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Seals and sea lions are what they eat, plus what? Determination of trophic discrimination factors for seven pinniped species

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RATIONALE: Mixing models are a common method for quantifying the contribution of prey sources to the diet of an individual using stable isotope analysis; however, these models rely upon a known trophic discrimination factor (hereafter, TDF) that results from fractionation between prey and animal tissues. Quantifying TDFs in captive animals is ideal, because diet is controlled and the proportional contributions and isotopic values of all prey items are known.

METHODS: To calculate TDFs for the Hawaiian monk seal, northern elephant seal, bearded seal, ringed seal, spotted seal, harbor seal, and California sea lion, we obtained whiskers, serum, plasma, red blood cells, and prey items from nine captive individuals. We obtained $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values using continuous-flow isotope-ratio mass spectrometry. The average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from bulk and lipid-corrected prey from the diet were subtracted from the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of each blood and whisker sample to calculate tissue-specific TDFs for each individual ($\Delta^{13}\text{C}$ or $\Delta^{15}\text{N}$).

RESULTS: The $\Delta^{13}\text{C}$ values ranged from +1.7 to +3.2‰ (bulk prey) and from +0.8 to +1.9‰ (lipid-corrected prey) for the various blood components, and from +3.9 to +4.6‰ (bulk prey) or +2.6 to +3.9‰ (lipid-corrected prey) for whiskers. The $\Delta^{15}\text{N}$ values ranged from +2.2 to +4.3‰ for blood components and from +2.6 to +4.0‰ for whiskers. The TDFs tended to group by tissue, with whiskers having greater $\Delta^{13}\text{C}$ values than blood components. In contrast, the $\Delta^{15}\text{N}$ values were greater in serum and plasma than in red blood cells and whiskers.

CONCLUSIONS: By providing the first TDF values for five seal species (family Phocidae) and one otariid species (family Otariidae), our study facilitates more accurate mixing models for these species. These values are particularly important for critically endangered Hawaiian monk seals and the three Arctic seal species (bearded, ringed, and spotted) that are faced with a rapidly changing environment. Copyright © 2016 John Wiley & Sons, Ltd.

Due to the challenges of directly observing foraging behavior in many cryptic species, biochemical approaches such as stable isotope analysis have emerged as advantageous methods for estimating proportional prey contributions to the diets of wild animals. Stable isotopes of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) are used to investigate food webs and establish the trophic positions and diets of predators. The heavier isotopes of carbon and nitrogen (^{13}C and ^{15}N), relative to the lighter isotopes (^{12}C and ^{14}N), increase with trophic level as they are enriched during the metabolic processing of prey into predator tissues (i.e., isotopic fractionation).^[1–4] Mixing models are used to quantify the probabilistic contribution of prey sources to the diet. These models rely upon the assumption that the isotopic ratios of an animal's tissues are a function of the isotopic ratios

of its diet components plus a trophic discrimination factor (TDF) that results from the isotopic fractionation between prey sources and animal tissues.^[4–6] As TDFs vary among taxa and tissues (e.g.,^[7,8]), it is necessary to apply an appropriate TDF or the interpretation of mixing model outputs may be spurious.^[9–11]

TDFs are calculated using the exact proportion and isotopic composition of each prey item contained within an individual's diet. Thus, TDFs are typically quantified in captive animals because the isotopic contribution of each prey item to the diet can be controlled. Furthermore, factors that fluctuate considerably in free-ranging animals and influence TDFs (i.e., animal diet and condition^[12–14]) can be manipulated in a captive setting. Dietary studies of free-ranging pinnipeds (seals, sea lions, fur seals, and walrus) report stable isotope values from multiple tissues (e.g., whiskers, red blood cells, serum),^[6,15–18] but the TDFs required to interpret these data are limited. TDFs have been quantified for seven of 33 extant pinniped species, and species-specific values have been reported for six of these seven species.^[15–19] In the absence of species-specific TDFs, researchers often apply TDF values from other species to their stable isotope analyses. However, it is unknown if and when such assumptions are appropriate.

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In this study, we calculated TDFs for whiskers and blood (red blood cells, serum, and plasma) in seven pinniped species: the Hawaiian monk seal (*Neomonachus schauinslandi*), northern elephant seal (*Mirounga angustirostris*), bearded seal (*Erignathus barbatus*), ringed seal (*Pusa hispida*), spotted seal (*Phoca largha*), harbor seal (*Phoca vitulina*), and California sea lion (*Zalophus californianus*). The resulting TDF values obtained from captive animals consuming known prey items will enable more accurate estimation of the diets of wild pinnipeds using stable isotope mixing models.

EXPERIMENTAL

Tissue samples were collected from nine captive individuals, representing seven pinniped species, at Long Marine Laboratory, University of California, Santa Cruz (Santa Cruz, CA, USA). Eight of the nine animals were held in captivity for at least 2 years prior to sample collection, and during that time were maintained on a mixed diet of Atlantic herring (*Clupea harengus*), Pacific herring (*C. pallasii*), and capelin (*Mallotus villosus*), with additional vitamin supplements (Vita-Zu 584Y; Mazuri, St. Louis, MO, USA). The remaining animal, a young-of-the-year bearded seal, was placed in captivity at the Alaska SeaLife Center (Seward, AK, USA) in October 2014 and fed a diet of Atlantic herring, capelin, and market squid (*Doryteuthis opalescens*). Records were kept of each animal's daily food intake, and prey samples were archived at -20°C for subsequent isotopic analysis. Blood samples were obtained during routine health examinations, centrifuged to separate blood components (serum, plasma, and red blood cells), and stored at -20°C . Whisker samples were obtained from the animals' living enclosures following natural shedding events and stored in plastic bags in ambient indoor conditions.

The blood and prey samples were freeze-dried for > 48 h after homogenization of prey samples using a blender and scalpels, and then thoroughly re-homogenized. The whiskers were rinsed with de-ionized water and mild detergent followed by a petroleum ether wash in an ultrasonic bath to remove exogenous debris. They were measured for total length and three sequential ~ 0.5 mg segments were sub-sampled starting 1 cm from the whisker base, to avoid isotopic complications associated with the whisker root. For the northern elephant seal, only one whisker segment was sub-sampled at 1 cm from the base. All the tissue and prey samples were weighed into tin boats (~ 0.5 mg) and analyzed for their C and N stable isotope ratios at the Light Stable Isotope Laboratory, University of California, Santa Cruz, using a NE2500 CHNS-O analyzer (Carlo Erba, Lakewood, NJ, USA) coupled to a Finnigan DELTAplus XP isotope ratio mass spectrometer (Thermo, Waltham, MA, USA). The standards were Vienna-Pee Belemnite Limestone for C and atmospheric N_2 (air) for N. The isotope ratios obtained are expressed in delta notation ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values) in units of parts per thousand (‰) using the following equation:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \quad (1)$$

where R is the ratio of heavy to light isotopes ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) in the sample or standard. The within-run precision was assessed using an internal laboratory standard (Pugel) and was 0.05‰ for $\delta^{13}\text{C}$ values and 0.10‰ for $\delta^{15}\text{N}$ values.

The lipid content of prey can influence diet-to-tissue TDFs because the synthesis of lipids discriminates against ^{13}C , resulting in more negative $\delta^{13}\text{C}$ values in lipid-rich than lipid-depleted tissues or prey.^[20–22] Prey samples are therefore often chemically treated to extract lipids before isotope analysis to allow comparisons of isotope ratios among prey with different lipid contents.^[23] Alternatively, mathematical corrections can be applied to the prey isotope ratio values after analysis to normalize for variation in prey lipid content.^[23,24] We chose to apply the mathematical correction from Post *et al.*^[23] to prey $\delta^{13}\text{C}$ values, and we report TDFs that were both uncorrected and corrected for lipid content.

Published turnover rates for each tissue were used in conjunction with sample collection dates and diet records to determine the contribution of each prey to the diet. The half-life of isotopes in plasma is typically very short (~ 4 days^[25]), whereas for red blood cells, hereafter RBCs, the average half-life is one to several months (~ 28 or ~ 42 days^[25,26]). We therefore calculated the diet composition using diet records from 7 (serum and plasma) or 60 days (RBCs) prior to sample collection. For whiskers, it was not possible to determine the exact date that the sampled segment was grown because the whiskers of phocids (true seals) exhibit asymptotic growth.^[27,28] Instead, we used the growth equation determined by Beltran *et al.*^[28] for northern elephant seals to approximate the age of each seal whisker and estimate the most likely date that the sampled whisker segment was grown. The diet composition was determined by averaging the diet over the 7 days following the estimated growth initiation date of the whisker segment, as we estimated that a 0.5 mg segment would represent no more than 7 days of growth. In general, the diet of each captive animal was relatively stable over time; therefore, differences between the estimated and actual growth date of the whisker segment should not have affected the diet-to-whisker TDFs.

We calculated the diet-to-tissue TDFs (ΔX) as the difference in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values between the mean isotopic composition of tissue samples and diet using the following equation:

$$\Delta X = \delta X_{\text{Tissue}} - \left[\sum_{i=1}^n (p_i \times \delta X_{pi}) \right] \quad (2)$$

where p_i is the proportion of diet (by mass) comprised of prey species i , δX_{pi} is the mean isotope composition ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) of prey species i , δX_{Tissue} is the isotope composition of the tissue, and n is the number of prey items in the diet. Diet-to-tissue TDFs were calculated using bulk and lipid-corrected prey $\delta^{13}\text{C}$ values.

We did not obtain RBCs from the captive northern elephant seal, and therefore followed the methods of Germain *et al.*^[29] to estimate a diet-to-RBC TDF (ΔX_{RBC}) for this individual. This method combined samples from free-ranging animals with the whisker sample from the captive seal. We used whisker and RBC samples collected from four free-ranging adult female northern elephant seals upon arrival to the Año Nuevo colony (California, USA) following a foraging trip. To obtain the estimated diet-to-RBC TDF, we combined the calculated diet-to-whisker TDF ($\Delta X_{\text{WHISKER}}$) from the captive northern elephant seal with the isotope values of whiskers ($\delta X_{\text{WHISKER}}$) and RBC samples (δX_{RBC}) from the free-ranging seals, as follows:

$$\Delta X_{RBC} = \Delta X_{WHISKER} - (\delta X_{WHISKER} - \delta X_{RBC}) \quad (3)$$

Isotope values for each free-ranging individual were input into Eqn.(3), resulting in four diet-to-RBC TDF estimates for this species, which were then averaged.

RESULTS

We obtained $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for 17 blood samples and 8 whiskers from 9 captive individuals, representing 7 pinniped species (Table 1). The mean isotope ratio values of bulk prey tissues fed to study animals ranged from -23.5 to -18.6% for

$\delta^{13}\text{C}$ and from 11.5 to 13.1‰ for $\delta^{15}\text{N}$ (Table 2); lipid correction resulted in prey $\delta^{13}\text{C}$ values of -21.0 to -18.1% . The isotope ratios of predator tissues ranged from -19.3 to -16.2% for $\delta^{13}\text{C}$ values and 14.2 to 16.6‰ for $\delta^{15}\text{N}$ values (Table 3).

The diet-to-tissue TDFs ranged from 1.7 to 4.6‰ for $\Delta^{13}\text{C}$ values and from +2.2 to +4.3‰ for $\Delta^{15}\text{N}$ values (Table 4). The use of lipid-corrected prey $\delta^{13}\text{C}$ values resulted in lower $\Delta^{13}\text{C}$ values for all tissues, ranging from +0.8 to +3.9‰. The diet-to-tissue TDFs tended to group by tissue type, regardless of species (Fig. 1), although the small sample size did not allow for statistical comparison. The diet-to-whisker $\Delta^{13}\text{C}$ values from both bulk and lipid-corrected prey were greater for whiskers than for serum, plasma, or RBCs (Fig. 1).

Table 1. Descriptive information for the captive individuals sampled, along with the corresponding sample size of tissues analyzed (whisker, red blood cells (RBC), serum, and plasma). Stable isotope analysis was conducted on tissues from a captive Hawaiian monk seal (*Neomonachus schauinslandi*), a northern elephant seal (*Mirounga angustirostris*), a bearded seal (*Erignathus barbatus*), two ringed seals (*Pusa hispida*), two spotted seals (*Phoca largha*), a harbor seal (*Phoca vitulina*), and a California sea lion (*Zalophus californianus*)

Species and ID	NOAA ID	Sex	Age class	Whisker	RBC	Serum	Plasma
Hawaiian monk seal	NOA0006781	M	Adult	1	1	-	1
Northern elephant seal	NOA0004829	F	Adult	1	-	1	1
Bearded seal	NOA0010177	M	Juvenile	1	-	1	-
Ringed seal #1	NOA0005618	M	Adult	1	1	-	1
Ringed seal #2	NOA0006783	F	Juvenile	1	-	-	-
Spotted seal #1	NOA0006674	M	Juvenile	1	1	-	1
Spotted seal #2	NOA0006675	M	Juvenile	1	1	-	1
Harbor seal	NOA0001707	M	Adult	1	1	-	1
California sea lion	NOA0004827	F	Adult	-	1	2 ^a	1

For species where more than one animal was sampled, individuals are denoted using numbers.

^aSerum samples were collected twice, separated by about 1 year.

Table 2. Dietary analyses for the captive pinnipeds listed in Table 1, including prey type, sample size (number of whole fish analyzed), prey isotope ratio values (mean \pm SD), carbon to nitrogen ratios (C:N), and proportion of total diet. For C, the bulk and lipid-corrected (LC) $\delta^{13}\text{C}$ values are shown. Prey species include capelin (*Mallotus villosus*), Atlantic herring (*Clupea harengus*), Pacific herring (*C. pallasii*), and market squid (*Doryteuthis opalescens*), with individuals grouped by similar diets. Capelin and herring fed to the animals were from different catch lots and are thus listed separately. Diet proportion values for each prey type are given as range for the individuals listed (minimum – maximum)

Predator and Prey	n	$\delta^{13}\text{C}$ (‰)	$\delta^{13}\text{C}$ LC (‰)	$\delta^{15}\text{N}$ (‰)	C:N	Diet proportion
Hawaiian monk seal						
capelin	3	-20.7 ± 0.2	-19.9 ± 0.1	11.9 ± 0.5	4.1 ± 0.2	0.51–0.74
Pacific herring	3	-23.5 ± 0.8	-21.0 ± 0.3	12.0 ± 0.1	5.9 ± 1.1	0.26–0.49
Northern elephant seal						
capelin	6	-21.1 ± 0.5	-20.4 ± 0.3	11.9 ± 0.5	4.1 ± 0.5	0.48–0.53
Atlantic herring	6	-20.2 ± 1.0	-19.6 ± 0.7	11.5 ± 0.4	4.0 ± 0.4	0.47–0.52
Bearded seal						
capelin	3	-21.0 ± 0.5	-20.1 ± 0.1	11.8 ± 0.3	4.3 ± 0.4	0.50–0.62
Atlantic herring	3	-21.3 ± 0.1	-18.1 ± 0.1	12.6 ± 0.3	6.7 ± 0.2	0.27–0.41
squid	3	-18.6 ± 0.1	-18.3 ± 0.2	13.1 ± 0.5	3.7 ± 0.2	0.09–0.11
Ringed seal, spotted seal, harbor seal, California sea lion						
capelin	7	-21.1 ± 0.4	-20.3 ± 0.2	11.7 ± 0.3	4.1 ± 0.4	0.34–0.81
Atlantic herring	7	-20.0 ± 0.6	-19.5 ± 0.3	11.6 ± 0.5	3.9 ± 0.5	0.00–0.66
Pacific herring	4	-21.2 ± 0.5	-18.7 ± 0.4	13.1 ± 0.4	5.9 ± 0.3	0.00–0.51

Table 3. Carbon and nitrogen stable isotope ratio values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, ‰) and carbon to nitrogen ratios (C:N) for four tissue types (whisker, red blood cells (RBC), serum, and plasma) analyzed from the captive pinnipeds listed in Table 1. Whisker samples are the average of the three most recently grown segments. Isotope values are reported as mean \pm SD for sample sizes >1 . Sample sizes are provided in Table 1

Species	Whisker			RBC			Plasma			Serum		
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N
Monk seal	-17.5 ± 0.04	14.5 ± 0.01	2.9	-19.1	14.2	3.3	-19.3	15.0	3.7	-	-	-
Elephant seal	-16.5^b	14.4^b	2.9	-	-	-	-18.3	15.5	3.7	-18.5	15.7	3.6
Bearded seal	-16.6 ± 0.13	15.4 ± 0.07	2.8 ± 0.07	-	-	-	-	-	-	-18.6	16.6	3.9
Ringed seal #1	-16.2 ± 0.04	14.8 ± 0.04	2.9	-18.3	14.4	3.2	-18.8	15.8	3.7	-	-	-
Ringed seal #2	-16.2 ± 0.06	14.6 ± 0.09	2.9	-	-	-	-	-	-	-	-	-
Spotted seal #1	-16.2 ± 0.04	15.6 ± 0.02	2.9	-18.3	14.4	3.3	-18.8	15.7	3.7	-	-	-
Spotted seal #2	-16.3 ± 0.06	15.7 ± 0.06	2.9	-18.3	14.9	3.2	-18.5	15.8	3.7	-	-	-
Harbor seal	-16.3 ± 0.04	15.3 ± 0.07	2.9	-18.2	14.2	3.3	-18.7	15.5	3.8	-	-	-
California sea lion ^a	-	-	-	-17.9	15.2	3.3	-18.3	16.3	3.8	-18.5	16.4	3.9

^aSerum samples were collected twice, approximately one year apart and used to calculate an average trophic discrimination factor. Here we present the isotope ratio values for the sample that was obtained at the same time as the plasma and RBCs from this animal.

^bOnly one whisker segment was analyzed for the elephant seal, whereas three consecutive segments were analyzed and the results then averaged for all other animals.

In all individuals, the $\Delta^{15}\text{N}$ values were greater for serum and plasma than for either whiskers or RBCs, and the TDFs for serum and plasma were similar to each other (Fig. 1). In addition, the $\Delta^{15}\text{N}$ values were greater for whiskers than for RBCs, with the exception of the estimated diet-to-RBC $\Delta^{15}\text{N}$ values for northern elephant seals.

DISCUSSION

Here we present the first species-specific TDF values for the Hawaiian monk seal, northern elephant seal, bearded seal, ringed seal, and California sea lion. While TDFs had been previously calculated for two ringed seals, the values were combined with those of harbor and harp seals (*Pagophilus groenlandicus*) and not reported separately.^[6] These results nearly double the number of species-specific TDFs available for pinnipeds, and provide valuable information for researchers investigating the foraging ecology of these species.

In general, the TDFs tended to be similar within a tissue type, regardless of species, although we did not have sufficient sample sizes to statistically test for tissue or species differences. The trends that we observed among tissues in TDFs were generally consistent with previous studies on marine and terrestrial carnivores, and seabirds, including those with sufficient sample sizes to statistically test for differences among tissues.^[6,11,15,16,30,31] The main exception to this is that several studies have reported higher $\Delta^{15}\text{N}$ values for keratinized tissues than for blood components,^[6,31,32] while we found higher $\Delta^{15}\text{N}$ values in the blood components than in the whiskers. Tissues reflect different time periods as a result of variation in tissue turnover rates^[25,33,34] and, because of this, the TDFs could vary among tissues due to temporal changes in diet, life history events (e.g., reproduction, molt), or physiology (e.g., nutritional stress). Dietary variation should have little influence on TDFs for captive animals held on constant

diets; instead differences in TDFs among tissue types may be driven by differences in biochemical composition among tissues.^[8] It is important to note that several of the animals in our study were juveniles, and that age class can affect $\Delta^{13}\text{C}$ or $\Delta^{15}\text{N}$ values, although the magnitude and direction of this change appear variable among species.^[30,35] We did not find any consistent trends in TDFs for juvenile animals, with the exception of diet-to-whisker TDFs; however, appropriate within-species comparisons cannot be made because of small sample sizes and the lack of TDFs for adult animals of these species.

Diet-to-whisker TDFs were the most variable of all the tissue types, which may have been in part due to the growth dynamics of pinniped whiskers. The use of whisker growth rates to calculate TDFs, similar to Tyrrell *et al.*,^[36] provides some increased confidence in the temporal period represented in a given whisker segment. The non-linear growth pattern exhibited by phocid seals,^[27,28] coupled with the general lack of data on whisker growth in mammalian carnivores, complicates the ability to accurately link whisker isotope ratio values with a specific time period. Both these factors could have influenced our estimation of the diet that corresponded to each whisker segment, especially because we applied estimates of whisker growth rates from northern elephant seals to all species. However, the typical diets of the study animals were relatively constant over time, so that inaccuracies in the estimated age or growth rate of the whisker should not significantly change the TDFs. The one exception was the bearded seal, which had only been in captivity for several weeks when the whisker used for TDF calculations was estimated to have started growing. Whisker loss and regrowth have been shown to overlap with annual pelage molt for some species,^[37] and physiological or behavioral changes during this period could also influence whisker isotope values,^[38] thereby contributing to among-individual variation in diet-to-whisker TDFs.

Table 4. Diet-to-tissue trophic discrimination factors (TDF) of carbon and nitrogen stable isotope ratios ($\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$, ‰) from this and other studies on captive-held (captive or rehabilitated) marine carnivores

Species	n	Whisker				RBC				Plasma				Serum			
		$\Delta^{13}\text{C}$ NLC	$\Delta^{13}\text{C}$ IC	$\Delta^{15}\text{N}$	$\Delta^{13}\text{C}$ LC	$\Delta^{13}\text{C}$ NLC	$\Delta^{13}\text{C}$ LC	$\Delta^{15}\text{N}$	$\Delta^{13}\text{C}$ LC	$\Delta^{13}\text{C}$ NLC	$\Delta^{13}\text{C}$ LC	$\Delta^{15}\text{N}$	$\Delta^{13}\text{C}$ LC	$\Delta^{13}\text{C}$ NLC	$\Delta^{13}\text{C}$ LC	$\Delta^{15}\text{N}$	
This study:																	
Monk seal	1	+3.9	+2.7	+2.6	+2.9	+1.4	+2.2	+2.8	+1.1	+3.1	-	-	-	-	-	-	
Elephant seal	1	+4.1	+3.5	+2.8	+1.7 ± 0.2 ^j	+1.1 ± 0.2 ^j	+3.1 ± 0.7 ^j	+2.3	+1.7	+3.8	+2.1	+1.5	+2.1	+0.8	+4.0	+4.3	
Bearded seal	1	+4.4	+2.6	+3.0	-	-	-	-	-	-	-	-	-	-	-	-	
Ringed seal #1	1	+4.6	+3.9	+3.0	+2.3	+1.6	+2.7	+1.8	+1.2	+4.1	-	-	-	-	-	-	
Ringed seal #2	1	+4.4	+3.8	+3.2	-	-	-	-	-	-	-	-	-	-	-	-	
Spotted seal #1	1	+4.3	+3.7	+4.0	+2.2	+1.5	+2.8	+1.7	+1.1	+4.0	-	-	-	-	-	-	
Spotted seal #2	1	+4.3	+3.7	+3.9	+2.2	+1.6	+3.3	+2.0	+1.4	+4.1	-	-	-	-	-	-	
Harbor seal	1	+4.4	+3.4	+3.4	+2.1	+1.6	+2.6	+1.8	+1.2	+3.9	-	-	-	-	-	-	
California sea lion	1	-	-	-	+3.2	+1.9	+3.2	+2.8	+1.5	+4.2	+2.6 ^k	+1.1 ^k	-	-	+4.3 ^k	-	
Other studies:																	
Harbor seal ^a	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Harbor seal ^b	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Harbor seal ^b	102	-	-	-	+1.8 [*]	-	+3.2 ^{*1}	-	-	-	-	-	-	-	-	-	
Harbor seal ^c	3	-	-	-	-	+1.6	+2.0 ⁱ	-	-	-	-	-	-	+0.8	+3.5 ⁱ	-	
Harbor seal ^c	3	-	-	-	-	+1.2	+1.5 ⁱ	-	-	-	-	-	-	+0.6	+2.7 ⁱ	-	
Harbor seal ^c	4	-	-	-	-	+1.1	+2.0 ⁱ	-	-	-	-	-	-	+0.7	+2.7 ⁱ	-	
Harbor, harp, ringed seal ^d	7	-	+3.2 ± 0.2	+2.8 ± 0.1 ⁱ	-	-	-	-	-	-	-	-	-	-	-	-	
Harbor, harp, ringed seal ^d	14	-	-	-	-	+1.7 ± 0.1	1.7 ± 0.1 ⁱ	-	-	-	-	-	-	-	-	-	
Harp seal ^c	8	-	-	-	-	+1.7	+1.7 ⁱ	-	-	-	-	-	-	+0.8	+3.3 ⁱ	-	
Gray seal ^c	5	-	-	-	-	+1.7	+1.7 ⁱ	-	-	-	-	-	-	+1.0	+3.4 ⁱ	-	
Gray seal ^c	2	-	-	-	-	+1.2	+1.6 ⁱ	-	-	-	-	-	-	+0.5	+2.9 ⁱ	-	
Northern fur seal ^e	6	-	-	-	-	+1.4	+4.1 ⁱ	-	-	+5.2 ⁱ	-	-	-	+0.6	+5.2 ⁱ	-	
South American sea lion ^f	2	-	-	-	-	+0.2 ± 0.1 [*]	+2.0 ± 0.1	-	+1.0 ± 0.1 [*]	+2.4 ± 0.2	-	-	-	+1.0 ± 0.1 [*]	+2.6 ± 0.3	-	
Steller sea lion ^g	4	+6.5 ± 0.3	+3.3 ± 0.3	+3.7 ± 0.3	-	-	-	-	-	-	-	-	-	-	-	-	
Southern sea otter ^h	5	+2.4 ± 0.2	+2.8 ± 0.2	+5.5 ± 0.2	-	-	-	-	-	-	-	-	-	-	-	-	

All values are separated by species and tissue, and $\Delta^{13}\text{C}$ values are further separated into estimates obtained from bulk or lipid-corrected/extracted prey (LC). Predator samples that were lipid extracted prior to the calculation of the TDF are marked with an asterisk (*). All values are given as mean ± SD (as available). Sample sizes (n) are provided for reference.

^aZhao *et al.*,^[17] ^bGermain *et al.*,^[29] ^cLesage *et al.*,^[15] ^dHobson *et al.*,^[6] ^eKurle,^[16] ^fDrago *et al.*,^[18] ^gStricker *et al.*,^[19] ^hTyrrell *et al.*,^[36]
ⁱLipid-extracted prey samples were used for calculation of $\Delta^{15}\text{N}$ values.
^jThe TDF was estimated using the average offset between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in whiskers and RBCs in free ranging northern elephant seals collected upon return to the colony from a foraging trip (the present study).
^kSerum samples were collected twice, approximately 1 year apart and used to calculate an average TDF.
^lSamples were collected from rehabilitated animals.

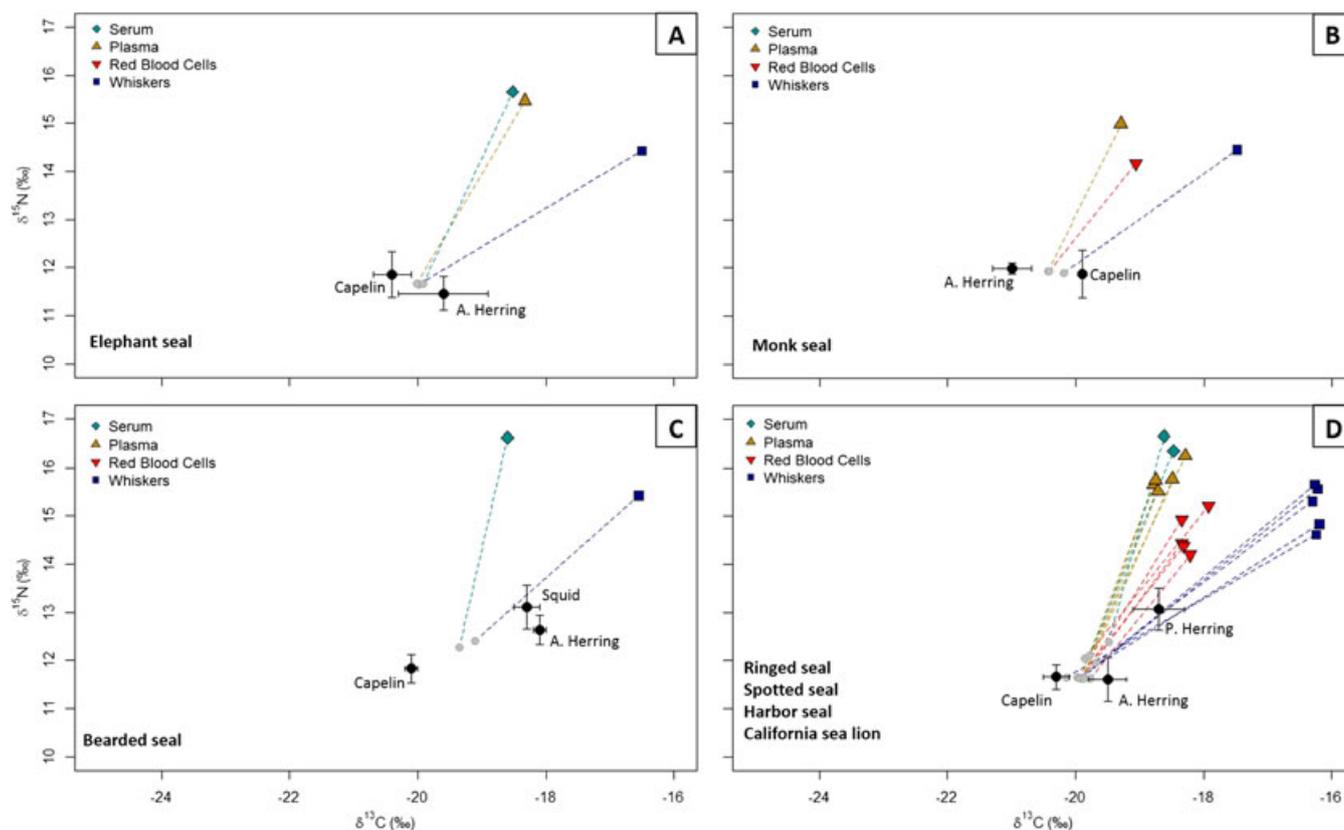


Figure 1. The relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SD) for lipid-corrected prey (black circles) and predator tissues (colored symbols) grouped by individuals with different food sources: (A) Northern elephant seal; (B) Hawaiian monk seal; (C) bearded seal; (D) ringed seal, spotted seal, harbor seal and California sea lion. Dashed lines represent the diet-to-tissue trophic discrimination factors ($\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$) by connecting calculated individual diet $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (gray circles) with individual tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

The diet-to-tissue $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values calculated from bulk prey were generally higher than reported in previous studies on pinnipeds (Table 4). The exception to this was that diet-to-blood $\Delta^{15}\text{N}$ values were $\sim 1\text{‰}$ lower than those reported by Kurle^[16] for northern fur seals (*Callorhinus ursinus*). The diet-to-whisker $\Delta^{15}\text{N}$ values (+2.6 to +4.0‰) of animals in our study were also lower than the mean value of +5.5‰ for captive sea otters (*Enhydra lutris*), but within the range reported for wild sea otters (+2.4 to +4.3‰).^[39] For all tissue types, the $\Delta^{13}\text{C}$ values calculated from lipid-corrected prey were within the range of calculated $\Delta^{13}\text{C}$ values from studies where the prey had been lipid-extracted, including the captive sea otter study.^[39] The one exception was that our estimates of the diet-to-RBC $\Delta^{13}\text{C}$ values (+1.4 to +1.9‰) were considerably greater than the value of +0.2‰ reported by Drago *et al.*^[18] for South American sea lions (*Otaria flavescens*). The similarities among ours and previous studies for estimates of $\Delta^{13}\text{C}$ values calculated using lipid-corrected prey suggest that differences among studies in prey lipid content may partially explain why we observed higher $\Delta^{13}\text{C}$ values from bulk prey than found in previous studies.

The noted differences between previously published $\Delta^{15}\text{N}$ values for marine carnivores and our $\Delta^{15}\text{N}$ values may have been related to a variety of factors associated with prey sampling or the prey themselves. We used whole prey instead of only fish muscle for the prey isotopic composition, whereas

several of these previous studies used only fish muscle.^[6,15,18] Fish muscle typically has higher $\delta^{15}\text{N}$ values than whole fish,^[31] which results in lower TDF values than those calculated using isotope ratios from whole fish.^[31] Lipid extraction of prey may also result in decreased $\Delta^{15}\text{N}$ values because common solvent-based methods also remove N-containing compounds, often resulting in higher $\delta^{15}\text{N}$ values in lipid-extracted than in non-lipid-extracted prey.^[22,23] Several studies appear to have used lipid-extracted prey for the calculation of both $\Delta^{13}\text{C}$ values and $\Delta^{15}\text{N}$ values,^[6,15,16] although the effect of lipid extraction on $\Delta^{15}\text{N}$ values may have been relatively small as lipid extraction has a larger effect on prey $\delta^{15}\text{N}$ values when whole prey and not muscle are used,^[22] and most of the previous studies used muscle. Lastly, differences in the isotope values or composition of the prey could have also caused variation in TDFs among studies. For example, negative relationships between the $\Delta^{15}\text{N}$ (and $\Delta^{13}\text{C}$) values and the $\delta^{15}\text{N}$ (and $\delta^{13}\text{C}$) values of prey have been found for a variety of taxonomic groups, including mammals.^[7,12] This relationship may explain why the diet-to-whisker $\Delta^{15}\text{N}$ values of captive sea otters were so much greater than for both captive and wild pinnipeds, as the $\delta^{15}\text{N}$ values of the top three sea otter prey items (6.1 to 10.5‰) were considerably lower than the prey $\delta^{15}\text{N}$ values in all pinniped studies. Alternatively, as suggested by Tyrrell *et al.*,^[36] these differences may have been due to differences in dietary protein quality or quantity.^[14,40,41]

CONCLUSIONS

The selection of TDFs for use in mixing models is not trivial and can significantly affect the estimated contribution of prey to the diet.^[10,11] By providing the first TDF values for five phocid and one otariid species, our study facilitates the use of mixing models for determining pinniped foraging patterns. These values are particularly important for Hawaiian monk seals, which are critically endangered, and the three Arctic seal species (bearded, ringed, and spotted) that are faced with a rapidly changing environment. Differences between our calculated $\Delta^{15}\text{N}$ values and those from previous studies highlight the need for researchers to carefully consider study methodology when selecting TDF values most appropriate for their study species. These differences also emphasize the need for additional studies to calculate TDFs for different age classes of pinnipeds under a range of dietary conditions, including those that closely mimic the diet of wild animals. Captive feeding studies have provided important information about the processes that govern the uptake of stable isotopes into tissues, and continue to enhance the use of stable isotope analysis as a powerful tool in ecology.

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REFERENCES

- [1] J. F. Kelly. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Can. J. Zool.* **2000**, *78*, 1.
- [2] K. Crawford, R. A. McDonald, S. Bearhop. Applications of stable isotope techniques to the ecology of mammals. *Mamm. Rev.* **2008**, *38*, 87.
- [3] B. S. Graham, P. L. Koch, S. D. Newsome, K. McMahon, D. Aurioles, Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems, in *Isoscapes: Understanding Movement, Pattern, and Process on Earth through Isotope Mapping*, (Eds: J. B. West, G. J. Bowen, T. E. Dawson, K. P. Tu). Springer, New York, New York, **2010**, pp. 1–487.
- [4] S. D. Newsome, M. T. Clementz, P. L. Koch. Using stable isotope biogeochemistry to study marine mammal ecology. *Mar. Mamm. Sci.* **2010**, *26*, 509.
- [5] L. Z. Gannes, D. M. O'Brien, C. Martínez del Rio. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology* **1997**, *78*, 1271.
- [6] K. A. Hobson, D. M. Schell, D. Renouf, E. Noseworthy. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Can. J. Fish. Aquat. Sci.* **1996**, *53*, 528.
- [7] S. Caut, E. Angulo, F. Courchamp. Variation in discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$): the effect of diet isotopic values and applications for diet reconstruction. *J. Appl. Ecol.* **2009**, *46*, 443.
- [8] C. Martínez del Rio, N. Wolf, S. A. Carleton, L. Z. Gannes. Isotopic ecology ten years after a call for more laboratory experiments. *Biol. Rev.* **2009**, *84*, 91.
- [9] S. Caut, E. Angulo, F. Courchamp. Caution on isotopic model use for analyses of consumer diet. *Can. J. Zool.* **2008**, *86*, 438.
- [10] A. L. Bond, A. W. Diamond. Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecol. Appl.* **2011**, *21*, 1017.
- [11] A. A. D. McLaren, G. J. Crawshaw, B. R. Patterson. Carbon and nitrogen discrimination factors of wolves and accuracy of diet inferences using stable isotope analysis. *Wildl. Soc. Bull.* **2015**, DOI <http://dx.doi.org/10.1002/wsb.599>.
- [12] S. Caut, E. Angulo, F. Courchamp. Discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$) in an omnivorous consumer: effect of diet isotopic ratio. *Funct. Ecol.* **2008**, *22*, 255.
- [13] K. A. Hobson, R. G. Clark. Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. *Condor* **1992**, *94*, 189.
- [14] C. T. Robbins, L. A. Felicetti, M. Sponheimer. The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. *Oecologia* **2005**, *144*, 534.
- [15] V. Lesage, M. Hammill, K. M. Kovacs. Diet-tissue fractionation of stable carbon and nitrogen isotopes in phocid seals. *Mar. Mamm. Sci.* **2002**, *18*, 182.
- [16] C. M. Kurle. Stable-isotope ratios of blood components from captive northern fur seals (*Callorhinus ursinus*) and their diet: applications for studying the foraging ecology of wild otariids. *Can. J. Zool.* **2002**, *80*, 902.
- [17] L. Zhao, D. M. Schell, M. A. Castellini. Dietary macronutrients influence ^{13}C and ^{15}N signatures of pinnipeds: captive feeding studies with harbor seals (*Phoca vitulina*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **2006**, *143*, 469.
- [18] M. Drago, V. Franco-Trecu, L. Cardona, P. Inchausti. Diet-to-female and female-to-pup isotopic discrimination in South American sea lions. *Rapid Commun. Mass Spectrom.* **2015**, *29*, 1513.
- [19] C. Stricker, A. Christ, M. Wunder, A. Doll, S. Farley, L. Rea, D. Rosen, R. Scherer, D. Tollit. Stable carbon and nitrogen isotope trophic enrichment factors for Steller sea lion vibrissae relative to milk and fish/invertebrate diets. *Mar. Ecol. Prog. Ser.* **2015**, *523*, 255.

- [20] N. Bodin, F. Le Loc'h, C. Hily. Effect of lipid removal on carbon and nitrogen stable isotope ratios in crustacean tissues. *J. Exp. Mar. Biol. Ecol.* **2007**, *341*, 168.
- [21] U. Focken, K. Becker. Metabolic fractionation of stable carbon isotopes: implications of different proximate compositions for studies of the aquatic food webs using $\delta^{13}\text{C}$ data. *Oecologia* **1998**, *115*, 337.
- [22] M. A. Sotiropoulos, W. M. Tonn, L. I. Wassenaar. Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissue: potential consequences for food web studies. *Ecol. Freshw. Fish* **2004**, *13*, 155.
- [23] D. M. Post, C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, C. G. Montaña. Getting to the fat of the matter: models, methods, and assumptions for dealing with lipids in stable isotope analysis. *Oecologia* **2007**, *152*, 179.
- [24] J. M. Logan, T. D. Jardine, T. J. Miller, S. E. Bunn, R. A. Cunjak, M. E. Lutcavage. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *J. Anim. Ecol.* **2008**, *77*, 838.
- [25] G. V. Hilderbrand, S. D. Farley, C. T. Robbins, T. A. Hanley, K. Titus, C. Servheen. Use of stable isotopes to determine diets of living and extinct bears. *Can. J. Zool.* **1992**, *74*, 2080.
- [26] S. Caut, S. Laran, E. Garcia-Hartmann, K. Das. Stable isotopes of captive cetaceans (killer whales and bottlenose dolphins). *J. Exp. Biol.* **2011**, *214*, 538.
- [27] D. K. Greaves, M. O. Hammill, J. D. Eddington, J. F. Schreer. Growth rate and shedding of vibrissae in the gray seal, *Halichoerus grypus*: a cautionary note for stable isotope diet analysis. *Mar. Mamm. Sci.* **2004**, *20*, 296.
- [28] R. Beltran, M. Connolly Sadou, R. Condit, S. Peterson, C. Reichmuth, D. Costa. Fine-scale whisker growth measurements can reveal temporal foraging patterns from stable isotope signatures. *Mar. Ecol. Prog. Ser.* **2015**, *523*, 243.
- [29] L. R. Germain, M. D. McCarthy, P. L. Koch, J. T. Harvey. Stable carbon and nitrogen isotopes in multiple tissues of wild and captive harbor seals (*Phoca vitulina*) off the California coast. *Mar. Mamm. Sci.* **2012**, *28*, 542.
- [30] J. D. Roth, K. A. Hobson. Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction. *Can. J. Zool.* **1996**, *78*, 848.
- [31] Y. Cherel, K. A. Hobson, S. Hassani. Isotopic discrimination between food and blood and feathers of captive penguins: implications for dietary studies in the wild. *Physiol. Biochem. Zool.* **2003**, *78*, 106.
- [32] S. Bearhop, S. Waldron, S. C. Votier, R. W. Furness. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiol. Biochem. Zool.* **2002**, *75*, 451.
- [33] K. A. Hobson, R. G. Clark. Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. *Condor* **1992**, *94*, 181.
- [34] L. L. Tieszen, T. W. Boutton, K. G. Tesdahl, N. A. Slade. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* **1983**, *57*, 32.
- [35] N. Lecomte, Ø. Ahlstrøm, D. Ehrich, E. Fuglei, R. A. Ims, N. G. Yoccoz. Intrapopulation variability shaping isotope discrimination and turnover: experimental evidence in arctic foxes. *PLoS One* **2011**, *6*, e21357.
- [36] L. P. Tyrrell, S. D. Newsome, M. L. Fogel, M. Viens, R. Bowden, M. J. Murray. Vibrissae growth rates and trophic discrimination factors in captive southern sea otters (*Enhydra lutris nereis*). *J. Mammal.* **2013**, *94*, 331.
- [37] L. Zhao, D. Schell. Stable isotope ratios in harbor seal *Phoca vitulina* vibrissae: effects of growth patterns on ecological records. *Mar. Ecol. Prog. Ser.* **2004**, *281*, 267.
- [38] S. Ashwell-Erickson, F. H. Fay, R. Elsner, D. Wartzok. Metabolic and hormonal correlates of molting and regeneration of pelage in Alaskan harbor and spotted seals (*Phoca vitulina* and *Phoca largha*). *Can. J. Zool.* **1986**, *64*, 1086.
- [39] S. D. Newsome, G. B. Bentall, M. T. Tinker, O. T. Oftedal, K. Ralls, J. A. Estes, M. L. Fogel. Variation in $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ diet-vibrissae trophic discrimination factors in a wild population of California sea otters. *Ecol. Appl.* **2010**, *20*, 1744.
- [40] S. F. Pearson, D. J. Levey, C. H. Greenberg, C. Martínez del Río. Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia* **2003**, *135*, 516.
- [41] S. T. Florin, L. A. Felicetti, C. T. Robbins. The biological basis for understanding and predicting dietary-induced variation in nitrogen and sulphur isotope ratio discrimination. *Funct. Ecol.* **2011**, *25*, 519.