Vol. 523: 255–266, 2015
doi: 10.3354/meps11205

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published March 16

Stable carbon and nitrogen isotope trophic enrichment factors for Steller sea lion vibrissae relative to milk and fish/invertebrate diets

C. A. Stricker[1,*], A. M. Christ[2], M. B. Wunder[3], A. C. Doll[3], S. D. Farley[2], L. D. Rea[2,4], D. A. S. Rosen[5], R. D. Scherer[3], D. J. Tollit[5,6]

[1]U.S. Geological Survey, Fort Collins Science Center, Denver, CO 80225, USA
[2]Alaska Department of Fish and Game, Division of Wildlife Conservation, Anchorage, AK 99518, USA
[3]Department of Integrative Biology, University of Colorado Denver, PO Box 173364, Denver, CO 80217-3364, USA
[4]Institute of Northern Engineering, University of Alaska Fairbanks, PO Box 755910, Fairbanks, AK 99775-5910, USA
[5]Marine Mammal Research Unit, University of British Columbia, 2202 Main Mall, Vancouver, British Columbia V6T 1Z4, Canada
[6]SMRU Canada Ltd, 3069 6th Avenue West, Vancouver, British Columbia V6K 1X4, Canada

ABSTRACT: Nutritional constraints have been proposed as a contributor to population declines in the endangered Steller sea lion *Eumetopias jubatus* in some regions of the North Pacific. Isotopic analysis of vibrissae (whiskers) is a potentially useful approach to resolving the nutritional ecology of this species because long-term (up to 8 yr) dietary information is sequentially recorded and metabolically inert once formed. Additionally, vibrissae are grown *in utero*, potentially offering indirect inference on maternal diet. However, diet reconstruction using isotopic techniques requires *a priori* knowledge of trophic enrichment factors (TEFs), which can vary relative to diet quality and among animal species. In this study, we provide new TEF estimates for (1) maternal relative to pup vibrissae during both gestation and nursing and (2) adult vibrissae relative to a complex diet. Further, we refine vibrissa–milk TEFs based on an additional 76 animals with an age distribution ranging from 1 to 20 mo. Mother–pup vibrissae TEF values during gestation and nursing were near zero for $\delta^{13}C$ and averaged 0.8 and 1.6‰, respectively, for $\delta^{15}N$. In contrast, vibrissa–fish/invertebrate TEFs averaged 3.3 (± 0.3 SD) and 3.7‰ (±0.3) for lipid-free $\delta^{13}C$ and $\delta^{15}N$, respectively. Average lipid-free $\delta^{13}C$ and $\delta^{15}N$ vibrissa–milk TEFs were 2.5 (±0.9) and 1.8‰ (±0.8), respectively, and did not differ among metapopulations. Empirically determined TEFs are critical for accurate retrospective diet modeling, particularly for evaluating the hypothesis of nutritional deficiency contributing to the lack of Steller sea lion population recovery in some regions of Alaska.

KEY WORDS: Pinniped · Stable isotopes · Fractionation · Diet · Vibrissae

INTRODUCTION

The western distinct population segment (DPS) of Steller sea lions *Eumetopias jubatus* (SSL), which ranges from the Gulf of Alaska to the Kuril Islands, has declined in recent decades and is currently protected under the Endangered Species Act (Bickham et al. 1996, Anonymous 1997, Loughlin 1997, Calkins et al. 1999). The cause of the decline is unknown; various inter-related hypotheses range from the ecosystem scale to individual SSL physiology (Guénette et al. 2006, Anonymous 2008, Atkinson et

*Corresponding author: cstricker@usgs.gov

al. 2008). Two of the more favored hypotheses relate to nutritional stress due to the inability of adult females to deliver adequate milk to large late-lactation pups or an inability of newly weaned pups and juveniles to acquire resources of sufficient quantity and quality while foraging (Merrick et al. 1997, Sease & Merrick 1997, Bickham et al. 1998, Atkinson et al. 2008, Rosen 2009; but see Calkins et al. 2013). Nutritional stress may or may not be related to anthropogenic impacts (Atkinson et al. 2008), but it could be the cause for decreased natality and/or juvenile survival rates for some metapopulations in the western DPS (Holmes et al. 2007). The effects of nutritional stress might be expressed as changes in nursing times, the timing of weaning, and foraging behavior by the young (Atkinson et al. 2008). It is critical to develop a more detailed understanding of SSL diets so that scientifically sound fisheries management policies can be developed. A number of techniques have been employed to meet this need by providing short-term (scat analysis) and long-term (fatty acids and stable isotopes) dietary inference (Newsome et al. 2010a, Tollit et al. 2010). However, these techniques are not without limitations and all require calibration coefficients (Stegall et al. 2008, Rosen & Tollit 2012).

The vibrissa of SSL grow sequentially over time, beginning *in utero*, and record dietary history for many years. Furthermore, vibrissa do not undergo reorganization once formed and, as such, are highly suitable for stable isotope analysis (Hirons et al. 2001, Stegall et al. 2008, Cherel et al. 2009, Habran et al. 2010, Newsome et al. 2010b). However, to exploit this tissue for isotope-based dietary inference, several key conditions are required. First, prey isotope spacing (i.e. endmember spacing) needs to be sufficiently broad such that quantitative statements about diet can be made (Newsome et al. 2007). Second, tissue–diet and tissue–tissue isotopic discrimination or trophic enrichment factors (TEFs) must be known. TEFs represent the net isotopic difference between consumer tissues and prey, which is the summation of the various vital isotope effects related to digestion, assimilation, and routing (Martínez del Rio et al. 2009, Wiley et al. 2010). For reasons of simplicity, we also refer to tissue–tissue isotope differences as TEFs but recognize that Δ may be more appropriate and therefore report tabular data in that format. Traditionally, TEF estimates are derived empirically from captive feeding studies or under well-constrained field conditions (Martínez del Rio et al. 2009, Wolf et al. 2009, Newsome et al. 2010b). The latter can prove challenging but likely yields more reliable estimates, as most laboratory-based experiments are focused on animals at basal metabolic activity and often lack dietary complexity. Lastly, the integration period of the focal tissue must be constrained, and in the case of sequentially growing tissues, such as vibrissa, growth rate information is required to properly reference diet history with respect to time, with recognition of potential ontogenetic rate changes (Hirons et al. 2001, Greaves et al. 2004, Robertson et al. 2013, Tyrrell et al. 2013, Rea et al. in press).

Gestation in SSL takes approximately 9 mo, during which time vibrissa grow *in utero*, followed by near synchronous range-wide births between 15 May and 15 July (Pitcher & Calkins 1981, Pitcher et al. 2001; Fig. 1A). Age of weaning has been more difficult to constrain but is thought to be between 9 mo and 3 yr, after which juvenile SSL free forage on a variety of fishes and cephalopods (Merrick et al. 1997, Sinclair & Zeppelin 2002, Winship & Trites 2003). Improvements in underwater capture techniques have aided researchers in studying sea lions younger than 3 yr old, but the capture of adult females remains difficult and expensive. However, adult female foraging history can be recovered indirectly from pup/juvenile vibrissa that record maternal dietary history during *in utero* growth and development through winter and spring (Fig. 1A). In order to estimate this, however, knowledge of the TEF for pup vibrissa relative to the adult female vibrissa (TEF_1) and of the TEF for adult female vibrissa relative to diet (TEF_2) is required (Fig. 1B). Nursing pups also potentially offer an opportunity to infer summer and fall maternal diet (Fig. 1A) if the TEF for pup vibrissa relative to milk (TEF_3) and for milk relative to the adult female diet (TEF_4) are known. Of all the potentially useful TEF values described, only TEF_3 has previously been estimated (Stegall et al. 2008). Further, building isotope chronologies (i.e. sampling along the vibrissa) from juvenile SSL vibrissa may also be used to infer age of weaning, as an isotopic shift is well documented in mammals when young are no longer nutritionally dependent (Jenkins et al. 2001, Polischuk et al. 2001, Stegall et al. 2008, Habran et al. 2010, Ben-David et al. 2012). Finally, the dietary habits of weaned SSL can also be reconstructed in older animals when samples are available from necropsy or if capture efforts are eventually extended to sub-adults, though this too requires the TEF for vibrissa relative to a complex fish and cephalopod diet (TEF_2). Hence, vibrissa potentially offer a wealth of dietary information, yet the assembly of long-term (1–8 yr) diet chronologies is relatively rare in the literature (Hobson et al.

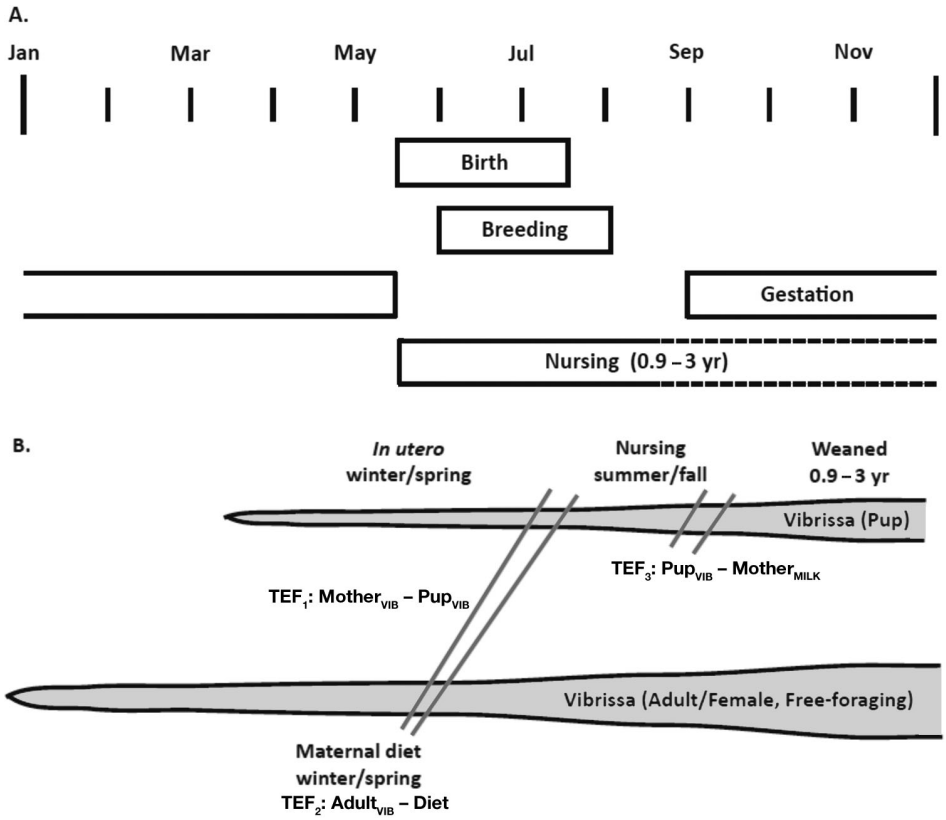

Fig. 1. (A) Generalized Steller sea lion reproductive phenology over a typical annual cycle. (B) Vibrissae-related trophic enrichment factors (TEFs) estimated in this study and required to derive dietary inference for free-ranging Steller sea lions. TEF_1: pup vibrissa – adult female (mother) vibrissa; TEF_2: adult female vibrissa – diet; TEF_3: pup vibrissa – mother milk. Headings above the pup whisker offer phenological context. Gray lines indicate hypothetical sampling and temporal alignment of pup and mother vibrissae sections

1996, Zhao & Schell 2004, Hall-Aspland et al. 2005, Lewis et al. 2006, Cherel et al. 2009, Newsome et al. 2009a, 2010b, Lowther et al. 2011, Newland et al. 2011, Kernaléguen et al. 2012). With the exception of Hirons et al. (2001), long-term chronologies have not yet been explored in detail for SSL but would be very informative for assessing hypotheses for population decline and lack of recovery related to nutritional stress.

The goal of this study was to estimate TEFs specific to vibrissa of pup and sub-adult/adult SSL. To accomplish this goal, we made empirical measurements on captive and free-ranging animals under well-constrained conditions, with the following objectives: First, derive TEF_1 from 4 mother–pup pairs captured on the northern Kuril Islands in the Russian Far East, where the pups were all less than 2 mo of age. Second, derive TEF_2 from a captive feeding study conducted on 4 sub-adult/adult SSL maintained on complex fish and squid diets at the Vancouver Aquarium (British Columbia, Canada). Lastly, revise previous estimates of TEF_3 (Stegall et al. 2008) by including an additional 76 milk–pup vibrissal root pairs.

MATERIALS AND METHODS

Mother–pup pairs

Vibrissae from 4 mother–pup SSL pairs captured on the northern Kuril Islands of the Russian Far East were collected in 2008 as part of a resource partitioning study (Waite et al. 2012). Isotopic analyses of vibrissae sections (0.2–4.0 mm) from these animals were used to derive TEF_1 (Fig. 1B). Vibrissae were cleaned using a 2:1 chloroform and methanol solution and air dried. The root bulbs were excised from individual vibrissae to avoid biasing (e.g. with inadvertently attached skin) isotopic measurements.

Stable carbon and nitrogen isotope analysis was conducted at the Alaska Stable Isotope Facility (University of Alaska Fairbanks, Fairbanks, Alaska, USA). Isotopic measurements were made using an elemental analyzer (Costech Analytical NC) interfaced to an isotope ratio mass spectrometer (Thermo Fisher, DeltaPlus XP) operated in continuous flow mode. Isotope values are expressed in delta (δ) notation:

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

where X represents ^{13}C or ^{15}N in parts per thousand deviation (‰) relative to a standard and R represents the isotope ratio of samples and standards, respectively. Isotopic data were normalized to Vienna PeeDee Belemnite (VPDB) and air using a calibrated internal peptone standard (Sigma Chemical; mean $\delta^{13}C = -15.8$‰ and mean $\delta^{15}N = 7.0$‰); precision was 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

Most adult female SSL vibrissae exhibit an annual cycle in C and N isotope profiles (Hirons et al. 2001, Rea et al. in press), similar to other pinnipeds (Hobson et al. 1996, Hall-Aspland et al. 2005, Lewis et al. 2006, Cherel et al. 2009, Lowther et al. 2011, Newland et al. 2011, Kernaléguen et al. 2012). Therefore, readily identifiable characteristics within the profiles, such as local maxima or minima, were used to calculate the length of vibrissae growth in an annual cycle (Fig. 2, see Appendix 1 in Rea et al. in press). Vibrissae growth rates (cm mo^{-1}) for each of the 4 adult females were calculated using this technique applied to the 2 most recent $\delta^{13}C$ peaks. Using this rate, we then calculated the date prior to capture corresponding to the location of the most recent $\delta^{13}C$ maximum preceding animal capture.

Steller sea lion pup vibrissae grow at a different rate than adult females and typically exhibit less distinct seasonal variation (Fig. 2; Rea et al. in press). Using methods similar to above, we identified the location of the pup isotope peak prior to capture. To this distance we assigned the same time as calculated for the associated mother. When rates were calculated assuming constant vibrissae growth between the peak location and capture, it was clear that the growth rate was not constant over this period because other identifiable features of the profiles were not co-located in time. To simplify the estimation of a growth model, we applied a piecewise-linear model that assumed an underlying quadratic function. Under such an assumption, the first two-thirds of the distance to the peak would grow on average at twice the rate as the last third, thus resulting in a simple algebraic solution to the growth rate over the 2 time periods. The growth rate for the remainder of the vibrissae toward the tip was not estimated because of uncertainty associated with early gestational growth.

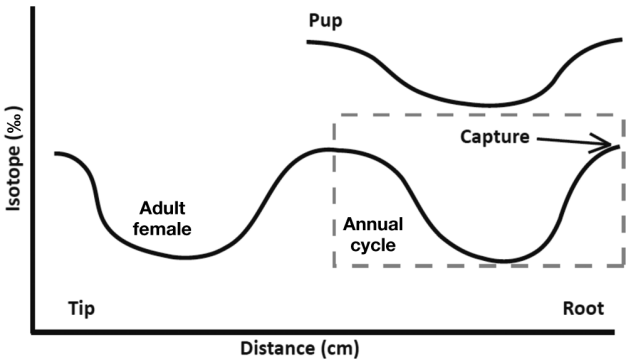

Fig. 2. Hypothetical vibrissae isotope profiles for adult female (mother)–pup pairs. Profiles illustrate a typical annual cycle (dashed gray box) observed in the vibrissae of Steller sea lions, though the amplitude is typically reduced in pups. Trophic enrichment factors for mother vibrissae relative to pup vibrissae (TEF_1; $\Delta X = \text{Mother}_{\text{vib}} - \text{Pup}_{\text{vib}}$) were estimated during gestation (1.6 to 4.7 cm from root; see 'Materials and methods') and nursing (near root)

Using the growth rates calculated for the mother–pup pairs, the vibrissae pairs were transformed from a measure of length to one of time. This allowed for a comparison of the profile values for sections approximately co-located in time between the root and the first peak in $\delta^{13}C$. We were interested in late gestational differences, so we derived TEF_1 corresponding to 3–4 mo prior to capture, or 1.6 to 4.7 cm from the pup's root depending upon vibrissa growth rates.

The points in time where each profile changed values usually did not overlap between the mother–pup pairs because of the nature of sampling in non-uniform vibrissae section lengths (i.e. vibrissae are tapered; thin sections required more length to achieve appropriate mass), in addition to the effect of transforming lengths to times. One could consider the union of cut points for the two as a shared set of cut points where the isotope value of at least one of the sections changes. Intuitively, TEF_1 would be calculated as a weighted average over each segment difference with the weights proportional to the relative size of each segment as determined by the shared set of cut points. It can readily be shown that this is mathematically equivalent to the difference between the weighted averages of each profile over the period of interest, and TEF_1 was calculated accordingly. It can similarly be shown that the measurement error of this weighted average is 2 times the measurement error common to each of the individual segments (±0.2‰).

Because isotope compositions were assessed as average values for a segment, there is an inherent loss of resolution of the underlying isotopic profile. To better account for the induced variability, we simulated the underlying profile as a smooth function based on the observed values with additional variance added as a function of the segment length (Rea et al. in press). These candidate profiles were then

accepted or rejected according to an importance sampling ratio which included the probability of observing the true data given the underlying candidate profile. Accepted candidates were then used to calculate TEF_1 by the method outlined previously. By generating numerous candidates for each of the pairs, we could then simulate a distribution for TEF_1, which would reflect potential error induced by incorrect alignment of the vibrissae due to misidentification of peak locations because we were observing segment-wise averages with known measurement error rather than the true underlying profile. We also calculated TEF_1 for nursing pups by comparing mother and pup profiles from the segments closest to the root. All modeling was performed in R (R Development Core Team 2008).

Controlled feeding experiment

One sub-adult (4 yr in age) and 3 adult (7 yr) female SSL were maintained on 1 of 2 controlled diets for 119–120 d (~4 mo) at the Vancouver Aquarium (British Columbia, Canada) from September 2006 through January 2007 (Rosen & Tollit 2012). The animals were captured as pups and trained to participate in a broad conservation research program. Group 1 animals (n = 2) were rationed a homogenate diet, which consisted of daily supplementation with specific proportions of Pacific herring (*Clupea pallasii*; 64% wet weight, see 'Results'), eulachon (*Thaleichthys pacificus*; 14%), rockfish (Pacific ocean perch *Sebastes alutus*; 7%), and squid (California market squid *Loligo opalescens*; 14%). Group 2 animals (n = 2) were rationed a mixed diet, which alternated within a week to consist of either solely Pacific herring, a mixture of herring and either squid or rockfish, or solely eulachon. The diet pattern of Group 2 was such that it matched the intake of Group 1 on a weekly basis. All daily rations were recorded and food intake (kJ $mass^{-0.7}$) was balanced between groups over 4 wk blocks (Rosen & Tollit 2012). Because this was not a classical diet switch experiment, we were only interested in deriving TEFs and the duration of the experiment (~4 mo) was sufficient such that the animal tissues of interest were assumed to have approached isotopic equilibrium with the respective diets. We did not attempt to derive isotopic half-life estimates for vibrissae. Trial durations were converted to mo, assuming an average of 30 d, to facilitate longitudinal interpretation of vibrissae. Vibrissae were collected from each animal at Day 279–280 or ~5 mo post-experiment.

Aliquots of diet sample homogenates (n = 5–10 per prey), archived frozen for each lot throughout the experiment, were freeze-dried and ground to a fine powder using a cryomill (Spex CertiPrep Freezer Mill 6750). Lipids were removed from a sub-sample by loading into 10 × 100 mm cellulose thimbles, followed by soxhlet extraction using an azeotropic solvent (2:1 chloroform and methanol) for a total duration of 6 h and air dried overnight. Vibrissa were cleaned using a 2:1 chloroform and methanol solution and air dried. Vibrissae were measured for total length and sectioned at 1.0–3.0 mm intervals using a chisel. Approximately 2 mg of sample material was transferred to a 5 × 9 mm tin capsule and crimp sealed.

Stable carbon and nitrogen isotope analysis of vibrissae from the controlled feeding study was conducted at the US Geological Survey Stable Isotope Laboratory (Denver, Colorado, USA). Isotopic measurements were made using an elemental analyzer (Carlo Erba NC1500) interfaced to an isotope ratio mass spectrometer (Micromass Optima) operated in continuous flow mode (Fry et al. 1992). Isotopic data, expressed as above, were normalized to VPDB and air using primary standards, USGS 40 (–26.24 and –4.52‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively) and USGS 41 (37.76 and 47.57 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively). Analytical error was < 0.2 ‰, assessed through replicate measures of primary standards across all analytical sequences. In-house standards and replicate measures of samples were used as quality control checks; reproducibility was better than 0.2 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. Accuracy was within 0.2 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ assessed by analysis of primary standards as unknowns.

The collection of vibrissae 5 mo post-experiment presented a challenge in the estimation of TEF_2. Vibrissa growth rates have been previously measured on 13 recaptured adult and sub-adult SSL between the 2 discrete capture events (Method B in Rea et al. in press). The growth rates ranged from 0.31 to 0.61 cm mo^{-1} and were re-sampled (with replacement; 10 000 iterations) to generate a vector of rates.

TEF_2 was derived in an iterative multi-step modeling approach, similar to that employed by Newsome et al. (2010b) but with the caveats of satisfying mass balance in isotope mixing space relative to empirically measured diet rations, and maintaining $\delta^{15}N$ and $\delta^{13}C$ covariance throughout the process. First, distributions of prey isotope values were generated by re-sampling (1000 iterations) the means and respective standard deviations. Daily rations (kg d^{-1}) over the duration of the experiment were collapsed into average weekly proportions and bootstrapped (1000 iterations) for each prey and for all 4 SSLs. Using the

distributions of proportional contributions and prey isotope values, composite diet isotope values were derived for each animal by re-sampling (1000 iterations). This assumed that all prey were equivalent in terms of digestibility and energy density; however, this has been shown not to be the case (Rosen & Trites 2000). From the distribution of vibrissa growth rates (see above) and SSL vibrissa isotope profiles, the distances from the vibrissal roots corresponding to the end of the experiment were estimated by re-sampling (1000 iterations) and the respective vibrissa isotope values extracted (Fig. 3). Because individual SSL diets were similar (proportionally and isotopically) and with no assumptions made with regard to isotopic routing and differential digestion, we then used the proportional prey data and vibrissa isotope data for each SSL (while maintaining $\delta^{13}C$ and $\delta^{15}N$ covariance) to generate a distribution (4000 iterations) of TEF_2 values that satisfied mass balance in bivariate isotope mixing space, where

$$Y + X = f_i P_i + f_j P_j + f_k P_k + f_l P_l \quad (2)$$

$$X = f_i P_i + f_j P_j + f_k P_k + f_l P_l - Y \quad (3)$$

and Y = vibrissa isotope value, $X = TEF_2$, f = proportional diet contributions, and P = prey isotope values for each respective prey (i, j, k, and l). This process was conducted on both bulk and lipid extracted prey C isotope data; only bulk N isotope data were used. All modeling was carried out in R (R Development Core Team 2008).

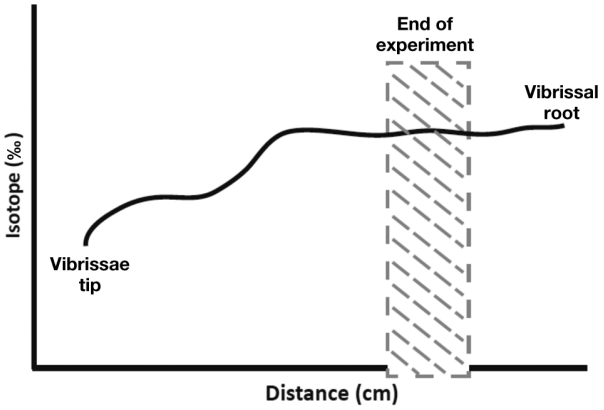

Fig. 3. The end point of the controlled feeding experiment (gray hatched area) was estimated by re-sampling the corresponding distance along the vibrissae using a growth rate (cm mo^{-1}) distribution (mean and SD) based on 2–5 yr old adult/sub-adult animals from which empirical estimates exist (see 'Materials and methods'). The 5 segments preceding the estimated distance from the root were then collated and used in the modeling of trophic enrichment factors for vibrissae relative to diet (TEF_2; see 'Materials and methods')

Refined vibrissa–milk discrimination estimates

Vibrissae and ingested milk samples were collected from free-ranging SSL pups as described in Stegall et al. (2008). All animals had recently suckled and milk samples were fresh. Including the data reported by Stegall et al. (2008), we provide revised estimates based on a total of 90 animals with an age range of ~1 to 20 mo: 13 from the Aleutian Islands, 29 from Prince William Sound, and 48 from southeast Alaska. Vibrissae were cleaned using the azeotropic solvent described above, skin excised from vibrissal roots, and ~1 mm sections cut using a chisel. The second section from the end of the root was used to represent the most recent diet since blood or other tissue could potentially be adhering to the growth surface of the root, which would confound the analysis. Milk samples were freeze-dried, ground to a fine powder using a glass stir rod, and a sub-sample was soxhlet extracted as described above to remove lipids. Aliquots of bulk milk were analyzed for $\delta^{15}N$ and $\delta^{13}C$; lipid-free milk was analyzed for $\delta^{13}C$ only. All isotopic analyses were conducted at the US Geological Survey Stable Isotope Laboratory as described above. TEF_3 was calculated by subtracting the milk from the vibrissal root isotope values, and we merged new data with those reported in Stegall et al. (2008). These data were then bootstrapped by region (n = 1000), and because 95% confidence intervals overlapped among regions, data were pooled to generate global TEF_3 estimates.

RESULTS

Mother–pup pairs

At late gestation, pup vibrissae were on average 0.8‰ higher for $\delta^{15}N$ and 0.4 ‰ lower for $\delta^{13}C$ compared to mother vibrissae (Table 1), though these data were not normally distributed, and the $\delta^{15}N$ TEF distribution in particular was strongly bimodal in one pair as an artifact of the particular observed data used to estimate peak location rather than as a result of an inherent bimodal structure. Results for TEF_1 of nursing pups (Table 1) indicated a slight decrease in $\delta^{13}C$ of ~0.1 ‰ and an increase in $\delta^{15}N$ of ~1.6 ‰ between late gestation and post parturition. This comparison is rather tentative, however, because the nursing values only represented single segment estimates from each of the 4 pairs. Although pups on average had lower $\delta^{13}C$ than the mothers, this was within analytical error, and the variance with only

Table 1. Mean and 95% confidence intervals for trophic enrichment factors (TEF_1) of pup vibrissae relative to adult female (mother) vibrissae during gestation (3–4 mo prior to capture; approx. March) and nursing (segment closest to root; n = 1), reported as capital delta values ($\Delta X = Mother_{vib} - Pup_{vib}$) in ‰

Mother–pup pair (Seal ID pair)	Gestation		Nursing	
	$\Delta^{13}C$	$\Delta^{15}N$	$\Delta^{13}C$	$\Delta^{15}N$
08–01	−0.7 (−1.0 to −0.6)	0.7 (0.5 to 1.1)	−0.3	1.5
08–11	−0.3 (−0.4 to −0.2)	1.1 (0.8 to 1.4)	−0.4	2.0
08–12	−0.3 (−0.5 to −0.1)	0.6 (−0.7 to 0.7)	0.2	1.5
08–13	−0.2 (−0.3 to 0.2)	0.8 (0.8 to 0.8)	0.0	1.6
Mean (95% CI)	−0.4 (−0.5 to −0.3)	0.8 (0.5 to 0.9)	−0.1 (−0.6 to 0.3)	1.6 (1.2 to 2.1)

4 samples is too great to statistically differentiate from zero (Table 1).

Controlled feeding experiment

The lipid content of prey was highest for eulachon and herring (~8–9% higher than rockfish and squid), but N content was 2–3% higher for rockfish and squid, resulting in molar C:N values of ~5.0 compared to ~7.5 for the former. The effect of lipid removal on median $\delta^{13}C$ was nearly double for eulachon and herring (~3 to 4‰) compared to rockfish and squid (~2‰). Re-sampled bulk prey isotope data were uniformly distributed and yielded median (median absolute deviation; MAD) prey $\delta^{13}C$ values that spanned a range of 2.3‰ (eulachon: −20.6 [0.3]‰, herring: −21.5 [0.4]‰, rockfish: −20.5 [0.5]‰, squid −19.2 [0.2]‰) and ranged 1.7‰ among median $\delta^{15}N$ values (eulachon: 14.7 [0.1]‰, herring: 13.0 [0.3]‰, rockfish: 13.9 [0.2]‰, squid: 13.4 [0.2]‰). Eulachon had the highest median (MAD) lipid-free $\delta^{13}C$ value (−17.3 [0.4]‰) followed by squid (−17.5 [0.3]‰), herring (−17.7 [0.3]‰), and rockfish (−18.7 [0.4]‰). Within homogenates (n = 5–10), isotopic variation depended on prey species but ranged from 0.9 to 1.8‰ and 1.0 to 1.4‰ for bulk and lipid-free $\delta^{13}C$, respectively, and between 0.4 and 1.3‰ for $\delta^{15}N$.

Bootstrapping of the proportional daily ration data yielded uniform distributions where the 2 diet treatments were equivalent for all animals: 0.143 (0.0) eulachon (median [MAD]), 0.643 (0.0) herring, 0.143 (0.0) rockfish, and 0.071 (0.0) squid. When combined with prey isotope values, median bulk and lipid-free composite diet isotope estimates did not differ among the animals.

Re-sampling of vibrissa growth rates from the 13 adult and sub-adult SSL resulted in a uniform distribution with a median value of 0.51 cm mo^{-1}; this distribution was used to place temporal constraints on the distance along the vibrissa (relative to the root) that corresponded to the end of the experiment, as vibrissae were pulled ~5 mo post-experiment. The modeled distances along the vibrissa were not normally distributed, but mean and median values did converge (2.9 and 3.0 cm, respectively) and were similar across all animals. Corresponding isotope values extracted from these regions of the vibrissa were also not normally distributed, though mean and median values converged, and distributions were similar among animals ($\delta^{13}C$ range: −14.2 to 14.8‰; $\delta^{15}N$ range: 16.6 to 17.5‰). TEF_2 model estimates were uniformly distributed though median and mean values converged; covariance was low between the 2 isotopes (Table 2). Lipid-free $\delta^{13}C$ TEF_2 estimates were nearly half that of bulk.

Refined vibrissa–milk discrimination estimates

There were no statistical differences among DPS for TEF_3, and pooling these data resulted in median (95% confidence interval) lipid-free $\delta^{13}C$ and bulk $\delta^{15}N$ TEF_3 estimates of 2.5‰ (1.0–4.3) and 1.6‰ (0.3–3.3), respectively (Table 3). These estimates were uniformly distributed and similar to those presented by Stegall et al. (2008). Milk was lipid-rich with median bulk $\delta^{13}C$ values 5.9‰ lower than lipid extracted splits.

Table 2. Mean (SD), median, and 95% confidence intervals for adult/sub-adult (n = 4) vibrissae (Vib) trophic enrichment factors (TEF_2) derived by satisfying mass balance of dietary proportions relative to bulk ($_B$) and lipid-extracted ($_{LE}$) diets. Summary statistics are reported as capital delta values (ΔX = Vib − Diet) in ‰

	Mean (SD)	Median	95% CI
$\Delta^{13}C_B$	6.5 (0.3)	6.5	5.8–7.2
$\Delta^{15}N_B$	3.7 (0.3)	3.6	3.1–4.4
$\Delta^{13}C_{LE}$	3.3 (0.3)	3.3	2.8–3.8

Table 3. Mean (SD), median, and 95% confidence intervals for trophic enrichment factors (TEF_3) derived from paired pup vibrissal (Vib) roots relative to bulk ($_B$) and lipid-extracted ($_{LE}$) milk samples (n = 90). Summary statistics are reported as capital delta values (ΔX = Vib − Diet) in ‰

	Mean (SD)	Median	95% CI
$\Delta^{13}C_B$	8.2 (0.8)	8.3	6.4–9.6
$\Delta^{15}N_B$	1.8 (0.8)	1.6	0.3–3.3
$\Delta^{13}C_{LE}$	2.5 (0.9)	2.5	1.0–4.3

DISCUSSION

The utility of stable isotopes to constrain diet or habitat use in free-ranging animals that utilize diverse and expansive ecosystems is well documented for fishes (Carlisle et al. 2012), reptiles (Reich et al. 2007, Vander Zanden et al. 2010, 2013), birds (Cherel & Hobson 2007, Wiley et al. 2012), and mammals (Cherel et al. 2009, Newsome et al. 2009b, Adams et al. 2010). However, knowledge of tissue–diet isotopic discrimination is a pre-requisite for making quantitative diet statements, and here we present new data specific to SSL vibrissae. Under carefully controlled field and laboratory conditions, we were able to estimate mother–pup vibrissae isotopic differences (TEF_1). Further, we estimated vibrissae–diet TEFs for mature animals maintained on a complex fish/squid diet (TEF_2) comparable to prey available to free-ranging western stock SSL. Lastly, TEF estimates for nursing pup vibrissal roots relative to milk have been revised by inclusion of an additional 76 paired samples (Stegall et al. 2008). Collectively, these data provide a more robust basis for the use of stable isotopes in reconstructing the diet of multiple age classes of SSL based on continuously growing vibrissae, which can record up to 8 yr of foraging history (Rea et al. in press). Such records have the potential to (1) improve our understanding of the nutritional ecology of this endangered species, (2) offer insight into habitat and resource use at the individual and DPS level, (3) fill knowledge gaps about seasonal and ontogenetic shifts in resource and habitat use, and (4) help identify key resources that may be in conflict with commercial fisheries and other marine resource industries.

Mother–offspring isotopic comparisons of paired tissues are seldom made (but see Jenkins et al. 2001, Dalerum et al. 2007, Miller et al. 2011), and this study represents only the second attempt for vibrissae (Lowther & Goldsworthy 2011). We have demonstrated that late gestation pup vibrissae were isotopically enriched in ^{15}N relative to maternal vibrissae (TEF_1), ~0.9‰ for $\delta^{15}N$. The offset for $\delta^{15}N$ is consistent with expectations for nursing animals (Jenkins et al. 2001, Polischuk et al. 2001, Stegall et al. 2008, Habran et al. 2010, Ben-David et al. 2012) but lower than a full trophic step for carnivores (i.e. 3–4‰), suggestive of potential biochemical routing differences specific to this tissue. In contrast, pup vibrissae $\delta^{13}C$ values were lower than maternal values by ~0.4‰. Whereas this difference approaches analytical uncertainty, the 95% percentile from simulations was −0.5 to −0.3‰. However, the estimated individual TEF_1 distributions were not always normally distributed. With one exception, which appeared multimodal, skewing was moderate. This result was not unexpected due to the variability in identifying peaks for alignment on a few of the profiles, which introduced error in aligning the mother–pup profiles as well as in estimating the mother's annual average vibrissa growth rate. The latter would have the effect of slightly changing the segments chosen for comparison. Although our late gestation simulations were based on only 4 mother–pup pairs, TEF_1 estimates were very similar to those for Australian sea lions based on 22 pairs, where average $\delta^{15}N$ and $\delta^{13}C$ differences were 1.3 and −0.2‰, respectively (Lowther & Goldsworthy 2011). Nursing pup vibrissae sections were similarly offset from maternal vibrissae, where $\delta^{15}N$ was on average 1.6‰ higher and −0.1‰ lower for $\delta^{13}C$; the subtle differences relative to late gestation likely reflects changes in dietary quality between *in utero* placental provisioning and milk ingestion during nursing (Jenkins et al. 2001).

Since pup vibrissae are markedly thinner than adult vibrissae, the larger sections necessary to achieve enough tissue mass for isotopic analysis, particularly toward the tip, result in increased uncertainty in locating alignment points with the maternal vibrissae. Simply using the midpoints of longer sections may introduce bias and does not account for the variability of using that location as an estimate of the peak location. This error is still present in sampling adult vibrissae but has a smaller effect because the lengths necessary to achieve mass requirements for isotope analyses are smaller.

A number of isotope-based captive feeding studies have been conducted on mature phocids (Hobson et al. 1996, Kurle 2002, Lesage et al. 2002, Zhao et al. 2006) and otariids (Kurle 2002) with an emphasis on blood components, but none involved SSL. Hobson et al. (1996) and Lesage et al. (2002) did make measurements for fur, but vibrissae–diet TEFs have only been estimated experimentally for phocid seals (Hobson et

al. 1996) and under controlled field conditions for sea otters *Enhydra lutris nereis* (Newsome et al. 2010b). Empirically measured vibrissae–diet TEF estimates for animals maintained on complex diets are very important for generalist predators like the SSL. While our experiment was originally conceived as a fatty acid calibration coefficient trial based on 2 discrete diet regimens (Rosen & Tollit 2012), we found no difference among diet groups and therefore pooled all 4 animals for TEF_2 estimation. Our results for sub-adult/adults indicate that vibrissae were isotopically enriched relative to diet by an average of 3.7 (±0.3 SD) and 3.3‰ (±0.3 SD) for $\delta^{15}N$ and lipid-free $\delta^{13}C$, respectively. Our estimates were similar to those for phocid seals but higher by 0.9 and 0.5‰ for $\delta^{15}N$ and lipid-free $\delta^{13}C$, respectively, though the reported phocid values represent pooled means for 3 species, all of which were maintained on a single prey (herring) diet (Hobson et al. 1996). Sea otter estimates derived from field observations were also similar for $\delta^{15}N$, where the population mean was 3.5‰ (±0.6 SD) (Newsome et al. 2010b). However, the bulk $\delta^{13}C$ TEF estimate for sea otters was 2.2‰ (±0.7 SD), which is very different than our estimate for SSL (6.5‰ [±0.4 SD]). We are not aware of other bulk $\delta^{13}C$ TEF estimates for marine mammals but contend that a relatively large TEF would be expected for keratinaceous tissues when animals are maintained on lipid-rich prey (but see Newsome et al. 2010b). Nevertheless, we present TEF_2 estimates relative to both bulk and lipid-free diet. In doing so, this will better facilitate diet estimation of free-ranging SSL based on literature-derived prey data, which typically employ lipid extraction.

To the best of our knowledge, our modeling approach for TEF_2 is novel and has not been applied in any other controlled feeding studies, though a similar extension was used to estimate the TEFs for sea otter vibrissae under carefully controlled field conditions (Newsome et al. 2010b). The real value in our approach relates to resolving TEFs for animals maintained on more than 2 prey for extended periods of time, which is uncommon in captive marine mammal studies (Hobson et al. 1996, Kurle 2002, Lesage et al. 2002, Zhao et al. 2006, Caut et al. 2011). For metabolically active tissues, captive animal studies typically emphasize an experimental design (i.e. diet switch) that facilitates the estimation of tissue turnover rates, though this has not been attempted for marine mammals. Further, in the case of metabolically inactive tissues, such as vibrissae, an experimental emphasis on TEFs rather than turnover is more appropriate.

There are a number of potential sources of error that may have affected our estimates of TEF_2. Similar to TEF_1, variation in vibrissae growth rates would influence the selection of intervals coinciding with the end of the controlled feeding experiment. We attempted to constrain growth rates (median = 0.51 cm mo^{-1}) using data from recaptured free-ranging adult and sub-adult females, and our empirical data were comparable to estimates for grey seals (mean = 0.72 cm mo^{-1}; Greaves et al. 2004), fur seals (mean = 0.22 cm mo^{-1}; Kernaléguen et al. 2012), harbor seals (range = 0.22–2.34 cm mo^{-1}, depending on season; Zhao & Schell 2004), and captive adult SSL (0.3–0.51 cm mo^{-1}; Hirons et al. 2001). However, because the captive animals in this study were maintained on complex diets for 4 mo, which was likely ample time to approach equilibrium conditions, some error in growth rate would not have had a major impact on our TEF estimates.

Other sources of error in the estimation of TEF_2 include 'tight' delta-space (i.e. prey isotope spacing), data distributions, and vibrissae sampling intervals. The difficulty in making quantitative statements about diet is well appreciated when endmembers are not distinctly different (Hopkins & Ferguson 2012). Our prey and isotopic data were relatively confined in bivariate space (3.2 and 2.0‰ for bulk $\delta^{13}C$ and $\delta^{15}N$, respectively, based on point estimates and SD), and this effect would typically influence the resolution of proportional diet estimates. However, because we knew the prey proportions *a priori*, the modeling approach was inverted to derive TEF_2 directly, though this approach remains no less susceptible to issues related to isotopic routing, particularly since the prey are known to differ with respect to digestibility and energy density (Rosen & Trites 2000). Nevertheless, our TEF_2 estimates are similar to other captive seal experiments. However, re-sampled data were typically uniform rather than normally distributed, which appears to be an artifact of physical sampling frequency along vibrissa (1–2 mm) and relatively low temporal variation in isotope values (collectively yielding longitudinal step functions rather than spline curves). Although this is not a major concern, it does impact our selection of metrics used to summarize those distributions.

Our refined TEF_3 estimates changed little from those previously published (Stegall et al. 2008), but error estimates are now more robust. In general, vibrissal roots were 2.5 ‰ higher for $\delta^{13}C$ compared to lipid-free milk and 1.8‰ higher for bulk $\delta^{15}N$. Whereas these vibrissae estimates are lower than those for adults/sub-adults maintained on a complex

fish/invertebrate diet, previous work on nursing pups hypothesized that the biochemical composition of SSL milk is near ideal, such that discrimination is comparatively low (Stegall et al. 2008). This disparity in TEFs related to diet composition also illustrates the critical need for empirically estimating TEFs to enhance the accuracy of diet reconstructions. Lastly, the lack of regional differences among TEF_3 estimates indicates that vibrissae–milk discrimination is invariant, regardless of differences in female dietary habits — a useful feature for future studies of SSL.

Using 3 independent data sets, we were able to estimate relevant TEFs for SSL vibrissae that will likely be very useful in diet reconstruction using stable isotope techniques. The emphasis on keratinaceous vibrissae owes to the fact that this tissue is metabolically inert once formed and provides a long-term record of diet. Serial sectioning of vibrissae has proven useful in the study of marine mammals (Lewis et al. 2006, Newsome et al. 2009a, 2010b), but it has been historically underutilized, particularly for SSL, yet holds great potential for recovering high resolution (i.e. days to weeks) information. It is this type of data that will facilitate formal hypothesis testing in the context of SSL population decline and recovery. Additionally, a number of studies have made inferences about seasonal movements based on vibrissae isotopic patterns, yet more robust growth rate estimates would increase temporal resolution on both diet and movement. Further, this could also be extended to important life history transitions, such as the age or timing of weaning. Lastly, at the individual level, vibrissae offer higher temporal resolution and increased data density relative to metabolically active tissues, which could be used to fill seasonal data gaps, infer diet diversity within and among DPS, quantify the degree of individual dietary specialization, and help identify potential commercial fishing conflicts which may impact prey availability.

Acknowledgements. We thank J. Waite, V. Burkanov and R. Andrews for logistical and financial support for the collection of samples in Russia. Research in Russia was conducted under permits from Russian permitting agencies, the Federal Veterinary and Agricultural Control Service (Rosselkhoznadzor, Sakhalin Region), Sakhalin-Kuril Territorial Department of the Federal Committee of Fisheries of Russia (Goskomrybolovstvo), and the Federal Environmental Control Service (Rosprirodnadzor) of the Ministry of Natural Resources of Russia. This research was approved by the Institutional Animal Care and Use Committee of the Alaska SeaLife Center, and was funded by grants from NOAA to the Alaska SeaLife Center and the National Marine Mammal Laboratory. Additional funding and logistical support was provided by North Pacific Wildlife Consulting. We are grateful for the assistance of the Marine Mammal Energetics and Nutrition Laboratory staff and thank the Vancouver Aquarium for their support. Funding was provided by the North Pacific Marine Science Foundation and NOAA, and studies were carried out under Animal Care Permits from the University of British Columbia. We appreciate the hard work of M. Drier (USGS) and the Alaska Stable Isotope Facility for sample preparation and stable isotope analyses. We also thank V. Stegall, J. Hayes, K. Kokx, and P. Rivera for laboratory preparation of free-ranging SSL vibrissae samples and the dedicated field teams from the Alaska Department of Fish and Game (ADFG) Steller Sea Lion Program, the Alaska SeaLife Center Transient Juvenile Program, and the Russian Marine Mammal Ecology Program for sample collection. Funding was provided through NOAA Cooperative Agreements. ADFG research was conducted under Marine Mammal Protection Act permits numbers 358-1564, 358-1769 and 358-1888 and ADFG ACUC #03-002 and #06-07. Peggy Ostrom and 3 anonymous reviewers provided welcome feedback on earlier drafts of this manuscript. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

LITERATURE CITED

Adams LG, Farley SD, Stricker CA, Demma DJ, Roffler GH, Miller DC, Rye RO (2010) Are inland wolf/ungulate communities in Alaska influenced by marine subsidies of Pacific salmon? Ecol Appl 20:251–262

Anonymous (1997) Threatened fish and wildlife: change in listing status of Steller sea lions under the Endangered Species Act. Fed Regist 62:24345–24355. http://epa.gov/fedrgstr/EPA-SPECIES/1997/May/Day-05/e11668.htm

Anonymous (2008) Recovery plan for the Steller sea lion (*Eumetopias jubatus*). Revision. National Marine Fisheries Service, Silver Spring, MD

Atkinson S, DeMaster DP, Calkins DG (2008) Anthropogenic causes of the western Steller sea lion *Eumetopias jubatus* population decline and their threat to recovery. Mammal Rev 38:1–18

Ben-David M, Newsome SD, Whiteman JP (2012) Lipid and amino acid composition influence incorporation and discrimination of ^{13}C and ^{15}N in mink. J Mammal 93: 399–412

Bickham JW, Patton JC, Loughlin TR (1996) High variability for control-region sequences in a marine mammal: implications for conservation and biogeography of Steller sea lions (*Eumetopias jubatus*). J Mammal 77:95–108

Bickham JW, Loughlin TR, Calkins DG, Wickliffe JK, Patton JC (1998) Genetic variability and population decline in Steller sea lions from the Gulf of Alaska. J Mammal 79: 1390–1395

Calkins DG, McAllister DC, Pitcher KW, Pendleton GW (1999) Steller sea lion status and trend in southeast Alaska: 1979–1997. Mar Mamm Sci 15:462–477

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

Carlisle AB, Kim SL, Semmens BX, Madigan DJ and others (2012) Using stable isotope analysis to understand the migration and trophic ecology of northeastern Pacific white sharks (*Carcharodon carcharias*). PLoS ONE 7: e30492

Caut S, Laran S, Garcia-Hartmann E, Das K (2011) Stable isotopes of captive cetaceans (killer whales and bottlenose dolphins). J Exp Biol 214:538–545

Cherel Y, Hobson KA (2007) Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. Mar Ecol Prog Ser 329:281–287

Cherel Y, Kernaléguen L, Richard P, Guinet C (2009) Whisker isotopic signature depicts migration patterns and multi-year intra- and inter-individual foraging strategies in fur seals. Biol Lett 5:830–832

Dalerum F, Bennett NC, Clutton-Brock TH (2007) Longitudinal differences in ^{15}N between mothers and offspring during and after weaning in a small cooperative mammal, the meerkat (*Suricata suricatta*). Rapid Commun Mass Spectrom 21:1889–1892

Fry B, Brand W, Mersch FJ, Tholke K, Garritt R (1992) Automated analysis system for coupled $\delta^{13}C$ and $\delta^{15}N$ measurements. Anal Chem 64:288–291

Greaves DK, Hammill MO, Eddington JD, Pettipas D, Schreer JF (2004) Growth rate and shedding of vibrissae in the gray seal, *Halichoerus grypus*: a cautionary note for stable isotope diet analysis. Mar Mamm Sci 20: 296–304

Guénette S, Heymans SJJ, Christensen V, Trites AW (2006) Ecosystem models show combined effects of fishing, predation, competition, and ocean productivity on Steller sea lions (*Eumetopias jubatus*) in Alaska. Can J Fish Aquat Sci 63:2495–2517

Habran S, Debier C, Crocker DE, Houser DS, Lepoint G, Bouquegneau JM, Das K (2010) Assessment of gestation, lactation and fasting on stable isotope ratios in northern elephant seals (*Mirounga angustirostris*). Mar Mamm Sci 26:880–895

Hall-Aspland SA, Rogers TL, Canfield RB (2005) Stable carbon and nitrogen isotope analysis reveals seasonal variation in the diet of leopard seals. Mar Ecol Prog Ser 305: 249–259

Hirons AC, Schell DM, St. Aubin DJ (2001) Growth rates of vibrissae of harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*). Can J Zool 79:1053–1061

Hobson KA, Schell DM, Renouf D, Noseworthy E (1996) 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 53:528–533

Holmes EE, Fritz LW, York AE, Sweeney K (2007) Age-structured modeling reveals long-term declines in the natality of western Steller sea lions. Ecol Appl 17: 2214–2232

Hopkins JB, Ferguson JM (2012) Estimating the diets of animals using stable isotopes and a comprehensive Bayesian mixing model. PLoS ONE 7:e28478

Jenkins SG, Partridge ST, Stephenson TR, Farley SD, Robbins CT (2001) Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. Oecologia 129:336–341

Kernaléguen L, Cazelles B, Arnould JPY, Richard P, Guinet C, Cherel Y (2012) Long-term species, sexual and individual variations in foraging strategies of fur seals revealed by stable isotopes in whiskers. PLoS ONE 7: e32916

Kurle CM (2002) 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 80:902–909

Lesage V, Hammill MO, Kovacs KM (2002) Diet-tissue fractionation of stable carbon and nitrogen isotopes in phocid seals. Mar Mamm Sci 18:182–193

Lewis R, O'Connell TC, Lewis M, Campagna C, Hoelzel AR (2006) Sex-specific foraging strategies and resource partitioning in the southern elephant seal (*Mirounga leonine*). Proc R Soc Lond B Biol Sci 273:2901–2907

Loughlin TR (1997) Using the phylogeographic method to identify Steller sea lion stocks. In: Dizon AE, Chivers SJ, Perrin WF (eds) Molecular genetics of marine mammals. Allen Press, Lawrence, KS, p 159–171

Lowther AD, Goldsworthy SD (2011) Detecting alternate foraging ecotypes in Australian sea lion (*Neophoca cinerea*) colonies using stable isotope analysis. Mar Mamm Sci 27: 567–586

Lowther AD, Harcourt RG, Hamer DJ, Goldsworthy SD (2011) Creatures of habit: foraging habitat fidelity of adult female Australian sea lions. Mar Ecol Prog Ser 443: 249–263

Martínez del Rio C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev Camb Philos Soc 84:91–111

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

Miller JF, Millar JS, Longstaffe FJ (2011) Stable nitrogen and carbon isotope discrimination between juveniles and adults in an income-breeding small mammal (*Peromyscus maniculatus*). Mamm Biol 76:563–569

Newland C, Field IC, Cherel Y, Guinet C, Bradshaw CJA, McMahon CR, Hindell MA (2011) Diet of juvenile southern elephant seals reappraised by stable isotopes in whiskers. Mar Ecol Prog Ser 424:247–258

Newsome SD, Martinez del Rio C, Bearhop S, Phillips DL (2007) A niche for isotopic ecology. Front Ecol Environ 5: 429–436

Newsome SD, Tinker MT, Monson DH, Oftedal O and others (2009a) Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). Ecology 90:961–974

Newsome SD, Etnier MA, Monson DH, Fogel ML (2009b) Retrospective characterization of ontogenetic shifts in killer whale diets via $\delta^{13}C$ and $\delta^{15}N$ analysis of teeth. Mar Ecol Prog Ser 374:229–242

Newsome SD, Clementz MT, Koch PL (2010a) Using stable isotope biogeochemistry to study marine mammal ecology. Mar Mamm Sci 26:509–572

Newsome SD, Bentall GB, Tinker MT, Oftedal OT, Ralls K, Estes JA, Fogel ML (2010b) Variation in $\delta^{13}C$ and $\delta^{15}N$ diet–vibrissae trophic discrimination factors in a wild population of California sea otters. Ecol Appl 20: 1744–1752

Pitcher KW, Calkins DG (1981) Reproductive biology of Steller sea lions in the Gulf of Alaska. J Mammal 62: 599–605

Pitcher KW, Burkanov VN, Calkins DG, Le Boeuf BJ, Mamaev EG, Merrick RL, Pendleton GW (2001) Spatial and temporal variation in the timing of births of Steller sea lions. J Mammal 82:1047–1053

Polischuk SC, Hobson KA, Ramsay MA (2001) Use of stable-carbon and nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. Can J Zool 79: 499–511

R Development Core Team (2008) R: a language and environment for statistical computing. Vienna. www.R-project.org

Rea LD, Christ AM, Hayden AB, Stegall V and others (in press) Age-specific vibrissae growth rates: a tool for determining the timing of ecologically important events in Steller sea lions. Mar Mamm Sci, doi:10.1111/mms.12221

Reich KJ, Bjorndal KA, Bolten AB (2007) The 'lost years' of green turtles: using stable isotopes to study cryptic lifestages. Biol Lett 3:712–714

Robertson A, McDonald RA, Delahay RJ, Kelly SD, Bearhop S (2013) Whisker growth in wild Eurasian badgers *Meles meles*: implications for stable isotope and bait marking studies. Eur J Wildl Res 59:341–350

Rosen DAS (2009) Steller sea lions *Eumetopias jubatus* and nutritional stress: evidence from captive studies. Mammal Rev 39:284–306

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

Rosen DAS, Trites AW (2000) Digestive efficiency and dry-matter digestibility of Steller sea lions fed herring, pollock, squid, and salmon. Can J Zool 78:234–239

Sease JL, Merrick RL 1997. Status and population trends of Steller sea lions. In: Stone J, Goebel G, Webster S (eds) Pinniped populations, eastern North Pacific: status, trends and issues. Symposium of the American Fisheries Society 127th Annual Meeting, Monterey, CA, 28 August 1997. American Fisheries Society, Bethesda, MD, p 22–30

Sinclair EH, Zeppelin TK (2002) Seasonal and spatial differences in diet in the western stock of Steller sea lions (*Eumetopias jubatus*). J Mammal 83:973–990

Stegall VK, Farley SD, Rea LD, Pitcher KW and others (2008) Discrimination of carbon and nitrogen isotopes from milk to serum and vibrissae in Alaska Steller sea lions (*Eumetopias jubatus*). Can J Zool 86:17–23

Tollit DJ, Pierce GJ, Hobson KA, Bowen WD, Iverson SJ (2010) Diet. In: Boyd IL, Bowen WD, Iverson SJ (eds) Marine mammal ecology and conservation: a handbook of techniques. Oxford University Press, Oxford, p 191–221.

Tyrrell LP, Newsome SD, Fogel ML, Viens M, Bowden R, Murray MJ (2013) Vibrissae growth rates and trophic discrimination factors in captive southern sea otters (*Enhydra lutris nereis*). J Mammal 94:331–338

Vander Zanden HB, Bjorndal KA, Reich KJ, Bolten AB (2010) Individual specialists in a generalist population: results from a long-term stable isotope series. Biol Lett 6: 711–714

Vander Zanden HB, Bjorndal KA, Bolten AB (2013) Temporal consistency and individual specialization in resource use by green turtles in successive life stages. Oecologia 173:767–777

Waite JN, Trumble SJ, Burkanov VN, Andrews RD (2012) Resource partitioning by sympatric Steller sea lions and northern fur seals as revealed by biochemical dietary analyses and satellite telemetry. J Exp Mar Biol Ecol 416–417:41–54

Wiley AE, Ostrom PH, Stricker CA, James HF, Gandhi H (2010) Isotopic characterization of flight feathers in two pelagic seabirds: sampling strategies for ecological studies. Condor 112:337–346

Wiley AE, Welch AJ, Ostrom PH, James HF and others (2012) Foraging segregation and genetic divergence between geographically proximate colonies of a highly mobile seabird. Oecologia 168:119–130

Winship AJ, Trites AW (2003) Prey consumption of Steller sea lions (*Eumetopias jubatus*) off Alaska: How much prey do they require? Fish Bull 101:147–167

Wolf N, Carleton SA, Martinez del Rio C (2009) Ten years of experimental animal isotopic ecology. Funct Ecol 23: 17–26

Zhao L, Schell DM (2004) Stable isotope ratios in harbor seal *Phoca vitulina* vibrissae: effects of growth patterns on ecological records. Mar Ecol Prog Ser 281:267–273

Zhao L, Schell DM, Castellini MA (2006) Dietary macronutrients influence $\delta^{13}C$ and $\delta^{15}N$ signatures of pinnipeds: captive feeding studies with harbor seals (*Phoca vitulina*). Comp Biochem Physiol A 143:469–478

Editorial responsibility: Yves Cherel, Villiers-en-Bois, France

Submitted: July 21, 2014; Accepted: January 13, 2015
Proofs received from author(s): March 6, 2015