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ARTICLE

Net energy gained by northern fur seals (*Callorhinus ursinus*) is impacted more by diet quality than by diet diversity

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Abstract: Understanding whether northern fur seals (*Callorhinus ursinus* (L., 1758)) 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 (*Gadus chalcogrammus* Pallas, 1814, formerly *Theragra chalcogrammus* (Pallas, 1814)), Pacific herring (*Clupea pallasii* Valenciennes in Cuvier and Valenciennes, 1847), capelin (*Mallotus villosus* (Müller, 1776)), and magister armhook squid (*Berryteuthis magister* (Berry, 1913))) 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 dietary protein content. Urinary energy loss (9.3%–26.7%) increased significantly for high-protein diets. Heat increment of feeding (4.3%–12.4%) was significantly lower for high-lipid diets. Overall, net energy gain (57.9%–83.0%) was affected by lipid content and varied significantly across diets. Mixed-species diets did not provide any energetic benefit over single-species diets. Our study demonstrates that diet quality was more important in terms of energy gain than diet diversity. These findings suggest that fur seals consuming low-quality prey in the Bering Sea would be more challenged to obtain sufficient energy to satisfy energetic and metabolic demands, independent of high prey abundance.

Key words: northern fur seal, *Callorhinus ursinus*, net energy, mixed-species diets, diet quality.

Résumé : La détermination d'une éventuelle incidence négative de changements de la qualité ou de la diversité des proies sur les otaries à fourrure (*Callorhinus ursinus* (L., 1758)) pourrait jeter un nouvel éclairage sur la baisse soutenue de leur population dans le centre de la mer de Behring. Nous avons examiné comment six otaries à fourrure femelles en captivité assimilaient l'énergie tirée de huit régimes alimentaires distincts composés de quatre espèces de proies (goberge de l'Alaska (*Gadus chalcogrammus* Pallas, 1814, anciennement *Theragra chalcogrammus* (Pallas, 1814)), hareng du Pacifique (*Clupea pallasii* Valenciennes in Cuvier et Valenciennes, 1847), capelan (*Mallotus villosus* (Müller, 1776)) et calmar rouge (*Berryteuthis magister* (Berry, 1913))) dispensées seules ou en combinaison. L'énergie nette a été quantifiée en mesurant la perte énergétique fécale, la perte énergétique urinaire et l'accroissement de la température dû à l'alimentation. L'énergie digestible (95,9 % – 96,7 %) était élevée (reflétant une perte énergétique fécale faible) et négativement influencée par la masse ingérée et le contenu en protéines alimentaires. La perte énergétique urinaire (9,3 % – 26,7 %) augmentait significativement pour les régimes à forte teneur en protéines. L'accroissement de la température dû à l'alimentation (4,3 % – 12,4 %) était significativement plus faible pour les régimes à forte teneur en lipides. Globalement, le gain d'énergie nette (57,9 % – 83,0 %) était influencé par la teneur en lipides et variait significativement selon le régime alimentaire. Les régimes composés de plusieurs espèces ne conféraient aucun avantage énergétique par rapport aux régimes composés d'une seule espèce. L'étude démontre que la qualité du régime alimentaire était plus importante en ce qui concerne les gains d'énergie que sa variété. Ces constatations donnent à penser que les otaries à fourrure qui consomment des proies de moins bonne qualité dans la mer de Behring auraient de la difficulté à obtenir assez d'énergie pour répondre à leurs demandes énergétiques et métaboliques, quelle que l'abondance de ces proies. [Traduit par la Rédaction]

Mots-clés : otarie à fourrure, *Callorhinus ursinus*, énergie nette, régimes à plusieurs espèces, qualité du régime alimentaire.

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, i.e., the energy remaining after 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

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conditions, foraging costs) and internal (e.g., developmental stage, nutritional state) factors (Raubenheimer and Simpson 1999). Pinnipeds are considered to be generalist predators, as reflected by the wide variety of prey species they typically consume (Riedman 1990). However, the diversity of prey consumed may not represent 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 theorized to enhance both the kinetic production rate of digestive products and the postdigestive 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 to a variety of predator species are consistent with the theory 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). The nutritional stress hypothesis 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; Calkins et al. 1998; Rosen and Trites 2000*a*; Trites et al. 2007).

Northern fur seals (*Callorhinus ursinus* (L., 1758)) inhabiting the North Pacific Ocean and Bering Sea have declined dramatically in the Eastern Pacific from 2.1 million in the late 1940s and early 1950s 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 (*Gadus chalcogrammus* Pallas, 1814, formerly *Theragra chalcogrammus* (Pallas, 1814)), Atka mackerel (*Pleurogrammus monopterygius* (Pallas, 1810)), capelin (*Mallotus villosus* (Müller, 1776)), Pacific herring (*Clupea pallasii* Valenciennes in Cuvier and Valenciennes, 1847), and various squid species (e.g., family 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 1970s (Swartzman and Haar 1983), concurrent with changes in the abundance of fish stocks (National Marine Fisheries Service 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 impacts on individual development, survival, reproductive fitness, and ultimately, population growth rates. However, the impact of the apparent 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 et al. (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. Nor did they 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 theory 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 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 individual 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 to 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 (Vancouver, 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* Berry, 1911), supplemented with vitamins, fed three times a day. The fur seals had access to continuous-flow seawater pools (with adjacent haul-out 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 3 weeks and were composed of four key prey items that fur seals encounter in the wild: Pacific herring, walleye pollock, capelin, and magister armhook squid (*Berryteuthis magister* (Berry, 1913)), fed alone or in combinations (Table 1). Animals were previously exposed to herring and capelin, but not to pollock or magister armhook squid. The amount of fish consumed by the fur seals was recorded daily.

The different prey items were chosen to represent a range of proximate compositions and energy densities (Table 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 GEIs 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 and 12 500 $kJ \cdot d^{-1}$. 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) +

Table 1. Mean proximate composition (percent crude protein and percent lipid content), mean energy density, mean manganese (Mn^{2+}) concentration, mean (±SD) body size (measured as length), and mean (±SD) body mass of a subsample of four species of prey (n = 12 of each) experimentally fed to six female northern fur seals (*Callorhinus ursinus*).

Experimental prey	Water (%)	Total lipid (%)	Crude protein (%)	Energy density ($kJ \cdot g^{-1}$)	Mn^{2+} (ppm)	Fish length (cm)	Fish mass (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 armhook squid diet only)	69.2	37.0	53.6	22.9	5.5	18.5±0.6	64.0±6.7
Walleye pollock (*Gadus chalcogrammus*)	75.3	32.8	57.5	22.1	2.4	24.5±2.2	134.0±33.2
Capelin (*Mallotus villosus*)	82.6	4.0	81.6	15.2	2.9	15.0±1.0	24.0±5.4
Magister armhook squid (*Berryteuthis magister*)	71.3	44.3	46.7	23.2	2.8	—	—

Note: Proximate composition, energy density, and Mn^{2+} concentration measured on a dry-weight basis.

magister armhook squid (50%), and (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 2 weeks and was only consumed by four of the fur seals due to a shortage of magister armhook squid. Additionally, all of the diets used herring from the same batch (batch A) with the exception of the magister armhook squid diet that used a different batch of herring (batch B; Table 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 haul-out 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, California, 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 were 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 h 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 × 0.61 m × 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 $L \cdot min^{-1}$ using a Sable Systems Model 500H Mass Flow Controller that continuously corrected the flow to standard temperature and pressure (Sable Systems, Las Vegas, Nevada, USA). Subsamples of excurrent air were then desiccated using anhydrous Drierite (Hammond Drierite, Xenia, Ohio, USA) and were analyzed afterwards to quantify O_2 and CO_2 concentrations the chamber via Sable Systems FC-1B and CA-1B gas analyzers, respectively (Sable Systems, Las Vegas, Nevada, USA). Gas concentrations were monitored and recorded to a portable computer every 0.5 s 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 with ambient air baselines were converted into $\dot{V}O_2$ (Withers 1977). RMR was determined as the lowest continuous mean $\dot{V}O_2$ 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. TC, i.e., the metabolic rate while in the water, was determined as the lowest continuous mean $\dot{V}O_2$ consumption maintained for ~10 min during the last 20 min of the 30 min trial. TC was calculated as the change in metabolic rate compared with 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 h 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, British Columbia, 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 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 analysis; the majority of the samples were collected 24 h 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 h to a constant mass (Freeze dryer Freezone 6; Labconco Corp., Kansas City, Missouri, USA). Dried samples were reweighed to determine dry matter and water content.

Homogenized dried samples were used to measure proximate composition, energy density, and Mn^{2+} concentration. Throughout all laboratory analyses, sample variation of ≤5% was considered 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, Illinois, 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 samples with the addition of 2 Kjeltabs Cu catalyst tablets (FOSS, Eden Prairie, Minnesota, USA), using the Kjeldahl method (AOAC 1990) via spectrophotometric flow injection analyzer (FOSS FIAstar 5000 TKN analyzer unit; FOSS, Eden Prairie, Minnesota, 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 contents from duplicate samples of approximately 2 g of feces and 1.5 g of fish were measured using a modified Bligh–Dyer 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 to ensure the accuracy of our techniques. Both protein and lipid contents 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, Montréal, Quebec, 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, and 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 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 (e.g., Fadely et al. 1990; Lawson et al. 1997; Rosen and Trites 2000*b*). 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:

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

where C is the concentration of Mn^{2+} in diet (i) and feces (f) (Schneider and Flatt 1975).

Digestible energy (DE%), i.e., the amount of energy assimilated, was calculated as

$$(2)\qquad \text{DE}\ (\%) = \left(1 - \frac{C_i \times E_f}{C_f \times E_i}\right) \times 100$$

where E is the energy density of the ingested diet (i) and feces (f) (Mårtensson et al. 1994).

Fecal energy loss (FEL%), i.e., the inverse of DE%, was calculated as

$$(3)\qquad \text{FEL}\ (\%) = 1 - \text{DE}\%$$

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

$$(4)\qquad \text{ANI}\ (\text{g}\cdot\text{d}^{-1}) = \frac{\text{total crude protein consumed} \times \text{digestibility of crude protein}}{6.25}$$

where digestibility of crude protein was calculated as

$$(5)\qquad \text{Protein digestibility}\ (\%) = \left(1 - \frac{C_i \times P_f}{C_f \times P_i}\right) \times 100$$

where P is the crude protein content of the ingested diet (i) and feces (f) (Mårtensson et al. 1994).

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

$$(6)\qquad \text{UEL}\ (\text{kJ}\cdot\text{d}^{-1}) = (6.128 \times \text{ANI} + 14.737) \times 4.186$$

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%), i.e., the energy that remains available after accounting for the energy lost through the excreta, expressed as a percentage of GEI, was calculated as

$$(7)\qquad \text{ME}\ (\%) = \left[\frac{\text{GEI} - (\text{FEL} + \text{UEL})}{\text{GEI}}\right] \times 100$$

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

$$(8)\qquad \text{NE}\ (\%) = \left[\frac{\text{GEI} - (\text{FEL} + \text{UEL} + \text{HIF})}{\text{GEI}}\right] \times 100$$

Table 2. Body mass of six captive female northern fur seals (*Callorhinus ursinus*) at the start of the feeding trial and ingested mass (wet) of the eight experimental diets with their respective proximate composition, energy density, and manganese (Mn^{2+}) concentration.

Diet	Fur seal body mass (kg)	Ingested diet mass (kg)	Water (%)	Total lipid (%)	Protein (%)	Energy density ($kJ \cdot g^{-1}$)	Mn^{2+} (ppm)
Pacific herring (*Clupea pallasii*)	23.9±3.5	1.6±0.3	68.5±3.6	38.0±0.01	47.1±0.01	24.3±0.01	5.1±0.01
Walleye pollock (*Gadus chalcogrammus*)	23.1±3.1	2.3±0.3	75.3±1.3	35.8±0.01	62.8±0.01	22.1±0.01	2.4±0.01
Capelin (*Mallotus villosus*)	23.2±3.3	3.3±0.5	82.6±1.4	3.3±0.01	67.6±0.01	15.2±0.01	2.9±0.01
Herring + pollock	23.1±3.2	2.0±0.1	72.4±0.04	37.0±0.01	54.6±0.1	23.1±0.01	3.8±0.02
Herring + capelin	23.0±2.7	2.9±0.3	79.0±0.04	15.9±0.1	60.2±0.08	18.7±0.04	3.7±0.01
Herring + magister armhook squid (*Berryteuthis magister*)	23.8±3.2	2.4±0.2	70.3±0.01	43.2±0.1	57.2±2.8	23.1±0.01	4.3±0.02
Pollock + capelin	22.9±2.8	3.0±0.5	79.9±0.5	15.9±2.2	65.8±0.3	18.3±0.5	2.7±0.03
Herring + pollock + capelin	23.1±2.8	2.6±0.5	77.9±0.06	21.0±0.2	60.7±0.9	19.7±0.05	3.4±0.01

Note: Proximate composition, energy density, and Mn^{2+} concentration measured on a dry-weight basis. Values are reported as means ± SD.

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 version 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). LME models were built in a stepwise 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's 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's 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 ($kg \cdot d^{-1}$), gross energy intake ($kJ \cdot d^{-1}$), diet lipid intake ($\% \cdot d^{-1}$ dry-weight), diet protein intake ($\% \cdot d^{-1}$ 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 armhook squid diet). The expected digestibility of energy for mixed-species diets (except for herring + magister armhook 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 (see Forster 1999) according to

$$\text{Expected DE (\%)} = \frac{(\text{mass}_{F1} \times \text{energy}_{F1} \times \text{DE\%}_{F1}) + (\text{mass}_{F2} \times \text{energy}_{F2} \times \text{DE\%}_{F2})}{(\text{mass}_{F1} \times \text{energy}_{F1} + \text{mass}_{F2} \times \text{energy}_{F2})} \quad (9)$$

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 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 (see also 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).

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). 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 armhook squid diet −2.3% ± 2.3%), there were no significant differences in percent body mass change due to diet (%) ($F_{[33]} = 0.4$, $p = 0.9$).

Prey item and dietary characteristics

Proximate composition, energy density, and Mn^{2+} concentration differed among the four experimental prey items (dry-weight basis; Table 1). Overall, magister armhook 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 armhook squid had the lowest (46.7%). Herring (batch A)

Table 3. 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 (*Callorhinus ursinus*) across the eight experimental diets.

Diet	DMD%	GEI (kJ·d^{-1})	FEL%	DE%	ANI (g·d^{-1})	UEL%	ME%	HIF%	NE%
Pacific herring (*Clupea pallasii*)	91.5±1.1	12 135.7 ± 2 412.5	3.1±0.3	96.9±0.3	39.4±7.9	9.9±0.1	87.2±0.2	4.3±1.0	83.0±1.0
Walleye pollock (*Gadus chalcogrammus*)	88.2±1.1	12 688.6 ± 1 570.4	3.7±0.5	96.3±0.5	50.9±6.1	10.3±0.1	86.4±0.5	6.5±3.8	80.0±3.5
Capelin (*Mallotus villosus*)	90.2±1.9	8 712.9 ± 1 409.7	4.0±0.8	96.0±0.8	72.3±11.6	26.7±1.9	70.3±1.5	12.4±2.0	57.9±2.6
Herring + pollock	90.1±0.4	12 482.5 ± 787.9	3.5±0.7	96.5±0.7	45.3±2.7	10.1±0.1	86.8±0.7	7.1±2.3	79.7±2.8
Herring + capelin	90.8±1.2	11 301.8 ± 1 245.9	3.5±0.4	96.5±0.4	65.3±6.8	18.6±0.1	78.5±0.3	7.9±3.0	70.6±3.1
Herring + magister armhook squid (*Berryteuthis magister*)	92.3±0.1	15 866.0 ± 1 426.8	3.9±0.2	96.1±0.2	55.1±4.8	9.3±0.1	87.1±0.1	6.0±1.5	81.1±1.5
Pollock + capelin	88.6±2.2	11 118.7 ± 1 613.2	4.1±0.6	95.9±0.7	66.3±10.9	18.1±1.2	78.5±1.1	6.9±2.0	71.6±1.2
Herring + pollock + capelin	91.0±1.0	11 472.6 ± 2 184.1	3.3±0.4	96.7±0.4	59.5±11.5	15.8±0.2	81.4±0.4	5.2±1.1	76.2±1.0

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

had the highest energy density (24.3 kJ·g^{-1}), while 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). Lipid content varied from 3.3% ± 0.01% (capelin diet) to 43.2% ± 0.1% (herring and magister armhook squid diet) (LRT = 265.3, $p < 0.001$). Diet protein content also differed significantly, ranging from 47.1% ± 0.01% (herring diet) to 67.6% ± 0.01% (capelin diet) (LRT = 366.9, $p < 0.001$), while energy density ranged from 15.2 ± 0.01 kJ·g^{-1} (capelin diet) to 24.3 ± 0.01 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).

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 3) due to two anomalies. The first was that the animals refused to eat sufficient levels 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 armhook squid diet had the highest GEI (15 866.0 ± 1 426.8 kJ·d^{-1}), a byproduct of an attempt to maximize the use of the available magister armhook squid because 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 3). DMD% was significantly lower for pollock (88.2% ± 1.1%) than most other diets, and highest for the herring and magister armhook squid diet (92.3% ± 0.1%). DMD% also decreased significantly with increased protein content of diets (%) (LRT = 9.9, $p = 0.002$; Fig. 1).

Fecal energy density was significantly different across diets, with the pollock diet having the lowest fecal energy density (7.7 ± 1.1 kJ·g^{-1}) and the herring and magister armhook 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 eq. 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.

FEL%, expressed as a percentage of GEI, ranged from 3.1% ± 0.3% to 4.1% ± 0.6% and was significantly different across diets (LRT = 19.6, $p = 0.006$; Table 3). 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 3). 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).

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. Although there was a significant positive relationship between DMD% and DE% across all eight experimental diets (DE% = 0.21·DMD% + 77.8, $r^2 = 0.38$) (LRT = 21.6, $p < 0.001$), the slope was 0.2 and therefore the measures are not interchangeable.

UEL%, expressed as percentage of GEI, ranged from 8.9% ± 0.1 to 22.0% ± 0.2% and was significantly different across diets (LRT = 247.9, $p < 0.001$; Table 3). The lowest UEL% was from the herring-only diet and the highest UEL% was from the capelin diet. There was a significant interaction between 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 content 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 3). 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 3). 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 significantly with the lipid content of the diets (%), where HIF% decreased as lipid content increased (LRT = 15.3, $p = 0.001$; Fig. 3).

Total net energy gain (kJ·d^{-1}) increased significantly with increases in GEI (kJ·d^{-1}) across experimental diets with a positive relationship (NE = 1.04·GEI – 3043.9, $r^2 = 0.93$). However, NE% as a proportion of GEI also differed by diet (LRT = 122.3, $p < 0.001$; Table 3). 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 NE%, such that fur seals retained the most NE% from fattier diets (LRT = 77.7, $p < 0.001$; Fig. 4).

Fig. 1. Changes in dry matter digestibility (DMD%) with the eight experimental diets tested in six captive female northern fur seals (*Callorhinus ursinus*). 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 quartiles ("hinges"), and 95% confidence intervals of the 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. Letters above boxes indicate significant differences between diets.

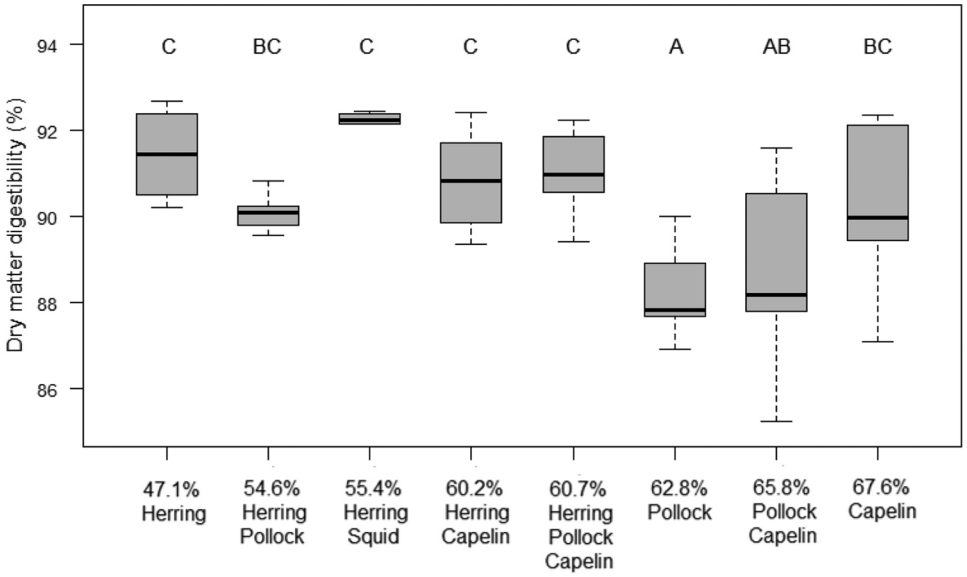
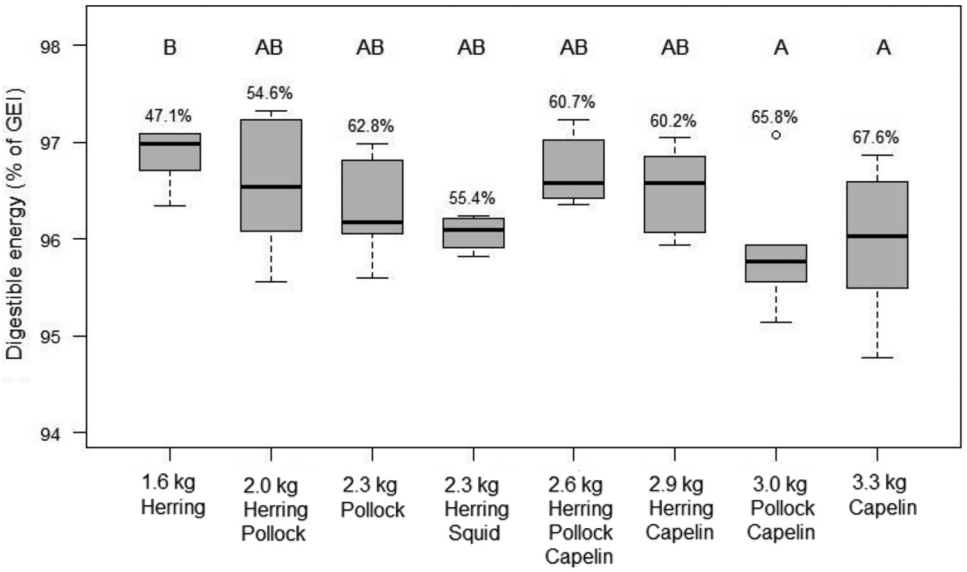


Fig. 2. Changes in digestible energy (DE%) over the eight experimental diets tested in six captive female northern fur seals (*Callorhinus ursinus*). Diets are arranged accordingly from low to high mean ingested mass (wet-weight) during experimental trials, as denoted above the diet labels. Each box 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.



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 compared with 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 mean mass-specific resting metabolic rate (RMR) while the fur seals were resting in ambient air across all diets was 10.0 ± 3.4 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$. While mean mass-specific RMR ranged from 8.2 ± 4.3 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ for the pollock diet to 11.9 ± 4.6 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ for the herring and magister armhook squid diet, it did not significantly differ among the experimental diets ($F_{[26]} = 2.2$, $p = 0.07$). Mean mass-specific metabolic rate while the fur seals were partially submerged in 2 °C water was 17.1 ± 4.1 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$, and this ranged from 13.8 ± 4.4 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$

Fig. 3. Changes in heat increment of feeding (HIF%) of the eight experimental diets consumed by six captive female northern fur seals (*Callorhinus ursinus*). 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.

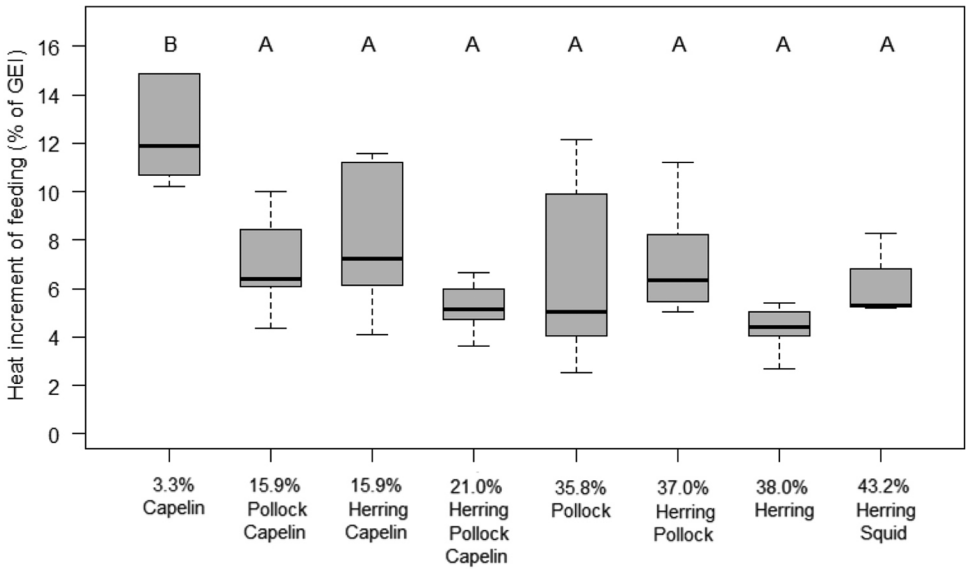
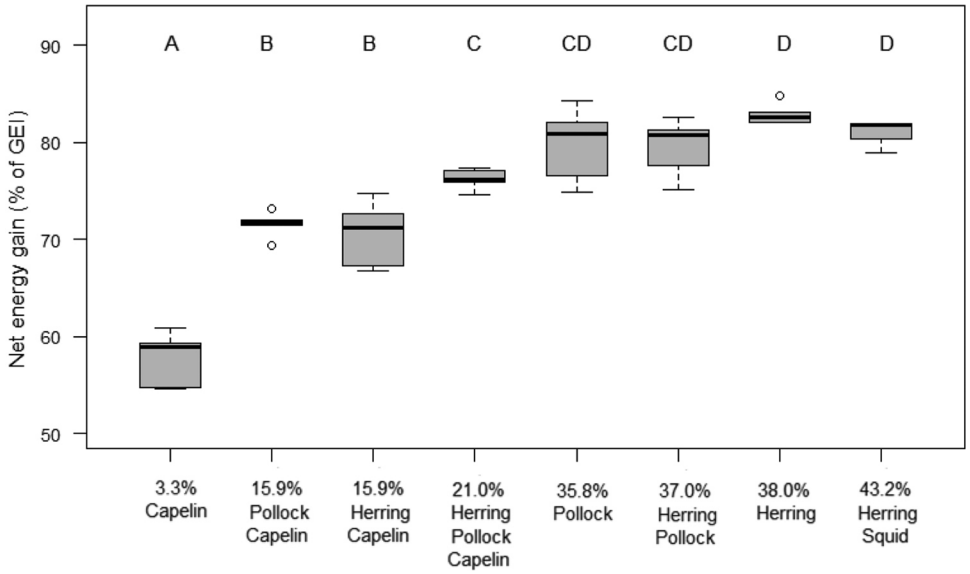


Fig. 4. Changes in net energy gain (NE%) from the eight experimental diets tested in six captive female northern fur seals (*Callorhinus ursinus*). Diets are arranged accordingly from low to high lipid content (%) in the experimental diets, as denoted above the diet labels. Each box represents one diet 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.



for the pollock diet to 18.9 ± 5.4 mL O_2·kg^{-1}·min^{-1} for the herring and pollock diet. The added thermoregulation cost (TC) of being partially submerged in 2 °C water, i.e., calculated as the mean 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 rate for capelin. 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 armhook 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 ($F_{[26]} = 1.2$, $p = 0.4$).

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 predator. 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 1 g of crude lipid contains 37.7 kJ of energy, whereas 1 g of protein provides 17.8 kJ (Blaxter 1989). However, the net energy gain (NE%), i.e., the biologically useful energy 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). 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%). Furthermore, 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, i.e., those normally classified as "low quality", yielded significantly less NE% to the fur seals than would be predicted solely on the basis of GEI. 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 theory, there appeared to be no benefit in terms of energy digestibility associated with consuming mixed-species diets.

Changes in digestibility and bioenergetics due to changes in diet

In the past, many studies have quantified the dry matter digestibility (DMD%), i.e., 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 3). DMD% was lowest for walleye pollock (Fig. 1), due to a combination of its high protein content and the fact that pollock has large bony structures compared with the other prey consumed, making it more challenging to digest.

Dry matter digestibility values in our study were consistent with previous pinniped studies (see Table 3 in Rosen and Trites 2000*b*). For example, our DMD values for the pollock diet (88.2% ± 1.1%) were 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 armhook squid diet (92.3% ± 0.1%), the DMD of our herring-only diet (91.5% ± 1.1%) was comparable with previous fur seal studies by both Miller (1978; 91.6%–93%) and Fadely et al. (1990; 90%) (Table 3). Even though DMD% values are often reported, they are not informative with respect to the energy absorbed from the various diets (Rosen and Trites 2000*b*). 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 3) and were comparable with previous pinniped studies (see Table 3 in Rosen and Trites 2000*b*), 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 converse, 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 with strong 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, 3). 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 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 with 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.

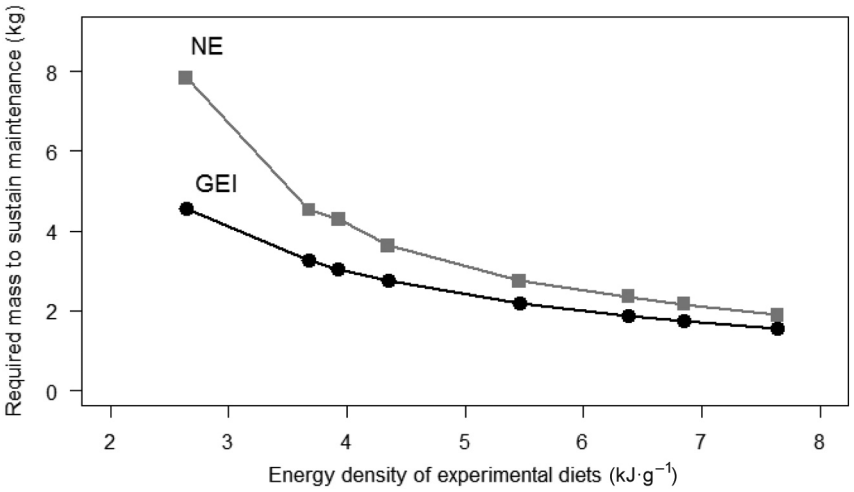
Urinary energy loss is notoriously difficult to directly measure from complete urine collection in large mammals. In our study, UEL% was estimated from the apparent digestible nitrogen intake of each diet, using equations generated from previous studies with phocid seals (Keiver et al. 1984; Goodman-Lowe 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 (i.e., 22.6 kJ·g^{-1} of nitrogen; 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* L., 1758) 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 (56.3% protein; 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 3), further demonstrating the high cost of disposal of nitrogenous waste products from protein sources.

Few past pinniped studies have measured metabolizable energy (ME%), the 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% to 92.5% for herring, from 85.9% to 89.4% for pollock, and from 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 3) and with ME% values from other carnivorous terrestrial mammals consuming either fish diet (Pritchard and Robbins 1990) or mammal meat diet (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

Fig. 5. Changes in required mass intake (kg) to sustain maintenance energetic level (12 000 kJ·d^{-1}) with the energy density (wet-basis) of the eight experimental diets (kJ·d^{-1}) tested in six captive female northern fur seals (*Callorhinus ursinus*). Data are presented as mean values 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 content of prey vs. the food intake level that take into account digestive energy losses (i.e., NE).



Hawaiian monk seals (*Monachus schauinslandi* Matschie, 1905). 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 2009). 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 2009).

Among pinnipeds, HIF% is known to range from 4.7% to 16.8% of GEI when animals are fed herring-only diets, 5.7% for pollock diets, and from 11.5% to 13.0% for capelin diets (see Table 3 in Rosen and Trites 1997). Estimates of heat increment of feeding for the fur seals in our study (Table 3) are comparable with 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 (Fig. 3). 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); 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. 3; Table 3). 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% to 83.0% for the fur seals (Table 3). 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 NE% was for the capelin diet (Table 3). Similar to these findings, Fisher et al. (1992) reported that walruses (*Odobenus rosmarus* (L., 1758)) feeding on lipid-rich herring diets had a higher apparent digestibility of lipids compared with 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. 4).

Robbins (1993) recognized that the amount of food that an animal must ingests 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 with estimates calculated on GEI alone (Fig. 5). 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. 5). This example highlights how the 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, 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, i.e., 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 theory 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 theory. The observed DE% and DMD% values of the mixed-species diets approximated the mean values of their single-species DE% and DMD% constituents and did not surpass them (Figs. 1, 2; Table 3). Similar results have been reported for harbour seals where the DE% of the mixed-species diet (30% herring, 30% capelin, 30% pollock, and 10% market 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, as well as ecological benefits overall (Stephens and Krebs 1986; Singer and Bernays 2003; Barbosa and Castellanos 2005).

Effect of diet on metabolism

Northern fur seals are known 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* (Schreber, 1776)) 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. 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 with metabolic costs when resting in ambient air. Similarly, Costa and Gentry (1986) found that the at-sea metabolic rate of fur seals (lactating and nonlactating) 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.

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, i.e., 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 groenlandicus* (Erxleben, 1777)), sea otters (*Enhydra lutris* (L., 1758)), and Atlantic spotted dolphins (*Stenella frontalis* (G. Cuvier, 1829)) 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 proper 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 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 (Table 1; Buckley and Livingston 1994; Kimura 2008). While whole-body proximate composition of young walleye pollock fluctuates across seasons, on average, young pollock range from 3.7 to 4.8 $kJ \cdot g^{-1}$, from 2.3% to 3.2% lipid, and from 14.1% to 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; Table 3).

Miller (1978) estimated that a wild fur seal of median mass (23 kg) require a daily energy intake of ~16 300 $kJ \cdot d^{-1}$. 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 $kg \cdot d^{-1}$ of fish (~27% of their body mass) 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 $kg \cdot d^{-1}$ of the lower quality fish (capelin). However, further research is required to specifically test such satiation limits (see Rosen et al. 2012; 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 content (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 theory 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.

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