

Converting isotope values to diet composition: the use of mixing models

DONALD L. PHILLIPS*

United States Environmental Protection Agency, National Health & Environmental Effects Research Laboratory,
Western Ecology Division, 200 SW 35th Street, Corvallis, OR 97330, USA

* Correspondent: phillips.donald@epa.gov

A common use of stable isotope analysis in mammalogy is to make inferences about diet from isotope values (typically $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) measured in tissues and food sources of a consumer. Mathematical mixing models are used to estimate the proportional contributions of food sources to the isotopic composition of the tissues of a consumer, which reflect the assimilated diet. This paper reviews basic mixing models and how they work; additional refinements also are described that include addressing uncertainty, larger numbers of sources, combining sources, concentration effects, and Bayesian statistical frameworks. Information is provided on where to access software for the various models. Numerous examples are cited to show application of these models in the mammal research literature.

Key words: diet, mixing model, stable isotopes

© 2012 American Society of Mammalogists

DOI: 10.1644/11-MAMM-S-158.1

In the past several decades, stable isotope analysis has become increasingly employed in a number of biological fields of study, including mammalogy (Ben-David and Flaherty 2012). One of the most common applications of stable isotope analysis is estimating the proportional contributions of sources to a mixture. There are a wide variety of ecological applications for this, including sorting out pollution sources to air or water bodies (Soto-Jimenez et al. 2008), carbon sources for soil organic matter (Vitorello et al. 1989), and water sources for plants (Li et al. 2007). However, the most frequent use involves reconstruction of animal diets, including those of mammals (Crawford et al. 2008; McKechnie 2004). In this application, the researcher measures the isotopic composition, such as $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ or both (Ben-David and Flaherty 2012), of diet items and tissue samples from the consumer to make inferences about the diet.

Mathematical mixing models are the tools used to convert isotopic data into estimates of source proportions, such as diet composition (Fry 2006). The objective of this paper is to describe stable isotope mixing models used for studying mammal diets, how they work, and how added features have expanded their scope and utility. Numerous illustrative examples involving mammals are given, but a comprehensive review of mammalian applications is beyond the scope of this paper. Some assumptions of mixing models and caveats about their use due to the complexity of metabolic physiology are briefly mentioned, but the reader is directed elsewhere for fuller discussion of these issues (Martínez del Río and Carleton 2012).

BACKGROUND

Mixing models for diet are based on the premise that “you are what you eat (plus a few permil)” (DeNiro and Epstein 1976). Per mil refers to parts per thousand, the units used for expressing stable isotope ratios in δ notation, a common form of isotopic measurement (Ben-David and Flaherty 2012). In other words, the isotopic composition of tissues of a consumer reflects the composition of its diet, weighted by the proportions of the dietary items. A few explanations are in order here. First, the tissues of the consumer will reflect the composition of the assimilated diet, not necessarily the ingested diet. Animals may ingest some items that are completely or partially indigestible, and the indigestible parts pass through the digestive tract without being absorbed. The rest is digested, absorbed, and with some additional losses to respiration and excretion, assimilated into animal tissues whose isotopic composition reflects the composition of this material. Second, physiological processes often occur at different rates for compounds containing light compared to heavy isotopes of carbon (^{12}C compared to ^{13}C) and nitrogen (^{14}N compared to ^{15}N) because of the difference in their chemical masses (Fry 2006). The resulting systematic isotopic difference between diet and consumer tissues has been variously referred to as diet–tissue discrimination (Arneson



and MacAvoy 2005), trophic shift (McCutchan et al. 2003), trophic enrichment (Hobson and Welch 1992), trophic discrimination (Newsome et al. 2010), trophic fractionation (Gorokhova and Hansson 1999), and other similar terms. Corrections for this systematic bias must be made to the diet and consumer isotope values before the mixing analysis is performed. This may be done either by subtracting the correction factor from the consumer tissue isotope values or adding it to the food source isotope values, although the latter allows the flexibility of using different corrections for different food sources (Ben-David et al. 1997b). Rather than focusing on the proximate prey for a consumer, some studies aim to define the importance to the consumer of food webs based on different basal resources. For example, Reid et al. (2008) examined the importance of allochthonous detritus, biofilm, macrophytes, and algae basal resources for consumers in streams. For this type of mixing analysis, the correction factors must be multiplied by the number of trophic levels between the basal resources and the consumer, which requires additional knowledge about consumer trophic levels.

In recent years, diet–tissue discrimination factors have been noted to vary among environments, trophic levels, physiological types (e.g., homeothermic or poikilothermic, ammonotelic, or ureotelic or uricotelic), taxa, tissues, sample treatment procedures (McCutchan et al. 2003), diet quality (Robbins et al. 2005), and other factors. Captive feeding studies (Hilderbrand et al. 1996) or situations where the diets of wild populations are well known (Fox-Dobbs et al. 2007; Newsome et al. 2010) can be particularly useful to establish the level of diet–tissue discrimination for the particular species, tissue, diet items, and other factors of interest. Otherwise the researcher must rely on values for similar taxa, tissues, and other factors from the literature (Dalerum and Angerbjörn 2005; McCutchan et al. 2003; Vanderklift and Ponsard 2003).

Although examination of mammal gut contents gives a snapshot of recently ingested food, stable isotope analysis of tissues gives a temporally integrated picture of assimilated diet. The period of integration depends on the particular tissue sampled and its turnover time. Different mammalian tissue types have isotopic half-lives ranging from around 1 day for plasma or serum to >100 days for tissues such as bone collagen and bat wing membranes (Dalerum and Angerbjörn 2005; Tieszen et al. 1983). Rapidly growing animals may have higher isotopic turnover rates due to synthesis of new growing tissue in addition to metabolic replacement (Carleton and Martínez del Rio 2010). When using isotopic mixing models to assess diet, it is important to keep in mind the period of time over which diet is being integrated, as well as whether it has stayed constant over that period (Newsome et al. 2007; Phillips and Eldridge 2006).

There are several other assumptions behind mixing models that need to be mentioned. The 1st is that all the food sources are included in the analysis; exclusion of a food source will bias the apparent proportions for the other sources and may even result in no combinations of the included sources being consistent with the isotopic composition of the consumer

(Phillips and Gregg 2003). The other assumption is that assimilated nutrients are completely homogenized in the body of the consumer prior to tissue synthesis (Martínez del Rio and Wolf 2005; Martínez del Rio et al. 2009). However, C that is part of amino acids resulting from protein digestion will be incorporated into protein in the consumer and not into other types of macromolecules such as lipids (Schwarcz 1991). Similarly, C in dietary lipids may be preferentially routed to synthesis of body fat (Stott et al. 1997). Depending on the dietary and consumer tissues sampled, this could lead to under- or overestimates of some food sources (Schwarcz 1991). The reader is referred to these references and Martínez del Rio and Carleton (2012) for further details on these physiological issues and caveats.

BASIC MIXING MODELS

Dietary mixing models consist of mathematical equations that explain the observed consumer isotopic composition as a simple mixture of the isotopic composition of its (assimilated) diet based on isotopic mass balance. In the simplest situation, a single isotope value (say $\delta^{13}\text{C}$) has been measured in tissue of the consumer and 2 prey items that constitute its diet. The mixing model for this consists of the following equations:

$$\begin{aligned}\delta^{13}\text{C}_{\text{mix}} &= f_1\delta^{13}\text{C}_1 + f_2\delta^{13}\text{C}_2 \\ f_1 + f_2 &= 1.\end{aligned}\quad (1)$$

The 1st equation expresses $\delta^{13}\text{C}$ of the consumer (with subscript mix) as a combination of the $\delta^{13}\text{C}$ of the 2 prey (subscripts 1 and 2), weighted by their diet fractions (f_1 and f_2 , respectively). The diet fractions are subject to the constraint that they sum to 1 (2nd equation). In mathematical terms, this is a system of 2 equations in 2 unknowns (f_1 and f_2) that has a unique solution. One way of solving is to algebraically rearrange the equations as:

$$\begin{aligned}f_1 &= \frac{\delta^{13}\text{C}_{\text{mix}} - \delta^{13}\text{C}_2}{\delta^{13}\text{C}_1 - \delta^{13}\text{C}_2} \\ f_2 &= 1 - f_1.\end{aligned}\quad (2)$$

The 1st equation can be solved for f_1 because the other values in it are known, and then the 2nd equation can be solved for f_2 .

As a hypothetical example, consider a bison that has muscle tissue with a $\delta^{13}\text{C}$ value of -21‰ ($\delta^{13}\text{C}_{\text{mix}}$) and consumes a mixture of C_3 grasses ($\delta^{13}\text{C}_1 = -25.5\text{‰}$) and C_4 grasses ($\delta^{13}\text{C}_2 = -15.5\text{‰}$). Applying a diet–tissue discrimination correction of 0.5‰ gives corrected $\delta^{13}\text{C}$ values of $-25.5\text{‰} + 0.5\text{‰} = -25\text{‰}$ for C_3 and $-15.5\text{‰} + 0.5\text{‰} = -15\text{‰}$ for C_4 . (In subsequent examples, assume that appropriate diet–tissue discrimination corrections have already been made.) Inserting these values in the mixing model equations results in the solution $f_1 = 0.6$ and $f_2 = 0.4$, so the assimilated diet of the bison consists of 60% C_3 grasses and 40% C_4 grasses. This makes intuitive sense because the $\delta^{13}\text{C}$ of the tissue of the bison is somewhat closer to that of C_3 grass than C_4 grass. In fact, this mixing model simply amounts to a linear

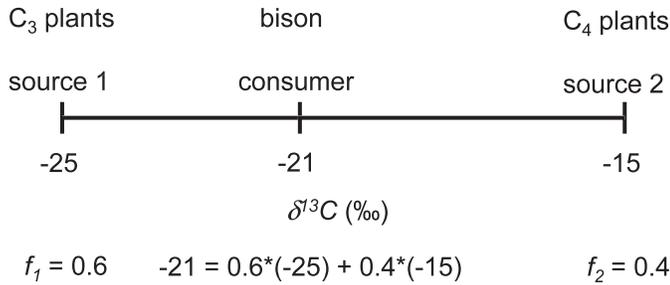


FIG. 1.—Mixing diagram for a simple mixing model utilizing 1 isotope value ($\delta^{13}\text{C}$) and 2 food sources (C_3 and C_4 plants) in the diet of a bison (*Bison bison*). In this case, the bison tissue represents a mixture of 0.6 C_3 plants and 0.4 C_4 plants as determined by the model in equation 1.

interpolation of $\delta^{13}\text{C}$ of the consumer between the $\delta^{13}\text{C}$ of the 2 sources. Fig. 1 shows a graphical representation of this example. Note that isotope value of the consumer must fall between those of the dietary end-members to be explained as a mixture of them. If it falls outside this mixing space, the mixing model can still find a mathematical solution of diet fractions that sum to 1, but one of them will be negative and the other will be >1 , neither of which makes biological sense. Such a situation might indicate the existence of an additional food source that was not considered, the use of inappropriate diet-tissue discrimination corrections, or possibly uncertainty in the isotope values of the sources or the consumer (see next section). Examples from the mammal literature of this type of mixing model analysis with 1 element and 2 sources include estimating the importance of C_3 and C_4 plants in the diets of African giraffes (*Giraffa camelopardalis*), zebras (*Equus burchellii*), impalas (*Aepyceros melampus*), and baboons (*Papio ursinus*—Codron et al. 2006), and plant and insect components of the diets of several bat species (*Monophyllus redmani* and *Phyllonycteris poeyi*) in Cuba (Mancina and Herrera 2010).

In diet reconstruction studies, a 2nd isotope value (usually $\delta^{15}\text{N}$) is often used as well. This allows for a more expansive mixing model with 2 isotope values and 3 food sources:

$$\delta^{13}\text{C}_{\text{mix}} = f_1\delta^{13}\text{C}_1 + f_2\delta^{13}\text{C}_2 + f_3\delta^{13}\text{C}_3$$

$$\delta^{15}\text{N}_{\text{mix}} = f_1\delta^{15}\text{N}_1 + f_2\delta^{15}\text{N}_2 + f_3\delta^{15}\text{N}_3 \quad (3)$$

$$f_1 + f_2 + f_3 = 1.$$

Again, the 1st equation expresses $\delta^{13}\text{C}$ of the consumer (subscript mix) as a combination of the $\delta^{13}\text{C}$ of 3 prey (subscripts 1, 2, and 3) weighted by their diet fractions (f_1 , f_2 , and f_3 , respectively), the 2nd equation does the same for $\delta^{15}\text{N}$, and the 3rd equation specifies that the fractions must sum to 1. In mathematical terms, this is a system of 3 equations in 3 unknowns (f_1 , f_2 , and f_3) that also has a unique solution. Fig. 2 shows a mixing diagram for this type of model. Again, the consumer must fall within the mixing space defined by the sources (triangle) in order for its isotopic composition to be explained as a mixture of the sources. It is always a good idea to plot data in this way before doing a mixing analysis to verify that this is the case. Recent applications of this model

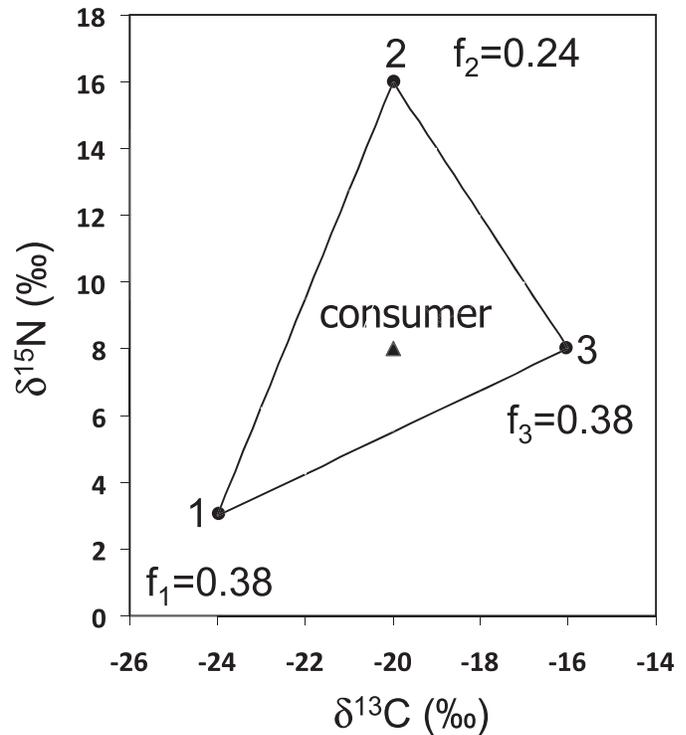


FIG. 2.—Mixing diagram for a mixing model utilizing 2 isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and 3 food sources (circles) for a consumer (triangle). The diet fractions for the 3 sources are shown as determined by the model in equation 3.

with 2 elements and 3 sources to study mammal diets include examining the use of freshwater vegetation, estuarine vegetation, and seagrass by manatees (*Trichechus manatus*) in the West Indies (Alves-Stanley et al. 2010) and ringed seals (*Pusa hispida*), bearded seals (*Erignathus barbatus*), and remains of bowhead whales (*Balaena mysticetus*) in diets of Alaskan polar bears (*Ursus maritimus*—Bentzen et al. 2007).

Mixing models may be expanded further to include a 3rd isotope value or even more if the data are available for the consumer and its food sources. One possibility is the sulfur isotope value $\delta^{34}\text{S}$, which generally has distinctly different ranges of values for terrestrial vegetation than for marine plankton and seaweeds (Fry 2006). Consequently, it is sometimes used as an additional discriminating factor in diet studies in coastal areas (Connolly et al. 2004; Granek et al. 2009) or in other situations where there are distinctive sulfur signatures for certain food sources, such as whitebark pine nuts, a food source for grizzly bears (*Ursus arctos*) in Yellowstone National Park (Felicetti et al. 2003). Addition of a 3rd isotope value allows unique identification of the dietary contributions of 4 food sources, and in general, the use of n isotope values in mixing models will permit estimation of unique diet proportions for $n + 1$ food sources (Phillips and Gregg 2003).

DEALING WITH UNCERTAINTY

So far this discussion has considered the isotope values of consumer and food sources that go into mixing models as if

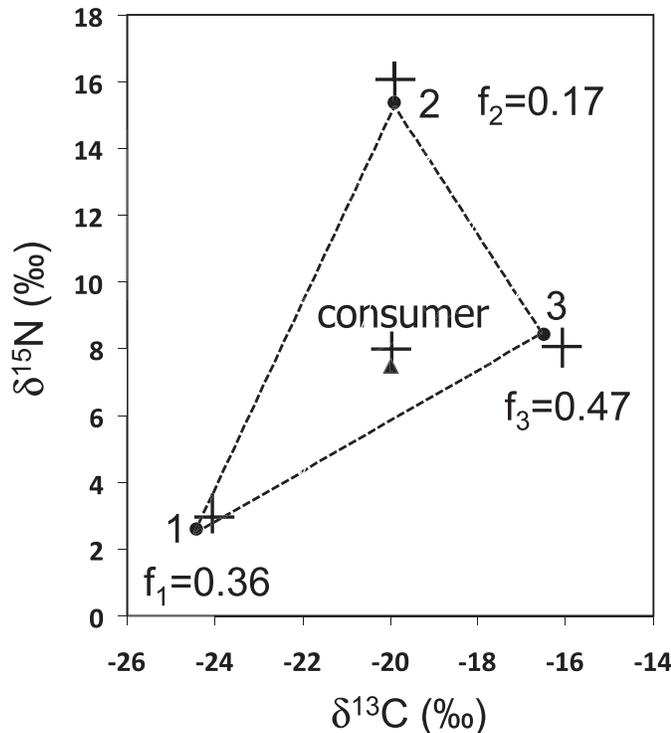


FIG. 3.—Illustration of the sensitivity of diet mixing model results to variability in isotope values of consumers and food sources. The error bars show the mean ± 1 SE for the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values for 3 food sources and a consumer as shown in Fig. 2. Alternative plausible values within these error ranges for the food sources (circles) and consumer (triangle) were used in a mixing model (note the offset from mean values), which resulted in different diet proportions (compare to Fig. 2, which used the mean values).

they were exact values. However, there may be substantial variability among individuals in isotopic composition of consumers and food sources, sampling error (i.e., variability among alternative random samples), as well as a small amount of analytical measurement error (Phillips and Gregg 2001a). Using somewhat different but plausible isotope values of consumers and food sources within their error bounds may result in somewhat different estimates of diet composition, as illustrated in Fig. 3.

How can uncertainty in mixing model inputs be reflected in the uncertainty of diet proportions that it provides? Phillips and Gregg (2001a, 2001b) performed error propagation calculations to provide statistical confidence intervals around the estimates of diet proportions, and incorporated these into an Excel (Microsoft Corporation, Redmond, Washington) mixing model called IsoError (www.epa.gov/wed/pages/models.htm; accessed 9 August 2011) for mixing models with 1 element and 2 sources or with 2 elements and 3 sources. They examined the effects of different factors on the uncertainty (SE) of the diet proportion estimates and found that the most important factor is the isotopic difference among sources. The more distinct the sources are from each other, the more precisely the mixing model is able to estimate diet proportions. The next most important factor in the precision of

estimates is the variability of isotope values within the consumer and food source populations, followed by the number of samples used to determine these isotope values. Analytical error is typically small, with standard deviations of a fraction of a per mil for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and is relatively unimportant, as is the evenness of the diet proportions (e.g., 90% and 10% compared to 50% and 50%). IsoError provides mean estimates of diet proportions and the 95% confidence intervals around them to quantify how precise the estimates are. It can also be useful for study design in determining the sample size required to meet desired precision criteria if the researcher has some idea of the isotopic composition and variability of the consumer and food source populations (Phillips and Gregg 2001a). Applications of this method for mammals include manatees (*T. manatus*—Alves-Stanley et al. 2010; Reich and Worthy 2006), harp seals (*Pagophilus groenlandicus*—Hammill et al. 2005), polar bears (*U. maritimus*—Bentzen et al. 2007), grizzly bears (*U. arctos*—Mowat and Heard 2006), and bats (*M. redmani* and *P. poeyi*—Mancina and Herrera 2010).

EXCESS SOURCES

We have seen that mixing models using n isotope values can determine the diet proportions for $n + 1$ different food sources, for example, 3 food sources with the typical $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements. A common situation, however, is that there are more food sources than this. For example, Ben-David et al. (1997b) measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from coastal mink (*Neovison vison*) in southeastern Alaska and 7 food sources that they used. The mixing model in equation 3 could be extended to include 7 sources instead of 3, but this would result in a system of 3 equations in 7 unknowns, which theoretically has an infinite number of solutions. However, mixing models, with their requirement for conservation of isotopic mass balance, can still be used to find multiple combinations of source proportions that are isotopically feasible solutions and at least put bounds on possible diet proportions.

Phillips and Gregg (2003) devised a procedure for this purpose and provided Visual Basic (Microsoft Corporation, Redmond, Washington) software called IsoSource (www.epa.gov/wed/pages/models.htm; accessed 9 August 2011). The 1st step is to iteratively create each possible combination of diet source proportions (that sum to 100%) by some small increment, such as 1%. Second, the predicted isotope values for the consumer are computed for each of these combinations (weighting the source isotope values by the proportions and summing these products as in equation 3). Third, these predicted consumer isotope values are compared with the observed consumer values. If they are equal, or within some small tolerance (e.g., 0.2‰, a typical value for analytical measurement error for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, or somewhat higher values to include sampling variability as well), this combination of source proportions represents a feasible solution that satisfies isotopic mass balance, and it is stored in a data set. Lastly, the distribution of all such feasible solutions in the data

set is described. The minimum and maximum values for each source define the range of its possible dietary contributions. Users are exhorted not to simply report the mean of these source distributions because it is only 1 of many possible solutions and gives the false impression of a unique answer (Phillips and Gregg 2003).

The mink study mentioned above was 1 example described in Phillips and Gregg (2003). Ben-David et al. (1997b) collected samples of coastal mink and 7 prey items: fishes, crabs, mussels, rodents, shrimp, ducks, and amphipods and measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for each of these. Phillips and Gregg (2003) analyzed this data set with IsoSource (using an increment of 1% and a tolerance of 0.1‰) and reported the ranges of possible diet contributions to the mink for each food source (Fig. 4). Note that in Fig. 4, the mixing space is shown as the convex hull defined by the food sources as vertices; sources may be on the interior if including them as vertices would result in a concave side, for example, amphipods and shrimp. Fishes represented the largest part of the diet with contributions of 49–63%, followed by crabs with 19–42%, and the other 5 prey were minor contributors. These results show that even though exact estimates of diet composition are not possible due to the excess number of sources and the underdetermined system of mixing model equations, putting upper and lower bounds on diet composition can still be informative. In this case, the 2 major diet items were clearly identified and the other 5 prey made up the balance. Such well-constrained results are not always the case, however, and depend on the geometry of the mixing space defined by the sources and where the consumer falls within it. In this instance, the mink fell close to the edge of the mixing polygon defined by the fish and crab isotopic composition, and thus its diet had to be largely explained by those 2 sources (Fig. 4). If the mink had fallen more in the center (e.g., $\delta^{13}\text{C} = -20\text{‰}$ and $\delta^{15}\text{N} = 12\text{‰}$), then many different combinations could be possible, such as a diet of roughly 50% ducks and 50% mussels, a diet of 50% fishes and 50% rodents, or a large number of other diets of 2–7 food sources. This would have led to much more diffuse solutions (e.g., 0–50%) for many of the foods and not provided much insight into the diet of the mink. Phillips and Gregg (2003) illustrate a number of such hypothetical situations that lead to relatively informative constrained solutions or uninformative diffuse solutions (their figure 6).

This method and the IsoSource software have been widely used in a number of studies reconstructing diets. Usually $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are the isotope values employed, although occasionally other isotope values such as $\delta^{34}\text{S}$ are used as well. Applications to extant mammals have included baboons (*P. ursinus*—Codron et al. 2008), badgers (*Meles meles*—Vulla et al. 2009), bats (*Euderma maculatum*—Painter et al. 2009), bears (*Ursus americanus*, *U. arctos*, and *Ursus thibetanus*—Ben-David et al. 2004; Edwards et al. 2011; Felicetti et al. 2003; Fortin et al. 2007; Mowat and Heard 2006; Narita et al. 2006; Vulla et al. 2009), beavers (*Castor canadensis*—Milligan and Humphries 2010), cetaceans (*B.*

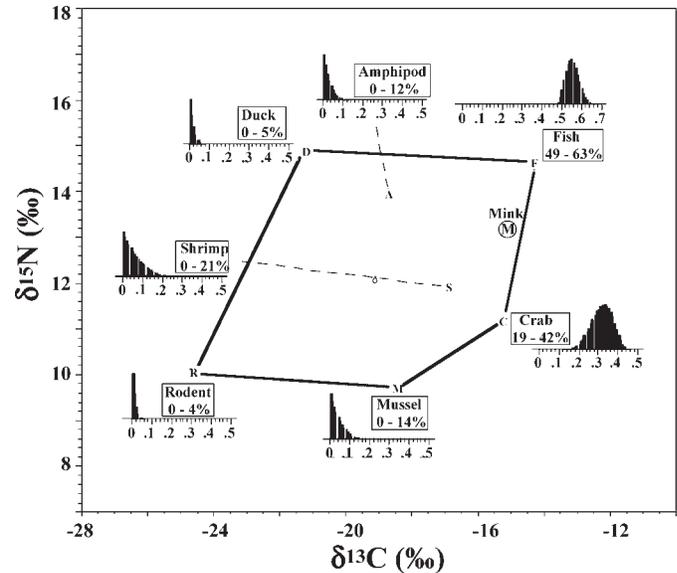


FIG. 4.—Mixing diagram for spring coastal mink (*Neovison vison*) example from Ben-David et al. (1997b). Histograms show the distribution of isotopically feasible contributions from each food source to the mink diet from IsoSource. Points plotted for each food source represent mean values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Note that these values for amphipods (A) and shrimp (S) lie within the mixing space defined as the convex hull encompassing all the sources. Values shown in the boxes are the 1–99 percentile ranges for these distributions. (Reproduced from Phillips and Gregg [2003] by permission of the publisher, Springer.).

mysticetus, *Balaenoptera acutorostrata*, *Balaenoptera physalus*, *Delphinapterus leucas*, *Megaptera novaeangliae*, and *Phocoena phocoena*—Lesage et al. 2010), foxes (*Vulpes lagopus*—Samelius et al. 2007), hyenas (*Crocuta crocuta*—Codron et al. 2007), kangaroos (*Macropus fuliginosus* and *Macropus giganteus*—Iles et al. 2010), mouse lemurs (*Microcebus*—Dammhahn and Kappeler 2010), lions (*Panthera leo*—Codron et al. 2007), martens (*Martes martes*—Vulla et al. 2009), rodents (*Neotoma fuscipes*, *Rattus norvegicus*, and *Rattus rattus*—Caut et al. 2009; Harper 2007; Major et al. 2007; McEachern et al. 2006), seals (*Mirounga leonina* and *P. hispida*—Eder et al. 2010; Sinisalo et al. 2006), and wolves (*Canis lupus*—Fox-Dobbs et al. 2007; Urton and Hobson 2005). In addition, this method has been applied to dietary studies of fossil mammals including various carnivores (Fox-Dobbs et al. 2007, 2008; Palmqvist et al. 2008a, 2008b), ungulates (Fox-Dobbs et al. 2008; Palmqvist et al. 2008b), and prehistoric humans (*Homo neanderthalensis* and *Homo sapiens*—Arnay-de-la-Rosa et al. 2010, 2011; Bocherens et al. 2005; Byers et al. 2011; Drucker and Henry-Gambier 2005; Naito et al. 2010; Newsome et al. 2004).

Combining sources.—When there is an excess number of sources that precludes unique solutions for diet proportions (i.e., $>n + 1$ sources when there are n isotope values being employed), there are several options for combining sources to reduce this number, as discussed by Phillips et al. (2005). Gannes et al. (1998) stated that in reconstructing animal diets, the sources examined must have isotopically distinct signatures.

In an a priori approach, one could do statistical tests for equality of means and combine sources before running the mixing model if they are not significantly different (Ben-David et al. 1997a, 1997b; Rosing et al. 1998). Interpretation of the results will be aided if the sources combined have some logical connection (e.g., same taxon or trophic guild) so that the combined source has some biological meaning (Phillips et al. 2005). Several recent papers used this a priori approach for combining sources in studies of diets for bears (*U. americanus* and *U. arctos*—Edwards et al. 2011; Fortin et al. 2007). Alternatively, if the sources all have significantly different isotope values, then the mixing analysis can be run on the full suite of sources, using a procedure such as IsoSource, and then combining sources a posteriori. In this procedure, each of the many isotopically feasible solutions is examined, and the diet proportions for chosen sources are summed to get a proportion for that combined source (Phillips et al. 2005). Then the range of feasible proportions for this combined source can be described, just as for individual sources.

An example of this a posteriori approach discussed in Phillips et al. (2005) comes from a study on diets of an early Holocene group (about 7,000 years ago) and a middle Holocene group (about 4,500 years ago) of humans on the central California coast (Newsome et al. 2004). The overall question was whether there was a shift between marine and terrestrial foods over this 2,500-year period. Three types of marine foods (shellfishes, fishes, and pinnipeds), 3 types of terrestrial plants (leafy plants, seeds or grains, and nuts), and terrestrial meat were included as food sources in a mixing model using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data. The ranges of possible diet proportions were fairly diffuse and did not lead to firm conclusions. For example, marine fishes could account for 0–68% of the early Holocene diet and 0–44% of the middle Holocene diet. However, food sources were combined a posteriori to define marine, terrestrial plant, and terrestrial meat groups. The distributions of proportions for these groups were much more sharply defined (Fig. 5). Marine foods decreased from a collective 70–84% of the early Holocene diet to 48–58% of the middle Holocene diet, with concomitant increases in terrestrial foods. The focusing of the dietary results by strategically combining sources aided in interpretation and allowed Newsome et al. (2004) to discuss the significance of these results in terms of climate-driven changes in marine upwelling patterns and terrestrial vegetation over this period, the development of the mortar and pestle, which allowed greater processing of terrestrial plant foods, and a general transition from a mobile to a more sedentary lifestyle. Other mammalian examples of this a posteriori approach to combining sources include studies on bats (*E. maculatum*—Painter et al. 2009), bears (*U. maritimus*—Cherry et al. 2011), and various fossil carnivores (Palmqvist et al. 2008a).

CONCENTRATION EFFECTS

The typical diet study with stable isotopes uses $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values to discern the contributions of various food sources, using a mixing model such as shown in equation

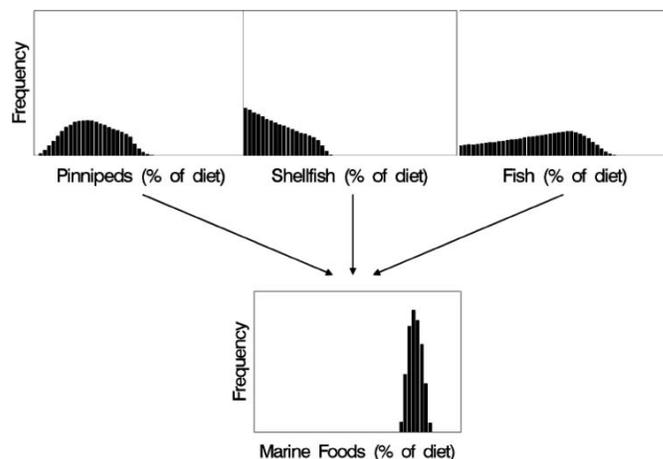


FIG. 5.—Ranges of feasible dietary biomass contributions of individual marine food sources for an early Holocene (about 7,000 years ago) group of humans on the central California coast (Newsome et al. 2004). Distributions shown reflect 11,345 dietary solutions found by the mixing model. Although wide ranges of use are possible for each individual food source, the combined marine food group is tightly constrained and represents 70–84% of the diet. (Reproduced from Phillips et al. [2005] by permission of the publisher, Springer.)

3 above. An implicit assumption of this model is that the proportional dietary contribution of a food source is the same for both elements, as can be seen from the common set of proportions used in equation 3. If the C and N concentrations are similar among all sources, this may be a reasonable assumption. However, 1 source may be particularly poor or rich in N, for example, which would logically result in a proportionate decrease or increase in its N contribution to the consumer compared with its C contribution. Phillips and Koch (2002) devised a concentration-weighted mixing model that assumes that for each element (e.g., C and N), the contribution of a source is proportional to the mass it contributes multiplied by the elemental concentration in that source. The model determines 3 separate sets of dietary proportions for the food sources on a biomass basis, a C basis, and an N basis. If the C and N concentrations are equal in all sources, these 3 sets of proportions are identical and the model mathematically reduces to the standard model that does not consider concentrations (equation 3). Phillips and Koch (2002) constructed an Excel model called IsoConc to perform these calculations (www.epa.gov/wed/pages/models.htm; accessed 9 August 2011).

If C and N concentrations do not vary markedly among food sources, there may be little need to consider the added complexity of this model. However, if there are considerable concentration differences, as might be expected for N between plant and animal foods in the diet of an omnivore, for example, a concentration-dependent model may be called upon to sort out the differential C and N contributions among food sources rather than having them confounded. Phillips and Koch (2002) presented an example from a captive mink feeding trial reported by Ben-David and Schell (2001). In this

TABLE 1.—Dietary reconstruction of data from captive feeding trials with mink (*Neovison vison*—Ben-David and Schell 2001) using standard and concentration-dependent (conc.-dep.) isotopic mixing models as discussed in Phillips and Koch (2002). Values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of foods have been corrected for discrimination factors between diet and assimilated consumer tissue (mink fat). The standard mixing model found no solutions that did not include negative proportions, whereas the concentration-dependent model reproduced closely the actual dietary proportions, not only for biomass, but also for carbon and nitrogen. See Phillips and Koch (2002) for further details.

	Salmon	Lean beef	Beef fat	Mink fat
Isotope values				
$\delta^{13}\text{C}$ (‰)	-23.02	-24.48	-25.28	-24.04
$\delta^{15}\text{N}$ (‰)	14.57	10.19	10.19	12.91
Concentrations				
Carbon (%)	55	56	72	
Nitrogen (%)	12	11.5	1.5	
Biomass proportions				
Actual diet biomass (%)	50	25	25	
Conc.-dep. model biomass (%)	49	28	23	
Standard model biomass (%)	62	-21	58	
Carbon proportions				
Actual diet C (%)	46	24	30	
Conc.-dep. model C (%)	45	27	28	
Nitrogen proportions				
Actual diet N (%)	65	31	4	
Conc.-dep. model N (%)	62	34	4	

study, mink were fed a mixture of salmon, lean beef, and beef fat that had widely varying C and N concentrations (Table 1). The standard mixing model (equation 3) was unable to find any solution that did not include negative proportions, whereas IsoConc reproduced closely the actual dietary proportions, not only for biomass, but also for C and N (Table 1).

For animals eating other animals, prey C and N in proteins, lipids, and carbohydrates are highly digestible (Koch and Phillips 2002). However, although some plant foods such as fruit also may be highly digestible, other plant foods may not be because of the presence of indigestible components such as fiber. This would affect primarily the C available to the consumer because plant N is almost entirely in proteins, which are highly digestible. Consequently, for mixed diets it may be necessary to adjust the C and N concentration data for the concentration-dependent model to reflect what is actually digested and available for assimilation, rather than using the raw ingested concentrations (Koch and Phillips 2002). This is because consumer tissues reflect the isotopic composition of what is assimilated, not what is ingested, as stated earlier.

Example applications of concentration-dependent models to mammals include mink and bear as discussed in Phillips and Koch (2002) and Koch and Phillips (2002). Cassaing et al. (2007) and Kurle (2008) used concentration-dependent mixing models to study rat (*R. rattus*) diets, as did Ramírez-Hernández and Herrera M. (2010) for mice (*Liomys pictus*). A number of studies also have applied these models to assessing human paleodiets (Bocherens and Drucker 2006;

Bocherens et al. 2006; Drucker and Bocherens 2004; Newsome et al. 2004; Ogrinc and Budja 2005).

MORE COMPLEX MIXING MODELS

This discussion has covered basic mixing models for assessing animal diets and several developments over the last decade that have extended their scope and utility, such as adding uncertainty calculations, concentration dependence, and the ability to address a larger number of sources or combining sources, or both. There are a few studies on mammals that have combined several of these aspects (Kurle 2008; Newsome et al. 2004; Painter et al. 2009), but for the most part these mixing model developments have addressed 1 issue at a time and combined approaches have required custom programming rather than using the stand-alone software products like IsoError, IsoConc, and IsoSource. Several new statistical mixing models recently have been devised (SIAR [<http://cran.r-project.org/web/packages/siar/index.html>]; accessed 9 August 2011; Parnell et al. 2010] and MixSIR [<http://conserver.iugo-cafe.org/user/brice.semmens/MixSIR>]; accessed 9 August 2011; Moore and Semmens 2008]) that have built upon these model developments and allow flexible specification of mixing models of varying complexity in a Bayesian statistical framework. Briefly, users specify any prior knowledge about mixing proportions (although noninformative priors can be specified to denote no such prior knowledge), the stable isotope data for consumers and sources, diet-tissue discrimination correction factors if needed, and, optionally, source elemental concentrations as well. Uncertainties (e.g., *SDs*) for stable isotope data and correction factors also can be specified. The model randomly generates a large number of possible mixing proportion sets (f_q); the “posterior” probability of each of these sets given the observed data ($P(f_q|\text{data})$) is estimated based on the likelihood of the observed data given this set of proportions ($L(\text{data}|f_q)$) and the set’s probability in the prior distribution ($P(f_q)$) as defined by Bayes’ theorem (Moore and Semmens 2008):

$$P(f_q|\text{data}) = \frac{L(\text{data}|f_q) \times P(f_q)}{\sum [L(\text{data}|f_q) \times P(f_q)]}. \quad (4)$$

In this way, Bayesian mixing models consider the joint probability distributions of all relevant variables and estimate the probability distribution of mixing proportions. Readers are referred to Moore and Semmens (2008), Parnell et al. (2010), and Ward et al. (2010) for further details about these methods. The advantage of such models is that they can explicitly account for uncertainty from various sources, and also consider other factors if desired (e.g., prior information and concentration dependence). Alternative groupings of sources to simplify the model also can be evaluated (Ward et al. 2011). A growing number of published studies on mammals have used these new mixing models for diet assessments of bats (*Noctilio albiventris*—Voigt et al. 2010), bears (*U. maritimus*—Cherry et al. 2011), dolphins (*Pontoporia blainvillei*, *Sotalia guianensis*, and *Tursiops truncatus*—Di Benedetto et al. 2011; Fernández et al. 2011), foxes (*Vulpes vulpes*—Killengreen et al. 2011), lions (*P.*

leo—Yeakel et al. 2009), mice (*Peromyscus leucopus*—Shaner and Macko 2011), rats (*R. rattus*—Pisanu et al. 2011; Ruffino et al. 2011), sea lions (*Otaria flavescens*—Drago et al. 2010), whales (*M. novaeangliae*—Witteveen et al. 2011), and wolves (*C. lupus*—Milakovic and Parker 2011; Semmens et al. 2009). The Bayesian models are likely to be more widely adopted for diet studies as they become better known.

CONCLUSION

Mixing models can be useful tools for examining assimilated diet in the study of mammals. A number of new features have been developed for mixing models over the last decade that increase their capabilities. Future areas for further development might include further elaborating Bayesian models; adapting for multiple compound-specific stable isotope analyses to get around problems of isotopic routing (Popp et al. 2007; Wolf et al. 2009); and incorporating additional nonisotopic information to improve diet estimates (Phillips et al. 2006; Yeakel et al. 2011). As with all tools, however, it is important to know how to use mixing models and what their assumptions and limitations are. This paper has attempted to serve as an introduction to mixing models and provide background on their judicious use in mammalogy.

ACKNOWLEDGMENTS

I thank M. Ben-David and E. Flaherty for organizing the stable isotope symposium at the annual meeting of the American Society of Mammalogists (2010) and for inviting my participation. M. Wunder, M. Ben-David, C. Kurle, B. Blake, and an anonymous reviewer provided helpful comments on the manuscript. The research in this document has been funded by the United States Environmental Protection Agency. It has been subjected to United States Environmental Protection Agency peer and administrative review, and it has been approved for publication as a United States Environmental Protection Agency document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

LITERATURE CITED

- ALVES-STANLEY, C., G. WORTHY, AND R. BONDE. 2010. Feeding preferences of West Indian manatees in Florida, Belize, and Puerto Rico as indicated by stable isotope analysis. *Marine Ecology Progress Series* 402:255–267.
- ARNAY-DE-LA-ROSA, M., E. GONZÁLEZ-REIMERS, Y. YANES, C. S. ROMANEK, J. E. NOAKES, AND L. GALINDO-MARTÍN. 2011. Paleonutritional and paleodietary survey on prehistoric humans from Las Cañadas del Teide (Tenerife, Canary Islands) based on chemical and histological analysis of bone. *Journal of Archaeological Science* 38:884–895.
- ARNAY-DE-LA-ROSA, M., E. GONZÁLEZ-REIMERS, Y. YANES, J. VELASCO-VÁZQUEZ, C. S. ROMANEK, AND J. E. NOAKES. 2010. Paleodietary analysis of the prehistoric population of the Canary Islands inferred from stable isotopes (carbon, nitrogen and hydrogen) in bone collagen. *Journal of Archaeological Science* 37:1490–1501.
- ARNESON, L. S., AND S. E. MACAVOY. 2005. Carbon, nitrogen, and sulfur diet–tissue discrimination in mouse tissues. *Canadian Journal of Zoology* 83:989–995.
- BEN-DAVID, M., AND E. A. FLAHERTY. 2012. Stable isotopes in mammalian research: a beginner's guide. *Journal of Mammalogy* 93:312–328.
- BEN-DAVID, M., R. W. FLYNN, AND D. M. SCHELL. 1997a. Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. *Oecologia* 111:280–291.
- BEN-DAVID, M., T. A. HANLEY, D. R. KLEIN, AND D. M. SCHELL. 1997b. Seasonal changes in diets of coastal and riverine mink: the role of spawning Pacific salmon. *Canadian Journal of Zoology* 75:803–811.
- BEN-DAVID, M., AND D. M. SCHELL. 2001. Mixing models in analyses of diet using multiple stable isotopes: a response. *Oecologia* 127:180–184.
- BEN-DAVID, M., K. TITUS, AND L. R. BEIER. 2004. Consumption of salmon by Alaskan brown bears: a trade-off between nutritional requirements and the risk of infanticide? *Oecologia* 138:465–474.
- BENTZEN, T. W., E. H. FOLLMANN, S. C. AMSTRUP, G. S. YORK, M. J. WOOLLER, AND T. M. O'HARA. 2007. Variation in winter diet of southern Beaufort Sea polar bears inferred from stable isotope analysis. *Canadian Journal of Zoology* 85:596–608.
- BOCHERENS, H., AND D. G. DRUCKER. 2006. Isotope evidence for paleodiet of late Upper Paleolithic humans in Great Britain: a response to Richards et al. (2005). *Journal of Human Evolution* 51:440–442.
- BOCHERENS, H., D. G. DRUCKER, D. BILLIOU, M. PATOU-MATHIS, AND B. VANDERMEERSCH. 2005. Isotopic evidence for diet and subsistence pattern of the Saint-Césaire I Neanderthal: review and use of a multi-source mixing model. *Journal of Human Evolution* 49:71–87.
- BOCHERENS, H., M. MASHKOUR, D. G. DRUCKER, I. MOUSSA, AND D. BILLIOU. 2006. Stable isotope evidence for palaeodiets in southern Turkmenistan during Historical period and Iron Age. *Journal of Archaeological Science* 33:253–264.
- BYERS, D. A., D. R. YESNER, J. M. BROUGHTON, AND J. B. COLTRAIN. 2011. Stable isotope chemistry, population histories and Late Prehistoric subsistence change in the Aleutian Islands. *Journal of Archaeological Science* 38:183–196.
- CARLETON, S. A., AND C. MARTÍNEZ DEL RIO. 2010. Growth and catabolism in isotopic incorporation: a new formulation and experimental data. *Functional Ecology* 24:805–812.
- CASSAING, J., C. DERRÉ, I. MOUSSA, AND G. CHEYLAN. 2007. Diet variability of Mediterranean insular populations of *Rattus rattus* studied by stable isotope analysis. *Isotopes in Environmental and Health Studies* 43:197–213.
- CAUT, S., E. ANGULO, AND F. COURCHAMP. 2009. Avoiding surprise effects on Surprise Island: alien species control in a multitrophic level perspective. *Biological Invasions* 11:1689–1703.
- CHERRY, S. G., A. E. DEROCHE, K. A. HOBSON, I. STIRLING, AND G. W. THIEMANN. 2011. Quantifying dietary pathways of proteins and lipids to tissues of a marine predator. *Journal of Applied Ecology* 48:373–381.
- CODRON, D., J. CODRON, J. A. LEE-THORP, M. SPONHEIMER, D. DE RUITER, AND J. S. BRINK. 2007. Stable isotope characterization of mammalian predator–prey relationships in a South African savanna. *European Journal of Wildlife Research* 53:161–170.
- CODRON, D., J. A. LEE-THORP, M. SPONHEIMER, D. DE RUITER, AND J. CODRON. 2006. Inter- and intrahabitat dietary variability of chacma baboons (*Papio ursinus*) in South African savannas based on fecal $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\% \text{N}$. *American Journal of Physical Anthropology* 129:204–214.

- CODRON, D., J. A. LEE-THORP, M. SPONHEIMER, D. DE RUITER, AND J. CODRON. 2008. What insights can baboon feeding ecology provide for early hominin niche differentiation? *International Journal of Primatology* 29:757–772.
- CONNOLLY, R. M., M. A. GUEST, A. J. MELVILLE, AND J. M. OAKES. 2004. Sulfur stable isotopes separate producers in marine food-web analysis. *Oecologia* 138:161–167.
- CRAWFORD, K., R. A. McDONALD, AND S. BEARHOP. 2008. Applications of stable isotope techniques to the ecology of mammals. *Mammal Review* 38:87–107.
- DALERUM, F., AND A. ANGERBJÖRN. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia* 144:647–658.
- DAMMHAHN, M., AND P. M. KAPPELER. 2010. Scramble or contest competition over food in solitarily foraging mouse lemurs (*Microcebus* spp.): new insights from stable isotopes. *American Journal of Physical Anthropology* 141:181–189.
- DENIRO, M. J., AND S. EPSTEIN. 1976. You are what you eat (plus a few per mil): the carbon isotope cycle in food chains. *Geological Society of America Abstracts with Programs* 8:834–835.
- DI BENEDETTO, A. P. M., C. M. M. DE SOUZA, H. A. KEHRIG, AND C. E. REZENDE. 2011. Use of multiple tools to assess the feeding preference of coastal dolphins. *Marine Biology* 158:2209–2217.
- DRAGO, M., L. CARDONA, E. A. CRESPO, N. GARCIA, S. AMEGHINO, AND A. AGUILAR. 2010. Change in the foraging strategy of female South American sea lions (Carnivora: Pinnipedia) after parturition. *Scientia Marina* 74:589–598.
- DRUCKER, D., AND H. BOCHERENS. 2004. Carbon and nitrogen stable isotopes as tracers of change in diet breadth during Middle and Upper Palaeolithic in Europe. *International Journal of Osteoarchaeology* 14:162–177.
- DRUCKER, D. G., AND D. HENRY-GAMBIER. 2005. Determination of the dietary habits of a Magdalenian woman from Saint-Germain-la-Rivière in southwestern France using stable isotopes. *Journal of Human Evolution* 49:19–35.
- EDER, E. B., M. N. LEWIS, C. CAMPAGNA, AND P. L. KOCH. 2010. Evidence of demersal foraging from stable isotope analysis of juvenile elephant seals from Patagonia. *Marine Mammal Science* 26:430–442.
- EDWARDS, M. A., A. E. DEROCHE, K. A. HOBSON, M. BRANIGAN, AND J. A. NAGY. 2011. Fast carnivores and slow herbivores: differential foraging strategies among grizzly bears in the Canadian Arctic. *Oecologia* 165:877–889.
- FELICETTI, L. A., ET AL. 2003. Use of sulfur and nitrogen stable isotopes to determine the importance of whitebark pine nuts to Yellowstone grizzly bears. *Canadian Journal of Zoology* 81:763–770.
- FERNÁNDEZ, R., ET AL. 2011. Stable isotope analysis in two sympatric populations of bottlenose dolphins *Tursiops truncatus*: evidence of resource partitioning? *Marine Biology* 158:1043–1055.
- FORTIN, J. K., S. D. FARLEY, K. D. RODE, AND C. T. ROBBINS. 2007. Dietary and spatial overlap between sympatric ursids relative to salmon use. *Ursus* 18:19–29.
- FOX-DOBBS, K., J. K. BUMP, R. O. PETERSON, D. L. FOX, AND P. L. KOCH. 2007. Carnivore-specific stable isotope variables and variation in the foraging ecology of modern and ancient wolf populations: case studies from Isle Royale, Minnesota, and La Brea. *Canadian Journal of Zoology* 85:458–471.
- FOX-DOBBS, K., J. A. LEONARD, AND P. L. KOCH. 2008. Pleistocene megafauna from eastern Beringia: paleoecological and paleoenvironmental interpretations of stable carbon and nitrogen isotope and radiocarbon records. *Palaeogeography, Palaeoclimatology, Palaeoecology* 261:30–46.
- FRY, B. 2006. *Stable isotope ecology*. Springer, New York.
- GANNES, L. Z., C. MARTÍNEZ DEL RIO, AND P. KOCH. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comparative Biochemistry and Physiology, A. Molecular & Integrative Physiology* 119:725–737.
- GOROKHOVA, E., AND S. HANSSON. 1999. An experimental study on variations in stable carbon and nitrogen isotope fractionation during growth of *Mysis mixta* and *Neomysis integer*. *Canadian Journal of Fisheries and Aquatic Sciences* 56:2203–2210.
- GRANEK, E. F., J. E. COMPTON, AND D. L. PHILLIPS. 2009. Mangrove-exported nutrient incorporation by sessile coral reef invertebrates. *Ecosystems* 12:462–472.
- HAMMILL, M. O., V. LESAGE, AND P. CARTER. 2005. What do harp seals eat? Comparing diet composition from different compartments of the digestive tract with diets estimated from stable-isotope ratios. *Canadian Journal of Zoology* 83:1365–1372.
- HARPER, G. A. 2007. Detecting predation of a burrow-nesting seabird by two introduced predators, using stable isotopes, dietary analysis and experimental removals. *Wildlife Research* 34:443–453.
- HILDERBRAND, G. V., S. D. FARLEY, C. T. ROBBINS, T. A. HANLEY, K. TITUS, AND C. SERVHEEN. 1996. Use of stable isotopes to determine diets of living and extinct bears. *Canadian Journal of Zoology* 74:2080–2088.
- HOBSON, K. A., AND H. E. WELCH. 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. *Marine Ecology Progress Series* 84:9–18.
- ILES, J., ET AL. 2010. Grazing kangaroos act as local recyclers of energy on semiarid floodplains. *Australian Journal of Zoology* 58:145–149.
- KILLENGREEN, S. T., N. LECOMTE, D. EHRICH, T. SCHOTT, N. G. YOCOZO, AND R. A. IMS. 2011. The importance of marine vs. human-induced subsidies in the maintenance of an expanding mesocarnivore in the arctic tundra. *Journal of Animal Ecology* 80:1049–1060.
- KOCH, P. L., AND D. L. PHILLIPS. 2002. Incorporating concentration dependence in stable isotope mixing models: a reply to Robbins, Hilderbrand and Farley (2002). *Oecologia* 133:14–18.
- KURLE, C. M. 2008. Investigating trophic impacts of invasive rats on islands. Ph.D. dissertation, University of California, Santa Cruz.
- LESAGE, V., Y. MORIN, E. RIOUX, C. POMERLEAU, S. H. FERGUSON, AND E. PELLETIER. 2010. Stable isotopes and trace elements as indicators of diet and habitat use in cetaceans: predicting errors related to preservation, lipid extraction, and lipid normalization. *Marine Ecology Progress Series* 419:249–265.
- LI, S. G., ET AL. 2007. Plant water sources in the cold semiarid ecosystem of the upper Kherlen River catchment in Mongolia: a stable isotope approach. *Journal of Hydrology* 333:109–117.
- MAJOR, H. L., I. L. JONES, M. R. CHARETTE, AND A. W. DIAMOND. 2007. Variations in the diet of introduced Norway rats (*Rattus norvegicus*) inferred using stable isotope analysis. *Journal of Zoology (London)* 271:463–468.
- MANCINA, C. A., AND L. G. M. HERRERA. 2010. Disparate feeding strategies used by syntopic Antillean nectarivorous bats to obtain dietary protein. *Journal of Mammalogy* 91:960–966.
- MARTÍNEZ DEL RIO, C., AND S. A. CARLETON. 2012. How fast and how faithful: the dynamics of isotopic incorporation into animal tissues. *Journal of Mammalogy* 93:353–359.
- MARTÍNEZ DEL RIO, C., AND B. O. WOLF. 2005. Mass-balance models for animal isotopic ecology. Pp. 141–174 in *Physiological and ecological adaptations to feeding in vertebrates* (J. M. Starck and T. Wang, eds.). Science Publishers, Enfield, New Hampshire.
- MARTÍNEZ DEL RIO, C., N. WOLF, S. A. CARLETON, AND L. Z. GANNES. 2009. Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews* 84:91–111.

- MCCUTCHAN, J. H., W. M. LEWIS, JR., C. KENDALL, AND C. C. MCGRATH. 2003. Variation in the trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102:378–390.
- MCEACHERN, M. B., C. A. EAGLES-SMITH, C. M. EFFERSON, AND D. H. VAN VUREN. 2006. Evidence for local specialization in a generalist mammalian herbivore, *Neotoma fuscipes*. *Oikos* 113:440–448.
- MCKECHNIE, A. E. 2004. Stable isotopes: powerful new tools for animal ecologists. *South African Journal of Science* 100:131–134.
- MILAKOVIC, B., AND K. L. PARKER. 2011. Using stable isotopes to define diets of wolves in northern British Columbia, Canada. *Journal of Mammalogy* 92:295–304.
- MILLIGAN, H. E., AND M. M. HUMPHRIES. 2010. The importance of aquatic vegetation in beaver diets and the seasonal and habitat specificity of aquatic–terrestrial ecosystem linkages in a subarctic environment. *Oikos* 119:1877–1886.
- MOORE, J. W., AND B. X. SEMMENS. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology Letters* 11:470–480.
- MOWAT, G., AND D. C. HEARD. 2006. Major components of grizzly bear diet across North America. *Canadian Journal of Zoology* 84:473–489.
- NAITO, Y. I., N. V. HONCH, Y. CHIKARAISHI, N. OHKOUCHI, AND M. YONEDA. 2010. Quantitative evaluation of marine protein contribution in ancient diets based on nitrogen isotope ratios of individual amino acids in bone collagen: an investigation at the Kitakogane Jomon site. *American Journal of Physical Anthropology* 143:31–40.
- NARITA, R., A. SUGIMOTO, AND A. TAKAYANAGI. 2006. Animal components in the diet of Japanese black bears *Ursus thibetanus japonicus* in the Kyoto area, Japan. *Wildlife Biology* 12:375–384.
- NEWSOME, S. D., ET AL. 2010. Variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ diet–vibrissae trophic discrimination factors in a wild population of California sea otters. *Ecological Applications* 20:1744–1752.
- NEWSOME, S. D., C. MARTÍNEZ DEL RIO, S. BEARHOP, AND D. L. PHILLIPS. 2007. A niche for isotopic ecology. *Frontiers in Ecology and the Environment* 5:429–436.
- NEWSOME, S. D., D. L. PHILLIPS, B. J. CULLETON, T. P. GUILDERSON, AND P. L. KOCH. 2004. Dietary reconstruction of an early to middle Holocene human population from the central California coast: insights from advanced stable isotope mixing models. *Journal of Archaeological Science* 31:1101–1115.
- OGRINC, N., AND M. BUDJA. 2005. Paleodietary reconstruction of a Neolithic population in Slovenia: a stable isotope approach. *Chemical Geology* 218:103–116.
- PAINTER, M. L., C. L. CHAMBERS, M. SIDERS, R. R. DOUCETT, J. O. WHITAKER, AND D. L. PHILLIPS. 2009. Diet of spotted bats (*Euderma maculatum*) in Arizona as indicated by fecal analysis and stable isotopes. *Canadian Journal of Zoology* 87:865–875.
- PALMQVIST, P., J. A. PEREZ-CLAROS, C. M. JANIS, B. FIGUEIRIDO, V. TORREGROSA, AND D. R. GROCKE. 2008a. Biogeochemical and ecomorphological inferences on prey selection and resource partitioning among mammalian carnivores in an early Pleistocene community. *Palaios* 23:724–737.
- PALMQVIST, P., J. A. PEREZ-CLAROS, C. M. JANIS, AND D. R. GROCKE. 2008b. Tracing the ecophysiology of ungulates and predator–prey relationships in an early Pleistocene large mammal community. *Palaeogeography, Palaeoclimatology, Palaeoecology* 266:95–111.
- PARNELL, A. C., R. INGER, S. BEARHOP, AND A. L. JACKSON. 2010. Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE* 5:e9672.
- PHILLIPS, D. L., AND P. M. ELDRIDGE. 2006. Estimating the timing of diet shifts using stable isotopes. *Oecologia* 147:195–203.
- PHILLIPS, D. L., AND J. W. GREGG. 2001a. Uncertainty in source partitioning using stable isotopes. *Oecologia* 127:171–179.
- PHILLIPS, D. L., AND J. W. GREGG. 2001b. Uncertainty in source partitioning using stable isotopes (erratum). *Oecologia* 128:304.
- PHILLIPS, D. L., AND J. W. GREGG. 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136:261–269.
- PHILLIPS, D. L., AND P. L. KOCH. 2002. Incorporating concentration dependence in stable isotope mixing models. *Oecologia* 130:114–125.
- PHILLIPS, D. L., S. D. NEWSOME, AND J. W. GREGG. 2005. Combining sources in stable isotope mixing models: alternative methods. *Oecologia* 144:520–527.
- PHILLIPS, D. L., E. A. G. SCHUUR, J. R. BROOKS, M. BEN-DAVID, AND B. FRY. 2006. When isotopes aren't enough: using additional information to constrain mixing problems. Pp. 59 in Conference abstracts and Programme—5th International Conference on Applications of Stable Isotope Techniques to Ecological Studies. Queen's University, Belfast, Northern Ireland.
- PISANU, B., S. CAUT, S. GUTJAHR, P. VERNON, AND J.-L. CHAPUIS. 2011. Introduced black rats *Rattus rattus* on Ile de la Possession (Iles Crozet, Subantarctic): diet and trophic position in food webs. *Polar Biology* 34:169–180.
- POPP, B. N., ET AL. 2007. Insight into the trophic ecology of yellowfin tuna, *Thunnus albacares*, from compound-specific nitrogen isotope analysis of proteinaceous amino acids. Pp. 173–190 in Stable isotopes as indicators of ecological change (T. E. Dawson and R. T. W. Siegwolf, eds.). Terrestrial Ecology Series. Elsevier/Academic Press, San Diego, California.
- RAMÍREZ-HERNÁNDEZ, G., AND L. G. HERRERA M. 2010. Nutritional importance of seeds and arthropods to painted spiny pocket mice (*Lyomys pictus*): the effects of season and forest degradation. *Canadian Journal of Zoology* 88:1226–1234.
- REICH, K. J., AND G. A. J. WORTHY. 2006. An isotopic assessment of the feeding habits of free-ranging manatees. *Marine Ecology Progress Series* 322:303–309.
- REID, D. J., G. P. QUINN, P. S. LAKE, AND P. REICH. 2008. Terrestrial detritus supports the food webs in lowland intermittent streams of south-eastern Australia: a stable isotope study. *Freshwater Biology* 53:2036–2050.
- ROBBINS, C. T., L. A. FELICETTI, AND M. SPONHEIMER. 2005. The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. *Oecologia* 144:534–540.
- ROSLING, M. N., M. BEN-DAVID, AND R. P. BARRY. 1998. Analysis of stable isotope data: a K nearest-neighbors randomization test. *Journal of Wildlife Management* 62:380–388.
- RUFFINO, L., J. RUSSELL, B. PISANU, S. CAUT, AND E. VIDAL. 2011. Low individual-level dietary plasticity in an island-invasive generalist forager. *Population Ecology* 53:535–548.
- SAMELIUS, G., R. T. ALISAUSKAS, K. A. HOBSON, AND S. LARIVIÈRE. 2007. Prolonging the arctic pulse: long-term exploitation of cached eggs by arctic foxes when lemmings are scarce. *Journal of Animal Ecology* 76:873–880.
- SCHWARCZ, H. P. 1991. Some theoretical aspects of isotope paleodiet studies. *Journal of Archaeological Science* 18:261–275.
- SEMMENS, B. X., E. J. WARD, J. W. MOORE, AND C. T. DARIMONT. 2009. Quantifying inter- and intra-population niche variability using hierarchical Bayesian stable isotope mixing models. *PLoS ONE* 4:e6187.

- SHANER, P.-J. L., AND S. A. MACKO. 2011. Trophic shifts of a generalist consumer in response to resource pulses. *PLoS ONE* 6:e17970.
- SINISALO, T., E. T. VALTONEN, E. HELLE, AND R. I. JONES. 2006. Combining stable isotope and intestinal parasite information to evaluate dietary differences between individual ringed seals (*Phoca hispida botnica*). *Canadian Journal of Zoology* 84:823–831.
- SOTO-JIMENEZ, M. F., ET AL. 2008. Lead pollution in subtropical ecosystems on the SE Gulf of California coast: a study of concentrations and isotopic composition. *Marine Environmental Research* 66:451–458.
- STOTT, A. W., E. DAVIES, R. P. EVERSLED, AND N. TUROSS. 1997. Monitoring the routing of dietary and biosynthesized lipids through compound-specific stable isotope ($\delta^{13}\text{C}$) measurements at natural abundance. *Naturwissenschaften* 84:82–86.
- TIESZEN, L. L., T. W. BOUTTON, K. G. TESDAHL, AND N. A. SLADE. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* 57:32–37.
- URTON, E. J. M., AND K. A. HOBSON. 2005. Intrapopulation variation in gray wolf isotope ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) profiles: implications for the ecology of individuals. *Oecologia* 145:317–326.
- VANDERKLIFF, M. A., AND S. PONSARD. 2003. Sources of variation in consumer–diet $\delta^{15}\text{N}$ enrichment: a meta-analysis. *Oecologia* 136:169–182.
- VITORELLO, V. A., C. C. CERRI, F. ANDREUX, C. FELLER, AND R. L. VICTORIA. 1989. Organic matter and natural carbon-13 distribution in forested and cultivated oxisols. *Soil Science Society of America Journal* 53:773–778.
- VOIGT, C. C., K. SÖRGEL, AND D. K. N. DECHMANN. 2010. Refueling while flying: foraging bats combust food rapidly and directly to power flight. *Ecology* 91:2908–2917.
- VULLA, E., ET AL. 2009. Carnivory is positively correlated with latitude among omnivorous mammals: evidence from brown bears, badgers and pine martens. *Annales Zoologici Fennici* 46:395–415.
- WARD, E. J., B. X. SEMMENS, D. L. PHILLIPS, J. W. MOORE, AND N. BOUWES. 2011. A quantitative approach to combine sources in stable isotope mixing models. *Ecosphere* 2:art19.
- WARD, E. J., B. X. SEMMENS, AND D. E. SCHINDLER. 2010. Including source uncertainty and prior information in the analysis of stable isotope mixing models. *Environmental Science and Technology* 44:4645–4650.
- WITTEVEEN, B. H., G. A. J. WORTHY, R. J. FOY, AND K. M. WYNNE. 2011. Modeling the diet of humpback whales: an approach using stable carbon and nitrogen isotopes in a Bayesian mixing model. *Marine Mammal Science*.
- WOLF, N., S. A. CARLETON, AND C. MARTÍNEZ DEL RIO. 2009. Ten years of experimental animal isotopic ecology. *Functional Ecology* 23:17–26.
- YEAKEL, J. D., ET AL. 2009. Cooperation and individuality among man-eating lions. *Proceedings of the National Academy of Sciences* 106:19040–19043.
- YEAKEL, J. D., ET AL. 2011. Merging resource availability with isotope mixing models: the role of neutral interaction assumptions. *PLoS ONE* 6:e22015.

Special Feature Editor was Barbara H. Blake.