (*Phoca vitulina*) fed a pollock only diet (90.6% protein) had a UEL% that was 1.5 times higher than when the seals were fed only herring (Ashwell-Erickson and Elsner 1981). Similarly, the UEL% of the fur seals fed the capelin diet (67.6% protein) was 2.7 times greater than the herring only diet (47.1% protein) (Table 2.4), further demonstrating the high cost of disposal of nitrogenous waste products from protein sources.

Few pinniped studies have measured metabolizable energy (ME%), the dietary energy remaining after accounting for the energy that is lost through the excreta. Most of these were obtained when the animals were fed single-species diets, and ranged from 82.7–92.5% for herring, 85.9–89.4% for pollock, and 78.3% for squid (Parsons 1977; Ashwell-Erickson and Elsner 1981; Keiver et al. 1984; Ronald et al. 1984; Costa 1988). The ME% of the fur seals in our study across all experimental diets fit well within these previous pinnipeds studies (Table

2.4), and with ME% values from other carnivorous terrestrial mammals consuming either fish (Pritchard and Robbins 1990) or mammal meat diets (Davison et al. 1978).

Overall, metabolizable energy values for the fur seals were significantly positively correlated to lipid content of the diet. In contrast, Goodman-Lowe et al. (1999) reported that diets higher in protein and lower in lipid content provided the greatest ME% to Hawaiian monk seals (*Monachus schauinslandi*). However, it is important to note that their comparisons were not calculated as proportions of the gross energy intake—and that their data supports the same pattern found in our study when recalculated appropriately.

Of all the digestive processes, one of the most studied and best understood is the heat increment of feeding (HIF%), also known as the specific dynamic action of feeding (Jobling 1983). HIF% across a wide range of vertebrate and invertebrate taxa has been found to depend upon various features of the ingested meal (composition, type, size, temperature), characteristics of the animal (body size, sex and age), and of the environment where the animal is found (ambient temperature) (Blaxter 1989; Secor 2008). In mammals, the main factors that affect HIF% are the consumer's body mass, the energetic content of the food, and the ingested mass. This latter factor can account for about 90% of the variation in some animal's HIF% (Secor 2008).

Among pinnipeds, HIF% ranges from 4.7–16.8% of GEI when animals are fed herring-only diets, 5.7% for pollock diets and 11.5–13.0% for capelin diets (Rosen and Trites 1997). Estimates of heat increment of feeding for the fur seals in our study (Table 2.4) are comparable to other pinniped values reported by Rosen and Trites (1997). Overall HIF% was significantly affected by lipid content in the diet, where the diets with the higher lipid content required the least amount of energy to digest and absorb (Fig. 2.4). This coincides with the fact that the specific dynamic action of proteins is 32% and only 16% for lipids (Forbes and Swift 1944). For example, the fur seal's HIF% for the capelin only diet was significantly higher than the other diets, most likely due to its high protein content. It should also be noted that the ingested mass of the capelin diet (to attain an equivalent GEI) was significantly higher than the other diets (Table 2.2)—a factor that has also been observed to increase HIF% overall.

Mixed-species diets were predicted to have lower heat increment of feeding costs than single-species diets (Forbes and Swift 1944). However, our test to quantify the cost of the HIF%

of mixed-species diets with a pinniped showed that mixed-species diets do not lower the cost of HIF% (Fig. 2.4; Table 2.4). There were thus no energetic savings due to eating more than one prey species together in terms of the costs of digestion.

The energy remaining in the energy transformation pathway after accounting for the cost of heat increment of feeding is the net energy, which ranged from 57.9–83.0% for the fur seals (Table 2.4). The only other study to estimate NE% on a pinniped was with harbour seals, which reported NE% of 80.0–80.2% (Ashwell-Erickson and Elsner 1981). While the high NE% for some of the fur seal diets agrees with other carnivorous terrestrial mammals (83.5 ± 5.3%) and birds (83.4%) (Robbins 1993), some of our diets yielded surprisingly lower estimates.

The differences in net energy gain across our experimental diets was influenced by their lipid content, such that the highest NE% was for the herring diet and the lowest was for the capelin diet (Table 2.4). Similar to these findings, Fisher et al. (1992) reported that walruses (*Odobenus rosmarus*) feeding on lipid-rich herring diets had a higher apparent digestibility of lipids compared to those feeding on clam diets (who subsequently had a higher energetic gain). The findings from our fur seals and from walrus (Fisher et al. 1992) indicate that marine mammals are particularly adapted for high-lipid diets, given that the energetic digestibility and NE% return is significantly higher from fattier diets than from leaner diets (Fig. 2.6).

Robbins (1993) recognized that the amount of food that an animal must ingest to meet a fixed energetic requirement should be directly proportional to the losses in digestion and metabolism. However, as demonstrated by our results, the amount of food that an animal must consume to meet energetic requirements should be reconsidered in terms of the net energy gain from the food rather than in terms of the gross energy density. For example, the estimated amounts of capelin required to meet the fur seals' energy requirements based upon NE% were twice the amount of fish (~6.0 kg) compared to estimates calculated on GEI alone (Fig. 2.9). In contrast, the amount of herring required based on NE% would only be 20% more than estimates based on GEI due to herring being more digestible (Fig. 2.9). This example highlights how the

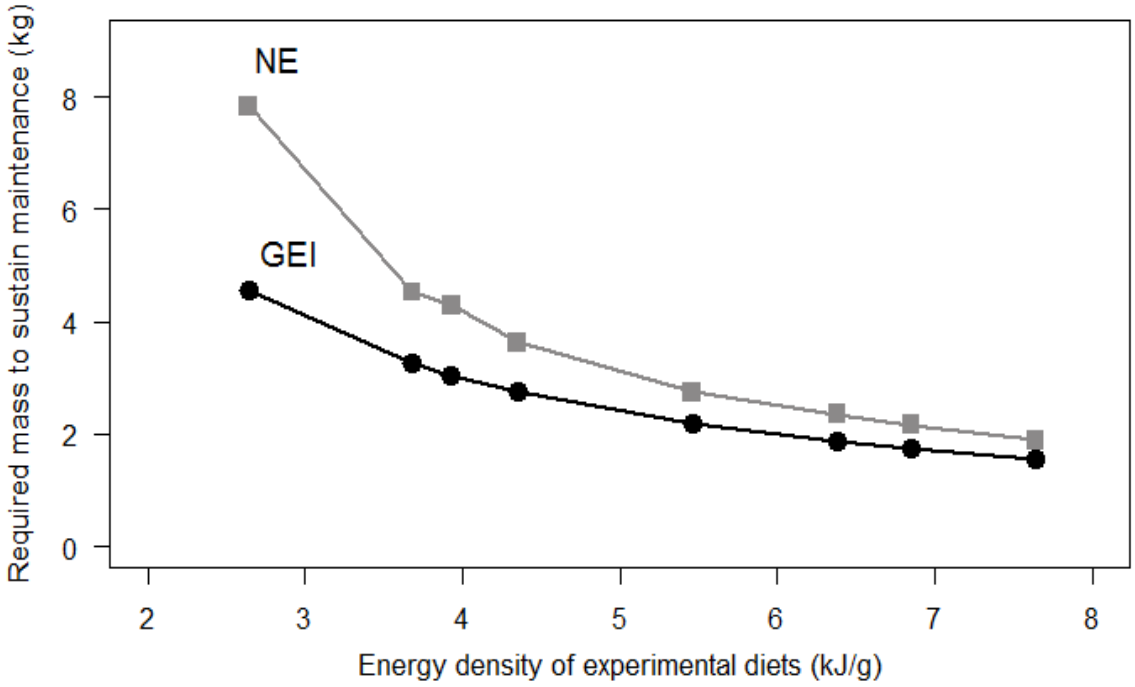

Figure 2.9 Changes in required mass intake (kg) to sustain maintenance energetic level (12,000 kJ/day) with the energy density (wet-basis) of the eight experimental diets (kJ/day) tested in six captive female northern fur seals. Data are presented as averages of the gross energy intake (GEI) in black circles and net energy gain (NE) in gray squares. The figure represents the differences between food intake levels required to meet energetic needs calculated on gross energy density of prey vs. the food intake level that take into account digestive energy losses (i.e. NE).

cost of energy transformation, and the variability of the digestive losses, can exaggerate the differences in the gross quality of the diet.

While the net energy gain by the fur seals was linearly related to the gross energy intake (Fig. 2.5), our study demonstrated that this relationship was driven by the lipid content of the diets, which was less costly to process and provided the fur seals with a greater energetic return per gram. These results emphasize how NE% depends upon the chemical nature of prey, and how the assimilation of these individual components can impact an animal's energy budget. It is nonetheless also important to recognize that the efficiency with which prey are assimilated is

dynamic over the course of an animal's life, and that it depends on the energetic, nutritional, and mineral and vitamin requirements of each stage of development (Reid et al. 1980).

Changes on digestive efficiency due to diet mixing

Mixed-species diets—those that consist of various prey items that differ qualitatively in their composition—are believed to provide a greater energetic benefit to the consumer than equivalent single-species diets (Penry and Jumars 1987; Singer and Bernays 2003). However, this hypothesis has only been tested on a few pinniped species. Experiments on captive harbour seals reported 30–40% higher digestible energy (DE%) values from mixed-species diets than from single-species diets (Trumble and Castellini 2005). While a similar difference was reported for the metabolizable energy (ME%) of Hawaiian monk seals (Goodman-Lowe et al. 1999), these differences were actually the result of differences in GEI and not proportional gains in ME%.

Our findings do not support the mixed-species diet hypothesis. The observed DE% and DMD% values of the mixed-species diets approximated the average values of their single-species DE% and DMD% constituents, and did not surpass them (Fig. 2.1; Fig. 2; Table 2.4). Similar results have been reported for harbour seals where the DE% of the mixed-species diet (30% herring, 30% capelin, 30% pollock and 10% *Loligo* squid) did not appear to be different from the herring-only diets (Yamamoto et al. 2009). While our results contradict the belief that mixed-species diets provide a significant advantage to fur seals to better assimilate either dry matter or energy, this does not imply that diet diversity is not important. Rather, the mixing of prey species is a fundamental component of foraging strategies and has ecological implications for predator-prey interactions, and ecological benefits overall (Stephens and Krebs 1986; Singer and Bernays 2003; Barbosa and Castellanos 2005).

Effect of diet on metabolism

Northern fur seals have been identified to change their foraging behaviour and dietary intake between seasons as they migrate through the North Pacific and Bering Sea (Kajimura 1984; Gentry and Kooyman 1986; Gentry 2002). Significant seasonal differences in resting metabolic rates (RMR) have also been identified in female fur seals (Dalton et al. 2014), but it is unclear how, if at all, these two seasonal changes are related. Some have suggested that diet quality can potentially affect physiological processes not directly associated with the digestive

process (Cruz-Neto and Bozinovic 2004). This is consistent with Steller sea lions (*Eumetopias jubatus*) significantly depressing their resting metabolism when consuming insufficient levels of low-energy diets (Rosen and Trites 1999). However, the dietary changes in our study had no impact on the fur seal's RMR (Table 2.5; Fig. 2.8). Nonetheless, mass-specific RMR values for the fur seals were within the range of RMR of other otariid and phocid seals (Miller 1978; Lavigne et al. 1986; Donohue et al. 2000; Dalton et al. 2014).

The fur seals in our study had thermoregulation cost (TC) rates that were 1.7 times higher when partially submerged in 2˚C water compared to metabolic costs when resting in ambient air (Table 2.5). Similarly, Costa and Gentry (1986)found that the at-sea metabolic rate of fur seals (lactating and non-lactating) was 1.8 times the on-shore fasting metabolic rate. This increase in metabolism in 2˚C water is similar to that reported for northern fur seal pups (Liwanag 2010; Rosen and Trites 2014). While the fur seals in our study exhibited a significant metabolic increase while in cold water, TC did not differ across the experimental diets (Fig. 2.9).

While diet did not directly affect resting metabolic rates or thermoregulation costs, it is possible that RMR and TC affect prey consumption by wild fur seals. Seasonal changes in energy intake requirements—due to seasonal requirements for growth or activity—could induce changes in diet to better fulfill those needs. In many marine mammal species, seasonal changes in energy requirements coincide with natural predictable changes in prey abundance or quality. Such is the case for pregnant or lactating harp seals (*Pagophilus groenlandica*), sea otters (*Enhydra lutris*) and Atlantic spotted dolphins (*Stenella frontalis*), that vary their prey consumption according to their reproductive condition (Ronald and Healey 1981; Riedman et al. 1988; Malinowski and Herzing 2015).

Energetic implications of consuming pollock

In our study, we chose prey species that allowed us to investigate the effects of prey composition (or quality) on digestibility and net energy gain. Our findings suggest that when adequate amounts of fish are available to fur seals, the quality of the diet is the major factor in determining the capacity of different prey to meet the fur seals' energetic requirements. These conclusions support optimal diet model predictions, where Estabrook and Dunham (1976) contended that small changes in the relative value of prey can be more effective in changing a predator's optimal diet than small changes in the relative abundance of the potential prey.

Another model of optimal digestion further indicates that the rate of efficiency of absorption of digestive products, and the rate of egestion of usable organic matter, both increase with food quality (Dade et al. 1990). This is important given that the quality of different prey species available to fur seals and other top predators likely differs significantly with both time of year and developmental stage (Van Pelt et al. 1997; Logerwell and Schaufler 2005; Vollenweider et al. 2011).

Measuring the digestive efficiency and net energy gain of northern fur seals consuming various prey species is important for evaluating whether the fur seal's current diet in the wild is negatively impacting their energy budgets. This entails using representative prey items that free-ranging fur seals may encounter or fish of comparable compositions. For example, the pollock we used was of relatively "high quality" and yielded a high net energy gain. However, the quality of pollock that the fur seals encounter in the wild usually differs considerably from this. Sinclair et al. (1994) reported that 65% of the fish in the stomachs of northern fur seals consisted of age-0 walleye pollock, and another 31% were of age-1 pollock. It appears that the pollock we fed our fur seals was about age-2 based on mean body size (Buckley and Livingston 1994). While whole-body proximate composition of young walleye pollock fluctuates across seasons, on average young pollock range from 3.7–4.8 kJ g^{-1}, 2.3–3.2% lipid, and 14.1–15.4% protein (wet-basis) (Van Pelt et al. 1997; Logerwell and Schaufler 2005; Vollenweider et al. 2011). These values are similar to the proximate composition values of the capelin used in our study, which had the highest HIF% cost and also exceedingly low NE% gain, and would be classified as "low quality" prey (Table 2.2; Table 2.4).

Miller (1978) estimated that a wild fur seal of median weight (23 kg) requires a daily energy intake of ~16,300 kJ d^{-1}. Combining the digestibility results from our study and the documented quality of the pollock that fur seals are currently consuming, fur seals foraging in the Bering Sea would need to consume ~6.2 kg of fish per day (~27% of their body weight) to obtain the required amount of energy, of which at least 4 kg would be pollock. However, our study suggests that free-ranging fur seals may be physically challenged to consume such amounts of fish, given that the fur seals in our study refused to eat more than 4 kg per day of the lower quality fish (capelin). However, further research is required to specifically test such satiation limits (Rosen et al. 2011; Calkins et al. 2013).

Our findings suggest that fur seals consuming primarily young pollock of poor quality could be nutritionally stressed despite there being a high biomass of pollock available to them. The higher digestive cost of processing large amounts of food with high protein and low energy density (such as young pollock) would increase the likelihood of the fur seals gaining less than the energy they ultimately require. Deriving sufficient energy from low quality prey sources becomes even more critical if stock densities are diminished, but the potential effect of changes in prey quality on nutritional status are independent of this consideration. Our study adds support to the hypothesis that the dominance of juvenile pollock in the current diet of wild fur seals in the Bering Sea is likely detrimental to their population health and reproductive fitness. Our results therefore have implications for the management of northern fur seals and the adult pollock fishery in the Eastern Bering Sea.

Conclusions

In summary, our study demonstrates that northern fur seals attained significant differences in net energy gain across experimental diets. These differences were driven by both the high energetic cost of protein digestion and the significantly higher energetic return of fattier diets. This highlights the importance of considering the individual digestion of each component of a diet to understand how fur seals obtain energy from particular prey items. Our study also highlights how differences in gross prey quality between prey items become exaggerated during the course of digestion. In addition, our results contradict the hypothesis that mixed-species diets provide an energetic advantage to fur seals over single-species diets. Furthermore, there was no effect of dietary changes on secondary metabolic costs of the fur seals, such as resting metabolism or the cost of thermoregulation. Collectively, our findings indicate that northern fur seals assimilate a higher proportion of the energy contained in high quality (lipid-rich) prey, and a lower proportion of the energy contained in lower quality prey such as pollock, particularly because of the higher digestive cost associated with handling large amounts of such low-quality prey. Our study therefore adds support to the nutritional stress hypothesis, by demonstrating the extent to which changes in prey quality result in proportionally larger changes in net energy gain. This has implications for population health, reproductive fitness, and determining why fur seal populations are declining in the central Bering Sea.

Chapter 3: High protein content in prey impacts macronutrient digestibility in northern fur seals

Summary

Pinnipeds, like all non-ruminant animals, have specific macronutrient requirements to satisfy physiological functions that are distinct from their energetic requirements. However, relatively little is known about the ability of pinnipeds to digest macronutrients, such as lipids and proteins, from different prey. We examined whether digestibility of macronutrients by northern fur seals (*Callorhinus ursinus*) differs between diets composed of different macronutrient ratios and to investigate the factors that affected digestibility. We fed eight experimental diets to six female captive fur seals, using four prey species (Pacific herring, walleye pollock, capelin and Magister squid) alone or in combination. Apparent digestibility was quantified by compositional differences between diet and fecal samples. Apparent digestibility coefficients (ADC) for both lipid and protein differed significantly among diets. Overall, lipid ADCs (96.0–98.4%) were significantly higher than protein ADCs (95.7–96.7%). We found that dietary protein level had the greatest influence on the ability of northern fur seals to digest both lipids and protein. Lipid digestibility was negatively correlated with dietary protein level, whereas, protein digestibility was positively correlated with dietary protein level. As a result, the fur seals displayed higher lipid assimilation without significantly compromising protein assimilation when consuming diets that had high lipid and low-to-moderate protein content. Our results refute the hypothesis that diets composed of multiple prey species provide greater macronutrient digestibility over single-species diets. Overall, our findings suggest that fur seals have an optimal macronutrient assimilation efficiency related to specific macronutrient ratios in the diet. This suggests that the high protein contained in walleye pollock that currently dominates the diet of declining populations of northern fur seals in the Bering Sea may be hindering their ability to digest and assimilate sufficient lipids.

Introduction

Modern approaches to nutritional ecology recognize that a consumer's macronutrient intake affects its nutritional state, which in turn impacts the consumer's physical ability to perform appropriately within its environment (Raubenheimer et al. 2009). Related theoretical frameworks aim to reveal the role that specific macronutrients play in the health and longevity of consumers, and demonstrate that proper balance between macronutrients in food are as important to a consumer as total energetic intake (Raubenheimer et al. 2009; Mair et al. 2011; Solon-Biet et al. 2015a). Acknowledging the importance of balanced macronutrient intake has led to new nutritional theories, such as the geometric theory of nutrition, to investigate the link between food selection and nutritional benefits (Raubenheimer and Simpson 1999).

Carnivores are traditionally thought to maximize rates of prey capture and energy intake irrespective of macronutrient return. However, recent research suggests that carnivores will in fact select foods that provide a balanced macronutrient intake when provided with complementary food choices in an attempt to satisfy their macronutrient needs (Mayntz et al. 2009; Hewson-Hughes et al. 2011; Kohl et al. 2015).

Proteins, lipids and essential fatty acids are different macronutrients that fulfill different physiological roles over the life history of an organism. Proteins, for example, provide the building blocks (i.e., amino acids) for enzymes, hormones, antibodies, etc.—with carnivores having the highest protein requirements (18–30% of dietary intake) among mammals (Robbins 1993). Lipids provide thermal insulation and are central to energy storage in anticipation of fasting, intense exercise, or periods of insufficient energy intake. Essential fatty acids typically impact the activity level of multiple basic tissue and must be ingested because they cannot be synthesized by the animal *de novo* (Burr and Burr 1930; Robbins 1993). Failure to acquire sufficient macronutrients will negatively affect a consumer's nutritional status and specific physiological functions (Anderson et al. 2005; Boersma and Elser 2006), while overconsumption of a particular macronutrient (Waldbauer 1968) could similarly lead to increased mortality (Raubenheimer et al. 2005).

Knowing the gross nutritional content of food consumed by an animal does not necessarily translate into what an animal will gain nutritionally because digestive processes affect the capability of consumers to absorb different digestive products. Among pinnipeds,

dietary studies have shown seals and sea lions to have generally high macronutrient digestive efficiencies (Parsons 1977; Keiver et al. 1984; Ronald et al. 1984; Fisher et al. 1992; Goodman-Lowe et al. 1999; Stanberry 2003; Trumble et al. 2003; Yamamoto et al. 2009). However, these studies typically used single-species experimental diets, and were unable to determine the potential factors (e.g., prey composition profiles, level of food intake) that may lead to differences in digestibility of macronutrients.

We investigated differences in digestibility of both lipid and protein across eight different diets in captive female northern fur seals (*Callorhinus ursinus*), which is a species whose central Bering Sea population has been declining since the late 1970s, due possibly to a shift in diet from high-lipid prey to low-lipid (high-protein) prey (Swartzman and Haar 1983; NMFS 2007). Unlike most previous pinniped feeding studies, we fed the fur seals four different prey species alone or in combination. This allowed us to create diets with a broad range of nutritional profiles, which were representative of the diet of wild fur seals in the Bering Sea (Sinclair et al. 1994; Call and Ream 2012). This allowed us to quantify digestibility of lipid and protein, as well as determine which dietary factors contributed to changes in rates of assimilation. Additionally, we tested the hypothesis that animals that feed on mixed-species diets benefit from a greater nutritional return than when feeding on equivalent single-species diets (Penry and Jumars 1987; Singer and Bernays 2003). Ultimately, our study investigated whether the interplay of shifts in dietary intake and differences in macronutrient digestibility is potentially impacting the nutritional budget of fur seals (and other pinnipeds) in the Bering Sea at the individual level, and possibly impacting population numbers.

Materials and methods

Animals

For our experimental feeding trials, we used six female northern fur seals that were housed at the University of British Columbia's Marine Mammal Energetics and Nutritional Laboratory located at the Vancouver Aquarium (British Columbia, Canada). The fur seals were 4.5 years of age and their body mass ranged from 19.5–28.9 kg at the beginning of our study. The fur seals were kept in holding pools with adequate haulout space, and with continuously-flowing filtered seawater. The fur seals were weighed daily on a platform scale (± 0.02 kg) prior

to feeding, and received a typical diet of thawed Pacific herring (*Clupea pallasii*) and market squid (*Loligo opalescens*), fed three times a day (supplemented with vitamins).

Experimental diets and study design

The feeding trials took place from November 2012–June 2013. All experimental manipulations were in accordance with the guidelines of the University of British Columbia Animal Care Committee (permit #A10-0342) and the Canadian Council on Animal Care.

The fur seals were subject to the same experimental diets as those previously detailed in Chapter 2. In brief, we used four prey species commonly consumed by fur seals in the wild that were either fed to the fur seals alone or in combination to generate eight diets. The prey species included Pacific herring, walleye pollock (*Theragra chalcogrammus*), capelin (*Mallotus villosus*), and Magister squid (*Berryteuthis magister*), chosen to create a wide spectrum of prey with different protein and lipid concentrations (Table 3.1). The herring + Magister squid trials used a different batch of herring (batch B) than the other diets that contained herring (batch A). Five of the dietary treatments consisted of multiple prey items that were fed in equal proportions according to their gross energy content (hence, actual fish mass differed between species).

Each experimental diet was fed for three weeks, with the exception of herring + Magister squid diet that was fed for only two weeks and was only consumed by four of the fur seals. The first week of the feeding trials was dedicated to acclimation, and the second week to fecal sample collection (the third week was for metabolic data collection for a concurrent study). The duration of the acclimation phase was designed to be sufficient for the fur seals to adjust to the new diet and eliminate any residues from previous diets (Robbins 1993). The diets were intended to be fed at equal levels of gross energetic intake with the goal of sustaining the animals at a maintenance state; i.e., maintaining relatively constant body mass (Kleiber 1975). As maintenance intake levels for each animal were estimated according to their individual body mass, the ingested mass per diet changed between animals and across experimental diets. To minimize potential effects due to season, the diet trials were randomly assigned to different pairs of study animals (three study groups with two animals in each group).

Table 3.1 Proximate composition (crude protein and lipid content), energy density, manganese (Mn^{2+}) concentration (dry-weight basis) and mean body size and weight (± SD) of a subsample of four species of prey (n=12 of each) experimentally fed to six female northern fur seals.

Experimental prey	Water (%)	Total lipid (%)	Crude protein (%)	Energy density (kJ g^{-1})	Mn^{2+} (ppm)	Fish length (cm)	Fish weight (g)
Pacific herring (*Clupea pallasii*)							
Batch A (main source)	68.5	41.6	51.4	24.3	5.1	19.9 (1.5)	93.0 (20.8)
Batch B (Magister diet only)	69.2	37.0	53.6	22.9	5.5	18.5 (0.6)	64.0 (6.7)
Walleye pollock (*Theragra chalcogramma*)	75.3	32.8	57.5	22.1	2.4	24.5 (2.2)	134.0 (33.2)
Capelin (*Mallotus villotus*)	82.6	4.0	81.6	15.2	2.9	15.0 (1.0)	24.0 (5.4)
Magister squid (*Berryteuthis magister*)	71.3	44.3	46.7	23.2	2.8	--	--

Feces collection and laboratory analysis of feces and prey

To differentiate feces from individuals among the six fur seals, gel capsules containing approximately 5-6 g of coloured Micro-grits markers (Micro Tracers Inc., 1370 Van Dyke Avenue, San Francisco, USA) were inserted into several of the prey fed throughout the day, with color unique to a specific fur seal. Feces were collected several times a day from the bottom of the holding pool or haulout area from the bottom of the pools. The samples were identified (by color), weighed, and frozen at - 20 °C until analyzed.

Methods of analysis of prey and fecal samples are detailed in Chapter 2. Briefly, 10 samples of each prey item were analyzed both in-house and by a commercial laboratory (SGS Canada Inc., Burnaby, Canada) for proximate composition (moisture, lipid, and crude protein), as well as energy density and manganese (Mn^{2+}) concentration. A total of 138 fecal samples (3 samples per diet per animal) were analyzed in-house for proximate composition and Mn^{2+} concentration. Additionally, 16 fecal samples were analyzed by SGS laboratory for Mn^{2+} to validate in-house measurements. Sample analyses were all done on freeze-dried samples, and total lipid and crude protein content are expressed as percentage of total dried sample.

To determine water and dry matter content from the prey and fecal subsample replicates were freeze-dried for 36 h to a constant mass (Freeze dryer Freezone 6, LABCONCO, Kansas City, USA). Replicates of dried homogenized samples (prey and feces) were then analyzed for proximate composition, energy density, and Mn^{2+} concentration. Energy density of replicates of dried samples was measured using an oxygen bomb calorimeter (6400 Automatic isoperibol calorimeter, Parr Instrument Company, Moline, Illinois USA). Total lipid content of dried feces and dried prey samples were measured by chloroform/methanol extraction (Bligh and Dyer 1959).

Crude protein was determined by the Kjeldahl method (AOAC 1990) on dried samples. Total Kjeldahl Nitrogen (TKN) concentration (mg L^{-1}) in digested samples was determined by spectrophotometric flow injection analyzer (FOSS FIAstar 5000 TKN analyzer unit, Eden Prairie, Minnesota, USA) measured at 590 nm. Nitrogen concentration was then multiplied by 6.25 to determine total crude protein as a percent (g/100 g) of sample weight (Robbins 1993).

Concentrations of Mn^{2+} (used as an inert biomarker – see below) were measured on replicate subsamples of dried feces (0.2 g) and prey items (0.4 g) that were digested via a wet oxidation in a similar manner as the Kjeldahl method (for details see Chapter 2). The Mn^{2+} concentration of resulting solutions were determined via an atomic absorption spectrophotometer (Perkin-Elmer 2380; 279.5 nm wavelength, slit width 0.2 nm, oxidizing air-acetylene flame; Perkin-Elmer, Montreal, Quebec, Canada).

Calculations of nutrient digestibility

When total fecal collection is possible the apparent digestibility of a nutrient can be calculated using the following formula (Schneider and Flatt 1975):

$$Apparent\ Digestibility\ of\ nutrient\ (\%) = \frac{N_i - N_f}{N_i} \times 100$$

where *N* is the nutrient concentration of the ingested diet (*i*) and in feces (*f*). Because the feces potentially contains nutrients from sources other than the diet (e.g., enzymes secreted into the gastro intestinal tract, cells sloughed off, or gut microflora), the digestibility coefficient calculated is termed “apparent digestibility”, unless these are accounted for.

Total fecal collection is challenging in large marine mammals and was not possible in our study. However, we calculated digestibility of nutrients using Mn^{2+} as a naturally occurring marker in both prey samples and fecal samples. This method has been widely accepted and used in digestibility studies with fur seals and other pinniped species (Fadely et al. 1990; Fadely et al. 1994; Lawson et al. 1997; Rosen and Trites 2000b a). Due to low concentrations of Mn^{2+} in prey samples, only the results from the SGS laboratory were used for this parameter. Using Mn^{2+} as a marker permitted the calculation of the apparent digestibility coefficient (ADC_n) of specific nutrients (n) using the following formula:

$$ADCn = \left(1 - \frac{C_i \times N_f}{C_f \times N_i}\right) \times 100$$

where *C* is the concentration of Mn^{2+} and *N* is the nutrient concentration of the ingested diet (*i*) and feces (*f*) (Schneider and Flatt 1975; Mårtensson et al. 1994). In essence, Mn^{2+} concentrations allowed us to determine the amount of prey that was represented by the fecal sample.

The mass of either lipid or protein in the food that is lost through the feces is measured as the fecal nutrient loss (FNL) for that specific component; i.e., as either fecal lipid loss (FLL) or fecal protein loss (FPL). FNL was calculated with the following formula:

$$FNL(g\ day^{-1}) = nutrient\ consumed - (ADC_N \times\ nutrient\ consumed)$$

Fecal nutrient loss can also be calculated as a proportion of the intake. These relative measures of FNL are the inverse of ADC of nutrients and are calculated as:

$$\%FNL = 1 - \%ADC_N$$

We tested whether mixed-species diets provided a greater nutrient digestibility for both lipid and protein than would be expected from a weighted average of single-species diets. The expected digestibilities of both lipid and protein of mixed-species diets were calculated (except for herring + Magister squid diet) from the observed ADC of the single-species diet counterparts, weighted by the proportion of the specific nutrient in each prey item of the diet (Forster 1999) according to:

$$Expected\ ADCn\ (\%) = \frac{(Mass_{F1} \times Nutrient_{F1} \times Observed\ ADCn_{F1}) + (Mass_{F2} \times Nutrient_{F2} \times Observed\ ADCn_{F2})}{(Total\ nutrient\ fed)}$$

Statistical analyses

Significant differences between experimental diets were examined via linear mixed-effect (LME) models using R 3.1.2 statistical software (R Core Team 2014) (as detailed in Chapter 2). In brief, we explored transforming the proportional data and concluded that the transformations were unnecessary because the residuals of the analytically robust LME models met the required assumptions of normality, homogeneity and independence (see below) (Chapter 2; Wilson et al. 2013). For models with multiple potential fixed effects, our analysis consisted of fitting various models using maximum likelihood estimates (as required for LME comparisons) with the *nlme* package (Pinheiro et al. 2015). LME models were built step-wise to assess which of the fixed factors could best explain the changes observed in the response variables, and included repeated measurements of animals as a random effect (Pinheiro and Bates 2000; Crawley 2007; Zuur et al. 2009; Galecki and Burzykowski 2013).

Initially, the influence of diet type was tested independently on the following response variables: lipid apparent digestibility, fecal lipid loss, protein apparent digestibility, and fecal protein loss. A *post-hoc* Tukey test adjusted with a Bonferroni correction was used to investigate specific differences among the diets. For response variables that changed significantly with diet, the nature of the significant differences in digestibility across diets was further investigated by analyzing which components of the diet were driving the relationship. Specifically, we tested the following fixed effects: food mass intake (kg d^{-1} wet-weight), gross energy intake (kJ d^{-1}), dietary lipid intake (% d^{-1}), dietary protein intake (% d^{-1}), and lipid to protein intake ratio. We compared Models with fewer fixed effects by likelihood ratio tests (LRT) and by Akaike information criterion (AIC) values. Overall, the best-fit model contained the fewest factors that best explained the trends observed in the response variables, while satisfying the LME models assumptions (Pinheiro and Bates 2000; Crawley 2007; Zuur et al. 2009; Galecki and Burzykowski 2013).

Preliminary analysis of lipid digestibility indicated that data from two of the fur seals (consuming different diets) were outliers because their inclusion failed to fulfill the assumptions of the LME models (also confirmed as outliers through the extreme studentized deviate method, known as Grubbs' test). Excluding these animals from lipid digestibility analyses resulted in all LME models meeting the assumptions of normality of the random effect and of the residual

errors and homogeneity of the variance (Pinheiro and Bates 2000; Crawley 2007; Zuur et al. 2009; Galecki and Burzykowski 2013).

Finally, within each diet, we also investigated the significance of differences in digestibility of lipid and protein by testing nutrient type as a fixed factor (2 levels LME). Additionally, statistical differences between the expected and observed digestibility of both lipid and protein from mixed-species diets were determined using a Welch two sample *t*-test.

For all tests, significance was determined at the 5% rate of error. Where appropriate, results are presented as mean ± Standard Deviation (SD).

Results

Prey item and dietary characteristics

The proximate composition of the four experimental prey items (Table 3.1, dry-weight basis) revealed that Magister squid had the highest lipid content (44.3%), and capelin had the lowest (4.0%). However, capelin had the highest protein content (81.6%), while Magister squid had the lowest (46.7%). Herring (batch A) had the highest energy density (24.3 kJ g^{-1}) and capelin had the lowest (15.2 kJ g^{-1}).

The proximate composition and energy density of the eight experimental diets differed significantly (dry-weight basis; Table 3.2). Total lipid content was lowest for the capelin diet (3.3 ± 0.0%) and highest for the herring + Magister squid diet (43.2 ± 0.1%) (LRT= 265.3, $p<0.001$). Total crude protein content also differed significantly between diets, with herring diet having the lowest content (47.1 ± 0.0%) and the capelin diet the highest (67.6 ± 0.0%) (LRT=366.9, $p<0.001$). Conversely, the capelin diet had the lowest energy density (15.2 ± 0.0 kJ g^{-1}) while the herring diet had the highest (24.3 ± 0.0 kJ g^{-1}) (LRT=266.7, $p<0.001$).

Although the experimental diets were intended to be balanced for gross energy intake (GEI) at maintenance levels, daily GEI differed significantly across diets (LRT= 61.1, $p<0.001$; Table 3.3) due to the fact that intake was significantly lower on the capelin diet (8,712.9 ± 1,409.7 kJ d^{-1}) and significantly higher on the herring + Magister squid diet (15,866.0 ± 1,426.8 kJ d^{-1}). These differences were due, respectively, to an unwillingness of the animals to consume sufficient quantities of capelin and an attempt to maximize the intake of Magister squid in a limited number of animals. These anomalies, combined with the overall differences in energy density of the diets, resulted in ingested mass (wet-weight) differing significantly across diets

Table 3.2 Mean (± S.D.) body mass of six captive female northern fur seals at the start of each feeding trial, and mean ingested mass (wet) for the eight experimental diets with their respective proximate composition (crude protein and lipid content), energy density, and manganese (Mn^{2+}) concentration (dry-weight basis).

Diet	Mean body mass (kg)	Mean ingested mass (kg)	Water (%)	Total lipid (%)	Crude protein (%)	Energy density (kJ g^{-1})	Mn^{2+} (ppm)
Herring	23.9	1.6	68.5	38.0	47.1	24.3	5.1
	(3.5)	(0.3)	(3.6)	(0)	(0)	(0)	(0)
Pollock	23.1	2.3	75.3	35.8	62.8	22.1	(2.4)
	(3.1)	(0.3)	(1.3)	(0)	(0)	(0)	(0)
Capelin	23.2	3.3	82.6	3.3	67.6	15.2	2.9
	(3.3)	(0.5)	(1.4)	(0)	(0)	(0)	(0)
Herring + Pollock	23.1	2.0	72.4	37.0	54.6	23.1	3.8
	(3.2)	(0.1)	(0.04)	(0.01)	(0.1)	(0.01)	(0.02)
Herring + Capelin	23.0	2.9	79.0	15.9	60.2	18.7	3.7
	(2.7)	(0.3)	(0.04)	(0.1)	(0.08)	(0.04)	(0.01)
Herring + Magister Squid	23.8	2.4	70.3	43.2	57.2	23.1	4.3
	(3.2)	(0.2)	(0.01)	(0.1)	(2.8)	(0)	(0.02)
Pollock + Capelin	22.9	3.0	79.9	15.9	65.8	18.3	2.7
	(2.8)	(0.5)	(0.5)	(2.2)	(0.3)	(0.5)	(0.03)
Herring + Pollock + Capelin	23.1	2.6	77.9	21.0	60.7	19.7	3.4
	(2.8)	(0.5)	(0.06)	(0.2)	(0.9)	(0.05)	(0.01)

(LRT= 76.9, $p<0.001$), ranging from 1.6 ± 0.3 kg (herring diet) to 3.3 ± 0.5 kg (capelin diet) (Table 3.2).

Crude protein digestibility

The differences in proximate composition across the eight experimental diets were substantial enough to yield significant differences in macronutrient losses in feces and, consequently, differences in digestibility of macronutrients. Total crude protein intake differed significantly among diets (LRT= 68.9, $p<0.001$; Table 3.3), with the capelin diet having the

Table 3.3 Mean (± S.D.) gross energy intake (GEI), mean intake, fecal loss and apparent digestibility (%AD) of total lipid and total crude protein of six captive female northern fur seals across the eight experimental diets (dry-weight basis).

Diet	GEI (kJ d^{-1})	Total lipid			Crude protein		
		Intake (g d^{-1})	Fecal lipid loss (g d^{-1})	AD_{lipid} (%)	Intake (g d^{-1})	Fecal protein loss (g d^{-1})	$AD_{protein}$ (%)
Herring	12135.7 (2412.5)	207.6 (41.3)	3.3 (0.9)	98.4 (0.2)	257.3 (51.2)	11.1 (2.9)	95.7 (0.7)
Pollock	12688.6 (1570.4)	188.2 (23.3)	4.4 (1.0)	97.7 (0.4)	330.0 (40.8)	11.7 (2.7)	96.5 (0.4)
Capelin	8712.9 (1409.7)	22.1 (3.3)	0.9 (0.4)	96.0 (0.3)	468.7 (75.8)	17.0 (5.2)	96.4 (0.8)
Herring + Pollock	12482.5 (787.9)	199.5 (12.7)	3.5 (0.8)	98.3 (0.3)	294.4 (18.4)	11.2 (1.8)	96.2 (0.5)
Herring + Capelin	11301.8 (1245.9)	111.8 (12.8)	2.7 (0.6)	97.6 (0.3)	423.4 (45.7)	15.4 (3.5)	96.4 (0.5)
Herring + Magister Squid	15866.0 (1426.8)	279.5 (25.3)	9.5 (1.3)	96.6 (0.1)	358.7 (31.9)	14.1 (2.2)	96.1 (0.2)
Pollock + Capelin	11118.7 (1613.2)	106.9 (13.7)	3.9 (0.8)	96.6 (0.7)	430.4 (70.5)	16.3 (4.1)	96.2 (0.7)
Herring + Pollock + Capelin	11472.6 (2184.1)	133.1 (25.1)	3.0 (0.7)	97.7 (0.4)	384.4 (73.7)	12.7 (2.4)	96.7 (0.4)

highest protein intake (486.7 ± 75.8 g d^{-1}) and the herring diet having the lowest (257.3 ± 51.2 g d^{-1}). Fecal protein loss (FPL) varied significantly across diets (LRT= 19.7, p= 0.006). FPL was highest when the fur seals consumed the herring diet (11.1 ± 2.9 g d^{-1}) and lowest when consuming the capelin diet (17.0 ± 5.2 g d^{-1}). FPL was significantly affected by the protein content (%) in the diets, such that FPL decreased as protein in the diet increased (LRT= 8.9, p= 0.003).

The mean apparent digestibility coefficient of crude protein (ADCcp) was 96.3 ± 0.6%, and was significantly different among the experimental diets (LRT= 19.9, p= 0.006; Table 3.3).

ADCcp was highest for the herring + pollock + capelin diet (96.7 ± 0.4%) and lowest for the herring diet (95.7 ± 0.7%). ADCcp were positively affected by the protein content (%) of the diets, such that the apparent digestibility of crude protein increased with increasing dietary protein content (LRT= 9.0, p= 0.003, Fig. 3.1).

When comparing the expected protein digestibility of the mixed-species diets against the observed protein digestibility, we found that none of the comparisons were significantly different (p>0.05). Therefore, there was no significant advantage to protein digestibility when fur seals consumed mixed-species diets over single-species diets.

Lipid digestibility

Total lipid intake was significantly different across diets (LRT= 125.4, p<0.001; Table 3.3), where the capelin diet had the lowest lipid consumption (22.1 ± 3.3 g d^{-1}) and the herring + Magister squid diet had the highest (279.5 ± 25.3 g d^{-1}). Fecal lipid loss (FLL) significantly varied among diets (LRT= 100.3, p<0.001; Table 3.3). Specifically, FLL during the capelin diet treatment was significantly lower than the rest of the experimental diets (0.9 ± 0.4 g d^{-1}), and higher during the herring + Magister squid diet treatment (9.5 ± 1.3 g d^{-1}). FLL was significantly affected by the protein content (%) of the diets, such that increases in protein content of diet caused increases in FLL (LRT= 30.3, p<0.001).

Mean apparent digestibility coefficient of lipid (ADC_{lipid}) was 97.4 ± 0.9%, and differed significantly across diets (LRT= 100.1, p<0.001; Table 3.3), and was lowest when fur seals consumed the capelin diet (96.0 ± 0.3%) and highest when consuming the herring diet (98.4 ± 0.2%). Similar to FLL, lipid digestibility was significantly affected by the protein content (%) in the diets, such that ADC_{lipid} decreased as the protein content in the diets increased (LRT= 30.3, p<0.001; Fig. 3.2). Comparisons within each diet showed that, for six out of the eight diets, the mean digestibility of lipid was significantly higher than the mean digestibility of protein (p<0.001) (with the exception of capelin only diet and pollock + capelin diet).

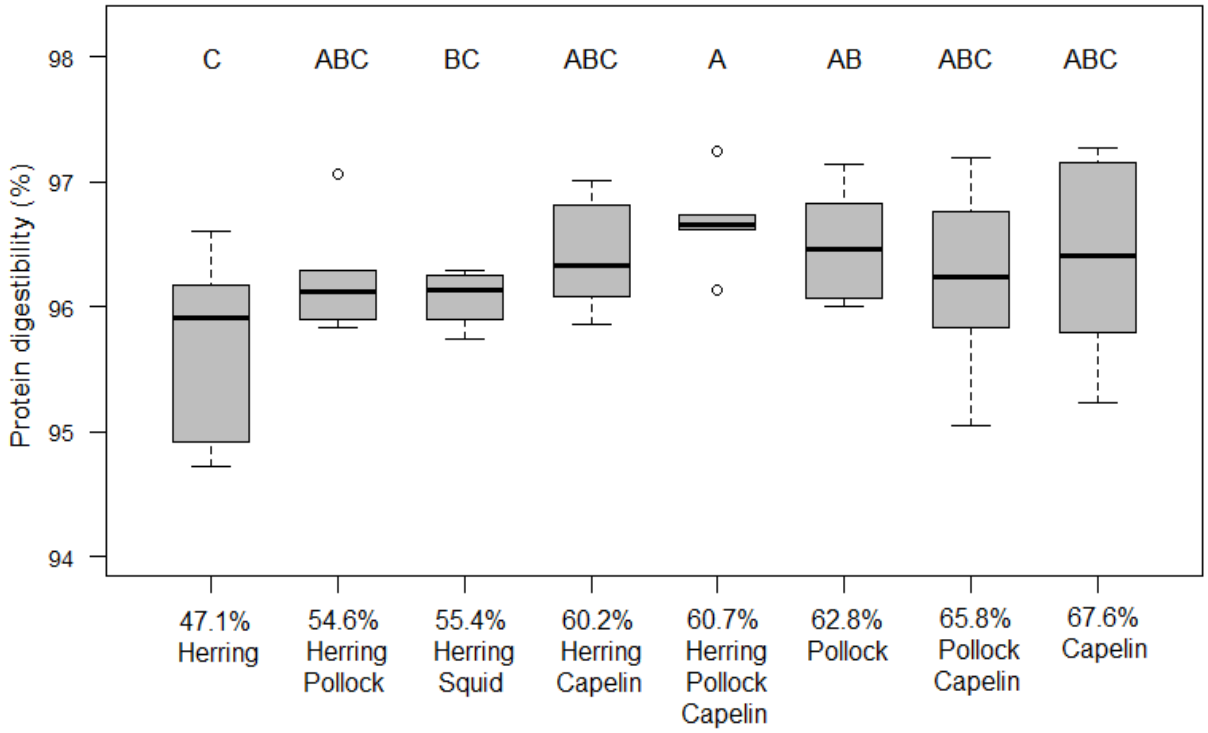

Figure 3.1 Apparent protein digestibility (%) of the eight experimental diets tested in six captive female northern fur seals. Diets are arranged from low to high protein content (% dry-weight basis), as denoted above the diet labels. Each box represents the median (thick horizontal line), first and third quartile ("hinges") and 95% confidence interval of median ("notches"). Data for each diet trial are from fur seals, with the exception of the herring and squid diet which was only consumed by four of the animals. Boxes not sharing letters indicate significant differences between diets ($p<0.05$).

There were significant differences between the observed and expected lipid digestibility of two of the mixed-species diets. Contrary to predictions however, the mean observed lipid digestibility for the herring + capelin diet (97.6%) was significantly lower than the expected (98.0%; $p< 0.001$), as was the mean observed lipid digestibility for the pollock + capelin diet (96.4% observed vs. 97.5% expected; $p= 0.01$).

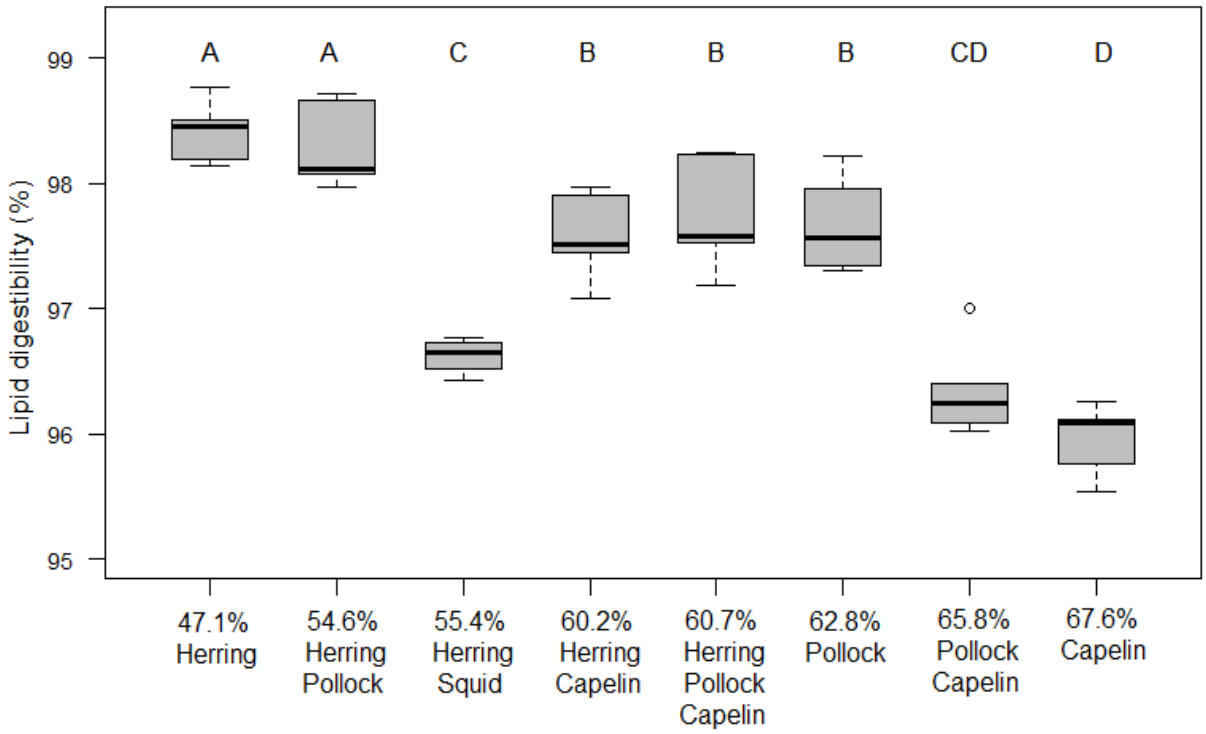

Figure 3.2 Apparent lipid digestibility (%) of the eight experimental diets tested in six captive female northern fur seals (except for the capelin only diet and the pollock and capelin diet for which n= 5, as well as the herring and squid diet where n=4). Diets are arranged from low to high protein content (% dry-weight basis), as denoted above the diet labels. Each box represents the median (thick horizontal line), first and third quartile ("hinges") and 95% confidence interval of median ("notches"). Boxes not sharing letters indicate significant differences between diets ($p<0.05$).

Discussion

Major factors that affect energy digestibility of prey have been studied for various groups of carnivores, including marine mammals (e.g., Best 1985; Pritchard and Robbins 1990; Robbins 1993; Rosen and Trites 2000b a). However, the factors that affect the ability of marine mammals to digest macronutrients are not well known. Understanding the potential effects of changes in prey composition on the digestive efficiency of macronutrients is important for predicting the impact of dietary changes on the nutritional status of individual animals and consequently of

populations. This is especially true for northern fur seals from the Pribilof Islands in Alaska which are currently experiencing a population decline (NMFS 2007), along with many other pinnipeds inhabiting the Bering Sea and the Gulf of Alaska (Pitcher 1990; Trites and Larkin 1996; National Research Council 1996). A shift in dietary intake has been hypothesized to be negatively impacting pinnipeds as they increasingly direct their foraging efforts towards prey that are more readily accessible but of less nutritional value (Swartzman and Haar 1983; Castellini 1993; Decker et al. 1995; Merrick et al. 1997; Calkins et al. 1998; Rosen and Trites 2000a b; Trites et al. 2007).

Our study investigated how macronutrient digestibility differs across diets in northern fur seals. In particular, we examined which dietary components (i.e., caloric intake, ingested prey mass, dietary lipid and protein content, and lipid-to-protein ratios) most influenced macronutrient digestibility. In contrast to previous macronutrient digestibility studies in pinnipeds that focused on single-species diets, our study explicitly tested macronutrient digestibility across both single-species and mixed-species diets. Our results demonstrate that dietary protein content is the most relevant factor affecting digestibility of prey by fur seals, with increasing protein content leading to increasing digestibility of protein and decreasing digestibility of lipid. Nonetheless, we found the apparent digestibility of lipid to be significantly higher than that of protein digestibility for six of the eight experimental diets. Lastly, we found no significant benefit associated with the consumption of mixed-species diets over single-species diets in terms of macronutrient return.

Experimental diets

Diet studies with pinnipeds have reported differences in macronutrient digestibility, but it is difficult to determine which factors specifically contributed to these differences because most studies used only one or two prey types fed independently, often at differing levels of intake (Parsons 1977; Keiver et al. 1984; Ronald et al. 1984; Fisher et al. 1992; Stanberry 2003; Trumble et al. 2003). Our combination of four prey species that are commonly consumed by free-ranging northern fur seals in the Bering Sea (Sinclair et al. 1994; Call and Ream 2012) allowed us to examine the effects of diet composition on macronutrient digestibility across a wide, representative array of diets. This resulted in a wider range of both lipid and protein intake that allowed us to directly examine which aspect of diet composition impacts macronutrient digestibility in fur seals.

Lipid digestibility vs. protein digestibility

It has been suggested that predators at higher trophic levels obtain most of their energy from protein (Robbins 1993; Raubenheimer et al. 2009). In addition, carnivores are hypothesized to be better adapted than herbivores or omnivores to utilize surplus amino acids from protein for energy and structural carbon (Russell et al. 2002; Russell et al. 2003). We found that, in six of our eight experimental diets, ADC for lipid was significantly greater than the ADC for protein. Of note, ADC for lipid was not significantly greater than for protein for those 2 diets with the highest dietary protein. This observed trend was anticipated, given that the long chains of amino acids with strong peptide bonds within protein molecules means that the breakdown and uptake of proteins requires a more complex chemical digestion than the easily digested and absorbed animal fats (Leoschke 1959; Best 1985; Blaxter 1989; Stevens and Hume 1995). Other pinniped studies have similarly reported higher digestibility of lipid relative to protein (Parsons 1977; Keiver et al. 1984; Ronald et al. 1984; Fisher et al. 1992; Goodman-Lowe et al. 1999; Stanberry 2003; Trumble et al. 2003; Yamamoto et al. 2009). Overall, the lipid and protein digestibility ranges in our study are comparable to those documented in pinniped studies, as well as for terrestrial mammalian carnivores (Clauss et al. 2010).

Changes in protein digestibility

The apparent digestibility of crude protein by the fur seals in our study was relatively high (95.7-96.7%; Table 3.3), and near the upper end of protein digestibility values reported for other pinniped species (61.5–98.3%) (Parsons 1977; Keiver et al. 1984; Ronald et al. 1984; Fisher et al. 1992; Goodman-Lowe et al. 1999; Stanberry 2003; Trumble et al. 2003; Yamamoto et al. 2009). We also found that dietary protein content has the strongest effect on protein digestibility, where protein digestibility increased as the proportion of dietary protein increased (Fig. 3.1).

Terrestrial carnivores have been similarly documented to have increased protein digestibility when the protein content of the consumed diets increased (Russell et al. 2002; Russell et al. 2003; Mayntz et al. 2009). Digestibility of protein has been described as a curvilinear function of dietary protein, such that at low protein concentrations protein digestibility increases exponentially with increasing levels and then reaches a plateau at higher dietary protein levels (Crampton and Rutherford 1954; Robbins 1993). Hence, protein

digestibility changes are more pronounced between diets with relatively low protein content (Schneider and Flatt 1975), while diets with higher protein content induce little change in protein digestibility. Our results corroborate such patterns, as all of our experimental diets had high protein levels and the change in protein digestibility across diets increased by only 1%. Thus, the fur seal's high protein digestibility values fit within the upper plateau of the expected curvilinear function of protein digestibility.

Changes in lipid digestibility

Lipid digestibility values for the fur seals across our experimental diets (96.0-98.4%; Table 3.3) were similar to lipid digestibility values reported by other pinniped studies (82-99%) (Parsons 1977; Keiver et al. 1984; Ronald et al. 1984; Fisher et al. 1992; Goodman-Lowe et al. 1999; Stanberry 2003; Trumble et al. 2003; Yamamoto et al. 2009). We found that dietary protein level significantly affected lipid digestibility and, similar to terrestrial carnivores, the fur seals' lipid digestibility decreased with high dietary protein and was highest for low-to-moderate dietary protein (Russell et al. 2002; Mayntz et al. 2009).

This opposing digestibility trends between protein and lipid may have been caused by the inverse relationship between lipid and protein in the tested diets. Unfortunately, since the protein and lipid levels are inversely related particularly in fish diets, it is often difficult to differentiate the effects of these factors. Thus, it is possible that lipid digestibility may have been physiologically regulated by lipid content itself, even though lipid digestibility was more strongly related to protein content of the diet. This would be consistent with pinniped studies that showed that higher lipid content in the diet leads to higher lipid digestibility (Parsons 1977; Keiver et al. 1984; Ronald et al. 1984; Fisher et al. 1992; Lawson et al. 1997; Goodman-Lowe et al. 1999), although many of these studies did not specifically examine the potential role of protein content. Alternatively, it is possible that lipid absorption was truly determined by protein content since in all our experimental diets the protein content was greater than the lipid content. This suggests that protein digestibility was "prioritized", leading to a parallel reduction in lipid digestibility.

The changes in protein and lipid digestibility with changes in diet composition suggest that the fur seals were displaying digestive plasticity. Of particular note is the increase that occurred in protein assimilation that is consistent with the adaptive modulation hypothesis

(Karasov 1992; Karasov 1996), which proposes that consumers should adjust their digestive system to new diets in order to optimize the potential returns by down-regulating the synthesis of unnecessary intestinal nutrient transporters and up-regulating required transporters based on dietary intake (Buddington et al. 1991; Karasov 1992; Karasov 1996). A review of 12 species demonstrated that animals across different taxa are capable of displaying such gastrointestinal adaptations when animals are fed diets of varying ratios of protein and carbohydrate (Karasov 1992). It is possible that under the high-protein diets in our study, the biosynthesis and maintenance of specific amino acid transporters beneficial to maximize protein uptake were up-regulated. However, more research is necessary to further investigate if the fur seals were actually engaging in such intestinal adaptation.

Differences in macronutrient digestibility of mixed-species diets

We found that observed protein digestibilities of mixed-species diets were not significantly different from expected values for equivalent single-species diets. Additionally, for two of our four mixed-species diets, the observed lipid digestibility was significantly lower than expected (herring + capelin diet and pollock + capelin diet). Although it is unclear why lipid assimilation was lower than expected, it is possible that the higher dietary protein content, and particularly, the specific lipid-to-protein proportions (~16% lipid: 65% protein) of these diets may have significantly impacted the digestive efficiency of lipids.

Overall, our results contradict the hypothesis that consumption of mixed-species diets is nutritionally more beneficial than consumption of single-species diets (Penry and Jumars 1987; Singer and Bernays 2003). We found no advantage in macronutrient gain when fur seals consumed mixed-species diets compared to single-species diets. While some studies have suggested that a mixed-diet might provide nutritional benefits to pinnipeds, they did not specifically examine macronutrient digestibility (Trumble and Castellini 2005). However, a recent study on harbour seals (*Phoca vitulina*) similarly reported equal macronutrient digestibility values between single-species diets and mixed-species diets (Yamamoto et al. 2009). Nonetheless, it is important to note that while our study demonstrates that diet diversity has no advantage in terms of macronutrient return, prey diversity could reflect other ecological advantages. Diet diversity may reflect ecosystem health and serve as an indicator of the ease with which predators can meet their nutritional requirements.

Additional factors affecting nutrient digestibility

Apart from being affected by diet composition, the digestibility of macronutrients is also influenced by a number of other factors, including the animal's level of food intake, nutritional state, growth rate, and reproductive status (especially lactation) (Schneider and Flatt 1975). Although we designed our experiment to control these factors, their effects can have significant impacts on digestibility. For example, when animals are fed at a higher level of intake than is required for a steady-state nutritional plane, digestive efficiency may decrease (Schneider and Flatt 1975). Developmental effects were not a concern in our study given that the fur seals were non-reproductive adult females and were not growing. We fed the fur seals at a constant level of energy intake sufficient to meet their maintenance needs, which resulted in ingested mass differences among diets. Even though the fur seals consumed different levels of intake, we found no significant impact of ingested food mass on macronutrient digestibility.

We conducted our experiment with fur seals that were in a stable nutritional state. Thus, our results reflect the digestibility of macronutrients for an overall healthy northern fur seal population. However, if free-ranging fur seals are subject to periodic nutritional stress, it is possible that the rate of macronutrient digestibility in the wild will be lower than the observed values in our study, although the differences between prey species would likely still be significant. However, more research is required to specifically test the effect of an animal's nutritional state on macronutrient digestibility before such conclusions can be drawn.

The sweet spot of optimal macronutrient digestibility

Our study is the first to identify which dietary components contribute to observed differences in macronutrient digestibility in northern fur seals, and in pinnipeds in general. Recent research in terrestrial animal nutrition suggests that carnivores forage in order to balance (rather than maximize) their macronutrient intake when provided a choice of foods with different nutritional qualities (Kohl et al. 2015). Based on the current research in combination with our findings, we propose that a link exists between carnivores selecting a particular lipid-to-protein ratio intake and their different digestive efficiencies to assimilate these macronutrients.

The geometric framework of nutrition proposes that predators actively balance macronutrient intake of both lipid and protein independently to satisfy an ideal intake target. While carnivores are also concerned with total energy gain, this framework suggests that they

channel their foraging efforts to provide an intrinsic ratio of macronutrients (Raubenheimer and Simpson 1999; Mayntz et al. 2009; Raubenheimer et al. 2009; Kohl et al. 2015). A classic example is the tendency for bears to disrupt their foraging on energy-rich salmon to supplement their diet with berries. Unfortunately, few tests of the geometric framework have been conducted on large carnivores. However, a recent study of free-ranging Atlantic spotted dolphins (*Stenella frontalis*) demonstrated that non-reproductive, pregnant, and lactating dolphins consumed significantly different prey, in terms of both species and macronutrient compositions, in order to satisfy their individual nutritional needs (Malinowski and Herzing 2015). Similarly, research comparing various cetacean species found a strong relationship between the quality of prey that these marine mammals targeted and their individual costs of living (Spitz et al. 2012). These studies suggest that marine mammals are capable of selecting different prey to prioritize their specific macronutrient demands.

Most studies of the foraging ecology of pinnipeds assume that they will preferentially consume prey with the highest energy content. Considering that lipid provides approximately 40% more energetic return per unit of weight compared to protein (Blaxter 1989), it could be expected that carnivores, including pinnipeds, would choose diets with greater lipid content if provided the choice. However, research testing the geometric framework hypothesis has demonstrated that this is not the case. Feeding choice studies indicated that when carnivores are allowed to regulate their own dietary intake, they frequently choose to consume diets that provide 36-50% lipid and 35-52% protein, rather than those diets with highest lipid ratios (Mayntz et al. 2009; Hewson-Hughes et al. 2011). This suggests that balancing the lipid-to-protein intake is more important for carnivores than previously believed.

Our study suggests an additional aspect to the geometric framework hypothesis by proposing that the frequently chosen optimal macronutrient ratio intake may be partly due to changes in assimilation. Our results demonstrated that the fur seals displayed higher lipid assimilation under low-to-moderate-protein content diets without significantly compromising protein assimilation. This suggests an optimal ratio between lipid and protein that allows fur seals to maximize their benefits from such macronutrients, while simultaneously fulfilling their energetic demands. However, this optimal lipid-to-protein intake may only be achievable when the diversity of available prey is extensive.

In the wild, predators may be limited by prey availability and quality, such that food selection is a luxury. As a result, predators may be forced to prioritize their energetic needs over macronutrient balancing. This may be the case for declining northern fur seal populations breeding on the Pribilof Islands, as well as other pinnipeds in the Bering Sea region (Pitcher 1990; Trites and Larkin 1996; Merrick et al. 1997; Rosen and Trites 2000a; Rosen 2009). Fur seals in the Bering Sea appear to have shifted from a high-lipid herring diet (i.e., high energy density or quality) to a high-protein pollock diet (i.e., low energy or quality) (Swartzman and Haar 1983). Coincidentally, pollock is the most abundant semi-pelagic fish in the Bering Sea (Kajimura 1984), and currently young pollock comprise approximately 60% of the fur seal's diet (Sinclair et al. 1994). Though it is possible that wild fur seals are increasing their intake of young pollock to achieve sufficient energy gain, our results suggest that this intake does not provide an optimal ratio of macronutrients. Moreover, our study shows that a high-protein diet makes it more difficult for fur seals to obtain sufficient net energy, not just by lowering gross energy density of prey, but also by decreasing digestibility of energy-dense lipids. This is of particular importance for free-ranging fur seals given that, based on mean seasonal proximate composition values of pollock in the Bering Sea, young pollock consumed by wild fur seals range from 3.7–4.8 kJ g^{-1}, 2.3–3.2% lipid, and 14.1–15.4% protein (wet-basis) (Van Pelt et al. 1997; Logerwell and Schaufler 2005; Vollenweider et al. 2011). This young pollock is therefore, comparable in terms of proximate composition to the capelin used in our study (Table 3.1), which had significantly low lipid digestibility due to the high protein content. Even though carnivores are supposed to be adapted to deal with high protein consumption rates, such dietary intakes are known to have long-term negative effects on overall health (see below).

Implications of over-digesting macronutrients

Much of the past nutritional research has been concerned with the consequences of animals being unable to obtain sufficient levels of specific macronutrients. However, there are also important effects of excess macronutrient intake that need to be considered when evaluating the potential impacts of nutritionally imbalanced diets on animal health. For example, research on mice has demonstrated that lifespan was greatest when mice were fed low protein and high carbohydrate diets (Solon-Biet et al. 2015b).

When terrestrial carnivores are provided only with high-protein foods, they actively select to consume foods beyond the estimated optimal protein intake amounts, presumably to increase their lipid and energy gain (Mayntz et al. 2009; Hewson-Hughes et al. 2011). As demonstrated by our findings with the fur seals, wild carnivores that opt to consume an excess of protein in order to increase their lipid intake are actually compromising lipid digestibility in exchange for a potentially greater energetic return. This foraging strategy may serve as a short-term solution to obtaining required energy, but may have long-term negative consequences.

Overconsumption of amino acids is detrimental to the performance and survival rates of both vertebrate and invertebrate consumers, due to the high breakdown cost and the challenge of discarding toxic remnants (Harper et al. 1970; Anderson et al. 2005; Raubenheimer et al. 2005). Additionally, excess protein consumption has been linked to decreased reproductive function and fertility of mice (Solon-Biet et al. 2015b). Hence, northern fur seals feeding on large amounts of pollock in the wild may be facing negative impacts to their overall performance and lifespan related to managing excess protein intake and waste products in an effort to obtain sufficient energy. Ultimately, the consumption of macronutrient imbalanced diets may be an important (and until now, overlooked) consideration within the broader context of the nutritional stress hypothesis, to provide a better understanding of the declines of various pinniped populations in the Bering Sea and Gulf of Alaska.

Conclusion

Our study demonstrated that lipid digestibility was significantly higher than protein digestibility for the majority of our experimental diets, but that digestibility of specific macronutrients varied significantly across diets. We also found that high levels of protein content in the diet caused an increase in protein digestibility for northern fur seals, but significantly decreased lipid digestibility. As a consequence, diets with low-to-moderate amounts of protein resulted in high levels of lipid digestibility, without compromising the digestibility of protein. These findings suggest that there may be an ideal lipid-to-protein ratio that allows fur seals to optimize their digestive efficiency to assimilate these macronutrients. Furthermore, we demonstrated that there is no significant benefit to consuming mixed-species diets over single-species diets in terms of macronutrient digestibility. However, diversity in available prey may be

important for fur seals to optimize their macronutrient intake in terms of nutritional requirements and maximizing assimilation efficiencies.

Given the potential conservation consequences, further research should test for potential changes in macronutrient digestibility when fur seals are in a nutritionally compromised state. Future research should also investigate the potential for and limits to intestinal plasticity that fur seals may display when switching between prey items with different macronutrient ratios. Finally, our study suggests that consuming low quality prey (high protein/low lipid), such as pollock, has negative consequences in terms of macronutrient digestion and assimilation by wild northern fur seals, that may ultimately impact the nutritional status of the population.

Chapter 4: Research conclusions

King (1974) stated that "the essential continuity between an animal and its environment is revealed most obviously in energy exchanges and transformations". My research aimed to increase understanding of how northern fur seals obtain energy and nutrients from their environment via their digestive physiology. I ultimately wanted to make accurate inferences regarding the effect of dietary changes on the nutritional state of declining fur seal populations in the Bering Sea, and define potential nutritional or energetic challenges they may be facing.

The overall goal of my thesis was to quantify how efficiently fur seals transform gross energy into net energy. I also wanted to determine the factors that contribute most significantly to any observed differences in digestive efficiency between different diets. This included quantify the fur seals' ability and efficiency to digest proteins and lipids — some of the most essential macronutrients contained in prey — and determining which component of their dietary intake contributed to changes in their ability to digest macronutrients. I also wanted to test the potential effect of dietary changes on other aspects of metabolism (i.e., resting metabolic rate, thermoregulatory cost) in northern fur seals.

To accomplish my research goals, I fed six captive female northern fur seals eight experimental diets. These were composed of four key prey species consumed by free-ranging northern fur seals (capelin, walleye pollock, Pacific herring, and Magister squid). I combined the prey species to create a wide range of representative diets to simulate dietary intake of wild fur seals. The diets were designed to contain different ratios of macronutrients and have different energy densities, but were fed at levels of equal gross energy content in order to test the importance of prey quality (e.g., energy-rich prey *vs.* energy-poor prey) independent of energy intake levels. Additionally, these different diets allowed me to test the hypothesis that mixed-species diets composed of various food items provide the consumer with a greater energetic and nutritional return than equivalent single-species diets (Penry and Jumars 1987; Singer and Bernays 2003). To quantify the fur seals' transformation pathway from gross to net energy (Fig.1.1), I collected individual fecal samples for proximate composition analyses (i.e., water, energy, lipid, protein, and manganese content). Comparisons, between the proximate compositions of both fecal and diet samples (using manganese content as a naturally occurring marker) allowed me to calculate the fur seals' digestibility of energy, lipid, and protein, as well

as dry matter digestibility. I also measured estimated losses from urinary energy loss from apparent nitrogen intake, and directly measured the heat increment of feeding.

I also examined the potential effects of diet changes on other aspects of their energy budget. This included measuring changes in body mass, resting metabolic rate, and their energy expenditure during a thermal challenge in 2 °C water.

Digestive efficiency: the importance of quality over quantity

Similar to previous feeding studies with northern fur seals, I measured dry matter digestibility (DMD%) of different diets. The DMD% values in my study for herring (91.5±1.1%), pollock (88.2±1.1%), and capelin (90.2±1.9%) diets, were similar to those previously reported (Miller 1978; Fadely et al. 1990). My DMD% values were significantly different across experimental diets and were most significantly influenced by protein content, such that DMD% decreased with increasing dietary protein. However, DMD% was not informative in regards to the fur seals' energy transformation capabilities, despite its prominence in the literature.

The amount of energy the fur seals initially gained after the first step of digestion was more directly measured as energy digestibility (DE%) and its counterpart, fecal energy loss (FEL%). My estimates of DE% (95.9–96.9%) and of FEL% (3.1–4.1%) were similar to those previously reported for other pinnipeds (Rosen and Trites 2000b; Stanberry 2003; Yamamoto et al. 2009) and varied significantly across diets. My analysis revealed that dietary protein and ingested mass had the greatest impact on DE%, and that diets with higher protein content also had greater levels of ingested food mass, which led to lower DE% values.

Determining urinary energy loss (UEL%) is ideally done by collecting all urine excreted over a period of time. However, because of the difficulty of collecting such excreta samples from fur seals, I was only able to estimate UEL% based on the fur seals' apparent digestible nitrogen intake from each diet. My estimates of UEL% (9.3–26.7%; as % of DE%) significantly differed among diets, and increased as dietary protein increased. This procedure allowed me to calculate metabolizable energy (ME%) by subtracting the energy lost through excreta (UEL% and FEL%) from the dietary GEI. My ME% values (70.3–87.2%) were significantly different among diets and also similar to those previously reported for pinnipeds. My analysis indicated that ME% values were positively correlated to dietary lipid.

To determine the heat increment of feeding (HIF%), the fur seals were fed test diets while inside a metabolic chamber where I could measure oxygen consumption to estimate energy expenditure over the full duration of digestion. The fur seals' HIF% values (4.3–12.4%) were comparable to those previously reported for other pinnipeds (Table 3 in Rosen and Trites 1997). HIF% varied significantly across diets and was positively correlated to dietary lipid. Previous nutrient studies have reported that the specific dynamic action of protein is twice as high as lipids (Forbes and Swift 1944).

Subtracting the energy lost through HIF% from the metabolizable energy allowed me to calculate the net energy gained (NE%) by the fur seals for each diet. My NE% values (57.9–83.0%) were similar to the only previous NE% estimate in a phocid seal (Ashwell-Erickson and Elsner 1981). Likewise, my NE% estimates fit within NE% values for terrestrial carnivores, as well as birds (Robbins 1993). However, the lower range of my NE% values was much lower than those of any other animal.

Additionally, my analysis showed that NE% was significantly influenced by lipid content of the diets. Overall, the significantly lower NE% gained by fur seals on high protein/low lipid diets such as the capelin diet (used in our study as a low-quality prey species) highlights how large amounts of dietary protein can exaggerate differences in gross energy composition through the energetic cost of energy transformation. Furthermore, my results demonstrated that—due to the high cost of the digestive processes—deriving sufficient net energy from low quality prey sources is energetically challenging to fur seals. My results also demonstrated that, contrary to theoretical beliefs (Penry and Jumars 1987; Singer and Bernays 2003), consumption of mixed-species diets do not provide a greater energetic return to a consumer compared to single-species diets.

To test the potential effect of dietary changes on other aspects of the metabolic pathway in northern fur seals, I measured both resting metabolic rate (RMR) in air, as well as thermoregulatory costs (TC) when the animals were partially submerged in a 2 ºC water bath. As per experimental design, average mass-specific RMR (10.0 ± 3.4 mL O_2 kg^{-1} min^{-1}) and TC (17.1 ± 4.1 mL O^2 kg^{-1} min^{-1}) were significantly different from each other (due to differences in thermal environment), but changes in dietary intake had no effect on either the RMR or TC of the fur seals.

A potential optimal macronutrient intake for digestibility

Nutrition is more than just energy, and my research is the first to measure the apparent digestibility coefficient (ADC_n) of specific macronutrients in fur seals and examine whether dietary changes relate to changes in digestive efficiency of macronutrients. I quantified the fur seals' efficiency in digesting lipid and protein via the analysis of lipid and protein contents in both fish and fecal samples. My results showed that both lipid and protein digestibility significantly varied across diets and that both were significantly influenced by protein content, but with opposing effects (see below). Additionally, my analysis indicated that the apparent digestibility of lipid was significantly higher than the apparent digestibility of crude protein for all but the lowest lipid diets, in accordance with previous research indicating that animal fats were more easily digested and assimilated than protein (Leoschke 1959; Best 1985; Blaxter 1989; Stevens and Hume 1995). My results for both lipid and protein digestibility were overall similar to those reported by previous pinniped feeding studies (Parsons 1977; Keiver et al. 1984; Ronald et al. 1984; Fisher et al. 1992; Goodman-Lowe et al. 1999; Stanberry 2003; Trumble et al. 2003; Yamamoto et al. 2009).

My estimates for apparent crude protein digestibility ranged from 95.7–96.7% and were significantly positively correlated to dietary protein intake. Estimates of apparent lipid digestibility ranged from 96.0–98.4% and, unlike protein digestibility, were negatively correlated with dietary protein content. The fact that crude protein was the largest component in all diets in combination with our observed apparent crude protein digestibility, could potentially be explained by the adaptive modulation hypothesis. This hypothesis suggests that animals up-regulate specific nutrient transporters in order to increase and optimize the gain of that nutrient (Karasov 1992; Karasov 1996). It is possible that the fur seals were up-regulating amino acid transporters in response to high protein loads and these might have competed with lipid assimilation. However, future research should directly test this potential gastrointestinal trade-off. My results on macronutrient digestibility also indicate the possibility that there is an optimal ratio between dietary lipid and protein that allows fur seals to optimize digestibility of both macronutrients. As a result, fur seals consuming diets of this particular protein-to-lipid ratio do not compromise digestibility of either of these macronutrients.

I also tested the hypothesis that mixed-species diets provide greater nutrient return than single-species diets. My results indicate that protein return did not significantly differ between single-species and mixed-species diets. Similarly, lipid digestibility values were not significantly different than the equivalent single-species diets for most diets. Furthermore, lipid digestibility of two experimental diets (herring + capelin and pollock + capelin) was significantly lower than expected.

Strengths and study limitations

Captive studies with marine mammals are inherently restrained to a small sample size, which limits statistical power. While my research only used six female fur seals, I was able to overcome some of the issues of low statistical power by using analytically robust mixed effects models. My study was also limited in testing only females of a specific age class. While this group might not be representative of the whole population, it did limit experimental variability by having a uniform test group. Furthermore, female fur seals are thought to have the greatest influence on overall population trends because of their polygynous mating system. Therefore, understanding how changes in diet affect females may provide the most relevant information on any potential link to their current nutritional status in the Eastern Bering Sea.

The issue of adequate nutrition for adult females may impact the population through inadequate allocation of energy from female fur seals to their pups. If lactating females are extending their at-sea time to acquire sufficient energy, this may be extending the pups' fasting times between suckling periods beyond their physiological capabilities, resulting in increased mortality due to emaciation syndrome (Keyes 1965; Costa and Gentry 1986). Hence, the fact that my research focuses solely on females may also be viewed as a strength of the study.

An assumption of my study was that the assimilation efficiency of nutrients from experimental diets that were hand fed to the animals is similar to those of free-ranging foraging fur seals. The energy transformation pathway is affected by several internal (e.g., nutritional state) and external (e.g., environmental conditions) factors. However, as an animal's basic gastrointestinal functions are the result of a long course of evolutionary adaptations and are not likely significantly to be altered by human interactions in an experimental set-up or as a result of captivity. Therefore, I feel that the digestive functions of these captive animals are comparable to their wild counterparts. My goals were to describe the fur seals' digestive efficiency patterns and

to test the effect that low quality prey would have on the animals' capacity to transform gross energy to net energy. Thus, I believe that my results can be used to infer the digestive efficiency trends of free-ranging fur seals.

A potential source of bias in my data relates to the in-house chemical analysis I conducted as part of my overall scientific education. However, given the difficulties of applying these techniques, I was careful to corroborate the validity of my in-house laboratory analyses with those of a private laboratory. When the differences were too great — such as with the very low manganese concentrations in the fish samples — I chose to rely on the values from the commercial laboratory. However, while there was greater agreement with the values derived for the proximate composition, differences still existed. Underestimates in the composition of lipid (~3%) and protein (~9%) in the fish samples may have led to overestimates in macronutrient digestibility. This potential bias could explain why my results for protein digestibility were on the upper range of those previously reported. While it might have been more accurate to analyze all samples through a private laboratory to prevent variability in results due to differences in the techniques utilized, I felt it was important for me to learn these techniques and gain an appreciation of their inherent difficulties and potential sources of error.

Another limitation presented in my study was related to the measurement of HIF%. Past HIF% studies on harp seals (*Phoca groelandica*) and harbour seals (*Phoca vitulina*) (Gallivan and Ronald 1981; Markussen and Øritsland 1991) that consumed fish diets of similar size (1.5-2.0 kg) to those I used in my study, reported that their HIF% trials were approximately 10 hrs in duration. In order to accommodate logistical limitations that limited my HIF% trials to 5 to 6 hrs, I fed the fur seals only half of their daily GEI during HIF% trials. This allowed me to capture the bulk of the increase in metabolism in the shorter amount of time. However, I also had to assume that the measured HIF% cost for half of the animals' daily GEI would be proportional to that of the total GEI the animals would eat in a day. In reality, animals in the wild may forage over extended periods during the day, but it is unfeasible to mimic this foraging pattern during HIF% trials. Rather, we chose to use standardized method to try to ensure our results were consistent within our study and reasonably comparable to studies of other pinnipeds.

The findings and conclusions reached in my study are most directly applicable to animals in the same setting that are in a similar nutritional and physical state. This, however, also raises

an interesting point regarding the implications of my findings for free-ranging fur seals that may have a different nutritional status. My study demonstrated that reliance by northern fur seals on high-protein, low-energy prey may make it more difficult for them to acquire sufficient prey to meet their energy requirements, leading them to experience episodes of nutritional stress. However, it is unclear how changes in nutritional status would subsequently affect their digestive efficiencies. It is possible that energy transformation and macronutrient digestibility would be improved in nutritionally stressed individuals (perhaps through some sort of digestive adaptation), thus assisting them in overcoming their nutritional deficits. However, it is also possible that the digestive efficiencies of wild fur seals under nutritional stress would, in fact, be even lower than those reported in my study due to some physiological impairment. In this case, this might lead to further potential negative effects on an animal's nutritional state. Clearly, this requires further study.

Lastly, one of my main suggestions is that consumption of low quality prey (high in protein and lipid/energy poor) that the fur seals encounter in the wild may be impairing their energy balance. This is based on the similarities of the proximate composition of my capelin diets and the young pollock that the fur seals are reported to consume in the Eastern Bering Sea (Sinclair et al. 1994; Call and Ream 2012). My conclusions are inferred from the low quality prey I used in my study, fed to relatively healthy individuals. However, to make more accurate conclusions, young pollock should be specifically included as part of the test prey items in future studies, perhaps in conjunction with studies of animals simultaneously undergoing a period of nutritional stress.

Future research

My research focused on describing the fur seals' ability to transform energy and digest lipid and protein. I also tested the factors that affected the fur seals' ability to transform such nutrients when consuming various prey that fur seals encounter in the Bering Sea. However, as previously noted, I recommend that future energetic studies use young pollock (such as age-0 and age-1 year olds) in order to make more direct conclusions about whether this forage fish may be the source behind the fur seals' nutritional stress. Most importantly, I recommend using samples of these fish obtained during the summer months, to specifically test the fish that the

female fur seals are acquiring during their foraging trips in the Bering Sea while caring for their pups.

I would further recommend that body composition parameters should be quantified for the duration of future feeding experiments. In my study, I tested changes in body weight as an indicator that the animals remained within their maintenance state. However, fur seals on the capelin diet had no significant change in body weight even though the HIF% cost to digest the high protein meal was the greatest and resulted in the lowest NE% gain. It is possible that the reason why I did not capture significant changes in body mass was because the fur seals utilized stored fats to offset the shortfall in required NE%, and thus maintained a relatively stable weight. Measurements of body composition would capture these potential changes in tissue use related to changes in net energy intake.

For my study, it was logistically unfeasible to obtain urine samples from the fur seals. However, as demonstrated in my results, the large levels of apparent digestible nitrogen intake from my diets may have possibly led to an overestimates of urinary energy loss. Therefore, I recommend that future studies obtain urine samples from the fur seals to quantify its energetic content, in order to make more accurate estimates of net energy gain.

Ultimately, it would also be important to investigate how changes in dietary intake (particularly the consumption of high levels of protein) impact the fur seals' reproductive functions. For example, a study on mice proposed that an optimal macronutrient-balanced diet allows mice to maximize reproduction success (Solon-Biet et al. 2015b). Testing the potential of reduced fertility in female fur seals due to consumption of low quality prey with high protein content may be a more direct test of the proposed link between dietary changes and population declines of wild northern fur seals.

Study implications for wild fur seals

The nutritional stress hypothesis has suggested that fur seals are being affected by changes in the quality and quantity of prey available in the Bering Sea, causing them, along with many other pinnipeds, to be energetically and nutritionally challenged. However, the nutritional stress hypothesis has never been tested on northern fur seals, and more importantly, no study has been undertaken to understand how fur seals transform ingested chemical energy and nutrients into biologically useful energetic (ATP) and nutritional gains. Thus, by testing the effects that

dietary changes may bring upon individual fur seals, my study has implications for making inferences about the status of the currently declining fur seal populations. As previously noted, my experimental diets were designed to represent a range of lipid and protein ratios, in order to provide a clearer picture of the effects of different proximate compositions on macronutrient and energy gain. Thus, the results from my study can also provide accurate estimates of energy and macronutrient gain across diets of different compositions, and could even be used as inputs for population-level models. Such models could be designed to make more precise inferences of the nutritional state of fur seals and other pinnipeds on a population scale.

In Chapter 2, I noted how the proximate composition of the capelin diet used in my study was comparable in quality to the young pollock (low quality prey) available to fur seals in the Bering Sea (Vollenweider et al. 2011; Call and Ream 2012). Therefore, my findings from the capelin diet suggest that fur seals consuming large amounts of young pollock may struggle to obtain sufficient net energy, despite the large biomass of pollock available to them. Furthermore, the greater cost of digesting a greater mass of prey due to their lower energy density further decrease the potential net energy that fur seals would gain to satisfy physiological needs. My study therefore adds support to the hypothesis that the dominance of juvenile pollock in the current diet of wild fur seals in the Eastern Bering Sea is detrimental to their population nutritional status and overall health.

My findings in Chapter 3 on the digestibility of macronutrients indicated that consumption of diets with high dietary protein are associated with low lipid digestibility. These findings have implications for lactating female fur seals in the Bering Sea because females must acquire enough energy to sustain their own needs and provide nutrient rich milk to their pups. If they are challenged to digest the little lipid available in their prey, they will not only receive low energetic return but may also provide less nutritious (fatty) milk for their pups.

Additionally, the results in Chapter 3 indicate that protein assimilation increased with increasing dietary protein, which may lead to an overconsumption of this nutrient. Other studies on different taxa have demonstrated that excess consumption of protein has negative long-term effects, such as reduction in fertility and lifespan (Harper et al. 1970; Elrod and Butler 1993; Anderson et al. 2005; Solon-Biet et al. 2015b; Solon-Biet et al. 2015a). Therefore, fur seals in

the Bering Sea consuming large amounts of young pollock may have to deal with excessive amino acids, which may as a consequence negatively impact their fitness.

In conclusion, I sought to increase knowledge on how northern fur seals and other pinnipeds exchange energy and nutrients with their ever-changing environment to contribute to the understanding of their nutritional ecology and to provide new avenues of research for future explorations.

References

Alverson DL (1992) A review of commercial fisheries and the Steller sea lion (*Eumetopias jubatus*) the conflict arena. Rev Aquat Sci 6:203–256

Anderson TR, Hessen DO, Elser JJ, Urabe J (2005) Metabolic stoichiometry and the fate of excess carbon and nutrients in consumers. Am Nat 165:1–15. doi: 10.1086/426598

AOAC (1990) Official methods of analysis, 15th edn. Association of Official Analytical Chemists, Arlington, V.A.

Ashwell-Erickson SM, Elsner R (1981) The energy cost of free existence of Bering Sea harbour and spotted seals. In: Hood DW, Calder JA (eds) The Eastern Bering Sea shelf: oceanography and resources. Seattle University of Washington Press, Seattle, pp 869–899

Barbiers RB, Vosburgh LM, Ku PK, Ullrey DE (1982) Digestive efficiencies and maintenance energy requirements of captive wild felidae: cougar (*Felis concolor*); leopard (*Panthera pardus*); lion (*Panthera leo*); and tiger (*Panthera tigris*). J Zoo Anim Med 13:32–37. doi: 10.2307/20094560

Barbosa P, Castellanos I (2005) Ecology of predator-prey interactions. Oxford University Press, Inc, New York, N.Y.

Barbour AS (1993) Heat increment of feeding in juvenile northern elephant seals. MSc thesis, University of California

Best RC (1985) Digestibility of ringed seals by the polar bear. Can J Zool 63:1033–1036. doi: 10.1139/z85-155

Blaxter SKL (1989) Energy metabolism in animals and man. Cambridge University Press, Cambridge, UK

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917. doi: 10.1139/o59-099

Boersma M, Elser JJ (2006) Too much of a good thing: on stoichiometrically balanced diets and maximal growth. Ecology 87:1325–1330. doi: 10.1890/0012-9658(2006)87[1325:TMOAGT]2.0.CO;2

Buckley TW, Livingston PA (1994) A bioenergetics model of walleye pollock (*Theragra chalcogramma*) in the Eastern Bering Sea: Structure and documentation. US Department of Commerce, NOAA Tech. Memo, pp 55

Buddington RK, Chen JW, Diamond JM (1991) Dietary regulation of intestinal brush-border sugar and amino acid transport in carnivores. Am J Physiol - Regul Integr Comp Physiol 261:R793–R801

Budge SM, Iverson SJ, Koopman HN (2006) Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Mar Mammal Sci 22:759–801. doi: 10.1111/j.1748-7692.2006.00079.x

Burr GO, Burr MM (1930) On the nature and role of the fatty acids essential in nutrition. J Biol Chem 86:587–621

Calkins DG, Atkinson S, Mellish JA, Waite JN, Carpenter JR (2013) The pollock paradox: juvenile Steller sea lions experience rapid growth on pollock diets in fall and spring. J Exp Mar Biol Ecol 441:55–61. doi: 10.1016/j.jembe.2013.01.011

Calkins DG, Becker EF, Pitcher KW (1998) Reduced body size of female Steller sea lions from a declining population in the Gulf of Alaska. Mar Mammal Sci 14:232–244. doi: 10.1111/j.1748-7692.1998.tb00713.x

Call KA, Ream RR (2012) Prey selection of subadult male northern fur seals (*Callorhinus ursinus*) and evidence of dietary niche overlap with adult females during the breeding season. Mar Mammal Sci 28:1–15. doi: 10.1111/j.1748-7692.2011.00463.x

Castellini MA (1993) Is it food? Addressing marine mammal and sea bird declines. University of Alaska Fairbanks, Fairbanks

Clauss M, Kleffner H, Kienzle E (2010) Carnivorous mammals: nutrient digestibility and energy evaluation. Zoo Biol 29:687–704. doi: 10.1002/zoo.20302

Costa DP (1988) Assessment of the impact of the California sea lion and northern elephant seal on commercial fisheries. University of California

Costa DP, Gentry RL (1986) Free-ranging energetics of northern fur seals. In: Fur seals: maternal strategies on land and sea. Princeton University Press, Princeton, pp 79–101

Crampton EW, Rutherford BE (1954) Apparent digestibility of dietary protein as a function of protein level. J Nutr 54:445–451

Crawley MJ (2007) The R book. Wiley, Chichester

Cruz-Neto AP, Bozinovic F (2004) The relationship between diet quality and basal metabolic rate in endotherms: insights from intraspecific analysis. Physiol Biochem Zool 77:877–889. doi: 10.1086/425187

Dade WB, Jumars PA, Penry DL (1990) Supply-side optimization: maximizing absorptive rates. In: Hughes RN (ed) Behavioural mechanisms of food selection. Springer Berlin Heidelberg, pp 531–556

Dalton AJM, Rosen DAS, Trites AW (2014) Broad thermal capacity facilitates the primarily pelagic existence of northern fur seals (*Callorhinus ursinus*). Mar Mammal Sci 30:994–1013. doi: 10.1111/mms.12103

Davison RP, Mautz WW, Hayes HH, Holter JB (1978) The efficiency of food utilization and energy requirements of captive female fishers. J Wildl Manag 42:811–821. doi: 10.2307/3800771

Decker MB, Hunt Jr G, Byrd GV (1995) The relationships among sea-surface temperature, the abundance of juvenile walleye pollock (*Theragra chalcogramma*), and the reproductive performance and diets of seabirds at the Pribilof Islands. In: Beamish RJ (ed) Climate change and northern fish populations. Canadian Special Publications in Fisheries and Aquatic Sciences, Ottowa, pp 425–437

Dierauf L, Gulland FMD (2001) CRC handbook of marine mammal medicine: health, disease, and rehabilitation, Second Edition. CRC Press, Boca Raton, F.L.

Donohue MJ, Costa DP, Goebel ME, Baker JD (2000) The ontogeny of metabolic rate and thermoregulatory capabilities of northern fur seal, *Callorhinus ursinus*, pups in air and water. J Exp Biol 203:1003–1016

Elrod CC, Butler WR (1993) Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. J Anim Sci 71:694–701. doi: /1993.713694x

Estabrook GF, Dunham AE (1976) Optimal diet as a function of absolute abundance, relative abundance, and relative value of available prey. Am Nat 110:401–413

Fadely BS, Worthy GAJ, Costa DP (1990) Assimilation efficiency of northern fur seals determined using dietary manganese. J Wildl Manag 54:246–251. doi: 10.2307/3809037

Fadely BS, Zeligs JA, Costa DP (1994) Assimilation efficiencies and maintenance requirements of California sea lions (*Zalophus californianus*) fed walleye pollock (*Theragra chalcogramma*) and herring (*Clupea harengus*). Final Report to the National Marine Mammal Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service 7600 (1994): 98115-0070

Fisher KI, Stewart REA, Kastelein RA, Campbell LD (1992) Apparent digestive efficiency in walruses (*Odobenus rosmarus*) fed herring (*Clupea harengus*) and clams (*Spisula* sp.). Can J Zool 70:30–36. doi: 10.1139/z92-005

Forbes EB, Swift RW (1944) Associative dynamic effects of protein, carbohydrate and fat. Science 99:476–478. doi: 10.1126/science.99.2580.476

Forster IP (1999) A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. Aquac Nutr 5:143–145. doi: 10.1046/j.1365-2095.1999.00082.x

Fortune SME, Trites AW, Mayo CA, et al (2013) Energetic requirements of North Atlantic right whales and the implications for species recovery. Mar Ecol Prog Ser 478:253–272

Fowler CW (1982) Interactions of northern fur seals and commercial fisheries. Publications, Agencies and Staff of the US Department of Commerce

French DP, Reed M (1990) Potential impact of entanglement in marine debris on the population dynamics of the northern fur seal, *Callorhinus ursinus*. In: Proceedings of the Second International Conference on Marine Debris, April 2–7, 1989, Honolulu, Hawaii. NOAA Technical Memorandum NMFS-SWFSC-154

Galecki A, Burzykowski T (2013) Linear mixed-effects models using R: a step-by-step approach, 2013 edition. Springer, New York, N.Y.

Gallivan GJ, Ronald K (1981) Apparent specific dynamic action in the harp seal (*Phoca groenlandica*). Comp Biochem Physiol A Physiol 69:579–581. doi: 10.1016/0300-9629(81)93024-3

Gentry RL (2002) Northern fur seal (*Callorhinus ursinus*). In: Encyclopedia of marine mammals. Academic Press, San Diego, C.A., pp 813–817

Gentry RL, Kooyman GL (1986) Fur seals: maternal strategies on land and at sea. Princeton University Press, Princeton, N.J.

Goodman-Lowe GD, Carpenter JR, Atkinson S (1999) Assimilation efficiency of prey in the Hawaiian monk seal (*Monachus schauinslandi*). Can J Zool 77:653–660. doi: 10.1139/z98-238

Gordon MS, Bartholomew GA, Grinnell AD, Jorgensen CB, White FN (1972) Animal physiology: principles and adaptation. Macmillan Publishing Company, New York, N.Y.

Grodzinski W (1975) Methods for ecological bioenergetics. Blackwell Scientific Publications, Oxford, UK

Harper AE, Benevenga NJ, Wohlhueter RM (1970) Effects of ingestion of disproportionate amounts of amino acids. Physiol Rev 50:428–558

Hewson-Hughes AK, Hewson-Hughes VL, Miller AT, Hall SR, Simpson SJ, Raubenheimer D (2011) Geometric analysis of macronutrient selection in the adult domestic cat, *Felis catus*. J Exp Biol 214:1039–1051. doi: 10.1242/jeb.049429

Iverson SJ, Lang SLC, Cooper MH (2001) Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. Lipids 36:1283–1287. doi: 10.1007/s11745-001-0843-0

Jobling M (1983) Towards an explanation of specific dynamic action (SDA). J Fish Biol 23:549–555. doi: 10.1111/j.1095-8649.1983.tb02934.x

Kajimura H (1984) Opportunistic feeding of the northern fur seal, *Callorhinus ursinus*, in the eastern North Pacific Ocean and eastern Bering Sea. U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Washington, D.C.

Karasov WH (1992) Tests of the adaptive modulation hypothesis for dietary control of intestinal nutrient transport. Am J Physiol - Regul Integr Comp Physiol 263:R496–R502

Karasov WH (1996) Digestive plasticity in avian energetics and feeding ecology. In: Carey C (ed) Avian Energetics and Nutritional Ecology. Springer US, pp 61–84

Keiver KM, Ronald K, Beamish FWH (1984) Metabolizable energy requirements for maintenance and faecal and urinary losses of juvenile harp seals (*Phoca groenlandica*). Can J Zool 62:769–776. doi: 10.1139/z84-110

Keyes MC (1965) Pathology of the northern fur seal. J Am Vet Med Assoc 1090–1095

King JR (1974) Seasonal allocation of time and energy resources in birds. In: Paynter RAJ (ed) Avian Energetics and Nutritional Ecology. Cambridge, Mass, pp 4–85

King JR, Murphy ME (1985) Periods of nutritional stress in the annual cycles of endotherms: fact or fiction? Am Zool 25:955–964. doi: 10.1093/icb/25.4.955

Kleiber M (1975) The fire of life: an introduction to animal energetics. R. E. Krieger Pub. Co., New York, N.Y.

Kohl KD, Coogan SCP, Raubenheimer D (2015) Do wild carnivores forage for prey or for nutrients? BioEssays 37:701–709. doi: 10.1002/bies.201400171

Lavigne DM, Barchard W, Innes S, Øritsland NA (1982) Pinniped bioenergetics. In: Mammals in the seas: Small cetaceans, seals, sirens, and sea otters. FAO, Rome, IT, pp 191–235

Lavigne DM, Innes S, Worthy GAJ, Kovacs KM, Schmitz OJ, Hickie JP (1986) Metabolic rates of seals and whales. Can J Zool 64:279–284. doi: 10.1139/z86-047

Lawson JW, Miller EH, Noseworthy E (1997) Variation in assimilation efficiency and digestive efficiency of captive harp seals (*Phoca groenlandica*) on different diets. Can J Zool 75:1285–1291. doi: 10.1139/z97-152

Leoschke WL (1959) The digestibility of animal fats and proteins by mink. Am J Vet Res 20:1086–1089

Liwanag HEM (2010) Energetic costs and thermoregulation in northern fur seal (*Callorhinus ursinus*) pups: the importance of behavioral strategies for thermal balance in furred marine mammals. Physiol Biochem Zool 83:898–910. doi: 10.1086/656426

Logerwell EA, Schaufler LE (2005) New data on proximate composition and energy density of Steller sea lion (*Eumetopias jubatus*) prey fills seasonal and geographic gaps in existing information. Aquat Mamm 31:62–82

Mair W, Morantte I, Rodrigues AP, Manning G, Montminy M, Shaw RJ, Dillin A (2011) Lifespan extension induced by AMPK and calcineurin is mediated by CRTC-1 and CREB. Nature 470:404–408. doi: 10.1038/nature09706

Malinowski CR, Herzing DL (2015) Prey use and nutritional differences between reproductive states and age classes in Atlantic spotted dolphins (*Stenella frontalis*) in the Bahamas. Mar Mammal Sci n/a–n/a. doi: 10.1111/mms.12238

Markussen NH, Øritsland NA (1991) Food energy requirements of the harp seal (*Phoca groenlandica*) population in the Barents and White Seas. Polar Res 10:603–608. doi: 10.1111/j.1751-8369.1991.tb00678.x

Mårtensson PE, Nordøy ES, Blix AS (1994) Digestibility of krill (*Euphausia superba* and *Thysanoessa* sp.) in minke whales (*Balaenoptera acutorostrata*) and crabeater seals (*Lobodon carcinophagus*). Br J Nutr 72:713–716. doi: 10.1079/BJN19940073

Mayntz D, Nielsen VH, Sørensen A, Toft S, Raubenheimer D, Hejlesen C, Simpson SJ (2009) Balancing of protein and lipid intake by a mammalian carnivore, the mink, *Mustela vison*. Anim Behav 77:349–355. doi: 10.1016/j.anbehav.2008.09.036

Merrick RL, Chumbley MK, Byrd GV (1997) Diet diversity of Steller sea lions (*Eumetopias jubatus*) and their population decline in Alaska: a potential relationship. Can J Fish Aquat Sci 54:1342–1348. doi: 10.1139/f97-037

Miller LK (1978) Energetics of the northern fur seal in relation to climate and food resources of the Bering Sea. U.S. Marine Mammal Commission, Washington, D.C.

Moyes CD, Schulte PM (2007) Principles of animal physiology, 2 edn. Pearson, San Francisco, C.A.

National Research Council (1996) The Bering Sea ecosystem. National Academies Press, Washington, D.C.

NMFS (1993) Conservation plan for the northern fur seal (*Callorhinus ursinus*). National Marine Fisheries Service, Seattle, W.A.

NMFS (2007) Conservation plan for the Eastern Pacific stock of northern fur seals (*Callorhinus ursinus*). National Marine Fisheries Service, Juneau, A.K.

Parsons JL (1977) Metabolic studies on ringed seals (*Phoca hispida*). M.Sc., Univeristy of Guelph

Penry DL, Jumars PA (1987) Modeling animal guts as chemical reactors. Am Nat 129:69–96

Perrin WF, Würsig BG, Thewissen JGM (2002) Encyclopedia of Marine Mammals. Gulf Professional Publishing

Pinheiro JC, Bates DM (2000) Mixed-effects models in S and S-PLUS. Springer, Verlag, N.Y.

Pinheiro J, Bates D, DebRoy S, Sarkar D, Team RC (2015) nlme: linear and nonlinear mixed effects models. R Package

Pitcher KW (1990) Major decline in number of harbor seals, *Phoca vitulina richardsi*, on Tugidak Island, Gulf of Alaska. Mar Mammal Sci 6:121–134. doi: 10.1111/j.1748-7692.1990.tb00234.x

Pritchard GT, Robbins CT (1990) Digestive and metabolic efficiencies of grizzly and black bears. Can J Zool 68:1645–1651. doi: 10.1139/z90-244

Raubenheimer D, Lee KP, Simpson SJ (2005) Does Bertrand's rule apply to macronutrients? Proc Biol Sci 272:2429–2434

Raubenheimer D, Simpson SJ (1999) Integrating nutrition: a geometrical approach. In: Simpson SJ, Mordue AJ, Hardie J (eds) Proceedings of the 10th International Symposium on Insect-Plant Relationships. Springer Netherlands, pp 67–82

Raubenheimer D, Simpson SJ, Mayntz D (2009) Nutrition, ecology and nutritional ecology: toward an integrated framework. Funct Ecol 23:4–16. doi: 10.1111/j.1365-2435.2009.01522.x

R Core Team (2014) R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria

Reid JT, White OD, Anrique R, Fortin A (1980) Nutritional energetics of livestock: some present boundaries of knowledge and future research needs. J Anim Sci 51:1393–1415

Reynolds JEI, Rommel SA (eds) (1999) Biology of Marine Mammals. Smithsonian, Washington

Riedman M (1990) The pinnipeds: seals, sea lions, and walruses. University of California Press, Berkeley/Los Angeles

Riedman M, Staedler M, Estes JA (1988) Behavioral and biological studies on the California sea otter in the northern part of its range. Research plan, Monterey Bay Aquarium, Monterey bay

Robbins CT (1993) Wildlife feeding and nutrition. Academic Press, San Diego, C.A.

Ronald K, Healey PJ (1981) Harp seal – *Phoca groenlandica*. In: Ridgway SH, Harrison J (eds) Handbook of marine mammals. Academic Press, London, U.K., pp 55–87

Ronald K, Keiver KM, Beamish FWH, Frank R (1984) Energy requirements for maintenance and faecal and urinary losses of the grey seal (*Halichoerus grypus*). Can J Zool 62:1101–1105. doi: 10.1139/z84-160

Rosen DAS (2009) Steller sea lions *Eumetopias jubatus* and nutritional stress: evidence from captive studies. Mammal Rev 39:284–306. doi: 10.1111/j.1365-2907.2009.00150.x

Rosen DAS, Gerlinsky CD, Trites AW (2015) Evidence of partial deferment of digestion during diving in Steller sea lions (*Eumetopias jubatus*). J Exp Mar Biol Ecol 469:93–97. doi: 10.1016/j.jembe.2015.04.017

Rosen DAS, Trites AW (1997) Heat increment of feeding in Steller sea lions, *Eumetopias jubatus*. Comp Biochem Physiol A Physiol 118:877–881. doi: 10.1016/S0300-9629(97)00039-X

Rosen DAS, Trites AW (1999) Metabolic effects of low-energy diet on Steller sea lions, *Eumetopias jubatus*. Physiol Biochem Zool 72:723–731. doi: 10.1086/316705

Rosen DA, Trites AW (2000a) Digestive efficiency and dry-matter digestibility in Steller sea lions fed herring, pollock, squid, and salmon. Can J Zool 78:234–239. doi: 10.1139/z99-201

Rosen DAS, Trites AW (2000b) Pollock and the decline of Steller sea lions: testing the junk-food hypothesis. Can J Zool 78:1243–1250

Rosen DAS, Trites AW (2003) No evidence for bioenergetic interaction between digestion and thermoregulation in Steller sea lions *Eumetopias jubatus*. Physiol Biochem Zool 76:899–906. doi: 10.1086/378140

Rosen DAS, Trites AW (2005) Examining the potential for nutritional stress in young Steller sea lions: physiological effects of prey composition. J Comp Physiol B 175:265–273. doi: 10.1007/s00360-005-0481-5

Rosen DAS, Trites AW (2014) Thermal limits in young northern fur seals, *Callorhinus ursinus*. Mar Mammal Sci 30:1014–1028. doi: 10.1111/mms.12097

Rosen DAS, Young BL, Trites AW (2011) Rates of maximum food intake in young northern fur seals (*Callorhinus ursinus*) and the seasonal effects of food intake on body growth. Can J Zool 90:61–69. doi: 10.1139/z11-112

Russell K, Lobley GE, Millward DJ (2003) Whole-body protein turnover of a carnivore, *Felis silvestris catus*. Br J Nutr 89:29–37. doi: 10.1079/BJN2002735

Russell K, Murgatroyd PR, Batt RM (2002) Net protein oxidation is adapted to dietary protein intake in domestic cats (*Felis silvestris catus*). J Nutr 132:456–460

Schneider BH, Flatt WP (1975) The evaluation of feeds through digestibility experiments. University of Georgia Press, Athens

Secor SM (2008) Specific dynamic action: a review of the postprandial metabolic response. J Comp Physiol B 179:1–56. doi: 10.1007/s00360-008-0283-7

Sergeant DE (1976) History and present status of populations of harp and hooded seals. Biol Conserv 10:95–118. doi: 10.1016/0006-3207(76)90055-0

Sinclair E, Loughlin T, Pearcy W (1994) Prey selection by northern fur seals (*Callorhinus ursinus*) in the eastern Bering Sea. Fish Bull 92:144–156

Singer MS, Bernays EA (2003) Understanding omnivory needs a behavioral perspective. Ecology 84:2532–2537

Solon-Biet SM, Walters KA, Simanainen UK, McMahon AC, Ruohonen K, Ballard JWO, Raubenheimer D, Handelsman DJ, Le Couteur DG, Simpson SJ (2015a) Macronutrient balance, reproductive function, and lifespan in aging mice. Proc Natl Acad Sci 112:3481–3486. doi: 10.1073/pnas.1422041112

Solon-Biet SM, Mitchell SJ, de Cabo R, Raubenheimer D, Le Couteur DG, Simpson S (2015b) Macronutrients and caloric intake in health and longevity. J Endocrinol JOE–15–0173. doi: 10.1530/JOE-15-0173

Spitz J, Trites AW, Becquet V, Brind'Amour A, Cherel Y, Galois R, Ridoux V (2012) Cost of living dictates what whales, dolphins and porpoises eat: the importance of prey quality on predator foraging strategies. PLoS ONE 7:e50096. doi: 10.1371/journal.pone.0050096

Stanberry K (2003) The effect of changes in dietary fat level on body composition, blood metabolites and hormones, rate of passage, and nutrient assimilation efficiency in harbor seals. MSc thesis, University of Hawaii

Stephens DW, Krebs JR (1986) Foraging theory. Princeton University Press, Princeton, N.J.

Stevens CE, Hume ID (1995) Comparative physiology of the vertebrate digestive system, 2nd edn. Cambridge University Press, Cambridge, U.K.

Strandberg U, Käkelä A, Lydersen C, Kovacs KM, Grahl-Nielsen O, Hyvärinen H, Käkelä R (2008) Stratification, Composition, and function of marine mammal blubber: the ecology of fatty acids in marine mammals. Physiol Biochem Zool 81:473–485. doi: 10.1086/589108

Swartzman GL, Haar RT (1983) Interactions between fur seal populations and fisheries in the Bering Sea. Fish Bull 81:121–132

Towell R, Ream RR, Bengtson J, Sterling J (2014) 2014 northern fur seal pup production and adult male counts on the Pribilof Islands, Alaska. Alaska Fisheries Science Center, National Marine Mammal Laboratory. Available at www.afsc.noaa.gov/nmml/PDF/2014-nfs-pup-counts-pribs.pdf

Trites AW (1992) Northern fur seals: why have they declined? Aquat Mamm 18:3–18

Trites AW, Calkins DG, Winship AJ (2007) Diets of Steller sea lions (*Eumetopias jubatus*) in Southeast Alaska, 1993−1999. Fish Bull 105:234–248

Trites AW, Donnelly CP (2003) The decline of Steller sea lions *Eumetopias jubatus* in Alaska: a review of the nutritional stress hypothesis. Mammal Rev 33:3–28. doi: 10.1046/j.1365-2907.2003.00009.x

Trites AW, Larkin PA (1996) Changes in the abundance of Steller sea lions (*Eumetopias jubatus*) in Alaska from 1956 to 1992: How many were there? Aquat Mamm 22:153–166

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. doi: 10.1007/s00360-003-0358-4

Trumble SJ, Castellini MA (2005) Diet mixing in an aquatic carnivore, the harbour seal. Can J Zool 83:851–859. doi: 10.1139/z05-069

Van Pelt TI, Piatt JF, Lance BK, Roby DD (1997) Proximate composition and energy density of some north pacific forage fishes. Comp Biochem Physiol A Physiol 118:1393–1398. doi: 10.1016/S0300-9629(97)00240-5

Vollenweider JJ, Heintz RA, Schaufler L, Bradshaw R (2011) Seasonal cycles in whole-body proximate composition and energy content of forage fish vary with water depth. Mar Biol 158:413–427. doi: 10.1007/s00227-010-1569-3

Waldbauer GP (1968) The consumption and utilization of food by insects. Adv Insect Physiol 5:229–288

Wilson E, Underwood M, Puckrin O, Letto K, Doyle R, Caravan H, Camus S, Bassett K (2013) The arcsine transformation: has the time come for retirement. Available at http://www.mun.ca/biology/dschneider/b7932/B7932Final10Dec2010.pdf

Winship AJ, Trites AW, Rosen DAS (2002) A bioenergetic model for estimating the food requirements of Steller sea lions *Eumetopias jubatus* in Alaska, USA. Mar Ecol Prog Ser 229:291–312

Withers PC (1977) Measurement of VO2, VCO2, and evaporative water loss with a flow-through mask. J Appl Physiol 42:120–123

Yamamoto KS, Carpenter JR, Atkinson S, Polasek L, Zaleski H (2009) Validation of acid insoluble ash (AIA) as an internal biomarker for digestibility studies in harbor seals (*Phoca vitulina*). Am Soc Anim Sci 60:199–203

Zuur A, Leno EN, Walker N (2009) Mixed effects models and extensions in ecology with R, 2009 edition. Springer, New York, N.Y.