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# Variation in $\delta^{13}C$ and $\delta^{15}N$ diet–vibrissae trophic discrimination factors in a wild population of California sea otters

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*Abstract*. The ability to quantify dietary inputs using stable isotope data depends on accurate estimates of isotopic differences between a consumer (c) and its diet (d), commonly referred to as trophic discrimination factors (TDFs) and denoted by $\Delta_{c\text{-}d}$. At present, TDFs are available for only a few mammals and are usually derived in captive settings. The magnitude of TDFs and the degree to which they vary in wild populations is unknown. We determined $\delta^{13}C$ and $\delta^{15}N$ TDFs for vibrissae (i.e., whiskers), a tissue that is rapidly becoming an informative isotopic substrate for ecologists, of a wild population of sea otters for which individual diet has been quantified through extensive observational study. This is one of the very few studies that report TDFs for free-living wild animals feeding on natural diets. Trophic discrimination factors of 2.2‰ ± 0.7‰ for $\delta^{13}C$ and 3.5‰ ± 0.6‰ for $\delta^{15}N$ (mean ± SD) were similar to those reported for captive carnivores, and variation in individual $\delta^{13}C$ TDFs was negatively but significantly related to sea urchin consumption. This pattern may relate to the lipid-rich diet consumed by most sea otters in this population and suggests that it may not be appropriate to lipid-extract prey samples when using the isotopic composition of keratinaceous tissues to examine diet in consumers that frequently consume lipid-rich foods, such as many marine mammals and seabirds. We suggest that inherent variation in TDFs should be included in isotopically based estimates of trophic level, food chain length, and mixing models used to quantify dietary inputs in wild populations; this practice will further define the capabilities and limitations of isotopic approaches in ecological studies.

*Key words:* Enhydra lutris nereis; *San Nicolas Island, California, USA; sea otter; stable isotopes; trophic discrimination factors (TDFs); vibrissae.*

## Introduction

Stable isotope analysis is rapidly becoming a common tool in ecology and has been especially useful in studies of trophic ecology, ecophysiology, population connectivity, and energy flux within and among ecosystems. Recent reviews (e.g., Martinez del Rio et al. 2009) highlight the exponential rate at which isotope-based studies are being published in ecological journals, but also appeal for more ground-truthing work in both the laboratory and field to better understand ecological and physiological factors that may cause variation in trophic discrimination factors (TDFs) used to estimate consumer diets. Trophic discrimination factors are defined as the difference between the isotope value of a consumer's bulk tissue (e.g., blood, muscle, liver) and the isotope value of the average prey consumed. Tissue-specific differences in amino acid composition result in TDF variation among commonly analyzed tissues, but TDFs have also been shown to vary with growth rate (Gaye-Siessegger et al. 2003), diet quality (Oelbermann and Scheu 2002, Pearson et al. 2003), changes in a consumer's physiological condition (i.e., nutritional stress, reproductive status; Fuller et al. 2004, 2005), and/or excretion pathways (Vanderklift and Ponsard 2003). Thus, the accurate application of isotopic tools to studies of foraging and trophic ecology not only requires estimates of mean TDFs, but also estimates of how much TDFs may vary in a free-ranging, wild population.

Isotopic investigations of animal foraging and/or trophic ecology that utilize stable isotope mixing models (e.g., Phillips and Koch 2002, Phillips and Gregg 2003, Moore and Semmens 2008) typically require analysis of frequently consumed prey sources for accurate interpretation. Consumer isotope values are then corrected for trophic discrimination using TDFs for the tissue of interest, if available. Often, TDFs have not been determined for the organism in question, and rarely

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are they available for closely related species. As the creators of popular stable isotope mixing models suggest (Phillips and Gregg 2003, Phillips et al. 2005), a major caveat in their use is the application of suitable TDFs to consumer isotope values. In some cases, researchers even perform sensitivity analyses to see how model results vary with respect to variation in TDFs or have recently developed mixing models that incorporate uncertainty in TDFs in the estimation of prey proportions (Moore and Semmens 2008).

All of the data compiled to date on TDFs for carnivores comes from controlled laboratory settings in which a limited number of animals (typically 3–5) are usually fed ad libitum an isotopically homogenous prey for a suitable length of time that ensures the tissue(s) of interest reach isotopic steady state with diet. For mammalian carnivores, we only have laboratory-derived TDFs for seals (Hobson et al. 1996, Kurle 2002, Lesage et al. 2002, Zhao et al. 2006) and red foxes (*Vulpes vulpes*; Roth and Hobson 2000). The $\delta^{13}C$ TDFs for commonly analyzed tissues such as blood, liver, and muscle range from +0‰ to +2‰ and are generally smaller than those for $\delta^{15}N$, which range from +2‰ to +5‰. Bone collagen and keratinaceous tissues (fur and vibrissae or whiskers) have unique amino acid compositions that result in larger TDFs for $\delta^{13}C$ (DeNiro and Epstein 1978). The $\delta^{13}C$ TDFs for bone collagen are typically in the +3–5‰ range, whereas fur and vibrissae are typically enriched by +2–3‰ in relation to diet.

These controlled laboratory experiments on a limited number of captive taxa show that $\delta^{13}C$ and $\delta^{15}N$ TDFs vary among tissue types, but at present limited data exist on the magnitude of TDFs for a wild carnivore population. To our knowledge the only published TDF estimates for a wild mammalian carnivore population are for bone collagen $\delta^{13}C$ and $\delta^{15}N$ TDFs in wolves (*Canis lupis*) from Isle Royale, Michigan, USA (Fox-Dobbs et al. 2007), and a few other studies have attempted to estimate TDFs for wild marine vertebrate and invertebrate animal populations (Fry et al. 1984, Fry 1988). Furthermore, we do not know how much interindividual variation in TDFs might be inherent in a wild population that is likely subject to greater physiological demands than captive animals. In this study, instead of focusing on captive subjects, we examined TDFs in a wild population of California sea otters (*Enhydra lutris nereis*) from San Nicolas Island (SNI) off southern California, for which extensive individual-level foraging data has been determined through observational study (Tinker et al. 2008). For each individual ($n = 10$), we compared mean vibrissae $\delta^{13}C$ and $\delta^{15}N$ values from serial sections of a vibrissa to a modeled diet value, which we calculated by combining observational foraging data with the isotopic composition of common sea otter prey also collected from SNI. The difference between the mean measured and modeled values provided an estimate of the $\delta^{13}C$ and $\delta^{15}N$ TDFs for each individual. This approach was designed not only to determine the mean $\delta^{13}C$ and $\delta^{15}N$ TDFs for vibrissae, a tissue that is rapidly becoming an informative isotopic substrate for ecologists (Lewis et al. 2006, Newsome et al. 2009), but also to examine the degree of variation in TDFs among individuals in a healthy, free-ranging population of mammalian carnivores.

## Materials and Methods

### *Observational dietary data*

In October of 2003, 16 sea otters were captured at San Nicolas Island (33°14′57″ N, 119°29′58″ W) using methods described in Ames et al. 1983, tagged with color-coded flipper tags, and fitted with intraperitoneal VHF radio transmitters (Advanced Telemetry Systems, Isanti, Minnesota, USA) and time–depth recorders (TDR; Model Mark IX, Wildlife Computers, Redmond, Washington, USA). Instrumented otters were located on a daily basis using standard telemetric techniques and those that were found foraging were targeted for observation. Foraging data were collected with high-powered (50–80×) spotting scopes (Questar, Norman, Oklahoma, USA) as described in Estes et al. (2003), with observations continuing until the otter finished feeding or visual contact was lost. The data from this bounded period defined a foraging "bout" (Estes et al. 2003, Tinker et al. 2008). Information recorded included date and time, precise GPS location of the focal otter, dive duration, surface interval duration, dive success (if prey captured), species of prey captured (to the lowest possible taxon), size of prey (maximum body dimension in 5-cm increments), number of prey, tool use, and handling time. Data were collected at SNI from November 2003 through September 2004. Consistent foraging data were obtained for 10 of the study animals for a total of 170 bouts (~5000 foraging dives with known outcome). All 10 study animals had >200 known outcome foraging dives recorded. We calculated diet composition as the proportion of each prey item in the diet over the entire period of observation, on the basis of consumed organic biomass. Following the analytical methods described by Tinker et al. (2008), prey ingestion rates (grams per minute of foraging time) were estimated for each prey species and study animal, with the associated variances accounting for temporal variation in diet as well as all quantifiable sources of known sampling and observer error. Specifically, the Monte-Carlo-based resampling analysis was stratified by dive duration, prey size, time at surface, and prey handling duration (Tinker et al. 2008) and thus explicitly corrected for recognized sources of bias such as lower recognition rates for very small prey types or prey captured far from shore.

### *Prey and sea otter tissue collection*

With the exception of abalone, all sea otter prey samples were collected during a series of diving and shore-based sampling trips on San Nicolas Island in 2004. The abalone samples were collected during diving-

based trips on the mainland coast of central California (USA) in 2004. We analyzed 12 prey species that together comprise >95% of prey consumed at the population level by sea otters at SNI based on observational data (Tables 1 and 2; Bentall 2005). Unfortunately, we were unable to obtain a permit to collect abalone at SNI in 2004 and therefore analyzed black and red abalone collected on the central California Coast from San Simeon (35°38′39″ N, 121°11′26″ W) to Monterey Bay between December 2004 and April 2005. We do not believe that the use of mainland abalone isotope values compromises our results because (1) with the exception of one individual (SNI-977; Table 1), abalone are minor dietary components (<5%) for most of the sea otters examined here based on observational data; (2) there were no significant differences in mean $\delta^{13}C$ and $\delta^{15}N$ between abalone collected from mainland sites at San Simeon and Monterey Bay (S. D. Newsome et al., *unpublished data*), which are separated by >200 km of coastline. Based on functional and isotopic similarities, these 12 species were classified into seven prey types: sea urchins, northern kelp crabs, *Cancer* crabs, spiny lobsters, herbivorous snails, planktivorous snails, and abalone; refer to Table 1 for scientific names of the species analyzed. Observational data show that red sea urchins are a more important prey item for the SNI sea otter population than purple sea urchins (Oftedal et al. 2007, Tinker et al. 2008). While red sea urchins comprised 16 of 21 individual urchins analyzed in this study, we report mean isotope values for all urchins because we found differences in isotopic composition between the two species to be insignificant.

Sea otter vibrissae were sampled from wild-caught animals collected during the 2003 population study. Details of the capture, handling, and radio-tagging of study animals are provided elsewhere (Bentall 2005, Tinker et al. 2006); all collection activities were authorized by federal, state, and institutional permits issued to J. A. Estes and M. T. Tinker.

### *Isotopic methods*

Prey specimens were rinsed of sediment and/or detritus, weighed, and measured using digital calipers. Inedible portions of prey (e.g., the spines and tests of sea urchins, carapace of large crabs and lobsters, snail and abalone shells) were removed prior to lyophilization. The dried edible portion was homogenized by grinding to a coarse powder in a Wiley mill. Subsamples of the dried material were further dried at 55°C and combusted in an adiabatic bomb calorimeter. The ash residue following combustion was used to correct dry matter (DM) to organic matter (OM = 100 − DM). For reasons discussed in detail below (see *Discussion*), we did not lipid-extract any of the prey samples except for sea urchins, which contain a substantial proportion of lipids (>17% by mass; Table 4; Oftedal et al. 2007), as suggested by their relatively high [C]/[N] ratios in comparison to protein-rich prey (Table 2).

Approximately 0.5 mg of the powdered sample of edible prey tissue was sealed into tin boats for isotopic analysis. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values were determined using a Carlo-Erba (Milan, Italy) or Costech (Valencia, California, USA) elemental analyzer (NC 2500) interfaced with a Finnegan Delta Plus XL mass spectrometer (Waltham, Massachusetts, USA) at the Carnegie Institution of Washington, D.C., USA and Stanford University, Stanford, California, USA. Isotopic results are expressed as δ values, $\delta^{13}C$ or $\delta^{15}N = 1000 \times [(R_{smpl} - R_{stnd})/R_{stnd}]$, where $R_{smpl}$ and $R_{stnd}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the sample and standard, respectively. The standards are Vienna-Pee Dee Belemnite limestone (VPDB) for carbon and atmospheric $N_2$ for nitrogen. The units are expressed as parts per thousand (‰). The within-run standard deviation of acetanilide and alanine standards was ≤0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

For $\delta^{13}C$ and $\delta^{15}N$ analysis, vibrissae were rinsed once with a 2:1 chloroform : methanol solution to remove surface contaminants. Cleaned vibrissae were then subsampled into ~0.5 mg segments using nail clippers and sealed into tin boats for isotopic analysis. Carbon and nitrogen isotope values were determined using the mass spectrometer system described for edible prey tissue. The number of vibrissae segments analyzed from each individual varied from 11 to 27, depending on the length of each vibrissa (Table 3). As a control for the quality of keratin, we measured the [C]/[N] ratios of each sample; atomic [C]/[N] ratios of all samples were 3.3–3.5, encompassing the theoretical atomic [C]/[N] ratio of keratin (3.4).

### *Modeling and statistical methods*

To calculate TDFs, we compared measured $\delta^{13}C$ and $\delta^{15}N$ values to modeled values, which we estimated by combining information on diet composition with prey isotopic composition data. We accounted for all recognized and quantifiable sources of uncertainty by using Monte Carlo resampling simulations. Specifically, five steps were carried out over a large number of iterations (50 000) for each study animal: (1) we generated a diet composition "point estimate" by randomly drawing species-specific prey consumption rates from lognormal sampling distributions with appropriate means and variances (see *Observational dietary data*, above); (2) for each prey type comprising >1% of the diet, we generated $\delta^{13}C$ and $\delta^{15}N$ point estimates by randomly drawing from normal distributions with appropriate means and variances (see *Isotopic methods*, above); (3) for each prey type, we multiplied the $\delta^{13}C$ and $\delta^{15}N$ point estimates by the proportional contribution of organic matter contributed by each prey type to the diet and then summed the products across all prey types to obtain point estimates for net consumed $\delta^{13}C$ and $\delta^{15}N$; (4) we generated point estimates for observed tissue $\delta^{13}C$ and $\delta^{15}N$ values by drawing

TABLE 1. Diet composition for individual sea otters (*Enhydra lutris nereis*; identified by tag number and sex) captured near San Nicolas Island, California, USA, as the percentage of each prey item in the diet over the entire period of observation, on the basis of consumed organic biomass (%).

| Sea otter | Sea urchins | Kelp crabs | *Cancer* crabs | Spiny lobsters | *Chlorostoma* snails | *Megastraea* snails | Abalone |
|---|---|---|---|---|---|---|---|
| ~30% sea urchin diet | | | | | | | |
| 965, F | 32 | 9 | 18 | 33 | 0 | 2 | 6 |
| 968, F | 32 | 11 | 20 | 28 | 2 | 5 | 3 |
| 977, F | 19 | 11 | 39 | 2 | 6 | 3 | 20 |
| Mean | 28 (7) | 10 (1) | 26 (11) | 21 (17) | 3 (3) | 4 (1) | 10 (9) |
| ~60% sea urchin diet | | | | | | | |
| 969, F | 57 | 26 | 5 | 0 | 0 | 13 | 0 |
| 978, F | 58 | 37 | 0 | 0 | 2 | 3 | 0 |
| 979, F | 61 | 32 | 0 | 0 | 4 | 3 | 0 |
| 961, M | 54 | 28 | 4 | 8 | 2 | 2 | 2 |
| 967, M | 58 | 37 | 0 | 0 | 0 | 3 | 2 |
| 976, M | 60 | 18 | 16 | 4 | 0 | 2 | 0 |
| 982, M | 71 | 24 | 2 | 0 | 2 | 2 | 0 |
| Mean | 60 (5) | 29 (7) | 4 (6) | 2 (3) | 1 (2) | 4 (4) | 1 (1) |

*Notes:* Sea otters are separated into two groups: those that consumed ~30% and those that consumed ~60% sea urchins. Key to abbreviations: F, female; M, male. Values in parentheses are SDs. Prey species include: red (*Strongylocentrotus franciscanus*) and purple (*S. pupuratus*) sea urchins; northern kelp crabs (*Pugettia producta*); red (*Cancer productus*) and Pacific (*C. antennarius*) rock crabs; spiny lobsters (*Panulirus interruptus*); black (*Chlorostoma funebralis*), banded (*C. eiseni*), and queen (*C. regina*) turban snails; wavy turban snail (*Megastraea undosa*); and black (*Haliotis cracherodii*) and red (*H. rufescens*) abalone.

randomly from the appropriate normal distributions (individual means and variances as shown in Table 3); and (5) we subtracted the estimates of net consumed $\delta^{13}C$ and $\delta^{15}N$ (step 3) from the estimates of tissue $\delta^{13}C$ and $\delta^{15}N$ values (step 4) to create TDF point estimates for carbon and nitrogen isotopes. We calculated the arithmetic mean and standard deviation of individual $\delta^{13}C$ and $\delta^{15}N$ TDFs using the 50 000 point estimates for each animal and similarly for the population using the combined point estimates (500 000) for all animals.

## RESULTS

Observational data for the San Nicolas population shows that two prey types, sea urchins (~70%) and kelp crabs (~12%), contribute 82% of edible biomass to the diet at the population level (Tinker et al. 2008). *Cancer* crabs are the third most consumed prey source (~8%), and the other four prey types (spiny lobsters, *Chlorostoma* snails, wavy turban snails, and abalone) on average represent small (≤3%) contributions to sea otter diets at SNI (Table 1).

Mean isotope values were different among most prey types (Fig. 1) and ranged from −19.1‰ to −14.3‰ for $\delta^{13}C$ and from 10.0‰ to 15.3‰ for $\delta^{15}N$ (Table 2). *Cancer* crabs and spiny lobsters have $\delta^{15}N$ values that are 2–5‰ higher than the sea urchins, kelp crabs, and snails analyzed in this study (Table 2 and Fig. 1), probably because they are benthic predators and generally feed one trophic level higher than the herbivorous invertebrates in kelp forest communities. A second interesting isotopic pattern is evident among the snail genera. Wavy turban snails (*Megastraea undosa*) often consume red algae, which typically have significantly lower $\delta^{13}C$ values than sympatric brown algae (e.g., Page et al. 2008). Overall, these patterns create a relatively large isotopic prey space that individual sea otters could potentially occupy.

At the population level, the sea otter vibrissae $\delta^{13}C$ and $\delta^{15}N$ values were −12.6‰ ± 0.6‰ and 15.1‰ ± 0.7‰, respectively (mean ± SD; Table 3). The overall range in mean vibrissae $\delta^{13}C$ and $\delta^{15}N$ values of only ~2‰ among individual sea otters is strikingly small in comparison to the overall range in mean isotope values among available prey (Table 3 and Fig. 1). In comparison to the actual vibrissae values, the mean modeled diet $\delta^{13}C$ and $\delta^{15}N$ values show a smaller overall range in mean values of <1‰ for $\delta^{13}C$ but a similar range of ~2‰ for $\delta^{15}N$. The mean modeled diet

TABLE 2. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values of sea otter prey collected near San Nicolas Island, California, USA.

| Metric | Sea urchin | Kelp crab | *Cancer* crab | Spiny lobster | *Chlorostoma* snails | *Megastraea* snails | Abalone |
|---|---|---|---|---|---|---|---|
| *n* | 18 | 5 | 8 | 5 | 5 | 5 | 12 |
| $\delta^{13}C$ | −14.4 (0.6) | −14.5 (0.8) | −15.1 (1.2) | −15.7 (0.7) | −14.3 (0.8) | −19.1 (0.5) | −15.4 (0.9) |
| $\delta^{15}N$ | 10.7 (0.7) | 11.3 (0.9) | 14.7 (0.3) | 15.3 (0.3) | 12.8 (0.9) | 11.2 (0.3) | 10.0 (0.9) |
| [C]/[N] | 7.0 (1.5) | 4.2 (0.2) | 3.9 (0.3) | 3.7 (0.2) | 3.9 (0.1) | 3.8 (0.1) | 3.6 (0.3) |

*Notes:* Values are given as mean with SD in parentheses. For some prey types (e.g., *Cancer* crabs), multiple species have been combined because there was a low amount of interspecific variation in isotope values. See Table 1 for prey species names. Abalone samples were collected from Monterey Bay and San Simeon, California, USA.

TABLE 3. Measured (using vibrissae) and modeled carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values for individual male (M) and female (F) sea otters in the San Nicolas Island population.

| Sea otter | $n$ | Length (cm) | $\delta^{13}C$ | | | $\delta^{15}N$ | | |
|---|---|---|---|---|---|---|---|---|
| | | | Vibrissae | Modeled diet | TDF | Vibrissae | Modeled diet | TDF |
| ~30% sea urchin diet | | | | | | | | |
| 965, F | 13 | 5.5 | −12.0 (0.8) | −15.1 (0.4) | 3.1 (0.9) | 15.5 (0.8) | 12.9 (0.4) | 2.6 (0.8) |
| 968, F | 18 | 5.4 | −13.1 (0.9) | −15.2 (0.4) | 2.1 (1.0) | 15.3 (0.8) | 12.9 (0.4) | 2.4 (0.9) |
| 977, F | 11 | 4.2 | −12.1 (0.3) | −15.1 (0.5) | 3.0 (0.6) | 16.4 (0.4) | 12.4 (0.3) | 4.0 (0.5) |
| Mean | | 5.0 (0.7) | −12.4 (0.6) | −15.1 (0.1) | 2.7 (0.6) | 15.7 (0.6) | 12.7 (0.3) | 3.0 (0.9) |
| ~60% sea urchin diet | | | | | | | | |
| 969, F | 12 | 4.4 | −13.3 (0.4) | −15.1 (0.5) | 1.8 (0.7) | 14.1 (0.3) | 11.1 (0.6) | 3.0 (0.6) |
| 978, F | 14 | 4.2 | −13.0 (0.4) | −14.6 (0.5) | 1.6 (0.7) | 14.1 (0.5) | 11.0 (0.6) | 3.1 (0.8) |
| 979, F | 12 | 4.7 | −12.9 (0.4) | −14.6 (0.5) | 1.7 (0.7) | 14.9 (0.2) | 11.0 (0.6) | 3.9 (0.6) |
| 961, M | 27 | 7.4 | −12.1 (0.4) | −14.7 (0.5) | 2.6 (0.7) | 15.4 (0.4) | 11.5 (0.5) | 3.9 (0.7) |
| 967, M | 15 | 4.8 | −12.0 (0.5) | −14.6 (0.5) | 2.6 (0.8) | 15.2 (0.5) | 10.9 (0.6) | 4.3 (0.8) |
| 976, M | 10 | 3.9 | −12.2 (0.5) | −14.7 (0.5) | 2.5 (0.8) | 15.1 (0.4) | 11.7 (0.6) | 3.4 (0.7) |
| 982, M | 18 | 5.2 | −13.4 (0.4) | −14.5 (0.5) | 1.1 (0.7) | 14.9 (0.3) | 11.0 (0.6) | 3.9 (0.7) |
| Mean | | 4.9 (1.2) | −12.7 (0.6) | −14.7 (0.2) | 2.0 (0.6) | 14.8 (0.5) | 11.2 (0.3) | 3.6 (0.5) |
| Population mean | | 5.0 (1.0) | −12.6 (0.6) | −14.8 (0.3) | 2.2 (0.7) | 15.1 (0.7) | 11.6 (0.8) | 3.5 (0.6) |
| Monte Carlo | | | | | 2.1 (1.0) | | | 3.6 (0.9) |

*Notes:* Values are given as mean with SD in parentheses. Sea otters are separated into two groups: those that consumed ~30% and those that consumed ~60% sea urchins (see Table 1). Also noted are the overall length (cm) and the number of segments ($n$) obtained from each vibrissa. TDFs are trophic discrimination factors.

isotope values also have a smaller degree of interindividual $\delta^{13}C$ variation; the population-level standard deviation of modeled $\delta^{13}C$ values was only 0.3‰.

We separated the SNI sea otter population into two groups based on observational dietary data; one group consumed ~60% and the other consumed ~30% sea urchins (Table 1). The mean vibrissae or modeled diet $\delta^{13}C$ values were not significantly different between these two groups (Table 3). In contrast, the mean vibrissae $\delta^{15}N$ values between these groups were significantly different, using either pooled mean values for all vibrissa segments in each group (ANOVA, $P < 0.05$) or mean vibrissae values for each individual sea otter ($t$ test, $P < 0.05$). Mean modeled diet $\delta^{15}N$ values were also significant between the two groups (ANOVA, $P < 0.01$).

Individual $\delta^{13}C$ TDFs ranged from +1.1‰ to +3.1‰, whereas $\delta^{15}N$ TDFs ranged from +2.4‰ to +4.3‰ (Table 3). The estimated population TDFs for $\delta^{13}C$ and $\delta^{15}N$ were +2.2‰ ± 0.7‰ and +3.5‰ ± 0.6‰, respectively (Table 3). There were no significant differences in TDFs for $\delta^{13}C$ ($t$ test, $P = 0.08$) or $\delta^{15}N$ ($t$ test, $P > 0.10$) between the high- and low-urchin-consuming groups. A linear regression between $\delta^{13}C$ TDFs and percentage of urchin consumption based on observation, however, was significant (Fisher's test $P = 0.03$, bootstrap $P = 0.01$). Monte Carlo simulations resulted in estimates of TDF SDs that incorporated both intra- and interindividual variation: 1.0‰ for $\delta^{13}C$ and 0.9‰ for $\delta^{15}N$ (Table 3).

## DISCUSSION

The overall magnitude and variation of the diet–vibrissae TDFs for the wild SNI sea otter population agrees with that found in controlled feeding experiments examining keratinaceous tissues of other mammalian carnivores. The population-level mean $\delta^{13}C$ TDF of +2.2‰ for SNI sea otters is similar to the range in mean carbon TDFs of +2.3–3.2‰ for fur and vibrissae of captive foxes (Roth and Hobson 2000) and phocid seals (Hobson et al. 1996, Lesage et al. 2002). For nitrogen, the mean TDF of +3.5‰ is also similar to the range in mean values of +2.8–3.3‰ found for fur and vibrissae in previous studies.

Controlled feeding experiments show that when analyzing a single tissue, interindividual variation in TDFs may result from high growth rates in young age classes, changes in nutritional status, and/or diet quality. Since all of the individual sea otters examined in our study were adults, effects due to growth rate were negligible. Likewise, the sea otters studied at SNI were in excellent body condition (Bentall 2005) and the population shows no evidence of nutritional stress (Tinker et al. 2008), thus effects of nutritional status likely do not explain the observed interindividual variations in TDFs. Furthermore, sea otters have extremely high metabolic rates and low lipid stores compared to other marine mammals (Reidman and Estes 1990). They are thus physiologically unable to withstand the extended periods

TABLE 4. Carbon isotope ($\delta^{13}C$) values of sea urchins collected near San Nicolas Island, California.

| Sea urchin tissue | $n$ | $\delta^{13}C$ | [C]/[N] | Lipids (%) |
|---|---|---|---|---|
| Bulk (no lipid-extraction) | 18 | −14.4 (0.6) | 7.1 (1.5) | 17.3 (6.0) |
| Lipid-extracted | 18 | −13.2 (0.6) | 5.0 (0.9) | ··· |
| Lipids | 6 | −16.6 (0.8) | ··· | ··· |

*Notes:* Lipid content is given as a percentage on a dry-mass basis (Oftedal et al. 2007). Values are given as mean with SD in parentheses.

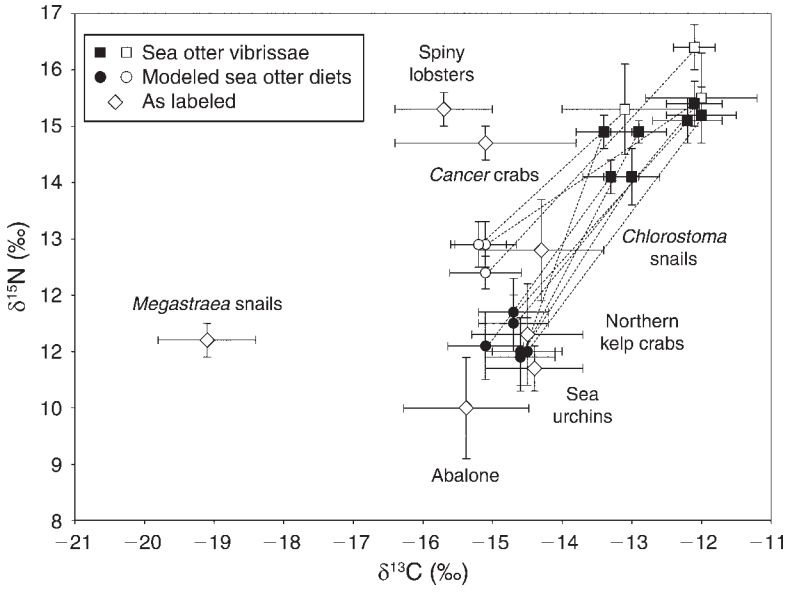


FIG. 1. Vibrissae and modeled diet $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD) for individual California sea otters (*Enhydra lutris nereis*) and common prey items (open diamonds) that represent >95% of consumed prey at the population level based on observational data (Table 1). Open symbols are sea otters that consumed ~30% sea urchins; solid symbols are sea otters that consumed ~60% sea urchins (Table 1). Refer to Tables 2 and 3 for sample sizes and mean isotope values of prey types and sea otters, respectively.

(weeks to months) of food deprivation and nutritional stress typically seen in many large marine mammals that lead to large changes in body protein stores (Oftedal 2000) that could result in significantly altered bulk tissue $\delta^{15}N$ values (e.g., Hobson et al. 1993, Cherel et al. 2005, Mekota et al. 2006).

Another source of interindividual variation in TDF estimates could be contributed by differences in nocturnal prey selection by some animals, leading to differing degrees of bias in observational diet estimates. Specifically, because our diet estimates are based only on daytime observations and since sea otters feed during the day and night, increased utilization of certain prey types at night such as decapod crustaceans, which are often more mobile during the night, could lead to biased estimates of diet and TDFs for some animals. At present we have no way to gauge the degree to which this may occur, although future analyses using archival time–depth data on 24-hour diving behavior may allow for examination of differences between diurnal and nocturnal feeding.

The degree of variation in $\delta^{15}N$ TDFs we observed for a healthy, free-ranging population suggests that variation in trophic discrimination must be included in the interpretation of isotopic data used to quantify trophic level in wild consumer populations. The difference in variation and spatial pattern between the mean vibrissae and modeled diet $\delta^{15}N$ values for SNI sea otters (Fig. 1) illustrates a limitation often confronted when using isotopes to quantify foraging and/or trophic ecology. The modeled diet $\delta^{15}N$ values clearly show two clusters (Fig. 1, open vs. solid circles); three sea otters that consumed ~30% sea urchins have $\delta^{15}N$ values that are significantly higher (by ~1‰) than the other seven individuals that consumed ~60% of this prey type (Tables 1 and 3). The three individuals that ate fewer sea urchins had a significant portion of *Cancer* crabs and spiny lobsters in their diet, which resulted in significantly higher mean modeled diet $\delta^{15}N$ values. The two clusters evident in the modeled diet $\delta^{15}N$ data are apparent in the mean vibrissae $\delta^{15}N$ values for the two sea otter groups (15.7‰ vs. 14.8‰; Table 3), but high interindividual variation results in an insignificant difference. Furthermore, higher interindividual variance in the measured vibrissae vs. modeled diet data blurs a clear distinction between high- and low-urchin-consuming otters in Fig. 1, likely because of inherent interindividual variation in TDFs for $\delta^{15}N$. This suggests that discriminating between two groups that are separated by only 1‰ in mean $\delta^{15}N$ values is problematic, but may be possible with large sample sizes and low amounts of isotopic variance at the consumer group level as well as in common prey.

Like TDFs for $\delta^{15}N$, the range in sea otter vibrissae $\delta^{13}C$ TDFs (2.0‰; Table 3) is similar to the range for serum $\delta^{13}C$ TDFs reported by Zhao et al. 2006, which is the only published experiment that has examined the effects of diet quality on TDFs in carnivores. Zhao et al. (2006) found that serum $\delta^{13}C$ TDFs for captive harbor seals varied by as much as 2.3‰, but found no predictable pattern between diet type (i.e., lipid- vs. protein-rich) and serum TDFs for $\delta^{13}C$ maybe because the study was limited by a small number of study subjects ($n = 3$). We speculate that variation in $\delta^{13}C$

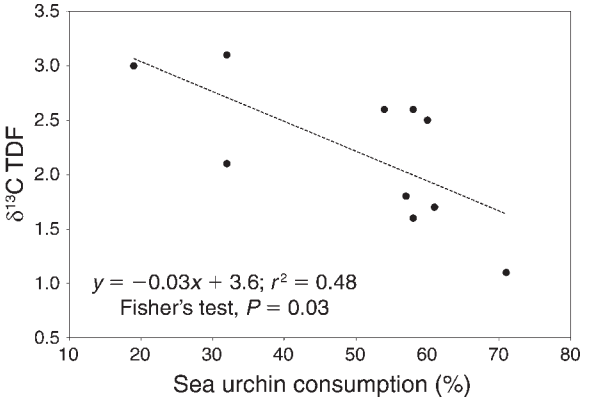


FIG. 2. Relationship between the $\delta^{13}C$ trophic discrimination factor (TDF) for individual San Nicolas sea otters and the percentage of sea urchins consumed (organic mass basis; see Table 1). Note that the $\delta^{13}C$ TDFs were calculated using non-lipid-extracted prey isotope values shown in Table 2.

TDFs of the SNI sea otter population examined here may relate to the high lipid content of their main prey source, red sea urchins, which contain on average >17% lipids on a dry mass basis (Table 4; Oftedal et al. 2007). While indispensable (i.e., essential) amino acids must be derived from diet or gut microbiota, dispensable (i.e., nonessential) amino acids may be synthesized de novo from other carbon-containing macromolecules (protein, lipids, carbohydrates) ingested by a consumer (e.g., O'Brien et al. 2002, Howland et al. 2003). Red sea urchin lipids have mean $\delta^{13}C$ values that are 2.2‰ lower than the bulk edible portion and 3.4‰ lower than the lipid-extracted portion of this prey type (Table 4) and lipids account for ~40% of the edible carbon in sea urchins on a per carbon basis. Furthermore, we documented a significant negative correlation between $\delta^{13}C$ TDFs and the fraction of sea otter diet composed of sea urchins (Fig. 2).

Mammalian vibrissae are constructed from α-keratin, which is primarily composed of glycine, serine, and glutamate (Marshall et al. 1991). Glycine is commonly synthesized from serine, which is synthesized via several steps from 3-phosphoglycerate during glycolysis. As such, the $\delta^{13}C$ of glycine and serine reflects whole-diet $\delta^{13}C$ values (Howland et al. 2003, Jim et al. 2006). For animals that consume lipid-rich diets, $^{13}C$-depleted glycerol (Weber et al. 1997) hydrolyzed from ingested lipids may provide a significant source of carbon for glycolysis and the eventual synthesis of glycine and serine used to build keratin. Likewise, glutamate, which is synthesized in the tricarboxylic acid cycle, typically has a carbon isotope composition reflecting bulk dietary carbon (Howland et al. 2003).

Since glycine is $^{13}C$ enriched relative to other amino acids (Hare et al. 1991, Howland et al. 2003, Jim et al. 2006), reported $\delta^{13}C$ TDFs for mammalian keratin (fur and vibrissae) range from 2‰ to 3‰ (Hobson et al. 1996, Roth and Hobson 2000, Lesage et al. 2002), which is larger than those reported for other commonly analyzed tissues (e.g., liver, muscle, blood) in isotope ecology. Published laboratory-based studies use lipid-extracted prey isotope values to calculate TDFs (Hobson et al. 1996, Roth and Hobson 2000, Zhao et al. 2006). Lipid extractions are often performed to remove an isotopic "bias" created by variation in prey or consumer lipid content, and isotope ecologists often gain analytical precision by extracting lipids. For example, the negative relationship shown in Fig. 2 is stronger ($P < 0.01$) if we use lipid-extracted prey values to determine $\delta^{13}C$ TDFs for sea otter vibrissae; however, the magnitude of the $\delta^{13}C$ TDFs ($y$-axis) are ~1‰ lower than those shown using non-lipid-extracted prey, especially for individual otters with lipid-rich diets (i.e., rich in sea urchins). If we use lipid-extracted prey to calculate $\delta^{13}C$ TDFs, several sea otters that consume >55% sea urchins have $\delta^{13}C$ TDFs near 0‰, which is significantly lower than the laboratory-based 2–3‰ TDF for $\delta^{13}C$ of keratin (Hobson et al. 1996, Roth and Hobson 2000) utilized by most applied studies in isotope ecology. If our speculation is correct, applying the accepted $\delta^{13}C$ TDF of 2–3‰ to sea otter vibrissae $\delta^{13}C$ values and then comparing them with lipid-extracted prey values would lead to erroneous inferences of diet composition for this sea otter population that consumes a high proportion of lipid-rich prey (i.e., sea urchins).

Sea otters that consume a high proportion of sea urchins (>55%) may synthesize a greater proportion of their nonessential amino acids (e.g., glycine and serine) from $^{13}C$-depleted lipids. Since glycine and serine are major components of mammalian α-keratin (Marshall et al. 1991), animals that consume lipid-rich diets may have smaller $\delta^{13}C$ TDFs for keratin. Our results suggest that diet quality and especially the consumption of a lipid-rich diet may affect $\delta^{13}C$ TDFs such that $^{13}C$-depleted lipids likely contribute to the amino acid pool used to synthesize proteinaceous tissues such as vibrissae. A similar pattern of lower $\delta^{13}C$ TDFs relative to expectations was observed in the bone collagen, another glycine-rich tissue (~33% by mass), of a captive wolf population fed a lipid-rich (~25% lipid by wet mass) diet (Fox-Dobbs et al. 2007). For many apex marine predators (e.g., marine mammals, seabirds) that commonly consume lipid-rich diets, dietary lipids might be more important in animal metabolism and tissue synthesis than most laboratory feeding experiments suggest. Our field study suggests an intriguing possibility that can be examined with laboratory studies at the compound-specific level (O'Brien et al. 2002, Howland et al. 2003, Jim et al. 2006). We hypothesize that $^{13}C$-depleted dietary lipids contribute more significantly than previously recognized to the synthesis of at least some nonessential amino acids that are major components of keratin.

Because most carnivore species are difficult to observe in their natural habitat(s), isotopically derived dietary

data show promise as a useful proxy for quantifying both dietary inputs and variation at the population and individual levels (e.g., Lewis et al. 2006, Newsome et al. 2009). The ability to accurately quantify dietary inputs, however, is dependent upon accurate estimates of TDFs for the tissue(s) of interest and the degree to which they vary under natural conditions. Previous work in the laboratory has highlighted the importance of diet quality (e.g., protein content), nutritional status (e.g., starvation), and growth rate on TDFs (see review by Martinez del Rio et al. 2009). Our results suggest that variation in TDFs must be incorporated into trophic-level and food chain length estimates derived from isotopic data (see reviews by Post 2002*a*, *b*). Our data also suggest that for consumers with lipid-rich diets such as the SNI sea otter population, variation in keratin $\delta^{13}C$ TDFs may relate to dietary lipid content and consumers with more lipid-rich diets may have smaller TDFs for $\delta^{13}C$. We suggest that the lipid content of common prey should be taken into consideration when using isotopes to characterize the diet of animals that frequently consume lipid-rich foods, such as many marine apex predators (e.g., marine mammals and seabirds). Stoichiometric calculations and previously published feeding experiments at the compound-specific (i.e., amino acid) level show that carbohydrate and lipid carbon contributes to the amino acid pool from which proteinaceous tissues are synthesized (Howland et al. 2003, Jim et al. 2006). Lastly, our data and theoretical considerations suggest that variation in $\delta^{15}N$ TDFs are not likely to be sensitive to prey lipid content, but others have shown that nitrogen isotope TDFs are influenced by other ecological (e.g., diet quality) and physiological (e.g., nutritional stress) factors commonly experienced by organisms in their natural habitats (Hobson et al. 1993, Gaye-Siessegger et al. 2003, Fuller et al. 2004, 2005, Cherel et al. 2005).

Ultimately, the degree of dispersion among isotopically distinct prey and the predators that consume them must be large enough such that interindividual variation in TDFs does not mask potential dietary variation. Until recently, stable isotope mixing models did not include variation in TDFs when calculating source proportions. Bayesian-based mixing models (e.g., MixSIR and SIAR) are now available that allow users to designate error terms in TDFs (Moore and Semmens 2008, Parnell et al. 2010), which will greatly benefit the accuracy of isotopically derived dietary information and help place statistically minded boundaries on the interpretive power of isotopic approaches in ecological studies.

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