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Applying stable isotopes to examine food-web structure: an overview of analytical tools

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ABSTRACT

Stable isotope analysis has emerged as one of the primary means for examining the structure and dynamics of food webs, and numerous analytical approaches are now commonly used in the field. Techniques range from simple, qualitative inferences based on the isotopic niche, to Bayesian mixing models that can be used to characterize food-web structure at multiple hierarchical levels. We provide a comprehensive review of these techniques, and thus a single reference source to help identify the most useful approaches to apply to a given data set. We structure the review around four general questions: (1) what is the trophic position of an organism in a food web; (2) which resource pools support consumers; (3) what additional information does relative position of consumers in isotopic space reveal about food-web structure; and (4) what is the degree of trophic variability at the intrapopulation level? For each general question, we detail different approaches that have been applied, discussing the strengths and weaknesses of each. We conclude with a set of suggestions that transcend individual analytical approaches, and provide guidance for future applications in the field.

Key words: Bayesian statistics, dietary variation, individual specialization, mixing model, predator-prey interactions, trophic structure.

CONTENTS

I. INTRODUCTION

Stable isotope analysis has emerged as one of the primary means to analyze the structure of food webs. Stable isotopes are especially useful because they provide time- and space-integrated insights into trophic relationships among organisms, and thus can be used to develop models of trophic structure. Many of the first applications of stable isotope data in a food-web context were critical advances, although largely qualitative, providing for broad inferences based on relative isotope values of consumers and resources (Haines & Montague, 1979; Peterson, Howarth & Garritt, 1985; Zieman, Macko & Mills, 1984). Over the past 10 years, a series of more quantitative approaches for analyzing stable isotope data has emerged. These approaches have dramatically improved our understanding of food webs, for example, providing new insight into food-chain length (Post, Pace & Hairston, 2000), niche variation (Martinez del Rio *et al.*, 2009*a*; Moore & Semmens, 2008; Semmens *et al.*, 2009*b*; Votier *et al.*, 2010), and human-driven shifts in community structure (Layman *et al.*, 2007*b*; Schmidt *et al.*, 2007).

The emergence of new analytical approaches has led to some debate about which method(s) is most appropriate to apply to stable isotope data (Hoeinghaus & Zeug, 2008; Jackson *et al*., 2009; Layman & Post, 2008; Newsome *et al*., 2007; Semmens, Moore & Ward, 2009*a*). At times, this discussion has focused on which analytical approach is "right" or "wrong". But a more useful perspective is recognizing the specific types of questions for which different approaches are best suited. Analogously, use of stomach contents to evaluate dietary breadth has some very well-understood limitations (Votier *et al*., 2003), but still provides critical insight into feeding relationships. Likewise, each stable isotope analytical approach has distinct strengths and weaknesses (Table 1), and each is more or less appropriate under specific circumstances. Information regarding these strengths and weaknesses is scattered among dozens of papers in the field, often rendering direct comparison among techniques difficult. Herein we provide a comprehensive review of these diverse approaches, structured around four core ecological questions: (1) what is the trophic position of an organism in a food web; (2) which resource pools support consumers; (3) what additional information does relative position of consumers in isotopic space reveal about food web structure; (4) what is the degree of trophic variability at an intrapopulation level?

This review is not intended to be a comprehensive catalogue of every food-web study that has employed stable isotopes, an endeavour which would be a monumental task given the rapid proliferation of such studies (Fig. 1). Instead, we emphasize those papers that are paradigmatic with respect to a particular analytical approach, as well as some of the most recent contributions to the field. Not expanded upon in this review are the many additional types of information that are necessarily relevant in interpreting isotope data sets (e.g. trophic discrimination factors, isotopic routing, tissue turnover rates, lipid extraction, etc.), as other reviews have discussed these topics thoroughly (e.g. Bearhop *et al*., 2004; Boecklen *et al*., in press; Martinez del Rio *et al*., 2009*b*; McCutchan *et al*., 2003; Oppel & Powell, 2011; Phillips & Eldridge, 2006; Post *et al*., 2007; Vanderklift & Ponsard, 2003). Our goal is to provide a single source that outlines analytical approaches currently being applied to answer questions about food-web structure, and provide guidelines as to which approaches are most appropriate with respect to a particular data set or question of interest.

II. STABLE ISOTOPE RATIOS AND FOOD WEBS

The two elements most commonly employed in a food-web context are nitrogen (N) and carbon (C), although sulphur (S), oxygen (O) and deuterium (D) are also useful in particular cases. The ratio of ^{15}N to ^{14}N (expressed relative to a standard, $\delta^{15}N$) exhibits stepwise enrichment with trophic transfers, and is a powerful tool for estimating trophic position of organisms (Minagawa & Wada, 1984; Peterson & Fry, 1987; Post, 2002*b*). Ratios of carbon isotopes ($\delta^{13}C$) vary substantially among primary producers with different photosynthetic pathways (e.g. C3 *versus* C4 photosynthetic pathways in plants), but change little with trophic transfers (DeNiro & Epstein, 1981; Inger & Bearhop, 2008; Peterson & Fry, 1987; Post, 2002*b*). Therefore, $\delta^{13}C$ can be used to determine original sources of dietary carbon. Similarly, the ratio of sulphur isotopes ($\delta^{34}S$) varies substantially among primary producers, but changes relatively little with progression through a food web, and also can be used to identify important resource pools. This has proven especially insightful in marine systems where the sulphur cycle often gives rise to distinct benthic and pelagic $\delta^{34}S$ values (Currin, Newell & Paerl, 1995; Peterson & Howarth, 1987) and along marine ecotones to differentiate marine and fresh-water (or terrestrial) sources (Jones *et al*., 2010). The $\delta^{18}O$ and $\delta^{2}H$ values of precipitation vary at multiple spatial scales, yielding insight into large-scale dietary patterns across geographic regions (Bowen &

Revenaugh, 2003), or across smaller-scale environmental gradients (Deines, Wooller & Grey, 2009; Finlay, Doucett & McNeely, 2010; Solomon *et al*., 2011, 2009). Newsome *et al.* (2007) and Oulhote *et al*. (2011) provide additional information regarding the insights that can be gleaned from various isotope tracers.

Most frequently, $\delta^{15}N$ and $\delta^{13}C$ (or one of these in combination with other elemental tracers) are plotted in bivariate fashion, a depiction that has been variously referred to as niche space, trophic space, isotope space, or the isotopic niche. Herein, we adopt the term "isotopic niche". In this sense, we view the ecological information contained in stable isotope plots as a proxy for a subset of the Hutchinsonian *n*-dimensional hypervolume (Hutchinson, 1957). We emphasize that the isotopic niche is distinct from, but in many circumstances should align closely with, aspects of the actual trophic niche (e.g. particular resource pools utilized or relative trophic position within a web).

Examining food-web structure involves analyzing and comparing the relative position of species, populations, or individuals within this niche space, i.e. concomitantly examining the relative positions along one (or more) isotopic axes. These data provide for inference regarding feeding relationships and food-web structure, but they are not direct characterizations of diet such as those provided by stomach-content analysis, feeding observations, or fecal analysis. Because of the indirect nature of the data, there are various sources of potential ambiguity in interpretation of isotope values that relate to all of the analytical approaches discussed herein.

Stable isotope values are a product not only of the actual trophic interactions, but are also driven by myriad underlying biological and chemical processes. For example, when isotopic routing occurs, i.e. when elemental isotopes from resources are broken down and assimilated differently among consumer tissue types, direct interpretation of the underlying trophic relationships may be more problematic (Martinez del Rio *et al.*, 2009*b*). In such a case, a consumer tissue does not reflect isotopic composition of the bulk diet, but rather the isotopic composition of the nutrient component of the diet from which the tissue was synthesized. This is especially important to consider when an individual consumes diverse resources (for example, feeding on both plants and animals, Kelly & Martinez del Rio, 2010; Martinez del Rio *et al.*, 2009*b*; Voigt *et al.*, 2008). Ignoring such biochemical processes driving variation in stable isotope values can result in biased interpretations of trophic interactions.

Emergent ecological factors also render δ values difficult to interpret in some circumstances. First, isotopic similarity does not necessarily mean ecological similarity, as two individuals may have the same isotopic niche, but distinct ecological niches. That is, even though trophic pathways that may support the two individuals are distinct, the different source pools are characterized by similar stable isotope values. Second, if different potential resource pools have overlapping δ values, stable isotopes alone may not be able to pinpoint the particular source pool being utilized. Different isotope values of source pools typically is essential for isotopes to be a useful analytical tool. Third, when using stable isotopes to reconstruct dietary relationships, both source and consumer pools must be sampled on spatial and temporal scales that reflect the relative incorporation rates of the elements and the turnover rates of tissues (Martinez del Rio *et al.*, 2009*b*; Post, 2002*b*). Yet this final point also underpins the strength of isotopes relative to direct dietary information: when sampled at appropriate scales, stable isotopes provide for time- and space-integrated representations of trophic relationships in food webs. Such data provide important insights into food-web structure not possible through snapshot characterizations of diet.

All of the analytical approaches discussed herein deal with either raw δ values, or values that are transformed to represent a specific ecological variable (e.g. trophic position or dietary proportions from different source pools) (Newsome *et al*., 2007). Analysis of raw δ values allows inferences regarding feeding relationships, but can be especially sensitive to the relative δ values of source pools. For example, broad dispersion among consumers in a $\delta^{15}N$ and $\delta^{13}C$ biplot would seem to imply diverse resource use among individuals, but this pattern may also be a result of high variance in isotope values of source pools. To this end, δ space can be transformed to "proportional" space using isotope values of known source pools (Newsome *et al*., 2007). Similarly, raw $\delta^{15}N$ values can be converted to direct trophic position estimates using assumed values of $\delta^{15}N$ discrimination with trophic transfers, as well as adequate characterization of isotopic baselines (Post, 2002*b*). Such transformations are often preferred because they are more ecologically meaningful than raw δ values. For example, transforming $\delta^{15}N$ values into trophic positions converts them into an actual characteristic of the organism. But such transformations require considerable additional *a priori* information, including temporally and spatially appropriate estimates of isotopic baselines and end members, as well as trophic discrimination factors. If this information is not available or of poor quality, the transformations may not accurately describe aspects of trophic structure.

III. INITIAL APPLICATIONS OF STABLE ISOTOPES IN A FOOD-WEB CONTEXT

In a food-web context, many of the first applications of stable isotope data were largely qualitative, i.e. making general inferences from the relative isotopic values of consumers and/or resources (Fry, Joern & Parker, 1978; Haines & Montague, 1979; Peterson *et al*., 1985; Zieman *et al.*, 1984). For example, Peterson *et al.* (1985) suggested the fundamental importance of *Spartina alterniflora* grass for marsh consumers by qualitatively comparing $\delta^{34}S$ and $\delta^{13}C$ values in producer and consumer tissue. Haines & Montague (1979) took a similar approach, using the variation in $\delta^{13}C$ among estuarine primary producers qualitatively to infer the most important sources for various estuarine consumer species. Hobson & Welch (1992) provided one of the first insights into the general structure of Arctic food webs using isotope values. All of these initial advances were critical to laying the foundation for the myriad stable isotope research programs that are now a fundamental part of the ecological sciences.

A logical progression from these early contributions was to apply basic statistics [e.g. *t*-tests, analysis of variance (ANOVA), multivariate models, etc.] to compare mean $\delta^{13}C$ and/or $\delta^{15}N$ values among groups, sites or time periods (Oulhote *et al.*, 2011). Some type of basic statistical comparison can be found in almost any current stable isotope paper in the field. Simple statistics provide the basic framework for interpreting isotope data, but can be limited as to the depth of ecological insight that they can provide. Often, basic statistics are used in conjunction with various other approaches outlined herein. Basic statistical approaches obviously were not developed for isotope data *per se*, so we focus the remainder of this review on analytical approaches that are targeted for isotope data sets specifically.

IV. WHAT IS THE TROPHIC POSITION OF AN ORGANISM IN A FOOD WEB?

(1) Species-specific baselines

One of the most important initial advances beyond basic statistics came from the realization that $\delta^{15}N$, because of the discrimination that occurs with trophic transfers, could be used as a proxy for trophic position (DeNiro & Epstein, 1981; Minagawa & Wada, 1984). In this context, δ^{15}N provided for a continuous measure of trophic position, a notable difference from simply assigning organisms to discrete trophic levels based on natural-history observations. Although early studies used the untransformed δ^{15}N values as a measure of trophic position, later work recognized that δ^{15}N is influenced by local biogeochemistry (baseline variation), trophic discrimination and the trophic position of an organism. Researchers have taken two approaches to address baseline variation: (1) using species-specific baselines to estimate relative trophic shifts and (2) using long-lived organisms or time-series baselines to estimate trophic position of higher order consumers.

Kling, Fry & Obrien (1992) and Post (2003) both used species-specific baselines to estimate relative differences in trophic position. Kling *et al*. (1992) used herbivorous copepods as a baseline to estimate the degree of trophic omnivory in copepods, and Post (2003) used largemouth bass (*Micropterus salmoides*) that had not transitioned to piscivory to estimate the degree of cannibalism in young-of-the-year individuals. The use of an ecologically relevant baseline in both of these examples minimized problems related to spatial and temporal differences between the baseline (herbivorous copepods and non-piscivorous bass) and the target organism (omnivorous copepods and cannibalistic bass). This baseline method works well for questions that do not require absolute estimates of trophic position and when the trophic position of the baseline organism is well understood (i.e. herbivorous copepods). It does not provide an absolute estimate of trophic position and is, therefore, limited to questions specific to individuals or a single species.

(2) Long-lived consumers as baselines

The second approach involves a more general baseline that allows for absolute estimates of trophic position, thereby facilitating comparisons among species and across ecosystems (Hobson, Piatt & Pitocchelli, 1994). Cabana & Rasmussen (1996) first suggested that long-lived primary consumers in temperate lakes (e.g. mussels) may be used to create an isotope baseline for fish. This was expanded to include multiple sources by Vander Zanden & Rasmussen (1999) and Post (2002*b*). Vander Zanden & Rasmussen (1999) proposed creating a baseline by fitting a logistic curve to the isotope values of primary consumers in the δ^{13}C-δ^{15}N bi-plot and using this baseline to calculate the trophic position of higher order consumers. Post (2002*b*) developed a more general solution by using a two-end member mixing model to create a baseline from which trophic position could be calculated (see Section V for detailed discussion of mixing models). Because the isotope estimates of trophic position calculated using these methods can be compared directly across diverse, complex food webs, this method has been widely adopted for calculating food-chain length, the number of transfers of energy from the base to the apex of a food web (Post, 2002*a*). The isotope method has allowed researchers to make considerable progress in addressing fundamental questions about variation in and environmental controls of food-chain length in lakes and ponds (Doi *et al.*, 2009; Post *et al.*, 2000), streams (McHugh, McIntosh & Jellyman, 2010; Sabo *et al.*, 2010; Walters & Post, 2008) and islands (Takimoto, Spiller & Post, 2008).

Trophic position estimates are perhaps the most widely reported metric in food-web studies employing stable isotopes. But these measures are characterized by fundamental limitations that are often not appreciated when trophic positions of individuals are calculated. First, trophic position calculation is dependent on establishing an adequate baseline. In some fresh-water ecosystems, basal resources are relatively easily isolated at a coarse level (e.g. seston and benthic microalgae in northern U.S. lakes; Post, 2002*b*), or can be aggregated into ecologically meaningful categories (e.g. autochthonous *versus* allochthonous pools in rivers; Layman *et al*., 2005*b*). But as food webs become more complex, and the number of potential basal resource pools increases, establishing an adequate baseline becomes more problematic. In systems with resource pools that have numerous and variable δ^{15}N and δ^{13}C values, it may be extremely difficult to establish an accurate baseline using just a few isotopes, rendering any trophic position estimates problematic (Layman, 2007). Second, and equally important, is identifying δ^{15}N discrimination values for each trophic transfer (Martinez del Rio *et al*., 2009*b*). Discrimination provides the stepwise correction that allows one to convert baseline isotope values into a trophic position for a consumer. Typically, this value is chosen based on available meta-analyses (Caut, Angulo & Courchamp, 2009; McCutchan *et al*., 2003; Post, 2002*b*; Vanderklift & Ponsard, 2003), but numerous physiological and environmental factors can affect discrimination in δ^{15}N (Martinez del Rio *et al*., 2009*b*). Values from the meta-analyses are valid approximations when averaged over a large number of trophic pathways, as is done for estimating food-chain length (Post, 2002*a*). But when used for estimating the trophic position of individuals or single species, literature values can prove misleading, and should be used with caution, until the causes of variation in trophic discrimination are better understood (Martinez del Rio *et al*., 2009*b*). Until recently, few studies propagated such error in assumed values in calculations (but see Vander Zanden & Rasmussen, 2001). Resampling from distributions of baselines and trophic discrimination factors to produce ranges of estimates for trophic position, with an associated error term, is now more commonly employed (including the frequently used Bayesian models, see Section V.3). Such estimates are more accurate depictions of possible solutions that account for potential variation in discrimination factors (Jackson *et al*., 2011).

V. WHICH RESOURCE POOLS SUPPORT CONSUMERS?

Stable isotope analysis can reveal dietary patterns by suggesting specific resources used by a consumer. In simple systems, where consumers only use two food resources, basic qualitative comparisons can be made using a single elemental tracer. For example, many of the first studies that applied stable isotope analysis in a food-web context capitalized on differentiation in carbon isotope ratios in various basal carbon resource pools (e.g. C3 *versus* C4 plants) to identify sources of primary productivity (Fry *et al*., 1978; Zieman *et al*., 1984). In some cases, traditional multivariate analyses (e.g. canonical discriminant analysis or non-metric multidimensional scaling) using $\delta^{15}N$, $\delta^{13}C$, and/or $\delta^{34}S$ may be sufficient to suggest source contributions (e.g. Litvin & Weinstein, 2004). But as the number of potential resources increases, the ability accurately to identify dietary contributions becomes more problematic. Over the last two decades, a number of isotope mixing models have been proposed to identify the relative contributions of various food resources to a consumer's diet.

(1) Geometric approaches

Early mixing models used geometric methods to estimate the proportional contribution of three or more food resources to a consumer's diet using δ values (BenDavid, Flynn & Schell, 1997; Kline *et al*., 1993; Peterson & Howarth, 1987; Whitledge & Rabeni, 1997). Euclidean distances between consumer and sources were calculated in isotopic niche space, and an inverse relationship was assumed between these distances and the relative contribution of each source to the consumer's diet. Although this method provides a visually appealing graphical representation of dietary contribution and is a useful heuristic tool (BenDavid *et al*., 1997; Kline *et al*., 1993; Peterson & Howarth, 1987; Whitledge & Rabeni, 1997), Phillips (2001) demonstrated that the equations used in these approaches failed accurately to identify dietary contributions. Euclidean methods underestimate commonly used food sources and overestimate rare food sources, and the equations provide inaccurate estimates when a consumed resource is excluded from the analysis. These Euclidean-based approaches have largely been supplanted by other mixing-model approaches (Phillips, 2001), but are still employed in isolated cases (e.g. Wengeler, Kelt & Johnson, 2010).

(2) Linear mixing models

Phillips (2001) suggested that partitioning of resources could most accurately be identified using a basic set of algebraic mass-balance equations (linear mixing model), and this has become a fundamental framework for understanding stable isotope data in a food-web context. A linear mixing model can determine the relative contribution of p unique food resources from the isotope ratios of q elemental tracers when $p \leq q + 1$ (i.e. the number of sources cannot exceed the number of elemental tracers by more than one) by solving a series of equations (Phillips, 2001). For example, in a simple system with only three possible food resources and two isotope tracers, solving a set of three linear mass-balance equations, containing three unknowns, will determine the exact proportional contribution of each resource. Assuming ^{15}N and ^{13}C are the two isotopes, the equations would be represented:

$$\delta^{13}C_T = f_A\,\delta^{13}C_A + f_B\,\delta^{13}C_B + f_C\,\delta^{13}C_C\,, \tag{1}$$

$$\delta^{15}N_T = f_A\,\delta^{15}N_A + f_B\,\delta^{15}N_B + f_C\,\delta^{15}N_C\,, \tag{2}$$

$$f_A + f_B + f_C = 1\,, \tag{3}$$

where δ_T is the isotopic composition of a consumer's tissue and f_A , f_B , and f_C are fractional contributions of sources A, B, and C. Although the linear mixing model and mass-balance equations had been previously used in palaeo-diet research (Schwarcz, 1991), Phillips (2001) was the first to promote their use in present-day diet studies. These linear mixing models have since been extended to account for uncertainty in source partitioning (Isoerror: Phillips & Gregg, 2001) and concentration dependence (Isoconc: Phillips & Koch, 2002). Nearly all of the more advanced models outlined below have their foundation in the same basic set of algebraic equations.

Most food webs are too complex to use simple linear mixing models because the number of source pools exceeds the number of useful isotope tracers by more than one. When this is the case, we move from a mathematically determined system to a mathematically undetermined system. The latter implies that there are multiple feasible solutions for combinations of source contributions. To this end, Phillips & Gregg (2003) developed the model IsoSource, which has become one of the most common analytical tools in the field. The model does not generate exact values for proportional contributions of each source, but instead provides a range of possible contributions or feasible solutions. The model examines every possible combination of source proportions (summing to 100%) incrementally (typically in increments of 1%), then calculates the predicted isotope value for each combination using linear mass-balance equations. These predicted values are then examined to determine which ones fall within some tolerance range (typically 0.1‰) of the observed consumer isotope value, and all of these feasible solutions are recorded. One of the main advantages of this model, besides its public availability and ease of use, is the relatively limited amount of input data required (average isotope values of the consumer and potential sources). Additionally, the model can be adjusted further to consider source pooling (Phillips, Newsome & Gregg, 2005). As with all mixing models, a series of critical assumptions must be made, and these will be discussed in detail below. But perhaps the most common problem with studies employing IsoSource is not related to the intrinsic structure of the model, but instead, to interpretation of its output. That is, researchers often interpret some measure of central tendency (e.g. the median or mode) as the definitive solution, a conclusion which is clearly not justified by the structure of the model (Phillips & Gregg, 2003).

Three other notable approaches have been developed to identify proportional source contributions. First, Lubtekin & Simenstad (2004) proposed two models (SOURCE and STEP) that are computationally less demanding than IsoSource. These models identify the outer bounds of possible mixtures in *n*-dimensional Euclidean space, instead of examining every single biologically possible solution. The output of these models is considerably reduced relative to that of IsoSource, but there is little reason to believe that the output of SOURCE and STEP would be significantly different from that of IsoSource (Maier & Simenstad, 2009). Second, the Moore-Penrose pseudoinverse model (Hall-Aspland, Hall & Rogers, 2005*a*; Hall-Aspland, Rogers & Canfield, 2005*b*) attempts to provide a unique solution of source contributions to a consumer using a single isotopic tracer and matrix algebra. Although output data often match up well with mean/modal resource values generated by IsoSource (S.A. Hall-Aspland, personal communication), this approach provides only a single solution and fails to acknowledge other feasible source combinations as provided by IsoSource. Third, a linear programming (LP) model employs linear algorithms instead of an iterative approach to determine the minimum and maximum possible proportions of each source to a consumer (Bugalho *et al*., 2008). The results are similar to those produced by IsoSource, with the LP model explicitly identifying which sources definitively do or do not contribute to an individual consumer. Likely because of the availability and ease of use of the IsoSource software, it is used much more frequently than SOURCE, STEP, Moore-Penrose, or LP models.

(3) Bayesian mixing models

A major limitation of all of the above mixing models (apart from Isoerror) is that they do not incorporate uncertainty and variation in input parameters (such as variation within source pools or trophic discrimination factors). In other words, much of the inherent variability in natural systems is ignored by use of mean resource isotope values or assumed trophic discrimination values. To this end, models (e.g. MixSIR and SIAR) have emerged, all of which are based on a series of related linear equations, that utilize Bayesian statistical techniques to identify proportional contributions of source pools (Jackson *et al.*, 2009; Moore & Semmens, 2008; Parnell *et al.*, 2010; Solomon *et al.*, 2011). Importantly, these approaches allow for incorporation of available prior information, thereby allowing for more realistic representations of variability in input terms. Outputs from the Bayesian models are in the form of true probability distributions, not just summaries of all feasible solutions. As such, unlike in IsoSource, measures of central tendency from the outputs can be used in subsequent analyses (Parnell *et al.*, 2010). Further, parameter transformations, as suggested by Semmens *et al.* (2009*b*), provide a framework for utilization of general linear model approaches. This allows for incorporation of fixed and random covariates into models, which can provide the ability to partition particular drivers of source contribution variation (Francis *et al.*, 2011). Largely because of the additional input data, the models often substantially narrow the reported ranges of source pool contributions to consumers (Moore & Semmens, 2008; Moreno *et al.*, 2010). Bayesian approaches are evolving rapidly, greatly expanding in capability and scope (Jackson *et al.*, 2011; Ward *et al.*, 2011), and are being applied to yield novel insights into aspects of trophic structure (e.g. Francis *et al*., 2011; Rutz *et al*., 2010; Solomon *et al*., 2011). As with IsoSource, the Bayesian models MixSIR and SIAR can be freely accessed online (Moore & Semmens, 2008; Parnell *et al*., 2010).

Solomon *et al.* (2011) provide one example of how Bayesian approaches can yield powerful insights into the contribution of sources to consumers. Their goal was to quantify resource use for zooplankton, zoobenthos, and fishes in four low-productivity lakes, using models that incorporated multiple sources of potential variance and error. Informative priors (and/or associated variance components) utilized in the model included the proportion of hydrogen in consumer tissues derived from environmental water, trophic position of organisms, trophic discrimination factors, source isotopic signatures and a term to estimate unexplained variation. The results provided strong evidence that both terrestrial and benthic basal resource pools were integral in supporting consumer production in the lake systems. That is, even when accounting for many of the sources of input error that could have biased model output, terrestrial and benthic basal resource pools were identified as particularly important contributors. More generally, even though determined source ranges may still be broad in Bayesian models, there is greater assurance in their validity because of the incorporated error terms.

It is important to recognize that all mixing models, including Bayesian-based approaches, are not a quick fix or a substitute for poor sampling strategy; moreover, they are not particularly useful for asking questions about systems where complementary information is largely lacking. Indeed, all of the mixing models described above are subject to a core set of limitations, with many of the guidelines in Section II applying here. First, some information on turnover rate, trophic discrimination and macronutrient composition (e.g. free lipid and carbonate content) associated with the consumer tissues is needed. Second, prey sources must have different isotope values. The more similar the resource pool isotope values, the less power the models have to delineate proportional contributions. Third, in many cases, *a priori* grouping may be necessary to constrain model outputs (Phillips *et al*., 2005; but Bayesian approaches may also be useful in this respect, see Ward *et al*., 2011), a decision that requires extensive knowledge of the basic natural history of the system. Fourth, prey should ideally be sampled on a time frame that coincides with the period during which the consumer tissue is synthesized, and all prey items must be known in order to provide the most meaningful results (although SIAR has an additional error term whereby the solution is not constrained to be merely a function of the identified sources, which would allow for some unknowns to be incorporated into the model). Fifth, as spatial and temporal variability in source pool values increases, so does the sampling effort necessary to determine adequately the appropriate input mean (and standard deviation in Bayesian models) values that should be used. As with any model, Bayesian tools such as MixSIR and SIAR are especially sensitive to the quality of the input data (Moore & Semmens, 2008). Finally, inclusion of prior information into models can lead to more uncertain outputs, depending on the nature of input data (Moore & Semmens, 2008).

Although still beholden to the quality of input data, the ability to incorporate prior information and propagate error using Bayesian frameworks holds much promise in the development of the field. One of the primary criticisms of isotope applications in food-web studies is the lack of specific information on the underlying biochemical processes that affect isotopic signatures (Martinez del Rio *et al*., 2009*b*); since Bayesian approaches allow for incorporation of uncertainty in input parameters, the models tacitly address this criticism by providing for more realistic estimates of source contributions to consumers.

(4) Spatially based approaches

Mixing models typically utilize values for source pools that have fixed, distinct isotope values, but continuous variables can also be incorporated into mixing-model formats (Francis *et al*., 2011; Gray *et al*., 2011; Rasmussen, 2010). For example, Rasmussen (2010) describes a model that can be applied when isotope signatures are not necessarily distinct (e.g. when δ^{13}C of sources overlap), but patterns of spatial variation occur. This model may be a useful tool in systems where source variability is predictable across some spatial axis (e.g. altitude, latitude, or river distance). The model uses the slopes of change along the spatial axis to estimate source contributions to the consumer by assuming that the consumer's isotope signature is a weighted mixture of the sources along the linear gradients. For example, aquatic and terrestrial resource pools may have the same mean isotope value across a distance gradient in river systems, but aquatic resources vary predictably with river distance (a predictable linear slope of distance *versus* δ^{13}C) while terrestrial sources remain consistent (Gray *et al*., 2011). These relationships between distance and δ^{13}C for terrestrial and aquatic resource pools allows the calculation of proportional resource contributions to the stream invertebrates (Rasmussen, 2010). The strengths of this approach are that it can overcome challenges involving overlap in resource-pool isotope values, and that it explicitly considers spatial variability. The main drawbacks are twofold. First, the proportions of the sources in a consumer's diet must be constant along the relevant gradient. Second, a detailed understanding of the underlying isotope gradients may be difficult to develop, if they exist at all, and the model will rapidly become mathematically intractable as the number of resource pools increases. As such, this approach may not be relevant in many systems.

Two other approaches also take advantage of spatial correlations to identify possible resource pools supporting consumers. Melville & Connolly (2003) sampled a consumer and its possible resource pools at many spatially distinct locations. For the isotopic niche, they calculated the Euclidean distances (*D*) between average consumer values and the resource pool averages at each location. Since the magnitude and directions of change of *D* were consistent across sampling locations, they suggested the consumer was “tracking” that resource pool and, therefore, it was an important part of the diet of that consumer. This approach does not provide estimates of the proportional contributions of sources, just an indication of which sources may or may not be important. In a similar across-site comparative approach, Vanderklift & Wernberg (2010) demonstrated, using partial regression analysis (controlling for within- and among-site source and consumer variation), that large-scale spatial variability in isotope signatures among sites can be used as a tool to identify diet sources of consumers. The strength of these two models is that they explicitly account for spatial variability in consumers and resource pools. There are two primary weaknesses. First, the models rest on the assumption that consumer diet items have unique isotope signatures and consumers have a relatively consistent, constrained, diet across sites. These factors must hold to link directly spatial variability in isotope values among source pools and consumer tissues. Second, many other ecological variables affect large-scale variability in isotope signatures, so ascertaining specific mechanisms giving rise to the isotopic niche may be difficult. These spatial-based approaches are likely to be most effective when used in conjunction with one of the aforementioned mixing models.

VI. WHAT ADDITIONAL INFORMATION DOES RELATIVE POSITION OF CONSUMERS IN ISOTOPIC SPACE REVEAL ABOUT FOOD-WEB STRUCTURE?

In addition to estimating vertical position in a web and quantifying proportional contributions of source pools to consumers, stable isotope data can provide more general depictions of food-web structure. That is, important information may be gleaned simply from relative spacing of target groups in isotopic niche space. Two general types of relative position metrics have been proposed: (1) one based on quantifying the amount of isotopic space occupied and (2) one quantifying relative change in position of target groups across temporal or spatial environmental gradients. These metrics often are applied in conjunction with trophic position and source contribution estimates to provide detailed information regarding trophic structure. However, they also may be informative even when limitations of particular data sets, e.g. lack of an adequate baseline or isotope source pools without distinct values, preclude precise calculations of trophic position and source contributions. In such instances, these two types of tools still allow for quantification of aspects of food-web structure, especially when these data are complemented with additional data sources (Layman & Post, 2008).

Layman *et al*. (2007*a*) proposed a series of metrics to quantify the area of isotopic space occupied by individuals or species. For example, the total area of a convex polygon encompassing all species within a community can be used as a measure of trophic diversity. That is, albeit with caveats associated with baseline resource pools, greater degree of isotopic niche space occupied relates to greater amount of trophic diversity among species (or individuals) in a community. In this way, overall trophic complexity is characterized by a single continuous variable which can be used to compare across systems or time periods. Likewise, other related metrics (e.g. mean nearest neighbour distance) further characterize spacing among individual data points in isotopic space, providing additional insight into trophic diversity and species packing within communities (Layman *et al*., 2007*a*).

Various modifications of these simple metrics have been proffered. For example, baseline-corrected trophic position estimates have been used instead of absolute δ^{15}N values in bivariate plots (Mercado-Silva, Helmus & Vander Zanden, 2009; Swanson, Kidd & Reist, 2010).

As mentioned above, raw isotope data also can be converted into proportion-space based on the contributions of the underlying resource pools (Newsome *et al*., 2007). With this transformation, traditional metrics (such as Shannon-Wiener diversity) may be used to compare aspects of niches across species and systems. When possible, such transformations are desirable, although they become more ambiguous with increasing numbers of potential resource pools. In fact, when resource pool diversity is substantial, as is the case in many complex food webs, the transformation to proportional space is impossible (Layman & Post, 2008).

Another set of metrics is used to quantify directional shifts within isotopic niche space. This set of approaches is based on computed vectors of the directional change between mean δ^{15}N and δ^{13}C values. Wantzen *et al*. (2002) analyzed these vectors across species using two-dimensional ANOVA. Schmidt *et al*. (2007) introduced the use of circular statistics in which changes in the angle and magnitude of vectors in isotope space can be quantified. These vector-based approaches should prove especially powerful in analyzing changes through time, reconstructing historical food-web structure, and/or predicting future food-web patterns (Mercado-Silva *et al*., 2009; Schmidt *et al*., 2007; Schmidt, Zanden & Kitchell, 2009).

Turner, Collyer & Krabbenhoft (2010) have taken area-based (Layman *et al*., 2007*a*) and directional (Schmidt *et al*., 2007) metrics a step further, specifically by using nested linear models and a residual permutation procedure to provide for a quantitative hypothesis-testing framework. Specifically, their model allows for testing of shifts in (1) location and dispersion between isotopic groups indicating potential differences in resource use and niche breath (e.g. because of ontogeny or movements between isotopically distinct habitats) and (2) magnitude and direction of changes in centroid position between isotopic samples. We recommend the quantitative approaches of Turner *et al*. (2010) be used in conjunction with the area-based

(Layman *et al*., 2007*a*) and directional (Schmidt *et al*., 2007) metrics to provide increased quantitative rigour.

The area-based (Layman *et al*., 2007*a*) and directional (Schmidt *et al*., 2007) metrics share a fundamental set of strengths and weaknesses that reflect the underlying nature of the isotope data. Both types of analytical approaches serve to reduce food-web complexity into continuous metrics, which can subsequently be compared across systems or time periods. The measures are relatively simple to compute, and provide for direct measures regarding specific aspects of trophic structure. An additional distinction is that $\delta^{15}N$ and $\delta^{13}C$ (or other elemental δ values) are simultaneously analyzed, revealing insights that may not be clear when focusing on variation in δ values for a single element. Further, intricacies of every factor affecting a single individual's isotope values (e.g. trophic discrimination) are not essential to elucidate general patterns in food-web structure.

Clear limitations of these approaches are apparent as well. First, as the number of consumer and resource species in a food web increases, sources of ambiguity become more likely. Second, comparisons among food webs become increasingly problematic as food webs become more dissimilar. For example, comparisons of niche width are not as meaningful when the focal food webs have very different basal resource pools (e.g. comparing a lake to a grassland food web). Third, the metrics are also especially sensitive to the sources of ecological ambiguity we introduced in the Section II. For example, similar food-web structures can give rise to very different metric values if the two webs have resource pools with underlying differences in relative δ values (although, in some cases, it may be possible to scale baseline variation among the food webs being compared). Finally, two issues apply explicitly to the convex hull-based measures (Layman, 2007). Sample sizes of the groups being compared can cause interpretation problems because the hull area will tend to increase with number of individual samples (Jackson *et al*., 2011), yet this can be addressed by running bootstrap procedures to ensure sample size is sufficient to characterize fully the isotopic niche (Vaudo & Heithaus, 2011). Finally, a few individual outliers may result in a relatively large convex hull in which much of the contained niche space is unoccupied. In such a case, evaluating the relative merits of different ways to characterize the isotopic niche is warranted (see discussion of convex hulls *versus* Bayesian ellipse models in Section VII.1). In summary, the quantitative metrics discussed in this section have a series of caveats but, if qualified appropriately and augmented with additional sources of data, provide useful insight into particular aspects of food-web structure.

VII. WHAT IS THE DEGREE OF TROPHIC VARIABILITY AT THE INTRAPOPULATION LEVEL?

(1) General approaches

There has been much renewed interest in the role of intrapopulation niche variation (Araújo, Bolnick & Layman, 2011; Bolnick *et al*., 2011), with stable isotopes emerging as one of the primary tools for analysis. Many of the techniques used to examine trophic structure at the intrapopulation level are extensions of those used to examine the overall structure of food webs (see above), with intrapopulation groups defined using categories of sex, stage of maturity, or habitat use. In fact, intraspecific variation in resource use was among the first applications of stable isotopes in food-web ecology. For example, Fry *et al*. (1978) showed that variance of individuals' $\delta^{13}C$ values was very low for some grasshopper species indicating no among-individual diet variation, whereas in other species variance was relatively large, suggesting that individuals consistently fed on either C3 or C4 plants. More recently, Martinez del Rio *et al*.

(2009*a*) adapted the Schmidt *et al.* (2007) vector-based approach, showing great variation in the magnitude and direction of changes in the isotopic niche of individual ovenbirds across seasons.

Two main categories of inquiry encompass many of the stable isotope applications in this context. First, many analyses are structured around using simple statistical tools (e.g. *t*-tests, ANOVA, linear regression) to examine ontogenetic diet shifts within populations. Specifically, $\delta^{15}N$ is used as a proxy to assess shifts in trophic position through ontogeny. For example, Jennings *et al.* (2002) examined the relationship between body size and trophic position for 31 fish taxa in the North Sea, and demonstrated the prevalence of increasing trophic position through ontogeny for most species. Second, stable isotopes are used to estimate relative niche width of populations, typically by analyzing individual-level dispersion. Approaches include range or variance in $\delta^{13}C$ and $\delta^{15}N$ (Bearhop *et al.*, 2004; McClellan *et al.*, 2010; Willson *et al.*, 2010), convex hulls calculated at the individual level (Layman *et al.*, 2007*b*; Quevedo, Svanback & Eklov, 2009), relative spacing among individuals (Martinez del Rio *et al.*, 2009*a*), two-dimensional confidence intervals based on mean $\delta^{13}C$ and $\delta^{15}N$ (Layman, Winemiller & Arrington, 2005*a*), and standard ellipse areas (SEA_B), i.e. bivariate equivalents to standard deviations in univariate analysis (Jackson *et al.*, 2011).

Each of these aforementioned approaches has strengths and weaknesses, and we provide one comparison as an example, i.e. between convex hulls (Layman *et al.*, 2007*b*) and the recently developed Bayesian approach (Jackson *et al.*, 2011). The convex hull approach is powerful because it incorporates each individual sampled, and thus includes information about every part of isotopic niche space occupied. Conversely, the Bayesian approach is targeted at niche widths of “typical” members in a population, which could be viewed as the mean or core isotopic niche of that population (Jackson *et al.*, 2011). The Jackson *et al.* (2011) approach generates standard ellipse areas as this core isotopic niche representation (SEA_B). Either the Bayesian approach or convex-hull-based quantitative analysis (Turner *et al.*, 2010) may be more appropriate with respect to a particular question of interest and/or the nature of the underlying data set. Convex hulls may be more appropriate when individual-level niche variation, and thus every niche position occupied by individuals, is central to the focal research question (Layman *et al.*, 2007*b*). When core aspects of a population's isotopic niche are of most interest, other prior information is available (e.g. on trophic discrimination rates), or error propagation is desirable, then the recently developed Bayesian-based approaches are preferable to characterize niche widths (Jackson *et al.*, 2011). In some cases, utilization of both of these analytical approaches may be desirable to reveal different aspects of trophic structure.

(2) Numerical simulations

Another research area that has developed rapidly in recent years has been examining incidence and causes of individual specialization, i.e. variation in resource use among individuals that is not attributable to age class, size or sex (Araújo *et al.*, 2011; Bolnick *et al.*, 2003, 2011). Individual specialists utilize a relatively narrow subset of the population's overall resource base so that there is substantial variability in the specific resources used among individuals. Such variation among individuals may have several evolutionary and ecological implications, including driving frequency-dependent disruptive selection (Bolnick *et al.*, 2011; Dieckmann & Doebeli, 1999; Roughgarden, 1972) or imparting population stability (Agashe, 2009; Bolnick *et al.*, 2011; Lomnicki, 1999). Quantification of individual specialization within populations would ideally be based on longitudinal samples in which the same individuals' diets are sampled repeatedly over time (Bryan & Larkin, 1972; Estes *et al.*, 2003; Werner & Sherry, 1987; West & Williams, 1986). However, in most cases, such longitudinal sampling schemes are too difficult to implement. Because of the time- and space-integrated insight provided by isotopes, they have become the primary way to investigate instances of individual specialization (Araujo *et al.*, 2007; Beaudoin *et al.*, 1999; Bolnick *et al.*, 2007; Cherel *et al.*, 2007; Herman *et al.*, 2005), and a new set of analytical tools have been developed to this end.

Matthews & Mazumder (2004) proposed a null-model approach to test directly for specialization, incorporating information from source pools that could otherwise lead to erroneous interpretations of consumer isotope values. That is, ostensible sources of dietary specialization can be inferred directly from measures of variation in individual isotope values and by carefully selecting among consumer tissues. But for a given degree of individual specialization, populations using resources that span a wider range of $\delta^{13}C$ or $\delta^{15}N$ will show higher variability in consumer isotopes (Bearhop *et al.*, 2004; Newsome *et al.*, 2007). Matthews & Mazumder (2004) used a null model approach in which individuals sample randomly from a common resource pool to generate a null distribution of variances in $\delta^{13}C$ among individuals. Empirical $\delta^{13}C$ variance is tested against the null distribution, providing a statistical test for the presence of individual specialization.

A subsequent advance was to quantify the degree of individual specialization in a population, not just identify its presence/absence. Araújo *et al.* (2007) proposed a method that uses the variance in individual $\delta^{13}C$ values in a population of consumers, and the $\delta^{13}C$ values of resources, to calculate two indices of individual specialization that can be compared across different populations (Bolnick *et al.*, 2002). In this approach, null populations with varying degrees of individual specialization are generated, for which both isotope variances and indices of individual specialization are calculated. A curve relating the expected isotopic variances and indices of individual specialization is built and used to interpolate a measure of individual specialization given an empirical variance in isotopes. This approach is especially useful for taxa which have relatively few items in their stomachs at any single time (e.g. piscivorous fish), for which estimations of dietary specialization would not be possible with direct diet analysis alone. The power of this approach has been illustrated in its first applications on frogs and birds (Araújo *et al*., 2007; Woo *et al*., 2008). However, this model has increased data input requirements, specifically, direct dietary information that corresponds to the time period that the isotope values of consumers and sources reflect. Again, collecting appropriately matched diet and isotope data sets can be difficult, especially as temporal and spatial heterogeneity in consumers (e.g. prey selection) and resources (e.g. seasonal variation in isotope values) increases. Such a null-model approach, however, is essential to identify true dietary specializations and should be employed in all cases in which isotope data are used directly to infer the degree of dietary specialization within a population.

Bayesian mixing models also can be used to examine niche variation and individual specialization within populations. The model of Semmens *et al*. (2009*b*) allows the partitioning of diet variation at different levels (e.g. individuals, sexes, morphs, age classes), providing insight that is not possible with other methods. The Bayesian framework also allows for incorporation of sources of variability that are not caused by diet variation, such as isotope variation within resources or variation in discrimination values among individual consumers. For populations of gray wolves (*Canis lupus*) in British Columbia, the model was used to show that not only do populations' diets differ because of geographic location, but diets also differed among packs and among individuals within packs (Semmens *et al*., 2009*b*). It is possible to use the outputs of Bayesian mixing models to identify more broad patterns of specialization. For example, SIAR was recently used to define a set of foraging specialists from a population of

Northern Gannets *Morus bassanus,* and the output was linked to differences in fitness and foraging tactics among the specialist group (Votier *et al*., 2010). Such ability to partition variance in isotope values across different hierarchical levels holds much potential.

(3) Different tissue types

Stable isotopes can also be used to track changes in individual-level resource use over time (Bearhop *et al*., 2004; Hobson, 1993; Tieszen *et al*., 1983). First, some tissues, such as hairs, feathers, and the dentine of teeth, are metabolically inert once they are deposited and therefore represent the isotope signature of a consumer's diet at the time of deposition. If the rate of tissue deposition is known, these tissues represent a timeline of the consumer's dietary history. For example, Hobson & Sease (1998) documented ontogenetic isotope shifts in Steller sea lions (*Eumetopias jubatus*) from tooth annuli. A more quantitative approach was proposed by Newsome *et al*. (2009) using small sections of Californian sea otter *Enhydra lutris nereis* whisker as a temporal series of resource use. They applied an ANOVA model to partition the variance in isotopes into a within-individual component (WIC, variation within an individual sea otter whisker) and a between-individual component (BIC, measured by differences between individual sea otter whiskers). Similarly, Jaeger *et al*. (2010) collected multiple feathers from individual seabirds to estimate Roughgarden's (1974) index of individual specialization (WIC/TNW where TNW is the total niche width of the seabird population), using the variation within an individual's feathers as an estimate of WIC and the total variation among individuals' feathers as an estimate of TNW. For such studies, some information on the nature of the inert tissue's deposition, e.g. whether it is continuous (e.g. the whiskers of some mammal species) or discontinuous (e.g. feathers), is necessary for appropriate analysis.

An alternative approach is based on the fact that different tissues have different turnover rates and therefore integrate resource use over different time scales (Hesslein, Hallard & Ramlal, 1993). For example, vertebrate blood plasma integrates diets over a time scale of days to weeks, whereas turnover in muscle tissue is on the scale of months (Dalerum & Angerbjorn, 2005; MacNeil, Drouillard & Fisk, 2006; Phillips & Eldridge, 2006). As a consequence, individuals that feed consistently on the same resource(s) over long time scales should have similar isotope values in tissues with different turnover rates, whereas individuals that switch their diets over time should show a mismatch between fast and slow turnover tissues. Martinez del Rio *et al.* (2009*a*) called the former "isotopic specialists" and the latter "isotopic generalists". They applied this framework to three species of ovenbirds and found that one species was made up of isotopic generalists that switch diets seasonally, another species had a mix of isotopic generalists and specialists, and the third species was composed of isotopic specialists. Likewise, Matich, Heithaus & Layman (2010) used different tissues to quantify differences in the degree of dietary specialization between bull sharks (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*). Importantly, information regarding turnover rates in the different tissues is needed to make inferences about the degree of dietary specialization. These approaches are especially sensitive to assumptions regarding isotopic routing and different discrimination factors among tissue types.

A general concern for all the methods outlined herein relates to the temporal and spatial scales at which individuals are sampled. Sampling individuals at different times or different locations might artificially inflate variation in isotope values if sources vary temporally and/or spatially. For example, individuals specialized on the same resource but feeding consistently in different areas may differ greatly in isotope values if there is spatial heterogeneity in resource isotope values, so that habitat-derived variation in consumers' isotopes will be mistaken as diet variation (Flaherty & Ben-David, 2010). As is often the case, knowledge of the temporal and spatial variation in sources' isotopes, as well as organism natural history, will greatly aid in the interpretation of isotope data.

VIII. CONCLUSIONS

(1) Quantitative analytical approaches for applying stable isotope data have proliferated rapidly over the past decades. The numerous choices for analyzing data bode well for the continued development of stable isotope analysis of food-web structure. We hope this review provides one framework from which researchers can select the most appropriate tools for particular questions of interest. Moving forward, we suggest the guidelines listed below for practitioners in the field using the analytical approaches discussed herein.

(2) Stable isotope analysis is not a substitute for a basic understanding of the natural history of the organism or ecosystem of interest. Stable isotopes are an important tool that can be used to provide insight into food-web structure, but these data alone cannot elucidate the complexities that are manifest in food webs.

(3) There are still huge gaps in the empirical data needed to support analytical approaches, including data on isotope incorporation rates and routing into tissues, tissue turnover rates, and trophic discrimination factors (Martinez del Rio *et al*., 2009*b*). Additional field and laboratory experiments are needed to this end.

(4) All models are beholden to the quality of input data available. Further, even basic stable isotope calculations that are well accepted in the literature, e.g. estimates of trophic position and food-chain length, should be qualified appropriately because of the lack of the underlying empirical data (e.g. on trophic discrimination and adequate baselines) necessary to produce adequate estimates.

(5) The validity of assumptions underlying analytical models will vary depending on the organism or system of interest. For example, the assumption that isotopic routing does not occur is especially problematic for omnivores (Kelly & Martinez del Rio, 2010; Martinez del Rio *et al.*, 2009b; Voigt *et al.*, 2008). As such, the appropriateness of a specific analytical approach must be evaluated on a case-by-case basis. By applying corrections, e.g. for concentration dependence or increasing uncertainty in the trophic discrimination factors, it is becoming increasingly possible to deal with these issues.

(6) Many of the ecological questions discussed herein are necessarily reliant on the fact that source pools must have distinct isotope values. When sources are not distinct (or overlap to some degree), stable isotopes may have little utility in answering questions about trophic relationships.

(7) Both source and consumer pools must be sampled on proper spatial and temporal scales that reflect the relative incorporation and turnover rates of tissues. Establishing appropriate isotope end members and baselines remains of core importance for many of the analytical techniques.

(8) Because of the different underlying structure of analytical models, multiple approaches on the same data set are often warranted. Different analytical approaches may give rise to conflicting

output data, the magnitude and importance of which will vary based on the nature of the underlying data sets (Ikeda *et al*., 2010; Maier & Simenstad, 2009; Moore & Semmens, 2008).

(9) While it is always tempting to favour approaches that provide analytical solutions, it is important to think about what the associated assumptions and simplifications might mean biologically.

(10) When possible, stable isotope analysis should always be augmented with additional data sets, particularly diet analysis or other data on feeding behaviour (Layman & Post, 2008). In fact, many of the approaches discussed herein require specific information on consumer's diets to parameterize models, *a priori* reduce the number of potential source pools, and define priors in Bayesian models. Stable isotope data in isolation cannot provide answers for all questions regarding food-web structure, and traditional dietary analysis will continue to be a core tool.

(11) In addition to diet data, stable isotopes used in conjunction with other dietary tracers (fatty acids, Boecklen *et al*., in press; Budge *et al*., 2008; Cheung & Sanyal, 2010) will likely provide new insights into food-web structure. Technological advances are expanding the potential suite of tools that can be employed. Compound-specific isotopic analysis may be one of the most important areas of future development (Chikaraishi, Ogaw & Ohkouchi, 2009*b*; Chikaraishi *et al*., 2009*a*; McMahon *et al*., 2010; Boecklen *et al*., in press).

(12) Our views of food-web structure have, until recently, been largely constrained to examining population "means". Stable isotopes have been a core tool in elucidating the importance of intrapopulation niche variation (Araujo *et al*., 2007; Layman *et al*., 2007*b*; Quevedo *et al*., 2009). Results of increasingly powerful analyses of stable isotope data sets will help reveal when intrapopulation niche variation is necessary to characterize adequately food-web structure.

(13) Stable isotopes, irrespective of the way they are analyzed, provide information regarding the flow of energy or nutrients through food webs. They do not provide definitive information as to the functional relationships among organisms (e.g. whether a predator controls the abundance of a given prey), information that typically necessitates controlled experimental manipulations. This distinction between energy flow and interaction food-web models must always be considered (Paine, 1980; Polis & Winemiller, 1996).

(14) The main caveat to using stable isotopes in a food-web context is that the data are only indirect indicators of feeding pathways. Nevertheless, the diversity and scope of papers included in this review reflects the important advances that stable isotopes have provided in food-web ecology. As analytical approaches become more advanced, stable isotopes should provide for many more important developments in the field.

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Fig. 1. Number of food web papers, as cataloged by *Web of Science,* employing stable isotopes published each year. Papers were identified using the key words "food web" and "isotope" or "diet" and "isotope".