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Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*)

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Abstract. Differences in diet composition among conspecifics (dietary specialization) have been documented across a broad range of taxonomic groups and habitats, and such variation at the individual level is increasingly recognized as an important component of diversity in trophic interactions. Accurate identification of individual dietary specialization, however, requires longitudinal dietary records that are labor-intensive and cost-prohibitive to obtain for many species. Here we explore the use of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) as a promising technique for detecting and quantifying patterns of individual dietary specialization. Southern sea otters (*Enhydra lutris nereis*) offer a unique opportunity for testing this approach because (1) they consume a wide variety of prey that span multiple trophic levels, habitats, and ecologically defined functional groups; and (2) individual diet specialization can be validated with existing observational data. We analyzed the isotopic composition of sea otter vibrissae ($n = 31$) in order to characterize inter- and intra-individual variation in sea otter diets at Monterey Bay, California, USA. At the population level, sea otters showed substantial variation in both $\delta^{13}C$ and $\delta^{15}N$ values, occupying nearly all of the "isotopic space" created by the diversity of isotopic signatures of potential prey taxa. Most of the variation in sea otter vibrissae was accounted for by differences between individuals, with much less contributed by within-individual variation. A majority of sea otters (~80%) showed relatively little temporal variability in isotopic composition, suggesting that the proportional composition of most individuals' diets is relatively constant over time; a few individuals (~20%) exhibited a high degree of intra-vibrissa isotopic variability, suggesting seasonal shifts in diet composition. These results and our interpretation of them were supported by long-term observational data on the diets of radio-tagged sea otters from the same population ($n = 23$). Our results demonstrate that stable isotopes can provide an efficient tool for measuring individual- and population-level dietary breadth and may be useful for studying populations where longitudinal data on individuals would otherwise be impossible to acquire. This will be critical for examining the causes and consequences of dietary variation within and among consumer populations, thereby improving our understanding of these important ecological and evolutionary processes at the community level.

Key words: dietary specialization; Enhydra lutris nereis; *isotopic niche; southern sea otter; stable isotopes; vibrissae.*

Introduction

There is growing evidence for individual variation in diet and foraging behavior across a wide range of species and ecosystems (reviewed in Bolnick et al. [2003]). Variation at this level is integral to conceptualizing and understanding process in various dimensions of ecology and evolutionary biology. For example, the complexity of process in food web dynamics will vary greatly depending upon the degree to which dietary variation is explainable at the levels of species vs. individuals. Similarly, the direction and magnitude of consumer population and behavioral responses to spatial and temporal variation in the abundance and quality of their prey differ greatly with the degree of individual variation in diet. Indeed, the extent to which population-level dietary patterns are dictated by the aggregate of similar vs. differing individuals has broad and potentially important implications to population biology, behav-

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ioral and evolutionary ecology, and ecosystem dynamics (Tinker et al. 2008).

At any given location and time, the realized niche of a consumer population represents the sum of all prey selection decisions by the constituent individuals: thus there is a fundamental link between foraging behavior at the individual level and trophic interactions defined at the population level. Nonetheless, the diets of consumers are typically considered only at the population or species level, in many cases ignoring or treating as statistical noise any intra-individual variability in diet. This bias likely reflects the limitations imposed by proxies conventionally used to define niche width, such as body or bill size, as well as the difficulties in obtaining quantitative and temporally integrated estimates of individual diets. Other sources of variation, such as temporal or seasonal dietary shifts by consumers, also have important implications for food web dynamics, but can be overlooked because of the same logistical constraints associated with measuring dietary specialization.

Definitions and criteria for the designation of individual dietary specialization vary, but here we will follow the conventions outlined in Bolnick et al. (2002), which define a foraging specialist as an individual whose dietary niche is considerably narrower than the total niche width (TNW) of the population. TNW measures the full spectrum of prey species consumed in a given population and is made up of two components: (1) a within-individual component (WIC), representing the dietary variation of a typical individual; and (2) a between-individual component (BIC), reflecting dietary variation among individuals. For a given value of TNW, the BIC:WIC ratio increases with the degree of individual specialization.

For most wild populations, accurate and representative estimates of WIC and BIC are difficult to obtain and usually involve time- and labor-intensive observational techniques. To properly quantify WIC, for example, one must collect repeated samples from known individuals over sufficiently long time periods to account for temporal variation, because individual diets can change over time in response to daily, seasonal, or even multiyear shifts in prey availability or profitability (Rosenthal and Janzen 1979, Bekoff and Wells 1986, Thomas 1987). An ideal methodology for determining the relative importance of WIC and BIC to TNW would therefore (1) identify effects of space (i.e., habitat), time, and individuality; (2) provide quantitative information on prey intake and assimilation; (3) be comparable across populations; (4) be noninvasive such that the collection of dietary information would not adversely affect subject behavior or survival; and, perhaps most importantly, (5) provide meaningful ecological data with less effort than required for observational techniques.

Our approach builds on a recent theoretical study by Bearhop et al. (2004) outlining the potential use of isotope biochemistry to study individual foraging specialization. To use this approach, three specific conditions of the consumer–prey system are necessary. First, specific prey species/types must have distinct isotopic values. Second, isotope signatures at the base of the food web must remain relatively constant over time. Seasonal variations in physical parameters such as temperature and nutrient availability can cause baseline changes in isotope values of primary producers, which cascade up food chains to primary and secondary consumers (Michener and Schell 1994, Kelly 2000). Third, in order to properly characterize the within-individual component (WIC) of niche width, the consumer must produce a continuously growing but metabolically inert tissue (e.g., feathers, vibrissae) that can be serially sampled to produce a temporal (longitudinal) record of dietary change. Studies of captive pinnipeds indicate that carbon and nitrogen isotopic variation along the length of individual whiskers was similar to the variance in isotopic composition of their diet (Hobson et al. 1996).

Here we assess the utility of this approach to characterize the degree of individual foraging specialization in a southern sea otter (*Enhydra lutris nereis*; see Plate 1) population from Monterey Bay, California, USA, using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values. For several reasons, sea otters are ideal for examining the utility of isotopes to assess dietary specialization. First, the diet of tagged, and thus individually recognizable, sea otters can be determined through direct field observations (Estes et al. 2003), providing context for interpreting isotopic results. Second, sea otters are known to consume a remarkably diverse array of prey species spanning multiple trophic levels and functionally defined prey groups (Reidman and Estes 1990), potentially resulting in a broad spectrum of isotopic signatures (Table 1, Fig. 2A). Third, adult sea otters tend to remain in the same general area for long periods (Ralls et al. 1996), which reduces the confounding influences of spatial differences in isotope values of primary producers at the base of the food web. Finally, it has already been determined that southern sea otters show a high degree of individual dietary specialization, with the bulk of a typical individual's diet consisting of just three to four prey types (Estes et al. 2003, Tinker et al. 2008).

As keystone predators (sensu Power et al. 1996) in nearshore marine communities of the North Pacific Ocean (Estes and Palmisano 1974, Estes et al. 2004), sea otter populations at high density are capable of limiting the abundance of key prey taxa (e.g., sea urchins, *Strongylocentrotus* spp. and abalone, *Haliotus* spp.), with the result that foraging success at the individual level tends to be negatively density dependent (Estes et al. 1982, Garshelis and Estes 1986, Estes 1990, 1996, Bodkin et al. 2000). As per capita food resources decline, diet diversity at the population level tends to increase (Estes et al. 1982, Ostfeld 1982), but it appears (for the southern sea otter at least) that this increase in niche

TABLE 1. Mean $\delta^{13}C$ or $\delta^{15}N$ values, standard error of the mean (SEM), and C:N ratios (mean ± SD) of sea otter prey collected along the central California coast (San Simeon and Monterey Bay), USA.

Common name	Species	n	$\delta^{13}C$		$\delta^{13}C_{LC}$	$\delta^{15}N$		C/N (SD)	Dietary %
			Mean	SE		Mean	SE		
Dungeness crab	*Cancer magister*	14	−15.6	0.2	−15.6	14.2	0.2	4.0 (0.1)	27.4†
Rock crab	*Cancer antennarius/productus*	15	−15.1	0.2	−15.1	13.9	0.2	4.0 (0.1)	27.4†
Northern kelp crab	*Pugettia producta*	16	−13.3	0.3	−13.0	11.4	0.2	4.6 (0.1)	15.8
Purple sea urchin	*Strongylocentrotus purpuratus*	6	−17.0	0.4	−16.5	9.3	0.2	5.0 (0.4)	11.1†
Red sea urchin	*Strongylocentrotus franciscanus*	9	−15.6	0.5	−14.9	10.4	0.2	5.7 (0.6)	11.1†
Clam	*Tresus/Saxidomus/Protothaca*	19	−15.4	0.2	−15.4	11.4	0.1	4.0 (0.1)	9.8
Snail	*Chlorostoma* spp.	12	−14.3	0.3	−14.0	10.4	0.2	4.5 (0.2)	7.9
Mussel	*Mytilus californianus*	8	−16.8	0.2	−16.8	9.6	0.2	3.9 (0.1)	7.3
Abalone	*Haliotis cracherodii/rufescens*	12	−15.4	0.2	−15.4	10.0	0.3	3.6 (0.1)	5.3
Innkeeper worm	*Urechis caupo*	6	−14.9	0.1	−14.5	11.8	0.2	4.7 (0.1)	3.0
Sea star	*Pisaster ochraceus/giganteus*	9	−19.0	0.5	−17.8	10.6	0.2	7.4 (0.6)	2.4
Sand crab	*Emerita analoga*	3	−18.5	0.4	−17.6	10.2	0.2	5.9 (0.4)	1.5

Notes: For some prey types (i.e., rock crab or clams), multiple species have been combined because there was low intraspecific variation in isotope values among species. For all prey types with average C:N ratios > 4.0, we have corrected measured $\delta^{13}C$ values for lipid content ($\delta^{13}C_{LC}$) using algorithms described in McConnaughey and McRoy (1979) and a mean difference of 3.5‰ between protein and lipid $\delta^{13}C$. Clam species include *Tresus nuttalli*, *Saxidomus nuttalli*, and *Protothaca staminea*. Snail species include *Chlorostoma funebralis*, *C. pulligo*, *C. brunnea*, and *C. montereyi*. Percentages represent the average diet composition of sea otters at the two sites based on extensive observational studies (Tinker et al. 2008). In the case of sea urchins, *Strongylocentrotus pupuratus* (purple sea urchins) represent >90% of sea urchins consumed along the central California coast (Tinker 2004, Oftedal et al. 2007).

† Percentages with daggers denote prey types that cannot be differentiated via remote observation. For example, *Cancer* crabs represent 27.4% of prey consumed at the population level; however, we do not know the exact dietary percentages of each *Cancer* species consumed (e.g., *Cancer magister* vs. *antennarius* vs. *productus*).

width occurs primarily via increased individuality (a greater BIC:WIC ratio) as opposed to an expansion of individual dietary breadth (Tinker et al. 2008).

It has taken decades of observational study to quantify the prevalence of individual dietary variation and explore how foraging individuality is created and maintained in wild sea otter populations. In this study, we show how stable-isotope-based dietary proxies can also be used to efficiently and effectively quantify this phenomenon in a southern sea otter population inhabiting Monterey Bay, California. We also demonstrate that isotopic proxies can provide data on temporal shifts in diet composition for individual animals. Comparison of our results with observational data on diet from a large, but mostly separate, group of individuals provides a unique opportunity to validate individual- and population-level foraging characteristics gathered from isotopic proxies.

MATERIALS AND METHODS

Prey and sea otter tissue collection

Sea otter prey samples were collected during a series of diving and shore-based sampling trips in 2004 and 2006 at two study sites in central California: the San Simeon/Cambria vicinity, and the Monterey Bay area. We analyzed 20 prey species that together comprise >90% of prey consumed at the population level by sea otters in central California, as based on observational data (Table 1; Estes et al. 2003, Tinker et al. 2008). Based on similarities in ecological characteristics and isotopic composition, these 20 species were collapsed into 12 taxonomic groups: Dungeness crab (*Cancer magister*), rock crabs (*Cancer antennarius* and *Cancer productus*), kelp crab (*Pugettia producta*), purple sea urchin (*Strongylocentrotus purpuratus*), red sea urchin (*Strongylocentrotus franciscanus*), clams (*Tresus nuttalli*, *Saxidomus nuttalli*, and *Protothaca staminea*), herbivorous marine snails (*Chlorostoma funebralis*, *Chlorostoma pulligo*, *Chlorostoma brunnea*, and *Chlorostoma montereyi*), mussel (*Mytilus californianus*), abalone (*Haliotis cracherodii* and *Haliotis rufescens*), innkeeper worm (*Urechis caupo*), sea star (*Pisaster ochraceus* and *Pisaster giganteus*), and sand crab (*Emerita analoga*). Purple sea urchins are more abundant in kelp forest communities along the central California mainland coast and probably represent a more important prey item for Monterey Bay sea otters in comparison to red sea urchins (Oftedal et al. 2007).

Sea otter vibrissae were sampled from both wild-caught animals and stranded carcasses collected within Monterey Bay, from Point Lobos (south) to the Santa Cruz Harbor (north). Vibrissae of wild-captured animals ($n = 9$) were collected during periodic population assessments by the U.S. Geological Survey (USGS) and California Department of Fish and Game from 2000 to 2004. Stranded carcasses ($n = 22$) were collected between 1998 and 2006 as part of the USGS carcass retrieval program. Details of the capture, handling, and radio-tagging of study animals are provided elsewhere (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

Fig. 1 presents a schematic of our isotopic approach, in which the *x*-axis represents the stable isotope "space"

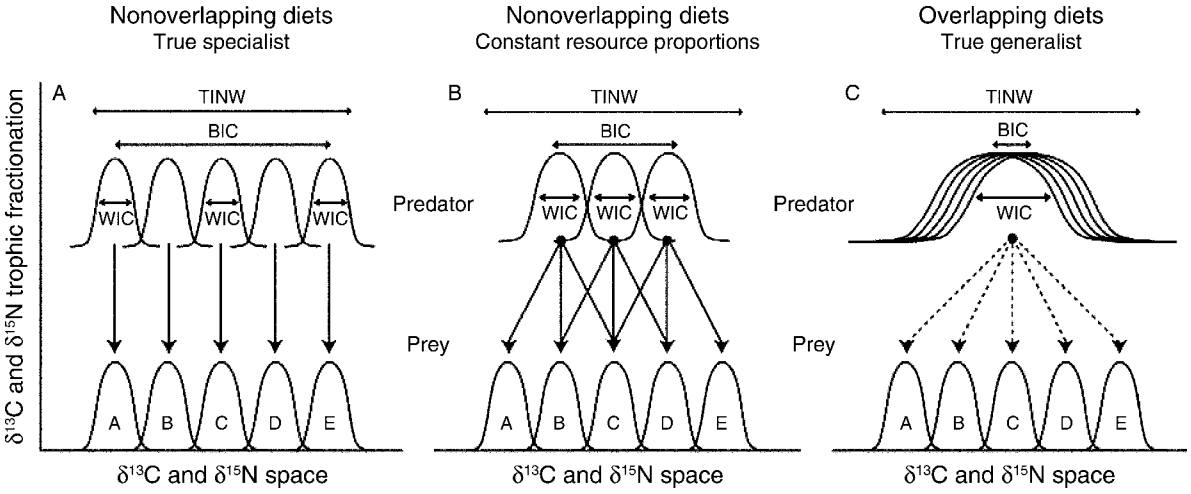

FIG. 1. A schematic diagram of how to determine the degree of specialization within animal populations using stable isotope values that adopts terms commonly used in the study of individuality (see Bolnick et al. 2003). Here, total isotopic niche width (TINW) is defined as the total variance of prey isotope values, which can be defined in two-dimensional space when using two isotope systems. Prey values are reflected in isotope values of consumers after appropriate trophic-dependent discrimination factors are applied to either consumer or prey values. Solid lines represent constant percentages of single or multiple food resource(s) in consumer diets over time; dashed lines represent cases when resource proportions shift over time. Isotopic variance within and between consumers, WIC and BIC, respectively, varies with the degree of individual specialization (i.e., nonoverlapping diets) in a given population. (A) Predictions for a population of true specialists in which the within-individual component (WIC) of niche space, as measured in two-dimensional isotope space, is relatively small in comparison to the variance in isotope values across prey types (i.e., TINW). (B) Predictions for a second type of specialist that forages on multiple food sources where the relative proportions of those prey do not change over time. Individuals in this scenario would have low isotopic variability and occupy a relatively small isotopic "space" in comparison to purely generalist individuals (C). (C) Predictions for a population of foraging generalists, in which the relative proportions of multiple prey in a given individual's diet do change over time. In this case, the variability in individual consumer isotope values (WIC) is a larger proportion of both the overall variance of prey isotope values (i.e., TINW) and the inter-individual variance in consumer isotope values (i.e., BIC).

of prey and individual consumers in a predator population. The isotope "space" here refers to the two-dimensional area in a $\delta^{13}C$ or $\delta^{15}N$ plot commonly used in isotope ecology, but for heuristic simplicity we represent the variation by a single dimension that can be thought of as either $\delta^{13}C$ or $\delta^{15}N$ values. The *y*-axis represents tissue-specific trophic discrimination in isotope values between prey and consumer. The three panels in Fig. 1 illustrate how different patterns of individual diet composition (ranging from complete specialization to complete generalization) will result in distinct and measurable patterns of variation in isotopic composition, represented as changes in the BIC:WIC ratio. The relative degree of isotopic variation within an individual sea otter vibrissa serves as a proxy for the within-individual component (WIC) of diet and also provides information on the degree of temporal consistency in diet composition. The between-individual component (BIC) is measured by differences between individual otter vibrissae; to serve as a proxy for the BIC:WIC ratio, we determined the relative contribution of different sources of isotopic variance (intra- vs. inter-vibrissae isotopic variability) using variance components analysis.

Prey specimens were rinsed of sediment or detritus, weighed, and measured using digital calipers. In many cases, the soft tissue of the prey was removed and sorted using a dissection microscope to separate edible from inedible matter. The edible portion was then lypholized and homogenized by grinding to a coarse powder using a freezer mill. Approximately 0.5 mg of the powdered tissue samples 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 elemental analyzer (NC 2500; Carlo Erba, Milan, Italy) interfaced with a Finnegan Delta Plus XL mass spectrometer (Carnegie Institution of Washington, Washington, D.C., USA).

Isotopic results are expressed as δ values, $\delta^{13}C$ or $\delta^{15}N = 1000\ [(R_{sam}/R_{std}) - 1]$, where R_{sam} and R_{std} 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 (V-PDB) for carbon and atmospheric N_2 for nitrogen. The units are expressed as parts per thousand, or per mil (‰). The within-run standard deviation of a gelatin standard ($n = 100$) 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 with a 2:1 chloroform : methanol mixture to remove surface contaminants. Cleaned vibrissae were subsampled into ~0.5-mg segments using nail clippers and were sealed into tin boats for isotopic analysis. Carbon and nitrogen isotope values were determined using the mass spectrometer system previously described. The number of

samples analyzed from each individual varied from 8 to 21 (mean = 16) depending on the length of its vibrissa. As a control for the quality of keratin, we measured the carbon-to-nitrogen ([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 (3.4:1).

To correct sea otter isotope data for trophic discrimination and plot consumers in dietary space (Figs. 2 and 3), we have subtracted 2.5‰ and 3.5‰ from mean individual $\delta^{13}C$ and $\delta^{15}N$ values, respectively, presented in Table 2. $\delta^{13}C$ and $\delta^{15}N$ trophic discrimination factors can vary (Vander Zanden and Rasmussen 2000, Vanderklift and Ponsard 2003) depending on prey type, prey quality, and the general metabolic pathway(s) consumers use to digest and assimilate food. Isotopic studies of captive red foxes (Roth and Hobson 2000), captive phocid seals (Hobson et al. 1996), and wild Steller sea lions (Stegall et al. 2008) suggest a $\Delta^{13}C_{diet\text{-}ker}$ discrimination factor of ~2.5–3.2‰ (subscript "ker" is keratin). To correct the nitrogen isotope data, we use a $\Delta^{15}N_{diet\text{-}ker}$ value of 3.5‰ commonly applied in isotopic studies of mammalian consumers (see reviews by Kelly [2000] and Vanderklift and Ponsard [2003]). Variations in sea otter $\Delta_{diet\text{-}ker}$ values for both carbon and nitrogen isotopes, however, do not compromise our conclusions regarding individuality, because characterizing within- (WIC) and between-individual (BIC) isotopic variation is more important than determining the true mean isotopic composition of each individual when attempting to assess the prevalence of dietary specialization in a single population.

We used linear mixed-effects models to examine the partitioning of variance in isotope signatures, assuming that variation in $\delta^{15}N$ and $\delta^{13}C$ measurements from individual vibrissae could be explained by a combination of fixed and random effects. Variance components were estimated for random effects using ANOVA methods with type-III sums of squares (Searle et al. 1992) and using Satterthwaite's (1946) method of denominator synthesis to test the significance of each potential source of variation. Nonsignificant variance components ($P > 0.05$) were assumed to be equal to zero. In our model, the random effects included geographic region (two levels: Monterey Peninsula, dominated by rocky substrate, and Monterey Bay, dominated by sandy substrate) and individual otters nested within region. The residual error term corresponded to within-individual variation, thus accounting for all remaining variance not explained by other terms. Sex was included in the model as a fixed effect, but the sex × region interaction represented an additional random effect and potential source of variation. We did not include age class as an effect because all animals in our sample were classified as adults. We estimated variance components separately for $\delta^{15}N$ and $\delta^{13}C$, but for simplicity of presentation we combined the results and we report average values as proportions of variance explained. We further assessed qualitative patterns of intra-individual variation through graphical examination of individual time series and variance/covariance patterns of $\delta^{13}C$ and $\delta^{15}N$ values.

Observational dietary data

Between February 2001 and August 2005 we collected observational information on the diets of 63 radiotagged study animals within the Monterey Bay National Marine Sanctuary, and most observations were conducted in one of two study sites: Monterey Peninsula in the north and San Simeon/Cambria in the south. With four exceptions, these were different individuals from those in which vibrissae were collected and isotopically analyzed. We restricted our analysis of the observational information to those animals with extensive dietary data sets collected across all seasons. Specifically, for each animal we first binned all foraging data by month, and then identified the number of months having at least three recorded foraging bouts consisting of at least 20 feeding dives each (≥60 dives per month). We then selected the 23 animals that met these criteria for at least four separate months within a period spanning at least eight months of one year; unfortunately, only one of these animals belonged to the same subset of animals for which we also acquired isotopic data. The resulting data set, consisting of 23 663 dives recorded over 516 foraging bouts and including 24 265 observed prey captures, provided a reliable basis for testing for both individual and seasonal variation in diet. As with the isotope analysis of prey, we combined related prey species into functional groups prior to analysis. These groupings were identical to those described for the isotopic analysis except that we combined red and purple sea urchins into one group ("urchins") due to the difficulties of reliably distinguishing between these species during field observations.

To assess the relative magnitude of within-individual variation vs. between-individual variation in diet composition, we estimated variance components using the methods previously described for the isotope analysis, except that in this case the raw data used were monthly estimates of prey-specific capture frequencies for individual otters, rather than stable isotope measurements. To simplify analysis, we first employed a principal components analysis (PCA) to collapse the monthly frequencies of all prey types into a smaller number of orthogonal factors. We limited further analysis to the first four principal components, which together accounted for most of the variance (63%) in all prey types. Using PCA factors as dependent variables, we again used ANOVA methods to examine variance partitioning. For this set of models, the random effects evaluated included geographic region (Monterey area vs. San Simeon area) and individual otters nested within regions. Any nonsignificant variance components ($P > 0.05$) were assumed equal to zero, and within-individual variation was represented by the residual error term. We did not

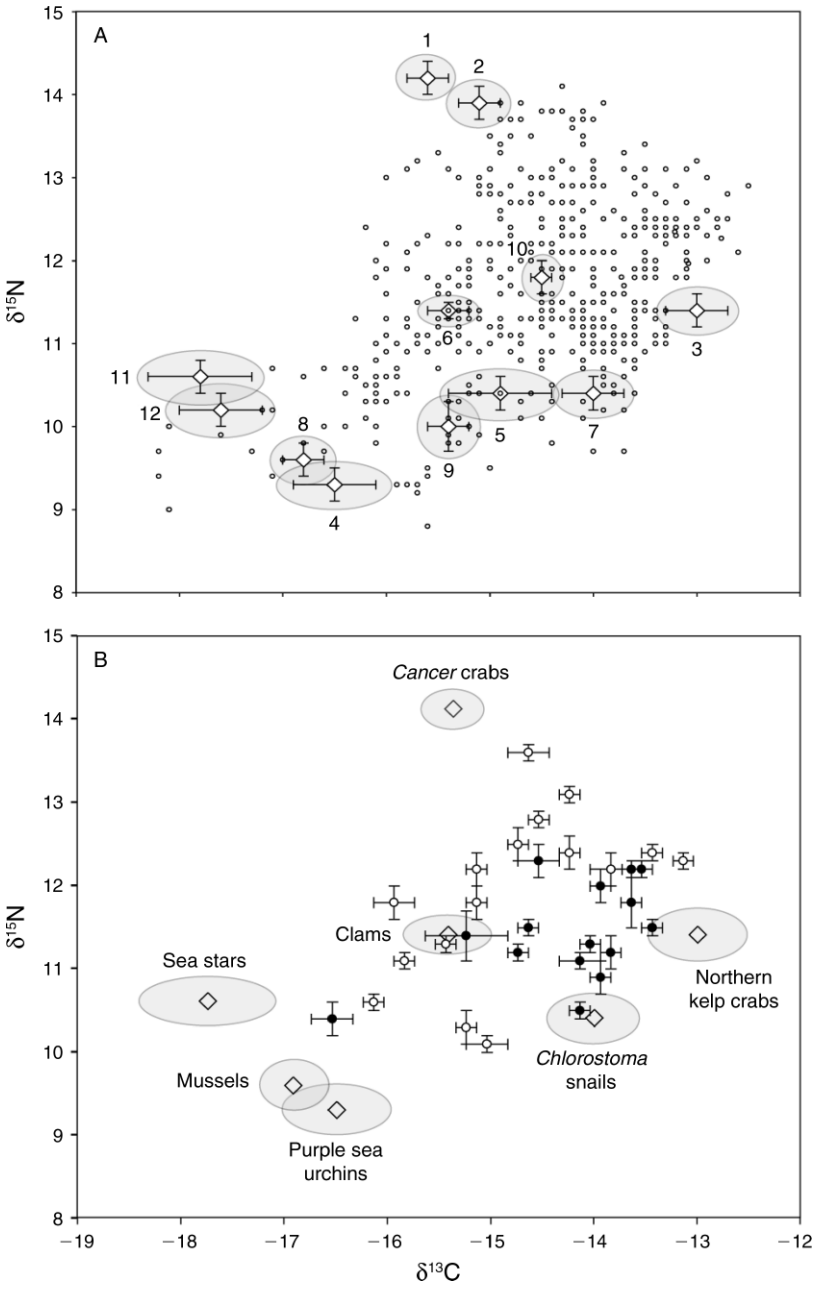

FIG. 2. (A) Open circles are $\delta^{13}C$ or $\delta^{15}N$ data of all serially sampled vibrissae segments from the Monterey Bay, California, USA population of sea otters (*Enhydra lutris nereis*; $n = 480$) corrected for trophic discrimination by subtracting 2.5‰ and 3.5‰ from carbon and nitrogen isotopic values, respectively. Open diamonds (in gray ovals) are mean (±SE) $\delta^{13}C_{LC}$ or $\delta^{15}N$ values for 12 sea otter prey taxa collected from different years and seasons at both localities; refer to Table 1 for scientific names and sample sizes. Individual prey species include: (1) Dungeness crabs, (2) rock crabs, (3) northern kelp crabs, (4) purple sea urchins, (5) red sea urchins, (6) clams, (7) snails, (8) mussels, (9) abalone, (10) fat innkeeper worms, (11) sea stars, and (12) sand crabs. Refer to Table 1 for scientific names, sample sizes, and mean isotope values (±SE) of prey species. (B) Mean keratin (vibrissae) $\delta^{13}C$ or $\delta^{15}N$ values of individual sea otters (Monterey Bay, California, USA) and common prey items that represent >90% of consumed prey at the population level based on observational data. Mean sea otter isotope values have been corrected for trophic discrimination by subtracting 2.5‰ and 3.5‰, respectively, from the carbon and nitrogen isotopic values reported in Table 2. Error bars and gray ovals represent standard error. Open circles are females; solid circles are males. Refer to Tables 1 and 2 for sample sizes and mean isotope values of individual otters and prey species. Isotopic data for Dungeness crabs (*Cancer magister*) and rock crabs (*Cancer antennarius*/*productus*) were combined because these species had similar isotope values.

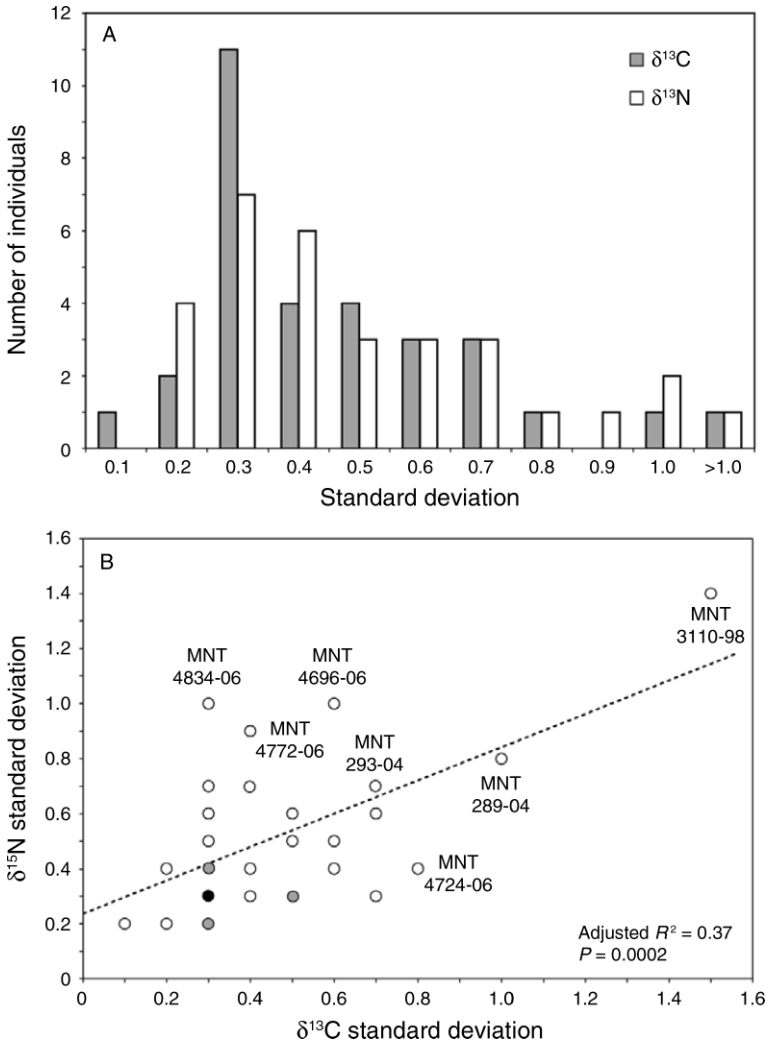

FIG. 3. (A) Frequency distribution of $\delta^{13}C$ or $\delta^{15}N$ standard deviations for individual sea otters ($n = 31$). (B) Covariance of intra-individual variability in $\delta^{13}C$ and $\delta^{15}N$ values. Open circles represent data for one individual; gray circles represent data for two individuals; the solid circle represents data for three individuals. Data labels identify seven individuals that have high isotopic variability in both or one isotope system (see Fig. 4).

include age class or sex as factors, because 21 of the 23 study animals were adult females. We averaged the variance component estimates for the four PCA factors, weighted by their associated eigenvalues, to obtain a single representative value for each variance component, which we report as the proportion of variance explained (specifically, the mean proportion of variance in the four PCA factors).

Next, for each study animal we identified the three most commonly consumed prey types (based on all data available for that animal) and then estimated the proportional contribution to the individual's monthly diet for each of these three types, in terms of total biomass consumed. This resulted in three generic variables (prey1, prey2, and prey3), measured across months and individuals. Note that the identity of prey types 1–3 differed from animal to animal, as we were interested here in the temporal patterns of variation in diet, irrespective of prey species. We collapsed the three prey types into a single composite variable using PCA: the first factor accounted for ~50% of the total variation in the data set, and was most closely correlated with the relative abundance of the most preferred prey type ($R = 0.928$, $P < 0.001$). For each individual we then calculated the magnitude of variance (across months) in PCA Factor 1, which we used as an index of seasonal dietary variation. We plotted a histogram of this index for all 23 study animals, to examine the distribution of individuals with respect to seasonal variation in diet composition.

All statistical tests were calculated using the software program JMP (version 7.0.1, 2007), with the exception of variance component analyses, which was conducted using the SYSTAT "MIXED" function, version 11

TABLE 2. Overall length (cm) and $\delta^{13}C$ or $\delta^{15}N$ values (mean and SE) of sea otter vibrissae from Monterey Bay, California, grouped according to sex.

Individual	*n*	Length (cm)	$\delta^{13}C$	SE	$\delta^{15}N$	SE	Sex
952-03	20	4.7	−12.6	0.1	15.7	0.2	F
953-03	11	3.9	−13.3	0.1	14.6	0.1	F
956-03	16	5.5	−12.6	0.1	15.3	0.2	F
959-03	14	5.6	−13.6	0.1	14.1	0.1	F
946-03	18	5.1	−12.0	0.1	16.3	0.1	F
960-03	13	3.9	−12.9	0.1	14.8	0.1	F
293-04	16	6.5	−13.4	0.2	15.3	0.2	F
4696-06	20	8.8	−12.7	0.1	13.8	0.2	F
4724-06	21	6.8	−12.6	0.2	13.5	0.1	F
4748-06	21	8.5	−10.6	0.1	15.8	0.1	F
4772-06	14	6.5	−12.2	0.1	16.0	0.2	F
4818-06	19	8.8	−11.3	0.2	15.7	0.1	F
4824-06	9	3.2	−10.9	0.1	15.9	0.1	F
4833-06	15	5.0	−12.1	0.2	17.1	0.1	F
4872-06	18	6.6	−11.7	0.1	16.6	0.1	F
4874-06	12	3.4	−11.7	0.1	15.9	0.1	F
Mean (SD)	16	5.8 (1.8)	−12.2 (0.9)		15.4 (1.0)		
938-03	19	6.6	−12.1	0.1	15.0	0.1	M
289-04	20	5.2	−14.0	0.2	13.9	0.2	M
3110-98	18	8.1	−12.7	0.4	14.9	0.3	M
4687-06	14	7.3	−11.4	0.1	14.4	0.2	M
4690-06	17	6.5	−10.9	0.1	15.0	0.1	M
4732-06	15	6.1	−11.3	0.1	14.7	0.2	M
4742-06	14	4.1	−11.4	0.1	15.5	0.2	M
4749-06	14	4.0	−11.0	0.1	15.7	0.2	M
4751-06	17	5.4	−12.2	0.1	14.7	0.1	M
4794-06	19	7.2	−11.1	0.1	15.7	0.1	M
4834-06	9	3.3	−11.1	0.1	15.3	0.3	M
4844-06	15	7.2	−11.6	0.2	14.6	0.1	M
4848-06	15	5.5	−11.5	0.1	14.8	0.1	M
4850-06	11	5.0	−12.0	0.2	15.8	0.2	M
4866-06	17	7.9	−11.6	0.1	14.0	0.1	M
Mean (SD)	15	6.0 (1.5)	−11.8 (0.8)		14.9 (0.6)		

Note: Sample size *n* is the number of segments sampled from each vibrissa, except where mean (and SD) values are given for all 16 females (F) and all 15 males (M).

(2006). Isotopic differences among prey types were determined via a multivariate analysis of variance (MANOVA) model of $\delta^{13}C$ and $\delta^{15}N$ values and an identity response function. Post hoc Wilks' lambda and Pillai's trace multivariate tests yielded significant differences ($P < 0.05$) among major prey types presented in Fig. 2B. When sample sizes were adequate, we also assessed whether prey isotope values were influenced by collection season and locality using a MANOVA model; neither of these factors had a significant effect on isotope values for individual prey types ($P > 0.10$).

RESULTS

Isotopic results

Isotopic analyses of common sea otter prey (Table 1) resulted in two patterns important to our approach in assessing foraging strategies in sea otters (Fig. 1). First is the exceptionally large range in isotope values of common prey taxa (Fig. 2A); mean $\delta^{13}C_{LC}$ values range from −17.8‰ to −13.0‰ and mean $\delta^{15}N$ values range from 9.3‰ to 14.2‰ (Table 1). Second is the significant isotopic separation among most prey types: variation in isotope values of individual prey types is relatively small in comparison to variation between prey types (we define a "prey type" here as either a single species of a group of ecologically or functionally similar species). These patterns create a relatively large isotopic prey space that individual sea otters could potentially occupy (Fig. 2).

Isotopic results of all sampled whisker segments ($n = 480$; Table 2, Fig. 2A) show that sea otters in the Monterey Bay population occupy a large percentage of available dietary space. If sea otters are diet generalists, we would expect that most of this variation reflects differences between whisker segments within individual vibrissae; conversely, in the case of diet specialization we would expect that most of the variation reflects differences between individual vibrissae. Examination of the means and variance of keratin $\delta^{13}C$ and $\delta^{15}N$ values for individual sea otter vibrissae revealed strong support for the latter scenario (Fig. 2B). Variance component analysis (Table 3) confirmed that the between-individual differences explained almost twice the amount of variation in isotopic values (48%) as did within-individual isotopic differences (28%). Effects related to geographic region and sex, respectively, accounted for 20% and 4% of the isotopic variance.

To further examine the variance/covariance patterns of within-individual $\delta^{13}C$ and $\delta^{15}N$ values, we plotted histograms of the standard deviation values for individual whiskers (Fig. 3). The variance structure was left-skewed for both carbon and nitrogen isotopes (Fig. 3A), indicating that most individuals analyzed had little isotopic variation along the length of a single whisker (Appendix), and within-individual $\delta^{13}C$ and $\delta^{15}N$ standard deviations for these animals were strongly and positively related (Fig. 3B).

Several individuals, however, did show significant isotopic variation in one or both isotope systems (Fig. 4; points are also labeled in Fig. 3B). Panels A–D of Fig. 4 present serially sampled data for four individuals that had high variability in both $\delta^{13}C$ and $\delta^{15}N$ values; panels E–G present serially sampled whisker data for three individuals that had high variance in just one of the isotope systems. In the case of the individuals that showed variability in both carbon and nitrogen (panels A–D), there was a positive correlation between $\delta^{13}C$ and $\delta^{15}N$ values and two of these individuals (panels A and B) show a cyclic, perhaps seasonal, pattern in ^{13}C and $\delta^{15}N$ values.

Observational dietary data

Patterns inferred from the isotopic data were generally supported by observational data collected from radio-tagged sea otters. Individual sea otter diets were highly variable, consistent with the strong degree of dietary specialization already reported for this population (Estes et al. 2003, Tinker et al. 2007, 2008). Variance component analysis indicated that between-individual differences explained a greater proportion of dietary variation (49%) than within-individual effects (44%), while differences between geographic regions contributed much less to the observed variation (7%). Similar to the pattern of temporal variation in isotopic ratios, the distribution of individuals with respect to monthly variance in diet composition was distinctly bimodal (Fig. 5). Five of the 23 individuals displayed strong seasonal variation in dietary composition, whereas the diets of the remaining 18 individuals remained largely unchanged over the same time period. The relative frequency of the most preferred prey type in the diets of these five animals over the course of one year is shown in Fig. 6. In each case there is a clear seasonal peak in the frequency of the prey, which generally corresponds to the reproductive season of that species and thus gonad size and net calorific density (Oftedal et al. 2007).

Discussion

Despite the growing evidence for dietary individuality and its relevance to understanding process in nature, the prevalence, effects, and limiting conditions of dietary variation among and within individuals are still poorly understood because of the long periods of time, high costs, and methodological impediments to obtaining the requisite longitudinal dietary records for all consumers using traditional observational techniques. Indirect dietary proxies, such as stable isotope and fatty acid analysis, offer manageable alternatives for documenting and understanding patterns of dietary variation in numerous wildlife species. For stable isotopes specifically, two fundamental conditions of the consumer and its prey resources are needed to effectively measure and analyze dietary individuality. One is that isotopic signatures must vary predictably among species in the consumer's prey field. The other is that the consumer must produce continuously growing, but metabolically inert, tissues, such as hair, nails/claws, teeth, scales, otoliths, or feathers that are retained by the consumer for sufficient lengths of time to provide a temporal record of isotopic variation (Furness et al. 2006, Lewis et al. 2006, Cherel et al. 2007, Harrison et al. 2007). Many of these tissues can be sampled directly from wild populations without endangering the health of study subjects.

Table 3. Percentage of variance in the vibrissae isotopic data set explained by four hierarchically nested effects (nANOVA): within-individual variation, between-individual variation, and variation attributable to geographic regions.

Effect	Variance explained (%)
Between-individuals	48.1
Within-individual	28.2
Geographic region	20.0
Sex × geographic region	3.7

Note: Sex was also included as a fixed effect, but the sex × region interaction represents an additional random effect.

For sea otters as well as many other mammalian species, the mustacial vibrissae are a potentially suitable candidate tissue. Our isotopic results from the vibrissae of California sea otters are consistent with findings from long-term field observational studies in showing that the proportional composition of diets varies strongly among individuals, and that the diets of most individuals remain largely unchanged over long periods of time (Estes et al. 2003). A majority of individuals (~80%) show a low degree of intra-individual isotopic variability, suggesting that the relative proportion of prey items in the diets of these animals does not vary substantially over time. In addition to low within-individual variation, variance component analyses suggest that a significantly greater proportion of the overall isotopic variance is explained by differences among (BIC) rather than within (WIC) individual whiskers (Table 3). Of the 31 individuals whose whiskers were serially sampled in this study, only seven (~23%) showed high intra-vibrissa isotopic variability (Figs. 5 and 6). This percentage is similar to that obtained independently from field observations, which also indicate that a minority (five of 23 individuals or ~22%) of individuals vary their diets seasonally (Fig. 6). Thus, observational and isotopic results both demonstrate that sea otters have highly individualized diets, that most individuals maintain

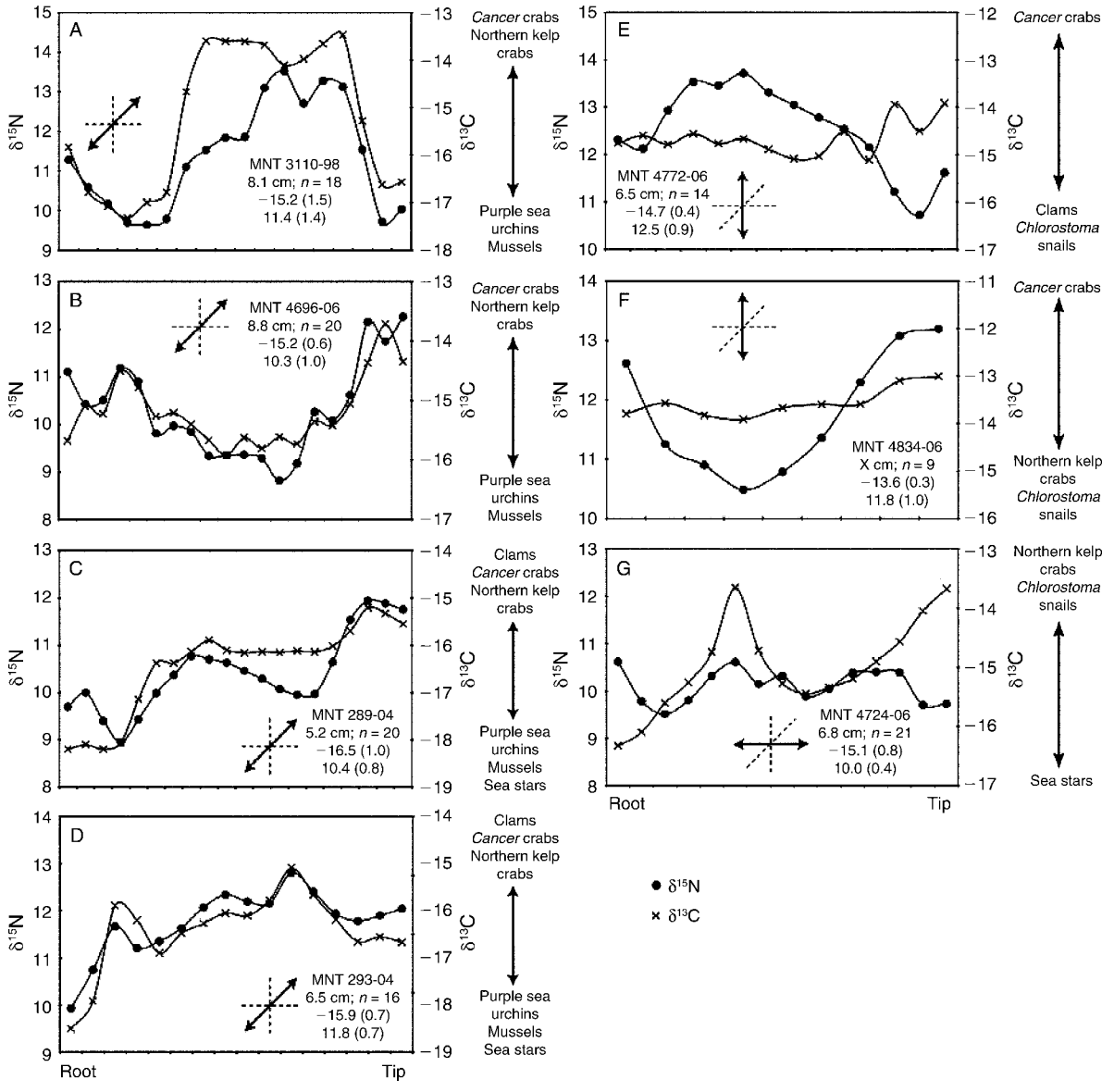

FIG. 4. The $\delta^{13}C$ or $\delta^{15}N$ values of sequential vibrissae segments for seven individuals that exhibit (A–D) exceptionally high isotopic variability in both $\delta^{13}C$ and $\delta^{15}N$ values or (E–G) high variance in one but not both isotope systems. For individuals with high variability in both $\delta^{13}C$ or $\delta^{15}N$ values (A–D), carbon and nitrogen isotope values are positively correlated. Sea otter isotope values have been corrected for trophic discrimination (see *Materials and Methods* for details). Vibrissae length (cm), the number of samples (*n*) analyzed from each vibrissa, and mean (SD) values are shown below sample identifications; root and tip denote the orientation of the vibrissae. Bold arrows on the schematic axes adjacent to sample information represent the direction in dietary space in which individual sea otters move through time. Prey information to the right of each panel shows likely dietary shifts through time based on the relative position of sea otter values with respect to those of potential dietary sources (Fig. 2).

unique dietary patterns through time, but that ~20% of the animals vary their diet in apparent response to seasonal fluctuations in prey quality and/or abundance.

For individuals with high intra-vibrissa variability in carbon and/or nitrogen isotopic composition, $\delta^{13}C$ and $\delta^{15}N$ values are sometimes positively correlated along the length of the vibrissa (Fig. 4A–D). In other cases, individuals show high variance in one, but not both, isotope systems (Fig. 4E–G). Besides temporal changes in diet composition, shifts in consumer isotope values can be influenced by two other factors: (1) temporal variation in isotope values at the base of the food web; and (2) changes in the consumer's physiological condition (e.g., anabolic vs. catabolic state). Neither of these two factors appears to explain the large isotopic shifts observed in some sea otter vibrissae. Although phytoplankton carbon and nitrogen isotope values are positively correlated in the upwelling system in Monterey Bay, California (Rau et al. 1998, 2001), the magnitude of seasonal isotopic variation in phytoplank-

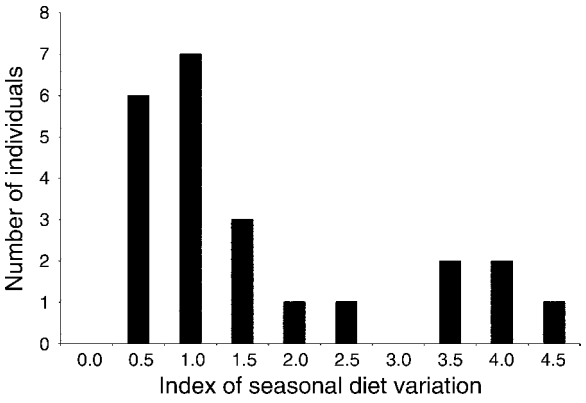

FIG. 5. Frequency distribution of individuals with respect to seasonal variation in diet composition as based on direct observation; see *Materials and methods* for details on how the diet variation index was calculated. Only five of 23 individuals (~22%) show a high degree of seasonal dietary variation (i.e., high seasonal index values).

ton is much lower than the large shifts of ~3–4‰ in $\delta^{13}C$ and/or $\delta^{15}N$ observed in some of the sea otter vibrissae. We know of no published data on seasonal variations in isotopic composition of macroalgae living along the central California coast. By analyzing common sea otter prey items collected over multiple seasons and in different regions, however, we have been able to characterize long-term and large spatial-scale variability in isotopic signatures of sea otter prey types due to variation in isotope values at the base of the food web. These data also provide little evidence for spatial or temporal variation in the isotopic composition of prey. Indeed, if seasonal variation in the isotopic signatures of autotrophs were responsible for the isotopic temporal variation observed in sea otter vibrissae, this pattern should appear in all of the individual sea otters, which was not the case.

Controlled laboratory studies have been used to explore the utility of stable isotopes as proxies of nutritional stress in numerous species (Hobson et al. 1993, Scrimgeour et al. 1995, Cherel et al. 2005, Boag et al. 2006). For experiments on vertebrates, most of which were conducted on birds, a few controlled feeding studies have found significant bulk tissue $\delta^{15}N$ enrichments of ~0.5–2.0‰ in nutritionally stressed animals (Hobson et al. 1993, Cherel et al. 2005); nutritional stress had little or no significant effect on bulk tissue $\delta^{13}C$ values. Temporal changes in physiological condition, however, do not explain the observed isotopic variation in sea otter whiskers for the following reasons. The magnitude of observed $\delta^{15}N$ variation in sea otter whiskers of ~3–4% is more than twice that found to result from nutritional stress in any experimental analysis we know of. Furthermore, carbon and nitrogen isotope values often covary in sea otters (Fig. 4A–D). If nutritional stress were the main driver of temporal isotopic variation in sea otters, this should be reflected in

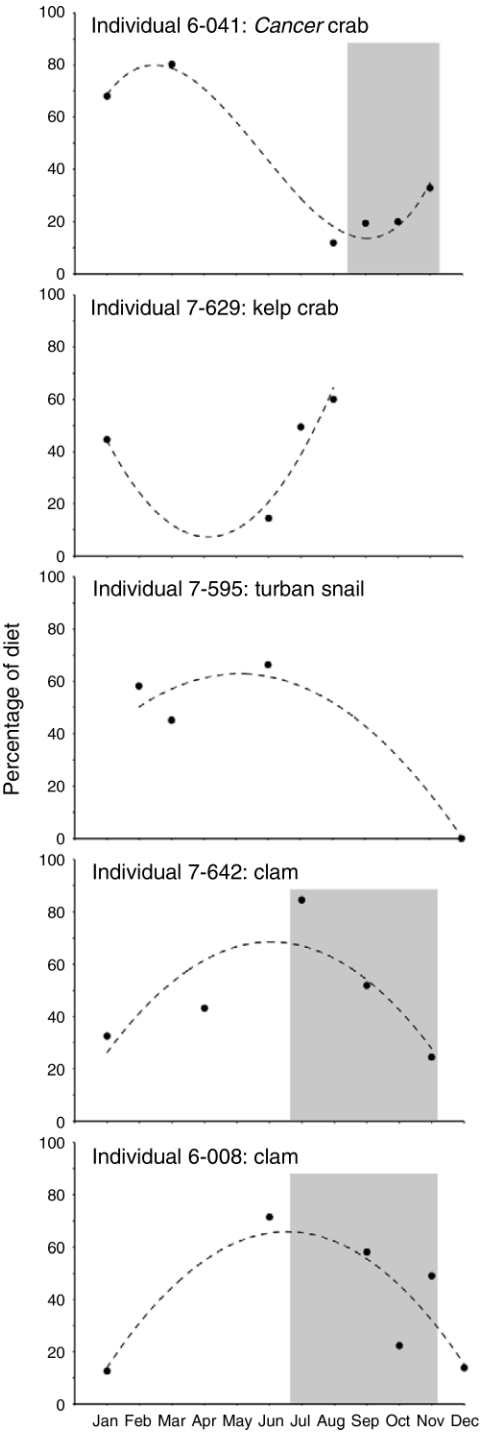

FIG. 6. Dietary frequency (percentage of observations) of the most preferred prey type for each of five individual sea otters that showed strong seasonal diet shifts (see *Results* for details). Shading indicates the season of peak energy content (based on a wet edible basis) available for some prey types (Oftedal et al. 2007).

PLATE 1. Territorial adult male California sea otter (*Enhydra lutris nereis*) grooming just before the start of a forage bout. Photo copyright © Jane Vargas, used by permission.

$\delta^{15}N$ but not $\delta^{13}C$. Lastly, sea otters, which have extremely high metabolic rates and low lipid stores compared to other marine mammals (Reidman and Estes 1990), are not physiologically designed to withstand the extended periods of nutritional stress (weeks to months) that would be required to alter bulk tissue values by the observed amounts.

The lack of an accurate vibrissa growth rate for sea otters makes it impossible to know with certainty the time frame over which serial vibrissae samples record diet composition. Observational evidence confirms that individual dietary preferences can persist for multiple years in the Monterey Bay sea otter population, even transcending generations through the cultural transmission of dietary preferences from mothers to their offspring (Estes et al. 2003). We have not yet measured vibrissae growth rates in sea otters; however, maximum vibrissae growth rates for other species of mammalian carnivores range from ~0.11 to ~0.37 cm/month (Hirons et al. 2001, Greaves et al. 2004, Zhao and Schell 2004, Hall-Aspland et al. 2005). As in pinnipeds, sea otter vibrissae function as sensory structures and are probably maintained from year to year with nearly continuous growth. Assuming that sea otters fall within this range, approximately 20 months would be required to attain a length of 5.9 cm, which is the average length of the vibrissae we have analyzed (Table 2). The aforementioned studies, however, suggest that growth rates for newly replaced vibrissae are probably faster than those for established vibrissae. Thus, growth rates are nonlinear: growth of the distal section near the tip of the sea otter vibrissa is likely to be higher than the proximate section near the base/root. Correcting for these differences would cause the average growth rate, as measured along the entire vibrissa (base to tip), to increase, thus decreasing the amount of time represented in each isotopic profile. Our weight-dependent sampling strategy, however, may help to offset potential problems related to this issue because the cross-sectional diameter of the whisker decreases in proportion to the distance from the base. Although growth rates may be higher for the distal portion of the whisker and thus represent a smaller temporal window in comparison to a similar length section near the base, fewer samples were collected in the distal section of each vibrissa in comparison to the proximal section.

Although isotopes do not typically reveal detailed information on dietary composition, they can offer a useful proxy for dietary variation. We have shown in this paper that stable isotope analysis can provide a cost-effective and efficient proxy for measuring individual- and population-level components of dietary variation in California sea otters. Because individual dietary variation in this species appears to be an epiphenomenon of food resource limitation (Tinker et al. 2008), isotopic characterizations of diet might be employed elsewhere to

evaluate population status relative to environmental carrying capacity. Although we have spent many thousands of hours over numerous years gathering observational data on diet and foraging behavior from known individual sea otters, not until we noted isotopic variation in some vibrissae did we realize that some of these observed individuals displayed seasonal dietary variation. The ease and simplicity with which isotopic data can be gathered and analyzed thus provided a window into foraging patterns that were not obvious from observational data collected in real time.

Isotopic profiles of dietary variation among and within individuals can provide various insights into dietary patterns and population status at a small fraction of the time required to characterize these same phenomena using standard observational techniques. Sea otters are one of the easier species of wild vertebrates to observe in nature. Information obtainable for most species is unsuitable for providing anything but the most superficial characterizations of diet. Yet many vertebrates, including most bird and mammal species, produce metabolically inert tissues in which time series of stable isotopic values easily could be obtained and quickly analyzed in comparison to the amount of time it takes to generate complimentary data from direct observation. The isotopic approach employed in this study could be used to characterize the degree of dietary variation among and within individuals for many, if not most, of these cryptic species. Future studies should be careful to test for sources of consumer isotopic variation that do not result from changes in diet. These include, but are not limited to, seasonal changes in the isotopic composition of food sources, shifts in diet-tissue fractionation patterns related to food quality, and potential isotopic variation resulting from temporal changes in consumer physiological condition. When carefully and properly applied, however, an isotopic approach could provide rapidly expanded insights into the various dimensions of ecology and evolutionary biology that are linked to temporal variation in food web patterns and dynamics.

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APPENDIX

Intra-vibrissae isotopic variation for individual sea otters from Monterey Bay, California, USA (*Ecological Archives* E090-062-A1).