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Source partitioning using stable isotopes: coping with too many sources

Abstract Stable isotopes are increasingly being used as tracers in environmental studies. One application is to use isotopic ratios to quantitatively determine the proportional contribution of several sources to a mixture, such as the proportion of various pollution sources in a waste stream. In general, the proportional contributions of $n+1$ different sources can be uniquely determined by the use of n different isotope system tracers (e.g., $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$) with linear mixing models based on mass balance equations. Often, however, the number of potential sources exceeds $n+1$, which prevents finding a unique solution of source proportions. What can be done in these situations? While no definitive solution exists, we propose a method that is informative in determining bounds for the contributions of each source. In this method, all possible combinations of each source contribution (0–100%) are examined in small increments (e.g., 1%). 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. To avoid misrepresenting the results, users of this procedure should report the distribution of feasible solutions rather than focusing on a single value such as the mean. We applied this method to a variety of environmental studies in which stable isotope tracers were used to quantify the relative magnitude of multiple sources, including (1) plant water use, (2) geochemistry, (3) air pollution, and (4) dietary analysis. This method gives the range of isotopically determined source contributions; additional non-isotopic constraints specific to each study may be used to further restrict this range. The breadth of the isotopically determined ranges depends on the geometry of the mixing space and the similarity of source and mixture isotopic signatures. A sensitivity analysis indicated that the estimated ranges vary only modestly with different choices of source increment and mass balance tolerance parameter values. A computer program (IsoSource) to perform these calculations for user-specified data is available at http://www.epa.gov/wed/pages/models.htm.

Keywords Stable isotope · Mixing model · Source partitioning

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Introduction

Natural abundance stable isotopes have become an important tool for determining water, carbon, nutrient and trace element fluxes and cycling in a variety of systems. This application uses mass balance equations and the distinct isotopic signatures of various sources to determine their relative contributions to the mixed signature in an end product. Deuterium isotope signatures (δ^2H) of ground versus rain waters, for example, can be used to determine the relative contribution of ephemeral versus permanent water sources for the sustenance of woody vegetation (Dawson 1993). In other applications, distinctive carbon and nitrogen isotope signatures ($\delta^{13}C$ and $\delta^{15}N$[1]) of various food sources can be used to determine their relative contribution to an animal's diet (Michener and Schell 1994), and heavy metal (e.g., lead) isotopes can identify natural versus anthropogenic sources of soil contaminants (Walraven et al. 1997).

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[1] $\delta^{13}C$ in ‰ is the deviation of the C isotope ratio of a sample from that of 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 $R=^{15}N/^{14}N$, and δ^2H is the deviation of the H isotope ratio of a sample from that of a standard (Standard Mean Ocean Water) where $R=^2H/^1H$.

Despite the general applicability of the use of mixing models across a range of systems and trophic levels, fully constrained mixing model applications are limited to solving for the contributions of $n+1$ sources when n isotope groups (usually separate chemical elements) are used (Phillips 2001). Distinct isotopic signatures generally persist for only one or two elements per system, so relative source contribution determinations are often limited to estimates for two or three sources. Although resolution of the contribution of relatively few sources can be useful for specific applications, the inherent complexity of natural systems often requires the inclusion of a larger number of sources. For example, rain waters could be obtained from a number of different depths within the evaporative gradient of the soil profile, animal diets often include numerous potential prey items, and the signatures of trace element contaminants can vary between a variety of natural and anthropogenic sources.

One common method of dealing with the limitations of stable isotope mixing models for systems with more than two or three sources is to assume that source signatures that fall closest to that of the mixture provide the greatest contribution. However, proportionate contributions of distal sources could also provide the isotopic mass balance necessary to explain the mixed signature, with little to no contribution from proximate sources (Fry and Sherr 1984). Vegetation with roots throughout the soil profile, for example, is unlikely to obtain water from a single depth with the most similar isotopic composition, but may also utilize other soil water components with both higher and lower signatures (Cramer et al. 1999).

In an effort to expand the use of mixing models to more complex systems, we developed a procedure to calculate the range of all possible source contributions for systems where the number of potential sources is greater than $n+1$. The procedures described in this paper are an expansion of those described in Phillips (2001), which demonstrated that a multiplicity of source contribution solutions were possible in these cases. Several different iterative procedures have been used in a few other isolated studies to determine contribution limits of three sources with a single isotope system (Dauby 1989; Zencich et al. 2002) or more than three sources with a dual isotope system (Minagawa 1992). To advance beyond these previous efforts, we sought to formalize a general procedure which could be used for any number of isotope systems and number of sources. The objectives of this paper were to: (1) describe a general method for determining the distribution of all possible source contributions where the number of sources precludes a unique solution, and provide publicly available software for this purpose; (2) demonstrate use of the method in a variety of ecological applications; (3) identify common patterns of source contribution distributions in different situations; and (4) test the sensitivity of results to model parameters.

Materials and methods

Procedure

When n isotope systems are used to determine the proportional contributions of $n+1$ sources to a mixture, standard linear mixing models can be used to mathematically solve for the unique combination of source proportions that conserves mass balance for all n isotopes (Phillips 2001). For example, with one isotope system and two sources, the following system of mass balance equations can be solved to determine the proportions (f_A, f_B) of source isotopic signatures (δ_A, δ_B) which coincide with the observed signature for the mixture (δ_M):

$$\delta_M = f_A\delta_A + f_B\delta_B$$
$$1 = f_A + f_B \tag{1}$$

This system of equations can be extended to include more than two sources. For example, with one isotope system and three sources, the following equations pertain:

$$\delta_M = f_A\delta_A + f_B\delta_B + f_C\delta_C$$
$$1 = f_A + f_B + f_C \tag{2}$$

This is a mathematically underdetermined system of two equations in three unknowns for which there is no unique solution. However, with n isotope systems and $>n+1$ sources, we can still use the requirement for mass balance conservation to find multiple combinations of source proportions which are feasible solutions.

The first step is to iteratively create each possible combination of source proportions (that sum to 100%) by some small increment (hereafter referred to as "source increment"), such as 1% or 2%. Second, the predicted isotopic signature(s) for the mixture are computed as each combination is created (as in Eq. 2 for the one isotope, three source case). Third, these predicted mixture signatures are compared with the observed mixture signatures. If they are equal, or within some small tolerance (hereafter referred to as "mass balance tolerance"), such as ±0.1‰, this combination of source proportions represents a feasible solution and is stored in a data set. Lastly, the distribution of all such feasible solutions in the data set is described. For example, the minimum and maximum proportion for each source defines the range of its feasible contribution to the mixture. However, because the range (minimum to maximum) is sensitive to small numbers of observations on the tails of the distribution, the trimmed 1–99th percentile range may also be a useful statistic which is more robust to these outliers.

A Visual Basic program called IsoSource was created to perform these procedures. The user supplies the isotopic signatures of the sources and the mixture, along with the desired source increment and the mass balance tolerance. Output files include all the feasible source combinations, with histograms and descriptive statistics on the distributions for each source. The IsoSource program is available for public use at http://www.epa.gov/wed/pages/models.htm.

Examples

To illustrate the utility of this procedure, we applied it to four data sets from the published literature which represent widely varying examples of stable isotope analyses in ecological studies. All of these examples included an overabundance of sources (n isotope systems with $>n+1$ sources) for which proportional contributions could not be uniquely determined without additional constraints. In some cases the authors were able to apply other non-isotopic constraints and reduce the range of possible source proportions; these additional constraints are not shown here. Data from published studies are used solely as examples to illustrate the method in a variety of environmental applications, and we do not intend to imply any reinterpretation of the authors' results.

1. Plant water use (one isotope system, three sources)—Zencich et al. (2002) studied water use by *Banksia* trees in southwest Australia using hydrogen isotope ratios (δ^2H) in twig xylem water, compared to δ^2H of three soil water sources in both summer and winter: surface soil, subsurface soil, and groundwater.
2. Geochemistry (one isotope system, three sources)—The relative contributions of geologic, atmospheric, and marine inputs to terrestrial nutrient pools were characterized along a soil chronosequence from 300 to 4.1 million years of age in the Hawaiian islands by Kennedy et al. (1998). They used Sr as a surrogate for other base cations of importance in terrestrial biogeochemistry such as Ca, K, P, and Mg. Kennedy et al. (1998) measured Sr isotopic ratios (^{87}Sr/^{86}Sr) for soil and for three soil nutrient input sources: basalt, atmospheric dust, and sea-salt aerosols.
3. Air pollution (two isotope systems, five sources)—Sturges et al. (1993) examined ^{206}Pb/^{207}Pb and ^{208}Pb/^{207}Pb lead isotopic ratios in air samples at Barrow, Alaska compared to air from 5 potential geographic sources of atmospheric lead: US, USSR, eastern Europe, western Europe, and northwestern Europe (Sweden and Norway) (see also Hopper et al. 1991).
4. Dietary analysis (2 isotope systems, 7 sources)—Ben-David et al. (1997) studied the diets of mink in southeast Alaska using C and N isotope ratios (δ^{13}C and δ^{15}N). For spring coastal mink, there were seven prey items as food sources: fish, mussels, crabs, shrimp, rodents, amphipods, and ducks. The isotopic signatures of each food source were corrected to account for fractionation during digestion and assimilation.

For all four examples we examined all possible source combinations using source increments of 1%. Combinations were considered to be feasible solutions if the predicted and observed mixture signatures matched within a mass balance tolerance of ±0.1‰ (δ notation) for the dietary and plant water use examples, or within ±0.001 and ±0.0001 (isotopic ratios) for the air pollution and geochemistry examples, respectively.

Sensitivity analysis

The choices of values for the source increments and mass balance tolerances could affect the distribution of feasible source proportions. To determine the magnitude of these effects, we performed a sensitivity analysis with the data from the mink dietary analysis example. The procedure for determining the range of source proportions was used with each combination of three source increments (0.5, 1, and 2%) and three mass balance tolerances (±0.05, 0.1, and 0.2‰).

The particular source increment values were chosen as follows. We view source increments of 1% as a convenient and appropriate level of precision for examining possible partitioning among sources, so we also chose precision levels two times higher and lower than this (0.5%, 2%). In addition, with larger numbers of sources, the number of possible combinations of sources to examine expands almost exponentially, and somewhat larger increments like 2% may be needed to keep the computing requirements down.

The mass balance tolerance values chosen (±0.05‰, 0.1‰, 0.2‰) likewise reflect a two-fold increase and decrease from a convenient middle value. However, each of these three values also reflects a particular benchmark for this example. If a 1% source increment is used, the largest incremental difference in the mink signature would be 1% of the maximum difference in signatures between sources, namely the ~10‰ δ^{13}C difference between rodent and fish food sources (Ben-David et al. 1997). Thus, a 1% decrease in fish utilization and a corresponding 1% increase in rodent utilization would affect the mink δ^{13}C by 1% of 10‰, or 0.1‰. In order to guarantee that no legitimate feasible source combinations are missed as the source proportions are iteratively changed, the mass balance tolerance must be no smaller than half this amount (0.5× increment × maximum difference between sources), or ±0.05‰ in this case. On the other hand, typical measurement errors

for δ^{13}C and δ^{15}N determinations are 0.1–0.2‰ (Ehleringer and Rundel 1989), and Ben-David et al. (1997) found population variability of 0.1–0.4‰ for the various mink food sources. Thus, mass balance tolerances of ±0.1‰ or ±0.2‰ may be more appropriate since they incorporate uncertainty of magnitudes similar to measurement error and source variability (Phillips and Gregg 2001).

Results and discussion

Examples

Plant water use (one isotope system, three sources)

Results for the plant water use example varied by season. In the summer, the ranges of feasible source contributions were narrow and informative (Fig. 1a). Groundwater use greatly predominated due to the dryness of surface and subsurface soils (Zencich et al. 2002). In the winter, when there was more precipitation, the surface soils accounted for 52–70% of the twig xylem water. However, there was a wide range of possible combinations of ground water and subsurface soil contributions which could explain the observed δ^2H of the twig xylem water, so the system was less well constrained (Fig. 1b). These results are consistent with those of Zencich et al. (2002), who performed a similar calculation to find the range of possible source contributions.

Geochemistry (one isotope system, three sources)

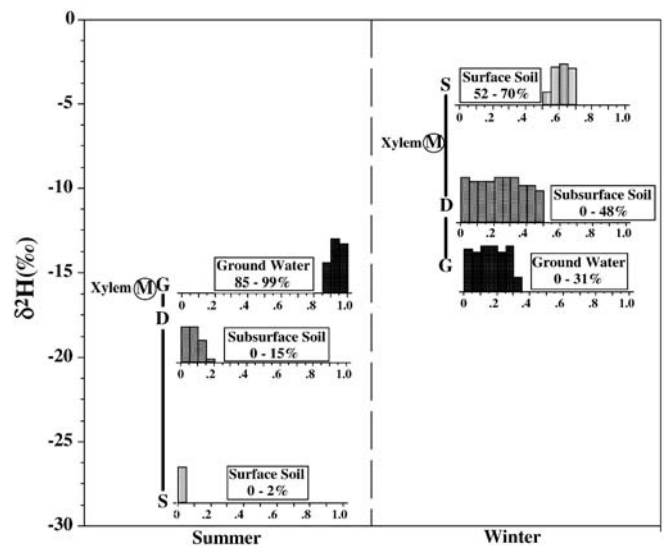The results for the geochemical example varied along the chronosequence in the Hawaiian Islands (Fig. 2). The

Fig. 1 Mixing lines for δ^2H signatures of water from three soil depths [surface soil (*S*), subsurface soil (*D*), and ground water (*G*)] for *Banksia* trees in Australia in **A** summer and **B** winter. Histograms show the distribution of feasible contributions from each source to the plant xylem water (*M*). Values shown in the boxes are 1–99 percentile ranges for these distributions

Fig. 2 Mixing lines for $^{87}Sr/$ ^{86}Sr isotopic ratios from three nutrient sources [geologic weathering of basalt (*B*), atmospheric dust (*D*), and sea-salt aerosols (*S*)] along a soil chronosequence in the Hawaiian islands. Histograms show the distribution of feasible contributions from each source to the soil (*M*) at the youngest (*Thurston*) and oldest (*Kauai*) sites. Values shown in the boxes are 1–99 percentile ranges for these distributions
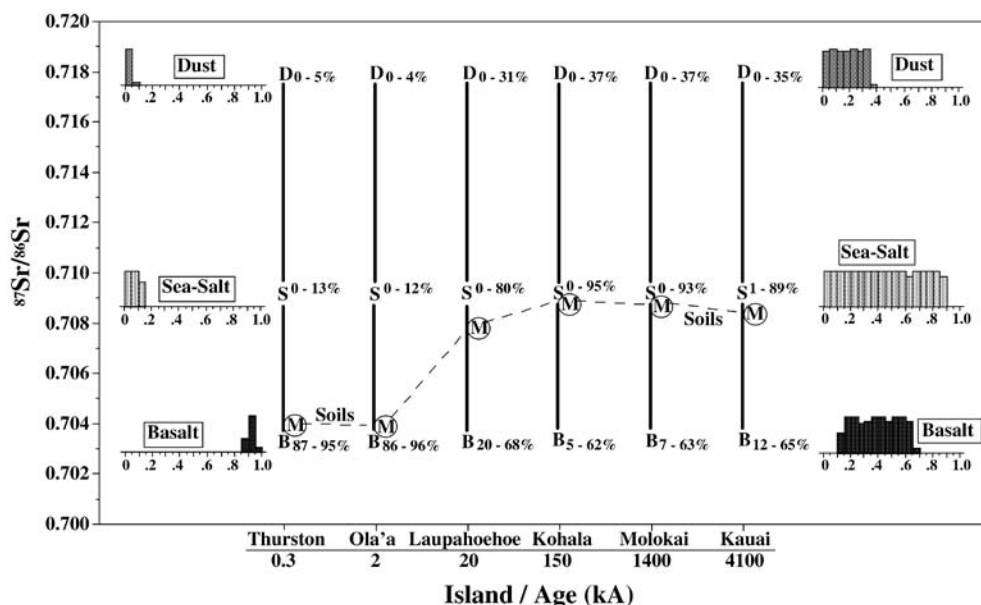
youngest sites (Thurston and Ola'a at 300 and 2,000 years of age, respectively) had soil isotopic signatures very close to the basalt end-member and the feasible range of source contributions was heavily weighted toward basaltic weathering. For sites at least 20,000 years of age, there was a progressive increase in the soil Sr signature toward atmospheric end-members (sea-salt aerosols and dust). However, the source distributions were broad and diffuse at these sites, indicating a variety of possible explanations for the observed Sr signatures (e.g., mostly sea-salt aerosols with little dust or basaltic weathering, a mixture of dust and basaltic weathering with little sea-salt influence, etc.). [Note: In their analysis, Kennedy et al. (1998) fixed the level of atmospheric dust input at 3% of the atmospheric contribution, based on concentrations of quartz in the soil, which is not derived from basalt. This additional restriction enabled calculation of proportional contributions of rock weathering vs atmospheric input (sea-salt aerosols plus a 3% contribution from atmospheric dust).]

Air pollution (two isotope systems, five sources)

In the lead deposition example, it was possible to determine a significant contribution from the USSR source (26–45%), but the feasible ranges for the other sources were diffuse and included zero contributions (Fig. 3). For most of the sources, only broad ranges of possible source contributions could be determined for two reasons. First, there was a high correlation of the two isotopic signatures, making the mixing polygon very narrow. Second, the mixture (Barrow air) fell near the center, and thus may be composed primarily of approximately equal mixtures of USSR and eastern Europe sources, or US and northwestern/western Europe sources plus some USSR contribution, or a combination thereof. [Note: In their analysis, Sturges
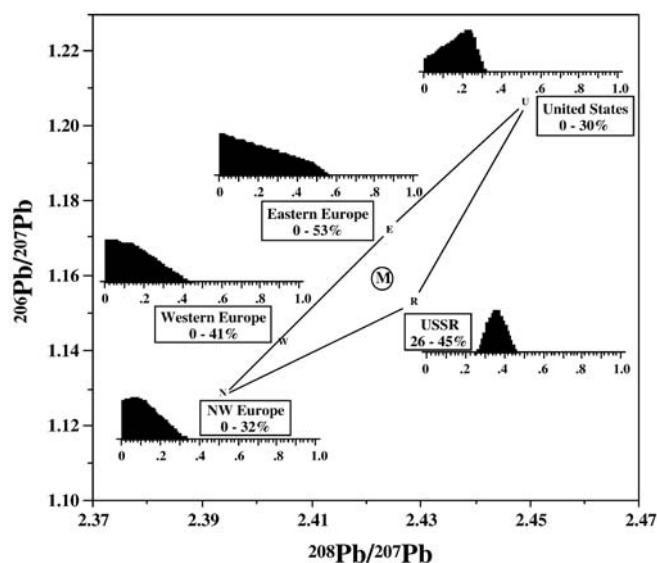

Fig. 3 Mixing polygon for $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ signatures of five regional sources of atmospheric lead deposition for Barrow, Alaska. Histograms show the distribution of feasible contributions from each source to the air at Barrow (*M*). Values shown in the boxes are 1–99 percentile ranges for these distributions

et al. (1993) used additional geographical and air circulation constraints to rule out US and northwestern Europe contributions, and calculated contributions for the western Europe source and a combined eastern Europe/USSR source based on $^{208}Pb/^{207}Pb$ alone.]

Dietary analysis (two isotope systems, seven sources)

In contrast to the air pollution example, the distributions of feasible diet proportions were well constrained and
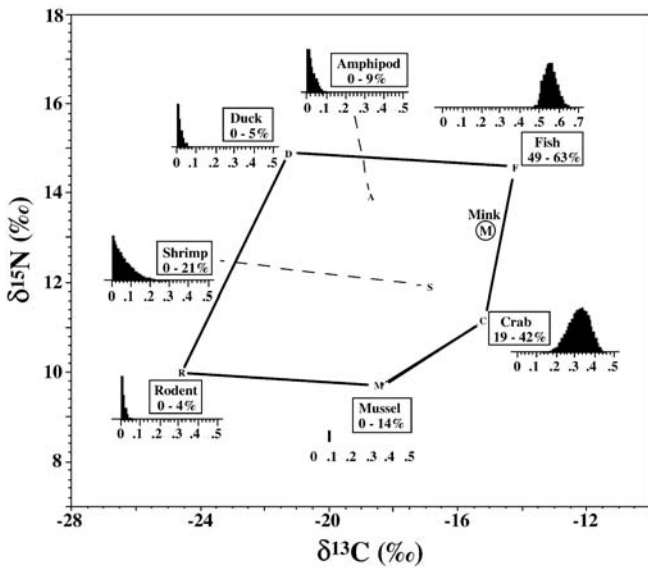
Fig. 4 Mixing polygon for δ^{13}C and δ^{15}N signatures of seven food sources for spring coastal mink in SE Alaska (after correcting for trophic fractionation). Histograms show the distribution of feasible contributions from each source to the mink diet (*M*). Values shown in the boxes are 1–99 percentile ranges for these distributions

appeared to constitute the majority of the diet (1–99th percentile: 49–63%), with crab an important secondary food source (19–42%), and the other potential food sources making up the remainder of the diet (Fig. 4). Rodents and ducks were definitely minor components (0–4% and 0–5%, respectively), while amphipods, mussels, and shrimp (0–9%, 0–14%, and 0–21%, respectively) had somewhat less precise small to medium dietary contributions. These results are consistent with the conclusions of Ben-David et al. (1997) about the diet of mink in this study.

Since each of these feasible source combinations is constrained to sum to 100%, there are tradeoffs among the sources within their feasible ranges. For example, if one source had the maximum feasible contribution consistent with the isotopic data, then some of the other food sources must have contributions closer to the lower end of their range. Figure 5 is a scatter plot matrix showing the joint distribution of food sources for all the feasible dietary compositions, which allows examination of these tradeoffs. Each plot shows a two-dimensional cross-section through the seven-dimensional cloud of points that represent isotopically feasible diets. If a particular proportion of one food is selected, the plots show what proportions or range of proportions the other food sources may have. For example, for the maximum feasible proportion of fish (68%) in the diet (circled data point in the top row), the mussel proportion would also be at the maximum of its feasible range (19%), and all other food

informative for spring coastal Alaska mink, despite the large number of sources. The mixing polygon was broad with the mixture falling near one end (Fig. 4). Fish

Fig. 5 Scatter plot matrix showing isotopically feasible contributions of seven food sources in the diet of spring coastal mink in SE Alaska. Each panel shows a scatter plot of the feasible contributions of two food sources on a 0–1 scale. For the panels on the diagonal, the lines show the range of feasible contributions for sources in that row and column. If a particular contribution of one food source is selected, the plots show how the contributions of the other food sources are constrained. For example, in the top row the point with the maximum feasible contribution of fish in the diet is circled. The coordinates for this point show the fish contribution (0.68) and the corresponding contributions of the other food sources: mussels contribute at the maximum of their feasible range (0.19), and all other food sources contribute at their minimum (0 for shrimp, rodent, amphipod, and duck; 0.13 for crab)
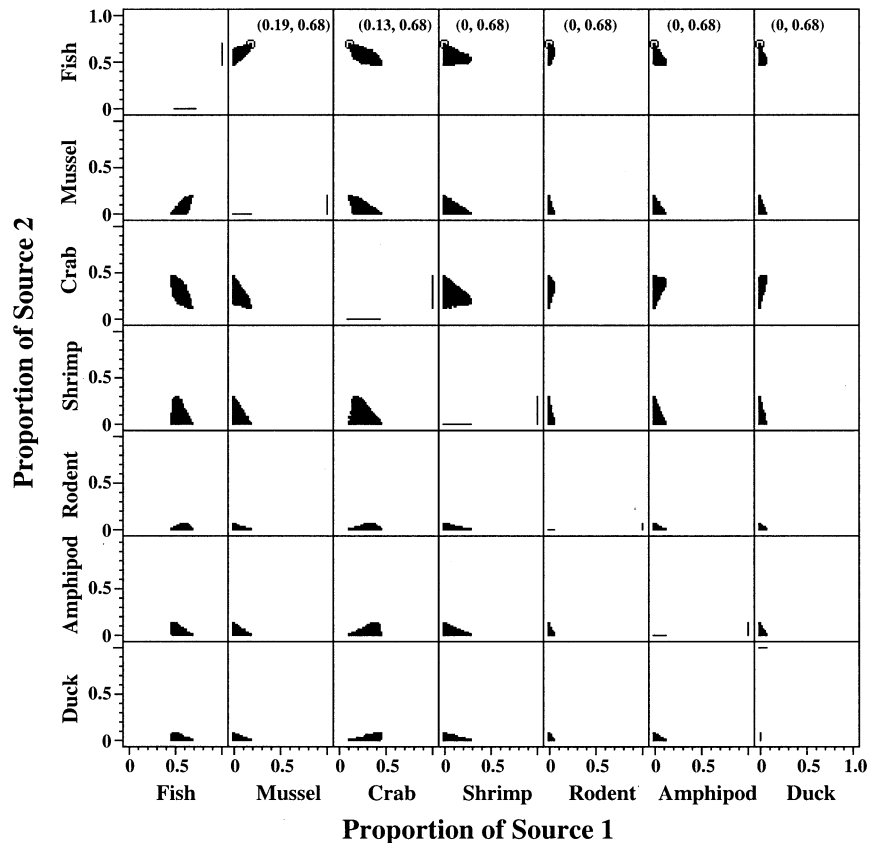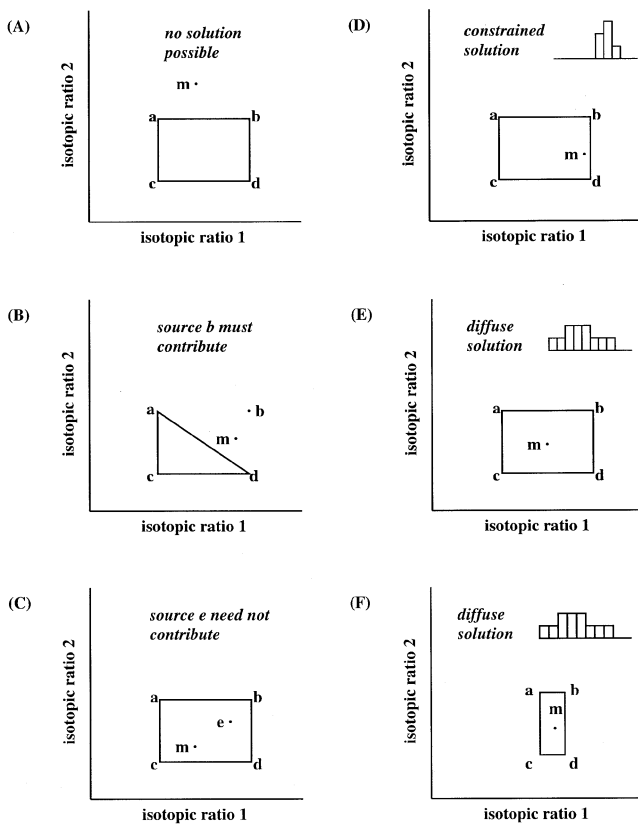
Fig. 6A–F Rules for contributions of sources (*a*, *b*, *c*, *d*, *e*) to mixture (*m*) using the standard linear mixing model. **A** No solution is possible if the mixture is outside the convex polygon bounded by all sources. **B** A source must contribute (cannot be 0) if the mixture is outside the convex polygon bounded by all other sources. **C** A source need not contribute (may be 0) if it is inside the convex polygon bounded by all other sources. **D** Mixtures near the periphery of the mixing polygon have well constrained solutions. **E** Mixtures near the center have more diffuse solutions. **F** Narrow mixing polygons with small differences between sources have diffuse solutions

that side of the mixing diagram predominate and the ranges of possible contributions from each source are well constrained. For single isotope systems, solutions are also well constrained when the mixture lies near one end of the mixing line (e.g., Fig. 1a). When the mixture lies near the center of the mixing polygon (e.g., Fig. 3) or mixing line (e.g., Fig. 1b), then the mixing proportions of the sources are largely indeterminate. Narrow mixing polygons where there is little isotopic differentiation among sources (e.g., Fig. 3) also create greater uncertainty in source proportion estimates (Phillips and Gregg 2001). These and other patterns inferred from graphical examination of mixing diagrams are illustrated in Fig. 6. The illustrations are for two dimensions (isotope systems) using the standard linear mixing model (Phillips 2001), but the same patterns hold true for other numbers of dimensions.

1. No set of source proportions is possible if the mixture is outside a convex polygon bounded by all sources (Fig. 6a).
2. A source must contribute (cannot be 0) if the mixture is outside the convex polygon bounded by all other sources (Fig. 6b).
3. A source need not contribute (may be 0) if it is inside the convex polygon bounded by all other sources (Fig. 6c).
4. Mixtures near the periphery of the mixing polygon have well constrained ranges of solutions (Fig. 6d), while mixtures near the center have more diffuse solutions (Fig. 6e).
5. Narrow mixing polygons, due to small differences among sources, have diffuse solutions (Fig. 6f).

Phillips and Koch (2002) outlined a more complex mixing model which factors in not only the isotopic signatures of the sources, but the elemental concentrations as well. While the IsoSource program provided here assumes the use of the standard linear mixing model, conceptually the iterative procedure described could be applied to other mixing models such as this concentration-dependent model. Due to the way in which concentration effects warp the usual mixing diagrams (Phillips and Koch 2002), the above rules would not necessarily apply in this case.

sources would be at their minimum (0% for shrimp, rodent, amphipod, and duck; 13% for crab).

Patterns of source contribution distributions

The procedure outlined is able to provide information about the range and distribution of possible source contributions where no unique solution exists because of an overabundance of sources. The usefulness of this information depends on how narrow the contribution ranges are for each source. In some cases, such as the mink dietary analysis example, the results were well constrained and informative. In other cases, such as the lead air pollution example, the ranges were so broad as to give little information about partitioning among many of the sources. The difference between these two outcomes depends on the geometry of the mixing diagrams. When the mixture lies near the periphery of the convex polygon connecting the sources (e.g., Fig. 4), then the sources on

Sensitivity analysis

Alterations in the mass balance tolerance did not affect the medians of the distributions of feasible mink food source contributions, but the distributions were somewhat wider for larger mass balance tolerance values (Fig. 7a). For 1% source increments, the 1–99th percentile width increased from 12% to 20% for fish and from 8% to 10% for amphipods as the mass balance tolerance was increased from 0.05 l to 0.2 l (Fig. 7a). Similarly, for a 0.1‰ mass balance tolerance value, alterations in source increment values from 0.5% to 2% had small effects on the width of the distributions of feasible source contributions (1–99th percentile width increased from 13.5% to
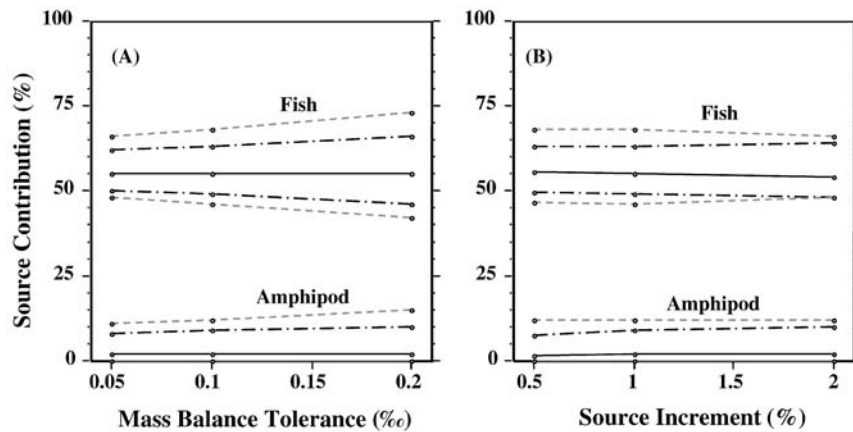
Fig. 7A, B Sensitivity of source contribution estimate distributions to parameter values of increment and tolerance. Two of the seven food sources for the mink dietary example are shown. *Dashed lines* indicate the minimum and maximum, *dotted/dashed lines* indicate the 1st and 99th percentiles, and *solid lines* indicate the median source contribution estimates for: **A** three levels of mass balance tolerance (0.05, 0.1, and 0.2‰), with source increments fixed at 1%; and **B** three levels of source increments (0.5, 1, and 2%), with mass balance tolerance fixed at 0.1‰

16% for fish and from 7.5% to 10% for amphipods) and did not affect the medians (Fig. 7b).

While the choice of the source increment only slightly affects the distributions of source contributions, practical computational limits can be encountered with larger numbers of sources, depending on the source increment chosen. The number of possible source combinations to examine is given by the formula:

$$\mathrm{No.\,combinations} = \binom{(100/i) + (s-1)}{s-1}$$

$$= \frac{[(100/i) + (s-1)]!}{(100/i)!\,(s-1)!} \qquad (3)$$

where i=increment (%) and s=number of sources. This increases almost exponentially as the number of sources increases, and requires impractical amounts of computation for larger numbers of sources at smaller source increments. In these cases, somewhat larger source increments of 2% or 2.5% can be used and still achieve satisfactory precision for determining the ranges of feasible source contributions. Larger source increments of 10% or even 5% would give a less precise picture of the distribution of possible source contributions, and are not recommended since they would increase the chance that other feasible solutions might fall between the cracks of such large incremental changes.

Measurement errors and sample variability of both sources and mixtures combine to create uncertainty in source contribution estimates even when the number of sources is not excessive and unique algebraic solutions are possible. Phillips and Gregg (2001) described procedures for determining statistical confidence limits for such estimates. While these statistical procedures cannot be directly applied to situations with excess numbers of sources, these sources of uncertainty can be implicitly incorporated by the choice of tolerance values for isotopic mass balance. The value of the mass balance tolerance parameter should be at least 0.5× source increment × maximum difference between sources to avoid missing any feasible solutions as source proportions are incrementally changed (see Materials and methods). However, the mass balance tolerance may be set higher to reflect the magnitude of measurement error and/or source and mixture sample variability. If this is done, the ranges of feasible source contributions are somewhat broader, reflecting uncertainty due to these important sources of variability, as discussed by Phillips and Gregg (2001).

Other considerations

Our procedure gives the distribution of feasible source contributions based entirely on isotopic constraints when the number of sources precludes a unique solution. Given this information, researchers may find it possible to apply other constraints to further reduce the range of contributions from each source. For example, in the Hawaiian islands geochemistry study, the authors used soil quartz content as an additional non-isotopic marker to fix the level of atmospheric dust input (Kennedy et al. 1998), effectively reducing the problem to a two end-member mixing model with a unique solution. Additional constraints are also possible in other types of studies. In dietary studies gut or scat contents could be used to confirm or rule out certain sources. Alternatively, considerations of protein requirements and energy demands may rule out infeasible dietary combinations (Minagawa 1992). Distributions of soil water content and rooting density among the different soil horizons may allow finer resolution of water sources in plant water use studies. One flexible way to incorporate such other factors is to use the IsoSource model to give the entire range of solutions using only isotopic constraints, and then extract

a subset of the output which also satisfies other non-isotopic constraints.

Other "inverse" methods of determining source contributions specifically for trophic webs use trophic relationships as non-isotopic constraints. However, these methods seek only a single solution which minimizes sums of squares of differences between predicted and observed isotopic signatures in each organism (e.g., Vezina and Platt 1988; Saito et al. 2001). The minimization criterion may be reasonable when trying to simultaneously solve dietary composition for a number of organisms at once (the entire food web). Yet for individual mixing problems, as we deal with here, there is little reason to prefer one solution over another if they both result in predicted mixture signatures within measurement error of the observed signatures, for example. Rather, the entire distribution of such solutions should be considered as feasible.

Previous related work on specific applications includes the use of iterative procedures to determine contribution limits of three sources with a single isotope system for dietary analysis (Dauby 1989) and plant water use (Zencich et al. 2002), and use of a Monte Carlo simulation method for bounding the contributions of more than three food sources in human diets based on dual isotopic signatures (Minagawa 1992). To advance beyond these previous efforts, we formalized a general procedure which could be used for any number of isotope systems and sources. In addition, this method incorporates a user-specified amount of mass balance tolerance which can reflect measurement error and population variability uncertainties. In contrast to Monte Carlo methods, this procedure uniformly covers the entire universe of possible source combinations to minimize the chance of overlooking feasible solutions. While more computations are required for this systematic approach compared to typical levels of Monte Carlo sampling, computer resources are much less limiting now than they were a decade ago. By using appropriate source increments of 1–2.5%, problems with up to ten sources can be run in minutes on a modern personal computer.

In some cases where the isotopic signatures of several sources are statistically indistinguishable, it may be possible to pool these sources together to reduce the number of sources to the number of isotopic tracers plus one. In this case, standard mixing models can be used to arrive at a unique solution of source contributions (e.g., using the IsoError software of Phillips and Gregg 2001 at http://www.epa.gov/wed/pages/models.htm). However, the contributions of the individual sources that were pooled together cannot be determined following this procedure.

In conclusion, the method outlined here and the associated IsoSource computer program provide a formalized, general procedure by which ranges of source contributions can be determined when the number of sources is too large to permit unique solutions from stable isotope mixing models. The breadth of these ranges depends on the geometry of the mixing space and the similarity of source and mixture isotopic signatures. The estimated ranges vary only modestly with different choices of source increment and mass balance tolerance parameter values. To avoid misrepresenting the uniqueness of the results, users should report the distribution of feasible solutions rather than focusing on a single value such as the mean.

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