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Incorporating concentration dependence in stable isotope mixing models: a reply to Robbins, Hilderbrand and Farley (2002)

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In Phillips and Koch (2002), we presented an isotopic mixing model for use in cases where elemental concentration differs greatly among potential contributors to the mixture. Our primary goal was to present the mathematics of this system for two isotopes and three sources, noting that it was generalizable to n isotopes and $n+1$ sources. To illustrate the magnitude of the differences that might result if elemental concentrations differed greatly among possible sources, we performed model sensitivity tests and examined examples involving mixing of different food sources in the diets of captive mink and wild bears. The response by Robbins et al. (2002) deals largely with our bear example and it makes five points we will address.

First, Robbins et al. (2002) argue that our example involving bears from the Kenai Peninsula, Alaska, which used data from their previously published work (Hilderbrand et al. 1996; Jacoby et al. 1999), is unrealistic in several ways, and therefore, that our results should not be used to make management decisions. We agree completely. We did not attempt to present a rigorous re-analysis of isotopic constraints on Kenai bear diets. Our only goal with the illustrative examples was to alert workers to an important complication that could crop up in dietary (and other) isotope analyses, and to offer a tool for addressing this complication in a quantitative manner.

Second, in addition to their concerns about our results for the coastal Kenai bears, Robbins et al. (2002) take issue with the single sentence in our paper on inland bear populations. We suggested that standard linear mixing “may be overestimating the amount of meat in

bear diets simply because bear tissue $\delta^{15}\text{N}$ values will strongly resemble those of terrestrial meat once this N-rich source makes up more than 10 or 20% of assimilated biomass”. Robbins et al. (2002) find this suggestion “baseless”, citing data from a subset of the inland populations with isotopic data suggesting nearly 100% plant diets. Yet these populations have no access to salmon and little access to large ungulates and, therefore, it is not especially surprising that they are essentially herbivorous. The existence of entirely herbivorous bear populations does not address the point we were raising. There are populations of brown (*Ursus arctos*) and black (*U. americanus*) bears that have access to terrestrial meat and apparently eat it. For the Greater Yellowstone ecosystem, Jacoby et al. (1999) used standard linear mixing and estimated that brown bears have a mean of 58% terrestrial meat in their diets, whereas black bears average 48% (all data from their Table 1). Likewise, brown bears from the southwestern United States have 88% terrestrial meat in their diets; black bears average 39%. Brown bears from the Blackfoot and Flathead Indian Reservations in Montana have estimated meat intakes of 69%. Clearly it is only for these populations, where there is a *mixture* of meat and plants in the diet, that our comments about biases in *isotope mixing* are relevant. Indeed, Jacoby et al. (1999) were surprised by the high dietary meat estimate for southwestern United States bears, and devoted two paragraphs to attempts to explain the result. We stand by our suggestion that concentration differences among leafy plants, nuts, and terrestrial meat should be investigated as a potential contributor to high dietary meat estimates.

Third, Robbins et al. (2002) present a strong case demonstrating that Kenai bears consume leafy plants rather than fruit, and argue that our model might yield different results with a more realistic leafy plant diet. They note that leafy plants tend to have higher N concentrations ([N]) and lower C:N ratios than fruit. And because plant protein digestion is efficient, whereas digestion of structural carbohydrates in leafy plants is not, the C:N ratio of assimilated leafy plant biomass may be

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Table 1 Diet composition, digestibility estimates, and isotopic data used in models. Crude protein data for meat and fish are determined as in Phillips and Koch (2002). Crude protein for fruit and leafy plants are from data compiled by Robbins et al. (2002). Digestible (Digest) protein is calculated from Crude protein, assuming 100% protein digestibility for meat and fish and 90% digestibility for fruit and leafy plants. Digest N is calculated from Digest protein assuming protein is 16% N. Digest dry matter (DM) is from data compiled by Robbins et al. (2002). Digest C data for

meat and fish are as determined in Phillips and Koch (2002). Digest C data for fruit and leafy plants are calculated by assuming Digest DM is 45% C. Units for Crude protein, and Digest protein, N, DM, and C are g per 100 g diet DM. Digest [N]=(Digest N/Digest DM)×100. Digest [C] is calculated in the same fashion. Units for Digest [N] and [C] are g per 100 g Digest DM. Dietary isotope values (δ) and diet-tissue fractionation factors (Δ) are from Phillips and Koch (2002). Units for isotope values and fractionations are ‰, relative to V-PDB for C and atmosphere N₂ for N

Diet	Crude protein	Digest protein	Digest N	Digest DM	Digest C	Digest [N]	Digest [C]	$\delta^{13}\text{C}/\Delta^{13}\text{C}$	$\delta^{15}\text{N}/\Delta^{15}\text{N}$
Meat	88.1±4.0	88.1±4.0	14.1±0.6	100	51.5±1.0	14.1±0.6	51.5±1.0	-21.5/+4.9	+3.9/+4.0
Fish	73.6±7.8	73.6±7.8	11.8±1.2	"	54.8±2.0	11.8±1.2	54.8±2.0	-20.5/+1.2	+13.2/+2.3
Fruit	5.0±3.5	4.5±3.2	0.7±0.5	63.4±9.8	28.5±4.4	1.2±1.0	45	-26.6/+3.3	-0.9/+4.1
Leafy	23.4±8.0	21.0±7.2	3.4±1.2	35.0±9.1	15.8±4.1	9.8±3.7	"	"	"

lower, comparable to that of meat or fish (see their Fig. 1). We agree with all these points. However Robbins et al. (2002) imply that similar C:N ratios would obviate the need for the concentration-weighted model, with which we disagree. Equal C:N ratios in assimilated food sources *might* reduce the impact of concentration differences, but if the absolute amount of assimilated C and N differs among foods, mixing may still be non-linear. Here, we evaluate the effects of a leafy plant diet and of digestibility differences with our concentration-weighted model using data from Fig. 1 of Robbins et al. (2002).

Data and calculations for our models are presented in Table 1. Briefly, isotope and fractionation values as well as composition data for meat and fish are from Phillips and Koch (2002)¹. Leafy plant and fruit composition and the dry matter digestibility of these diets are from Robbins et al. (2002). Prior work indicates that C and N in meat and fish are 100% digestible (Pritchard and Robbins 1990). We estimate that plant protein is 90% digestible and that protein is 16% N (Robbins 1993). Finally, we follow Robbins et al. (2002) in assuming that digested dry matter is 45% carbon (i.e., that digested dry matter has the stoichiometry of carbohydrate).

We first consider a realistic fruit diet that is corrected for digestibility. The concentrations of C and N assimilated from this more natural fruit diet are similar to that in Phillips and Koch (2002). This occurs because macromolecular characteristics of the USDA fruit data used by

Phillips and Koch (2002) are similar to those of natural fruits. In addition, because fruit protein and dry matter (largely soluble carbohydrate) are highly digestible, correcting for digestibility has little impact. Consequently, the dietary proportions from the concentration-weighted model of Phillips and Koch (2002) are identical to those obtained here (Fig. 1B, Table 2). Thus the key conclusion of the bear example in Phillips and Koch (2002) is supported by this more realistic treatment. Given these dietary isotope values, if an omnivore consumes a significant amount of a sugar-rich/N-poor food (such as fruit or honey), use of a model that fails to account for stoichiometry may lead to distorted estimates of dietary proportions. Kenai bears do not consume large amounts of fruit, but these foods may be important for other populations and species. While we have not investigated the stoichiometry or digestibility of lipid- or starch-rich plant foods (nuts, tubers, etc.), these too may have digestible [C] and [N] and C:N ratios very different than meat or fish.

Next we consider how dietary estimates change for a leafy plant diet corrected for digestibility. As suggested by Robbins et al. (2002), the C:N ratio of the assimilated portion of the leafy plants is low (4.6:1), similar to that of meat and fish. As important for estimates of dietary proportions is the fact that the average assimilated [N] for leafy plants is high, almost 10% of digested dry matter. Given almost equal absolute concentrations of C and N for assimilated biomass for all dietary sources, the concentration-weighted model provides estimates nearly identical to the standard mixing model (Fig. 1A, C; Table 1). Yet while the standard and the concentration-weighted mixing models yield the same answer in this case, there is no reason to expect this a priori. Here the effects of food stoichiometry (low [N] in plants compared to meat) and of the differential digestibility of elements in plants (low C digestibility, high N digestibility) are compensatory. The standard mixing model yields correct results even though it relies on the incorrect assumptions of equal elemental composition and equal assimilation. It yields the right answer for the wrong reasons. A similar compensation may be at work in the approach to estimate dietary proportions used by Jacoby et

¹ We must note that the $\delta^{13}\text{C}$ value for meat in Phillips and Koch (2002) is almost certainly different than the value used in the study by Jacoby et al. (1999), which was not reported. Our reading of their paper suggested they had estimated the $\delta^{13}\text{C}$ of plants from the $\delta^{13}\text{C}$ of herbivores using the fractionation relationship in Fig. 2 of Hilderbrand et al. (1996). We reversed this process to estimate the $\delta^{13}\text{C}$ of herbivores from the reported $\delta^{13}\text{C}$ value of plants to obtain a value of -21.5‰. However, further analyses conducted while preparing this reply indicate that Jacoby et al. (1999) must have used a value close to -27‰ for terrestrial meat. Because of differences in the treatment of diet-tissue fractionations and especially the large uncertainties about the isotopic composition of potential food sources for Kenai bears, our results cannot be directly compared with those of Jacoby et al. (1999) and *must not* be viewed as supplying realistic estimates of dietary proportions.

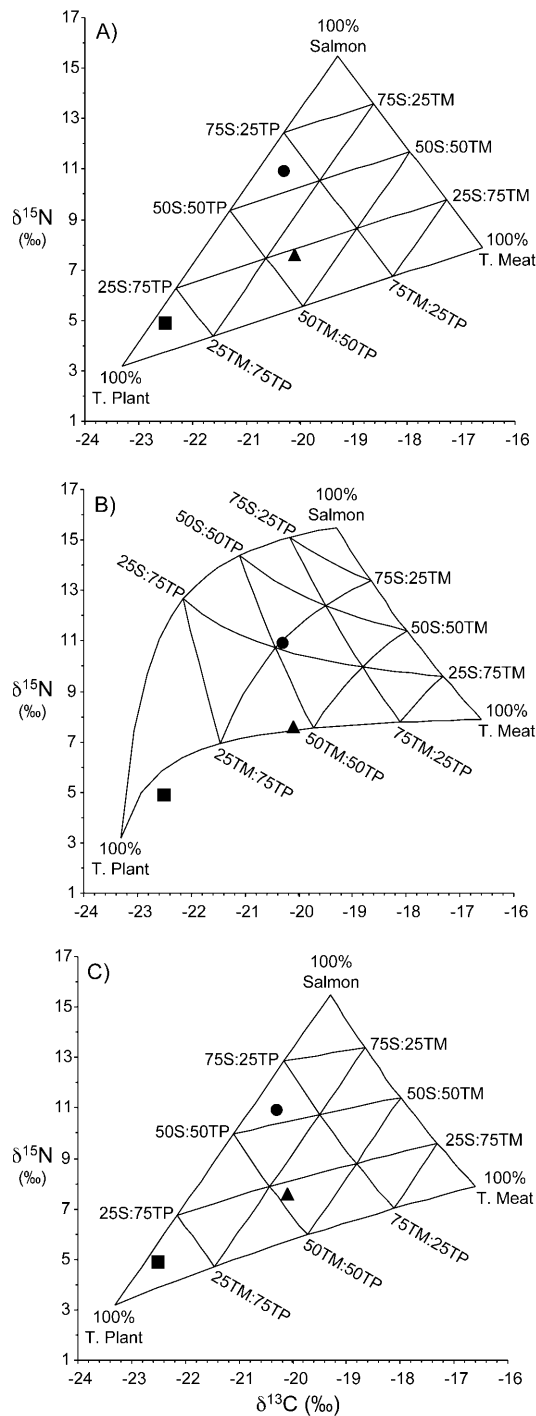


Fig. 1A–C Dietary mixing triangles for Kenai bears for different models. Isotopic values for pure diets at the vertices of each triangle have been corrected for trophic fractionation (Phillips and Koch 2002). Variations in proportional contribution of terrestrial plants (*TP*), salmon (*S*), and terrestrial meat (*TM*) are shown along the edges of the mixing triangles, and serve as labels for iso-diet lines that intersect each edge. **A** Mixing triangle for the standard linear mixing model. **B** Mixing triangle for the concentration-weighted model using a realistic fruit diet that is corrected for digestibility. **C** Mixing triangle for the concentration-weighted model using a leafy plant diet that is corrected for digestibility. Key: *star* sympatric black bears; *triangle* allopatric black bears (*Ursus americanus*); *circle* sympatric brown bears (*U. arctos*)

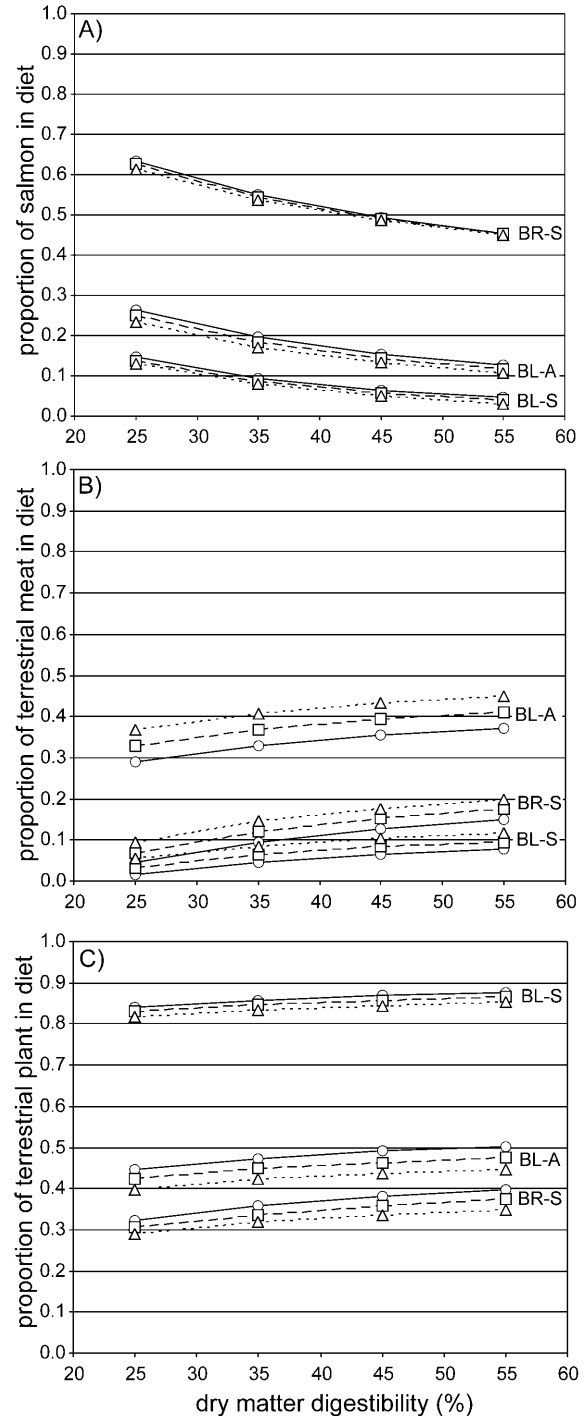


Fig. 2A–C Results of sensitivity studies that alter dry matter digestibility and the [C] of digested dry matter for leafy plants. **A** Effects on estimated proportions of salmon in diet. **B** Effects on estimated proportions of terrestrial meat in diet. **C** Effects on estimated proportions of terrestrial plants in diet. Key: *BR-S* sympatric brown bears; *BL-S* sympatric black bears; *BL-A* allopatric black bears; *circle with solid line* digested [C] = 45% (carbohydrate); *square with long dashed line* digested [C] = 52% (protein); *triangle with short dashed line* digested [C] = 60% (mix of protein and lipid)

Table 2 Estimates of diet biomass proportions for different plant diets and models. *Conc WT?* – was the model concentration-weighted? *Digest Cor?* – was the model corrected for digestibility differences? $f_{S,B}$, $f_{TM,B}$, f_{TPB} – proportions of dietary biomass from salmon, terrestrial meat and terrestrial plants, respectively. We re-

port mean \pm 1 SD above, and range of values in parentheses below. These values were calculated from data generated by obtaining separate concentration-weighted model estimates for each fruit or leafy plant considered in Robbins et al. (2002)

Plant Diet	Conc WT?	Digest Cor?	Brown bears sympatric			Black bears sympatric			Black bears allopatric		
			$f_{S,B}$	$f_{TM,B}$	f_{TPB}	$f_{S,B}$	$f_{TM,B}$	f_{TPB}	$f_{S,B}$	$f_{TM,B}$	f_{TPB}
Any ^a	No	No	0.59	0.10	0.31	0.12	0.05	0.83	0.23	0.34	0.43
Fruit ^a	Yes	No	0.26	0.26	0.48	-0.03	0.12	0.91	0.01	0.43	0.56
Fruit ^b	Yes	Yes	0.27 \pm 0.04 (0.24,0.36)	0.26 \pm 0.02 (0.21,0.27)	0.47 \pm 0.02 (0.44,0.49)	-0.03 \pm 0.02 (-0.04,0.0)	0.12 \pm 0.01 (0.10,0.13)	0.90 \pm 0.01 (0.89,0.91)	0.01 \pm 0.03 (-0.01,0.06)	0.44 \pm 0.02 (0.41,0.45)	0.55 \pm 0.01 (0.53,0.56)
Leafy ^c	Yes	Yes	0.55 \pm 0.09 (0.41,0.70)	0.10 \pm 0.05 (0.01,0.18)	0.36 \pm 0.04 (0.29,0.42)	0.10 \pm 0.05 (0.03,0.20)	0.05 \pm 0.03 (-0.01,0.09)	0.86 \pm 0.02 (0.82,0.88)	0.20 \pm 0.07 (0.10,0.32)	0.33 \pm 0.04 (0.26,0.39)	0.47 \pm 0.03 (0.42,0.52)

^a Results from the standard and concentration-weighted mixing models, as reported in Phillips and Koch (2002)

^b Results obtained using the concentration-weighted mixing model and data for meat, fish and fruit in Table 1

^c Results obtained using the concentration-weighted mixing model and data for meat, fish and leafy plants in Table 1

al. (1999), though this is difficult to evaluate without data on the isotopic composition of all dietary end-members.

The fourth point of Robbins et al. (2002) is to question the usefulness of the concentration-weighted model when dealing with a system as complex as the diets of omnivorous bears. They argue that the model requires so much information and/or so many assumptions about bear food sources and their digestibilities that it is useless in all but trivially simple cases. We disagree. When confronted with a problem that exhibits potentially confounding complexity with respect to inputs, their stoichiometry and digestibility, we believe that a sensible strategy is to sensitivity test using a model where poorly constrained variables are explicit terms that are allowed to vary around plausible values. The concentration-weighted mixing model allows such testing. Standard linear mixing models implicitly assume identical stoichiometry and equal digestibility among all diets, and thus can only be used to test the sensitivity to the isotope composition of dietary end members (as for example in Hobson et al. 2000).

The bear examples in Table 1 required data on the isotopic and elemental composition, protein and dry matter digestibility, and assimilated [C] of food sources. Dietary isotope values have been determined in many ecological studies (e.g., Ostrom et al. 1997; Ben-David et al. 1998; Hobson et al. 2000; Harding and Stevens 2001), and elemental compositions are collected simultaneously in laboratories using elemental analyzers interfaced with gas source mass spectrometers. Protein and dry matter digestibility can be measured in both the laboratory and the field (Robbins 1993). Assumptions about the high digestibility of plant protein and the 100% digestibility of meat and animal tissue are extremely robust, having been verified repeatedly in a diverse set of taxa (Robbins 1993). In contrast, plant dry matter digestibility varies greatly among animals and for different types of plants, and as Robbins et al. (2002) note, it is not feasible to

measure this parameter for all plant foods in all field studies. Furthermore, the assumption that the [C] of assimilated plant dry matter is 45% is hard to verify and certainly wrong in many cases. For example, when dry matter is highly indigestible, the only fraction of leafy plants that bears will assimilate is protein, so digested [C] will approach the stoichiometry of protein (52%). When bears consume lipid-rich seeds or nuts, digested [C] might be higher, near the stoichiometry of fat (ca. 75%).

We illustrate how to evaluate the effects of these uncertainties on dietary estimates by varying leafy plant dry matter digestibility and digested [C] through a range of plausible values (25–55% for digestibility; 45–60% for [C]) (Fig. 2). We find that dietary estimates are insensitive to assumptions about digested [C], varying by just 5–10% across the wide range of values explored. There is greater sensitivity to dry matter digestibility, particularly for the salmon estimate for Kenai brown bears, which varies by ca. 20%. This reflects the sensitivity of the salmon estimate to dietary [N]. As digestibility drops and protein comes to dominate digested dry matter, digested plant [N] rises and the C:N ratio falls, leading to the increase in the estimated proportion of salmon in the diet.

This sensitivity study demonstrates that explicit assumptions and inputs are strengths of the concentration-weighted model, not weaknesses. Such models are especially important because not every study will have the luxury of the experiments supporting the bear research. Using the concentration-weighted model forces workers to move beyond statistical prediction models based on empirically derived relationships to consider the theory about digestion and physiology that underpin such approaches. Our results emphasize that progress in this field hinges on the existence of robust data on food stoichiometry and the digestibility of dietary constituents. In any case, the general claim by Robbins et al. (2002) that accuracy is *necessarily* compromised by in-

creased model complexity is not a truism. A simple model that makes invalid assumptions is as likely to produce errors as a more complex model that makes valid assumptions.

The fifth point that Robbins et al. (2002) make also relates to model complexity. They note that our approach might fail to yield reliable dietary estimates because it does not account for metabolic factors that might be important for bears (substrate routing, non-linear mixing, etc.). These issues were discussed in Phillips and Koch (2002), but were excluded from our mixing models for two reasons. So little is currently known about these processes that including them in a model would be premature. We agree with Robbins et al. (2002) and Ben-David and Schell (2001) in calling for more experimentation and study of these issues, and have stated so in press (Gannes et al. 1998). Given our rudimentary state of knowledge of these processes, we think it best to treat isotope mixing models as a type of null model. They explain the distribution of two or more isotopes that would be expected if more complex digestive and physiological processes are not at work. For example, Ben-David and Schell (2001) offered a reasonable hypothesis when they suggested that the standard mixing model failed to explain their mink fat data because dietary lipid might be routed to form body lipid. In essence, they suggested that an assumption of the model had been violated by a complex physiological process. Our contribution was to point out that a simple model that accounts for differences in stoichiometry can explain their results without invoking routing (Phillips and Koch 2002).

The application of stable isotopes to nutritional ecology is a discipline that has recently attained majority. It was born in a pulse of seminal papers in the late 1970s that resulted from productive collaborations among archaeologists, geochemists, ecologists, and physiologists (Boutton et al. 1978; DeNiro and Epstein 1978, 1981; Fry et al. 1978; Tieszen 1978; van der Merwe and Vogel 1978; Estep and Dabrowski 1980). Our presentation of the concentration-weighted mixing model was entirely in this spirit, bringing a way of thinking about isotopic mixing that is common in the Earth sciences to ecologists in a new, quantitative formulation that was (we hoped) user-friendly. Detailed models that explicitly account for metabolic processes, perhaps through analysis of individual amino acids or fatty acids, would be welcomed. However concentration is such a basic part of any mixing problem that even compound-specific approaches will need to account for it when it is variable. We contend that a great deal can be learned about ecology and physiology by exploring models at the level of complexity of our concentration-weighted model, for example to disentangle effects of concentration differences from those due to physiological routing. At present, the only alternative when confronted with a complex mixing problem is to ignore stoichiometric differences and hope that it does not seriously affect the outcome, a situation that we hoped to rectify by proposing this model.

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