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Donald L. Phillips · Paul L. Koch

Incorporating concentration dependence in stable isotope mixing models

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Abstract Stable isotopes are often used as natural labels to quantify the contributions of multiple sources to a mixture. For example, C and N isotopic signatures can be used to determine the fraction of three food sources in a consumer's diet. The standard dual isotope, three source linear mixing model assumes that the proportional contribution of a source to a mixture is the same for both elements (e.g., C, N). This may be a reasonable assumption if the concentrations are similar among all sources. However, one source is often particularly rich or poor in one element (e.g., N), which logically leads to a proportionate increase or decrease in the contribution of that source to the mixture for that element relative to the other element (e.g., C). We have developed a concentration-weighted linear mixing model, which assumes that for each element, a source's contribution is proportional to the contributed mass times the elemental concentration in that source. The model is outlined for two elements and three sources, but can be generalized to *n* elements and *n*+1 sources. Sensitivity analyses for C and N in three sources indicated that varying the N concentration of just one source had large and differing effects on the estimated source contributions of mass, C, and N. The same was true for a case study of bears feeding on salmon, moose, and N-poor plants. In this example, the estimated biomass contribution of salmon from the concentration-weighted model was markedly less than the standard model estimate. Application of the model to a captive feeding study of captive mink fed on salmon, lean beef, and C-rich, N-poor beef fat reproduced very closely the known dietary proportions, whereas the standard model failed to yield a set of positive source proportions. Use of this concentration-weighted model is recommended whenever the elemental concentrations vary substantially among the sources, which may occur in a variety of ecological and geochemical applications of stable isotope analysis. Possible examples besides dietary and food web studies include stable isotope analysis of water sources in soils, plants, or water bodies; geological sources for soils or marine systems; decomposition and soil organic matter dynamics, and tracing animal migration patterns. A spreadsheet for performing the calculations for this model is available at http://www.epa.gov/wed/pages/models.htm.

Keywords Mixing model · Stable isotopes · Carbon:nitrogen ratios · Concentration · Dietary analysis

D.L. Phillips (✉)
U.S. Environmental Protection Agency,
Office of Research and Development,
National Health and Environmental Effects Research Laboratory,
Western Ecology Division, 200 SW 35th Street, Corvallis,
OR 97333, USA
e-mail: phillips.donald@epamail.epa.gov
Fax: +1-541-7544799

P.L. Koch
Department of Earth Sciences, University of California,
1156 High Street, Santa Cruz, CA 95064, USA

Introduction

Over the last several decades, stable isotope analyses have increasingly been used to determine the relative contributions of several sources to a mixture in ecological and geochemical research. Analysis of food sources in a consumer's diet is one example (Vogel and van der Merwe 1977; Boutton et al. 1978; Kelly 2000), but there are a number of other ecological and geochemical applications (Griffiths 1998). In this method for dietary analysis, isotopic values serve as naturally occurring labels of the different food sources. Isotopic values are determined for the tissues of a consumer and its food sources, and similarity in isotopic composition between the consumer's tissues and its food sources (after adjustment for isotopic sorting or fractionation during digestion, metabolism, and assimilation) gives an index of the relative importance of each item in the consumer's diet.

Specifically, linear mass balance mixing models have been used to quantify the fractional contribution of elemental mass from each food source to a consumer's diet (Schwarcz 1991; Phillips 2001). For example, two sourc-

es can be partitioned using a single isotope system (e.g., $\delta^{13}C$[1]). A two source, mass balance mixing model using mass balance for C isotopes is:

$$\delta^{13}C_M = f_X(\delta^{13}C_X + \Delta^{13}C_{tissue\text{-}X}) + f_Y(\delta^{13}C_Y + \Delta^{13}C_{tissue\text{-}Y});$$
$$1 = f_X + f_Y \quad (1)$$

where the subscripts X, Y, and M represent two food sources and the mixture (i.e., the consumer), respectively, f represents the fractional contribution of C from each food source to the consumer's diet, and $\Delta^{13}C_{tissue-X}$ is the trophic fractionation (change in $\delta^{13}C$ during assimilation) between food source X and the consumer's tissue. Note that f represents the proportion of C mass from a food source and not necessarily the proportion of biomass. Also, due to possible differences in digestibility, f represents the proportion of C assimilated rather than C consumed from a particular food source.

Simultaneous use of two isotopes (e.g., $\delta^{13}C$ and $\delta^{15}N$) allows the contributions of three sources to be estimated. Using C and N isotopes, for example, a simple three source mixing model based on mass balance equations is:

$$\delta^{13}C_M = f_X\,\delta^{13}C_{X'} + f_Y\,\delta^{13}C_{Y'} + f_Z\,\delta^{13}C_{Z'} \quad (2)$$
$$\delta^{15}N_M = f_X\,\delta^{15}N_{X'} + f_Y\,\delta^{15}N_{Y'} + f_Z\,\delta^{15}N_{Z'} \quad (3)$$
$$1 = f_X + f_Y + f_Z \quad (4)$$

where X, Y, M, and f are as defined above, and Z is the third food source (Schwarcz 1991; Phillips 2001). As for Eq. 1, isotopic values for food sources must be adjusted by the appropriate Δ values to account for trophic fractionation, but we have dropped these terms here and in subsequent equations for simplicity of presentation, and have designated fractionation corrected signatures with the prime ($'$) symbol (e.g., $\delta^{13}C_{X'}=\delta^{13}C_X+\Delta^{13}C_{tissue-X}$). This standard linear mixing model is a system of three equations in three unknowns (f_X, f_Y, and f_Z), which can be solved to determine the contribution of each food source to the diet (if the equations are linearly independent). Natural variations in C and N isotope ratios are generated by different biochemical and ecological processes, so the assumption of independence is probably valid in most cases.

Phillips (2001) has demonstrated the properties of this simple linear mixing model for source partitioning in comparison to Euclidean distance methods used in some ecological studies, and Schwarcz (1991) and Phillips (2001) have described situations under which the linear mixing model will fail to provide robust estimates of dietary proportions. Obviously, the approach only works if all the important dietary sources have been measured, and if there are no more than three such sources. The method works best when the food sources differ substantially in isotopic composition, but show low variance (Phillips and Gregg 2001). Finally, in a bivariate plot of the two isotopes, the composition of the mixture must fall within the triangular space enclosed by lines connecting the three food sources (once the values for these sources have been corrected for trophic fractionation). We refer to this space as a mixing triangle. Failure to fall within the mixing triangle indicates that either: (1) an important food source has been missed, (2) an incorrect Δ value has been used, or (3) an assumption of the mixing model has been violated.

Accurate estimation of Δ is complicated by several conceptual and analytical issues. For some systems, Δ values are relatively invariant (e.g., Cerling and Harris 1999; Kelly 2000). Yet in other cases of interest to ecologists, Δ values vary within species depending on the macromolecular composition of diet (Ambrose and Norr 1993; Tieszen and Fagre 1993). Differences in protein catabolism and N excretion may generate differences in $\Delta^{15}N$ values among animals consuming plant versus meat-rich diets or in wet versus dry regions (Ambrose and DeNiro 1986; Sealy et al. 1987; Hobson and Clark 1992; Hobson et al. 1993; Hobson 1995; Schoeller 1999). Similarly, animals mobilize endogenous nutrient stores (lipids and the C skeletons of amino acids) as sources of energy and as substrates for tissue formation when exogenous nutrients are limiting. Because endogenous lipids and amino acids can have different $\delta^{13}C$ values than exogenous C sources, mobilization and redeposition of endogenous sources may contribute to differences in $\delta^{13}C$ between tissues and exogenous nutrient sources when animals are fasting, starving, or otherwise deprived of essential nutrients (e.g., Hobson and Stirling 1997; O'Brien et al. 2000).

A key assumption of the linear mixing model is that C and N isotopes from all dietary sources are completely homogenized in the consumer's body prior to tissue synthesis. When dietary sources provide an element in just one macromolecular form that may be assimilated and metabolized in a uniform fashion, this assumption may be valid. For example, dietary N is supplied by protein, which may be digested in a broadly similar fashion regardless of diet type. In contrast, dietary C can exist as carbohydrate, lipid or protein, each with a distinct $\delta^{13}C$ value (Schoeller et al. 1984). Dietary proteins and lipids may be preferentially "routed" to synthesis of body proteins or lipids, respectively (Krueger and Sullivan 1984; Ambrose and Norr 1993; Tieszen and Fagre 1993). In such a situation, C isotopes in body proteins would be disproportionately labeled by dietary proteins, leading to an over-estimate of the fraction of protein-rich foods in the consumer's diet. Similarly, dietary lipids may be routed to synthesis of body fat (Stott et al. 1997).

Variations in Δ values and substrate routing can be accommodated through careful study in the laboratory or field. For example, Hilderbrand et al. (1996) studied the fractionation of C and N isotopes between different tissues and food sources for bears. Their Δ values for different food sources incorporate fractionation effects related to digestion and assimilation, any use of endoge-

[1] $\delta^{13}C$ in ‰ is the deviation of the C isotope ratio of a sample from that for a standard (PeeDee Belemnite). $\delta^{13}C=(R_{sample}/R_{standard}-1)\times1{,}000$, where $R=^{13}C/^{12}C$. Similarly, $\delta^{15}N$ is the deviation (in ‰) of the N isotope ratio of a sample from that of a standard (atmospheric N_2), where $R=^{15}N/^{14}N$.

nous resources, and any effects due to substrate routing. As long as animals in the wild consume foods with the same isotopic and macromolecular compositions as those used in laboratory studies and are of the same general condition with respect to nutrient supply and body condition, these Δ values should provide appropriate transformations for mixing models. Unfortunately, Δ values are lacking for many taxa, particularly those with diets that contain a wide mixture of resources.

The impacts of variable Δ values and the influences of substrate routing have been discussed by previous workers. Here, we explore the profound effects generated if another assumption of the linear mixing model represented by Eqs. 2, 3, 4 is violated. In this model, there is an implicit assumption that the proportion of C that the consumer derives from source X is the same as the proportion of N that it derives from source X (i.e., that f_X is the same in Eqs. 2, and 3), and similarly for sources Y and Z. If the food sources have similar C and N concentrations, this assumption is reasonable. For example, some carnivores, such as piscivores, eat just one class of foods (fish) which may exhibit a fairly restricted range of C and N concentrations on a whole body basis. At the other extreme, omnivores consume both plant and animal food sources which may differ greatly in C and N concentrations. One might expect that the proportion of N derived from a N-rich food source might be higher than the proportion of C derived from that food source. The standard mixing model does not account for how variations among the sources in elemental concentrations for the two isotope systems might affect the source proportions derived for the two elements. Rather, for each source, the proportions are assumed to be equal for the two elements.

The purpose of this paper is to develop an alternative mixing model for two isotopes and three sources that does incorporate concentration variation. Geochemical analyses of mixing of two sources that differ in isotope composition and elemental concentration have appeared in the geological literature (Faure 1986, chapter 9), but to our knowledge, this approach has not been generalized to more sources and used in ecological and dietary studies. After developing this concentration-weighted mixing model, we will explore the effects of concentration differences on estimates of source proportions, through examination of two case studies using data on omnivorous bears and carnivorous mink and through a sensitivity analysis.

Materials and methods

The concentration-weighted mixing model

Consider the case of two isotopic signatures (e.g., $\delta^{13}C$ and $\delta^{15}N$) being used to determine the proportional contributions of three sources (X, Y, and Z) to a mixture (M). We will discuss primarily the example of food sources in an animal's diet, but other ecological and geochemical applications might include partitioning of soil organic matter inputs (Nadelhoffer and Fry 1988), pollutant source identification (Aravena et al. 1993), and geochemical sources of minerals and nutrients (Faure 1986; Chadwick et al. 1999). Let $f_{X,B}$, $f_{Y,B}$, and $f_{Z,B}$ represent the fractions of assimilated biomass (B subscript) of sources X, Y, and Z, respectively, in the mixture M; and $f_{X,C}$, $f_{Y,C}$, $f_{Z,C}$, $f_{X,N}$, $f_{Y,N}$, and $f_{Z,N}$ similarly represent the fractions of assimilated C (C subscript) or N (N subscript) of the individual sources in the mixture. Mass balance equations can be written for each element (C and N in this example):

$$\delta^{13}C_M = f_{X,C}\delta^{13}C_{X'} + f_{Y,C}\delta^{13}C_{Y'} + f_{Z,C}\delta^{13}C_{Z'} \quad (5)$$

$$\delta^{15}N_M = f_{X,N}\delta^{15}N_{X'} + f_{Y,N}\delta^{15}N_{Y'} + f_{Z,N}\delta^{15}N_{Z'} \quad (6)$$

where $\delta^{13}C_X$ and $\delta^{15}N_X$ represent the C and N isotopic signatures for source X, and similarly for sources Y and Z and the mixture M. Isotopic signatures for the sources have been corrected for trophic fractionation as designated by the prime (′) symbol. The source fractional contributions for C, N, and biomass are constrained to sum to 1:

$$1 = f_{X,C} + f_{Y,C} + f_{Z,C} \quad (7)$$

$$1 = f_{X,N} + f_{Y,N} + f_{Z,N} \quad (8)$$

$$1 = f_{X,B} + f_{Y,B} + f_{Z,B} \quad (9)$$

The model assumes that for each element, the contribution of a food source to a consumer is proportional to the assimilated biomass times the elemental concentration in that source. So, letting $[C]_X$, $[C]_Y$, $[C]_Z$, $[N]_X$, $[N]_Y$, and $[N]_Z$ represent the C and N concentrations in food sources X, Y, and Z, then:

$$f_{X,C} = \frac{f_{X,B}[C]_X}{f_{X,B}[C]_X + f_{Y,B}[C]_Y + f_{Z,B}[C]_Z} \quad (10)$$

$$f_{Y,C} = \frac{f_{Y,B}[C]_Y}{f_{X,B}[C]_X + f_{Y,B}[C]_Y + f_{Z,B}[C]_Z} \quad (11)$$

$$f_{Z,C} = \frac{f_{Z,B}[C]_Z}{f_{X,B}[C]_X + f_{Y,B}[C]_Y + f_{Z,B}[C]_Z} \quad (12)$$

The latter equation (Eq. 12) is not independent of the previous equations because $f_{Z,C}$ is completely dependent on the values of $f_{X,C}$ and $f_{Y,C}$ as seen in Eq. 2, and can be determined by subtraction. Likewise for N:

$$f_{X,N} = \frac{f_{X,B}[N]_X}{f_{X,B}[N]_X + f_{Y,B}[N]_Y + f_{Z,B}[N]_Z} \quad (13)$$

$$f_{Y,N} = \frac{f_{Y,B}[N]_Y}{f_{X,B}[N]_X + f_{Y,B}[N]_Y + f_{Z,B}[N]_Z} \quad (14)$$

$$f_{Z,N} = \frac{f_{Z,B}[N]_Z}{f_{X,B}[N]_X + f_{Y,B}[N]_Y + f_{Z,B}[N]_Z} \quad (15)$$

As was the case for C, the latter equation is not independent because the value of $f_{Z,N}$ is dependent on the values of $f_{X,N}$ and $f_{Y,N}$ (see Eq. 8) and can be determined by subtraction.

Equations 5–11 and 13, 14 represent a set of nine independent equations in nine unknowns (the *f* variables). This can be reduced to a set of three equations to solve for the source fractional contributions for assimilated biomass ($f_{X,B}$, $f_{Y,B}$, and $f_{Z,B}$), which can then be plugged into the original equations to calculate the source contributions for C and N. Substituting Eqs. 10–12 into Eq. 5 and rearranging terms yields:

$$\left(\delta^{13}C'_X - \delta^{13}C_M\right)[C]_X f_{X,B} + \left(\delta^{13}C'_Y - \delta^{13}C_M\right)[C]_Y f_{Y,B} + \left(\delta^{13}C'_Z - \delta^{13}C_M\right)[C]_Z f_{Z,B} = 0 \quad (16)$$

Similarly, substituting Eqs. 13, 14, 15 into Eq. 6 and rearranging terms gives:

$$\left(\delta^{13}N'_X - \delta^{13}N_M\right)[N]_X f_{X,B} + \left(\delta^{13}N'_Y - \delta^{13}N_M\right) \cdot [N]_Y f_{Y,B} + \left(\delta^{13}N'_Z - \delta^{13}N_M\right)[N]_Z f_{Z,B} = 0 \quad (17)$$

Reiterating Eq. 9:

$$f_{X,B} + f_{Y,B} + f_{Z,B} = 1 \quad (18)$$

gives a system of three equations (16–18) in three unknowns ($f_{X,B}$, $f_{Y,B}$, and $f_{Z,B}$). The system can be solved algebraically but the terms are long and complex, and the solution is much more tractable using matrix algebra. In matrix notation Eqs. 16–18 can be written:

$$AF = B$$

$$A = \begin{bmatrix} (\delta^{13}C'_X - \delta^{13}C_M)[C_X] & (\delta^{13}C'_Y - \delta^{13}C_M)[C_Y] \\ (\delta^{15}N'_X - \delta^{15}N_M)[N_X] & (\delta^{15}N'_Y - \delta^{15}N_M)[N_Y] \\ 1 & 1 \end{bmatrix}$$

$$\begin{matrix} (\delta^{13}C'_Z - \delta^{13}C_M)[C_Z] \\ \cdot (\delta^{15}N'_Z - \delta^{15}N_M)[N_Z] \\ 1 \end{matrix} \Bigg]$$

$$F = \begin{bmatrix} f_{X,B} \\ f_{Y,B} \\ f_{Z,B} \end{bmatrix} \quad (19)$$

$$B = \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix}$$

To solve for $f_{X,B}$, $f_{Y,B}$, and $f_{Z,B}$ (vector **F**), both sides of the matrix equation are pre-multiplied by the inverse of **A** to give: $\mathbf{F=A^{-1}B}$. These values for $f_{X,B}$, $f_{Y,B}$, and $f_{Z,B}$ can then be inserted in Eqs. 10, 11, 12 to solve for $f_{X,C}$, $f_{Y,C}$, and $f_{Z,C}$, and in Eqs. 13, 14, 15 to solve for $f_{X,N}$, $f_{Y,N}$, and $f_{Z,N}$.

While the model above is outlined for determining the contributions of three sources to a mixture in terms of biomass and two elements, it can be generalized to any number *n* of elements and *n*+1 sources (e.g., using C, N, and O concentrations and isotopic signatures for four different sources).

Illustrative examples

To demonstrate our approach, we compare the results of the concentration-weighted model with those from the standard mixing model for two test cases. We sought example studies where elemental concentrations were likely to differ among food sources. Bears provide an ideal test case. They consume diverse resources, including marine salmon, terrestrial plants and terrestrial meat, their feeding ecology has received frequent isotopic study (Hobson and Welch 1992; Hilderbrand et al. 1999a, 1999b; Jacoby et al. 1999; Hobson et al. 2000), and Δ values are known for their food sources (Hilderbrand et al. 1996). Our first example is based on food source and hair isotope data for brown bears (*Ursus arctos*) and black bears (*Ursus americanus*) from the Kenai Peninsula, Alaska (Jacoby et al. 1999).

Our second example is based on food source and body fat isotope data from mink (*Mustela vison*) raised in a controlled feeding experiment that was presented as an independent test of the performance of linear mixing and Euclidean distance models for source partitioning (Ben-David and Schell 2001). In this study, mink were fed diets containing 50% salmon, 25% lean beef, and 25% beef fat by weight, and two tissues were analyzed: blood and body fat. Mink blood fell within the mixing triangle, and for this tissue, the standard linear mixing model provided more reasonable and interpretable estimates of biomass fractions than distance methods. Results for mink body fat were more problematic. Body fat data fell outside the mixing triangle, leading to nonsensical

Table 1 Data used in bear and mink examples of dietary analyses using the standard mixing model and the concentration-dependent model

	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\Delta^{13}C_{tissue\text{-}diet}$ (‰)	$\Delta^{15}N_{tissue\text{-}diet}$ (‰)	[C] (%)[f]	[N] (%)[f]
Kenai, Alaska, bear hair[a]							
Brown bear – sympatric	38	–20.3	10.9				
Black bear – sympatric	37	–22.5	4.9				
Black bear – allopatric	9	–20.1	7.6				
Kenai, food sources and fractionations							
Salmon	8	–20.5[c]	13.2[c]	1.2	2.3	55	12
Terrestrial meat	5	–21.5[d]	3.9[d]	4.9	4.0	51.5	14
Terrestrial plants	18	–26.6[e]	-0.9[e]	3.3	4.1	44	1
Captive mink[b]							
Mink fat	7	–24.04	12.91				
Captive mink, food sources and fractionations							
Salmon	?	–20.82	12.27	–2.2	2.3	55	12
Lean beef	?	–22.28	6.19	–2.2	4.0	56	11.5
Beef fat	?	–26.18	6.19	0.9	4.0	72	1.5

[a] Diet and tissue isotope data from Jacoby et al. (1999)
[b] Diet and tissue isotope data from Ben-David and Schell (2001)
[c] Estimated from isotopic measurements on unspecified tissue from chinook (*Oncorhynchus tshawytscha*) and sockeye (*O. nerka*) salmon
[d] Estimated from isotopic measurements on hair from moose (*Alces alces*), assuming that hair and muscle have the same isotopic composition
[e] Estimated from herbivore hair data, using tissue-diet fractionation relationships in Hilderbrand et al. (1996)
[f] [C] and [N] concentrations were determined using the following data from the USDA Nutrient Database (NDB). For salmon, we averaged data for raw chinook (*Oncorhynchus tshawytscha*, NDB 15078), chum (*O. keta*, NDB 15079), coho (*O. kisutch*, NDB 15081), pink (*O. gorbuscha*, NDB 15083), and sockeye (*O. nerka*, NDB 15085) salmon. For terrestrial vegetation, we averaged data for raw, whole fruit and one leafy plant, including apples (*Malus sylvestris*, NDB 9003), blackberries (*Rubus* sp., NDB 9042), cranberries (*Vaccinium macrocarpon*, NDB 9078), raspberries (*Rubus* sp., NDB 9302), and rhubarb (*Rheum rhabarbarum*, NDB 9307). For typical terrestrial meat, we averaged data for raw meat from caribou (*Rangifer* sp., NDB 17162), deer (*Odocoileus* sp., NDB 17164), elk (*Cervus elaphus*, NDB 17166), and moose (*A. alces*, NDB 17172). For lean beef, we averaged the data for various grades of trimmed retail beef cuts (NDB 13011, 13013, 13015 and 13017). For beef fat, we used data for fat trimmed from retail cuts of beef (NDB 13019)

negative biomass estimates for lean beef in the diet using the standard mixing model, and distance models gave inaccurate biomass estimates as well. Ben-David and Schell (2001) evaluated several causes for the failure of the standard linear mixing model (e.g., incorrect Δ values, slow turnover), but concluded that routing of dietary fat to body fat was the most likely explanation. We explored the body fat example to determine if strong differences in dietary source elemental concentrations can account for failure of the standard mixing model and can be corrected using the concentration-weighted model.

A summary of the data used in each example is provided in Table 1. Isotopic values for food and tissues were taken from Jacoby et al. (1999) and Ben-David and Schell (2001), but Δ values and elemental concentrations require some explanation. Hilderbrand et al. (1996) suggest that Δ values for bear hair and bear blood are similar, so for the bear example, we used Δ values determined for blood for bears fed diets of salmon, terrestrial meat (mule deer) and terrestrial plants (apples). As noted earlier, their Δ values for different food sources incorporate both fractionation and substrate-routing effects.

For mink fat relative to diet, like Ben-David and Schell (2001), we assumed that protein in mink adipose tissue would have the same $\Delta^{15}N$ value relative to diet as protein in mink blood. Ben-David and Schell (2001) also showed that Δ values were similar for bear and mink blood, so we used the value determined by Hilderbrand et al. (1996) for a salmon diet for $\Delta^{15}N_{\text{mink fat-salmon}}$, and the value for a terrestrial meat diet for both $\Delta^{15}N_{\text{mink fat-lean beef}}$ and $\Delta^{15}N_{\text{mink fat-beef fat}}$. Ben-David and Schell (2001) stated that $\Delta^{13}C$ values between body fat and diet were poorly known (citing early work by DeNiro and Epstein 1978), and they chose to assume no fractionation for this relationship. However, more recent work not cited by these authors has revealed consistent fractionations for body fat relative to bulk diet and dietary lipids, though no studies have been conducted on mink. Schoeller et al. (1984) report a $\Delta^{13}C_{\text{body fat-bulk diet}}$ value for humans of –2.2‰, similar to the body fat-bulk diet estimate for pigs reported in Tieszen and Fagre (1993). We used this for the $\Delta^{13}C$ value between mink fat and diets of salmon and lean beef. For mink fat relative to a beef fat diet, we used the $\Delta^{13}C_{\text{body fat-diet fat}}$ value for humans (Schoeller et al. 1984), which is slightly less than the average for pigs (Tieszen and Fagre 1993).

Dietary elemental concentrations were not reported in Jacoby et al. (1999) or Ben-David and Schell (2001). To estimate concentrations, we used data from the USDA Nutrient Database (NDB) (www.nal.usda.gov/fnic/foodcomp/index.html). The USDA NDB provides information on weight percent (wt %) water, protein, fat, carbohydrate, and ash. We used these data to calculate wt % C and N in each diet, assuming that protein is 52 wt % C, 16 wt % N; lipid is 75 wt % C, 0 wt % N; and carbohydrate (including fiber) is 45 wt % C, 0 wt % N (Robbins 1993).

Sensitivity analysis

In addition to the two case studies just described, we also created hypothetical source and mixture scenarios in order to vary conditions in a controlled manner and test the responsiveness of the model to these changes. A sensitivity analysis was performed to determine the magnitude of the effect of variations in elemental concentrations among the sources on the estimates of the proportions of biomass and the two elements attributed to each source. An infinite variety of combinations of source and mixture isotopic signatures and elemental concentrations can be devised. However, in order to control much of this variation and explore the effect of simple, single changes in concentrations, two source and mixture scenarios using C and N were constructed as shown in Fig. 1. Both scenarios had three sources that were equally spaced in the $\delta^{15}N$ vs. $\delta^{13}C$ plane, with the mixture in the center of this triangle. This situation reflects equal contributions from each source using the standard three source, dual isotope mixing model (Eqs. 2, 3, 4). The first scenario (Fig. 1a) had two sources (X and Y) with equal low values of $\delta^{15}N$ and the third source (Z) had a higher

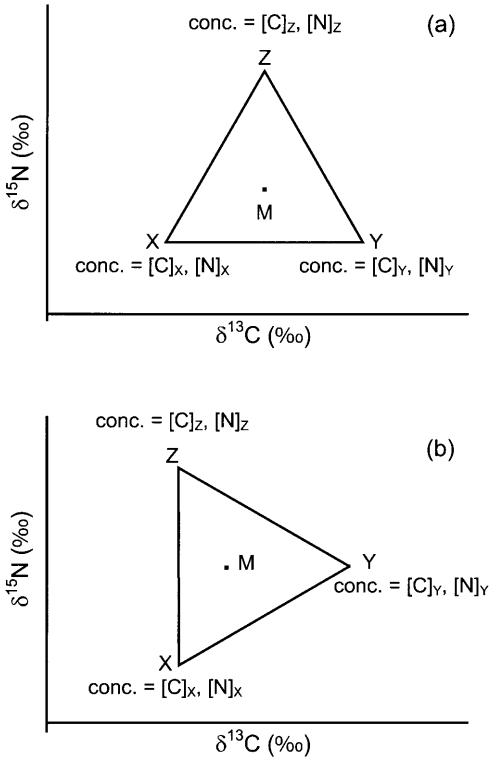

Fig. 1 Sensitivity analysis scenarios (**a**, **b**) with equally spaced sources (*X*, *Y*, *Z*) with equal contributions to the mixture (*M*) according to the standard mixing model (Eq. 1). In each scenario, [C] was held constant and equal for each source and the [N] for one source was varied while holding the other two constant and equal

$\delta^{15}N$. The second scenario (Fig. 1b) was similar but with the triangle rotated, showing two sources (X and Z) with equal low values of $\delta^{13}C$ and the third source (Y) had a higher $\delta^{13}C$. In each scenario, the C concentrations were assigned to be equal for the three sources. The N concentrations were equal for two of the sources and were varied 0.2–5 times this concentration for the third source. In turn each of the three sources was varied in this way, with the other two sources held constant. As in the bear and mink illustrative examples, this reflects a situation in which there is one particularly N-poor or N-rich food source that may cause different source strengths for C and N.

Results and discussion

Illustrative examples

Jacoby et al. (1999) conducted an isotopic study of brown and black bear diets (past and present) from habitats in the western contiguous U.S.A. and Alaska to document patterns and changes in trophic relations of these populations to provide a baseline for wildlife management planning. They reported data for three groups of bears from the Kenai Peninsula: sympatric brown and black bears and allopatric black bears. Figure 2a is $\delta^{13}C$-$\delta^{15}N$ plot of each group of bears and their potential dietary sources (corrected for trophic fractionation using the values in Table 1), as well as the mixing triangle for the

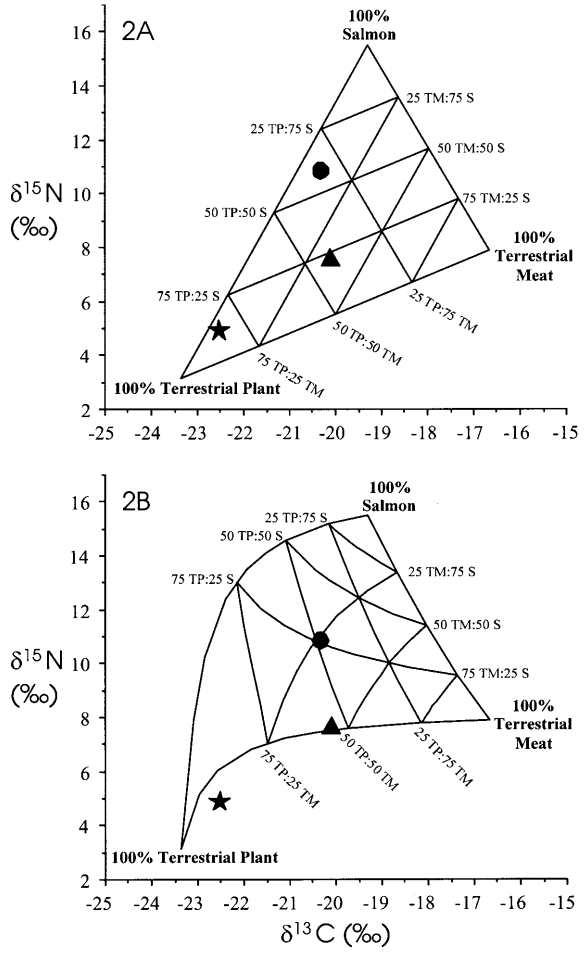

Fig. 2a, b Dietary mixing triangles for bears from the Kenai Peninsula, Alaska (Jacoby et al. 1999). Isotopic values for pure diets at the vertices of each triangle have been corrected for trophic fractionation using the values in Table 1. Variations in % contribution of terrestrial plants (*TP*), salmon (*S*), and terrestrial meat (*TM*) are shown along the edges of the mixing triangles, and serve as labels for iso-diet lines that intersect the edge. **a** Mixing triangle for the standard model. **b** Mixing triangle for the concentration-weighted model. H Sympatric black bears, s allopatric black bears, l sympatric brown bears

standard mixing model (Eqs. 2, 3, 4). In this space, the lines connecting the vertices of the triangle are simple, two-source mixing lines. For example, a point halfway between two vertices along such a line corresponds to a consumer diet containing 50% of each source. Lines within the triangle are "iso-diet" lines, along which the proportion of one dietary component is invariant. Iso-diet lines increase from 0% on the side of the triangle opposing a vertex to 100% at the vertex.

Sympatric black and brown bear data plot near the line joining salmon and terrestrial plant diets (Fig. 2a), thus the calculated proportions of terrestrial meat in their diets are small (<10%) using the standard mixing model (Table 2). The main difference between sympatric bears is a greater proportion of salmon in brown bear diets vs. more terrestrial plants in black bear diets. Data for allopatric black bears fall toward the center of the mixing triangle, consequently estimated dietary proportions are more balanced, including significant fractions of all three food sources (Table 2).

Terrestrial plants, a potential food source for Kenai bears (and all bears in the study by Jacoby et al. 1999), have a much lower [N] and higher C:N ratio than the other two sources (Table 1), which violates the assumption of constant elemental concentrations entailed by the standard mixing model. The concentration-weighted model must be applied to obtain accurate dietary biomass proportions in this situation. Figure 2b again shows the placement of each group of bears and their potential dietary sources in the $\delta^{13}C$-$\delta^{15}N$ plane. It also presents the appropriate triangular mixing space for the concentration-weighted mixing model. Lines connecting the vertices of this space are again two-source mixing lines, but they are now curved, and percent contribution is not linearly related to distance along the line between the vertices. As with Fig. 2a, the curved lines within the triangle are iso-diet lines.

The reason for non-linearity is intuitively clear. Consider an animal eating only salmon and terrestrial plants, whose diet would fall somewhere on the line connecting plant and salmon diets (Fig. 2b). Because salmon has a much higher [N] than terrestrial plants, even a small fraction of salmon in the diet causes the $\delta^{15}N$ of consumer tissues to shift strongly towards the $\delta^{15}N$ of salmon. Because [C] concentrations are almost equal in salmon and terrestrial plants, $\delta^{13}C$ values exhibit a more linear response to changes in dietary proportions along this mixing line. The same situation applies along the line joining terrestrial plant and terrestrial meat diets. Now consider a bear feeding only on salmon and terrestrial meat. The line between salmon and terrestrial meat diets is nearly straight because [C] and [N] are almost the same in these two food sources (Table 1). This non-linear response of isotope tracers in mixing models where elemental concentrations vary greatly has long been recognized in the geochemical literature (Faure 1986; Banner and Hanson 1990).

Use of the appropriate concentration-weighted mixing model has profound effects on estimates of dietary biomass proportions for Kenai bears. First, data for sympatric black bears fall outside the appropriate mixing space, suggesting that some aspect of the new model has been violated for this group (i.e., incorrect Δ value, substrate routing, or poorly constrained dietary sources) (Fig. 2b). (It should be noted that the mixture falling within the mixing space is no guarantee that none of these violations have occurred, since multiple errors could possibly compensate for each other, or individual errors might shift the mixture but still within the borders of the mixing space.) This model violation is signaled by the negative values for salmon in sympatric black bear diets (Table 2). A second obvious difference is that estimated proportions of salmon biomass in the diet are lower for all three groups, with nearly equal corresponding increases in the biomass estimates of terrestrial plants and terrestrial meat in the diet. For exam-

Table 2 Food source proportions estimated by standard mixing model and concentration-weighted model. *f* Fractional contribution, *B* biomass, *S* salmon, *TM* terrestrial meat, *TP* salmon, *LB* lean beef, *BF* beef fat

	Biomass			C			N		
Kenai bear	$f_{S,B}$	$f_{TM,B}$	$f_{TP,B}$	$f_{S,C}$	$f_{TM,C}$	$f_{TP,C}$	$f_{S,N}$	$f_{TM,N}$	$f_{TP,N}$
Standard model									
Brown bear – sympatric	0.59	0.10	0.31						
Black bear – sympatric	0.12	0.05	0.83						
Black bear – allopatric	0.23	0.34	0.43						
Concentration-weighted model									
Brown bear – sympatric	0.26	0.26	0.48	0.30	0.27	0.43	0.44	0.50	0.07
Black bear – sympatric	–0.03	0.12	0.91	–0.03	0.14	0.89	–0.15	0.75	0.40
Black bear – allopatric	0.01	0.43	0.56	0.01	0.47	0.52	0.01	0.91	0.08
Captive mink	$f_{S,B}$	$f_{LB,B}$	$f_{BF,B}$	$f_{S,C}$	$f_{LB,C}$	$f_{BF,C}$	$f_{S,N}$	$f_{LB,N}$	$f_{BF,N}$
Standard model									
Mink fat	0.62	–0.21	0.58						
Concentration-weighted model									
Mink fat	0.49	0.28	0.23	0.46	0.27	0.28	0.62	0.34	0.04
Actual ingested amounts	0.50	0.25	0.25	0.46	0.24	0.30	0.65	0.31	0.04

ple, the estimated contribution of salmon to allopatric black bear diets drops from 23% under the standard mixing model to 1% under the concentration-weighted mixing model.

Finally, the concentration-weighted model provides information on elemental contributions from each dietary source in addition to biomass proportions. The contribution of C from each dietary source is very similar to the biomass fraction from that source for all three groups of bears, because the C concentrations are similar among the sources. N contributions are more complex. For example, while terrestrial meat never accounts for >43% of the estimated biomass assimilated by any group of bears, it always accounts for ≥50% of the N assimilated by bears. And as might be expected, even when estimated terrestrial plant biomass assimilation is high, this N-poor source always accounts for ≤40% of the N assimilated by bears. Thus, the concentration-weighted model provides additional information that may be important in assessing feeding ecology.

The mink fat example provides a more direct test of the differences between the two models because dietary proportions are known in this study. Our test of the standard mixing model differs from that presented by Ben-David and Schell (2001) in that we used different Δ values. Still, the basic problem they identified persists. Mink body fat falls well outside the standard mixing triangle for salmon, lean beef, and beef fat diets (Fig. 3a), resulting in negative values for % lean beef in the diet (Table 2). As mentioned above, Ben-David and Schell (2001) offer the very reasonable speculation that the mixing model failed because of preferential routing of dietary lipids to body fat. Since lean beef has very little fat, it would be under-represented in mink body fat if routing were strong.

Results from the concentration-weighted model suggest an alternative explanation. Figure 3b shows the placement of mink fat in the appropriate triangular mixing space for the concentration-weighted mixing model. As expected from the previous discussion, the mixing line linking salmon with lean beef is straight, because [C] and [N] are nearly identical between these two food sources (Table 1). In contrast, the mixing line connecting beef fat and salmon is strongly curved, and percent contribution is not linearly related to distance along the line between the sources. This occurs because beef fat has such a low [N] and such a high [C] when compared to salmon. The mixing line connecting lean beef and beef fat must be straight, because these dietary sources have the same $\delta^{15}N$ value.

Mink fat falls very near the intersection of three plotted iso-diet lines with the concentration-weighted model (Fig. 3b). The estimated proportions of biomass contributing to mink fat (Table 2 : 49% salmon, 28% lean beef, 23% beef fat) are extremely close to the known fractions in the controlled diets (50%, 25%, 25%). Thus the problem with the standard mixing model seemed to be primarily the imbalance in C and N concentrations among the food sources, rather than dietary routing of fat. Mink were fed a diet with 25% beef fat by weight, and from mink fat isotope values, we reconstructed a diet with 23% beef fat on a biomass basis. Dietary routing may be a secondary factor which helps explain the residual errors of 1–3% in the estimates of biomass proportions in this example. Indeed, the fact that the protein-rich components of diet (salmon, lean beef) are nearly proportionally represented in mink fat reveals that C skeletons from dietary amino acids are used for energy storage and metabolism in these carnivorous mammals and that dietary protein is not routed solely to body proteins.

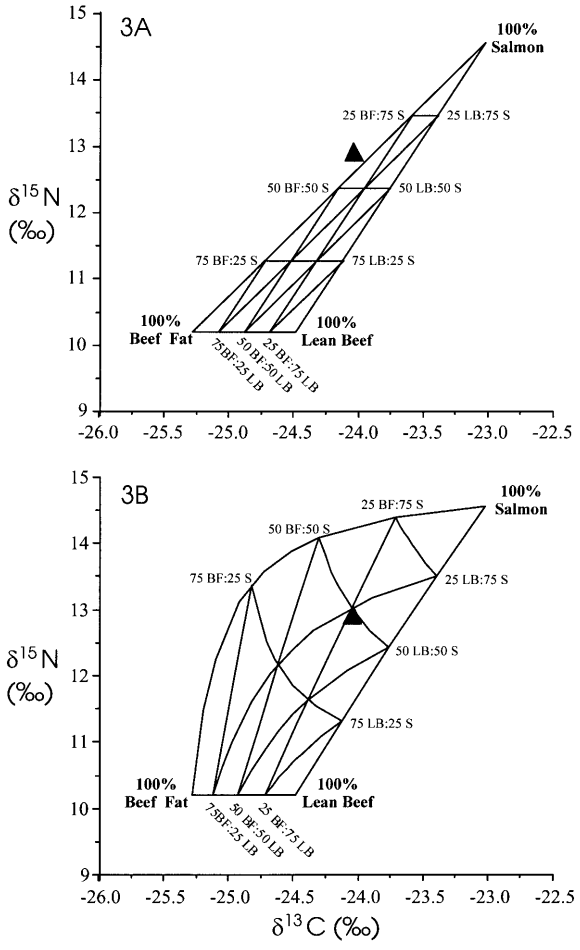

Fig. 3a, b Dietary mixing triangles for captive mink in feeding experiment by Ben-David and Schell (2001). Isotopic values for pure diets at the vertices of each triangle have been corrected for trophic fractionation using the values in Table 1. Variations in % contribution of lean beef (*LB*), beef fat (*BF*), and S are shown along the edges of the mixing triangles, and serve as labels for isodiet lines that intersect the edge. **a** Mixing triangle for the standard model. **b** Mixing triangle for the concentration-weighted model. s Mink body fat

Not only did the concentration-weighted model provide estimates very close to the correct biomass proportions, but the C and N proportions for each food source's contribution to mink fat were also within 0–3% of their actual representation in the mixed diet (Table 2). Because of beef fat's high [C] and low [N], it accounted for 30% of the dietary C but only 4% of the dietary N, compared to 25% of the diet on a biomass basis (Table 2). It is precisely this kind of imbalance in C and N contents of food sources which causes problems with the standard mixing model, since it assumes C and N partition equally.

Our examples illustrate three important points for workers planning to use isotopes to reconstruct the diet or trophic relations. The first is that whenever potential food sources have substantially different C and N concentrations, dietary proportions must be estimated with a model that takes concentration differences into account. Failure to do so may lead to large errors in estimated proportions of assimilated biomass. For example, Jacoby et al. (1999) used a more restrictive variant of the standard mixing model and slightly different Δ values[2], but they reached roughly the same conclusions about the diets of Kenai bears that we obtained with the standard mixing model. In particular, they estimated relatively high amounts of salmon in the diets of sympatric brown bears and allopatric black bears. We believe this result may be an artifact, produced by the strongly curved nature of the mixing line connecting N-poor terrestrial plant diets and N-rich salmon diets. Likewise, their analyses of diets from the lower 48 states, where bears have access to only terrestrial meat and plants, may be overestimating the amount of meat in bear diets simply because bear tissue $\delta^{15}N$ values will strongly resemble those of terrestrial meat once this N-rich source makes up >10% or 20% of assimilated biomass. Use of the standard mixing model when concentration variations are great may produce misleading results with major ramifications on assessments of feeding ecology and management strategies.

The second point is that the sides of mixing triangles will be strongly curved when connecting dietary sources that show great differences in elemental concentration. Indeed, at its most extreme, the mixing line between two sources might form nearly a right angle. This situation might apply for C and N isotope curves in birds that feed on nectar chiefly for carbohydrates (C rich, N poor) and some other substance (e.g., insects) for protein (e.g., Hobson et al. 1999). When the food sources have basically the same [C] and [N] concentrations, the edges of the triangle will be straight lines, and the standard mixing model will provide reasonable dietary estimates.

The final point is that assessing when isotopic data from consumers "fit" with potential dietary resources is more complicated than initially envisioned. Data from all three Kenai bears fell within the standard mixing triangle, but this provided no guarantee that they would provide accurate dietary estimates or even that they would fall within the appropriate, concentration-weighted mixing space. Similarly, mink fat data fell outside the standard mixing triangle, but were well within the appropriate concentration-weighted triangle, and they yielded proportions that match those in the known diet very closely. Prior to construction of the appropriate mixing triangle, the only sure sign that consumer data will not fit the concentration-weighted model is the presence of data with isotope values outside the range of values encompassed by

[2] Jacoby et al. (1999) used Eqs. 1 and 2 in Hilderbrand et al. (1996) to estimate the proportions of salmon, terrestrial meat, and plants in bear diets. They assume that terrestrial meat and plants have the same $\delta^{13}C$ value, which differs from that of salmon. They solve for % salmon in the diet using a two-component C isotope mass balance equation identical to our Eq. 1. Once they have % salmon, they solve for % plant and % terrestrial meat using a N isotope mass balance equation identical to our Eq. 3. This approach fails to account for concentration differences, and it entails the assumption that meat and plants have the same $\delta^{13}C$ value, which differed by >5‰ for the Kenai study.

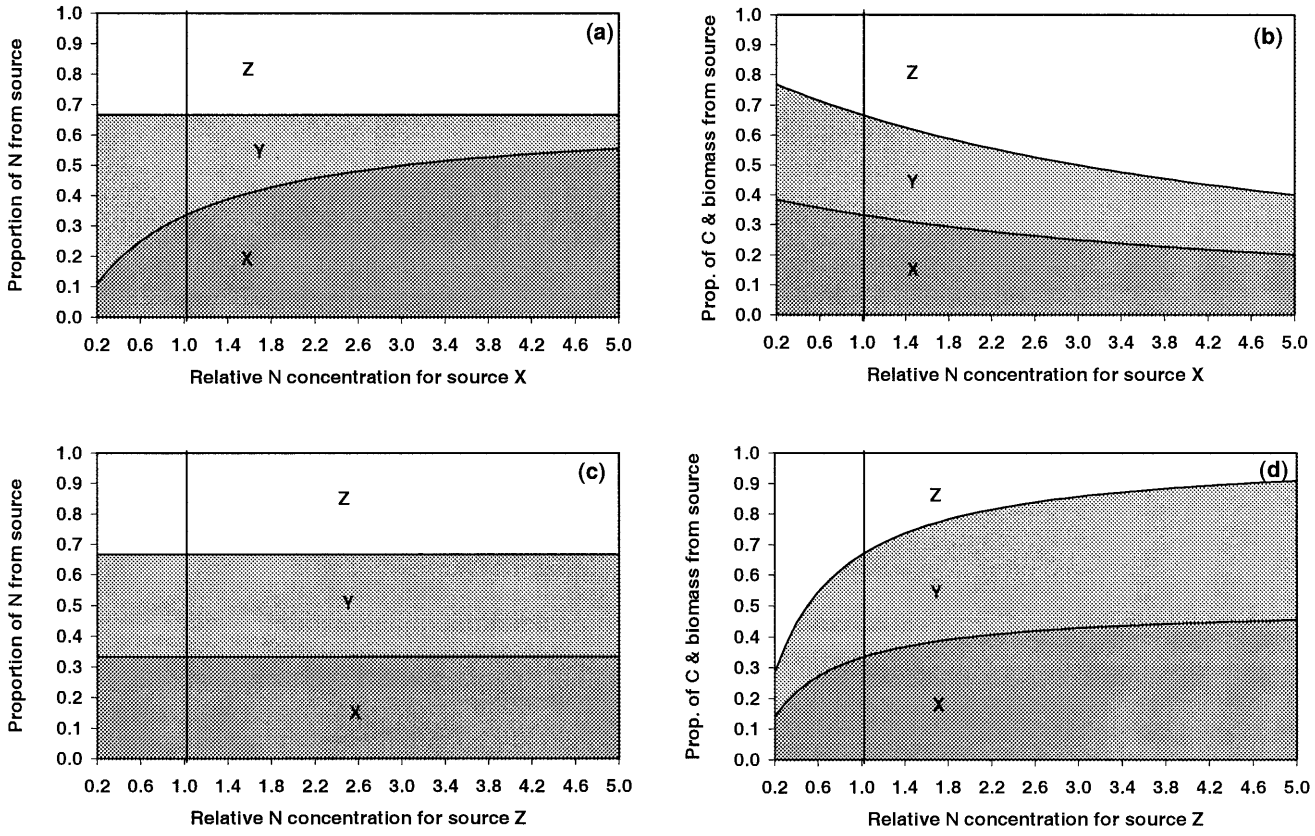

Fig. 4a–d Results of sensitivity analyses for the first scenario. **a** and **b** represent the partitioning of N and both C and biomass, respectively, among the three sources as a function of [N] for source X. **c** and **d** represent the same thing as a function of [N] for source Z. Effects for variation of [N] for source Y were similar to those for X and are not shown

potential diets. Because of the angular nature of mixing lines, the range of possible consumer values is the rectangle that has sides at the $\delta^{15}N$ and $\delta^{13}C$ extrema for potential diets. For example, mink fat data from Ben-David and Schell (2001) fall well within this rectangle. In contrast, the northern interior wolves from Szepanski et al. (1999; their Fig. 3) have $\delta^{13}C$ values higher than those for any measured food item, even after correction for trophic fractionation. These wolves may have been consuming a ^{13}C-enriched food source that was not measured; the authors mention ground squirrels, snowshoe hares, and beaver as other possible food items for northern interior wolves which were not included in the mixing model.

Sensitivity analysis

For fixed source and mixture signatures, the sensitivity analysis scenarios show how the proportions of C, N, and biomass assimilated from each source vary as [N] is changed for one source (with equal [C] for all sources). By design, the standard mixing model (Eqs. 2, 3, 4) gave estimates of 1/3 from each source for both scenarios. The concentration-weighted mixing model (Eqs. 3) also gave this result for both scenarios when [N] was equal for all sources (vertical lines at 1.0 on Figs. 4 and 5). Thus, when both [C] and [N] are equal among sources, the concentration-weighted mixing model gives identical results to the standard mixing model, which assumes that C and N partition equally. Examination of Eqs. 10, 11, 12, 13, 14, 15, 16, 17, 18 verifies that when $[C]_X=[C]_Y=[C]_Z$ and $[N]_X=[N]_Y=[N]_Z$, then $f_{X,C}=f_{X,N}=f_{X,B}$ and similarly for sources Y and Z. This demonstrates that the standard mixing model may be considered as a special case of the more general model which allows for concentration-dependent partitioning of the two elements among sources.

As the relative [N] for one source varied on either side of 1.0, the source proportions for assimilated biomass ($f_{X,B}$, $f_{Y,B}$, and $f_{Z,B}$), C ($f_{X,C}$, $f_{Y,C}$, and $f_{Z,C}$), and N ($f_{X,N}$, $f_{Y,N}$, and $f_{Z,N}$) changed in a variety of patterns (Figs. 4, 5). The source proportions for assimilated biomass and C were equivalent in all cases because [C] was assumed to be the same for all sources and are shown on the same graphs (Fig. 4b, d; 5b, d). The variety of responses to increasing the [N] of one source can be summarized as either: (1) the proportion of N derived from that source in the mixture stays constant while the biomass and C proportions from the source decrease (e.g., Fig. 4c, d); (2) the proportion of N from that source increases while the biomass and C proportions from the source remain constant (e.g., Fig. 5c, d); or (3) the source proportion of N from that source increases *and* the biomass and C proportions from the source decrease (e.g., Figs. 4a, b; 5a, b). These patterns of response emphasize the importance of considering the source ele-

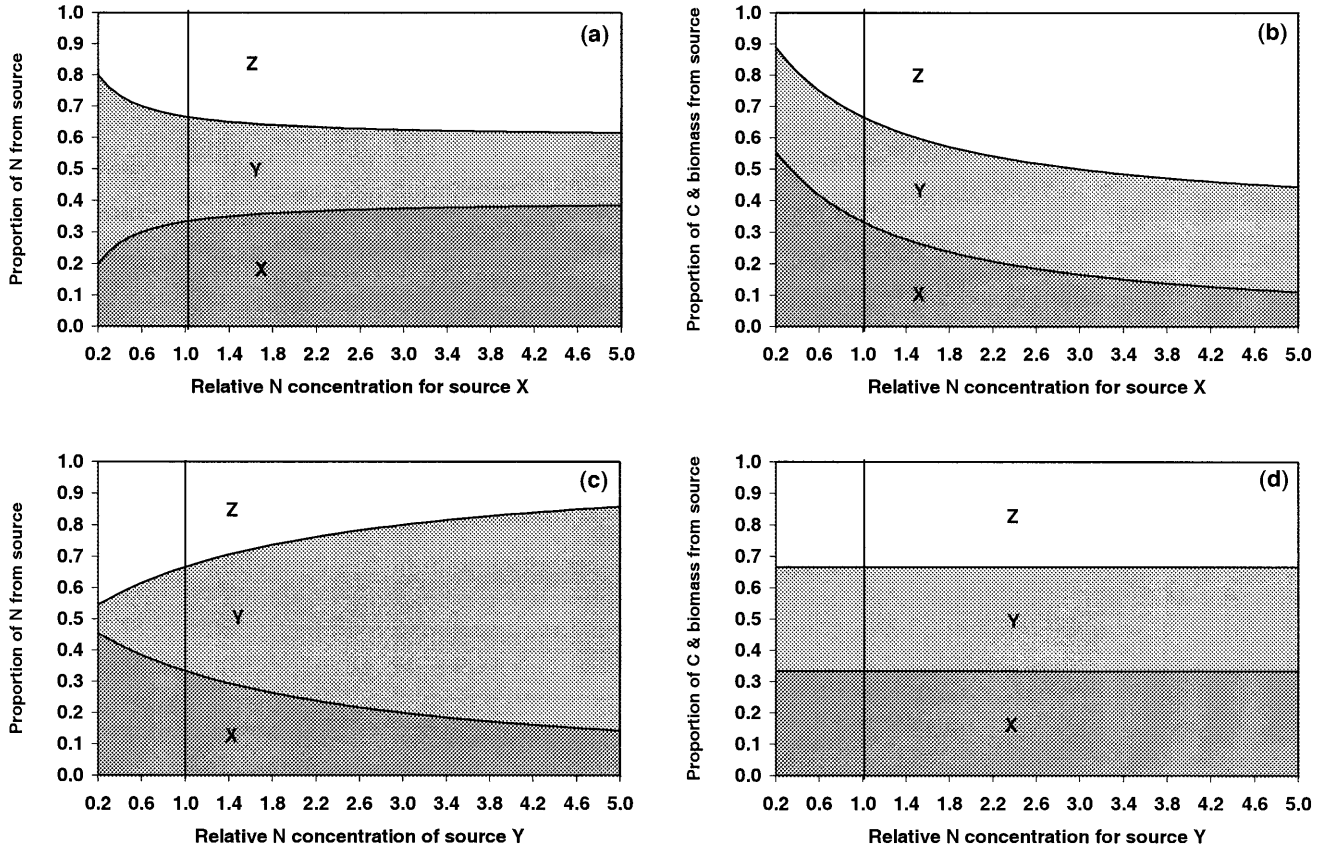

Fig. 5a–d Results of sensitivity analyses for the second scenario. **a** and **b** represent the partitioning of N and both C and biomass, respectively, among the three sources as a function of [N] for source X. **c** and **d** represent the same thing as a function of [N] for source Y. Effects for variation of [N] for source Z were similar to those for X and are not shown

mental concentrations when using stable isotope analyses to determine proportional source utilization.

The detailed differences in the patterns of response to changing [N] of one source depend on the details of the scenarios: which source is changed, and how its N and C isotopic signatures compare to the other sources and the mixture. For example, in the first scenario (Fig. 1a), as $[N]_X$ is increased relative to $[N]_Y$ and $[N]_Z$, the proportion of N derived from that source ($f_{X,N}$) increased, with a commensurate decrease in $f_{Y,N}$, and no change in $f_{Z,N}$ (Fig. 4a). It seems intuitively clear that the proportion of N derived from X ($f_{X,N}$) should increase as its N concentration increased. But why did the N contribution of one source (Y) decrease, while that of the other source (Z) remained constant? The explanation lies in the fact that X and Y have equal $\delta^{15}N$ values in this scenario (Fig. 1a). Thus, no matter how the N contribution of X and Y is split among those two sources, their joint effect on the $\delta^{15}N$ value of the mixture is the same. The problem essentially reduces to a two-endmember mixing model between Z and (X+Y), which satisfies mass balance with a N contribution of 1/3 from Z and 2/3 from (X+Y). Similar lines of reasoning explain the detailed patterns of response observed in the other parts of the sensitivity analysis.

Additional analyses (results not shown) were conducted to determine the effect on source proportion estimates if [C] and [N] concentrations were not equal among sources, but C:N ratios were. In this case, for each source the estimates of proportions of C and N derived from that source were equal, and were the same as provided by the standard mixing model. However, the concentration-weighted model gave additional information on the proportions of assimilated biomass from each of the sources, which were different from the C and N proportions. Sources with higher [C] and [N] had reduced biomass proportions compared to their C and N proportions. Thus, while equal C:N ratios among sources can be taken as a less stringent condition than equal [C] and [N] for the determination of C and N proportions, biomass proportions still vary when there is not equality of [C] and [N] among sources.

Conclusions

Numerous situations in which multiple stable isotope systems are used to partition the source contributions to a mixture fail to meet the assumption of equal elemental concentrations among the sources. This may lead to substantial over- or under-estimates of the source mass contributions to the mixture, and a failure to recognize that the source proportions may be different for each element. The

concentration-weighted mixing model outlined here accounts for differences in elemental concentrations among sources in estimating source contributions. This model reduces to the standard mixing model when all sources have equal elemental concentrations. When concentration equality is not the case, both sensitivity analyses and examples from the ecological research literature using C and N isotopes demonstrated substantial and distinct effects on the estimated proportions of source contributions for biomass, C, and N. The concentration-weighted model was able to successfully match the known proportions of food sources in a captive feeding study in which one source had markedly different C and N concentrations, while the standard mixing model failed to do so.

The weakest link in the application of mixing models to a dietary reconstruction relates to the estimation of appropriate Δ values. To date, very few species have been examined, and it is unclear if Δ values measured in captive feeding experiments, where animals are typically well fed and in good condition, are applicable in the wild, where animals may be under nutritional stress. Still, our study shows that diet-to-tissue routing or the use of endogenous stores under nutritional stress can only be detected in a reliable and quantifiable fashion once elemental concentration differences in potential food sources have been accounted for in an appropriate mixing model. A second weakness of many studies we examined in our search for case studies is a failure to rigorously quantify the isotopic composition of dietary plants. Finally, because workers have not been cognizant of the potentially large effects of elemental concentration differences, they have not reported elemental concentrations in food sources, though such data are easily obtained using continuous flow, elemental analyzer-isotope ratio monitoring mass spectrometry.

While we have presented only dietary analysis examples, this model may be applicable to a number of other ecological and geochemical applications of stable isotope analysis. Examples might include: (1) water mixing in soils, aquifers, plants, or estuaries when measured by independent tracers such as NO_3^--N, Sr, and water H or O (e.g., Aravena et al. 1993; Ingram and DePaolo 1993); (2) quantifying different geological sources for soils or marine systems using Sr, Pb, and Nd isotopes (e.g., Gosz et al. 1983; Borg and Banner 1996; Chadwick et al. 1999); (3) decomposition and soil organic matter dynamics (Nadelhoffer and Fry 1988); and (4) tracing animal migrations using multiple isotopes (e.g., Koch et al. 1995; Chamberlain et al. 1997; Hobson et al. 1999). Use of the concentration-weighted model is not necessary in situations where the concentrations of the tracer elements are invariant, e.g., when δ^2H and $\delta^{18}O$ are used together as tracers for water sources.

We suggest the following guidelines when conducting multi-element stable isotope studies to partition the contributions of sources to a mixture:

1. For clarity, always report the isotopic signatures and fractionation values used for each source and the mixture.
2. Always measure and report elemental concentrations for each source.
3. If the concentrations differ substantially among the sources, use a concentration-weighted mixing model to account for these differences.
4. For unique solutions of the contributions from *n* sources, *n*–1 isotope systems (elements) must be used. The concentration-weighted mixing model can be generalized to any number of isotope systems.

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References

Ambrose SH, DeNiro MJ (1986) The isotopic ecology of East African mammals. Oecologia 69:395–406

Ambrose SH, Norr L (1993) Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In: Lambert JB, Grupe G (eds) Prehistoric human bone: archaeology at the molecular level. Springer, Berlin Heidelberg New York, pp 1–37

Aravena R, Evans ML, Cherry JA (1993) Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. Ground Water 31:180–186

Banner JL, Hanson GN (1990) Calculation of simultaneous isotopic and trace-element variations during water-rock interaction with applications to carbonate diagenesis. Geochim Cosmochim Acta 54:3123–3137

Ben-David M, Schell DM (2001) Mixing models in analyses of diet using multiple stable isotopes: a response. Oecologia 127: 180–184

Borg LE, Banner JL (1996) Neodymium and strontium isotopic constraints on soil sources in Barbados, West Indies. Geochim Cosmochim Acta 60:4193–4206

Boutton TW, Cameron CN, Smith BN (1978) Insect herbivory on C3 and C4 grasses. Oecologia 36:21–32

Cerling TE, Harris JM (1999) Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. Oecologia 120:347–363

Chadwick OA, Derry LA, Vitousek PM, Huebert BJ, Hedin LO (1999) Changing sources of nutrients during four million years of ecosystem development. Nature 397:491–497

Chamberlain CP, Blum JD, Holmes RT, Sherry TW, Graves GR (1997) The use of isotope tracers for identifying populations of migratory birds. Oecologia 109:132–141

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

Faure G (1986) Principles of isotope geology, 2nd edn. Wiley, New York

Gosz JR, Brookins DG, Moore DI (1983) Using strontium isotope ratios to estimate inputs to ecosystems. BioScience 33:23–30

Griffiths H (1998) Stable isotopes: integration of biological, ecological and geochemical processes. Environmental Plant Biology Series. Bios, Oxford

Hilderbrand GV, Farley SD, Robbins CT, Hanley TA, Titus K, Servheen C (1996) Use of stable isotopes to determine diets of living and extinct bears. Can J Zool 74:2080–2088

Hilderbrand GV, Hanley TA, Robbins CT, Schwartz CC (1999a) Role of brown bears (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. Oecologia 121:546–550

Hilderbrand GV, Schwartz CC, Robbins CT, Jacoby ME, Hanley TA, Arthur SM, Servheen C (1999b) The importance of meat, particularly salmon, to body size, population productivity, and conservation of North American brown bears. Can J Zool 77:132–138

Hobson KA (1995) Reconstructing avian diets using stable-carbon and nitrogen isotope analysis of egg components: patterns of isotopic fractionation and turnover. Condor 97:752–762

Hobson KA, Clark RW (1992) Assessing avian diets using stable-carbon and nitrogen isotopes. II. Factors influencing diet-tissue fractionation. Condor 94:189–197

Hobson KA, Stirling I (1997) Terrestrial foraging by polar bears during the ice free period in western Hudson Bay: metabolic pathways and limitation of the stable isotope approach. Mar Mamm Sci 13:359–367

Hobson KA, Welch HE (1992) Determination of trophic relationships within a high arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar Ecol Prog Ser 84:9–18

Hobson KA, Alisauska RT, Clark RW (1993) Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analysis of diet. Condor 95:388–394

Hobson KA, Wassenaar LI, Taylor OR (1999) Stable isotopes ($\delta\Delta$ and $\delta^{13}C$) are geographic indicators of natal origins of monarch butterflies in eastern North America. Oecologia 120: 397–404

Hobson KA, McLellan BN, Woods JG (2000) Using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes to infer trophic relationships among black and grizzly bears in the upper Columbia River basin, British Columbia. Can J Zool 78:1332–1339

Ingram BL, DePaolo DJ (1993) A 4,300 year strontium isotope record of estuarine paleosalinity in San Francisco Bay, California. Earth Plant Sci Lett 119:103–119

Jacoby ME, Hilderbrand GV, Servheen C, Schwartz CC, Arthur SM, Hanley TA, Robbins CT, Michener R (1999) Trophic relations of brown and black bears in several western North American ecosystems. J Wildl Manage 63:921–929

Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can J Zool 78:1–27

Koch PL, Heisinger J, Moss C, Carlson RW, Fogel ML, Behrensmeyer AK (1995) Isotopic tracking of change in diet and habitat use in African elephants. Science 267:1340–1343

Krueger HW, Sullivan CH (1984) Models for carbon isotope fractionation between diet and bone. In: Turnland JR, Johnson PE (eds) Stable isotopes in nutrition. American Chemical Society, symposium series 258. American Chemical Society, Washington, D.C., pp 205–220

Nadelhoffer KJ, Fry B (1988) Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. Soil Sci Soc Am J 52:1633–1640

O'Brien DM, Schrag DP, Rio CM del (2000) Allocation to reproduction in a hawkmoth: a quantitative analysis using stable carbon isotopes. Ecology 81:2822–2831

Phillips DL (2001) Mixing models in analyses of diet using multiple stable isotopes: a critique. Oecologia 127:166–170

Phillips DL, Gregg JW (2001) Uncertainty in source partitioning using stable isotopes. Oecologia 127:171–179

Robbins CT (1993) Wildlife feeding and nutrition, 2nd edn. Academic Press, San Diego, Calif.

Schoeller DA (1999) Isotope fractionation: Why aren't we what we eat? J Archaeol Sci 26:667–673

Schoeller DA, Minagawa M, Slater R, Kaplan IR (1984) Stable isotopes of carbon, nitrogen and hydrogen in the contemporary North American human food web. Ecol Food Nutr 18:159–170

Schwarcz HP (1991) Some theoretical aspects of isotope paleodiet studies. J Archaeol Sci 18:261–276

Sealy JC, Merwe NJ van der, Lee-Thorp JA, Lanham JL (1987) Nitrogen isotopic ecology in southern Africa: implications for environmental and dietary tracing. Geochim Cosmochim Acta 51:2707–2717

Stott AW, Davies E, Evershed RP, Tuross N (1997) Monitoring the routing of dietary and biosynthesised lipids through compound-specific stable isotope ($\delta^{13}C$) measurements at natural abundance. Naturwissenschaften 84:82–86

Szepanski MM, Ben-David M, Van Ballenberghe V (1999) Assessment of anadromous salmon resources in the diet of the Alexander Archipelago wolf using stable isotope analysis. Oecologia 120:327–335

Tieszen LL, Fagre T (1993) Effect of diet quality and composition on the isotopic composition of respiratory CO_2, bone collagen, bioapatite, and soft tissues. In: Lambert JB, Grupe G (eds) Prehistoric human bone: archaeology at the molecular level. Springer, Berlin Heidelberg New York, pp 121–155

Vogel JC, Merwe NJ van der (1977) Isotopic evidence for early maize cultivation in New York State. Am Antiq 42:238–242