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A critical evaluation of intrapopulation variation of $\delta^{13}\text{C}$ and isotopic evidence of individual specialization

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Abstract Individual variation in the diet of consumers is common in many ecological systems and has important implications for the study of population dynamics, animal behavior, and evolutionary or ecological interactions. Ecologists frequently quantify the niche of a population by intensive analyses of gut contents and feeding behaviors of consumers. Inter-individual differences in $\delta^{13}\text{C}$ signature can indicate long term differences in feeding behavior, often unattainable by a single snapshot analysis of gut contents. If a consumer's food sources have unique $\delta^{13}\text{C}$ signatures, then the intrapopulation variation in $\delta^{13}\text{C}$ may be useful for quantifying diet variation and detecting isotopic evidence of individual specialization. However, intrapopulation variation in $\delta^{13}\text{C}$ can underestimate or overestimate dietary variation, and therefore is not directly equivalent to a dietary based niche. In this paper we show that intrapopulation variability of $\delta^{13}\text{C}$ in consumers critically depends on the isotopic range and distribution of food sources. Our analyses fundamentally challenge how we interpret the intrapopulation isotopic variance of $\delta^{13}\text{C}$, and how we evaluate isotopic evidence of individual specialization.

Keywords Stable isotopes · Niche · Individual diet variation · Habitat specialization · Random diet

Introduction

Intrapopulation variation in resource use is common in ecological communities, and has important implications for community and evolutionary ecology (Smith and Skulason 1996; Bolnick et al. 2003). Resource poly-

morphisms are extreme cases of intrapopulation variation, but are common in a wide range of taxa (Smith and Skulason 1996). However, intrapopulation variation is not limited to discrete resource polymorphisms. For example, stickleback populations exhibit a discrete polymorphism in some populations (McPhail 1993), but continuous variation between morphs in others (Robinson 2000). Evolutionary biologists are particularly attentive to intrapopulation variation, because of its implications for speciation. Many of the candidate cases for a sympatric phase of speciation involve individual specialization, and the establishment of a resource polymorphism (Via 2001). In the early stages of speciation, individual specialization may lead to a niche expansion, resulting in reduced intraspecific competition (Van Valen 1965). Individual specialization is at the interface between community and evolutionary ecology, and yet there are few methods available for its detection or accurate description (but see Bolnick et al. 2002).

Within a population, different sexes, age classes, morphs or individuals may specialize at acquiring different resources. Extensive literature on sexual dimorphisms (Slatkin 1984), ontogenetic niche shifts (Werner and Gilliam 1984; Polis 1984), and resource polymorphisms (Smith and Skulason 1996) illustrates the high frequency and prominence of intrapopulation variation in resource use (Bolnick et al. 2003). Bolnick et al. (2003) claim that individual specialization can account for a significant part of the residual variation of a population's diet, after accounting for sex, age, and morph. For example, freshwater fish species often have benthic and limnetic forms that specialize in littoral or pelagic habitats, respectively (Robinson and Wilson 1994). If we assume there are no dietary differences due to age or sex, the residual variation in diet within each morph may be related to individual specialization. Indeed, within pelagic and littoral habitats, individual planktivorous fish can specialize on different food types (Werner et al. 1981), or adjust their foraging time in response to predation risk (Wilson 1998).

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The dietary niche of a population is the variation in diet resulting from differences in sex, age, morph, and individual specialization. Though “niche” is typically a population parameter, variation in an individual’s niche contributes to the width of a population’s niche. A population’s total niche width (TNW) is the sum of the within individual component (WIC), and the between individual component (BIC) (Roughgarden 1974; Bolnick et al. 2003). In a population of individual generalists, the TNW is made up mostly of the WIC, whereas in a population of individual specialists the TNW is made up mostly of the BIC. Typically, we measure the niche width of a population using diet compositions, and infer individual specialization from indices of diet similarity and overlap (Bolnick et al. 2002). For example, the WIC/TNW ratio may indicate the degree of individual specialization in a population of consumers (Bolnick et al. 2002).

Several authors have recently associated intrapopulation variability in stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) with a population’s niche (Levesque et al. 2003; Grey et al. 2004; Bearhop et al. 2004) or with individual differences in diet (Post 2003; Bolnick et al. 2003). The isotope ^{13}C may indicate dietary sources of carbon because there is little trophic enrichment between an organism and its diet (Vander Zanden and Rasmussen 2001; Post 2002). In comparison, ^{15}N may indicate trophic position because of a predictable enrichment of ^{15}N between adjacent trophic levels (Vander Zanden and Rasmussen 2001; Post 2002). Stable isotopes can provide temporal consistency to diet analyses, because they provide a longer integrative history of feeding compared to traditional gut contents analysis (Hesslein et al. 1993). These features of stable isotopes make them appealing to study inter-individual differences in feeding behavior. For example, Post (2003) found that differences in $\delta^{15}\text{N}$ among largemouth bass juveniles reflected individual variation in the timing of an ontogenetic shift to piscivory. Post (2003) confirmed the shift to piscivory by concurrently analyzing gut contents and otolith microstructures. In a recent review of individual specialization, Bolnick et al. (2003) suggested that the large isotopic variance found in several different consumer populations (Fry et al. 1978; Angerbjorn et al. 1994; Gu et al. 1997; Ben David et al. 1997; Beaudoin et al. 1999; Fry et al. 1999) could provide evidence of individual specialization. However, none of these studies considered how the isotopic variability among food sources could affect intrapopulation isotopic variability of consumers. In addition, no one considered individual specialization as part of the residual variance in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ after accounting for differences in age, sex, and morph. Despite these deficiencies, Bolnick et al. (2003) used species that had “unexpectedly large isotopic differences between individuals” as evidence of individual specialization. This inference assumes that isotopic variation in the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ of a consumer population reflects the degree of dietary variation among its individuals.

The purpose of this paper is to evaluate current interpretations of isotopic variance as a measure of dietary variation. Specifically, does large isotopic variance imply

evidence of individual specialization (sensu Bolnick et al. 2003), and what factors contribute to isotopic variation that are unrelated to individual differences in diet? We use three approaches to evaluate intrapopulation isotopic variation of $\delta^{13}\text{C}$. First, we develop a simple null model (NULL_U; using a uniform random variate) to explicitly test whether the intrapopulation variation in $\delta^{13}\text{C}$ of a consumer population is larger than we would expect based on the variation in $\delta^{13}\text{C}$ among food sources (Fry et al. 1978), or among foraging habitats (Gu et al. 1997; Fry et al. 1999). Second, we develop a more biologically reasonable null model (NULL_M), which uses a multinomial sampling procedure to produce randomized diets. Third, we use an *F*-test approach to compare the observed variation in $\delta^{13}\text{C}$ of a population to an estimate of the expected variance resulting from variation in ^{13}C fractionation between consumers and their diets. The two null models compare an observed isotopic variance (σ_{obs}^2) with a test distribution of predicted variances (σ_{pred}^2), which are generated using randomizations of consumers’ diets and the measured $\delta^{13}\text{C}$ signatures of their food sources. The main difference between the null models is that NULL_U produces randomized diet compositions with more variance among individuals, and as a result is less likely to detect cases of individual specialization compared to NULL_M. We chose to use NULL_U and the *F*-test to evaluate the case studies because of the limited data available and the uncertainty about individual variation in fractionation. We then used NULL_M to explore how the isotopic range and distribution of food sources can affect the intrapopulation variation of $\delta^{13}\text{C}$ of consumers.

Materials and methods

The uniform null model (NULL_U)

The uniform null model (NULL_U) is a simple null model that tests whether variation in $\delta^{13}\text{C}$ of a population of consumers is larger than we would expect given the variability of $\delta^{13}\text{C}$ among food sources. The model inputs are the average diet composition of the population, and the average $\delta^{13}\text{C}$ of each diet category. The model simulates random diet compositions by choosing a random number from a uniform distribution (min=0, max=100) for each diet category. To simulate random variation around a population’s average diet, the model multiplies the actual diet composition by the random diet composition. Each diet category is then converted to a dietary proportion. The model calculates the $\delta^{13}\text{C}$ of each individual as the sum of the product of the random diet composition and the $\delta^{13}\text{C}$ of each diet category, as shown in Eq. 1:

$$\sum_{i=0}^n (DC_i)\delta^{13}C_i \quad (1)$$

where n is the number of diet categories in the diet that have different $\delta^{13}\text{C}$ signatures, DC_i is the biomass proportion of the i th diet category in the diet composition, and $\delta^{13}C_i$ is the isotopic signature of the i th diet category. Each simulation mimicked the sampling design of the comparison study. For example, if a study collected 15 fish to calculate the observed variance (σ_{obs}^2) in $\delta^{13}\text{C}$, the model would generate 15 individuals with random diets to calculate the predicted variance (σ_{pred}^2) in $\delta^{13}\text{C}$. To see if σ_{obs}^2 is different than what we would expect by chance, the model simulates

10,000 sampling events and generates a distribution of σ_{pred}^2 . We calculated a P -value for the simulation as the number of σ_{pred}^2 greater than σ_{obs}^2 divided by 10,000.

In making the null model, we made several simplifications. First, we assumed that each diet category had the same percentage of carbon per unit biomass. Second, we assumed that each diet category was a homogeneous mixture of ^{13}C , which may not be the case if lipids are present (Van Dongen et al. 2002). If this assumption is violated, then the model will not work for consumers that selectively assimilate different isotopic components of a food source (Cowie and Hedges 1996). In other words, the model does not account for isotopic routing (Gannes et al. 1997). Third, we assumed that there was no temporal or spatial variability in the $\delta^{13}\text{C}$ of diet categories. If spatial variation is present, the model can be adapted to test for individual specialization in habitat use, provided that the variation in the $\delta^{13}\text{C}$ among food sources within a habitat is small relative to differences in $\delta^{13}\text{C}$ among habitats. Finally, we assumed that the intrapopulation variation in the fractionation of ^{13}C between a consumer and its diet is small compared to differences in $\delta^{13}\text{C}$ among food sources. Overall, these simplifications tend to reduce the expected variation in the $\delta^{13}\text{C}$ of a consumer population, and increase the likelihood of detecting isotopic evidence of individual specialization. In testing these case studies, we are assuming that the residual isotopic variation, unaccounted for by NULL_U , is a result of diet or habitat variation. Validating this assumption is a continuing challenge for stable isotope studies of dietary variation.

The multinomial null model (NULL_M)

NULL_M generates random diet compositions for each individual in a simulation using a multinomial model. Consider a consumer population whose average diet composition consists of five diet categories ($N_e=5$) in equivalent proportions ($\pi_{1-5}=0.2$).

Over a series of 25 feeding events ($N_e=25$), one could argue that the diet composition of a randomly feeding individual results from a series of random realizations of the average diet composition of the population. Using the above parameters the expected number of items in each diet would be five (i.e. $N_e \times \pi_i$), and the expected variance around a given diet item would be $\pi_i(1-\pi_i)/N_e$. As we increase the number of feeding events, individual variability around the average population diet composition decreases. Eventually, as the number of feeding events increase, the diet composition of individuals will converge, and the resulting predicted isotopic variance (σ_{pred}^2) will approach zero.

NULL_M differs from NULL_U in that the randomized diet composition of each individual is based on the multinomial sampling approach described above, rather than sampling from a uniform distribution. The critical difference between the null models is that NULL_U does not take into account the biological process of random feeding behavior among individuals, which inevitably leads to random variation around the average diet composition of the population. Therefore NULL_U makes no prior assumption about how individuals acquire different resources, and instead uses an uninformed uniform distribution around the average population's diet. Though NULL_U is the best available approach to evaluate the current case studies from the literature, and helps to illustrate different applications of the null modeling approach, NULL_M provides a more biologically reasonable model and should be used to test future cases for isotopic evidence of individual specialization. In either case, the null models only provide isotopic evidence of diet variation, and may not be sufficient to confirm a specific case of individual specialization.

The F -test approach: comparing σ_{obs}^2 to the variance in isotopic fractionation (σ_{Δ}^2)

By leaving the variability in fractionation out of the null modeling approach we are likely underestimating the population's expected

variance using NULL_U . Incorporating variation in fractionation into a more complex null model would provide a more robust means to detect individual specialization. However, this is beyond the scope of this paper, primarily because estimates of variation in fractionation among individuals are not available for the taxa being considered. Instead, to balance our analysis, we used an F -test approach as a second statistical test to accompany NULL_U in our evaluation of the case studies.

The F -test allows us to determine if the observed variance in $\delta^{13}\text{C}$ of a consumer population is larger than we would expect based on interspecific variation in fractionation (mean $_{\Delta\text{C}}=0.39$, $\sigma_{\Delta\text{C}}^2=1.74$, $n=107$; Post 2002). Since $\sigma_{\Delta\text{C}}^2$ is an interspecific estimate, we also tried to find an estimate of the variance in isotopic fractionation (σ_{Δ}^2) that was more specific to the organism in the case study of interest. In cases where the F -ratio ($\sigma_{\text{obs}}^2/\sigma_{\Delta\text{C}}^2$) was less than 1, we reversed the F -test. In several comparisons, σ_{obs}^2 was significantly smaller than $\sigma_{\Delta\text{C}}^2$, suggesting that $\sigma_{\Delta\text{C}}^2$ might overestimate σ_{Δ}^2 of a given consumer. In the same way, if σ_{obs}^2 is significantly smaller than the distribution of σ_{pred}^2 then this would indicate that the null model is inappropriate to evaluate a particular case study. None of the σ_{obs}^2 in our analyses were significantly less than the test distributions generated by NULL_U . However, this could certainly arise when using the NULL_M , and could provide a validity check for a particular formulation of NULL_M .

Using an interspecific estimate ($\sigma_{\Delta\text{C}}^2$) in the null model approach would restrict our detection of individual specialization to cases where intrapopulation variance in $\delta^{13}\text{C}$ is extremely large. Ideally, we would use a species-specific estimate of σ_{Δ}^2 , but these are rarely available for the species being considered. For the purposes of this paper we left fractionation out of our null models, so that we can illustrate how the null modeling approach works without the uncertainty of fractionation. In this formulation, the null models are very sensitive, and can detect evidence of individual specialization even when the intrapopulation variation in $\delta^{13}\text{C}$ is significantly less than we would expect based on $\sigma_{\Delta\text{C}}^2$. In general, a case study should pass both the F -test and the null model test to provide any isotopic evidence of individual specialization (IEIS).

Results

Testing the case studies for IEIS

Dietary reconstruction in a grasshopper food web

Fry et al. (1978) measured the $\delta^{13}\text{C}$ of 25 grasshopper species, and the $\delta^{13}\text{C}$ of 61 grass species found in the grasshoppers' gut contents. This example is unique among our case studies because in addition to measuring the $\delta^{13}\text{C}$ signatures of each food source, they independently determined the diet composition of each grasshopper species (albeit in a semi-quantitative fashion). The grasses, collected from the study site in western Texas, had either a C_3 or C_4 photosynthetic pathway. Since C_4 plants readily incorporate ^{13}C during photosynthesis, their $\delta^{13}\text{C}$ signatures are typically enriched compared to C_3 plants (Smith and Epstein 1971). Fry et al. (1978) classified grasshopper species as either C_4 or C_3 specialists, or as mixed feeders on both C_4 and C_3 grasses. Fry et al. (1978) only reported the variation in $\delta^{13}\text{C}$ for 10 of the 25 species considered. For each of these species (Table 4 in Fry et al. 1978), we calculated the σ_{obs}^2 and the distribution of σ_{pred}^2 using NULL_U .

Bolnick et al. (2003) reported that 4 of these 10 grasshopper species showed evidence of individual spe-

cialization (Table 1). The results using NULL_U match the conclusions of Bolnick et al. (2003) in two cases, *Heliaula rufa* and *Melanus lakinus* (Table 1). Our analysis indicates that the large isotopic variance of *M. arizonae* and *M. gladstoni* does not provide IEIS. Since these species consume both C_3 and C_4 plants, their diet has a large range in $\delta^{13}\text{C}$, such that we would expect large intrapopulation variance in $\delta^{13}\text{C}$. Specialist species (specializing on C_3 or C_4) feed on grasses with a small range in $\delta^{13}\text{C}$, and thus have a much smaller σ_{pred}^2 than mixed feeders. In fact, NULL_U revealed that three of the four C_4 specialists showed IEIS. As expected, the variability in $\delta^{13}\text{C}$ among food sources affects our interpretation of the variation in $\delta^{13}\text{C}$ of a consumer population. For example, *M. gladstoni* (a mixed feeder) has $\sigma_{\text{obs}}^2 = 4.5$ and does not show IEIS, whereas *Philobostroma quadrimaculatum* (a C_4 specialist) has $\sigma_{\text{obs}}^2 = 0.078$ but does show IEIS using NULL_U .

Conclusions from the F -test approach differ from those reached by the null model approach. For this case study we did not use $\sigma_{\Delta C}^2$ for the F -test because Fry et al. (1978) provided an estimate of σ_{Δ}^2 that was specific to their 25 grasshopper species ($\sigma_{\Delta_{\text{res}}}^2 = 1.4$; see Table 2). In three of the six cases where $\sigma_{\text{obs}}^2 / \sigma_{\Delta_{\text{res}}}^2$ was less than 1, σ_{obs}^2 was significantly less than $\sigma_{\Delta_{\text{res}}}^2$. This suggests that even $\sigma_{\Delta_{\text{res}}}^2$ may overestimate the actual fractionation for a given grasshopper species. Using only the F -test approach, and not NULL_U , we would have missed two possible candidates for IEIS (*Philobostroma quadrimaculatum* and *Sybula fuscovittata*), and incorrectly identified two species as having IEIS (*M. arizonae* and *M. gladstoni*) (Table 2). This indicates that the F -test alone is insufficient to test for IEIS, and that the variation in $\delta^{13}\text{C}$ of food sources is critical to the interpretation of the intrapopulation variance of $\delta^{13}\text{C}$.

Table 1 Application of the null model approach to dietary reconstruction in a grasshopper food web. Isotopic evidence for individual specialization (IEIS) is confirmed if the observed variance σ_{obs}^2 is greater than 95% of the 10,000 simulations. Each simulation gave a predicted variance σ_{pred}^2 generated from randomly feeding individuals. The number of individuals in each simulation was equal to the sample size (n) used by Fry et al. (1978)

Species	$\delta^{13}\text{C}$ range of consumer (min, max)	$\delta^{13}\text{C}$ range of food sources	Number of prey items	σ_{obs}^2 $\delta^{13}\text{C}$ (n)	Calculated P -value	IEIS? (Bolnick et al. 2003)	IEIS? (this study)
C_4 specialists							
<i>Philobostroma quadrimaculatum</i>	-13.1, -11.7	-14.2, -11.7	6	0.078 (7)	0.018	No	Yes
<i>Boopedon nubilum</i>	-13.5, -12.3	-14.7, -11.7	6	0.25 (6)	0.065	No	No
<i>Sybula fuscovittata</i>	-13.7, -12.6	-14.2, -13.7	3	0.21 (6)	<0.0001	No	Yes
<i>Heliaula rufa</i>	-20.4, -10.6	-25.9, -11.7	10	18.2 (5)	<0.0001	Yes	Yes
C_3 specialists							
<i>Boottettix argentatus</i>	-24.3, -23.0	-25.5	1	0.18 (6)	–	No	No
<i>Dactylotum variegatum</i>	-24.4, -23.0	-26.8, -13.4	19	0.29 (6)	0.165	No	No
<i>Tropidolophus formosus</i>	-25.0, -23.4	-25.9	1	0.50 (5)	–	No	No
Mixed C_3 and C_4 feeders							
<i>Melanus lakinus</i>	-20.1, -13.3	-25.2, -12.6	11	7.29 (5)	0.0014	Yes	Yes
<i>Melanoplus arizonae</i>	-21.4, -17.6	-25.9, -12.6	8	1.76 (6)	0.668	Yes	No
<i>Melanoplus gladstoni</i>	-24.3, -18.7	-27.1, -12.6	13	4.5 (6)	0.091	Yes	No

Intrapopulation feeding diversity in blue tilapia

Gu et al. (1997) found an extraordinarily large range and variance in $\delta^{13}\text{C}$ (-25.9 to -9.5‰ , $\sigma_{\text{obs}}^2 = 20.706$, $n=31$) for blue tilapia (*Oreochromis aureus*) in Lake Apopka, Florida. The range in $\delta^{13}\text{C}$ was nearly double the range of all other fish species collected in Lake Apopka at the same time (Gu et al. 1996), and is significantly greater than $\sigma_{\Delta C}^2$ ($F_{31,107} = 11.88$, $P < 0.05$). Blue tilapia can feed on zooplankton and algae in the pelagic or littoral zone of lakes (Zohary et al. 1994). Since littoral and pelagic habitats often have different $\delta^{13}\text{C}$ signatures (France 1995), we might expect a population that feeds in both habitats to have large intrapopulation variability in $\delta^{13}\text{C}$. However, in contrast with the previous case study, Gu et al. (1997) did not provide a detailed description of gut contents, or diet composition, for blue tilapia. Instead, Gu et al. (1997) reported $\delta^{13}\text{C}$ signatures for a range of possible carbon sources found in the lake at the same time. Therefore, we split the possible food sources into four categories depending on their $\delta^{13}\text{C}$, and used NULL_U with a diet composition of 25% for each of the four categories. The four categories were (1) cattail (-27.5‰), (2) sediments (-22.5‰), (3) diatoms, nanophytoplankton, and zooplankton (-15‰), and (4) *Microcystis* (-2.5‰). These categories are only estimates of the $\delta^{13}\text{C}$ of possible food sources, and are not necessarily food items themselves.

Using this formulation of the null model, we generated a distribution of σ_{pred}^2 and used two different observed variances to test for IEIS. First, we used a variance in $\delta^{13}\text{C}$ calculated from the entire collection of blue tilapia (σ_{obs}^2). In this case, σ_{obs}^2 was higher than all 10,000 estimates of σ_{pred}^2 ($P < 0.0001$), which provides some IEIS. However,

to compute σ_{obs}^2 . When the number of food sources is 1, all individuals should feed on the same food source, so $\sigma_{\text{pred}}^2 = 0$. In this case the observed variance is the sum of individual variance in isotopic fractionation, individual differences in the temporal and spatial integration of the $\delta^{13}\text{C}$ of the food source, and measurement error (see text and Table 2). $\delta^{13}\text{C}$ values are in ‰ units

Table 2 Application of the *F*-test approach to dietary reconstruction in a grasshopper food web. Isotopic evidence for individual specialization (IEIS) is rejected if *F*-test ($\sigma_{obs}^2/\sigma_{\Delta res}^2$) is less than *F*-critical with degrees of freedom determined by the sample size used to estimate σ_{obs}^2 . Here $\sigma_{\Delta res}^2$ is the estimated variance in individual ^{13}C fractionation. It is calculated as the variance in the residuals from a regression between the $\delta^{13}C$ of the diet and the

$\delta^{13}C$ of the grasshopper species ($\sigma_{\Delta res}^2=1.4$). Residuals are from a regression of 25 grasshopper species in west Texas (grasshopper $^{13}C = 0.97 \times \text{diet } \delta^{13}C$), summarized in Table 3 of Fry et al. (1978). *Asterisks* denote cases where the observed variance is significantly less than $\sigma_{\Delta res}^2$, which implies that $\sigma_{\Delta res}^2$ is an overestimate of individual variance in fractionation for a given species

Species	IEIS? (Bolnick et al. 2003)	IEIS? (Table 1)	$\delta^{13}C \sigma_{obs}^2 (n)$	<i>F</i> -test ($\sigma_{obs}^2/\sigma_{\Delta res}^2$)	<i>F</i> -critical	Variance greater than $\sigma_{\Delta res}^2$
C₄ specialists						
<i>P. quadrimaculatum</i>	No	Yes	0.078 (7)	<1	<i>F</i> _{6,24} =2.87	No*
<i>B. nubilum</i>	No	No	0.25 (6)	<1	<i>F</i> _{5,24} =3.15	No
<i>S. fuscovittata</i>	No	Yes	0.21 (6)	<1	<i>F</i> _{5,24} =3.15	No*
<i>H. rufa</i>	Yes	Yes	18.2 (5)	53.4	<i>F</i> _{5,24} =3.15	Yes
C₃ specialists						
<i>B. argentatus</i>	No	No	0.18 (6)	<1	<i>F</i> _{5,24} =3.15	No*
<i>D. variegatum</i>	No	No	0.29 (6)	<1	<i>F</i> _{5,24} =3.15	No
<i>T. formosus</i>	No	No	0.50 (5)	<1	<i>F</i> _{4,24} =3.38	No
Mixed C₃ and C₄ feeders						
<i>M. lakinus</i>	Yes	Yes	7.29 (5)	21.4	<i>F</i> _{4,24} =3.38	Yes
<i>M. arizonae</i>	Yes	No	1.76 (6)	5.2	<i>F</i> _{5,24} =3.15	Yes
<i>M. gladstoni</i>	Yes	No	4.5 (6)	13.2	<i>F</i> _{5,24} =3.15	Yes

the length of blue tilapia varied from 10 to 35 cm (Gu et al. 1997), such that variation in age class may have contributed to intrapopulation variation in $\delta^{13}C$. Recall that Bolnick et al. (2003) defined individual specialization

as the residual variance in diet after we account for age. Therefore, we used $\sigma_{size-res}^2$ as a second variance to confirm individual specialization, where $\sigma_{size-res}^2$ is the variance of the residuals from a regression between length

Table 3 Application of the null model approach to the spatial variation in the intrapopulation variability of $\delta^{13}C$ in littoral and pelagic fish of Lake Okeechobee. Samples are taken from three main habitat zones: marsh [Moore haven (MH)], nearshore [Cochranes pass (CP), NLS and L005] and offshore (LZ40). The sites were divided into three groups that had distinct $\delta^{13}C$ signatures: MH, CP, and sites NLS, L005, LZ40 together (Fig. 1, Fry et al. 1999). Here,

comparing the observed variance against the simulated distribution of variances tests the hypothesis that individuals in the population feed randomly in the three isotopic zones. If the observed variance is greater than 95% of the predicted variances (*P*-value<0.05), then individuals within the population may specialize with respect to isotopic zone. *Asterisks* denote collections with σ_{obs}^2 significantly less than $\sigma_{\Delta C}^2$

Fish type	Site	Month	$\delta^{13}C \sigma_{obs}^2 (n)$	<i>P</i> -value	IEIS in habitat use?
Bluegill	Moore haven	Aug	0.96 (2)	<0.0001	Yes
	Cochranes Pass	Aug	0.99 (10)	<0.0001	Yes
		Jan	1.96 (10)*	<0.0001	Yes
		NLS	Aug	0.21 (5)*	0.011
	LZ40	Jan	0.02 (4)*	0.873	No
		Jan	0.11 (10)	0.153	No
Redear sunfish	Moore haven	Aug	1.56 (11)	<0.0001	Yes
	Cochranes Pass	Aug	1.45 (10)	<0.0001	Yes
		Jan	2.1 (10)	<0.0001	Yes
	L005	Aug	0.64 (5)	<0.0001	Yes
	NLS	Jan	0.05 (3)	0.547	No
Largemouth bass	Moore Haven	Aug	0.94 (9)	<0.0001	Yes
	Cochranes Pass	Aug	0.50 (6)	<0.0001	Yes
		Jan	2.1(10)	<0.0001	Yes
	NLS	Aug	0.018 (4)*	0.888	No
		Jan	0.135 (3)	0.177	No
Bowfin	Moore haven	Aug	0.33 (6)	<0.0001	No
	Cochranes Pass	Jan	2.27 (10)	<0.0001	Yes
	NLS	Jan	3.2 (9)	<0.0001	Yes

and $\delta^{13}\text{C}$ for individuals between 20 and 30 cm. The observed variance in the residuals ($\sigma_{\text{obs-res}}^2=13.64$, $n=26$) was also greater than all estimates of σ_{pred}^2 ($P<0.0001$). Based on these two tests, the large variation in $\delta^{13}\text{C}$ of blue tilapia is consistent with the hypothesis of individual specialization. However, this result should be treated with caution, because we only estimated the variability in $\delta^{13}\text{C}$ of potential food sources based on the best available data. A more definitive conclusion would require a comprehensive gut content analysis, and an average $\delta^{13}\text{C}$ signature for each diet category.

Spatial variation in the intrapopulation variability of littoral and pelagic fish

Fry et al. (1999) measured the $\delta^{13}\text{C}$ of 29 fish species in Lake Okeechobee, Florida, from five different sites, including (1) Moore Haven (MH), a marsh, (2) Cochrane's Pass (CP), at the boundary between the marsh and open water habitat, (3) North Lake Shoal (NLS), an open water site 1 km from the marsh, (4) L005, an open water site 1 km from the marsh, and (5) LZ40, an offshore site in the middle of the lake. Bolnick et al. (2003) selected 10 fish species from this study as evidence for individual specialization because the intrapopulation isotopic range was $>1.6\text{‰}$ (personal communication), a criterion originally set by Fry et al. (1978). Here we consider 4 of these species that are found in the marsh, boundary zone, and open water habitat of Lake Okeechobee (Table 3) to test for IEIS in habitat foraging.

We used two approaches to test for IEIS in the fish collections of Fry et al. (1999). First, we classified foraging habitats into three isotopic zones according to their $\delta^{13}\text{C}$: MH (-26.0‰), CP (-24.8‰), and the open water sites (NLS, L005, LZ40) (-23.3‰). We then used the NULL_U approach to test for individual specialization in foraging habitat for the four fish species that were caught in all three isotopic zones. We compared σ_{obs}^2 to a distribution of σ_{pred}^2 for each date that fish were collected. Bluegill caught in MH and CP had a larger σ_{obs}^2 than we would expect if each individual fish fed randomly among habitats; however, this was not the case for bluegill caught in the open water sites (Table 3). It is possible that proximity to two habitats with different $\delta^{13}\text{C}$ signatures increased the intrapopulation variation in $\delta^{13}\text{C}$ of bluegill. This pattern was the same for redear sunfish (except for L005), and largemouth bass. All collections of bowfin had a larger σ_{obs}^2 than we would expect based on random feeding in all three habitats (Table 3). This example illustrates that significant spatial variation in $\delta^{13}\text{C}$ of food sources may increase the intrapopulation variation in $\delta^{13}\text{C}$ of consumers.

Using NULL_M to explore sources of intrapopulation isotopic variability

Based on simulations of NULL_M we identified four dominant factors that affect intrapopulation isotopic variability of a consumer population; isotopic range of food sources, number of feeding trials, number of diet categories, and isotopic spacing among diet categories. In reviewing the cases cited by Bolnick et al. (2003), we found that the isotopic variance of a consumer population is positively correlated with the isotopic range of available food sources (Pearson's $R=0.75$, $n=21$) (Fig. 1a). We then used this range to constrain our simulations of NULL_M . In all simulations of NULL_M we used 1,000 individuals to generate each σ_{pred}^2 , and generated 1,000 σ_{pred}^2 for each test distribution. In the first set of simulations, we varied the number of feeding events (N_e) for each individual from 10 to 500, and the isotopic range of food sources ($F_{\text{‰}}$) from 2 to 32‰ (Fig. 1b). For these simulations we fixed the number diet categories (N_c) at five, maintained an evenly spaced isotopic distribution among food sources over $F_{\text{‰}}$, and monitored the change in the 95th percentile of the σ_{pred}^2 distribution. Recall that an σ_{obs}^2 greater than this 95th percentile is necessary for isotopic evidence of individual specialization. Changing the number of feeding trials from 500 to 10 at $F_{\text{‰}}=32\text{‰}$ increased the 95th percentile of the σ_{pred}^2 distribution from 0.046 to 22. This encompasses almost the entire range of the observed isotopic variances in Fig. 1a.

To illustrate the effect of changing the number of diet categories, we ran simulations with $N_e=10$ and $F_{\text{‰}}=24\text{‰}$, and varied the number of diet items that were evenly distributed over an isotopic range. Decreasing the number of diet items from 17 to 3 increased the 95th percentile of the σ_{pred}^2 distribution by 84%. To illustrate the effect of the isotopic spacing among three food sources, we set $N_c=3$, $N_e=10$, and $F_{\text{‰}}=24\text{‰}$ and varied the $\delta^{13}\text{C}$ of the second food source (Pos_2 in Fig. 1b). As we moved Pos_2 from -12‰ (even distribution) to -2‰ (skewed distribution) the 95th percentile of the σ_{pred}^2 distribution increased by about 20% (smallest squares in Fig. 1b). It is important to note that the effect of the number and isotopic spacing of diet categories on intrapopulation isotopic variability critically depends on the isotopic range and the number of feeding events. This is why NULL_M must be specifically tailored for the consumer population that is being tested for IEIS.

Discussion

Evaluating the case studies using NULL_U and the F -test approach revealed that the intrapopulation isotopic variance in a population of consumers depends on the isotopic composition of food sources. None of the authors of the cases cited by Bolnick et al. (2003) considered this issue, nor did Bolnick et al. (2003) discuss how it might affect the identification of individual specialization. To further clarify this issue we simulated a population of individual

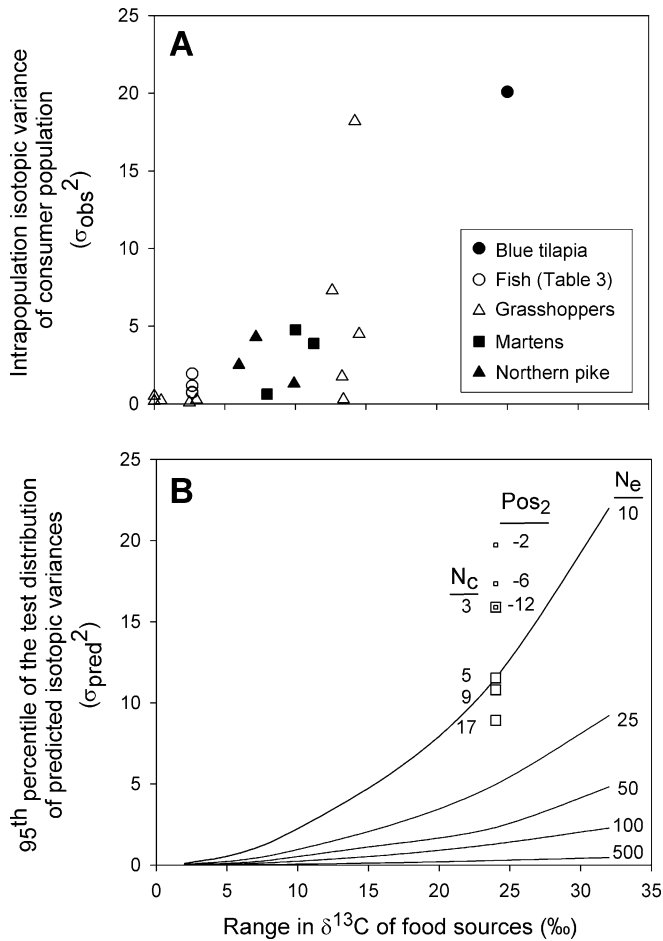


Fig. 1 **a** Results from reviewing some of the cases cited by Bolnick et al. (2003) as providing isotopic evidence of individual specialization. The blue tilapia (filled circle) is from Gu et al. (1997), the fish (open circle) are the average variance in Table 3 from Fry et al. (1999), the grasshoppers (open triangle) are from Fry et al. (1978), the martens (filled square) are from Ben-David et al. (1997), and the northern pike (filled triangle) are from Beaudoin et al. (1999). **b** Results from simulations of the multinomial null model ($NULL_M$). Lines result from varying the number of feeding events per individual (N_c : 10–500) as a function of the isotopic range of food sources ($F_{\%}$: 2–32‰) among five diet items ($N_e=5$). Setting $N_e=10$, the large squares result from varying the number of diet categories (N_c : 3–17) for a given isotopic range ($F_{\%}=24\%$). The small squares result from changing the isotopic spacing among three food sources ($N_e=3$), by moving the position of the second food source (Pos_2) from -12% (even), to -2% (skewed)

generalists and specialists that fed on either a large or small isotopic range of food sources. This allows us to compare the effect of feeding behavior (individual generalists or specialists) and the effect of isotopic variability among food sources (large and small isotopic range) on the intrapopulation variation of $\delta^{13}C$ in a consumer population. For this simulation we consider generalists and specialists in relation to the degree of individual specialization (high or low WIC/TNW respectively), and not in the classical sense of the total niche width.

For this simulation each population fed on up to five diet categories with unique $\delta^{13}C$ signatures (Fig. 2). Each

generalist had random diet compositions that included five food sources, whereas each specialist had a random diet composition that included two, three, four, or five diet categories. To simplify the analysis we used $NULL_U$ to generate random diets. We balanced the population of specialists so that the same number of individuals fed on each diet category. This approach simulated individual specialization in a population of specialists, but kept the total niche width the same as the simulated population of generalists. To confirm this, we used the methods from Bolnick et al. (2002) to calculate TNW and WIC. A ratio of WIC/TNW close to unity indicates a population of generalists. Our populations of generalists and specialists had the same TNW, but different WIC/TNW ratios (Fig. 2).

In the population of individual generalists we would expect a small range of $\delta^{13}C$ values, because each individual is feeding on similar diet categories in similar proportions (Fig. 2a). If we keep the population's feeding behavior constant and increase the range in $\delta^{13}C$ of food sources, then the intrapopulation variation in $\delta^{13}C$ increases (Fig. 2b). We see the same pattern for the simulated population of individual specialists (Fig. 2c,d), but the intrapopulation variation in $\delta^{13}C$ is generally larger for these populations given the same $\delta^{13}C$ signatures

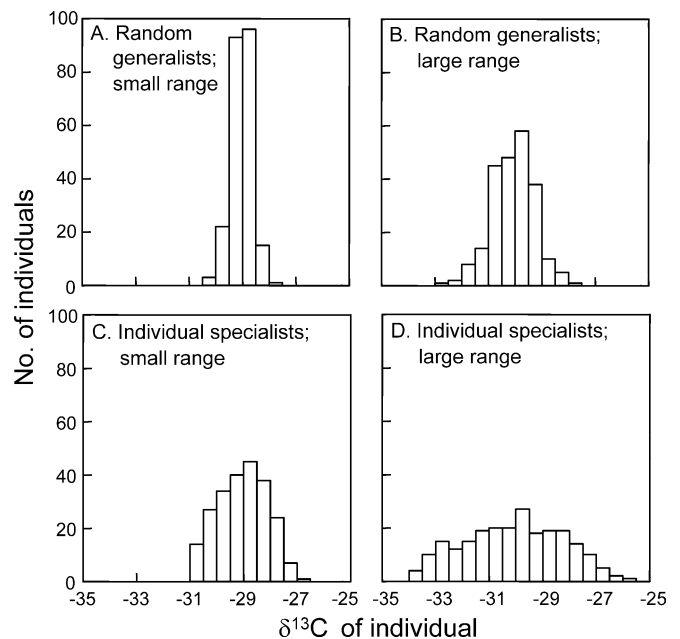


Fig. 2a–d Simulated effect of increasing the range of prey $\delta^{13}C$ for a population of (c, d) individual specialists, and for a population of (a, b) generalists that feed randomly on all five prey items available to the population. The five prey items had either $\delta^{13}C$ signatures with a small range ($-31, -30, -29, -28, -27\%$) or a large range ($-34, -32, -30, -28, -26\%$). The TNW is 1.61 for all populations, but the ratio of the WIC to the TNW is different for the individual specialists (WIC/TNW=0.587) and generalists (WIC/TNW=0.904). A WIC/TNW ratio close to unity indicates a population of generalists with little individual specialization (Bolnick et al. 2003). WIC and TNW were calculated using the program IndSpec1 (Bolnick et al. 2002). In these cases the variation in $\delta^{13}C$ of consumers alone does not provide isotopic evidence of individual specialization in the population

among food sources (compare panel a to c, and b to d in Fig. 2). Thus, even though the TNW is the same for all simulated consumer populations, the variation in $\delta^{13}\text{C}$ is very different (Fig. 2). Clearly, intrapopulation variation in $\delta^{13}\text{C}$ depends not only on intrapopulation variation in feeding behavior (i.e. generalists or specialists), but also on the variability in $\delta^{13}\text{C}$ among food sources. Among our case studies, the most powerful test for individual specialization was possible when a study defined both the diet composition and the $\delta^{13}\text{C}$ of each diet category (sensu Fry et al. 1978). Without an isotopic composition of food sources, it is extremely difficult to determine whether the variation in the $\delta^{13}\text{C}$ of a consumer population is due to individual specialization, or variation in the $\delta^{13}\text{C}$ of the population's food sources.

Insights from simulations of NULL_M

Simulations of NULL_M further demonstrate that isotopic variation in consumers depends on the isotopic variation of food sources, but also revealed that the detection of individual specialization critically depends on formulating an appropriate null model to the specific context of the study organism. How do we model a population of randomly feeding individuals that can provide us with a suitable null model to test for individual specialization using stable isotopes? Consider an idealized case where there is no spatial variability in the isotopic signature of diet categories, but large isotopic differences between diet categories. If we assume that all individuals experience the same period of isotopic integration of food sources, then isotopic differences among individuals will depend on the isotopic composition of the food sources and the number of consumed diet items. Individuals in a randomly feeding population will eventually converge on nearly identical isotopic signatures, as predicted by the multinomial model. In comparison, individuals that specialize on different diet categories might be isotopically different and contribute to intrapopulation isotopic variability. If there are more food sources than we can differentiate using stable isotopes (Phillips and Gregg 2003), then we may not be able to resolve exact dietary dependencies. For example, if individuals can feed on three different food sources with unique isotopic signatures (-20 , -25 , and -30‰), then two individuals may have the same $\delta^{13}\text{C}$ signature (-25‰) even though one feeds exclusively on one food source (-25‰), and the other one feeds on a 50:50 mixture of the other two food sources (-20‰ and -30‰). In this case, intrapopulation variance of $\delta^{13}\text{C}$ will underestimate the degree of dietary variation. However, we can still identify individuals that are isotopically distinct from the average population, and use other means, such as gut content analysis, to confirm individual specialization (Post 2003). In this manner, stable isotopes can provide independent evidence of individual dietary variation, but may not stand alone as the sole evidential source of diet variation.

Isolating dietary variation from total isotopic variation in field populations is fundamental for a null modeling approach, and is a significant challenge for stable isotopic analysis of diet variation. Spatial and temporal isotopic variation in food sources, and inter-individual variation in fractionation are not directly related to dietary variation but can contribute to intrapopulation isotopic variation, and may lead to an overestimate of dietary variation. These issues must be considered in any analysis of individual specialization using stable isotopes.

Spatial variability of food source $\delta^{13}\text{C}$

Spatial variation in the $\delta^{13}\text{C}$ of primary producers and consumers is common in a wide range of ecosystems, including lakes (France 1995; Post 2002), rivers (Wainright et al. 1996), estuaries (Fry 1999), and oceans (Jennings et al. 1997). Typically, spatial variability is highest in populations whose individuals are not very motile but widely distributed in a heterogeneous habitat (e.g. mussels) (McKinney et al. 1999). The range of variability in $\delta^{13}\text{C}$ depends on the spatial scale. For example, the $\delta^{13}\text{C}$ of particulate organic matter in the ocean declines as you move from the equator ($\sim -20\text{‰}$) to the poles ($\sim -35\text{‰}$) (Hollander and Smith 2001). In comparison, there is a large inter-lake range of $\delta^{13}\text{C}$ signatures in littoral (-27 to -14‰) and pelagic habitats (-34 to -20‰) (Post 2002). Fish populations with individuals that forage across these habitat boundaries may have a wide variation in $\delta^{13}\text{C}$ signatures, even though individual fish may feed on the same food sources in the same dietary proportions. In this case, isotopic variation may reflect variation in habitat use rather than dietary variation.

Temporal variability of food source $\delta^{13}\text{C}$

Researchers commonly consider temporal variation in the context of stable isotope analysis (O'Reilly et al. 2002; Post 2002; Schmidt et al. 2003). Temporal variability in the $\delta^{13}\text{C}$ of food sources has implications for intrapopulation isotopic variability of consumers. Since the $\delta^{13}\text{C}$ of producers can change quickly over time (Cabana and Rasmussen 1996), the $\delta^{13}\text{C}$ of producers and consumers collected at the same time may not match as expected (Post 2002). Temporal variability of plankton in lakes can be substantial, and amplitudes of seasonal variation of $\delta^{13}\text{C}$ varies from <3 to 20‰ (Zohary et al. 1994; Post 2002; Matthews and Mazumder 2003). If two consumers have different growth rates, and there is directional isotopic change in the food source, then their isotopic signatures may be different even if their diets are identical. Though there is little empirical evidence, temporal variability in food sources can contribute to intrapopulation variability, and may help account for high intraspecific variability in $\delta^{13}\text{C}$ among or within fish species (such as Beaudoin et al. 1999; Fry et al. 1999).

Cabana and Rasmussen (1996) found that the temporal variation of $\delta^{13}\text{C}$ is larger in primary producers than in primary or secondary consumers. This is not surprising given that the temporal variation of $\delta^{13}\text{C}$ for a consumer depends, in part, on the rate of tissue turnover (Tieszen et al. 1983). Simulations of the NULL_M model provide a link between isotopic tissue turnover and the expected intrapopulation isotopic variability. As we increase the number of feeding trials in our simulations, the expected intrapopulation isotopic variability decreases (Fig. 1b). This may increase the likelihood of detecting individual specialization. To confirm individual specialization using a null model approach, we must carefully choose a model that reflects the spatial and temporal variability of the food sources, and the feeding behavior and life history of the consumer.

Intrapopulation variation in fractionation

Accounting for fractionation processes is a common challenge in many stable isotope studies. Several recent reviews of stable isotope methodology provide laboratory and field estimates of ^{13}C fractionation between consumers and their food sources (France and Peters 1997; Vander Zanden and Rasmussen 2001; Post 2002; McCutchan et al. 2003). An average interspecific estimate may suffice for the analysis of whole food webs (Post 2002), but the variance of this estimate is not appropriate to test for individual specialization. Instead, we need an estimate of variation in ^{13}C fractionation among individuals for the species of interest (or a similar species), based on feeding trials in a controlled setting (DeNiro and Epstein 1978). Alternatively, we could compare the observed variance in a species with an unknown diet, to the variance of another species with a similar biology and a known diet (sensu Fry et al. 1978; Kling et al. 1992).

Currently there is little data on how ^{13}C fractionation varies in a given species under different food conditions. Adams and Sterner (2000) found that fractionation of ^{15}N by *Daphnia* increased by $\sim 3\%$ with an increase in the carbon to nitrogen ratio of *Scenedesmus acutus*. Oelbermann and Scheu (2002) found that a generalist predatory spider (*Pardosa lugubris*) had a consistently higher $\delta^{13}\text{C}$ signature when fed high quality prey. These studies provide a significant caveat for being able to detect individual specialization using stable isotopes, because the degree of fractionation depends on the quality of the diet. Currently, the sensitivity of the null model approach is limited by unknown variation in ^{13}C fractionation. Without reliable estimates of σ_{Δ}^2 , it is difficult to make inferences about the sources of intrapopulation variation in $\delta^{13}\text{C}$ from field observations.

Interpreting intrapopulation isotopic variance

The stable isotopes $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are potentially valuable for quantifying individual differences in diet, however

there are some significant challenges in using stable isotopes to define dietary variation. First, without isotopic variation among food sources stable isotopes provide little information about dietary variation among individuals. Comparing the isotopic variance of consumers among lakes (sensu Beaudoin et al. 1999) faces a similar problem as comparing average isotopic signatures among lakes. Inter-lake variability in $\delta^{15}\text{N}$ at the base of the food chain is unlikely related to trophic variation (Vander Zanden and Rasmussen 1999; Post 2002; Matthews and Mazumder 2003). Likewise, interlake variation in the isotopic range of prey items may not be related to dietary variation (using $\delta^{13}\text{C}$) or trophic variation (using $\delta^{15}\text{N}$). It is still an open question to what extent intrapopulation isotopic variation reflects dietary and trophic variation. Second, when there are n food sources for the consumer, we need at least $n-1$ isotopes to resolve dietary dependencies (Phillips and Gregg 2003). If we consider the variation of an isotope as a niche axis, then we may need multiple isotopes to resolve dietary based niche differences and infer individual specialization. Clearly, our ability to define a population's niche is limited by the number of available isotopic axes. If individual consumers have persistent feeding preferences for different prey types, and there is known or temporally consistent isotopic variation among prey, then variation in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ may help indicate intrapopulation variation in diet. However, isotopic variation is not necessarily synonymous with dietary variation, and therefore not directly equivalent to a dietary based niche. If we use stable isotopes to define a population's niche, and attribute high isotopic variance to individual specialization, then it is critical to isolate the isotopic variation that directly results from dietary and trophic variation among individuals.

Conclusions

Stable isotopes are useful tools for the study of ecological systems, but their relative ease of use, and assumed simplicity in interpretation, make them vulnerable to misinterpretation (Gannes et al. 1997). If we want to relate the variation in $\delta^{13}\text{C}$ to individual specialization, we must first account for variation in $\delta^{13}\text{C}$ resulting from differences in age, sex, and ecomorph, as Bolnick et al. (2003) eloquently outlined as a model to detect individual specialization based on diet composition. We can then establish a null model based on the diet composition, the isotopic composition of food sources, the life history of the consumer population, and the ^{13}C fractionation between the consumer and its diet. If we carefully consider temporal and spatial variability in $\delta^{13}\text{C}$ of the prey and the consumer, then we are better equipped to use stable isotopes to detect individual specialization.

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