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Combining sources in stable isotope mixing models: alternative methods

Abstract Stable isotope mixing models are often used to quantify source contributions to a mixture. Examples include pollution source identification; trophic web studies; analysis of water sources for soils, plants; or water bodies, and many others. A common problem is having too many sources to allow a unique solution. We discuss two alternative procedures for addressing this problem. One option is a priori to combine sources with similar signatures so the number of sources is small enough to provide a unique solution. Aggregation should be considered only when isotopic signatures of clustered sources are not significantly different, and sources are related so the combined source group has some functional significance. For example, in a food web analysis, lumping several species within a trophic guild allows more interpretable results than lumping disparate food sources, even if they have similar isotopic signatures. One result of combining mixing model sources is increased uncertainty of the combined end-member isotopic signatures and consequently the source contribution estimates; this effect can be quantified using the IsoError model (http://www.epa.gov/wed/pages/models/isotopes/isoerror1_04.htm). As an alternative to lumping sources before a mixing analysis, the IsoSource mixing model (http://www.epa.gov/wed/pages/models/ isosource/isosource.htm) can be used to find all feasible solutions of source contributions consistent with isotopic mass balance. While ranges of feasible contributions for each individual source can often be quite broad, contributions from functionally related groups of sources can be summed a posteriori, producing a range of solutions for the aggregate source that may be considerably narrower. A paleohuman dietary analysis example illustrates this method, which involves a terrestrial meat food source, a combination of three terrestrial plant foods, and a combination of three marine foods. In this case, a posteriori aggregation of sources allowed strong conclusions about temporal shifts in marine versus terrestrial diets that would not have otherwise been discerned.

Keywords Stable isotopes · Mixing model

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Introduction

In recent years, stable isotopes have increasingly been used as environmental tracers (Lathja and Michener 1994). One common application uses isotope mixing models to quantify source contributions to a mixture. Examples include pollution inputs to air, soil, or water bodies; food sources in animal diets; plant water use from different soil depths; and many others. Linear mixing models based on isotopic mass balance have long been used for this purpose (Schwarcz 1991; Phillips 2001). A typical formulation using two isotopic signatures (δ^1 and δ^2) to partition the contributions (f) of three sources (a, b, c) to a mixture (m) is

$$\delta_m^1 = f_a\delta_a^1 + f_b\delta_b^1 + f_c\delta_c^1,$$
$$\delta_m^2 = f_a\delta_a^2 + f_b\delta_b^2 + f_c\delta_c^2,$$
$$1 = f_a + f_b + f_c.$$

Here, the δ's represent isotopic signatures that may be expressed in several different ways: fraction of atoms,

which are the heavier isotope (atom percent), or δ notation $\left(\left(R_{\text{sample}}/R_{\text{standard}}-1\right)\times 1,000\right)$. Fractional isotopic abundance is the most technically correct expression, but use of δ notation entails only very small errors at natural abundances (Fry 2003).

The number of sources that can be partitioned is limited by the number of isotopic signatures employed. For the dual isotope example above, the mixing model is a system of three equations in three unknowns (f_a, f_b, f_c), for which there is a unique solution. In general, with n isotope signatures, contributions for $n+1$ sources can be determined. If the number of sources exceeds $n+1$, then the model is mathematically underdetermined, with more unknowns than equations and no unique solution (Phillips and Gregg 2003).

This surplus of sources commonly occurs in environmental studies using stable isotope analysis, such as an abundance of pollutant sources for regional air pollution (Sturges et al. 1993), prey items in an animal's diet (Ben-David et al. 1997b), and isotopically distinct sources of soil water for plant uptake (Cramer et al. 1999). One way to simplify a mixing analysis is to delete some sources from consideration. However, this should not be done unless there is reason to believe that they do not significantly contribute to the mixture, because their deletion would bias the apparent contributions of remaining sources. Another approach has been to combine sources to simplify the analysis and estimate source contributions. The objective of this paper is (1) to examine two alternative methods of combining sources in stable isotope mixing models; and (2) to provide guidelines on when they are appropriate.

Method 1: a priori aggregation

One obvious solution to an overabundance of sources in mixing analyses is a priori to combine sources down to a number that allows mixing models to find a unique solution. The first criterion for combining sources is similarity of isotopic signatures. Graphing sources along isotopic composition axes is a convenient way to visually examine for clustering of sources. Gannes et al. (1998) stated that in reconstructing animal diets, the sources examined must have isotopically distinct signatures. Thus, one logical procedure that some authors have used is to test for significant differences among food source isotopic signatures, and combine sources if they are not statistically distinguishable (Ben-David et al. 1997a, b). Rosing et al. (1997) provided a K nearest-neighbor randomization test for this analysis, although other statistical methods might also be used.

A second criterion for combining several sources into a single end-member for a mixing model is that the sources be logically related. While this is not a mathematical necessity, interpretation of the estimated source contribution may be difficult if the combination is comprised of extremely dissimilar components (e.g., elephants and amoebae). In food web studies, for example, lumping sources in the same trophic guild and/or taxon would allow inferences about the dietary importance of a logically defined aggregate source.

One consequence of combining several sources is increased isotopic variability of the aggregate compared to its individual component sources. Greater variability in source isotopic signatures translates into greater uncertainty in estimates of source contributions from mixing models (Schwarcz 1991; Phillips and Gregg 2001). However, quantitative confidence intervals around these estimates that reflect pooling of sources can be made using the IsoError procedure described by Phillips and Gregg (2001) and available at http://www.epa.gov/wed/pages/models/isotopes/isoerror1_04.htm. As input to this procedure, standard deviations of the isotopic signatures for the aggregate source must be computed by pooling data from the sources being combined. While these standard deviations will be higher than those for each individual source, the effect on confidence interval widths is somewhat mitigated by the increased sample size resulting from pooling the data.

Method 2: a posteriori aggregation

While a priori combination of related sources that have similar isotopic signatures may sometimes reduce the number of end-members sufficiently to allow mixing models to find a unique solution, this is certainly not always the case. Phillips and Gregg (2003) outlined a procedure for determining upper and lower limits for the contributions of each source in such situations. In this "IsoSource" method, all possible combinations of each source contribution (0–100%) are examined in small increments (e.g., 1–2%). Combinations that sum to the observed mixture isotopic signatures within a small tolerance (e.g., 0.1‰) are considered to be feasible solutions, from which the frequency and range of potential source contributions can be determined. Recent applications of this method include food web studies on bears (Felicetti et al. 2003; Ben-David et al. 2004), fish (Melville and Connolly 2003; Sara et al. 2004), molluscs (Sara et al. 2003), shrimp (Burford et al. 2004), and humans (Newsome et al. 2004).

The range of potential contributions from each source in some cases is narrow and constrained, allowing for easy interpretation, while in other cases the range is broad and diffuse, which limits meaningful conclusions about source contributions (Phillips and Gregg 2003). Phillips and Gregg (2003) presented examples of analysis with IsoSource for a number of different applications from the literature, which illustrate the variety of results. In one example (Ben-David et al. 1997b), fish was found to constitute 49–63% of the diet of mink in Alaska, a tight range which clearly indicates its major importance as a food source. In contrast, a lead pollution example (Sturges et al. 1993)

showed 0–53% of the lead in Barrow, Alaska air samples was derived from eastern Europe, an equivocal result.

When the IsoSource method produces such indeterminate results, a posteriori aggregation of sources can be a useful procedure to aid interpretation. For each individual solution (isotopically feasible combination of source contributions summing to 100%), sources can be combined into logical groups to reduce the number of sources and hopefully give more constrained, interpretable results. Each line of the IsoSource output file gives the proportion for each source (which sum to one) for one feasible source combination. This file can be read into other software such as SAS, Excel, etc., where new variables can be created, which are the sums of sources to be combined (e.g., $X = A + B$ and $Y = C + D$). The distributions of new aggregated sources (e.g., X and Y) can then be examined. For further details on file structure see http://www.epa.gov/wed/pages/models/isosource/isosource.htm.

Examples

Method 1: a priori aggregation

A number of studies in the ecological literature have used a priori source aggregation to allow mixing model estimation of source contributions. To determine the relative contributions of C_3 and C_4 plants to ecosystem respiration in a tallgrass prairie, Still et al. (2004) created a combined C_3 mixing model end-member from leaf biomass $\delta^{13}C$ signatures of 12 C_3 plant species, and similarly for four C_4 species. They did not state whether they tested for significant $\delta^{13}C$ differences among species in each group. However, the $\delta^{13}C$ ranges of 1.1‰ for C_4 plants and 4.3‰ for C_3 plants were small compared to the sizable $\delta^{13}C$ difference between groups ($\sim16‰$), which is typical of C_3–C_4 comparisons (Dawson et al. 2002). Phillips and Gregg (2001) demonstrated that large isotopic differences among sources is the most important factor in controlling uncertainty in estimates of source proportions, which helps to justify combining sources with comparatively smaller differences in signatures.

In food web studies, isotopically similar food sources are often combined if they represent logical groupings. For example, Hobson (1990) measured $\delta^{13}C$ of several freshwater and several marine fish species that were typical prey for marbled murrelets. He combined these into freshwater and marine food groups since the individual species had $\delta^{13}C$ values that were not significantly different, and used a two-source mixing model to determine the importance of freshwater versus marine feeding. In contrast, a similar type study on cormorants by Bearhop et al. (1999) found a wide range of potential freshwater end-members. Thus, they were unable to simplify the situation to run a simple mixing model to distinguish freshwater versus marine inputs.

Method 2: a posteriori aggregation

Newsome et al. (2004) provided an example of a posteriori source aggregation that illustrates the procedure and its advantages. In this study, human (*Homo sapiens*) bones from an archaeological site near Monterey Bay on the central California coast were analyzed. The human remains segregated into two temporally defined groups, an early Holocene group (EHG; $\sim7,000$ bp) and a middle Holocene group (MHG; $\sim4,500$ bp). The carbon and nitrogen stable isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of human bone collagen as well as archaeological and modern samples of potential food sources were determined. Modern sample $\delta^{13}C$ values were corrected for the Suess effect (isotopic depletion of surface C reservoirs due to fossil fuel burning) to estimate Holocene $\delta^{13}C$ values (Sonnerup et al. 1999). Seven food sources were chosen to represent the range of available foods: terrestrial meat (deer and elk), marine fish, shellfish, pinnipeds, leafy plants, nuts, and seeds/grains. Details on sample preparation, analysis, isotopic signatures, tissue-diet fractionation factors, specific taxa used, etc. are given in Newsome et al. (2004). Although leafy plants, nuts, and seeds/grains had similar $\delta^{13}C$ and $\delta^{15}N$ values, they varied considerably in C/N ratios and were treated as separate sources (Fig. 1). Because of this variation, a concentration-dependent stable isotope mixing model [IsoConc; (Phillips and Koch 2002)] was used within the IsoSource framework to determine the range of contributions of each of the seven food sources consistent with both elemental concentrations and isotopic mass balance. This model gives separate estimates for source contributions of assimilated C and N, as well as assimilated biomass taking C and N concentrations into account (Phillips and Koch 2002). For simplicity, only biomass contributions are discussed here.

There was a wide range of possible diets that were consistent with observed C and N concentrations and isotopic signatures: 11,345 combinations for EHG and 35,021 combinations for MHG (Newsome et al. 2004). Ranges of biomass contribution to prehistoric human diets for the seven food sources varied from as narrow as 0–16% (terrestrial meat, EHG) to as wide as 0–68% (marine fish, EHG). Since the ranges of dietary proportions were relatively large for most individual sources, the sources were combined into three distinct categories: terrestrial plants, terrestrial meat, and marine foods. Thus, for each feasible combination of the seven food sources, the terrestrial plant aggregate was defined as the sum of the three plant types (leafy plants, nuts, and seeds/grains). Similarly, shellfish, marine fish, and pinniped contributions were summed to form the marine aggregate. The terrestrial meat food source formed its own category. Table 1 shows an example of how this was done for each feasible combination of the seven individual food sources. In this manner, ranges of feasible dietary utilization of terrestrial plants, terrestrial meat, and marine foods were determined for both human groups.

Fig. 1 Mean isotopic composition ($\pm 1\sigma$) of food sources and human (*Homo sapiens*) groups used in mixing models by Newsome et al. (2004). *EHG*, early Holocene group (~7,000 bp), *MHG*, middle holocene group (~4,500 bp). Numbers in parentheses are C/N ratios associated with each food source. Isotopic signatures have been corrected for tissue-diet discrimination and other factors as described by Newsome et al. (2004)
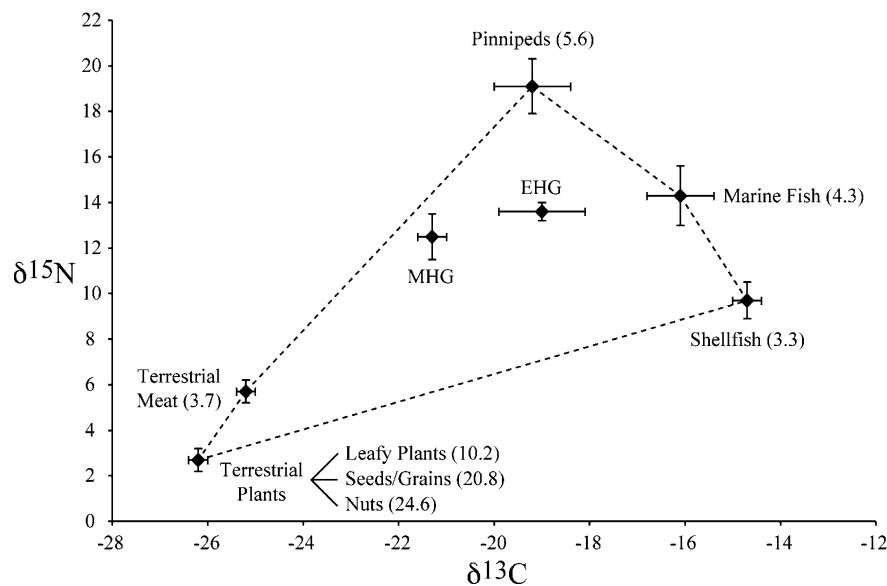
Dietary contributions of terrestrial plant and marine aggregate food groups had substantially more constrained ranges than their individual component food sources. For example, the EHG diet consisted of 4–48% pinnipeds, 0–36% shellfish, and 0–68% marine fish (first to ninety-ninth percentile values for dietary biomass), but the combined contribution of this marine food group had a narrow range of 70–84% (Fig. 2). Dietary contributions of these aggregate food groups showed clear changes over time (Fig. 3). While the EHG diet was composed of about three-fourths marine foods (70–84%), this decreased to about half (48–58%) by the middle Holocene, with concomitant increases in terrestrial food sources. Without a posteriori combination of food sources, the results would have been much less clear, because many individual terrestrial plant and marine food sources had very diffuse distributions of dietary importance, which broadly overlapped between early and middle Holocene groups.

Similarity of isotopic composition and elemental concentrations among members of the combined food groups was responsible for narrower ranges of dietary importance for food groups compared to individual food sources. For example, all the terrestrial plants had indistinguishable low $\delta^{13}C$ and $\delta^{15}N$ values, and while their C/N ratios were variable, they were all high compared to other food sources (Fig. 1). Similarly, the marine food sources were all enriched in $\delta^{13}C$ and $\delta^{15}N$ relative to other food sources. Thus, there was a terrestrial to marine continuum from lower to higher $\delta^{13}C$ and $\delta^{15}N$ values. Consequently, certain proportions of marine and terrestrial foods must be included in the diet to account for the human isotopic signatures, which were intermediate between terrestrial and marine ends of the spectrum. A variety of combinations of pinnipeds, shellfish, and fish could constitute the marine dietary contribution. Each of these could range anywhere from zero to the majority of the marine contribution, but their

Table 1 Calculation of dietary contributions of three aggregate food groups (terrestrial plants, marine, and terrestrial meat) from seven individual food sources

Biomass Percentage of Diet						
(A) Leafy Plants	(B) Nuts	(C) Seeds & Grains	(D) Pinnipeds	(E) Shellfish	(F) Marine Fish	(G) Terrestrial Meat
6	18	2	6	2	66	0
(A + B + C) Terrestrial Plants 26			(D + E + F) Marine 74			(G) Terrestrial Meat 0

This example is for one of 11,345 feasible dietary biomass solutions for the early Holocene group (~7,000 bp) of *Homo sapiens* from Newsome et al.(2004). The procedure is repeated for each solution to generate distributions of feasible terrestrial plant, marine, and terrestrial meat biomass contributions to the diet. Similar calculations can be made for C and N contributions

Fig. 2 Ranges of feasible dietary biomass contributions of individual marine food sources and aggregated marine food sources for the early Holocene group (∼7,000 bp) *Homo sapiens* diet (Newsome et al. 2004). Distributions shown reflect 11,345 dietary solutions found by the IsoConc/IsoSource mixing model. While wide ranges of utilization are possible for each individual food source, the combined marine food group is tightly constrained and represents 70–84% of the diet
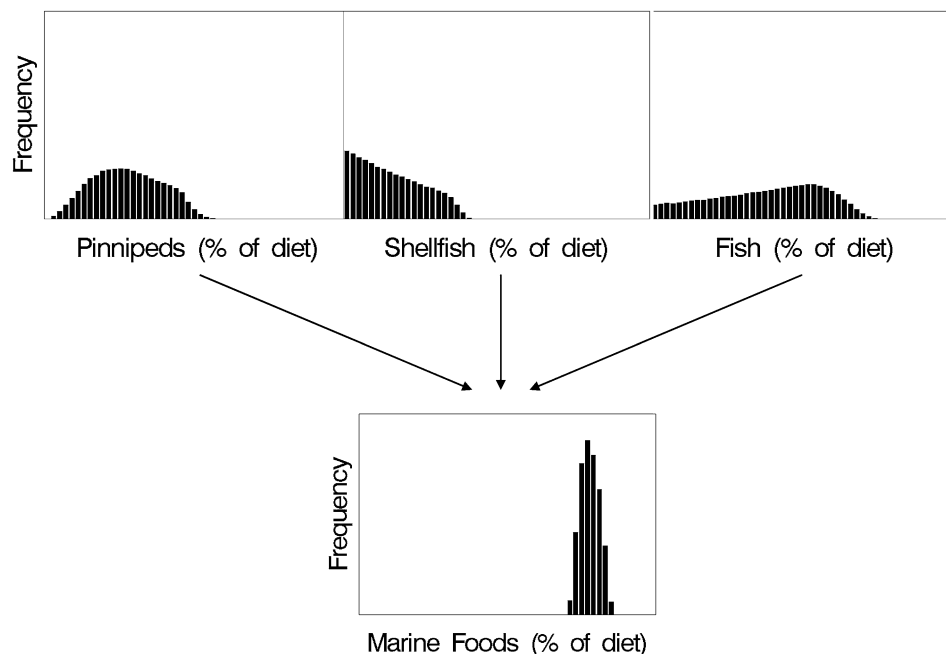
sum was narrowly constrained to balance terrestrial contributions in a way that accounted for the observed human isotopic composition. If one of these three sources has a large contribution, then the others must be small or the human isotopic composition would have been shifted further to the marine end than the observed value actually was. Hence, the contributions of the three marine sources are compensatory and the range of the combined marine food group is well constrained. A similar argument can be made for the three plant types that made up the combined terrestrial plant group.

Discussion

We have outlined methods for a priori and a posteriori combining of sources to simplify mixing analyses in the common situation where there is an overabundance of sources preventing a unique mixing solution. Whether several sources could be combined, and which method to use if so, depends on the particular situation. If all sources are widely distributed along isotopic axes, with little evidence of forming clusters with similar isotopic signatures, then combining sources by any method is probably unwarranted. In this case, the IsoSource method (Phillips and Gregg 2003) can be used to bound the range of source contributions consistent with isotopic mass balance. But if some sources form clusters with similar isotopic signatures, then some lumping procedure might be considered. Figure 4 presents a decision tree to provide guidelines for how to proceed.

If clustered sources do not significantly differ in isotopic composition and they are somehow related to form a logical grouping, then they may be combined a priori before the mixing analysis (Fig. 4—leftmost path). Requiring no statistically significant difference among

source signatures may be a stringent condition. However, combining sources and using their mean signature in a mixing model implies that the individual sources all contribute equally (or weighted by sample size, depending on how mean signatures are calculated) to the combined source contribution, which may not be the case. But if the signatures are statistically indistinguishable, the mean signature is common to all individual sources and this point becomes moot. Because of the relationship of statistical significance with sample size, a judgment of the practical significance of differences of the observed magnitude may also be prudent. With large sample sizes, mean differences of a small fraction of 1‰ may be statistically significant, but of no practical import. Conversely, source means differing by several ‰ may not be statistically significant, even though this may represent a substantial portion of the variation among all the sources. If one decides to combine sources a priori, then estimation uncertainty of contributions of these combined sources can be evaluated using the IsoError procedure (Phillips and Gregg 2001) after recalculating standard deviations of source signatures to reflect source aggregation.

Regardless of whether there are groups of logically related sources, or whether they have isotopic signatures not significantly different from each other, the IsoSource method (Phillips and Gregg 2003) can always be used to provide bounds on each source's contribution to the mixture (Fig. 4—all paths). If several sources are isotopically clustered and form a logical group for interpretation (e.g., similar taxonomic or trophic classes in food web studies), then they may be lumped a posteriori (after the IsoSource mixing analysis). This aggregation of sources is optional, but often results in much more constrained ranges for aggregate contributions than for each individual source. It is not strictly necessary that

Fig. 3 Dietary biomass proportions of three aggregate sources for early Holocene (~7,000 bp) and middle Holocene (~4,500 bp) groups of *Homo sapiens* (Newsome et al. 2004). The aggregate solutions are sums of biomass contributions for food sources in each category (e.g., the marine aggregate includes pinnipeds, shellfish, and marine fish). The aggregate contributions are calculated for all model iterations (in 2% increments), and are expressed as percent frequency of all possible solutions. The mean proportion of each aggregate distribution is labeled
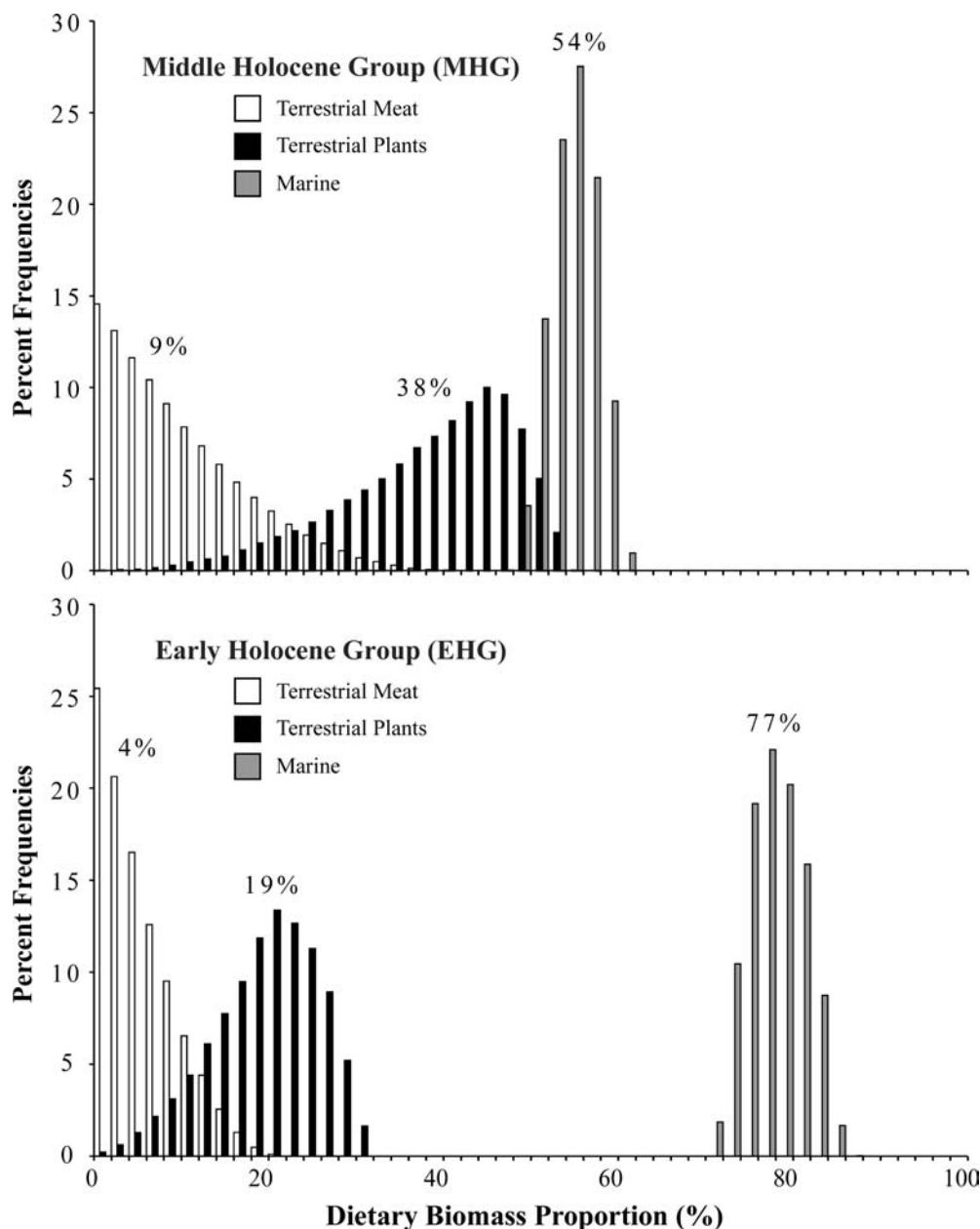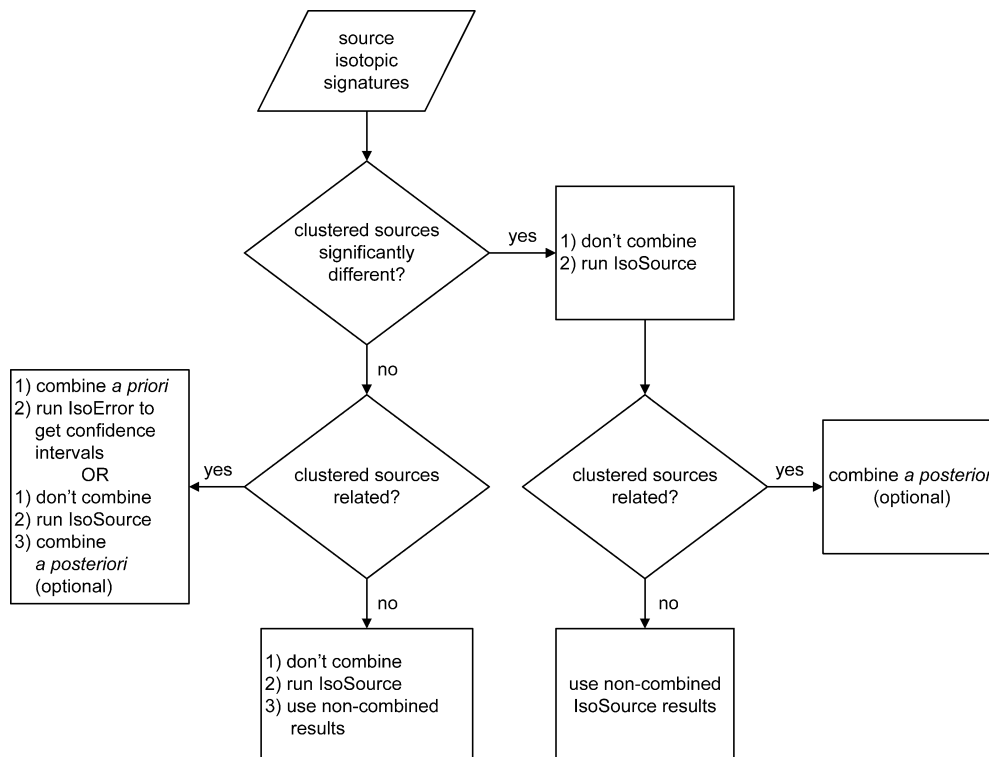

sources have isotopic signatures that are not significantly different in order to combine them, because IsoSource keeps the sources separate during the analysis, and provides all combinations of sources that preserve isotopic mass balance, regardless of the degree of similarity or difference among sources. However, the more similar the signatures of sources that are combined, the narrower will be the range of the combined source contribution to the mixture. Thus, the isotopic signatures of the sources to be combined should be somewhat clustered together for this method to be fruitful.

While unrelated sources that are isotopically similar could be combined, this creates difficulties in interpretation of the results. As a hypothetical example, consider a dietary study on fish, in which amphipods and

algae are each found (with IsoSource) to constitute 0–40% of the diet. They have similar isotopic signatures and are combined a posteriori into a single group, which has a feasible range of 35–45% of the diet. While such a narrow range of results is desirable, one would still want to know how this breaks down between amphipods and algae, since they differ so greatly in their taxonomic groups (animal vs. plant) and trophic levels (consumer vs. producer). Little interpretable information is gained by combining such disparate sources. In this case, it may be best to leave the sources separate and accept the wide potential range of dietary importance for each. This is probably an unrealistic example, since fractionation tends to create isotopic differences between trophic levels. However, it is pre-

Fig. 4 Decision tree for
combining sources in stable
isotope mixing analyses.
Assumptions here are: (1) an
excess number of sources
(>1 + number of isotopic
signatures, so there is no unique
solution); and (2) some
clustering of source signatures.
The diagram shows decision
points for determining whether
a priori or a posteriori lumping
of sources may be appropriate

sented simply as an example of sources that could be combined from an isotopic perspective, where the combined result does not provide any ecological insight.

There are trade-offs inherent in the decision to aggregate sources in a mixing analysis, namely loss of the ability to make inferences about individual sources that are combined. The combined contribution to the mixture can be estimated, but at the cost of detail about how this partitions among the members. For the a priori approach, however, the alternative is not being able to perform a mixing analysis that gives a unique solution. For the a posteriori approach, information is available on the range of possible contributions for each source before they are combined, but the chief reason for aggregating sources is that the ranges may be so broad as to be uninformative. In the paleohuman example, increased precision in estimating dietary importance of terrestrial plant and marine food groups warranted combining sources because little specificity was possible about contributions of individual food sources otherwise.

In summary, two alternative methods of combining sources, before or after mixing analysis, may be used to deal with an overabundance of sources that prevents a unique mixing solution. Each method has certain strengths and conditions under which it is appropriate (Fig. 4). Examples of a priori source aggregation are found in the ecological literature, while a posteriori aggregation represents a new approach building on availability of the IsoSource procedure (Phillips and Gregg 2003) for determining the range of possible source contributions when unique solutions are not possible.

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