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Vibrissae growth rates and trophic discrimination factors in captive southern sea otters (*Enhydra lutris nereis*)

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Isotopic analysis of serially sampled vibrissae (whiskers) is a powerful method to investigate changes in an individual's resource and habitat use over time, which is difficult or impossible to accomplish using traditional dietary proxies such as observation or scat analysis. A vibrissae-based isotopic approach is limited by knowledge of vibrissae growth rates, which are required to determine the time period represented by each subsampled segment. Likewise, determining the magnitude of, and variation in, isotopic differences between a consumer and its diet, commonly referred to as trophic discrimination factors (TDFs), is a crucial step in quantifying diet composition using stable isotopes. TDF estimates are available only for a few mammalian taxa. We measured vibrissae growth rates and $\delta^{13}C$ and $\delta^{15}N$ TDFs in captive southern sea otters (*Enhydra lutris nereis*). Sea otters were administered ^{15}N-enriched glycine intravenously and vibrissae were collected periodically and serially sampled for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis. Growth of adult sea otter vibrissae was linear with a mean ($\pm SD$) rate of 7.7 ($\pm$ 1.2) cm/year. Mean ($\pm SD$) whole diet–vibrissae TDFs were 2.8‰ ($\pm$0.2‰) for $\delta^{13}C$ and 5.5‰ ($\pm$0.2‰) for $\delta^{15}N$. Mean ($\pm SD$) lipid-extracted diet–vibrissae TDFs were 2.4‰ ($\pm$0.2‰) for $\delta^{13}C$ and 4.9‰ ($\pm$0.3‰) for $\delta^{15}N$. $\delta^{13}C$ TDFs were similar to previously reported values for mammalian carnivores, but $\delta^{15}N$ TDFs were higher than expected. These results will increase the accuracy of isotopic diet analyses of mustelids and other carnivores for which there are few estimates of TDFs and no estimates of vibrissae growth rates.

Key words: *Enhydra lutris*, sea otters, stable isotopes, trophic discrimination factor, vibrissae growth rate, whiskers

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Stable isotope analysis has quickly become a useful tool in studies of diet composition, nutritional status, and trophic ecology (e.g., Wolf et al. 2009). Stable isotope analysis is a valuable complement to traditional techniques (e.g., observation and stomach and fecal contents) used to study diet, especially for elusive species for which large dietary data sets using these other methods are time consuming and expensive to collect. Another valuable aspect of stable isotope analysis is that it can provide ecological information over a variety of temporal scales. For example, splanchnic organs (e.g., liver and plasma proteins) have high protein turnover rates, thus yielding ecological information on the scale of days to weeks (Hobson and Clark 1993). In contrast, tissues such as bone collagen have low protein turnover rates, and provide dietary information on the scale of months to years (Koch 2008; Tieszen et al. 1983). Metabolically inert tissues that continuously grow (e.g., pelage, vibrissae, and teeth) can be serially sampled to provide a time line of ecological information where the proximal end represents recent isotopic incorporation and the distal end represents isotopic incorporation from further in the past. For mammals, vibrissae (whiskers) are rapidly becoming an informative isotopic substrate for analyzing temporal dietary variation, individual dietary special-

ization, and habitat use in wild mammal populations (Cherel et al. 2009; Lewis et al. 2006; Newsome et al. 2009).

The accurate interpretation of isotopically derived temporal information along a serially sampled vibrissa requires estimates of vibrissae growth rates and whether vibrissae grow in a linear versus nonlinear pattern. In linear growth, segments of equal length represent equal time, but in nonlinear von Bertalanffy growth, segments near the distal tip represent higher temporal resolution than segments of equal length near the proximal root. ^{13}C-, ^{15}N-, and deuterium-enriched isotopic labels have been used to determine isotopic incorporation rates in metabolically active tissues (Doherty et al. 2005; Wu et al. 2004) and growth rates of metabolically inert tissues, including vibrissae (Greaves et al. 2004; Hall-Aspland et al. 2005; Hirons et al. 2001; Zhao and Schell 2004). To our knowledge, studies of growth rates or growth patterns of vibrissae in mammalian carnivores are limited to pinnipeds. These studies reported simple linear growth rates in *Eumetopias jubatus* (Hirons et al. 2001) and *Phoca vitulina* (Hirons et al. 2001; Zhao and Schell 2004) and nonlinear von Bertalanffy growth rates in *Halichoerus grypus* (Greaves et al. 2004) and *Hydrurga leptonyx* (Hall-Aspland et al. 2005).

Accurate application of isotopic tools in ecology also requires knowledge of physiologically mediated isotopic differences between a consumer and its diet, known as trophic discrimination factors (TDFs), for the specific organism and tissue (e.g., blood, muscle, and pelage) being studied and analyzed. TDFs are the difference between the isotope value of a consumer's tissue and the average isotope value of dietary sources, and are typically positive for carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopes because animals preferentially excrete the lighter isotope (^{12}C or ^{14}N) during respiration and protein metabolism (Blair et al. 1985; Deniro and Epstein 1981). TDFs differ among tissues due to variations in amino acid composition (Schmidt et al. 2004), and also can vary within tissues as a function of growth rate (Gaye-Siessegger et al. 2003), nutritional status (Fuller et al. 2004), reproductive status (Fuller et al. 2005), or nitrogen excretion pathways (e.g., ureotelic versus ammonotelic—Vanderklift and Ponsard 2003).

Trophic discrimination factors have been measured in a number of mammalian carnivores in both captive and wild settings (captive pinnipeds [Hobson et al. 1996; Kurle 2002; Lesage et al. 2002; Zhao et al. 2006], captive red foxes [*Vulpes vulpes*—Roth and Hobson 2000], wild wolves [*Canis lupus*—Fox-Dobbs et al. 2007], and wild sea otters [*Enhydra lutris*—Newsome et al. 2010]). In these studies, δ^{13}C TDFs for tissues such as blood, liver, and muscle typically range from +0‰ to 2‰, but increase to +2–3‰ for keratinous tissues (e.g., pelage and vibrissae) and +3–5‰ for bone collagen. δ^{15}N TDFs are generally higher than those for δ^{13}C, and vary among the species in these studies, ranging from +2‰ to 5‰.

Vibrissae provide ecologists with a noninvasive, efficient, and cost-effective tool for analyzing variation, seasonality, and specialization in the diets of mammals (Lowther et al. 2011; Newsome et al. 2009). Because sea otters are a protected keystone species (sensu Power et al. 1996), it is especially useful to have a reliable, noninvasive tool to assess individual- and population-level diet, which could aid management efforts and inform questions regarding the ecological causes and consequences of dietary variation and specialization. But accurate interpretation of vibrissae isotope data requires growth rates to place ecological information within the appropriate time frames and known TDFs of δ^{13}C and δ^{15}N to match consumer isotope values with those of their potential food. Here we use a ^{15}N-enriched glycine label in an experiment to determine vibrissa growth rates in captive southern sea otters (*E. lutris nereis*) at the Monterey Bay Aquarium (Monterey, California). We also quantify diet–vibrissae TDFs in a controlled feeding study where the relative proportions of food and diet isotopic composition were quantified on a monthly basis.

Materials and Methods

Vibrissae growth rates.—Four adult female captive southern sea otters (*E. lutris nereis*) at the Monterey Bay Aquarium (Monterey, California) were intravenously administered a glycine label enriched in ^{15}N (98%; Cambridge Isotope Laboratories, Andover, Massachusetts), in a solution of 100 mg/ml sterile saline at a dosage of 5 mg glycine/kg body mass. One adult otter (Rosa) was injected only once, and the other 3 adult otters (Joy, Maggie, and Mae) where injected twice, the 2nd injection coming 4 months after the initial injection. Vibrissae were plucked 4, 6, 8, 12, and 16 months after the initial glycine injection. All procedures were approved by the Institutional Animal Care and Use Committee of Monterey Bay Aquarium (IACUC permit 93-R-0476), and adhered to guidelines for animal care and use adopted by the American Society of Mammalogists (Sikes et al. 2011).

Vibrissae were washed in a 2:1 chloroform : methanol solution to remove surficial contaminants and cut into 0.30 ($\pm$0.04 *SD*)-mg segments using nail clippers from base to tip. Length of vibrissae was measured every 3rd segment. Carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope values were determined using a Carlo Erba elemental analyzer (NC 2500; Carlo Erba, Milan, Italy) interfaced with a Thermo Finnigan Delta^{PLUS} XL mass spectrometer (Thermo Electron Corp., Waltham, Massachusetts) or a 4010 Costech elemental analyzer (Costech, Valencia, California) interfaced with a Thermo Finnigan Delta^{PLUS} XP (Thermo Electron Corp.). Isotopic results are expressed as δ values, where $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = 1{,}000 \times [(R_{sample}/R_{standard}) - 1]$, where R_{sample} and $R_{standard}$ are the ^{13}C/^{12}C or ^{15}N/^{14}N ratios of the sample and standard, respectively.

The sharp increase in δ^{15}N values caused by glycine label served as a marker indicating its injection date, allowing us to measure new growth from vibrissa follicle to δ^{15}N spike over a known time frame. Vibrissae growth rates are expressed as centimeters per year by the equation:

$$\frac{365}{\text{days since injection}} \times \text{base to spike length.}$$

Trophic discrimination factors.—In a captive feeding experiment, monthly diets were recorded for 1 female pup

(Kit) and 5 adult female (Maggie, Mae, Rosa, Joy, and Toola) captive southern sea otters at the Monterey Bay Aquarium (Monterey, California). Otters were fed a combination of live prey, including blue mussels (*Mytilus edulis*), manila clams (*Tapes philippinarum*), and red rock crabs (*Cancer productus*) and also thawed, frozen food, including Atlantic surf clams (*Spisula solidissima*), California market squid (*Loligo opalescens*), Pacific white shrimp (*Penaeus vannamei*), and black tiger prawn (*Penaeus monodon*). The pup also was fed formula consisting of Atlantic surf clam (*Spisula solidissima*), Zoologic 33/40 (PetAg, Hampshire, Illinois), and water for 4 weeks from 5 January 2010 to 3 February 2010. All food items were weighed prior to being offered to otters. When on exhibit, otters were fed directly to their chest. But when housed off exhibit, food was scatter distributed. Uneaten food (~8% of food offered) was recovered during tank cleanings every 4–5 days, and amount of food consumed was calculated as grams of food offered less grams of uneaten food recovered during tank cleanings.

Twenty samples of whole surf clam and surf clam foot, 5 samples of formula, and 10 samples from every other food type were prepared for isotopic analysis. Samples that were analyzed in whole, including their lipid components, were freeze-dried and weighed to 0.5–0.6 mg and placed in tin capsules for isotopic analysis. Identical samples also were lipid extracted in 2:1 chloroform : methanol solution for ~24 h, rinsed with deionized water, and again extracted in 2:1 chloroform : methanol. Lipid-extracted samples were then rinsed with deionized water, freeze-dried, weighed to 0.5–0.6 mg, and finally placed in tin capsules for isotopic analysis. One vibrissa from each of the 6 otters was plucked, washed, and subsampled as described above. We calculated TDFs from both lipid-extracted and whole (i.e., non–lipid-extracted) food as the difference in $\delta^{13}C$ or $\delta^{15}N$ values between the mean isotopic composition of diet and vibrissae. Mean isotopic composition of diet was calculated for each individual using a concentration-dependent mixing model (Phillips and Koch 2002).

Trophic discrimination factors also were calculated on a monthly basis. We used our data for mean vibrissae growth rate to determine the length of vibrissae grown in a month and which segment(s) of a vibrissa was grown during a specific month. We then calculated TDFs as the mean isotope value of the segment(s) minus the mean isotopic composition of diet for the same month.

Results

Vibrissae growth rates.—Mean (±*SD*) vibrissae growths of the 4 adult sea otters used in this study were 7.7 (±1.2) cm/year (Table 1). We found no significant relationship between growth rate and vibrissa length ($F_{1,11} = 1.40$, $P = 0.262$, $R^2 = 0.11$). We collected a 6.6-cm-long vibrissa from the 6-month-old pup. The pup's diet changed between months 1 and 2, and this change was represented by a +4.2‰ and +1.7‰ change in dietary $\delta^{13}C$ and $\delta^{15}N$ values, respectively. But isotope values of the pup's vibrissa remained consistent along its entire length, with a mean (±*SD*) of –14.9‰ (±0.2‰) and 15.4‰ (±0.1‰) for $\delta^{13}C$ and $\delta^{15}N$, respectively. Therefore, the sampled vibrissa was grown out to 6.6 cm in no more than 5-months time, which means the pup grew the vibrissa at a minimum rate of 15.8 cm/year over that 5-month period.

Trophic discrimination factors.—Isotope values for dietary items fed to adults ranged from –20.3‰ to –14.3‰ for $\delta^{13}C$ and 6.4‰ to 15.1‰ for $\delta^{15}N$ (Table 2). The formula fed to the pup for her 1st month in captivity had bulk (i.e., non–lipid-extracted) values of –25.7‰ (±0.2‰) and 6.9‰ (±0.1‰) for $\delta^{13}C$ and $\delta^{15}N$, respectively. Sea otters' diet compositions were composed of ~62% Atlantic surf clam, ~15% Pacific white shrimp, ~11% California market squid, and ~12% other (Table 2). Modeled diet $\delta^{13}C$ and $\delta^{15}N$ values for adults ranged from –18.1‰ to –17.5‰ and from 9.7‰ to 10.2‰, respectively (see Supporting Information S1, DOI:10.1644/12-MAMM-A-035.1.S1). Vibrissae isotope values were similar among adult otters, ranging from –15.1‰ to –15.0‰ for $\delta^{13}C$ and from 15.2‰ to 15.8‰ for $\delta^{15}N$. In this study, we calculated TDFs in 2 ways: lipid-extracted diet–vibrissae TDF, and whole diet–vibrissae TDF. Mean (±*SD*) lipid-extracted diet–vibrissae TDFs were 2.4‰ (±0.2‰) and 4.9‰ (±0.3‰) for $\delta^{13}C$ and $\delta^{15}N$, respectively. Mean whole diet–vibrissae TDFs were 2.8‰ (±0.2‰) and 5.5‰ (±0.2‰) for $\delta^{13}C$ and $\delta^{15}N$, respectively (Fig. 1; Supporting Information S1). Lipid-extracted diet–vibrissae TDFs differed significantly from whole diet–vibrissae TDFs for both $\delta^{13}C$ (paired *t*-test, $t_4 = -21.00$, $P < 0.001$) and $\delta^{15}N$ (paired *t*-test, $t_4 = -26.13$, $P < 0.001$). $\delta^{15}N$ TDFs in this study were significantly higher (independent samples *t*-test, $t_{34} = -8.12$, $P < 0.001$) than previously reported values of fur and vibrissae in mammalian carnivores (Hobson et al. 1996; Lesage et al. 2002; Newsome et al. 2010; Roth and Hobson 2000). Whole-diet TDFs that also were calculated on a month-to-month basis using vibrissae growth rate data ranged from 1.8‰ to 3.6‰ with a mean (±*SD*) of 2.7‰ (±0.4‰) for $\delta^{13}C$ and from 4.4‰ to 6.6‰ with a mean (±*SD*) of 5.5‰ (±0.5‰) for $\delta^{15}N$ (Fig. 2).

Discussion

Vibrissae growth rates.—Southern sea otter vibrissae exhibit a simple linear growth pattern like that seen in the otariid *E. jubatus* (Hirons et al. 2001) rather than the nonlinear von Bertalanffy pattern found in the phocids *H. grypus* and *H. leptonyx* (Greaves et al. 2004; Hall-Aspland et al. 2005). If vibrissae were being grown nonlinearly, we would expect a significant negative relationship between vibrissa growth rate and vibrissa length, but that is not the case ($R^2 = 0.11$, $P = 0.262$). No significant seasonality patterns were found in growth rates that would indicate an endogenous annual cycle, and there was no regular seasonal shedding of vibrissae (Table 1); however, we realize that we have limited ability to recognize such patterns because of the small number of individuals examined in this study. Last, sea otters did not show continued enrichment over preinjection values following

Table 1.—Vibrissae lengths and growth rates of adult sea otters (*Enhydra lutris nereis*). Samples are organized by the seasons in which growth was measured (i.e., between the date of the glycine label injection and the date the vibrissa was sampled).

Sea otter*	Glycine injection	Sampled	Vibrissa length (cm)	Growth rate (cm/year)
Winter–spring				
Mae	30 October 2008	23 February 2009	4.0	7.6
Mae	30 October 2008	18 March 2009	9.0	7.1
Maggie	22 October 2008	19 February 2009	7.4	8.2
Rosa	13 November 2008	11 May 2009	5.4	6.2
$\bar{X}$ (*SD*)			6.5 (2.2)	7.3 (0.9)
Summer–autumn				
Mae	23 February 2009	27 October 2009	8.6	9.4
Joy	21 August 2008	20 December 2009	10.7	6.8
$\bar{X}$ (*SD*)			9.7 (1.5)	8.1 (1.8)
Full year				
Joy	22 December 2008	29 December 2009	9.5	9.3
Maggie	19 February 2009	1 February 2010	7.8	6.6
$\bar{X}$ (*SD*)			8.7 (1.2)	8.0 (1.9)
Total $\bar{X}$ (*SD*)			7.8 (2.2)	7.7 (1.2)

* The animals are named in support of the Monterey Bay Aquarium staff's training efforts.

the initial decline in isotope peaks that Hirons et al. (2001) found in harbor seal (*P. vitulina*) vibrissae.

The minimum vibrissae growth rate (15.8 cm/year) of the pup was higher than the mean ($\pm SD$) rate for the adults (7.7 ± 1.2 cm/year). Hirons et al. (2001) found that juvenile Steller sea lion (*E. jubatus*) vibrissae growth rates were twice those of adults. These similar results suggest that juvenile vibrissae growth rates are likely higher than those of the adults. However, we are aware of our limited ability to say anything definitive because of the low sample size.

Sex differences in vibrissae growth rates of fur seals (*Arctocephalus gazella* and *A. tropicalis*) result in males having longer whiskers than females (Kernaléguen et al. 2012). Although our study is limited to female sea otters, we feel comfortable applying our estimates of vibrissae growth rates to both male and female sea otters in the wild because sea otters show no sex differences in vibrissae length (Newsome et al. 2009).

Vibrissae $\delta^{13}C$ TDFs.—We found a mean whole diet–vibrissae $\delta^{13}C$ TDF of +2.8‰ (±0.2‰) for captive otters, which falls within the range of mean TDFs of +2.2–3.2‰ for keratinous tissues in other mammalian carnivores (Hobson et al. 1996; Lesage et al. 2002; Newsome et al. 2010; Roth and Hobson 2000). There is a relatively small but significant difference (0.4‰; $P < 0.001$) between the mean lipid-extracted diet–vibrissae $\delta^{13}C$ TDF of +2.4‰ (±0.2‰) and the mean whole diet–vibrissae $\delta^{13}C$ TDF of +2.8‰ (±0.2‰). We speculate that this difference is small because the captive sea otters analyzed here consume a protein-rich diet; most non–lipid-extracted prey items have low [C]/[N] ratios in the 3.0–3.5 range indicative of pure protein (Table 2). A study of a wild population of California sea otters that consume a high proportion of lipid-rich sea urchins found greater variability within whole diet–vibrissae $\delta^{13}C$ TDFs ($SD = 0.7$—Newsome et al. 2010) than we found among whole diet– and lipid-extracted diet–vibrissae $\delta^{13}C$ TDFs ($SD = 0.3$). Because lipids are ^{13}C-depleted relative to proteins (Tieszen et al. 1983), greater variability in $\delta^{13}C$ TDFs would be expected for wild populations that consume diets that are more variable in their macromolecular composition (i.e., protein to lipid ratio) than the captive sea otters examined in this study.

The $\delta^{13}C$ TDFs for vibrissae are larger than TDFs for other commonly analyzed tissues (e.g., blood, liver, and muscle) because they are constructed from α-keratin, which is synthesized primarily from the nonessential amino acids glycine, serine, and glutamate (Marshall et al. 1991). The amino acids glycine and serine are naturally enriched in ^{13}C relative to other amino acids in many animals, including carnivores (Hare et al. 1991; Howland et al. 2003; Jim et al. 2006). Glycine is commonly synthesized from serine, which in turn is synthesized via several steps from the glycolysis intermediate, 3-phosphoglycerate. For animals that consume lipid-rich diets, ^{13}C-depleted glycerol (Weber et al. 1997) can be a significant contributor of carbon to glycolysis (Tao et al. 1983), and thus glycine and serine. Therefore, the $\delta^{13}C$ values of glycine and serine are more similar to whole diet $\delta^{13}C$ values (Howland et al. 2003; Jim et al. 2006; Newsome et al. 2011) rather than dietary protein $\delta^{13}C$ values. Glutamate, which is synthesized from the tricarboxylic acid cycle, also reflects whole diet $\delta^{13}C$ values (Howland et al. 2003; Newsome et al. 2011).

Many marine mammals (e.g., sea otters, seals, and polar bears [*Ursus maritimus*]) and seabirds consume lipid-rich diets (e.g., sea urchins, herring, and seal fat—Dehn et al. 2007; Newsome et al. 2010; Thiemann et al. 2008). Because keratinous tissues are composed of a large proportion of nonessential amino acids (~70%—Marshall et al. 1991), which can be synthesized from lipids, we recommend that whole diet–vibrissae TDFs be applied in studies concerning animals with lipid-rich diets (Newsome et al. 2010). Application of lipid-extracted diet–vibrissae TDFs could lead to misinterpretation of dietary sources in cases where ^{13}C-depleted lipids are a carbon substrate for vibrissa synthesis.

Vibrissae $\delta^{15}N$ TDFs.—We found a mean lipid-extracted diet–vibrissae $\delta^{15}N$ TDF of +4.9‰ and a mean whole diet–vibrissae $\delta^{15}N$ TDF of +5.5‰. Previous work shows that

TABLE 2.—The $\delta^{13}C$ and $\delta^{15}N$ values of whole and lipid-extracted (LE) prey and diet composition of prey items fed to otters (*Enhydra lutris nereis*) at Monterey Bay Aquarium (Monterey, California). Values are expressed as mean (*SD*).

	Atlantic surf clam (whole) ($n = 20$)	Atlantic surf clam (food) ($n = 20$)	Pacific white shrimp ($n = 10$)	California market squid ($n = 10$)	Manila clam ($n = 10$)	Red rock crab ($n = 10$)	Blue mussel ($n = 10$)	Black tiger prawn ($n = 10$)	Pup formula ($n = 5$)
Whole $\delta^{13}C$	−16.9 (0.2)	−18.3 (0.5)	−20.3 (0.7)	−17.8 (0.4)	−17.1 (0.3)	−14.7 (0.4)	−19.5 (0.7)	−19.0 (1.0)	−25.7 (0.2)
LE $\delta^{13}C$	−16.5 (0.4)	−18.5 (0.5)	−20.4 (0.8)	−16.2 (0.3)	−16.4 (0.4)	−14.3 (0.5)	−18.6 (0.4)	−19.0 (1.0)	−22.9 (0.1)
Whole $\delta^{15}N$	10.5 (0.4)	10.1 (0.4)	6.1 (0.3)	13.1 (0.5)	9.9 (0.6)	14.3 (0.5)	8.4 (0.6)	6.7 (0.3)	6.9 (0.1)
LE $\delta^{15}N$	11.3 (0.5)	10.9 (0.5)	6.4 (0.3)	13.5 (1.2)	10.3 (0.6)	15.1 (0.5)	8.9 (0.6)	6.9 (0.2)	7.5 (0.1)
Whole [C]/[N]	3.6 (0.2)	3.5 (0.1)	3.3 (0.1)	3.7 (0.3)	4.0 (0.2)	3.3 (0.1)	4.2 (0.5)	3.3 (0.0)	8.7 (0.5)
LE [C]/[N]	3.3 (0.3)	3.5 (0.3)	3.0 (0.1)	3.2 (0.3)	3.4 (0.2)	3.1 (0.1)	3.6 (0.7)	3.0 (0.0)	3.4 (0.0)
				Diet composition (% consumed organic biomass)					
Sea otter*									
Maggie	47	23	10	8	9	2	1	1	0
Mae	37	22	16	13	7	1	3	1	0
Rosa	37	21	19	13	5	2	2	0	0
Joy	55	21	7	7	7	1	2	1	0
Toola	35	21	19	13	7	1	3	0	0
Kit	30	21	18	13	9	2	3	0	4
$\bar{X}$ (*SD*)	40 (9)	22 (1)	15 (5)	11 (3)	7 (2)	2 (1)	2 (1)	1 (1)	1 (2)

* The animals are named in support of the Monterey Bay Aquarium staff's training efforts.

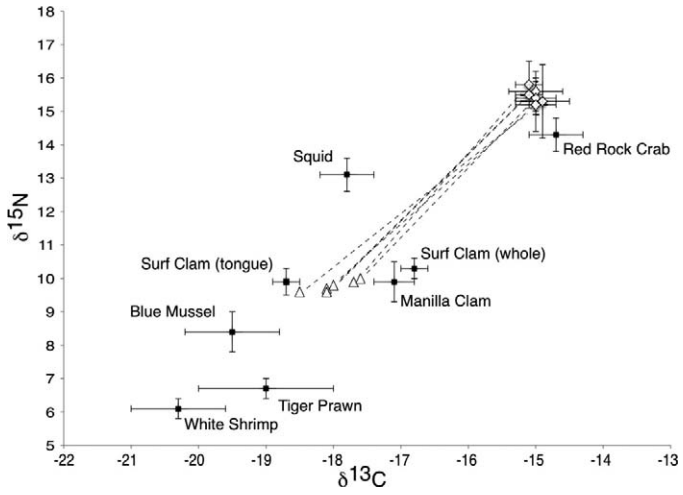

FIG. 1.—Mean (±*SD*) vibrissae (open diamonds) and modeled diet (open triangles) $\delta^{13}C$ and $\delta^{15}N$ values for individual sea otters (*Enhydra lutris nereis*) and whole food items (solid squares). Dashed lines connect the vibrissa value to the diet value of an individual otter.

chloroform : methanol solvents extract some nonlipid material (~18% of the extract) in addition to lipids (Dobush et al. 1985), which may include amino acids. Because amino acid $\delta^{15}N$ values can range by 20–30‰ in a single tissue of an apex predator (Popp et al. 2007), changes in amino acid composition resulting from solvent-based lipid extraction could yield a difference in bulk tissue $\delta^{15}N$ values (Post et al. 2007). Sweeting et al. (2006) found that muscle treated with chloroform : methanol had $\delta^{15}N$ values that were 0.7‰ higher than those of nontreated whole muscle. Thus, the small but significant difference (0.6‰, $P < 0.001$) we observed between TDFs calculated using lipid-extracted versus whole dietary samples may be explained by the solvents we chose for lipid extraction.

Regardless of the preparation method used, the $\delta^{15}N$ TDFs we observed were higher than previously reported TDFs of +2.8–3.5‰ ($P < 0.001$) in fur and vibrissae of mammalian carnivores (Hobson et al. 1996; Lesage et al. 2002; Newsome et al. 2010; Roth and Hobson 2000). Nutritional stress, growth rate, and dietary protein quality and quantity are all factors that influence $\delta^{15}N$ TDF values (Fuller et al. 2004; Gaye-Siessegger et al. 2003; Robbins et al. 2005). Nutritional stress and growth rate were not factors in our experiment because the captive sea otters examined in this study were adults that were well maintained by the Monterey Bay Aquarium staff and were fed diets that exceed their caloric requirements. Furthermore, sea otters have high metabolic rates and low lipid stores (Reidman and Estes 1990), and thus are physiologically unable to undergo long periods (weeks to months) of nutritional stress that would alter protein balance (Oftedal 2000) and result in elevated $\delta^{15}N$ TDFs (Hobson et al. 1993). Nutritional stress resulting from pregnancy also has been identified as a cause of elevated $\delta^{15}N$ values (Fuller et al. 2004), but it was not a factor here because none of the otters in this study were pregnant.

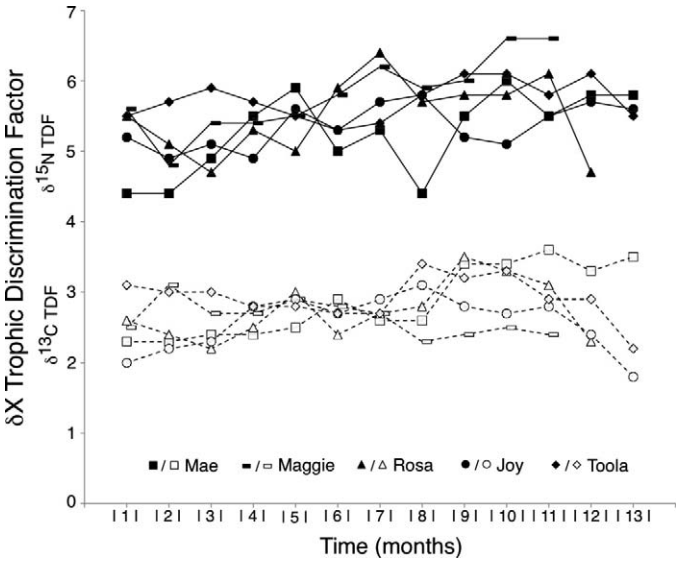

Fig. 2.—Whole diet–vibrissae trophic discrimination factors (TDFs) for $\delta^{13}C$ (open) and $\delta^{15}N$ (solid) calculated on a monthly basis in sea otters (*Enhydra lutris nereis*). Using mean vibrissae growth rate to determine the length of vibrissae grown in a month, we then calculated TDFs as the mean isotopic value of the vibrissa segment for a month minus the mean isotopic composition of the diet for the same month.

Dietary protein quality and quantity can substantially influence $\delta^{15}N$ TDFs (Florin et al. 2011; Pearson et al. 2003; Robbins et al. 2005, 2010). Diets with high protein quality, where amino acid intake matches a consumer's amino acid requirements, have been shown to coincide with lower TDFs (Robbins et al. 2005). Lower TDFs are caused by dietary amino acids being conserved in tissue synthesis (Roth and Hobson 2000). In contrast, when consuming a diet with a low protein quality, a greater proportion of amino acids is catabolized and exposed to transamination and deamination processes, which results in elevated TDFs (Robbins et al. 2005). The protein quality of the food fed to the captive otters in this study is unknown, so we can't rule out protein quality as a potential reason why the $\delta^{15}N$ TDFs we observed in captive sea otters were higher than those found in a population of wild sea otters (Newsome et al. 2010) and other controlled feeding experiments on mammalian carnivores (Hobson et al. 1996; Kurle 2002; Lesage et al. 2002; Roth and Hobson 2000; Zhao et al. 2006).

Previous studies investigating the effects of dietary protein quantity on $\delta^{15}N$ TDFs show mixed results. Pearson et al. (2003) suggested that animals with excess dietary protein will have higher $\delta^{15}N$ TDFs, because transamination and deamination processes that discriminate against heavy nitrogen (^{15}N) have more ^{14}N available for excreta formation, therefore reducing the likelihood that ^{15}N will be excreted. But some empirical studies have found evidence to the contrary, that increasing dietary protein quantity can actually decrease $\delta^{15}N$ TDFs (Mirón et al. 2006; Tsahar et al. 2008). A study investigating both protein quantity and quality found that a combination of low protein quality and high protein quality has the greatest potential to increase $\delta^{15}N$ TDFs, by increasing protein turnover (Florin et al. 2011). Although the protein quality of food in this study is unknown, the high protein intake of sea otters in this study (Table 2) could be responsible for the high $\delta^{15}N$ TDFs we observed relative to previous studies.

Stable isotope analysis of vibrissae has been successfully used to characterize many aspects of foraging ecology and niche variation, including individual dietary specialization, temporal dietary variation, sex-specific resource partitioning, and habitat use within and across populations (Cherel et al. 2009; Lewis et al. 2006; Newsome et al. 2009, 2010). Such studies can yield valuable information on species, such as sea otters, that are known to play an important role in the ecosystems they inhabit. The results of this study will increase both the trophic and temporal resolution with which sea otter foraging ecology, and that of closely related mammalian carnivores for which we have no estimates of TDFs, can be analyzed.

Supporting Information

Supporting Information S1.—Vibrissae $\delta^{13}C$ and $\delta^{15}N$ values, lipid–extracted (LE) and whole (NLE) diet $\delta^{13}C$ and $\delta^{15}N$ values, LE diet–vibrissae and NLE diet–vibrissae $\delta^{13}C$ and $\delta^{15}N$ values, and LE and NLE diet carbon : nitrogen ratios.
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