On the Use of Stable Isotopes in Trophic Ecology

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Abstract

Stable isotope analysis (SIA) has proven to be a useful tool in reconstructing diets, characterizing trophic relationships, elucidating patterns of resource allocation, and constructing food webs. Consequently, the number of studies using SIA in trophic ecology has increased exponentially over the past decade. Several subdisciplines have developed, including isotope mixing models, incorporation dynamics models, lipid-extraction and correction methods, isotopic routing models, and compound-specific isotopic analysis. As with all tools, there are limitations to SIA. Chief among these are multiple sources of variation in isotopic signatures, unequal taxonomic and ecosystem coverage, over-reliance on literature values for key parameters, lack of canonical models, untested or unrealistic assumptions, low predictive power, and a paucity of experimental studies. We anticipate progress in SIA resulting from standardization of methods and models, calibration of model parameters through experimentation, and continued development of several recent approaches such as isotopic routing models and compound-specific isotopic analysis.

INTRODUCTION

SIA: stable isotope analysis

Stable isotope analysis (SIA) has been accepted broadly by ecologists, evolutionary biologists, wildlife biologists, and conservation biologists as an important tool to examine animal migration and movement (Hobson 1999, Rubenstein & Hobson 2004); resource partitioning (Jackson et al. 1995, Young et al. 2010); host-parasite interactions (El-Hajj et al. 2004, Neilson et al. 2005); plant water use and nutrient status (Flanagan & Ehleringer 1991, Dawson et al. 2002); ecophysiological processes (Gannes et al. 1998, Cernusak & Hutley 2011); and ecosystem fluxes of carbon, nitrogen, and water (Peterson & Fry 1987).

The focus of this review is on the application of SIA to trophic ecology. SIA is used to reconstruct diets (Minson et al. 1975, Tieszen et al. 1983, Samelius et al. 2007), to assign species to trophic positions in food webs (Minagawa & Wada 1984, Fry 1991, Post 2002; see also Hoeinghaus & Zeug 2008), to elucidate patterns of resource acquisition and allocation (O'Brien et al. 2000, Cherel et al. 2005, Waas et al. 2010), and to characterize niche properties (Genner et al. 1999, Bearhop et al. 2004, Newsome et al. 2007). It is not surprising that SIA has had a major impact on these research areas, as SIA offers significant advantages over traditional methods that may be unethical (destructive sampling of endangered species), impractical (quantifying complex food webs over large temporal and spatial scales), prohibitively expensive (observational studies of ocean-going and deep-sea organisms), or simply impossible (reconstructing diets of long-extinct species).

The central conjecture of SIA in trophic ecology is perhaps best represented by the observation, "You are what you eat (plus a few per mil)" (DeNiro & Epstein 1976). This conjecture follows from the pioneering work of Smith & Epstein (1970), Minson et al. (1975), and Haines (1976), which suggested that the isotopic signatures (expressed as δ, the ratio of heavy to light isotope, and reported in parts per thousand as per mil) of consumers resembled the isotopic signatures of associated plants. This conjecture was given greater exposition by Fry et al. (1978) and DeNiro & Epstein (1978, 1981), who noted consistent differences between the isotopic signatures of consumers and their dietary resources. Elucidating the sources of variation in the isotopic signatures of species and understanding the magnitudes and causes of the differences between consumer and resource isotopic signatures (consumer-resource discrimination, expressed as Δ) are the centerpieces of SIA in trophic ecology.

Three decades of work and several thousand papers have identified many factors that contribute to variation in isotopic signatures and consumer-resource discrimination (**Table 1**). These factors have been addressed largely in an effort to define more precisely those ". . . few per mil." We consider two sources of variation as emergent factors: diet and trophic position. These are the principal factors that SIA in trophic ecology hopes to explain. We submit that variation in these factors is driven at a fundamental level by isotopic differences among resources, such as in photosynthetic pathways (Park & Epstein 1960, Smith & Epstein 1971) and by consumer-resource discrimination (DeNiro & Epstein 1978, 1981). We regard these factors as principal mechanistic factors. Secondary mechanistic factors include biotic and abiotic factors that can be partitioned according to properties of the consumer, properties of the dietary resources, properties of the environment, and properties of SIA analysis. Properties of the consumer can be partitioned further to reflect variation in tissue properties, life histories, physiological condition, and ecological and evolutionary history and circumstance.

The large number of factors generating variation in isotopic signatures makes comparison across studies problematic. Conceptually, each study must be placed in a multidimensional space defined by the factors. The high dimensionality of this space means that each study will likely occupy a unique position and that many coordinates in the space (combinations of factors) will be empty. Consequently, it will be difficult to generate large samples of studies that match in even a

Table 1 Sources of variation in the isotopic signatures of organisms

Factors			Source of variation	Reference
Emergent factors			Diet	Smith & Epstein 1970
			Trophic position	Haines 1976
Principal mechanistic factors			Consumer-resource discrimination	DeNiro & Epstein 1978
			Photosynthetic pathway	Park & Epstein 1960
Secondary mechanistic factors	Properties of the organism	Tissue level	Tissue examined	Tieszen et al. 1983
			Lipid content	McConnaughey & McRoy 1979
			Carbon:nitrogen ratio	Mintenbeck et al. 2008
			Uric acid and urea content	Bearhop et al. 2000
		Life-history level	Ontogenetic stage	Tibbets et al. 2008
			Body size	Fry & Arnold 1982
			Gender	Mariano-Jelicich et al. 2008
			Reproductive status	Fuller et al. 2004
		Physiological level	Metabolic rate	MacAvoy et al. 2006
			Starvation and nutrient stress	Hobson et al. 1993
			Water stress	Ambrose & DeNiro 1986
			Isotopic routing	Schwarcz 1991
			Trophic versus source amino acids	McClelland & Montoya 2002
			Excretion dynamics	Olive et al. 2003
		Ecological/ evolutionary level	Diet switch	Tieszen et al. 1983
			Feeding guild	Hobson & Clark 1992
			Taxonomic identity	Vanderklift & Ponsard 2003
			Symbionts and parasites	Miura et al. 2006
			Migratory status	Hobson 1999
			Intraspecific competition	Forero et al. 2002
	Properties of the resource		Lipid content	Gaye-Siessegger et al. 2004
			Protein content	Kelly & Martinez del Rio 2010
			Elemental concentration	Pearson et al. 2003
			Isotopic signatures	Caut et al. 2009
	Properties of the environment	Biome level	Marine versus terrestrial	Michener & Schell 1994
			Tropical versus temperate	Martinelli et al. 1999
			Benthic versus pelagic	Hobson et al. 1994
			Inshore versus offshore	Cherel & Hobson 2007
		Habitat level	Drought	Peuke et al. 2006
			Latitude	Kelly & Finch 1998
			Season	Perga & Gerdeax 2005
			Temperature	Bosley et al. 2002
			Humidity	Murphy et al. 2007
			Elevation	Graves et al. 2002
			Pollution	Schlacher et al. 2005
			Fire	Grogan et al. 2000

(*Continued*)

Table 1 *(Continued)*

Factors			Source of variation	Reference
			Surface runoff	McClelland et al. 1997
			El Niño southern oscillation	Stapp et al. 1999
	Analytical properties		Mass-spectrometer bias	Mill et al. 2008
			Tissue preservation	Kelly et al. 2006
			Lipid correction method	Sweeting et al. 2006
			Acidification	Jaschinski et al. 2008

small subset of factors. Without sufficient sample sizes, statistically robust models are not possible and those "few per mil" will not be estimated precisely.

Several factors listed in **Table 1** have attracted sufficient research interest to have produced several subdisciplines in trophic ecology SIA. These include trophic positioning and food web reconstruction, lipid correction of tissue samples, mixing models and diet reconstruction, isotope incorporation dynamics, and most recently, isotopic routing and single-compound-specific SIA. Disagreements over the conjectures, assumptions, models, and caveats attendant to each subdiscipline have fueled vigorous research programs.

The remainder of this review is in five parts. The first is a meta-analysis of SIA studies published between 2007 and 2009, inclusive. Here, we address issues such as taxonomic and ecosystem coverage and prevalence of experimental studies. We examine variation in isotopic signatures with respect to trophic position, ontogenetic stage, biome, taxonomic group, and tissue type. The second part analyzes mixing models (Phillips & Gregg 2001, 2003; Ward et al. 2010) used to reconstruct diets of consumers that use two or more resources. We discuss several mathematical and statistical constraints inherent in diet reconstruction based on SIA. We also examine the predictive power of mixing models. The third part reviews isotope incorporation dynamics models (Fry & Arnold 1982, Hobson & Clark 1992, Hesslein et al. 1993, Martinez del Rio & Wolf 2005). These models describe the change in isotopic signatures of consumers following a diet switch and are used to estimate isotopic turnover rates in consumer tissues. The fourth part of the review addresses the practice of lipid correction of tissue samples prior to the determination of isotopic signatures (McConnaughey & McRoy 1979, Post et al. 2007, Mintenbeck et al. 2008). Last, we examine two emerging areas of SIA in trophic ecology: isotopic routing (Podlesak & McWilliams 2007, Kelly & Martinez del Rio 2010) and compound-specific SIA (Chamberlain et al. 2004).

META-ANALYSIS OF STABLE ISOTOPE ANALYSIS (2007–2009)

We were motivated to conduct this meta-analysis simply because we wanted to understand the characteristics of the typical study using SIA in trophic ecology. In particular, we are interested in the extent of taxonomic and ecosystem coverage. We are particularly interested in the prevalence of experimental studies. This review follows other such reviews (e.g., Kelly 2000, Dawson et al. 2002, McCutchan et al. 2003, Vanderklift & Ponsard 2003, Caut et al. 2009, Martinez del Rio et al. 2009) and assesses progress in the field.

Methods

We examined the ISI Web of Knowledge using the key phrase "stable isotopes." The papers returned were then filtered by subject area. We restricted the analysis to papers in the following

disciplines: biochemistry and molecular biology; biodiversity conservation; biotechnology and applied microbiology; ecology; entomology; environmental sciences; evolutionary biology; fisheries; limnology; marine and freshwater biology; microbiology; ornithology; paleontology; parasitology; plant sciences; zoology; and agriculture, dairy and animal science. We eliminated papers from this analysis when unsuccessful in obtaining electronic copies. These were relatively few in number, and we are confident that their omission does not significantly bias our analysis with respect to taxonomic group or ecosystem type. We used the biome classification provided by the University of California Museum of Paleontology (**http://www.ucmp.berkeley.edu/exhibits/biomes**). In total, we compiled data from 249 studies, representing 1,720 species and species groups, and 3,791 separate determinations of carbon and/or nitrogen isotopic signatures.

Taxonomic and Ecosystem Coverage

The isotopic literature examined a diverse collection of organisms, with 39 major taxonomic groups represented. However, the literature was dominated by fish, mammals, plants, crustaceans, birds, and mollusks (**Figure 1*a***). These taxa collectively constituted approximately 76% of all isotopic determinations. A total of 16 major biomes were represented in the literature. There was a significant bias in ecosystem coverage (**Figure 1*b***): Approximately 72% of all isotopic samples were collected from marine, estuarine, and freshwater ecosystems. The bias toward marine, estuarine, and freshwater studies is not surprising given that these systems are the least amenable to traditional methods of trophic analysis. Isotopic determination involving direct experimentation constituted less than 2% of all records.

Tissue and Trophic Level Coverage

A diverse set of tissue types were represented in the literature (**Figure 1*c***). Approximately 61% of all isotopic determinations involved two tissue types: whole body and muscle. Carnivores were the most common trophic level represented (**Figure 1*d***), whereas detritivores and parasites were largely neglected.

MIXING MODELS AND DIET RECONSTRUCTION

A variety of isotope mixing models have been proposed to reconstruct consumer diets. The simplest ones are one-isotope, two-source linear models (e.g., France 1996, Raikow & Hamilton 2001, Dawson et al. 2002, Doi et al. 2008, Marquez & Boecklen 2010). They generally take the form

$$\delta' X = p\delta_1 X + (1 - p)\delta_2 X + \Delta,$$

where $\delta' X$ is the isotopic ratio of element X in the consumer's tissue, p is the percentage contribution of source 1, Δ is the consumer-resource discrimination, and $\delta_1 X$ and $\delta_2 X$ are the isotopic ratios of element X in source 1 and 2, respectively. Models of this type are fully determined as the number of equations equals the number of unknowns. Kwak & Zedler (1997) extended linear models to two isotopes and three sources.

End-member models (Forsberg et al. 1993, Těšitel et al. 2010) are generally rearrangements of one-isotope, two-source linear models and are of the form

$$\%E_1 = [\delta' X - \delta_2 X]/[\delta_1 X - \delta_2 X] \times 100,$$

where $\%E_1$ is the percent elemental contribution of source 1 to the consumer, and $\delta' X$, $\delta_1 X$, and $\delta_2 X$ are as defined above. End-member models have been applied widely to terrestrial and

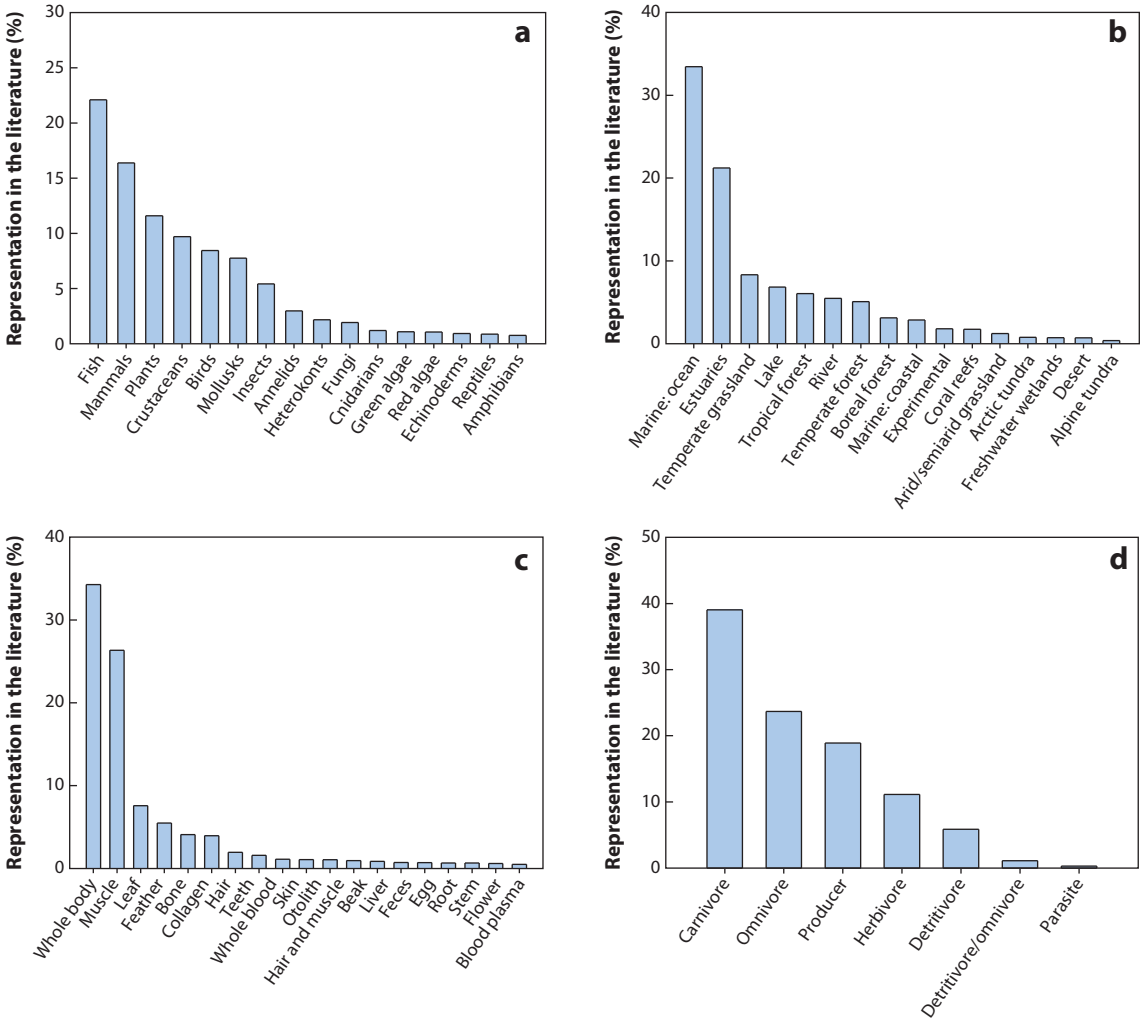

Figure 1

Relative frequency histogram of metadata from 249 studies published on stable isotope analysis (SIA) in 2007–2009. The studies provided 3,791 separate determinations of carbon and/or nitrogen isotopic signatures. These determinations are sorted by (*a*) taxonomic group (greater than 0.7% representation), (*b*) biome, (*c*) tissue examined (greater than 0.47% representation), and (*d*) trophic level.

aquatic ecosystems and to both plant and animal consumers. For example, Doucett et al. (1996) use an end-member model to determine the relative amount of carbon in fish tissues derived from allochthonous versus autochthonous sources. Millett et al. (2003) used an end-member model to determine the relative contribution of insect prey to the nitrogen content of sundews.

Euclidean-distance models (Kline et al. 1993, Ben-David et al. 1997) were proposed to distinguish among three or more sources using two isotopes. However, Phillips (2001) notes several shortcomings with these models. First, when the number of sources exceeds the number of isotopes by more than one, the models are underdetermined. Underdetermined systems of equations have an infinite number of solutions. Second, the models underestimate the contributions of the most commonly used source and overestimate the contributions of less used sources. The models

assume that all sources are actually consumed and that the partitioning of sources is the same for both elements. Last, the models return estimates of source contributions even when the consumer's isotopic ratios do not overlap those of the sources.

Phillips & Gregg (2001) present a mixing model that addresses most, but not all, the shortcomings identified for the Euclidean-distance models. The Phillips & Gregg (2001) model is structurally the same as the linear model proposed by Kwak & Zedler (1997). Perhaps the major advance over previous models is that the Phillips & Gregg (2001) model generates error estimates about predicted source contributions (**http://www.epa.gov/wed/pages/models.htm**). It is important to note, however, that the error estimates assume no covariation between isotopic ratios. This assumption is unlikely to be met in most systems. In addition, the Phillips & Gregg (2001) model assumes that the partitioning of sources is the same for all elements. Phillips & Koch (2002) relax this assumption by incorporating concentration dependency.

As noted above, when the number of unknowns (sources) exceeds the number of equations (number of isotopes + 1), the system of equations is underdetermined and has an infinite number of solutions. Two strategies have been proposed to address undetermined models: combining sources and the IsoSource mixing model (Phillips & Gregg 2003). Phillips et al. (2005) present two strategies for combining sources and discuss the relative merits of both approaches. The IsoSource model has, in many ways, become the workhorse of isotopic diet reconstruction. It is the most commonly used model by ecologists (see below). The IsoSource model considers all possible contributions of each source. These are incremented in small units (typically 1%), and combinations that sum to the consumer's isotopic signature (within a tolerance of 0.1 per mil) are considered feasible solutions. A frequency distribution of the percentage contribution of each source is generated. Of course, this approach does not solve the problems inherent with undetermined systems of equations. Phillips & Gregg (2003) clearly recognize the problem as they encourage researchers to report the range of percentage contribution of each source rather than simply reporting point estimates.

Wilson et al. (2009) adapted the IsoSource model to account for concentration dependency. The IsoSource model and the concentration-dependent IsoSource model (two isotopes and four sources) were applied to a variety of consumers inhabiting Apalachicola Bay, FL, and substantial differences were observed between the models. Wilson et al. (2009) conclude that concentration effects are a significant factor in determining source contributions and that mixing models should be modified to incorporate them.

Rasmussen (2009) presents a spatially explicit, gradient-based mixing model to examine contributions of sources whose isotopic signatures vary little but exhibit spatial gradients. Rasmussen (2009) argues that this approach may be useful especially in river systems where isotope ratios of autochthonous carbon exhibit gradients along rivers, but the isotope ratios of allochthonous contributions do not.

Bayesian Models

Bayesian models offer an alternative to the IsoSource family of models. Proponents of Bayesian models argue that such models better accommodate uncertainties regarding consumer-resource discrimination, variation in consumer and source isotopic signatures, and external sources of variation not directly connected to isotopic variation per se (Ogle et al. 2004, Moreno et al. 2010). Proponents also claim that Bayesian models can solve the problem of undetermined systems of equations common in mixing models (Moore & Semmens 2008, Parnell et al. 2010). We disagree. Bayesian methods may represent an alternative to other methods (e.g., maximum entropy methods) for constraining the set of feasible solutions in undetermined systems of equations, but they do not

eliminate the problem. Freely available Bayesian mixing model software includes MixSIR (Moore & Semmens 2008, Jackson et al. 2009, Semmens et al. 2009) and SIAR (Parnell et al. 2010).

Model Selection and Application

An electronic literature search using the ISI Web of Knowledge with the keywords "stable isotope mixing models" yielded 119 studies that form the basis of the following analyses. These studies generated 1,481 separate mixing models. In terms of model selection, the literature is dominated by the IsoSource model (Phillips & Gregg 2003), as 37% (47/127; several studies used more than one model) of all studies used it (**Figure 2*a***). End-member models were used in roughly 15% of studies, whereas Bayesian models were used in only approximately 5% of studies. We are not surprised by the low representation of Bayesian models in the literature, as these models are relatively new (e.g., Semmens et al. 2009, Ward et al. 2010).

The studies exhibited substantial differences in their use of consumer-resource discrimination values, treatment of source categories, and inclusion of supporting evidence. Most studies (approximately 92%) used mixing models that incorporated values of consumer-resource discrimination (**Figure 2*b***). Of these, only approximately 8% (9/110) used estimates derived directly from the study organisms; the majority used values extracted from the literature. Most studies (roughly 83%) used sources that represented a combined category of separate dietary items (**Figure 2*c***). For example, Reich & Worthy (2006) used a two-isotope, three-source model to characterize the diets of manatees. The three sources were marine, estuary, and freshwater vegetation. Each vegetation category represented the average isotopic signature of 7–13 species. In most instances, an isotopic ratio was estimated for a mixture of dietary items treated as a single sample. It was common practice to present diet reconstructions based solely on the results of mixing models and without benefit of supporting evidence (**Figure 2*d***). Only about 21% of studies complemented the results of the mixing model analysis with gut-content or fecal data, and only about 4% provided experimental evidence.

The studies covered a broad taxonomic range of consumers (**Figure 2*e***), but were dominated by fish, crustaceans, mollusks, and aquatic insects. In fact, the studies were directed primarily at wet things and wet places—approximately 71% of the studies examined marine or aquatic taxa and only approximately 20% of the studies represented the terrestrial biome (**Figure 2*f***). Terrestrial insects are clearly underrepresented in the SIA literature (Yarnes et al. 2005), whereas vertebrates may be overrepresented.

At the level of individual mixing models, the literature is dominated by one-isotope, two-source models and two-isotope, four-source models (**Figure 3*a***). Of the two-isotope models, approximately 68% of models are underdetermined. Fish, crustaceans, mollusks, and aquatic insects collectively represent roughly 65% of all mixing models (**Figure 3*b***).

Predictive Power

Mixing models return estimates of the percentage contribution of each dietary source to the consumer's overall diet. Consequently, the predictive power of mixing models will be related directly to the precision of these point estimates. Studies using linear models report standard deviations as estimates of the variation about point estimates. Bayesian models typically report 95% confidence intervals or ranges, whereas IsoSource models (Phillips & Gregg 2003) most frequently report ranges (**Table 2**). Models based on the Phillips & Gregg (2001) model report standard errors, standard deviations, or 95% confidence intervals. It is surprising that roughly 43% of studies do not present error estimates of any sort. We have excluded Euclidian-distance models

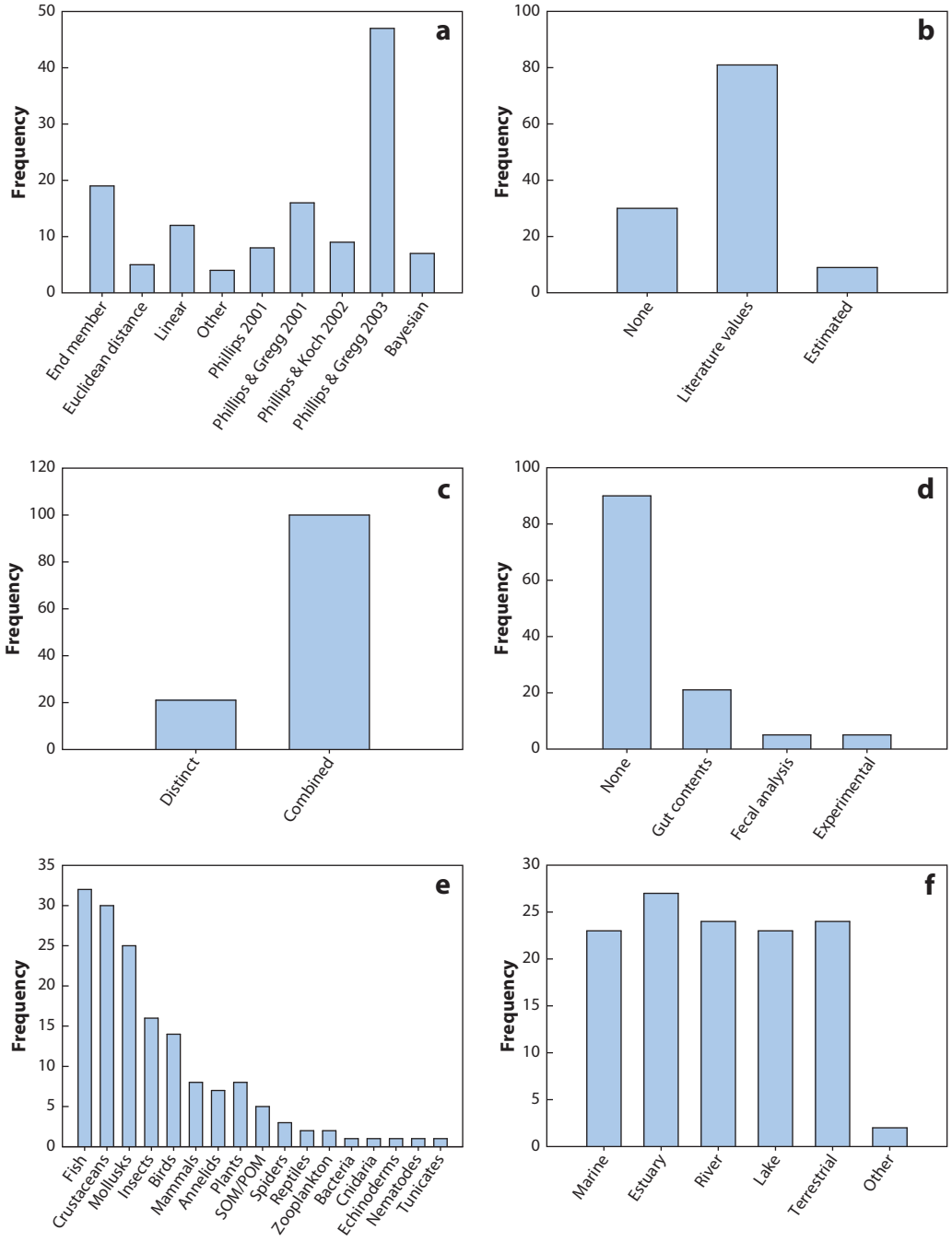

Figure 2

Frequency histogram of metadata from studies using isotope mixing models for diet reconstruction. Models are characterized by (*a*) type of model, (*b*) type of consumer-resource discrimination value, (*c*) source category, (*d*) type of supporting evidence, (*e*) taxonomic group, and (*f*) ecosystem. Abbreviations: POM, particulate organic matter; SOM, soil organic matter.

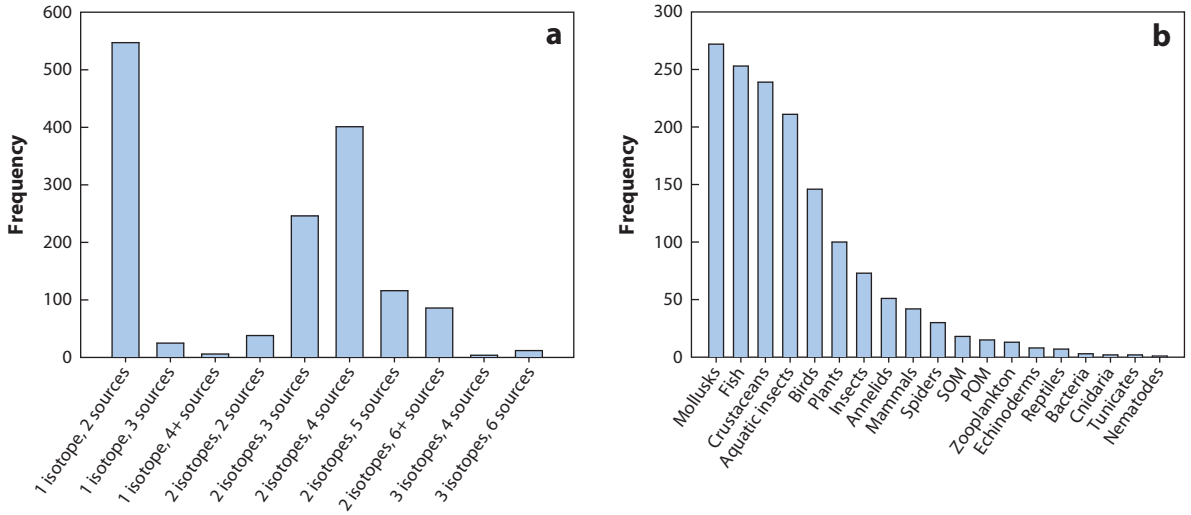

Figure 3

Frequency histograms of isotope mixing models by (*a*) number of isotopes and sources and (*b*) taxonomic groups. Abbreviations: POM, particulate organic matter; SOM, soil organic matter.

from this analysis because of their limited use in the literature and because of many concerns raised by Phillips & Gregg (2001) regarding their construction and use.

Direct comparisons of the predictive power of the various models are complicated by differences in the type of error estimate used. Nevertheless, several generalizations obtain. First, the one-isotope, two-source models [linear, end-member (Phillips & Gregg 2001, 2003; Phillips & Koch 2002)] exhibit roughly similar performance. These models generally estimate the percentage contribution of dietary items with a 95% confidence interval that spans approximately 33% (weighted average of 95% confidence intervals and 4× standard errors). Second, the two-isotope, three-source models based on the Phillips & Gregg (2001) model generally exhibit less precision than do their one-isotope, two-source counterparts. Third, the two-isotope, three-source IsoSource models (Phillips & Gregg 2003) return relatively precise estimates with a median range ($n = 291$) that spans only 12%. Last, as the IsoSource models become increasingly underdetermined, the median of range estimates generally increases (**Figure 4**).

Phillips & Gregg (2001) present a sensitivity analysis that indicates that the level of precision of point estimates should be inversely related to the magnitude of the difference in isotopic signatures of the sources. We have sufficient data to test this proposition for two-isotope IsoSource models with three, four, and five-or-more sources. We used linear regression with the average range for sources returned by a given model as the response variable, and range in source carbon signatures and range in source nitrogen signatures as predictor variables. We found no significant relationship for the three-source models ($p = 0.38$, $R^2 = 3.3\%$). We found a significant relationship with low explanatory power for the four-source models ($p = 0.031$, $R^2 = 12.1\%$), and a highly significant relationship with moderate explanatory power for the five-or-more source models ($p < 0.001$, $R^2 = 33.5\%$). In all cases, differences in source nitrogen signatures had the stronger effect. Although we consider these results to be highly preliminary, they do suggest that further experimental investigation is warranted.

We also have sufficient data to test for an effect of type of fraction estimates (none, estimated, literature) on the average range returned by two-isotope IsoSource models with three and four

Table 2 Number and type of error estimates (variation about point estimates) associated isotope mixing models

Model	Number of isotopes	Number of sources	Error estimate	*N*	Median	Q1	Q3	Min	Max
Linear	1	2	SD	14	19	12	23	3	33
End member	1	2	Range	8	39	15	49	6	66
			95% CI	2	27			27	27
			SD	2	13			11	15
			SE	4	4	3	11	3	13
Phillips & Gregg 2001	1	2	95% CI	11	35	17	45	1	63
			SD	5	17	5	17	4	17
			SE	9	10	10	30	1	43
	2	3	95% CI	27	53	38	69	15	93
			SD	49	13	8	17	0	31
			SE	27	17	11	22	6	34
Phillips & Koch 2002	1	2	SE	6	5	4	7	4	7
	2	3	SE	18	5	3	7	3	8
Phillips & Gregg 2003	1	2	SE	12	10	6	12	5	29
	1	3	Range	48	32	17	46	0	92
	1	4	Range	12	28	15	56	6	86
	2	2	Range	6	10	8	23	3	30
	2	3	Range	291	12	6	22	0	100
			SD	15	7	4	12	3	27
	2	4	Range	828	15	6	31	0	100
			SD	136	5	2	8	0	15
	2	5	Range	348	27	14	45	1	100
			SD	205	6	3	10	0	24
			Quart	5	20	13	28	12	32
	2	6	Range	364	22	12	35	0	78
	2	7+	Range	29	40	29	58	12	82
			SE	17	7	5	11	3	14
	3	6	Range	12	56	41	71	21	82
Bayesian	2	4	Range	92	29	18	51	6	82
	2	6	95% CI	18	7	3	18	1	33
	2	7	95% CI	21	14	11	18	5	20
	2	6+	95% CI	39	12	7	17	1	33

N is the number of error estimates calculated per model type (one for each source) pooled over all studies. Median values are the medians of those estimates. Q1 and Q3 are the first and third quartiles of those error estimates, respectively. Abbreviations: CI, confidence interval; SD, standard deviation; SE, standard error.

sources. We used a general linear model with number of sources and type of fractionation estimate as main effects. There were significant effects due to number of sources ($p = 0.011$), type of fraction estimate ($p = 0.032$), and their interaction ($p = 0.048$). Average ranges were smallest in models using literature estimates (mean = 20.8), intermediate in models using no fractionation estimate (mean = 28.8), and largest in models using fractionation estimates derived internally (mean = 37.3). These results are highly preliminary; the general linear model explained only about 6%

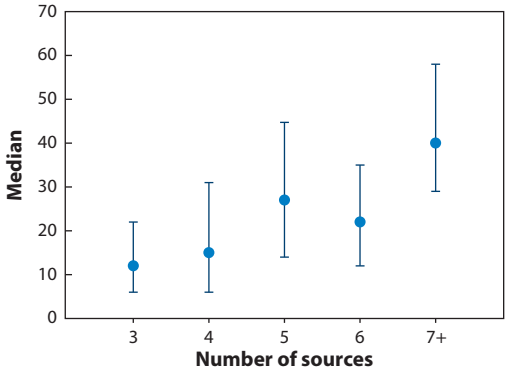

Figure 4

Performance of two-isotope, IsoSource mixing models (Phillips & Gregg 2003) as a function of the number of diet sources. Solid circles represent median values for the ranges in source contributions. Vertical bars represent the interquartile range.

of the variation in average ranges, and sample sizes were relatively small for models using no estimates and for models using internal estimates.

Ikeda et al. (2010) constructed two-isotope, four-source mixing models for 14 species of harpaline ground beetles using both IsoSource and a Bayesian model [MixSIR (Semmens et al. 2009)] and concluded that the models gave similar results, but that the IsoSource models tended to return higher percentages for principal food items and smaller ranges for the estimates than did the MixSIR models. We can directly compare the performance of the IsoSource and MixSIR models by first taking the difference of paired range estimates, and then testing whether the median paired difference is different from zero with a one-sample Wilcoxon Signed-Rank Test. MixSIR models returned ranges that were significantly ($p < 0.001$) larger than those returned by IsoSource. The median paired difference was 17.5%. A determination regarding the accuracy of the point estimates of both models must await laboratory experiments using known diets.

ISOTOPE INCORPORATION DYNAMICS

The isotopic signatures of a consumer's tissues are assumed to be in equilibrium with the isotopic signature of its diet (plus consumer-resource discrimination). When a consumer first switches to a new diet that has an isotopic signature distinct from that of the original diet, the consumer's tissues will be in disequilibrium. Isotope incorporation models track the changes in isotopic signature in a consumer's tissue as it approaches a new equilibrium consistent with the new diet.

The degree of consumer-resource discrimination varies by tissue type (Hobson & Clark 1992). In addition, tissues vary with respect to metabolic rate and turnover. Consequently, different tissues are assumed to change isotopic signatures at different rates following a diet switch (Teiszen et al. 1983, Bauchinger & McWilliams 2009). In other words, tissues with distinct rates of turnover may represent diets integrated over various temporal scales (Hesslein et al. 1993, Dalerum & Angerbjorn 2005, Wolf et al. 2009). For example, isotopic signatures in bone may integrate diets over the course of a year, hair over a few months, muscle over several weeks, and blood plasma over several days.

Understanding the dynamics of isotopic turnover may allow ecologists to detect seasonal, or even episodic, changes in an organism's diet. It might also be possible to back-calculate and

determine the timing of a diet switch (Phillips & Eldridge 2006, Klaassen et al. 2010, Oppel & Powell 2010). Of course, to make these approaches operational, the dynamics of isotopic turnover must be modeled, and the models must be calibrated to the particulars of the system examined.

Parameter estimates from incorporation models can be used to estimate tissue-specific isotopic half-lives. It is important to note, however, that the concept of half-life in isotope incorporation models differs from the more widely used concept used in physics and other disciplines. The traditional interpretation of half-life is the time required, assuming exponential decay, to reduce the initial amount of something by one half. In isotope incorporation models, half-life measures the time required for an isotope ratio to change halfway from its initial equilibrium value to its new equilibrium value. (We suggest the use of isotopic half-life in order to avoid confusion.) Because equilibrium values are determined by the isotopic compositions of the diets, estimates of isotopic half-lives are a function of the tissue and element examined and of the isotopic magnitude of the diet shift. Experimenters have used a wide range of isotopic diet shifts, introducing an additional source of variation in estimates of tissue-specific isotopic half-lives. This variation makes comparisons across studies complicated and cautions against the use of literature values in modeling isotopic turnover.

Models and Assumptions

There are four major classes of isotope incorporation models: growth-dependent models (Fry & Arnold 1982, Maruyama et al. 2001), time-dependent models (Hobson & Clark 1992, O'Brien et al. 2000, Martinez del Rio & Wolf 2005), growth-and-time-dependent models (Hesslein et al. 1993, Carleton & Martinez del Rio 2010), and multi-compartment models (Ayliffe et al. 2004, Cerling et al. 2007, Carleton et al. 2008). Within each major class there are variations.

Growth-dependent models. These models track changes in isotopic ratios as a function of the growth of the consumer. Fry & Arnold (1982) and Maruyama et al. (2001) present models of the following forms:

$$\delta X(t) = \delta X(\infty) - [\delta X(0) - \delta X(\infty)][w(t)/w(0)]^C \text{ and}$$
$$\delta X(t) = \delta X(\infty) - [\delta X(0) - \delta X(\infty)][w(0)/w(t)]C^t,$$

respectively. $\delta X(t)$ is the isotopic ratio of element X in the consumer's tissue at time t, $\delta X(\infty)$ is the asymptotic isotopic ratio, $\delta X(0)$ is the initial isotopic ratio, $w(t)$ is the body mass at time t, and $w(0)$ is the initial body mass. In the Fry & Arnold (1982) model, C is the metabolic decay constant. C is dimensionless and represents the relative contribution of metabolic turnover to changes in isotopic ratios. A value of $C = -1$ indicates turnover due to growth only (simple dilution), whereas greater contributions of metabolic turnover are indicated as C becomes progressively more negative. It is possible to solve for half-lives in these models if one knows the parameters of the growth curve as a function of time.

Time-dependent models. These models track changes in isotopic ratios as a function of time and have the general form

$$\delta X(t) = \delta X(\infty) - [\delta X(\infty) - \delta X(0)]e^{-\lambda t},$$

where λ is the turnover rate constant and has units of $(\text{time})^{-1}$. Isotopic half-life is calculated as $\ln(2)/\lambda$. Martinez del Rio & Anderson-Sprecher (2008) suggest a model where λ is replaced by $1/\text{T}$. T is the average residence time of an element. Isotopic half-lives can be calculated as $\ln(2)\text{T}$.

Growth-and-time-dependent models. Hesslein et al. (1993) suggest a modification of the above model to account for the joint contributions of growth and metabolism to isotopic turnover. In particular, λ is replaced by $(k + m)$, where k is the isotopic turnover constant owing to growth and m is the turnover constant owing to metabolism. The estimation of k typically assumes that growth is related to time as a function of e^{-kt} and is determined either by nonlinear regression of growth versus time or by solving the equation, $k = \ln[w(f)/w(0)]/t$, where t is the time between initial and final measurements, and $w(0)$ and $w(f)$ are the initial and final masses, respectively. Both k and m are rate constants with units of $(\text{time})^{-1}$. Nevertheless, many authors report k as grams per day. Isotopic half-lives can be calculated as $\ln(2)/(k + m)$.

The model assumes that growth and metabolism act independently. This assumption is unlikely to be true in real systems as covariation between body size and metabolism has long been recognized (e.g., Kleiber 1932). This assumption can also cause problems in parameter estimation. Typically, nonlinear regression is used to fit a model with the exponential term, $e^{-\beta t}$. Once β is estimated, the metabolic turnover constant is calculated as $m = \beta - k$, where k has been estimated as described above. Unless the effects of growth and metabolism act independently on isotopic turnover, then estimates of m are incorrect. In fact, we have seen models that have returned values of $m < 0$. Attempts to partition isotopic turnover into the percentage contributions of growth and metabolism from these models (e.g., MacAvoy et al. 2005, Reich et al. 2008, Buchheister & Latour 2010) are likewise suspect.

Multicompartment models. Ayliffe et al. (2004) developed a time-dependent, exponential model with three distinct isotope pools to examine carbon isotopic turnover in tail hair and breath of horses following a change from a C_3 to C_4 diet. The dynamics of both tail hair and breath were best described by three isotopic pools that exhibited short-, intermediate-, and long-term turnover. Cerling et al. (2007) proposed the reaction progress variable as a means of determining the number of isotopic pools. The reaction progress variable (F) measures the degree of progress made in the approach of the system ($F = 0$ at $t = 0$) to a new isotopic equilibrium ($F = 1$). For a one-pool system, $\ln(1 - F) = -\lambda t$, where λ is the isotopic turnover rate constant. Consequently, a plot of $\ln(1 - F)$ versus time should be linear with slope $= -\lambda$. Deviations from a linear relationship are assumed to indicate the presence of more than one isotope pool (see also Martinez del Rio & Anderson-Sprecher 2008). Carleton et al. (2008) tested the generality of multicompartmental models on a variety of tissues from house sparrows (*Passer domesticus*). Some tissues were better described by one-compartment models, others by two-compartment models. Significantly, estimates of isotopic half-lives varied by model type. Recently, Carleton & Martinez del Rio (2010) presented a multicompartment model that includes both growth-dependent and metabolic-dependent turnover.

We can identify several potentially problematic issues with the application and testing of multicompartmental models. First, a nonlinear relationship between $\ln(1 - F)$ and time may obtain for a variety of reasons other than the presence of multiple isotope pools. For example, values of λ may not be invariant temporally as assumed by the models. A nonlinear relationship might obtain simply from model lack-of-fit—isotopic turnover may be better modeled by higher powered exponential functions (e.g., $e^{-\beta t}$, where $\beta = \lambda^x$ and $x > 1$). Second, Akaike's information criteria are used for model selection. The models are hierarchical in nature, and we suggest using the principle of conditional error to determine whether the inclusion of terms representing additional isotope pools significantly improves the one-pool model. Last, the models assume that the isotope pools act independently. Perhaps a more realistic model would allow the pools to interact.

Model Application and Isotopic Half-Life Estimates

An electronic literature search returned 75 studies that modeled isotope incorporation dynamics. Collectively, these studies generated 262 separate models for carbon isotopes, 174 for nitrogen, and 15 for sulfur. Of the 75 studies, 37 used time-dependent models, 28 used growth-and-time-dependent models, 9 used compartmental models, and 8 used growth-dependent models. There was substantial bias in taxonomic coverage for isotopic half-life determination (growth-dependent models excluded), as approximately 80% of studies and 85% of individual models were directed at birds, mammals, and fish (**Table 2**). Muscle, liver, whole blood, and whole body were the most common tissues examined.

There was considerable variation in carbon isotopic half-lives between taxa (birds, mammals, and fish) and between tissue types (**Figure 5**). We had estimates of median half-lives for 9 tissues common to birds and mammals. The half-lives for mammals were significantly longer than those for birds ($p = 0.030$; $n = 9$) as indicated by a Wilcoxon Signed-Rank Test on paired differences. Small sample sizes did not permit a meaningful test for nitrogen isotopic half-lives between mammals and birds ($n = 3$) or for comparisons of birds versus fish or mammals versus fish.

Overall, there was little consistency within a tissue type across taxa. For example, half-lives for bird and mammal livers were similar (3–7 days), but those for fish were quite distinct (1–7 weeks). Mammal muscles exhibited half-lives on the order of 1–3 months, birds 1–3 weeks, and fish 2–8 weeks. We have sufficient data to test for differences in carbon isotopic half-lives between taxa (birds, mammals, and fish) and tissue type (muscle, whole blood, liver). There were significant effects due to taxa ($p < 0.001$), tissue type ($p = 0.002$), and their interaction ($p = 0.007$) as determined by a general linear model. For fish and bird tissues, isotopic half-lives were ordered: liver < muscle < whole blood. For mammals the order was liver < whole blood < muscle. Differences between tissues were greatest for fish and least for birds.

Fisk et al. (2009) suggest that rates of isotopic change may depend on the direction (enriched versus depleted) of the diet switch. Juvenile corn snakes exhibited larger metabolic rate constants (thus, smaller isotopic half-lives) when switched to depleted diets (elimination phase) than they did when switched to enriched diets (uptake phase). We tested for this pattern in birds, in mammals, and in fish. For each taxon, we restricted the analysis to tissue types that had replication at the level of direction of diet switch. We used general linear models with tissue and direction as main effects for both carbon and nitrogen isotopic half-lives. We found little evidence to support the pattern observed by Fisk et al. (2009). Only mammalian carbon half-lives varied significantly with respect to direction of diet switch ($p = 0.010$). This effect was largely due to several unusually large half-lives for muscle. When muscle was excluded from the analysis, there was no longer a significant effect for direction ($p = 0.124$). Of the six possible comparisons (three taxa by two elements), switches to enriched diets had lower half-lives than did switches to depleted diets—a pattern qualitatively at odds with that observed by Fisk et al. (2009). Of course, we may have introduced excessive variation into these tests by combining data from multiple studies. Consequently, we feel that directionality effects remain an open question.

LIPID EXTRACTION AND CORRECTION

Individuals and tissues within individuals vary in lipid content. Lipids tend to be depleted in heavier ^{13}C isotopes (DeNiro & Epstein 1977, 1978; Focken & Becker 1998). Consequently, comparisons of carbon isotopic ratios between individuals or across species may be confounded by variation in lipid content (Post et al. 2007). Several chemical procedures have been suggested to extract lipids from tissue samples prior to isotopic determination (Logan et al. 2008). However, these procedures may introduce their own artifacts as they may affect nitrogen isotopic signatures

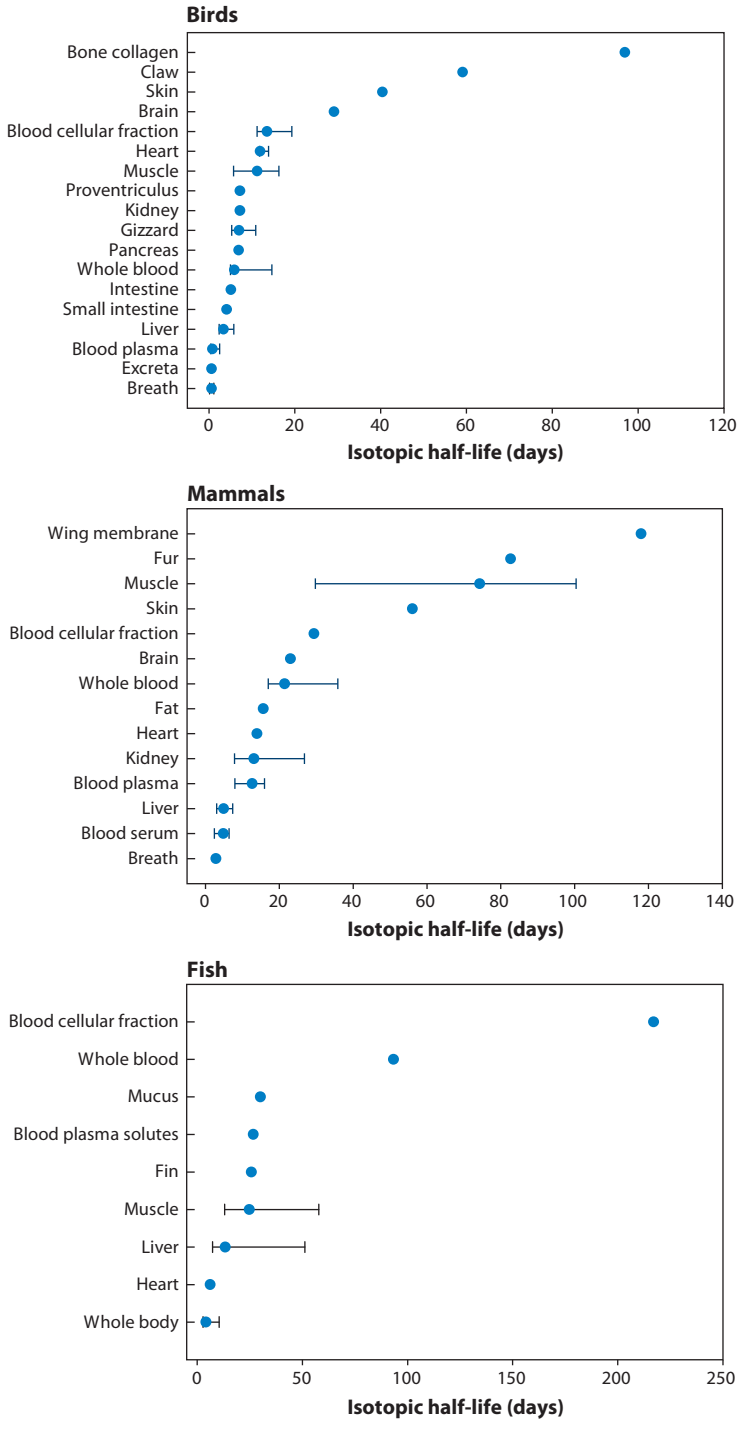

Figure 5

Median isotopic half-lives by taxa and by tissue types. Horizontal bars represent the interquartile range.

(Sotiropoulos et al. 2004). To avoid these complications, several mathematical models have been proposed to correct, or normalize, tissues for lipid content after isotopic determination (Logan & Lutcavage 2008). Despite several recent reviews (Kelly 2000, Logan et al. 2008, Post et al. 2007, Mintenbeck et al. 2008), no consensus has emerged regarding the use and efficacy of lipid extraction. In fact, Mateo et al. (2008) suggested that the literature is replete with "... abundant puzzling and contradictory results ...." Our focus here is to address how carbon isotopic correction varies by lipid extraction method, how nitrogen isotopic ratios are affected by lipid extraction, and how lipid extraction may introduce additional variation into carbon isotopic signatures.

Lipid extraction methods: accelerated solvent extraction (A), Bligh & Dyer (and with modifications) (B), Soxhlet (S), ultrasonication (U), and other (O)

C:N: carbon-to-nitrogen ratio

LE: lipid extracted

NLE: not lipid extracted

Methods

An electronic literature search (ISI Web of Knowledge) returned 20 studies that provided data on tissue carbon-to-nitrogen ratios (C:N) and on carbon ($\delta^{13}C_{LE}$–$\delta^{13}C_{NLE}$) and nitrogen ($\delta^{15}N_{LE}$–$\delta^{15}N_{NLE}$) isotopic ratios prior to and after lipid extraction. Collectively, these studies provide data on 289 tissue samples. We characterized lipid extraction methods according to criteria listed in Smedes (1999), Manirakiza et al. (2001), and Iverson et al. (2001). We identified six major methods: accelerated solvent extraction (A; Ramos et al. 2002), Bligh & Dyer (1959) with modifications (B), Soxhlet (S), B plus ultrasonication (B+U), B plus Soxhlet (B+S), and other (O; incomplete information or not specified). Tissue samples were sorted by taxonomic group and tissue type.

All combinations of extraction method by taxonomic group by tissue type were not well represented. In fact, roughly 60% of the estimates of $\delta^{13}C_{LE}$–$\delta^{13}C_{NLE}$ and 71% of $\delta^{15}N_{LE}$–$\delta^{15}N_{NLE}$ are from fish. Of these, roughly 64% are from muscle. Consequently, we had to partition the data set in various ways in order to test specific hypotheses regarding the effects of lipid extraction on isotopic signatures.

Effects on Carbon

There was considerable variation in values of $\delta^{13}C_{LE}$–$\delta^{13}C_{NLE}$ (**Figure 6*a***). Values ranged from −1.80‰ (a depletion) to 6.78‰. The median enrichment of $\delta^{13}C$ due to lipid extraction was

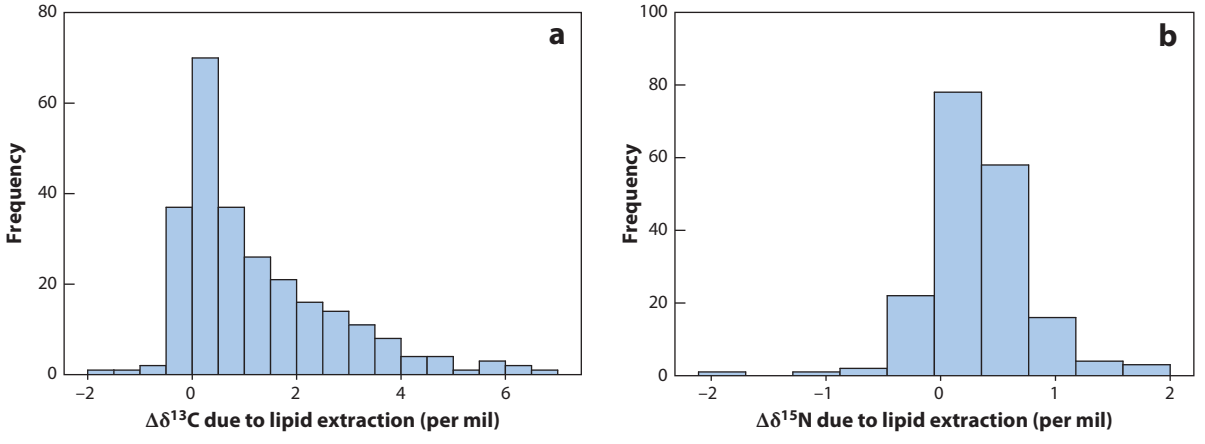

Figure 6

Frequency histograms of the effects of lipid extraction on (*a*) carbon and (*b*) nitrogen isotopic ratios. Values are pooled over taxonomic groups and tissue types.

0.975 $\delta^{13}C$ (Wilcoxon Signed-Rank Test $p < 0.001$; 95% Wilcoxon confidence interval: 0.800‰, 1.170‰).

KW: Kruskal-Wallis test

We suspect that some of this variation may be attributable to the lipid extraction method. We had sufficient data to test for differences in extraction methods (A, B, S, B+U, B+S) for fish muscle. There were marginally significant differences between methods as determined by a Kruskal-Wallis Test (KW) ($p = 0.065$; $n = 117$). The greatest differences in $\delta^{13}C_{LE}$–$\delta^{13}C_{NLE}$ were with B and B+U, while the smallest were with S.

A substantial part of the variation in $\delta^{13}C_{LE}$–$\delta^{13}C_{NLE}$ is due to differences in tissue type. There were highly significant differences between liver, bone, and muscle within fish (KW; $p < 0.001$; $n = 133$). The median difference was 0.19‰ for bone, 0.64‰ for muscle, and 4.98‰ for liver. The small median difference for muscle is surprising, given how often researchers correct for lipids in muscle samples and given the widely held assumption that fish tissues are relatively lipid-rich (Portner 2002). Differences of magnitudes observed here call into question the need for lipid correction of muscle and bone tissues.

There was also substantial variation in $\delta^{13}C_{LE}$–$\delta^{13}C_{NLE}$ due to taxonomic group. We had sufficient data to test for differences between birds, fish, and mammals with respect to muscle. A Kruskal-Wallis Test indicated significant differences between taxa ($p < 0.001$; $n = 142$). The median difference was 0.70‰ for fish, 0.20‰ for birds, and −0.04‰ for mammals. Once again, these results challenge the need to lipid-correct muscle tissues.

Lastly, we have sufficient data to test whether variation in $\delta^{13}C_{LE}$–$\delta^{13}C_{NLE}$ can be attributed to habitat. There were significant differences between marine, terrestrial, and freshwater birds in median differences in muscle (KW; $p = 0.028$; $n = 31$). Median differences were 0.60‰ for marine birds, 0.02‰ for freshwater birds, and −0.01‰ for terrestrial birds. There were also significant differences between marine, estuarine, and freshwater fishes (KW; $p = 0.036$; $n = 94$). Median differences were 1.10‰ for marine fish, 0.49‰ for estuarine fish, and 0.50‰ for freshwater fish.

Effects on Nitrogen

There was substantial variation in $\delta^{15}N_{LE}$–$\delta^{15}N_{NLE}$ owing to lipid extraction (**Figure 6*b***). Values ranged from −2.11‰ to 2.00‰. The median difference (0.30‰) was significantly different from 0.0‰, as determined by a Wilcoxon Signed-Rank Test ($p < 0.001$; $n = 185$). A 95% Wilcoxon confidence interval spanned 0.25‰ to 0.35‰.

We had sufficient data to test for differences in $\delta^{15}N_{LE}$–$\delta^{15}N_{NLE}$ between taxa (fish, birds, and mammals) and between extraction methods (B and S). A general linear model indicated significant differences between extraction methods ($p = 0.012$) and marginally significant differences between taxa ($p = 0.074$). The interaction effect was not significant ($p = 0.294$).

The magnitude of $\delta^{15}N$ enrichment following lipid extraction was greatest for the Bligh & Dyer (1959) method with ultrasonication (B+U) for data pooled across taxa and tissue types. The median enrichment for method B was 0.30‰, while that for B+U was 0.71‰. The difference in these medians (0.41‰) represents the added enrichment due to ultrasonication alone. While we consider these results to be highly preliminary, they do suggest that further experimental investigation is warranted.

Lipid Correction Models

Various mathematical corrections, or lipid normalizations, have been suggested to "correct" nonlipid-extracted tissue samples in postisotopic analysis (Kiljunen et al. 2006, Post et al. 2007).

The models relate $\delta^{13}C_{LE}$–$\delta^{13}C_{NLE}$ to C:N ratios (or in a few cases, %lipids or %C) and fall into three major categories: linear, mass-balance, and nonlinear models. Linear models are most appropriate for relatively low values of C:N (i.e., $2 < C:N < 10$), while nonlinear models are used over a broader range of C:N values ($2 < C:N < 63$). Mass-balance models typically assume that tissue samples are composed of only protein and lipids and require estimates of C:N ratios of pure proteins specific to a given tissue (Sweeting et al. 2006). Lipid correction models vary greatly in terms of predictive power ($0.25 < R^2 < 0.96$) and in terms of generality; many authors add additional parameters to fine-tune a more general model to their specific systems. Consequently, the number of parameters varies from two in linear models to eight in some nonlinear models. It is not uncommon to use literature values for several of these parameters.

CSIA: compound-specific isotope analysis

IRMS: isotope-ratio mass spectrometer

EA-IRMS: elemental analyzer-IRMS

COMPOUND-SPECIFIC ISOTOPE ANALYSIS

Analytical techniques are now readily available for the measurement of isotope ratios from individual compounds (Krummen et al. 2004, Sessions 2006), known as compound-specific isotope analysis (CSIA), primarily through the coupling of capillary gas-chromatography or liquid-chromatography systems to an isotope-ratio mass spectrometer (IRMS). Applications of CSIA in trophic ecology center on the stable isotopic measurement of two of the major biological macromolecules: amino acids (e.g., Fantle et al. 1999, Fogel & Tuross 2003) and fatty acids (e.g., Hammer et al. 1998, Morrison et al. 2010). As the metabolism and biochemistry of these compounds are generally well known, CSIA has the potential to increase greatly the resolution of SIA in foraging studies and to elucidate underlying physiological assumptions concerning the stable isotope ecology of organisms.

Advantages

In general, CSIA may provide greater clarification of foraging studies than bulk analysis alone (elemental analyzer isotope-ratio mass spectrometry, EA-IRMS). First, isotopic signatures estimated by averaging over individual constituent compounds closely agree with those estimated directly from bulk tissues (Popp et al. 2007, McMahon et al. 2010, Morrison et al. 2010). However, isotopic variation among individual compounds produces a much greater range of isotopic values, and therefore, the potential for additional ecological information. Second, the interpretation of CSIA requires fewer assumptions than bulk isotopic values. Specifically, the metabolic and physiological forces affecting isotopic values of a single group of compounds are less numerous, and often better understood, than the diversity of forces that are known to affect bulk tissues. Further, because the sample matrix is less complicated and individual compounds are separated, concerns about sample contamination are often less grave.

Amino Acids

The carbon and nitrogen isotopes found in amino acids reflect both diet composition and metabolic processes. Additionally, hydrogen isotopes of amino acids may also provide source information regarding drinking water, and ultimately, geographic origin. To date, applications using oxygen isotopes of amino acids have not been published.

Average amino acid $\delta^{15}N$ mirrors bulk $\delta^{15}N$ (McClelland & Montoya 2002); however, the range of $\delta^{15}N$ is much greater for amino acids than for bulk tissues. For example, Lorrain et al. (2009) report a $\delta^{15}N$ range of 3.4‰ for blood samples from penguin chicks, but a 26.2‰ $\delta^{15}N$ range for amino acids in the same experimental group, as some amino acids are strongly fractionated relative

FAME: fatty acid methyl ester

to diet, whereas others are not. The pattern of isotopic discrimination between amino acids appears to be predictable based on differences in metabolic processing (Chikaraishi et al. 2009). This has led to the categorization of trophic and source amino acids (McClelland & Montoya 2002, Popp et al. 2007). Trophic amino acids are strongly fractionated relative to diet and include glutamic acid, alanine, aspartic acid, leucine, isoleucine, and proline, whereas source amino acids are not strongly fractionated relative to diet and include glycine, phenylalanine, and histidine. Importantly, Chikaraishi et al. (2009) and others (e.g., Olson et al. 2010) suggest that the comparison of trophic and source amino acid $\delta^{15}N$ may allow for the trophic placement of organisms in cases where the direct measurement of producers is not feasible.

Average amino acid $\delta^{13}C$ also mirrors that of bulk tissues (McMahon et al. 2010). The carbon isotopic signatures of amino acids are largely defined by the differences between essential and nonessential amino acids. Essential amino acids appear to be very conservative indicators of diet, while nonessential amino acids may show increased or decreased isotopic discrimination, reflecting variation in amino acid catabolism as well as the various origins of carbon skeletons (metabolites of glycolysis or the citric acid cycle) used during de novo amino acid synthesis. In general, synthesis of nonessential amino acids should decrease as the protein content of diet increases (Jim et al. 2006), reflecting rates of direct incorporation, but exceptions have been found (McMahon et al. 2010). Amino acid $\delta^{13}C$ may also indicate the degree of carnivory in omnivores, as essential amino acids should be preferentially derived from high-protein animal sources (Fogel & Tuross 2003).

Presently, only scant effort has been directed at understanding the δD of amino acids. However, early studies indicate that hydrogen isotopes of amino acids may be particularly useful. Using bacterial cultures grown with deuterium-labeled water and growth media, Fogel and colleagues determined that while δD of essential amino acids matched the δD of diet, the δD of nonessential amino acids instead matched that of the supplied water (Fogel et al. 2010). Although additional study of δD of amino acids in other organisms and natural conditions is required, the utility of the dual tracer presented by hydrogen isotopes of amino acids would add a desirable spatial component to stable isotope applications in foraging studies.

Fatty Acids

Fatty acid profiling of fatty acid methyl esters (FAMEs) has been used extensively in foraging studies, especially in marine systems (Iverson et al. 2004, Loseto et al. 2009). However, until recently (Hammer et al. 1998, Chamberlain et al. 2006, Budge et al. 2008), the CSIA of FAME has been restricted to a handful of marine (e.g., Uhle et al. 1997, McLeod & Wing 2007, Pace et al. 2007) and soil ecosystem studies (e.g., Zak & Kling 2006). CSIA of FAME has the advantage of providing both fatty acid profiles and isotopic information for dietary studies. The $\delta^{13}C$ of bulk carbon is similar to the pooled isotopic value of the individual FAME (Morrison et al. 2010); however, individual storage fatty acid will differ from dietary fatty acid due to subsequent chain elongation and dehydrogenation as well as turnover processes (Stott et al. 1997, Hammer et al. 1998). This is not the case for essential fatty acids, such as omega fatty acids (e.g., linoleic acid 18:2n–6), as this group of fatty acids is directly incorporated and represents dietary fatty acids (Stott et al. 1997).

Limitations

CSIA is not, however, a panacea and bears some notable limitations. First, in contrast to bulk isotope studies, CSIA applications have been limited to the stable isotopes of carbon, nitrogen

and hydrogen; oxygen and sulfur techniques have not been well demonstrated and pose significant analytical problems to date (Sessions 2006).

The precision of CSIA also often varies dramatically between compounds, presumably through differences in combustion and chromatography. For example, Popp et al. (2007) report a reproducibility range of 0.1–4.4‰ for $\delta^{15}N$ measurements of individual amino acids (average: 1.4‰); similar values have been reported elsewhere for both amino acids and other compounds (Fogel & Tuross 2003, McCarthy et al. 2007). In contrast, the precision of $\delta^{15}N$ from bulk analysis via EA-IRMS is routinely near 0.2‰. Sufficient separation of compounds and strict data processing are critical (Ricci et al. 1994). Additionally, for many nonvolatile compounds, compounds must be derivatized prior to analysis by gas chromatography. Problematically, the derivative molecule will inevitably contain some nonanalyte carbon and/or nitrogen, forcing the measured isotope-ratio to be corrected, thereby leading to additional measurement error.

DEB: dynamic energy budget

DIB: dynamic isotope budget

ISOTOPIC ROUTING

The differential incorporation of dietary macronutrients into various tissues poses problems for diet reconstruction using stable isotopes (Schwarcz 1991). One example of isotopic routing involves the carbon isotope variation of bone. Carbon in bone collagen originates disproportionately from dietary protein due to the necessary recycling of essential amino acids, whereas the apatite carbon that originates from blood bicarbonate should be isotopically similar to bulk diet (Ambrose & Norr 1992). Unfortunately, the problem of isotopic routing is not limited to bone. For example, Podlesak & McWilliams (2006) demonstrate that the $\delta^{13}C$ of protein-rich tissues of yellow-rumped warblers (*Dendroica coronata*) fed a high-protein diet was derived equally from protein and nonprotein dietary sources, while those fed a low-protein diet derived most carbon from sources other than dietary protein. Additionally, variation in consumer-diet discrimination was amplified for $\delta^{15}N$ in low-protein diets, presumably due to the homogenization of amine nitrogen during transamination reactions. A companion study of $\delta^{13}C$ of storage lipids (Podlesak & McWilliams 2007) also demonstrated that consumer-diet discrimination was highest in low-lipid diets. High-lipid diets result in storage lipids with similar isotopic composition (Stott et al. 1997, Podlesak & McWilliams 2007). Similarly, Voigt et al. (2008) found that the $\delta^{13}C$ of breath CO_2 from short-tailed bats, *Carollia perspicillata*, was derived primarily from nectar and fruit sources, while the $\delta^{13}C$ of bat wing tissue matched the $\delta^{13}C$ from protein-rich insect prey. Significant isotopic routing of dietary protein has been demonstrated in omnivorous fish, as well (Kelly & Martinez del Rio 2010). These findings pose serious problems for foraging studies that include omnivores, as diet estimation from certain tissues may require prior knowledge of dietary protein content and quality.

Isotopic routing also poses significant problems for the application of mixing models, yielding biased results that may over- or underestimate the contribution of various sources to diet. While isotopic routing can be easily estimated by measuring different tissues under controlled-diets and conditions, only limited attempts have been made to model the phenomenon. The primary difficulties come in modeling the complexity of diet variation and metabolism, and the parameterization of subsequent models. Pecquerie and colleagues (2010) extend dynamic energy budget (DEB) modeling (Kooijman 2010) to explore the impact of metabolism on the flux of stable isotopes within organisms. Their dynamic isotope budget (DIB) specifically defines points of isotopic discrimination during metabolism, while incorporating the processes of turnover and isotopic routing. A promising application of DIB modeling involves the identification of key metabolic processes and physiological traits associated with patterns of isotopic variation in organisms, including isotopic routing. Another, presently more utilitarian, model presented by Martinez del Rio & Wolf

(2005) attempts to account for isotopic routing within the framework of a linear mixing model. In principle, this model adjusts the mixing line to account for the fractional abundance of protein in the diet, as well as the quality of protein. However, additional research is still needed to determine the rates of the direct incorporation and endogenous production of amino acids under different dietary compositions (e.g., Jim et al. 2006).

CONCLUSIONS

The eminent statisticians George Box and Norman Draper once remarked, "Remember that all models are wrong; the practical question is how wrong do they have to be to not be useful" (Box & Draper 1987). In many ways, similar considerations face stable isotope ecologists. On one hand, SIA offers great promise to trophic ecology, allowing investigation of species that would not be tractable using traditional techniques. On the other hand, much variation in stable isotope metrics remains unexplained, hindering the development of statistically robust predictive models. All things considered, we suspect that SIA in trophic ecology will remain a vigorous research program.

SIA in trophic ecology works best for systems for which key isotopic parameters have been calibrated with laboratory and field data. As literature values are substituted for these parameters, the predictive power of models declines and inferences become increasingly limited; the models may serve to illustrate broad patterns, but it is unlikely that they can define diets and trophic positions reliably and precisely. A fundamental challenge facing stable isotope ecology is an understanding of how error (variation) in isotopic signatures propagates throughout models and ecological systems, and to what degree this error limits inferences derived from SIA.

SUMMARY POINTS

1. Experimentation is still a minor component of SIA in trophic ecology. This is disappointing given the numerous and repeated calls for more laboratory experiments (e.g., Haines 1976, Gannes et al. 1997, Martinez del Rio et al. 2009).
2. SIA in trophic ecology is unequally focused across taxonomic groups and across major habitats. In fact, the literature appears inordinately concerned with wet things and wet places.
3. The use of mixing models for diet reconstruction is characterized by an excessive use of underdetermined models, overreliance on literature values for key parameters, and a general lack of experimental or complementary evidence.
4. There is substantial unexplained variation in isotopic half-lives within a given tissue, across taxa, and across tissue types. The assumption that a given tissue type will integrate diets over a consistent time frame for a diverse set of systems appears to be unwarranted.
5. The appropriateness of lipid correction varies according to taxonomic groups, method of extraction, habitat, and tissue type. The potential of lipid extraction to alter nitrogen isotopic ratios may introduce unwanted bias into SIA.
6. Compound-specific stable isotope analysis is a promising development in SIA. However, refinement of analytical techniques and increased experimental study may be necessary to make the approach operational for field-based research.

FUTURE ISSUES

1. We anticipate more efforts directed at quantifying intrapopulation variation in key isotopic variables, including isotopic ratios, values of consumer-resource discrimination, source utilization, and isotopic turnover rates (Grey et al. 2004, Urton & Hobson 2005, Hatase et al. 2006, Barnes et al. 2008, Anseeuw et al. 2009).
2. At some point, the generation of new models (and variants of existing models) may become counterproductive. The field may be best served by a few well-tested models that have sufficient generality. This may be especially true for the areas of lipid correction and isotopic incorporation.
3. Continued application and development of compound-specific stable isotope analysis in trophic ecology will not only expand the tools and library of SI information available to ecologists but also likely shed light on variation in bulk SIA, as well as inform emerging issues such as isotopic routing, turnover, and incorporation dynamics.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

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

Ambrose SH, Norr L. 1992. On stable isotopic data and prehistoric subsistence in the Soconusco Region. *Curr. Anthropol.* 33:401–4

Anseeuw D, Guelinckx J, Snoeks J. 2009. Differences in stable isotope composition within and among zooplanktivorous Utaka cichlid populations from Lake Malawi. *Afr. J. Ecol.* 48:378–85

Ayliffe LK, Cerling TE, Robinson T, West AG, Sponheimer M, et al. 2004. Turnover of carbon isotopes in tail hair and breath CO_2 of horses fed an isotopically varied diet. *Oecologia* 139:11–22

Barnes C, Jennings S, Polunin NVC, Lancaster JE. 2008. The importance of quantifying inherent variability when interpreting stable isotope field data. *Oecologia* 155:227–35

Bauchinger U, McWilliams S. 2009. Carbon turnover in tissues of a passerine bird: allometry, isotopic clocks, and phenotypic flexibility in organ size. *Physiol. Biochem. Zool.* 82:787–97

Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *J. Anim. Ecol.* 73:1007–12

Bearhop S, Teece MA, Waldron S, Furness RW. 2000. Influence of lipid and uric acid on $\delta^{13}C$ and $\delta^{15}N$ values of avian blood: implications for trophic studies. *Auk* 117:504–7

Ben-David M, Flynn RW, Schell DM. 1997. Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. *Oecologia* 111:280–91

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37:911–17

Bosley KL, Witting DA, Chambers RC, Wainright SC. 2002. Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder *Pseudopleuronectes americanus* with stable isotopes. *Mar. Ecol. Prog. Ser.* 236:233–40

Box G, Draper N. 1987. *Empirical Model-Building and Response Surfaces*. New York: John Wiley
Buchheister A, Latour RJ. 2010. Turnover and fractionation of carbon and nitrogen stable isotopes in tissues of a migratory coastal predator, summer flounder (*Paralichthys dentatus*). *Can. J. Fish. Aquat. Sci.* 67:445–61
Budge SM, Wooller MJ, Springer AM, Iverson SJ, McRoy CP, Divoky GJ. 2008. Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. *Oecologia* 157:117–29
Carleton SA, Kelly L, Anderson-Sprecher R, Martínez del Rio C. 2008. Should we use one-, or multi-compartment models to describe ^{13}C incorporation into animal tissues? *Rapid Commun. Mass Spectrom.* 22:3008–14
Carleton SA, Martínez del Rio C. 2010. Growth and catabolism in isotopic incorporation: a new formulation and experimental data. *Funct. Ecol.* 24:805–12
Caut S, Angulo E, Courchamp F. 2009. Variation in discrimination factors ($\delta^{15}N$ and $\delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. *J. Appl. Ecol.* 46:443–53
Cerling TE, Ayliffe LK, Dearing MD, Ehleringer JR, Passey BH, et al. 2007. Determining biological tissue turnover using stable isotopes: the reaction progress variable. *Oecologia* 151:175–89
Cernusak LA, Hutley LB. 2011. Stable isotopes reveal the contribution of corticular photosynthesis to growth in branches of *Eucalyptus miniata*. *Plant Physiol.* 155:515–23
Chamberlain PM, Bull ID, Black HIJ, Ineson P, Evershed RP. 2004. Lipid content and carbon assimilation in Collembola: implications for the use of compound-specific carbon isotope analysis in animal dietary studies. *Oecologia* 139:325–35
Chamberlain PM, Bull ID, Black HIJ, Ineson P, Evershed RP. 2006. Collembolan trophic preferences determined using fatty acid distributions and compound-specific stable carbon isotope values. *Soil Biol. Biochem.* 38:1275–81
Cherel Y, Hobson KA. 2007. Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. *Mar. Ecol. Prog. Ser.* 329:281–87
Cherel Y, Hobson KA, Weimerskirch H. 2005. Using stable isotopes to study resource acquisition and allocation in procellariiform seabirds. *Oecologia* 145:533–40
Chikaraishi Y, Ogawa NO, Kashiyama Y, Takano Y, Suga H, et al. 2009. Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. *Limnol. Oceanogr. Meth.* 7:740–50
Dalerum F, Angerbjorn A. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia* 144:647–58
Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP. 2002. Stable isotopes in plant ecology. *Annu. Rev. Ecol. Syst.* 33:507–59
DeNiro M, Epstein S. 1976. You are what you eat (plus a few per mil): the carbon isotope cycle in food chains. *Geol. Soc. Am.* 8:834–35 (Abstr.)
DeNiro MJ, Epstein S. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* 197:261–63
DeNiro MJ, Epstein S. 1978. Influence of diet on distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* 42:495–506
DeNiro MJ, Epstein S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim. Cosmochim. Acta* 45:341–51
Doi H, Yurlova NI, Vodyanitskaya SN, Kikuchi E, Shilkano S, et al. 2008. Parasite-induced changes in nitrogen isotope signatures of host tissues. *J. Parasitol.* 94:292–95
Doucett RR, Power G, Barton DR, Drimmie RJ, Cunjak RA. 1996. Stable isotope analysis of nutrient pathways leading to Atlantic salmon. *Can. J. Fish. Aquat. Sci.* 53:2058–66
El-Hajj Z, Kavanagh K, Rose C, Kanaan-Atallah Z. 2004. Nitrogen and carbon dynamics of a foliar biotrophic fungal parasite in fertilized Douglas-fir. *New Phytol.* 163:139–47
Fantle MS, Dittel AI, Schwalm SM, Epifanio CE, Fogel ML. 1999. A food web analysis of the juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. *Oecologia* 120:416–26
Fisk AT, Sash K, Maerz J, Palmer W, Carroll JP, MacNeil MA. 2009. Metabolic turnover rates of carbon and nitrogen stable isotopes in captive juvenile snakes. *Rapid Commun. Mass Spectrom.* 23:319–26

Flanagan LB, Ehleringer JR. 1991. Stable isotope composition of stem and leaf water: applications to the study of plant water use. *Funct. Ecol.* 5:270–77

Focken U, Becker K. 1998. Metabolic fractionation of stable carbon isotopes: implications of different proximate compositions for studies of the aquatic food webs using $\delta^{13}C$ data. *Oecologia* 115:337–43

Fogel ML, Griffin P, Newsome SD. 2010. *Hydrogen isotopes in amino acids trace food and water*. Presented at Int. Conf. Appl. Stable Isotope Tech. Ecol. Stud., 7th, Fairbanks, AK

Fogel ML, Tuross N. 2003. Extending the limits of paleodietary studies of humans with compound specific carbon isotope analysis of amino acids. *J. Archaeol. Sci.* 30:535–45

Forero MG, Hobson KA, Bortolotti GR, Donazar JA, Bertellotti M, Blanco G. 2002. Food resource utilisation by the Magellanic penguin evaluated through stable-isotope analysis: segregation by sex and age and influence on offspring quality. *Mar. Ecol. Prog. Ser.* 234:289–99

Forsberg BR, Araujolima C, Martinelli LA, Victoria RL, Bonassi JA. 1993. Autotrophic carbon sources for fish of the central Amazon. *Ecology* 74:643–52

France RL. 1996. Carbon-13 conundrums: limitations and cautions in the use of stable isotope analysis in stream ecotonal research. *Can. J. Fish. Aquat. Sci.* 53:1916–19

Fry B. 1991. Stable isotope diagrams of fresh-water food webs. *Ecology* 72:2293–97

Fry B, Arnold C. 1982. Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). *Oecologia* 54:200–4

Fry B, Joern A, Parker PL. 1978. Grasshopper food web analysis: use of carbon isotope ratios to examine feeding relationships among terrestrial herbivores. *Ecology* 59:498–506

Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, Hedges REM. 2004. Nitrogen balance and $\delta^{15}N$: why you're not what you eat during pregnancy. *Rapid Commun. Mass Spectrom.* 18:2889–96

Gannes LZ, Martínez del Rio C, Koch P. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comp. Biochem. Physiol. A* 119:725–37

Gannes LZ, O'Brien DM, Martínez del Rio C. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78:1271–76

Gaye-Siessegger J, Focken U, Abel H, Becker K. 2004. Dietary lipid content influences the activity of lipogenic enzymes in the liver and on whole body delta^{13}C values of Nile tilapia, *Oreochromis niloticus* (L.). *Isot. Environ. Health Stud.* 40:181–90

Genner MJ, Turner GF, Barker S, Hawkins SJ. 1999. Niche segregation among Lake Malawi cichlid fishes? Evidence from stable isotope signatures. *Ecol. Lett.* 2:185–90

Graves GR, Romanek CS, Navarro AR. 2002. Stable isotope signature of philopatry and dispersal in a migratory songbird. *Proc. Natl. Acad. Sci. USA* 99:8096–100

Grey J, Waldron S, Hutchinson R. 2004. The utility of carbon and nitrogen isotope analyses to trace contributions from fish farms to the receiving communities of freshwater lakes: a pilot study in Esthwaite Water, UK. *Hydrobiologia* 524:253–62

Grogan P, Bruns TD, Chapin FS. 2000. Fire effects on ecosystem nitrogen cycling in a Californian bishop pine forest. *Oecologia* 122:537–44

Haines EB. 1976. Relation between the stable carbon isotope composition of fiddler crabs, plants, and soils in a salt marsh. *Limnol. Oceanogr.* 21:880–83

Hammer BT, Fogel ML, Hoering TC. 1998. Stable carbon isotope ratios of fatty acids in seagrass and redhead ducks. *Chem. Geol.* 152:29–41

Hatase H, Sato K, Yamaguchi M, Takahashi K, Tsukamoto K. 2006. Individual variation in feeding habitat use by adult female green sea turtles (*Chelonia mydas*): Are they obligately neritic herbivores? *Oecologia* 149:52–64

Hesslein RH, Hallard KA, Ramlal P. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. *Can. J. Fish. Aquat. Sci.* 50:2071–76

Hobson KA. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120:314–26

Hobson KA, Alisauskas RT, Clark RG. 1993. Stable nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. *Condor* 95:388–94

Hobson KA, Clark RG. 1992. Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. *Condor* 94:181–88

Hobson KA, Piatt JF, Pitocchelli J. 1994. Using stable isotopes to determine seabird trophic relationships. *J. Anim. Ecol.* 63:786–98

Hoeinghaus DJ, Zeug SC. 2008. Can stable isotope ratios provide for community-wide measures of trophic structure? Comment. *Ecology* 89:2353–57

Ikeda H, Kubota K, Kagawa A, Sota T. 2010. Diverse diet compositions among harpaline ground beetle species revealed by mixing model analyses of stable isotope ratios. *Ecol. Entomol.* 35:307–16

Iverson SJ, Field C, Bowen WD, Blanchard W. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecol. Monogr.* 74:211–35

Iverson SJ, Lang SLC, Cooper MH. 2001. Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. *Lipids* 36:1283–87

Jackson AL, Inger R, Bearhop S, Parnell A. 2009. Erroneous behaviour of MixSIR, a recently published Bayesian isotope mixing model: a discussion of Moore & Semmens (2008). *Ecol. Lett.* 12:E1–E5

Jackson PC, Cavelier J, Goldstein G, Meinzer FC, Holbrook NM. 1995. Partitioning of water resources among plants of a lowland tropical forest. *Oecologia* 101:197–203

Jaschinski S, Hansen T, Sommer U. 2008. Effects of acidification in multiple stable isotope analyses. *Limnol. Oceanogr. Meth.* 6:12–15

Jim S, Jones V, Ambrose SH, Evershed RP. 2006. Quantifying dietary macronutrient sources of carbon for bone collagen biosynthesis using natural abundance stable carbon isotope analysis. *Brit. J. Nutr.* 95:1055–62

Kelly B, Dempson JB, Power M. 2006. The effects of preservation on fish tissue stable isotope signatures. *J. Fish Biol.* 69:1595–611

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

Kelly JF, Finch DM. 1998. Tracking migrant songbirds with stable isotopes. *Trends Ecol. Evol.* 13:48–49

Kelly LJ, Martínez del Rio C. 2010. The fate of carbon in growing fish: an experimental study of isotopic routing. *Physiol. Biochem. Zool.* 83:473–80

Kiljunen M, Grey J, Sinisalo T, Harrod C, Immonen H, Jones RI. 2006. A revised model for lipid-normalizing $\delta^{13}C$ values from aquatic organisms, with implications for isotope mixing models. *J. Appl. Ecol.* 43:1213–22

Klaassen M, Piersma T, Korthals H, Dekinga A, Dietz MW. 2010. Single-point isotope measurements in blood cells and plasma to estimate the time since diet switches. *Funct. Ecol.* 24:796–804

Kleiber M. 1932. Body size and metabolism. *Hilgardia* 6:315–51

Kline TC, Goering JJ, Mathisen OA, Poe PH, Parker PL, Scalan RS. 1993. Recycling of elements transported upstream by runs of Pacific salmon: II. $\delta^{15}N$ and $\delta^{13}C$ evidence in the Kvichak River Watershed, Bristol Bay, Southwestern Alaska. *Can. J. Fish. Aquat. Sci.* 50:2350–65

Kooijman S. 2010. *Dynamic Energy Budget Theory for Metabolic Organisation*. Cambridge, UK: Cambridge Univ. Press

Krummen M, Hilkert AW, Juchelka D, Duhr A, Schluter HJ, Pesch R. 2004. A new concept for isotope ratio monitoring liquid chromatography/mass spectrometry. *Rapid Commun. Mass Spectrom.* 18:2260–66

Kwak TJ, Zedler JB. 1997. Food web analysis of southern California coastal wetlands using multiple stable isotopes. *Oecologia* 110:262–77

Logan JM, Jardine TD, Miller TJ, Bunn SE, Cunjak RA, Lutcavage ME. 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *J. Anim. Ecol.* 77:838–46

Logan JM, Lutcavage ME. 2008. A comparison of carbon and nitrogen stable isotope ratios of fish tissues following lipid extractions with non-polar and traditional chloroform/methanol solvent systems. *Rapid Commun. Mass Spectrom.* 22:1081–86

Lorrain A, Graham B, Menard F, Popp B, Bouillon S, et al. 2009. Nitrogen and carbon isotope values of individual amino acids: a tool to study foraging ecology of penguins in the Southern Ocean. *Mar. Ecol. Prog. Ser.* 391:293–306

Loseto LL, Stern GA, Connelly TL, Deibel D, Gemmill B, et al. 2009. Summer diet of beluga whales inferred by fatty acid analysis of the eastern Beaufort Sea food web. *J. Exp. Mar. Biol. Ecol.* 374:12–18

MacAvoy SE, Arneson LS, Bassett E. 2006. Correlation of metabolism with tissue carbon and nitrogen turnover rate in small mammals. *Oecologia* 150:190–201

MacAvoy SE, Macko SA, Arneson LS. 2005. Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis. *Can. J. Zool.* 83:631–41

Manirakiza P, Covaci A, Schepens P. 2001. Comparative study on total lipid determination using Soxhlet, Roese-Gottlieb, Bligh & Dyer, and modified Bligh & Dyer extraction methods. *J. Food Compos. Anal.* 14:93–100

Mariano-Jelicich R, Botto F, Martinetto P, Iribarne O, Favero M. 2008. Trophic segregation between sexes in the Black Skimmer revealed through the analysis of stable isotopes. *Mar. Biol.* 155:443–50

Marquez MJ, Boecklen WJ. 2010. Isotopic shift in an introduced population of gemsbok (*Oryx gazella*). *J. Arid Environ.* 74: 928–32

Martinelli LA, Piccolo MC, Townsend AR, Vitousek PM, Cuevas E, et al. 1999. Nitrogen stable isotopic composition of leaves and soil: tropical versus temperate forests. *Biogeochemistry* 46:45–65

Martínez del Rio C, Anderson-Sprecher R. 2008. Beyond the reaction progress variable: the meaning and significance of isotopic incorporation data. *Oecologia* 156:765–72

Martínez del Rio C, Sabat P, Anderson-Sprecher R, Gonzalez SP. 2009. Dietary and isotopic specialization: the isotopic niche of three *Cinclodes* ovenbirds. *Oecologia* 161:149–59

Martínez del Rio C, Wolf BO. 2005. Mass-balance models for animal isotopic ecology. In *Physiological and Ecological Adaptations to Feeding in Vertebrates*, ed. JM Starck, T Wang, pp. 141–74. Enfield, NH: Sci. Publ.

Maruyama A, Yamada Y, Rusuwa B, Yuma M. 2001. Change in stable nitrogen isotope ratio in the muscle tissue of a migratory goby, *Rhinogobius* sp., in a natural setting. *Can. J. Fish. Aquat. Sci.* 58:2125–28

Mateo MA, Serrano O, Serrano L, Michener RH. 2008. Effects of sample preparation on stable isotope ratios of carbon and nitrogen in marine invertebrates: implications for food web studies using stable isotopes. *Oecologia* 157:105–15

McCarthy MD, Benner R, Lee C, Fogel ML. 2007. Amino acid nitrogen isotopic fractionation patterns as indicators of heterotrophy in plankton, particulate, and dissolved organic matter. *Geochim. Cosmochim. Acta* 71: 4727–44

McClelland JW, Montoya JP. 2002. Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. *Ecology* 83:2173–80

McClelland JW, Valiela I, Michener RH. 1997. Nitrogen-stable isotope signatures in estuarine food webs: a record of increasing urbanization in coastal watersheds. *Limnol. Oceanogr.* 42:930–37

McConnaughey T, McRoy CP. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Mar. Biol.* 53:257–62

McCutchan JH, Lewis WM, Kendall C, McGrath CC. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102:378–90

McLeod RJ, Wing SR. 2007. Hagfish in the New Zealand fjords are supported by chemoautotrophy of forest carbon. *Ecology* 88:809–16

McMahon KW, Fogel ML, Elsdon TS, Thorrold SR. 2010. Carbon isotope fractionation of amino acids in fish muscle reflects biosynthesis and isotopic routing from dietary protein. *J. Anim. Ecol.* 79:1132–41

Michener RH, Schell DM. 1994. Stable isotope ratios as tracers in marine aquatic food webs. In *Stable Isotopes in Ecology and Environmental Science*, ed. K Lajtha, RH Michener, pp. 138–57. Boston: Blackwell Sci.

Mill AC, Sweeting CJ, Barnes C, Al-Habsi SH, MacNeil MA. 2008. Mass-spectrometer bias in stable isotope ecology. *Limnol. Oceanogr. Meth.* 6:34–39

Millett J, Jones RI, Waldron S. 2003. The contribution of insect prey to the total nitrogen content of sundews (*Drosera* spp.) determined in situ by stable isotope analysis. *New Phytol.* 158:527–34

Minagawa M, Wada E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. *Geochim. Cosmochim. Acta* 48:1135–40

Minson DJ, Ludlow MM, Troughton JH. 1975. Differences in natural carbon isotope ratios of milk and hair from cattle grazing tropical and temperate pastures. *Nature* 256:602

Mintenbeck K, Brey T, Jacob U, Knust R, Struck U. 2008. How to account for the lipid effect on carbon stable-isotope ratio ($\delta^{13}C$): sample treatment effects and model bias. *J. Fish Biol.* 72:815–30

Miura O, Kuris AM, Torchin ME, Hechinger RF, Chiba S. 2006. Parasites alter host phenotype and may create a new ecological niche for snail hosts. *Proc. R. Soc. B Biol. Sci.* 273:1323–28
Moore JW, Semmens BX. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. *Ecol. Lett.* 11:470–80
Moreno R, Jover L, Munilla I, Velando A, Sanpera C. 2010. A three-isotope approach to disentangling the diet of a generalist consumer: the yellow-legged gull in northwest Spain. *Mar. Biol.* 157:545–53
Morrison DJ, Cooper K, Preston T. 2010. Reconstructing bulk isotope ratios from compound-specific isotope ratios. *Rapid Commun. Mass Spectrom.* 24:1799–804
Murphy BP, Bowman D, Gagan MK. 2007. The interactive effect of temperature and humidity on the oxygen isotope composition of kangaroos. *Funct. Ecol.* 21:757–66
Neilson R, Boag B, Hartley G. 2005. Temporal host-parasite relationships of the wild rabbit, *Oryctolagus cuniculus* (L.) as revealed by stable isotope analyses. *Parasitology* 131:279–85
Newsome SD, Martínez del Rio C, Bearhop S, Phillips DL. 2007. A niche for isotopic ecology. *Front. Ecol. Environ.* 5:429–36
O'Brien DM, Schrag DP, Martínez del Rio C. 2000. Allocation to reproduction in a hawkmoth: a quantitative analysis using stable carbon isotopes. *Ecology* 81:2822–31
Ogle K, Wolpert RL, Reynolds JF. 2004. Reconstructing plant root area and water uptake profiles. *Ecology* 85:1967–78
Olive PJW, Pinnegar JK, Polunin NVC, Richards G, Welch R. 2003. Isotope trophic-step fractionation: a dynamic equilibrium model. *J. Anim. Ecol.* 72:608–17
Olson RJ, Popp BN, Graham BS, Lopez-Ibarra GA, Galvan-Magana F, et al. 2010. Food-web inferences of stable isotope spatial patterns in copepods and yellowfin tuna in the pelagic eastern Pacific Ocean. *Prog. Oceanogr.* 86:124–38
Oppel S, Powell AN. 2010. Carbon isotope turnover in blood as a measure of arrival time in migratory birds using isotopically distinct environments. *J. Ornithol.* 151:123–31
Pace ML, Carpenter SR, Cole JJ, Coloso JJ, Kitchell JF, et al. 2007. Does terrestrial organic carbon subsidize the planktonic food web in a clear-water lake? *Limnol. Oceanogr.* 52:2177–89
Park R, Epstein S. 1960. Carbon isotope fractionation during photosynthesis. *Geochim. Cosmochim. Acta* 21:110–26
Parnell AC, Inger R, Bearhop S, Jackson AL. 2010. Source partitioning using stable isotopes: coping with too much variation. *PLoS One* 5:e9672
Pearson SF, Levey DJ, Greenberg CH, Martínez del Rio C. 2003. Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia* 135:516–23
Pecquerie L, Nisbet RM, Fablet R, Lorrain A, Kooijman S. 2010. The impact of metabolism on stable isotope dynamics: a theoretical framework. *Philos. Trans. R. Soc. B* 365:3455–68
Perga ME, Gerdeaux D. 2005. 'Are fish what they eat' all year round? *Oecologia* 144:598–606
Peterson BJ, Fry B. 1987. Stable isotopes in ecosystems. *Annu. Rev. Ecol. Syst.* 18:293–320
Peuke AD, Gessler A, Rennenberg H. 2006. The effect of drought on C and N stable isotopes in different fractions of leaves, stems and roots of sensitive and tolerant beech ecotypes. *Plant Cell Environ.* 29:823–35
Phillips DL. 2001. Mixing models in analyses of diet using multiple stable isotopes: a critique. *Oecologia* 127:166–70
Phillips DL, Eldridge PM. 2006. Estimating the timing of diet shifts using stable isotopes. *Oecologia* 147:195–203
Phillips DL, Gregg JW. 2001. Uncertainty in source partitioning using stable isotopes. *Oecologia* 127:171–79
Phillips DL, Gregg JW. 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136:261–69
Phillips DL, Koch PL. 2002. Incorporating concentration dependence in stable isotope mixing models. *Oecologia* 130:114–25
Phillips DL, Newsome SD, Gregg JW. 2005. Combining sources in stable isotope mixing models: alternative methods. *Oecologia* 144:520–27
Podlesak DW, McWilliams SR. 2006. Metabolic routing of dietary nutrients in birds: effects of diet quality and macronutrient composition revealed using stable isotopes. *Physiol. Biochem. Zool.* 79:534–49

Podlesak DW, McWilliams SR. 2007. Metabolic routing of dietary nutrients in birds: effects of dietary lipid concentration on $\delta^{13}C$ of depot fat and its ecological implications. *Auk* 124:916–25

Popp BN, Graham BS, Olson RJ, Hannides CCS, Lott MJ, et al. 2007. Insight into the trophic ecology of yellowfin tuna, *Thunnus albacares*, from compound-specific nitrogen isotope analysis of proteinaceous amino acids. In *Terrestrial Ecology*. Vol. 1: *Stable Isotopes as Indicators of Ecological Change*, ed. T Dawson, R Siegwolf, pp. 173–90. Amsterdam: Elsevier/Academic

Portner HO. 2002. Physiological basis of temperature-dependent biogeography: trade-offs in muscle design and performance in polar ectotherms. *J. Exp. Biol.* 205:2217–30

Post DM. 2002. The long and short of food-chain length. *Trends Ecol. Evol.* 17:269–77

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montana CG. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152:179–89

Raikow DF, Hamilton SK. 2001. Bivalve diets in a midwestern US stream: a stable isotope enrichment study. *Limnol. Oceanogr.* 46:514–22

Ramos L, Kristenson EM, Brinkman UAT. 2002. Current use of pressurised liquid extraction and subcritical water extraction in environmental analysis. *J. Chromatogr. A* 975:3–29

Rasmussen JB. 2009. Estimating terrestrial contribution to stream invertebrates and periphyton using a gradient-based mixing model for $\delta^{13}C$. *J. Anim. Ecol.* 79:393–402

Reich KJ, Bjorndal KA, Martínez del Rio C. 2008. Effects of growth and tissue type on the kinetics of ^{13}C and ^{15}N incorporation in a rapidly growing ectotherm. *Oecologia* 155:651–63

Reich KJ, Worthy GAJ. 2006. An isotopic assessment of the feeding habits of free-ranging manatees. *Mar. Ecol. Prog. Ser.* 322:303–9

Ricci MP, Merritt DA, Freeman KH, Hayes JM. 1994. Acquisition and processing of data for isotope-ratio-monitoring mass-spectrometry. *Org. Geochem.* 21:561–71

Rubenstein DR, Hobson KA. 2004. From birds to butterflies: animal movement patterns and stable isotopes. *Trends Ecol. Evol.* 19:256–63

Samelius G, Alisauskas RT, Hobson KA, Lariviere S. 2007. Prolonging the arctic pulse: long-term exploitation of cached eggs by arctic foxes when lemmings are scarce. *J. Anim. Ecol.* 76:873–80

Schlacher TA, Liddell B, Gaston TF, Schlacher-Hoenlinger M. 2005. Fish track wastewater pollution to estuaries. *Oecologia* 144:570–84

Schwarcz HP. 1991. Some theoretical aspects of isotope paleodiet studies. *J. Archaeol. Sci.* 18:261–75

Semmens BX, Ward EJ, Moore JW, Darimont CT. 2009. Quantifying inter- and intra-population niche variability using hierarchical bayesian stable isotope mixing models. *PLoS One* 4:e6187

Sessions AL. 2006. Isotope-ratio detection for gas chromatography. *J. Sep. Sci.* 29:1946–61

Smedes F. 1999. Determination of total lipid using non-chlorinated solvents. *Analyst* 124:1711–18

Smith BN, Epstein S. 1970. Biogeochemistry of stable isotopes of hydrogen and carbon in salt marsh biota. *Plant Physiol.* 46:738–42

Smith BN, Epstein S. 1971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. *Plant Physiol.* 47:380–84

Sotiropoulos MA, Tonn WM, Wassenaar LI. 2004. Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: potential consequences for food web studies. *Ecol. Freshw. Fish* 13:155–60

Stapp P, Polls GA, Pinero FS. 1999. Stable isotopes reveal strong marine and El Niño effects on island food webs. *Nature* 401:467–69

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

Sweeting CJ, Polunin NVC, Jennings S. 2006. Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Commun. Mass Spectrom.* 20:595–601

Těšitel J, Plavcová L, Cameron DD. 2010. Heterotrophic carbon gain by the root hemiparasites, *Rhinanthus minor* and *Euphrasia rostkoviana* (Orobanchaceae). *Planta* 231:1137–44

Tibbets TM, Wheeless LA, Martínez del Rio C. 2008. Isotopic enrichment without change in diet: an ontogenetic shift in $\delta^{15}N$ during insect metamorphosis. *Funct. Ecol.* 22:109–13

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. *Oecologia* 57:32–37

Uhle ME, Macko SA, Spero HJ, Engel MH, Lea DW. 1997. Sources of carbon and nitrogen in modern planktonic foraminifera: the role of algal symbionts as determined by bulk and compound specific stable isotopic analyses. *Org. Geochem.* 27:103–13

Urton EJM, Hobson KA. 2005. Intrapopulation variation in gray wolf isotope ($\delta^{15}N$ and $\delta^{13}C$) profiles: implications for the ecology of individuals. *Oecologia* 145:317–26

Vanderklift MA, Ponsard S. 2003. Sources of variation in consumer-diet δ15N enrichment: a meta-analysis. *Oecologia* 136:169–82

Voigt CC, Rex K, Michener RH, Speakman JR. 2008. Nutrient routing in omnivorous animals tracked by stable carbon isotopes in tissue and exhaled breath. *Oecologia* 157:31–40

Waas S, Werner RA, Starck JM. 2010. Fuel switching and energy partitioning during the postprandial metabolic response in the ball python (*Python regius*). *J. Exp. Biol.* 213:1266–71

Ward EJ, Semmens BX, Schindler DE. 2010. Including source uncertainty and prior information in the analysis of stable isotope mixing models. *Environ. Sci. Technol.* 44:4645–50

Wilson RM, Chanton J, Lewis G, Nowacek D. 2009. Combining organic matter source and relative trophic position determinations to explore trophic structure. *Estuar. Coasts* 32:999–1010

Wolf N, Carleton SA, Martínez del Rio C. 2009. Ten years of experimental animal isotopic ecology. *Funct. Ecol.* 23:17–26

Yarnes CT, Rockwell JN, Boecklen WJ. 2005. Patterns of trophic shift in $\delta^{15}N$ and $\delta^{13}C$ through a cynipid gall wasp community (*Neuroterus* sp.) in *Quercus turbinella*. *Environ. Entomol.* 34:1471–76

Young HS, McCauley DJ, Dirzo R, Dunbar RB, Shaffer SA. 2010. Niche partitioning among and within sympatric tropical seabirds revealed by stable isotope analysis. *Mar. Ecol. Prog. Ser.* 416:285–94

Zak DR, Kling GW. 2006. Microbial community composition and function across an arctic tundra landscape. *Ecology* 87:1659–70

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