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

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19 ABSTRACT

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

33

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

36

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62 I. INTRODUCTION

63 Stable isotope analysis has emerged as one of the primary means to analyze the structure
64 of food webs. Stable isotopes are especially useful because they provide time- and space-
65 integrated insights into trophic relationships among organisms, and thus can be used to develop
66 models of trophic structure. Many of the first applications of stable isotope data in a food-web
67 context were critical advances, although largely qualitative, providing for broad inferences based
68 on relative isotope values of consumers and resources (Haines & Montague, 1979; Peterson,
69 Howarth & Garritt, 1985; Zieman, Macko & Mills, 1984). Over the past 10 years, a series of
70 more quantitative approaches for analyzing stable isotope data has emerged. These approaches
71 have dramatically improved our understanding of food webs, for example, providing new insight
72 into food-chain length (Post, Pace & Hairston, 2000), niche variation (Martinez del Rio *et al.*,
73 2009a; Moore & Semmens, 2008; Semmens *et al.*, 2009b; Votier *et al.*, 2010), and human-driven
74 shifts in community structure (Layman *et al.*, 2007b; Schmidt *et al.*, 2007).

75 The emergence of new analytical approaches has led to some debate about which
76 method(s) is most appropriate to apply to stable isotope data (Hoeinghaus & Zeug, 2008;
77 Jackson *et al.*, 2009; Layman & Post, 2008; Newsome *et al.*, 2007; Semmens, Moore & Ward,
78 2009a). At times, this discussion has focused on which analytical approach is “right” or
79 “wrong”. But a more useful perspective is recognizing the specific types of questions for which
80 different approaches are best suited. Analogously, use of stomach contents to evaluate dietary
81 breadth has some very well-understood limitations (Votier *et al.*, 2003), but still provides critical
82 insight into feeding relationships. Likewise, each stable isotope analytical approach has distinct
83 strengths and weaknesses (Table 1), and each is more or less appropriate under specific
84 circumstances. Information regarding these strengths and weaknesses is scattered among dozens
85 of papers in the field, often rendering direct comparison among techniques difficult. Herein we
86 provide a comprehensive review of these diverse approaches, structured around four core
87 ecological questions: (1) what is the trophic position of an organism in a food web; (2) which
88 resource pools support consumers; (3) what additional information does relative position of
89 consumers in isotopic space reveal about food web structure; (4) what is the degree of trophic
90 variability at an intrapopulation level?

91 This review is not intended to be a comprehensive catalogue of every food-web study that has
92 employed stable isotopes, an endeavour which would be a monumental task given the rapid
93 proliferation of such studies (Fig. 1). Instead, we emphasize those papers that are paradigmatic
94 with respect to a particular analytical approach, as well as some of the most recent contributions
95 to the field. Not expanded upon in this review are the many additional types of information that
96 are necessarily relevant in interpreting isotope data sets (e.g. trophic discrimination factors,
97 isotopic routing, tissue turnover rates, lipid extraction, etc.), as other reviews have discussed

98 these topics thoroughly (e.g. Bearhop *et al.*, 2004; Boecklen *et al.*, in press; Martinez del Rio *et*
99 *al.*, 2009b; McCutchan *et al.*, 2003; Oppel & Powell, 2011; Phillips & Eldridge, 2006; Post *et*
100 *al.*, 2007; Vanderklift & Ponsard, 2003). Our goal is to provide a single source that outlines
101 analytical approaches currently being applied to answer questions about food-web structure, and
102 provide guidelines as to which approaches are most appropriate with respect to a particular data
103 set or question of interest.

104 **II. STABLE ISOTOPE RATIOS AND FOOD WEBS**

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

121 Revenaugh, 2003), or across smaller-scale environmental gradients (Deines, Wooller & Grey,
122 2009; Finlay, Doucett & McNeely, 2010; Solomon *et al.*, 2011, 2009). Newsome *et al.* (2007)
123 and Oulhote *et al.* (2011) provide additional information regarding the insights that can be
124 gleaned from various isotope tracers.

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

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

140 Stable isotope values are a product not only of the actual trophic interactions, but are also
141 driven by myriad underlying biological and chemical processes. For example, when isotopic
142 routing occurs, i.e. when elemental isotopes from resources are broken down and assimilated
143 differently among consumer tissue types, direct interpretation of the underlying trophic

144 relationships may be more problematic (Martinez del Rio *et al.*, 2009b). In such a case, a
145 consumer tissue does not reflect isotopic composition of the bulk diet, but rather the isotopic
146 composition of the nutrient component of the diet from which the tissue was synthesized. This is
147 especially important to consider when an individual consumes diverse resources (for example,
148 feeding on both plants and animals, Kelly & Martinez del Rio, 2010; Martinez del Rio *et al.*,
149 2009b; Voigt *et al.*, 2008). Ignoring such biochemical processes driving variation in stable
150 isotope values can result in biased interpretations of trophic interactions.

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

166 All of the analytical approaches discussed herein deal with either raw δ values, or values
167 that are transformed to represent a specific ecological variable (e.g. trophic position or dietary
168 proportions from different source pools) (Newsome *et al.*, 2007). Analysis of raw δ values
169 allows inferences regarding feeding relationships, but can be especially sensitive to the relative δ
170 values of source pools. For example, broad dispersion among consumers in a $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$
171 biplot would seem to imply diverse resource use among individuals, but this pattern may also be
172 a result of high variance in isotope values of source pools. To this end, δ space can be
173 transformed to “proportional” space using isotope values of known source pools (Newsome *et*
174 *al.*, 2007). Similarly, raw $\delta^{15}\text{N}$ values can be converted to direct trophic position estimates using
175 assumed values of $\delta^{15}\text{N}$ discrimination with trophic transfers, as well as adequate
176 characterization of isotopic baselines (Post, 2002*b*). Such transformations are often preferred
177 because they are more ecologically meaningful than raw δ values. For example, transforming
178 $\delta^{15}\text{N}$ values into trophic positions converts them into an actual characteristic of the organism.
179 But such transformations require considerable additional *a priori* information, including
180 temporally and spatially appropriate estimates of isotopic baselines and end members, as well as
181 trophic discrimination factors. If this information is not available or of poor quality, the
182 transformations may not accurately describe aspects of trophic structure.

183

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

185 In a food-web context, many of the first applications of stable isotope data were largely
186 qualitative, i.e. making general inferences from the relative isotopic values of consumers and/or
187 resources (Fry, Joern & Parker, 1978; Haines & Montague, 1979; Peterson *et al.*, 1985; Zieman

188 *et al.*, 1984). For example, Peterson *et al.* (1985) suggested the fundamental importance of
189 *Spartina alterniflora* grass for marsh consumers by qualitatively comparing $\delta^{34}\text{S}$ and $\delta^{13}\text{C}$ values
190 in producer and consumer tissue. Haines & Montague (1979) took a similar approach, using the
191 variation in $\delta^{13}\text{C}$ among estuarine primary producers qualitatively to infer the most important
192 sources for various estuarine consumer species. Hobson & Welch (1992) provided one of the
193 first insights into the general structure of Arctic food webs using isotope values. All of these
194 initial advances were critical to laying the foundation for the myriad stable isotope research
195 programs that are now a fundamental part of the ecological sciences.

196 A logical progression from these early contributions was to apply basic statistics [e.g. *t*-
197 tests, analysis of variance (ANOVA), multivariate models, etc.] to compare mean $\delta^{13}\text{C}$ and/or
198 $\delta^{15}\text{N}$ values among groups, sites or time periods (Oulhote *et al.*, 2011). Some type of basic
199 statistical comparison can be found in almost any current stable isotope paper in the field.
200 Simple statistics provide the basic framework for interpreting isotope data, but can be limited as
201 to the depth of ecological insight that they can provide. Often, basic statistics are used in
202 conjunction with various other approaches outlined herein. Basic statistical approaches
203 obviously were not developed for isotope data *per se*, so we focus the remainder of this review
204 on analytical approaches that are targeted for isotope data sets specifically.

205

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

207 **(1) Species-specific baselines**

208 One of the most important initial advances beyond basic statistics came from the
209 realization that $\delta^{15}\text{N}$, because of the discrimination that occurs with trophic transfers, could be

210 used as a proxy for trophic position (DeNiro & Epstein, 1981; Minagawa & Wada, 1984). In this
211 context, $\delta^{15}\text{N}$ provided for a continuous measure of trophic position, a notable difference from
212 simply assigning organisms to discrete trophic levels based on natural-history observations.

213 Although early studies used the untransformed $\delta^{15}\text{N}$ values as a measure of trophic position, later
214 work recognized that $\delta^{15}\text{N}$ is influenced by local biogeochemistry (baseline variation), trophic
215 discrimination and the trophic position of an organism. Researchers have taken two approaches
216 to address baseline variation: (1) using species-specific baselines to estimate relative trophic
217 shifts and (2) using long-lived organisms or time-series baselines to estimate trophic position of
218 higher order consumers.

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

231 **(2) Long-lived consumers as baselines**

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

250 Trophic position estimates are perhaps the most widely reported metric in food-web
251 studies employing stable isotopes. But these measures are characterized by fundamental
252 limitations that are often not appreciated when trophic positions of individuals are calculated.
253 First, trophic position calculation is dependent on establishing an adequate baseline. In some
254 fresh-water ecosystems, basal resources are relatively easily isolated at a coarse level (e.g. seston

255 and benthic microalgae in northern U.S. lakes; Post, 2002b), or can be aggregated into
256 ecologically meaningful categories (e.g. autochthonous *versus* allochthonous pools in rivers;
257 Layman *et al.*, 2005b). But as food webs become more complex, and the number of potential
258 basal resource pools increases, establishing an adequate baseline becomes more problematic. In
259 systems with resource pools that have numerous and variable $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, it may be
260 extremely difficult to establish an accurate baseline using just a few isotopes, rendering any
261 trophic position estimates problematic (Layman, 2007). Second, and equally important, is
262 identifying $\delta^{15}\text{N}$ discrimination values for each trophic transfer (Martinez del Rio *et al.*, 2009b).
263 Discrimination provides the stepwise correction that allows one to convert baseline isotope
264 values into a trophic position for a consumer. Typically, this value is chosen based on available
265 meta-analyses (Caut, Angulo & Courchamp, 2009; McCutchan *et al.*, 2003; Post, 2002b;
266 Vanderklift & Ponsard, 2003), but numerous physiological and environmental factors can affect
267 discrimination in $\delta^{15}\text{N}$ (Martinez del Rio *et al.*, 2009b). Values from the meta-analyses are valid
268 approximations when averaged over a large number of trophic pathways, as is done for
269 estimating food-chain length (Post, 2002a). But when used for estimating the trophic position of
270 individuals or single species, literature values can prove misleading, and should be used with
271 caution, until the causes of variation in trophic discrimination are better understood (Martinez del
272 Rio *et al.*, 2009b). Until recently, few studies propagated such error in assumed values in
273 calculations (but see Vander Zanden & Rasmussen, 2001). Resampling from distributions of
274 baselines and trophic discrimination factors to produce ranges of estimates for trophic position,
275 with an associated error term, is now more commonly employed (including the frequently used
276 Bayesian models, see Section V.3). Such estimates are more accurate depictions of possible
277 solutions that account for potential variation in discrimination factors (Jackson *et al.*, 2011).

278

279 V. WHICH RESOURCE POOLS SUPPORT CONSUMERS?

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

292

293 (1) Geometric approaches

294 Early mixing models used geometric methods to estimate the proportional contribution of
295 three or more food resources to a consumer's diet using δ values (BenDavid, Flynn & Schell,
296 1997; Kline *et al.*, 1993; Peterson & Howarth, 1987; Whitley & Rabeni, 1997). Euclidean
297 distances between consumer and sources were calculated in isotopic niche space, and an inverse
298 relationship was assumed between these distances and the relative contribution of each source to
299 the consumer's diet. Although this method provides a visually appealing graphical representation

300 of dietary contribution and is a useful heuristic tool (BenDavid *et al.*, 1997; Kline *et al.*, 1993;
 301 Peterson & Howarth, 1987; Whitley & Rabeni, 1997), Phillips (2001) demonstrated that the
 302 equations used in these approaches failed accurately to identify dietary contributions. Euclidean
 303 methods underestimate commonly used food sources and overestimate rare food sources, and the
 304 equations provide inaccurate estimates when a consumed resource is excluded from the analysis.
 305 These Euclidean-based approaches have largely been supplanted by other mixing-model
 306 approaches (Phillips, 2001), but are still employed in isolated cases (e.g. Wengeler, Kelt &
 307 Johnson, 2010).

308 (2) Linear mixing models

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

$$319 \quad \delta^{13}\text{C}_T = f_A \delta^{13}\text{C}_A + f_B \delta^{13}\text{C}_B + f_C \delta^{13}\text{C}_C, \quad (1)$$

$$320 \quad \delta^{15}\text{N}_T = f_A \delta^{15}\text{N}_A + f_B \delta^{15}\text{N}_B + f_C \delta^{15}\text{N}_C, \quad (2)$$

$$321 \quad f_A + f_B + f_C = 1, \quad (3)$$

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

330 Most food webs are too complex to use simple linear mixing models because the number
331 of source pools exceeds the number of useful isotope tracers by more than one. When this is the
332 case, we move from a mathematically determined system to a mathematically undetermined
333 system. The latter implies that there are multiple feasible solutions for combinations of source
334 contributions. To this end, Phillips & Gregg (2003) developed the model IsoSource, which has
335 become one of the most common analytical tools in the field. The model does not generate exact
336 values for proportional contributions of each source, but instead provides a range of possible
337 contributions or feasible solutions. The model examines every possible combination of source
338 proportions (summing to 100%) incrementally (typically in increments of 1%), then calculates
339 the predicted isotope value for each combination using linear mass-balance equations. These
340 predicted values are then examined to determine which ones fall within some tolerance range
341 (typically 0.1‰) of the observed consumer isotope value, and all of these feasible solutions are
342 recorded. One of the main advantages of this model, besides its public availability and ease of
343 use, is the relatively limited amount of input data required (average isotope values of the
344 consumer and potential sources). Additionally, the model can be adjusted further to consider

345 source pooling (Phillips, Newsome & Gregg, 2005). As with all mixing models, a series of
346 critical assumptions must be made, and these will be discussed in detail below. But perhaps the
347 most common problem with studies employing IsoSource is not related to the intrinsic structure
348 of the model, but instead, to interpretation of its output. That is, researchers often interpret some
349 measure of central tendency (e.g. the median or mode) as the definitive solution, a conclusion
350 which is clearly not justified by the structure of the model (Phillips & Gregg, 2003).

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

368 availability and ease of use of the IsoSource software, it is used much more frequently than
369 SOURCE, STEP, Moore-Penrose, or LP models.

370 **(3) Bayesian mixing models**

371 A major limitation of all of the above mixing models (apart from Isoerror) is that they do
372 not incorporate uncertainty and variation in input parameters (such as variation within source
373 pools or trophic discrimination factors). In other words, much of the inherent variability in
374 natural systems is ignored by use of mean resource isotope values or assumed trophic
375 discrimination values. To this end, models (e.g. MixSIR and SIAR) have emerged, all of which
376 are based on a series of related linear equations, that utilize Bayesian statistical techniques to
377 identify proportional contributions of source pools (Jackson *et al.*, 2009; Moore & Semmens,
378 2008; Parnell *et al.*, 2010; Solomon *et al.*, 2011). Importantly, these approaches allow for
379 incorporation of available prior information, thereby allowing for more realistic representations
380 of variability in input terms. Outputs from the Bayesian models are in the form of true
381 probability distributions, not just summaries of all feasible solutions. As such, unlike in
382 IsoSource, measures of central tendency from the outputs can be used in subsequent analyses
383 (Parnell *et al.*, 2010). Further, parameter transformations, as suggested by Semmens *et al.*
384 (2009b), provide a framework for utilization of general linear model approaches. This allows for
385 incorporation of fixed and random covariates into models, which can provide the ability to
386 partition particular drivers of source contribution variation (Francis *et al.*, 2011). Largely
387 because of the additional input data, the models often substantially narrow the reported ranges of
388 source pool contributions to consumers (Moore & Semmens, 2008; Moreno *et al.*, 2010).
389 Bayesian approaches are evolving rapidly, greatly expanding in capability and scope (Jackson *et*
390 *al.*, 2011; Ward *et al.*, 2011), and are being applied to yield novel insights into aspects of trophic

391 structure (e.g. Francis *et al.*, 2011; Rutz *et al.*, 2010; Solomon *et al.*, 2011). As with IsoSource,
392 the Bayesian models MixSIR and SIAR can be freely accessed online (Moore & Semmens,
393 2008; Parnell *et al.*, 2010).

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

407 It is important to recognize that all mixing models, including Bayesian-based approaches,
408 are not a quick fix or a substitute for poor sampling strategy; moreover, they are not particularly
409 useful for asking questions about systems where complementary information is largely lacking.
410 Indeed, all of the mixing models described above are subject to a core set of limitations, with
411 many of the guidelines in Section II applying here. First, some information on turnover rate,
412 trophic discrimination and macronutrient composition (e.g. free lipid and carbonate content)
413 associated with the consumer tissues is needed. Second, prey sources must have different

414 isotope values. The more similar the resource pool isotope values, the less power the models
415 have to delineate proportional contributions. Third, in many cases, *a priori* grouping may be
416 necessary to constrain model outputs (Phillips *et al.*, 2005; but Bayesian approaches may also be
417 useful in this respect, see Ward *et al.*, 2011), a decision that requires extensive knowledge of the
418 basic natural history of the system. Fourth, prey should ideally be sampled on a time frame that
419 coincides with the period during which the consumer tissue is synthesized, and all prey items
420 must be known in order to provide the most meaningful results (although SIAR has an additional
421 error term whereby the solution is not constrained to be merely a function of the identified
422 sources, which would allow for some unknowns to be incorporated into the model). Fifth, as
423 spatial and temporal variability in source pool values increases, so does the sampling effort
424 necessary to determine adequately the appropriate input mean (and standard deviation in
425 Bayesian models) values that should be used. As with any model, Bayesian tools such as
426 MixSIR and SIAR are especially sensitive to the quality of the input data (Moore & Semmens,
427 2008). Finally, inclusion of prior information into models can lead to more uncertain outputs,
428 depending on the nature of input data (Moore & Semmens, 2008).

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

436 **(4) Spatially based approaches**

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

457 Two other approaches also take advantage of spatial correlations to identify possible
458 resource pools supporting consumers. Melville & Connolly (2003) sampled a consumer and its
459 possible resource pools at many spatially distinct locations. For the isotopic niche, they

460 calculated the Euclidean distances (D) between average consumer values and the resource pool
461 averages at each location. Since the magnitude and directions of change of D were consistent
462 across sampling locations, they suggested the consumer was “tracking” that resource pool and,
463 therefore, it was an important part of the diet of that consumer. This approach does not provide
464 estimates of the proportional contributions of sources, just an indication of which sources may or
465 may not be important. In a similar across-site comparative approach, Vanderklift & Wernberg
466 (2010) demonstrated, using partial regression analysis (controlling for within- and among-site
467 source and consumer variation), that large-scale spatial variability in isotope signatures among
468 sites can be used as a tool to identify diet sources of consumers. The strength of these two
469 models is that they explicitly account for spatial variability in consumers and resource pools.
470 There are two primary weaknesses. First, the models rest on the assumption that consumer diet
471 items have unique isotope signatures and consumers have a relatively consistent, constrained,
472 diet across sites. These factors must hold to link directly spatial variability in isotope values
473 among source pools and consumer tissues. Second, many other ecological variables affect large-
474 scale variability in isotope signatures, so ascertaining specific mechanisms giving rise to the
475 isotopic niche may be difficult. These spatial-based approaches are likely to be most effective
476 when used in conjunction with one of the aforementioned mixing models.

477

478 **VI. WHAT ADDITIONAL INFORMATION DOES RELATIVE POSITION OF** 479 **CONSUMERS IN ISOTOPIC SPACE REVEAL ABOUT FOOD-WEB STRUCTURE?**

480 In addition to estimating vertical position in a web and quantifying proportional
481 contributions of source pools to consumers, stable isotope data can provide more general
482 depictions of food-web structure. That is, important information may be gleaned simply from

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

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

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

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

513 Another set of metrics is used to quantify directional shifts within isotopic niche space.
514 This set of approaches is based on computed vectors of the directional change between mean
515 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values. Wantzen *et al.* (2002) analyzed these vectors across species using two-
516 dimensional ANOVA. Schmidt *et al.* (2007) introduced the use of circular statistics in which
517 changes in the angle and magnitude of vectors in isotope space can be quantified. These vector-
518 based approaches should prove especially powerful in analyzing changes through time,
519 reconstructing historical food-web structure, and/or predicting future food-web patterns
520 (Mercado-Silva *et al.*, 2009; Schmidt *et al.*, 2007; Schmidt, Zanden & Kitchell, 2009).

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

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

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

541 Clear limitations of these approaches are apparent as well. First, as the number of
542 consumer and resource species in a food web increases, sources of ambiguity become more
543 likely. Second, comparisons among food webs become increasingly problematic as food webs
544 become more dissimilar. For example, comparisons of niche width are not as meaningful when
545 the focal food webs have very different basal resource pools (e.g. comparing a lake to a grassland
546 food web). Third, the metrics are also especially sensitive to the sources of ecological ambiguity
547 we introduced in the Section II. For example, similar food-web structures can give rise to very
548 different metric values if the two webs have resource pools with underlying differences in
549 relative δ values (although, in some cases, it may be possible to scale baseline variation among
550 the food webs being compared). Finally, two issues apply explicitly to the convex hull-based
551 measures (Layman, 2007). Sample sizes of the groups being compared can cause interpretation

552 problems because the hull area will tend to increase with number of individual samples (Jackson
553 *et al.*, 2011), yet this can be addressed by running bootstrap procedures to ensure sample size is
554 sufficient to characterize fully the isotopic niche (Vaudo & Heithaus, 2011). Finally, a few
555 individual outliers may result in a relatively large convex hull in which much of the contained
556 niche space is unoccupied. In such a case, evaluating the relative merits of different ways to
557 characterize the isotopic niche is warranted (see discussion of convex hulls *versus* Bayesian
558 ellipse models in Section VII.1). In summary, the quantitative metrics discussed in this section
559 have a series of caveats but, if qualified appropriately and augmented with additional sources of
560 data, provide useful insight into particular aspects of food-web structure.

561

562 **VII. WHAT IS THE DEGREE OF TROPHIC VARIABILITY AT THE** 563 **INTRAPOPULATION LEVEL?**

564 **(1) General approaches**

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

575 (2009a) adapted the Schmidt *et al.* (2007) vector-based approach, showing great variation in the
576 magnitude and direction of changes in the isotopic niche of individual ovenbirds across seasons.

577 Two main categories of inquiry encompass many of the stable isotope applications in this
578 context. First, many analyses are structured around using simple statistical tools (e.g. *t*-tests,
579 ANOVA, linear regression) to examine ontogenetic diet shifts within populations. Specifically,
580 $\delta^{15}\text{N}$ is used as a proxy to assess shifts in trophic position through ontogeny. For example,
581 Jennings *et al.* (2002) examined the relationship between body size and trophic position for 31
582 fish taxa in the North Sea, and demonstrated the prevalence of increasing trophic position
583 through ontogeny for most species. Second, stable isotopes are used to estimate relative niche
584 width of populations, typically by analyzing individual-level dispersion. Approaches include
585 range or variance in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Bearhop *et al.*, 2004; McClellan *et al.*, 2010; Willson *et al.*,
586 2010), convex hulls calculated at the individual level (Layman *et al.*, 2007b; Quevedo, Svanback
587 & Eklov, 2009), relative spacing among individuals (Martinez del Rio *et al.*, 2009a), two-
588 dimensional confidence intervals based on mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Layman, Winemiller &
589 Arrington, 2005a), and standard ellipse areas (SEA_B), i.e. bivariate equivalents to standard
590 deviations in univariate analysis (Jackson *et al.*, 2011).

591 Each of these aforementioned approaches has strengths and weaknesses, and we provide
592 one comparison as an example, i.e. between convex hulls (Layman *et al.*, 2007b) and the recently
593 developed Bayesian approach (Jackson *et al.*, 2011). The convex hull approach is powerful
594 because it incorporates each individual sampled, and thus includes information about every part
595 of isotopic niche space occupied. Conversely, the Bayesian approach is targeted at niche widths
596 of “typical” members in a population, which could be viewed as the mean or core isotopic niche
597 of that population (Jackson *et al.*, 2011). The Jackson *et al.* (2011) approach generates standard

598 ellipse areas as this core isotopic niche representation (SEA_B). Either the Bayesian approach or
599 convex-hull-based quantitative analysis (Turner *et al.*, 2010) may be more appropriate with
600 respect to a particular question of interest and/or the nature of the underlying data set. Convex
601 hulls may be more appropriate when individual-level niche variation, and thus every niche
602 position occupied by individuals, is central to the focal research question (Layman *et al.*, 2007b).
603 When core aspects of a population's isotopic niche are of most interest, other prior information is
604 available (e.g. on trophic discrimination rates), or error propagation is desirable, then the recently
605 developed Bayesian-based approaches are preferable to characterize niche widths (Jackson *et al.*,
606 2011). In some cases, utilization of both of these analytical approaches may be desirable to
607 reveal different aspects of trophic structure.

608 **(2) Numerical simulations**

609 Another research area that has developed rapidly in recent years has been examining
610 incidence and causes of individual specialization, i.e. variation in resource use among individuals
611 that is not attributable to age class, size or sex (Araújo *et al.*, 2011; Bolnick *et al.*, 2003, 2011).
612 Individual specialists utilize a relatively narrow subset of the population's overall resource base
613 so that there is substantial variability in the specific resources used among individuals. Such
614 variation among individuals may have several evolutionary and ecological implications,
615 including driving frequency-dependent disruptive selection (Bolnick *et al.*, 2011; Dieckmann &
616 Doebeli, 1999; Roughgarden, 1972) or imparting population stability (Agashe, 2009; Bolnick *et al.*,
617 2011; Lomnicki, 1999). Quantification of individual specialization within populations would
618 ideally be based on longitudinal samples in which the same individuals' diets are sampled
619 repeatedly over time (Bryan & Larkin, 1972; Estes *et al.*, 2003; Werner & Sherry, 1987; West &
620 Williams, 1986). However, in most cases, such longitudinal sampling schemes are too difficult to

621 implement. Because of the time- and space-integrated insight provided by isotopes, they have
622 become the primary way to investigate instances of individual specialization (Araujo *et al.*, 2007;
623 Beaudoin *et al.*, 1999; Bolnick *et al.*, 2007; Cherel *et al.*, 2007; Herman *et al.*, 2005), and a new
624 set of analytical tools have been developed to this end.

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

636 A subsequent advance was to quantify the degree of individual specialization in a
637 population, not just identify its presence/absence. Araújo *et al.* (2007) proposed a method that
638 uses the variance in individual $\delta^{13}\text{C}$ values in a population of consumers, and the $\delta^{13}\text{C}$ values of
639 resources, to calculate two indices of individual specialization that can be compared across
640 different populations (Bolnick *et al.*, 2002). In this approach, null populations with varying
641 degrees of individual specialization are generated, for which both isotope variances and indices
642 of individual specialization are calculated. A curve relating the expected isotopic variances and
643 indices of individual specialization is built and used to interpolate a measure of individual

644 specialization given an empirical variance in isotopes. This approach is especially useful for
645 taxa which have relatively few items in their stomachs at any single time (e.g. piscivorous fish),
646 for which estimations of dietary specialization would not be possible with direct diet analysis
647 alone. The power of this approach has been illustrated in its first applications on frogs and birds
648 (Araújo *et al.*, 2007; Woo *et al.*, 2008). However, this model has increased data input
649 requirements, specifically, direct dietary information that corresponds to the time period that the
650 isotope values of consumers and sources reflect. Again, collecting appropriately matched diet
651 and isotope data sets can be difficult, especially as temporal and spatial heterogeneity in
652 consumers (e.g. prey selection) and resources (e.g. seasonal variation in isotope values)
653 increases. Such a null-model approach, however, is essential to identify true dietary
654 specializations and should be employed in all cases in which isotope data are used directly to
655 infer the degree of dietary specialization within a population.

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

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

670 (3) Different tissue types

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

689 An alternative approach is based on the fact that different tissues have different turnover
690 rates and therefore integrate resource use over different time scales (Hesslein, Hallard & Ramlal,
691 1993). For example, vertebrate blood plasma integrates diets over a time scale of days to weeks,
692 whereas turnover in muscle tissue is on the scale of months (Dalerum & Angerbjorn, 2005;
693 MacNeil, Drouillard & Fisk, 2006; Phillips & Eldridge, 2006). As a consequence, individuals
694 that feed consistently on the same resource(s) over long time scales should have similar isotope
695 values in tissues with different turnover rates, whereas individuals that switch their diets over
696 time should show a mismatch between fast and slow turnover tissues. Martinez del Rio *et al.*
697 (2009a) called the former “isotopic specialists” and the latter “isotopic generalists”. They applied
698 this framework to three species of ovenbirds and found that one species was made up of isotopic
699 generalists that switch diets seasonally, another species had a mix of isotopic generalists and
700 specialists, and the third species was composed of isotopic specialists. Likewise, Matich,
701 Heithaus & Layman (2010) used different tissues to quantify differences in the degree of dietary
702 specialization between bull sharks (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*).
703 Importantly, information regarding turnover rates in the different tissues is needed to make
704 inferences about the degree of dietary specialization. These approaches are especially sensitive
705 to assumptions regarding isotopic routing and different discrimination factors among tissue
706 types.

707 A general concern for all the methods outlined herein relates to the temporal and spatial
708 scales at which individuals are sampled. Sampling individuals at different times or different
709 locations might artificially inflate variation in isotope values if sources vary temporally and/or
710 spatially. For example, individuals specialized on the same resource but feeding consistently in
711 different areas may differ greatly in isotope values if there is spatial heterogeneity in resource

712 isotope values, so that habitat-derived variation in consumers' isotopes will be mistaken as diet
713 variation (Flaherty & Ben-David, 2010). As is often the case, knowledge of the temporal and
714 spatial variation in sources' isotopes, as well as organism natural history, will greatly aid in the
715 interpretation of isotope data.

716

717 **VIII. CONCLUSIONS**

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

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

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

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

737 (5) The validity of assumptions underlying analytical models will vary depending on the
738 organism or system of interest. For example, the assumption that isotopic routing does not occur
739 is especially problematic for omnivores (Kelly & Martinez del Rio, 2010; Martinez del Rio *et al.*,
740 2009b; Voigt *et al.*, 2008). As such, the appropriateness of a specific analytical approach must
741 be evaluated on a case-by-case basis. By applying corrections, e.g. for concentration dependence
742 or increasing uncertainty in the trophic discrimination factors, it is becoming increasingly
743 possible to deal with these issues.

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

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

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

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

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

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

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

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

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

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

786

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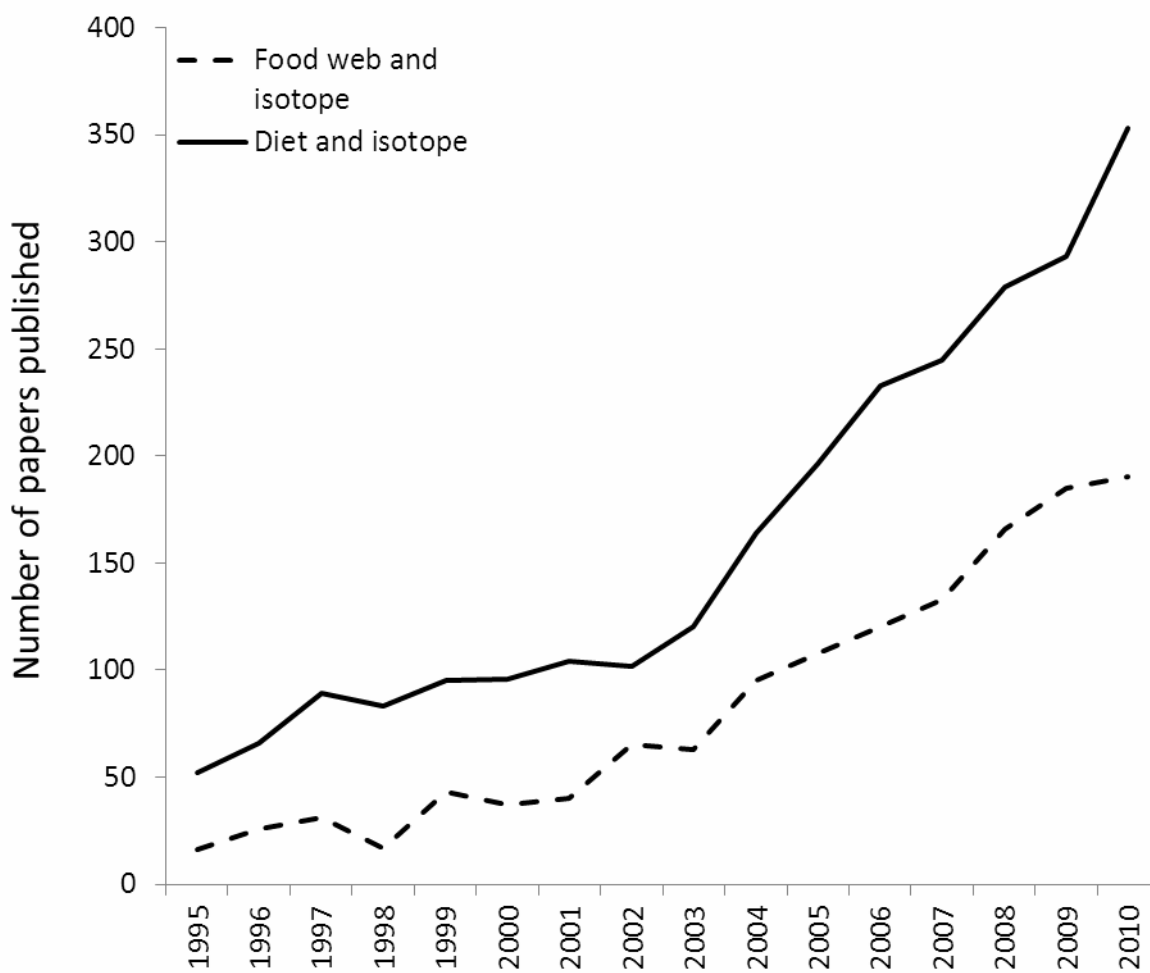
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1150 **Fig. 1.** Number of food web papers, as cataloged by *Web of Science*, employing stable isotopes
1151 published each year. Papers were identified using the key words “food web” and “isotope” or
1152 “diet” and “isotope”.

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