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Prey composition impacts lipid and protein digestibility in northern fur seals (*Callorhinus ursinus*)

Mariana Diaz Gomez, David A.S. Rosen, Ian P. Forster, and Andrew W. Trites

Abstract: Pinnipeds have specific macronutrient (protein, lipid) requirements to satisfy physiological functions, yet little is known about how diet characteristics affect macronutrient digestibility. We measured relative and absolute lipid and protein digestibility in six female northern fur seals (*Callorhinus ursinus* (Linnaeus, 1758)) fed eight experimental diets composed variously of four prey species (Pacific herring, *Clupea pallasii* Valenciennes in Cuvier and Valenciennes, 1847; walleye pollock, *Gadus chalcogrammus* Pallas, 1814 (formerly *Theragra chalcogramma* (Pallas, 1814)); capelin, *Mallotus villosus* (Müller, 1776); magister armhook squid, *Berryteuthis magister* (Berry, 1913)). We quantified how digestibility was affected by proximate composition of the diet (% lipid or % protein), levels of food mass and macronutrient intake, and tested for any potential benefit of multi-species diets. Overall, digestibility of both protein and lipid were high across diets, although macronutrient retention of lipids (96.0%–98.4%) was significantly higher than protein (95.7%–96.7%) for all but the two highest protein diets. Increased levels of protein intake resulted in increased protein retention, but decreased lipid digestibility. There was no evidence that mixed-species diets provide greater macronutrient digestibility over single-species diets. The results suggest that high to moderate lipid diets are more beneficial to northern fur seals because they lead to increased levels of lipid retention without large decreases in protein digestibility. This raises concerns that dietary factors may be contributing to the population declines of northern fur seals in the Bering Sea.

Key words: northern fur seal, *Callorhinus ursinus*, diet composition, macronutrients, lipid digestibility, protein digestibility.

Résumé : Si les pinnipèdes ont des exigences précises en matière de macronutriments (protéines, lipides) pour soutenir leurs fonctions physiologiques, les connaissances sur l'incidence des caractéristiques du régime alimentaire sur la digestibilité des macronutriments sont limitées. Nous avons mesuré la digestibilité relative et absolue des lipides et des protéines chez six otaries à fourrure (*Callorhinus ursinus* (Linnaeus, 1758)) femelles nourries de huit régimes alimentaires expérimentaux différents composés de diverses proportions de quatre espèces de proies (hareng du Pacifique, *Clupea pallasii* Valenciennes dans Cuvier et Valenciennes, 1847; goberge de l'Alaska, *Gadus chalcogrammus* Pallas, 1814 (anciennement *Theragra chalcogramma* (Pallas, 1814)); capelan, *Mallotus villosus* (Müller, 1776); calmar rouge, *Berryteuthis magister* (Berry, 1913)). Nous avons quantifié l'incidence de la composition proximale du régime alimentaire (% de lipides ou % de protéines), la masse des aliments et l'apport de macronutriments sur la digestibilité et vérifié l'existence d'éventuels avantages de régimes alimentaires plurispécifiques. Dans l'ensemble, la digestibilité tant des protéines que des lipides est élevée pour tous les régimes alimentaires, la rétention de lipides (96,0 % – 98,4 %) étant toutefois significativement plus élevée que celle des protéines (95,7 % – 96,7 %) pour tous les régimes alimentaires à l'exception des deux plus riches en protéines. Des apports en protéines accrus se traduisent par une plus grande rétention de protéines, mais réduisent la digestibilité des lipides. Rien n'indique que des régimes alimentaires plurispécifiques produisent une meilleure digestibilité des macronutriments que les régimes monospécifiques. Les résultats indiqueraient que des régimes à teneur élevée à modérée en lipides sont plus bénéfiques pour les otaries à fourrure, puisqu'ils se traduisent par une rétention accrue de lipides sans réduction importante de la digestibilité des protéines. Ainsi, des facteurs associés au régime alimentaire pourraient jouer un rôle dans les déclins de populations d'otaries à fourrure dans la mer de Behring. [Traduit par la Rédaction]

Mots-clés : otarie à fourrure, *Callorhinus ursinus*, composition du régime alimentaire, macronutriments, digestibilité des lipides, digestibilité des protéines.

Introduction

Classical optimal foraging theory models for carnivores, such as pinnipeds, are constructed in terms of maximizing net energetic gain from prey while minimizing the predator's energetic expenses (reviewed in Pyke et al. 1977). Hence, studies of the digestive efficiency of different prey types in pinnipeds have primarily focused on overall net energy gain. For example, studies with northern fur seals (*Callorhinus ursinus* (Linnaeus, 1758)) have demonstrated that higher energy density prey provide relatively higher net energy gain (Fadely et al. 1990; Diaz Gomez et al. 2016). However, it is important to consider that the energy contained in ingested prey is a product of its macronutrient composition (lipid, protein, carbohydrate) and that total energetic gain is similarly a product of the digestibility of each one of these individual com-

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ponents. Yet, little consideration has been given in past pinniped diet studies to the importance of the specific role of prey proximate composition on these digestive processes. The efficiency with which protein and lipid are digested and assimilated are important for understanding the overall nutritional value of prey beyond its energetic yield and also to comprehend potential inherent digestive constraints.

There is a traditional perception that prey with higher lipid content — whether fish or copepods — are considered "better" for marine mammals. This belief is largely founded in the basic biochemical fact that higher lipid prey will (with all other things being equal) have a higher energy density (National Research Council 1981). It is also based on the predominance of lipids as a metabolic fuel source and its central role in thermoregulation and energy storage. Despite the importance of lipids to marine mammals, it is not known whether digestive capabilities are similarly lipid-focused or how digestive efficiencies of lipid (or protein) might change with differences in prey composition. The few studies that have specifically measured rates of lipid and protein absorption by marine mammals have all used simple diets (see Table 29.1 in Rosen and Worthy 2018). This is problematic as macronutrient concentrations and total loads are known to affect digestive efficiency in other mammals (e.g., Crampton and Rutherford 1954). Furthermore, it is theorized that there is a nutritional benefit (via greater digestive efficiency) of mixed-species diets over single-species diets (Penry and Jumars 1987; Singer and Bernays 2003; Trumble and Castellini 2005). What are required are studies measuring macronutrient assimilation over a range of naturalistic diet regimes.

The effects of prey composition and intake levels on total energy absorption can be discerned by examining several studies quantifying the energetic digestive efficiency of different prey in marine mammals (reviewed in Rosen and Worthy 2018). Although understanding the rates of absorption of specific macronutrients is useful for estimating the energetic benefit of diets, it is also important for predicting the effect of different prey on an animals' overall nutritional state, which in turn impacts the animal's physiological ability to perform within its environment (Raubenheimer et al. 2009). Modern approaches to nutritional ecology, including the geometric theory of nutrition, hypothesize that proper balance between macronutrients in food are as important to an animal's longevity and overall health as total energetic intake (Raubenheimer and Simpson 1999; Raubenheimer et al. 2009; Mair et al. 2011; Solon-Biet et al. 2015a). The observation that carnivores actively select foods that provide a particular proportion of macronutrient intake when provided with complementary food choices is interpreted as an attempt to satisfy their specific macronutrient needs given that proteins and lipids fulfill different physiological and biochemical roles over the life history of an organism (Mayntz et al. 2009; Hewson-Hughes et al. 2011; Kohl et al. 2015). 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 certain macronutrients (i.e., where the rate of intake and utilization of nutrients are incomparable; Waldbauer 1968) could similarly lead to increased risk of mortality (Raubenheimer et al. 2005).

Quantification of macronutrient digestive efficiency in pinnipeds is also central for understanding how changes in consumption of diverse prey with different macronutrient profiles could potentially impact the overall nutritional status of a population. For example, the population of northern fur seals from the Pribilof Islands in the central Bering Sea has been declining since the late 1970s, possibly due to a shift in diet from high-lipid prey to low-lipid (high-protein) prey (Swartzman and Haar 1983; NMFS 2007; Towell et al. 2014). Knowledge of the macronutrient digestibility of different prey items is essential for evaluating whether nutritional inadequacies are negatively impacting northern fur seal populations.

We investigated differences in digestibility of both lipids and proteins across experimental diets in trained female northern fur seals under human care. The northern fur seals were fed eight diets composed of different prey types, representing a larger range of macronutrient profiles than previous pinniped feeding studies. The experimental diets comprised representative prey of wild northern fur seal diets in the Bering Sea (Sinclair et al. 1994; Call and Ream 2012). This allowed the digestibility of lipids and proteins to be quantified, the dietary factors that contributed to changes in rates of assimilation (such as nutrient load and food mass intake) to be examined, and the potential benefit of mixed-species diets over single-species diets to be tested. Ultimately, our study investigated whether the interplay of shifts in prey intake and differences in macronutrient digestibility impact the nutritional budget of northern fur seals, and whether this could potentially affect population numbers in the Bering Sea.

Materials and methods

Animals

The experimental feeding trials used six female northern fur seals that were housed at the The University of British Columbia's Marine Mammal Energetics and Nutrition Laboratory located at the Vancouver Aquarium (Vancouver, British Columbia, Canada). The animals had been brought from the Pribilof Islands, Alaska, USA, at weaning (~4 months) as part of a long-term conservation research program. The northern fur seals were 4.5 years of age and their body mass ranged from 19.5 to 28.9 kg at the beginning of our study. The northern fur seals were kept in holding pools with adequate haul-out space and continuously flowing filtered ambient seawater. Prior to the experiments, animals received a typical diet of thawed Pacific herring (*Clupea pallasii* Valenciennes in Cuvier and Valenciennes, 1847; henceforth herring) and market squid (*Loligo opalescens* Berry, 1911) three times a day (supplemented with Mazuri vitamins). During the experimental period, the northern fur seals were weighed daily on a platform scale (±0.02 kg) prior to the morning feeding.

Experimental diets and study design

The feeding trials occurred from November 2012 to June 2013. All experimental manipulations were conducted in accordance with the guidelines of The University of British Columbia Animal Care Committee (permit No. A10-0342) and the Canadian Council on Animal Care.

The northern fur seals were subject to the same experimental diets as those previously detailed in Diaz Gomez et al. (2016). In brief, four prey species commonly consumed by northern fur seals in the wild were fed to the fur seals either singly or in combination to generate eight diets. The prey species included Pacific herring, capelin (*Mallotus villosus* (Müller, 1776); henceforth capelin), walleye pollock (*Gadus chalcogrammus* Pallas, 1814 (formerly *Theragra chalcogramma* (Pallas, 1814)); henceforth pollock), and magister armhook squid (*Berryteuthis magister* (Berry, 1913); henceforth magister squid). The latter two species were caught in the Bering Sea and were provided by the At-Sea Processers Association, while the other two species were caught locally. These species were chosen to compose diets with a varied spectrum of protein and lipid concentrations (Table 1). Three diets consisted of single-species diets (herring, pollock, capelin) and five diets consisted of multiple prey items that were fed in equal proportions according to their gross energy content (Table 2).

Each experimental diet was fed for 3 weeks, with the exception of the herring + magister squid diet that (due to limited supplies) was fed for 2 weeks and was consumed by only four of the six animals. The first week of the feeding trial was dedicated to acclimation, the second week to fecal sample collection, and the third

Table 1. Mean (±SD) ingested food mass (wet) for the eight experimental diets (Pacific herring, *Clupea pallasii*; walleye pollock, *Gadus chalcogrammus* (formerly *Theragra chalcogramma*); capelin, *Mallotus villosus*; magister armhook squid, *Berryteuthis magister*) with their respective proximate composition (total lipid content and crude protein content), and energy density (dry-weight basis).

Diet	Ingested mass (kg)	Water (%)	Total lipid (%)	Crude protein (%)	Energy density (kJ·g⁻¹)
Herring	1.6 ± 0.3	68.5 ± 3.6	38.0 ± 0.01	47.1 ± 0.01	24.3 ± 0.01
Pollock	2.3 ± 0.3	75.3 ± 1.3	35.8 ± 0.01	62.8 ± 0.01	22.1 ± 0.01
Capelin	3.3 ± 0.5	82.6 ± 1.4	3.3 ± 0.01	67.6 ± 0.01	15.2 ± 0.01
Herring + pollock	2.0 ± 0.1	72.4 ± 0.04	37.0 ± 0.01	54.6 ± 0.1	23.1 ± 0.01
Herring + capelin	2.9 ± 0.3	79.0 ± 0.04	15.9 ± 0.1	60.2 ± 0.08	18.7 ± 0.04
Herring + magister squid	2.4 ± 0.2	70.3 ± 0.01	43.2 ± 0.1	57.2 ± 2.8	23.1 ± 0.01
Pollock + capelin	3.0 ± 0.5	79.9 ± 0.5	15.9 ± 2.2	65.8 ± 0.3	18.3 ± 0.5
Herring + pollock + capelin	2.6 ± 0.5	77.9 ± 0.06	21.0 ± 0.2	60.7 ± 0.9	19.7 ± 0.05

Table 2. Mean (±SD) gross energy intake (GEI), mean food intake, fecal loss, and apparent digestibility (ADC) of total lipid and total crude protein across the eight experimental diets (Pacific herring, *Clupea pallasii*; walleye pollock, *Gadus chalcogrammus* (formerly *Theragra chalcogramma*); capelin, *Mallotus villosus*; magister armhook squid, *Berryteuthis magister*), on a dry-weight basis, fed to six captive female northern fur seals (*Callorhinus ursinus*).

Diet	GEI (kJ·d⁻¹)	Total lipid			Crude protein		
		Intake (g·d⁻¹)	Fecal lipid loss (g·d⁻¹)	ADC lipid (%)	Intake (g·d⁻¹)	Fecal protein loss (g·d⁻¹)	ADC protein (%)
Herring	12 135.7 ± 2 412.5	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	12 688.6 ± 1 570.4	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	8 712.9 ± 1 409.7	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	12 482.5 ± 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	11 301.8 ± 1 245.9	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	15 866.0 ± 1 426.8	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	11 118.7 ± 1 613.2	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	11 472.6 ± 2 184.1	133.1 ± 25.1	3.0 ± 0.7	97.7 ± 0.4	384.4 ± 73.7	12.7 ± 2.4	96.7 ± 0.4

week to metabolic data collection (see Diaz Gomez et al. 2016). The duration of the acclimation phase was designed to be sufficient for the northern 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). Maintenance intake levels for each animal thus differed across diets and also across animals due to differences in body mass and innate energy expenditures. To minimize potential seasonal effects, the diet trials were randomly assigned to different pairs of study animals over the course of the experimental period.

Feces collection and laboratory analysis of feces and prey

To match fecal samples to individual northern fur seals, gel capsules containing approximately 5–6 g of colored, non-digestible Microgrits markers (Micro Tracers Inc., 1370 Van Dyke Avenue, San Francisco, California, USA) were inserted into several of the prey fed throughout the day, with each color assigned to a specific northern fur seal. Feces were collected several times a day from the bottom of the holding pool or haul-out area. The samples were identified by marker color, weighed, and frozen at −20 °C until analyzed.

Methods of analysis of prey and fecal samples are detailed in Diaz Gomez et al. (2016) and are detailed briefly below. Ten samples of each prey item were analyzed both in-house and by a commercial laboratory (SGS Canada Inc., Burnaby, British Columbia, Canada) for proximate composition (moisture, lipid, and crude protein) and energy density. A total of 138 fecal samples (3 samples per diet per animal) were analyzed in-house for proximate composition and Mn²⁺ concentration. Additionally, 16 fecal samples were analyzed by SGS laboratory for Mn²⁺ to validate in-house measurements. Given the low concentration of Mn²⁺ in prey samples, only prey Mn²⁺ concentrations obtained from SGS were used in subsequent calculations. Sample analyses were all

done on freeze-dried samples, and total lipid content and total crude protein content are expressed as percentage of total dried sample.

For analysis preparation and to determine dry matter content from the prey and feces, replicates were freeze-dried for 36 h to a constant mass (Freeze dryer Freezone 6; Labconco Corporation, Kansas City, Missouri, USA). Replicates of dried homogenized samples (prey and feces) were then analyzed for proximate composition, energy density, and Mn²⁺ concentration. Energy density of replicates of dried prey samples was measured using an oxygen bomb calorimeter (6400 Automatic isoperibol calorimeter; Parr Instrument Company, Moline, Illinois, USA). Total lipid content of dried fecal and prey samples was measured by chloroform to methanol extraction (Bligh and Dyer 1959). Crude protein content of dried fecal and prey samples was determined by the Kjeldahl method (AOAC 1990). Total Kjeldahl nitrogen (TKN) concentration (mg·L⁻¹) of samples was determined by spectrophotometric flow injection analyzer (FOSS FIAstar 5000 TKN analyzer unit; FOSS North America, Eden Prairie, Minnesota, USA) measured at 590 nm. Nitrogen concentration was then multiplied by 6.25 to determine total crude protein as a percentage (g/100 g) of sample weight (Robbins 1993). Concentrations of Mn²⁺ (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 Diaz Gomez et al. 2016). The Mn²⁺ concentration in resulting solutions was determined via 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).

Calculations of nutrient digestibility

Total fecal collection is challenging in large marine mammals and was not possible in our study. We therefore calculated digest-

ibility of nutrients and energy indirectly using Mn^{2+} as a naturally occurring marker with minimal biological absorption in both prey samples and fecal samples. In essence, Mn^{2+} concentrations allowed us to determine the amount of prey that was represented by the fecal sample (see formulae below). This method has been widely accepted and used in digestibility studies with northern fur seals and other pinniped species (Fadely et al. 1990; Fadely et al. 1994; Lawson et al. 1997; Rosen and Trites 2000a).

The loss or retention of a specific nutrient can be expressed in terms of either absolute amounts (g·d⁻¹) or as a proportion of nutrient intake. The apparent digestibility coefficient (ADC_n) of specific nutrients (n) expresses the proportion of a specific nutrient that is absorbed during digestion (i.e., not lost in feces). The term "apparent digestibility" reflects the fact that feces contains nutrients and energy from sources other than the diet (e.g., enzymes secreted into the gastrointestinal tract, intestinal cells sloughed off, or gut microflora). ADC_n is calculated using the following formula:

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

where C is the concentration of Mn^{2+} and N is the nutrient concentration of the ingested diet (i) and feces (f) (see eq. XXI in Schneider and Flatt 1975; Mårtensson et al. 1994). These calculations yield the apparent digestibility coefficient of lipid (ADC_L) and the apparent digestibility coefficient of crude protein (ADC_{CP}).

The absolute dried mass (g·d⁻¹) of either lipid or crude protein ingested from the food that is lost through the feces is expressed as the fecal nutrient loss (FNL) for that specific component (i.e., as either fecal lipid loss (FLL) or fecal crude protein loss (FPL)). FNL was calculated with the following formula:

$$FNL = \text{nutrient consumed} - (ADC_n \times \text{nutrient consumed})$$

where FNL and nutrient consumed are measured in g·d⁻¹.

The expected digestibility coefficients of both lipid and crude 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

$$\text{Expected } ADC_n \, (\%) = \frac{(\text{mass}_1 \times \text{nutrient}_1 \times \text{observed } ADC_{n,1}) + (\text{mass}_2 \times \text{nutrient}_2 \times \text{observed } ADC_{n,2})}{\text{total nutrient fed}}$$

where 1 and 2 refer to the specific prey items fed in the combined diet; $mass_1$ and $mass_2$ are the amounts of prey item 1 and prey item 2 fed, respectively; $nutrient_1$ and $nutrient_2$ are the proportions of lipid or protein (n) in prey item 1 and prey item 2, respectively; and $ADC_{n,1}$ and $ADC_{n,2}$ are the calculated apparent digestive coefficients for specific nutrient n when fed solely prey item 1 and prey item 2, respectively.

The expected ADC_n was then compared with the observed ADC_n to determine if there was any synergistic effect of macronutrient digestibility from mixed-species diets relative to single-species diets.

Statistical analyses

Preliminary analysis of lipid digestibility indicated that data from two of the northern fur seals (consuming different diets) were outliers and their inclusion failed to fulfil the assumptions of the linear mixed-effects (LME) models; this was also confirmed through the extreme Studentized deviate method, known as Grubbs' test (Grubbs 1969). Excluding these mean values (one per diet per northern fur seal) of one animal consuming the capelin diet, and one animal from the pollock–capelin diet 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 variance (Pinheiro and Bates 2000; Crawley 2007; Zuur et al. 2009; Galecki and Burzykowski 2013; Pinheiro et al. 2015).

Preliminary results also showed that transforming the proportional ADC data did not change results because the residuals of the analytically robust LME models met the required assumptions of normality, homogeneity, and independence (Wilson et al. 2013). Where LME models indicated diet type was a significant factor, a post hoc Tukey's test adjusted with a Bonferroni correction (Zar 2010) was used to investigate where specific differences among the diets occurred.

We first tested for the effect of diet type on absolute values of digestive efficiency and prey characteristics (i.e., ingested mass, FLL, and FPL), as well as on relative measures such as lipid and protein diet content and digestibility (i.e., ADC_L and ADC_{CP}), using LME models in R version 3.1.2 statistical software (R Core Team 2014) as detailed in Diaz Gomez et al. (2016).

The potential explanatory cause of any differences in lipid and crude protein digestibility across diets was further investigated by analyzing which components of the diet were driving the relationship using LME models (Pinheiro and Bates 2000; Crawley 2007; Zuur et al. 2009; Galecki and Burzykowski 2013; Pinheiro et al. 2015). Specifically, we tested the following fixed factors: food mass intake (kg·d⁻¹; wet weight), absolute levels of macronutrient intake (lipid and protein intake; dry g·d⁻¹), and two measures of relative intake — with the component expressed either as a proportion of total intake (% protein and % lipid intake) or expressed as the ratio of lipid to protein intake. These last two measures are similar but not identical, given differing levels of other components (measured as % ash) in the prey species. Additionally, we also tested for statistical differences between the expected digestibility (expected ADC_n; %) and the observed digestibility (observed ADC_n; %) of both lipid and protein from the mixed-species diets using a Welch two-sample t test.
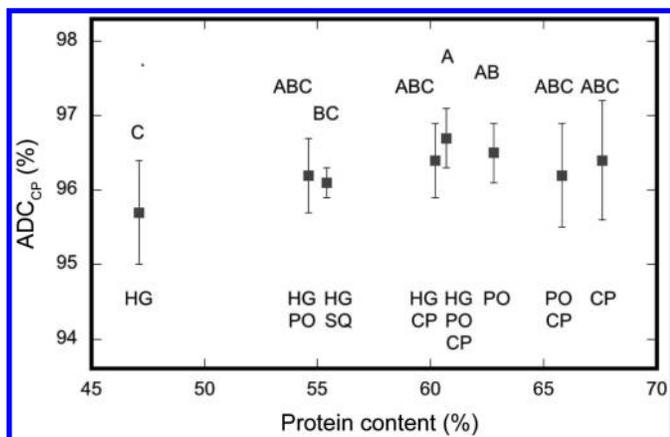
For all statistical tests, significance was determined at the 5% rate of error. Where appropriate, mean values are calculated for each individual northern fur seal and results are presented as mean ± SD among animals.

Results

Experimental diet characteristics

As per the overall goal of the experimental design, the proximate composition (dry-weight basis) of the eight experimental diets differed significantly from each other (Table 1). Total lipid content was lowest for the capelin diet (3.3% ± 0.0% dry weight) and highest for the herring + magister squid diet (43.2% ± 0.1%; likelihood ratio test (LRT) = 265.3, $p < 0.001$). Total crude protein content was lowest for the herring diet (47.1% ± 0.0%) and highest for the capelin diet (67.6% ± 0.0%; LRT = 366.9, $p < 0.001$). As a result of these proximate composition differences and the need to maintain constant gross energy intake, ingested mass (wet) was also significantly different across diets (Table 1). Mass intake was highest when northern fur seals were consuming the capelin diet (3.3 ± 0.5 kg) and lowest while on the herring diet (1.6 ± 0.3 kg; LRT = 76.9, $p < 0.001$).

Fig. 1. Apparent digestibility coefficient of crude protein (ADC$_{CP}$; %) of the eight experimental diets tested in six captive female northern fur seals (*Callorhinus ursinus*). Results from individual diets composed variously of Pacific herring (*Clupea pallasii*; HG), walleye pollock (*Gadus chalcogrammus* (formerly *Theragra chalcogrammus*); PO), capelin (*Mallotus villosus*; CP), and magister armhook squid (*Berryteuthis magister*; SQ) (see Table 1) are arranged from low to high protein content (%; dry-weight basis). Data are represented as mean ± SD. Data for each diet trial are from six northern fur seals, with the exception of the HG and SQ diets that were only consumed by four of the animals. Data not sharing letters above indicate significant differences between diets (*p* < 0.05).

Fig. 2. Apparent digestibility coefficient of lipid (ADC$_L$; %) of the eight experimental diets tested in six captive female northern fur seals (*Callorhinus ursinus*). Results from individual diets composed variously of Pacific herring (*Clupea pallasii*; HG), walleye pollock (*Gadus chalcogrammus* (formerly *Theragra chalcogrammus*); PO), capelin (*Mallotus villosus*; CP), and magister armhook squid (*Berryteuthis magister*; SQ) (see Table 1) are arranged from low to high lipid content (%; dry-weight basis). Data are represented as mean ± SD. Data for each diet trial are from six northern fur seals, with the exception of HG and SQ diets that were only consumed by four of the animals. Data not sharing letters above indicate significant differences between diets (*p* < 0.05).

Crude protein apparent digestibility

Total crude protein intake differed significantly among diets (LRT = 68.9, *p* < 0.001; Table 2), with the highest intake during the capelin diet (468.7 ± 75.8 g·d^{-1}) and the lowest intake during the herring diet (257.3 ± 51.2 g·d^{-1}). FPL also varied significantly across diets (LRT = 38.1, *p* = 0.001). FPL increased with increased food mass intake (LRT = 42.4, *p* < 0.001), as well as with increased protein content (%) of diet (LRT = 14.3, *p* < 0.001). For example, the amount of crude protein lost in the feces was highest when the northern fur seals consumed the capelin diet (17.0 ± 5.2 g·d^{-1}) and lowest when consuming the herring diet (11.1 ± 2.9 g·d^{-1}). In contrast, FPL decreased significantly (*p* < 0.05) as both lipid content (%) in the diet (LRT = 18.6, *p* < 0.001) and lipid to protein ratio increased (LRT = 18.8, *p* < 0.001).
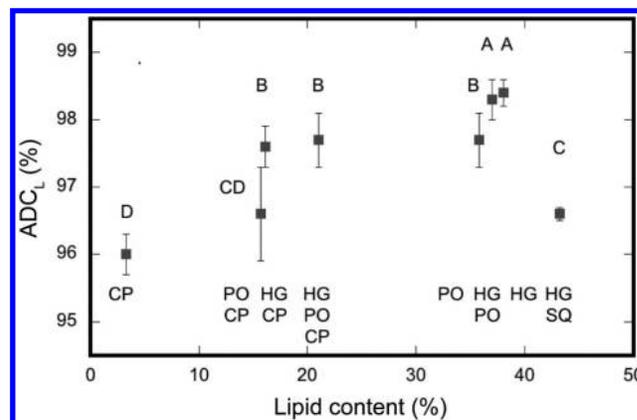
Whereas FPL is a measure of absolute levels of crude protein loss, ADC$_{CP}$ describes the relative efficiency of protein retention (i.e., retention as a proportion of intake). ADC$_{CP}$ was high across all experimental diets (96.3% ± 0.6%) and differed statistically among the diets (LRT = 19.9, *p* = 0.006; Table 2). ADC$_{CP}$ was highest for the herring + pollock + capelin diet (96.7% ± 0.4%) and lowest for the herring diet (95.7% ± 0.7%). Although differences in ADC$_{CP}$ between diets were small, ADC$_{CP}$ significantly increased as both food mass intake (LRT = 4.6, *p* = 0.03) and dietary protein content (%) increased (LRT = 9.0, *p* = 0.003; Fig. 1), although the diet with the highest ADC$_{CP}$ (herring + pollock + capelin diet) had a moderate protein content. Furthermore, ADC$_{CP}$ was observed to significantly decrease as both lipid content (%) in the diet (LRT = 4.4, *p* = 0.04) and lipid to protein ratios increased (LRT = 7.0, *p* = 0.008).

No significant differences (*p* > 0.05) were found when comparing the expected ADC$_{CP}$ (based on individual species ADC$_{CP}$) of the mixed-species diets against the observed ADC$_{CP}$, where the observed were within 0.3% of expected values. Therefore, there was no significant advantage to protein digestibility when northern fur seals consumed mixed-species diets over equivalent single-species diets.

Lipid digestibility

Total lipid intake was significantly different across diets (LRT = 134.7, *p* < 0.001; Table 2), where the capelin diet had the lowest

lipid intake (22.1 ± 3.3 g·d^{-1}) and the herring + magister squid diet had the highest lipid intake (279.5 ± 25.3 g·d^{-1}). FLL varied significantly among diets (LRT = 112.0, *p* < 0.001; Table 2). Specifically, FLL was significantly lower during the capelin diet (0.9 ± 0.4 g·d^{-1}) and higher during the herring + magister squid diet (9.5 ± 1.3 g·d^{-1}) than the rest of the experimental diets. This is consistent with the finding that FLL significantly increased as both lipid content (%) of the diets (LRT = 25.0, *p* < 0.001) and lipid to protein ratio increased (LRT = 18.8, *p* < 0.001). Unlike FPL, FLL was not significantly affected by food mass intake or protein content (%) in the diet.

As with protein, ADC$_L$ is a measure of relative lipid retention rates. ADC$_L$ was generally high across all diets (97.4% ± 0.9%) but varied statistically across diets (LRT = 100.1, *p* < 0.001; Table 2). ADC$_L$ was lowest when northern fur seals were consuming the capelin diet (96.0% ± 0.3%) and highest when consuming the herring diet (98.4% ± 0.2%). ADC$_L$ significantly increased as both relative lipid content (%) (LRT = 18.4, *p* < 0.001; Fig. 2) and lipid to protein ratio (LRT = 21.0, *p* < 0.001) increased in the diets. Conversely, ADC$_L$ significantly decreased as both food mass intake (LRT = 26.2, *p* < 0.001) and protein content (%) of diet increased (LRT = 30.3, *p* < 0.001).

When testing for an effect for diet mixing, there were no significant differences between observed and expected values of ADC$_L$ for two of the four mixed-species diets (differences were less than 0.2%, *p* > 0.05). Contrary to predictions, however, observed ADC$_L$ was lower than the expected values for both the herring + capelin diet (97.6% observed vs. 98.0% expected; *p* < 0.001) and the pollock + capelin diet (96.4% observed vs. 97.5% expected; *p* = 0.01), indicating a decreased lipid digestive efficiency for these mixed diets.

Overall, comparisons within each diet showed that, for six out of the eight diets, the mean ADC$_L$ was significantly higher than the mean ADC$_{CP}$ (*p* < 0.001). The two exceptions were the capelin-only diet and the pollock + capelin diet, which also were the diets with the highest protein and lowest lipid intake levels.

Discussion

Macronutrient digestibility not only affects the overall energetic gain from a given diet, but also the specific assimilation of required proteins and lipids. Past dietary studies on pinnipeds

have shown that seals and sea lions have comparatively 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 experimental diets consisting of single-prey species and were thus unable to determine the potential effect of critical factors — such as prey composition profiles or level of food intake — that may lead to significant differences in digestibility of macronutrients. Our study used a broad range of prey items to compose various diets that would allow us to specifically quantify the effects of different lipid and protein profiles and levels of intake on macronutrient digestibility in northern fur seals.

Understanding the root causes of differences in macronutrient digestibility is made difficult by the innate relationships between many of the potential explanatory factors. The relative amounts of lipid and protein tend to be inversely proportional to each other in fish, given the almost complete lack of carbohydrates. Thus, fish with a lower relative lipid content or higher protein content will also have lower energy densities. Consequently, animals consuming a given gross energy intake from a high protein diet would be ingesting a greater mass of fish and have a higher protein intake and a lower lipid intake than when achieving the same gross energy intake via a lipid-rich diet. Despite their interrelatedness, our study provided insight into how many of these dietary factors of diet composition — relative and absolute macronutrient intakes, ingested food mass, and gross energy intake — affect macronutrient digestibility and overall digestive efficiencies.

Ingested protein is digested to amino acids in the gastrointestinal tract through the action of a variety of proteolytic enzymes excreted into the gastrointestinal tract under specific pH conditions. The liberated amino acids are then readily absorbed from the intestine; however, some proteins are resistant to these digestive enzymes. It is thus the relative amount of proteins that are either susceptible or resistant to the action of proteolytic enzymes that affects the digestibility of dietary protein. Excretion rates of the proteolytic enzymes can be substrate dependent, whereby diet composition or consumption rates can stimulate greater excretion of certain enzymes. In our study, the excretion rates of proteolytic enzymes were not measured, because doing so is a challenge in marine mammals. Such information would contribute to a more complete understanding of the biological implications of the influence of diet on digestibility.

Lipids are also complex to digest since they involve emulsification by a combination of bile and salts secreted by the liver. Lipids are broken down into small droplets, known as micelles, that simply diffuse across the cell membrane of the small intestine. This is in sharp contrast to absorption of amino acids that requires special transport proteins and adenosine triphosphate. Thus, proteins not only take longer to digest, but they are also energetically costly to break down compared with lipids, which already provide a higher caloric intake.

Digestibility of protein and lipids

We found that high protein diets and increasing food mass intake resulted in higher rates of total FPL. However, this does not mean that these diets decrease the efficiency of protein absorption, but rather that greater absolute protein intake inevitably leads to greater absolute protein loss. In fact, the ADC_{CP} of the northern fur seals increased (i.e., absorption became more efficient) as the proportion of dietary protein increased, as well as with increasing ingested food mass. As previously discussed, protein and total food intake are intricately linked.

In our study, more high-protein prey was required to meet gross energy intake requirements, resulting in increased food mass intake. We propose that the increased efficiency in protein digestion was likely the result of increased protein intake rather than increased food mass intake per se. Since protein intake and lipid intake are also closely related, we similarly suggest the apparent increases in FPL and decreases in ADC_{CP} with decreasing ingested lipid and lipid to protein ratios are a side effect of the overall inverse relationship between lipid and protein within prey items rather than a direct effect of lipid content. It is important to note that the changes we observed in protein digestibility, while statistically significant, may not have a substantial biological effect. ADC_{CP} for all diets in our study was high (95.7%–96.7%; Table 2) and was 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 observed that ADC_L varied more than for protein digestibility, ranging from 96.0% to 98.4% across our experimental diets (Table 2). Again, our results were towards the high end of lipid digestibility values reported from 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). Designing isocaloric diets (i.e., similar gross energy intake across diets) meant that diets containing lipid-rich prey resulted in higher lipid intake and lower food mass intake, whereas diets composed of protein-rich prey had the opposite trend. As with FPL, FLL increased with increased lipid intake levels, as well as with increasing relative amounts of lipid to protein ratios in the diet. However, unlike FPL, FLL did not significantly increase with increasing ingested food mass. This supports our suggestion that absolute levels of FNL are primarily determined by nutrient intake levels.

ADC_L increased with increasing relative lipid content. This is consistent with the conclusions of other pinniped studies that reported high lipid digestibility from high lipid diets (Parsons 1977; Keiver et al. 1984; Ronald et al. 1984; Fisher et al. 1992; Lawson et al. 1997; Goodman-Lowe et al. 1999). We also observed that ADC_L increased with decreases in protein content of the diet, similar to changes reported in terrestrial carnivores (Russell et al. 2002; Mayntz et al. 2009), although it is unclear the extent to which this is an artefact of the innate inverse relationship between lipid and protein contents of diets. In contrast to protein digestibility, ADC_L significantly decreased with increasing food mass intake. This lends additional support to our hypothesis that changes in nutrient digestibility are driven by differences in the load of that specific nutrient and that any relationships to food intake levels are secondary in nature.

Overall, the lipid and protein digestibility ranges that we found are comparable with those documented elsewhere for pinnipeds, as well as for terrestrial mammalian carnivores (Clauss et al. 2010). We also found that apparent lipid digestibility was significantly greater than the apparent digestibility of protein in all diets except two (capelin-only diet and capelin + pollock diet). The generally lower digestibility of protein compared with lipid was expected, given that the breakdown of long chains of amino acids with strong peptide bonds within protein molecules cause the digestion of protein and absorption of amino acids to require a more complex chemical digestion than the easily digested and absorbed animal fats (Leoschke 1959; Best 1985; Blaxter 1989; Stevens and Hume 1995). Studies with other pinnipeds have similarly reported lower digestibility of protein relative to lipid (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). For the two cases where lipid digestibility was not different from protein digestibility, it was likely because those two diets had the lowest lipid content. As noted previously, lower lipid digestibility was related to low dietary lipid content (high dietary protein) across all diets.

Is there an optimal food choice?

Given the differences in macronutrient digestibility across different diets, it is reasonable to ask whether there is an "optimal"

diet for northern fur seals? Part of the difficulty in answering this question is that, as demonstrated in our study, there is a general inverse relationship between changes in protein and lipid digestibility with changes in proximate composition of prey. For example, shifting from a high-lipid to a high-protein prey may increase protein digestibility, but at the expense of decreasing lipid digestibility.

Terrestrial carnivores have been similarly documented to have increased protein digestibility when consuming diets with higher protein content (Russell et al. 2002, 2003; Mayntz et al. 2009). Digestibility of protein has been described as a curvilinear function of dietary protein, such that protein digestibility increases exponentially at low protein concentrations with increasing levels and then reaches a plateau at higher dietary protein levels (Crampton and Rutherford 1954; Robbins 1993). Hence, differences in protein digestibility are more pronounced between diets with relatively low protein content (Schneider and Flatt 1975), whereas smaller changes in protein digestibility are seen among diets with higher protein content. The northern fur seal's high values of protein digestibility fit within the upper plateau of the expected curvilinear function of protein digestibility, as all of our experimental diets had relatively high protein levels (47.1%–67.6%; Table 1), and the range in mean protein digestibility was only 1% across all diets.

Our study also confirmed that lipid digestibility increased with increased lipid content of the diet. It has been demonstrated across multiple phyla that lipid absorption generally varies with both the amount and the specific type of fatty acids in the diet (Mu and Høy 2004). In our study, differences in lipid digestibility were greater between diets than differences in protein digestibility, suggesting that lipid content of prey may be more important to determining overall nutritional benefit. This is emphasized by the fact that lipid provides more energy, such that northern fur seals require less prey mass to fulfill their energy requirements.

The net result of these assimilation trends is that the northern fur seals in our study displayed higher lipid assimilation under diets of moderate protein content without significantly compromising protein assimilation. This suggests an optimal intake ratio between lipid and protein that allows northern fur seals to maximize their digestibility benefits from such macronutrients while simultaneously fulfilling their energetic demands. By these criteria, high-lipid prey would seem to be "better" for northern fur seals than high-protein prey. Of course, this simple evaluation does not take into account specific nutrient requirements, which vary with life-history stage and season. Other considerations include specific fatty acid and amino acid to satisfy particular lipid and protein requirements, as well as specific micronutrient requirements (e.g., vitamins, minerals).

Finally, it should be noted that the energetic benefits of these changes in digestive efficiency are relatively minor. Total fecal energy loss derives directly from lipid and protein loss. Our results indicate that overall fecal energy loss ranges from 2.7% of gross energy intake (herring-only diet and herring + pollock diet) to 4.0% (pollock-only diet and herring + magister squid diet). These estimates of fecal energy loss based on lipid and protein loss agree very closely with previous direct measures of fecal energy loss that ranged from 3.1% to 4.1% (Diaz Gomez et al. 2016). These differences in energetic value of prey items may be magnified by the composition-dependent effects of energy loss through the heat increment of feeding (Secor 2009), but may still be minor compared with the effects of ecological availability on net energetic benefits to individual northern fur seals.

Mixed diets have been proposed as a way of both maximizing digestive efficiencies and facilitating absorption of required macro- and micro-nutrients (Penry and Jumars 1987; Singer and Bernays 2003; Trumble and Castellini 2005). In our study, we found no significant benefit associated with the consumption of mixed-species diets over single-species diets in terms of macronutrient return, as also reported for harbor seals (*Phoca vitulina* Linnaeus, 1758) (Yamamoto et al. 2009). The observed apparent protein and lipid digestibility coefficients of mixed-species diets in our study were not significantly different from the expected values for equivalent single-species diets. In fact, the observed lipid digestibility for two of our four mixed-species diets was significantly lower than expected (herring + capelin diet and pollock + capelin diet).

Mixed-species diets may confer other nutritional advantages, such as providing a specific proportion of ingested macronutrients. The geometric framework of nutrition hypothesis proposes that, when presented with various food choices, predators actively balance (rather than maximize) macronutrient intake of both lipid and protein independently to satisfy an ideal intake target (Raubenheimer and Simpson 1999; Mayntz et al. 2009; Raubenheimer et al. 2009; Kohl et al. 2015). Unfortunately, few tests of the geometric framework have been conducted on large carnivores, and none specifically on marine mammals. However, a recent study of free-ranging Atlantic spotted dolphins (*Stenella frontalis* (G. Cuvier, 1829)) demonstrated that non-reproductive, pregnant, and lactating dolphins consumed significantly different prey, in terms of both species and macronutrient compositions, presumably to satisfy their disparate nutritional needs (Malinowski and Herzing 2015). Similarly, a strong relationship has been demonstrated between prey composition of various cetacean species 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.

Implications for northern fur seals in the Bering Sea

In theory, animals should target prey that are abundant, provide the greatest net energetic return, and provide the required level of macro- and micro-nutrients. In the wild, diets of predators may be limited by prey availability, such that preferential food selection is not possible. As a result, predators may be forced to prioritize their basic energetic needs over macronutrient balancing. This may be the case for declining northern fur seal populations on the Pribilof Islands (National Research Council 1996; Towell et al. 2014), as well as other pinnipeds in the Bering Sea region (Pitcher 1990; Trites and Larkin 1996; Merrick et al. 1997; Rosen and Trites 2000*b*; Rosen 2009).

Northern fur seals breeding on the Pribilof Islands appear to have shifted from a high-lipid, high-energy northern smooth-tongue (*Leuroglossus schmidti* Rass, 1955) diet to a high-protein pollock diet (low energy) (Swartzman and Haar 1983; Perez and Bigg 1986), with juvenile pollock comprising approximately 60% of their diet (Sinclair et al. 1994; Call and Ream 2012). This diet shift coincides with the observation that pollock is the most abundant semi-pelagic fish in the Bering Sea (Kajimura 1984; Ianelli et al. 2010).

Our study confirms that the previously reported lower energetic returns of low lipid, high protein diets (Diaz et al. 2016) are directly the result of differential digestion of lipids and proteins. Although differences in overall energy efficiency were small, it does raise the question of whether the increased reliance of wild northern fur seals on young pollock to achieve sufficient energy gain may be challenging their overall nutritional state. The proximate composition of young pollock in the Bering Sea (Van Pelt et al. 1997; Logerwell and Schaufler 2005; Vollenweider et al. 2011) are comparable with the high protein content of the capelin used in our study (Table 1). This high protein diet was associated with the lowest lipid digestive efficiency, as well as the highest urinary energy loss and heat increment of feeding (Diaz et al. 2016).

Ensuring sufficient intake, digestion, and absorption of specific macronutrients is important from a nutritional perspective, as the macronutrients perform different physiological functions. Consuming a high protein diet will result in low lipid intake, which may result in related nutritional deficiencies. However,

high protein diets also necessitate greater protein intake (and fish prey mass) to meet energy requirements.

Although carnivores have the highest protein requirements (18%–30% of dietary intake) among mammals (Robbins 1993), and are generally adapted to deal with high rates of protein consumption (Russell et al. 2002, 2003), excess macronutrient intake is known to have long-term negative effects on overall health. For example, the 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). In addition, excess protein consumption has been linked to decreased reproductive function and fertility in mice (Solon-Biet et al. 2015b). Therefore, it is reasonable to hypothesize that the overall performance and vital rates of wild northern fur seals in the Bering Sea is negatively affected by the long-term high protein intake and waste products associated with feeding on large amounts of young, high protein pollock.

A shift in dietary intake towards prey that are more readily accessible but of lower nutritional value has been hypothesized to be negatively impacting other species in the Bering Sea and Gulf of Alaska, such as Steller sea lions (*Eumetopias jubatus* (Schreber, 1776)) and sea birds (Swartzman and Haar 1983; Castellini 1993; Decker et al. 1995; Merrick et al. 1997; Calkins et al. 1998; Rosen and Trites 2000b; Trites et al. 2007). As with northern fur seals, the consumption of macronutrient-imbalanced diets may be an important and previously understudied consideration within the broader context of the relationship between nutritional status and population declines of these top predators.

Acknowledgements

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