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ORIGINAL PAPER

The isotopic composition and insect content of diet predict tissue isotopic values in a South American passerine assemblage

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Abstract We analyzed the carbon and nitrogen isotopic values of the muscle, liver, and crop contents ("diet") of 132 individuals of 16 species of Chilean birds. The nitrogen content of diet was tightly correlated with the fraction of gut contents represented by insects relative to plant material. The carbon and nitrogen isotopic values of diet, liver, and muscle were all linearly correlated, implying high temporal consistency in the isotopic value of the diet of these birds. However, δ^{15}N was not significantly related with the percentage of insects in diet. These results cast doubt on the applicability of the use of ^{15}N enrichment to diagnose trophic level in, at least some, terrestrial ecosystems. However, the residuals of the relationship relating the isotopic value of bird tissues with those of their diet were weakly negatively correlated with insect intake. We hypothesize that this negative correlation stems from the higher quality of protein found in insects relative to that of plant materials. Finally, our data corroborated a perplexing and controversial negative relationship between tissue to diet isotopic discrimination and the isotopic value of diet. We suggest that this relationship is an example of the commonly observed regression to the mean effect that plagues many scientific studies.

Keywords Stable isotope · Diet · Birds

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Introduction

What animals eat determines their physiological, morphological and behavioral traits (Klasing 1998; McNab 2002). It is also a major factor in the role that animals play in biotic communities and ecosystems. Therefore, it is not surprising that the study of dietary habits has been a central focus of the attention of animal ecologists (Bolnick et al. 2003; Karasov 2011). Traditionally, an animal's diet was characterized by the analyses of stomach content, by fecal analysis, and by direct observation (Stephens et al. 2007). However, those methods have restrictions and may be costly in effort and time (Araújo et al. 2007; Martínez del Rio 2007). Moreover, fecal samples and stomach contents provide only snapshots of what animals just ate and defecated and thus cannot be used to infer animal diets at broader temporal scales without incurring in high economic and time costs. Recently, the use of stable isotope analyses (SIA) of animal tissues has facilitated the study of animal diets (Herrera et al. 2001; Sabat and Martínez del Rio 2002; Bearhop et al. 2003; Akamatsu et al. 2004; Sabat et al. 2006a, b; Araújo et al. 2007; Fox-Dobbs et al. 2007). The cost of isotope analyses has decreased steadily, automation has greatly facilitated the method's use and accessibility (Martínez del Rio et al. 2009), and stable isotope analyses have the major advantage of allowing determining animal diets at a variety of temporal scales (Dalerum and Angerbjörn 2005).

The use of isotope ratio of a consumer's tissues to assess diet relies on two observations: (1) tissues reflect the isotopic composition of an animal's diet (Hobson and Clark 1992), and (2) primary producers at the base of food webs often differ in carbon, nitrogen, and hydrogen isotopic composition (Farquhar et al. 1989; Robinson 2001; Hobson 1987; Martínez del Rio et al. 2009). Although the isotopic value of animal tissues reflects that of diet, the transfer of the isotopic composition between diet and tissues is not perfectly faithful. The difference in isotopic value between tissues and diet is called the discrimination factor (DF) if it is estimated in the laboratory or the trophic enrichment factor (TEF) if it is measured in the field (Caut et al. 2009). DFs and TEFs are denoted by a capital Δ ($\Delta X_{\text{tissues-diet}} = \delta X_{\text{tissues}} - \delta X_{\text{diet}}$, where X is an isotope). Estimates of tissue to diet DF/TEF values are needed for diet reconstruction (Vander Zanden and Rasmussen 2001). Several studies have demonstrated consistent differences in isotopic values, and hence discrimination factors, among different tissues (Carleton and Martínez del Rio 2005; Voigt et al. 2008). These differences have been explained as the result of variation in macromolecular composition such as lipid content and in protein in amino acid composition (Florin et al. 2011). With few exceptions (see Newsome et al. 2010), tissue to diet discrimination factors are measured in the laboratory under controlled conditions (DeNiro and Epstein 1978; Tieszen et al. 1983; Bearhop et al. 2003; Pearson et al. 2003; Carleton et al. 2006), Even controlled experiments sometimes reveal large variation in tissue to diet discrimination. Sponheimer et al. (2003) documented tissue δ^{15}N values that differed by as much as 3.6 ‰ in mammalian herbivores eating identical diets. There are fewer data available for trophic enrichment factors measured in the field.

Animal tissues differ not only in discrimination factors but also in the rate at which they incorporate new materials (Hobson and Clark 1992). In fact, different tissues differ in the rate at which they incorporate new components (Martínez del Rio and Wolf 2005). Some tissues, such as liver and pectoralis muscle, have faster turnover rates, and their isotopic composition reflects integration of recent diets (days to weeks), whereas others, such as bone, exhibit slow incorporation rates and their isotopic composition reflects dietary components over longer time periods (months, Tieszen et al. 1983; Carleton and Martínez del Rio 2005). Some inert tissues, such as feathers, retain the isotopic composition of resources incorporated during the molt period (Bearhop et al. 2003, 2004). Thus, the isotopic variation among tissues within a single individual permits inferring the temporal variation in use of resources with distinct isotopic composition—or isotopic niche breadth—of individuals and populations (Bearhop et al. 2004; Newsome et al. 2007; Martínez del Rio et al. 2009).

One of the most common uses of stable isotope analysis is the estimation of trophic position (Ehleringer et al. 1986; Peterson and Fry 1987; Post 2002). This method is dependent on the observation that because the tissues of consumers are enriched in ^{15}N relative to those of their diets (i.e. $\Delta^{15}\text{N} > 0$), ^{15}N appears to become biomagnified across trophic levels (Gannes et al. 1997; Robbins et al. 2005). Vander Zanden et al. (1997) tested this conjecture in aquatic ecosystems by comparing the results of stable isotope analyses with those of gut contents. They found a good correspondence between trophic level estimates obtained by measuring δ^{15}N and those estimated by direct diet analyses. Although the correlation between δ^{15}N and trophic level is deemed to be generally valid, to our knowledge a test similar to that of Vander Zanden et al. (1997) has not been done in a terrestrial ecosystem. For example, Schondube et al. (2001) differentiated between animal and plant-eating bats using the δ^{15}N of blood samples. Herrera et al. (2003) examined the avian trophic patterns in a tropical rain forest in Mexico, and concluded that stable nitrogen isotope analysis separated birds into three distinct trophic levels. These studies assumed that δ^{15}N could be used to separate trophic levels but did not use an independent measure of trophic level to test whether their isotopic estimation of trophic position actually matched that estimated by other means.

One of the purposes of this study is to assess whether there are differences in ^{15}N content in the tissues of birds that feed on different amounts of animal, relative to plant, material and hence on different trophic levels. We report the δ^{15}N and δ^{13}C values of tissues in 134 individuals from 16 passerine species from a Mediterranean ecosystem in Chile. Our aim was to determine whether there were differences in the isotopic values of the tissues of birds that ingest different amounts of animal and plant material. In addition to measuring isotopic values, we collected data on gut contents to give more quantitative assessment of degree of ingestion of animal material, and thus of higher trophic level. By measuring both the isotopic composition of tissues and diets, we were able not only to examine whether ^{15}N increases with trophic level but also to estimate tissue to diet enrichment factors in the field. The magnitude of the nitrogen isotope tissue to diet discrimination factor has been hypothesized to increase with diet protein content and to decrease with protein quality (Martínez del Rio and Wolf 2005). Animal tissues generally have higher protein quality than plant foods (Boyd and Goodyear 1971). Hence in our study, both protein content and quality increased with the fraction of diet comprised by animal matter, and would support to discriminate between these two alternative hypotheses. Thus, an increase of TEF with animal matter in the gut provides support to the protein content hypothesis, whereas an decrease in TEF with animal prey

would be expected if the protein quality hypothesis is correct. Because we measured the isotopic values of two tissues (liver and muscle) with contrasting turnover rates (see Boecklen et al. 2011 for a review), our measurements allowed us to find out the degree of temporal constancy in diet among the bird species in this assemblage.

Caut et al. (2009, 2010) reported a roughly linear decrease in $\Delta^{13}C_{\text{tissue-diet}}$ and $\Delta^{15}N_{\text{tissue-diet}}$ with the carbon and nitrogen isotopic signature of diet, respectively, in a variety of taxa. This pattern is potentially useful as it suggests a way to estimate trophic enrichment from data on the isotopic value of diet when no direct estimates are available (Caut et al. 2009). However, the pattern has been criticized as the results of either an unaccounted biological effect (Perga and Grey 2010) or a statistical artifact (Auerswald et al. 2010). Our data set allowed us to assess the generality of Caut et al.'s (2009) controversial pattern.

Methods

Birds (134 specimens from 16 passerine species) were captured at Quebrada de la Plata (33°31′S, 70°50′W), central Chile. This study site has Mediterranean climate characterized by hot, dry summers and cold, rainy winters (mean annual precipitation = 367 mm, di Castri and Hajek 1976). All birds were captured with Ecotone mist nets in the summer of 2008–2009 (November to February). Immediately after capture, birds were killed by CO_2 asphyxiation and their proventriculus and gizzard contents were removed and frozen in liquid nitrogen. We were able to obtain gut content data for only 54 of all specimens collected. Gut contents were thawed, prey items were separated into animal and plant components, dried to constant mass at 60 °C, and weighed (±0.0005 g). Because all animal materials were represented by insect parts, we refer to the animal fraction as the insect content of diet. The whole gut contents of each specimen were re-mixed, homogenized with a Potter–Elvehjem tissue grinder, freeze-dried, and ground into a fine powder. Bird tissues (liver and *pectoralis* muscle) were freeze-dried, ground into a fine powder, and lipid extracted with a Soxhlet apparatus. We also collected a set of 19 plant species present in the area of study which were processed as above for isotope determination. All samples were loaded into pre-cleaned tin capsules (ca. 0.15 mg) for carbon and nitrogen isotopic analysis.

Isotope ratios were measured on a continuous flow isotope ratio mass spectrometer (Finnigan Delta + XP; University of Wyoming's Light Stable Isotope Facility) in line with a Costech elemental analyzer, which measured the percent of carbon and nitrogen in our samples. The precision of isotopic analyses was ± 0.2 % for both isotopes. Our standards were vacuum oil [$\delta^{13}C = -27.5$ %, Vienna Pee Dee belemnite (VPDB)] and ANU sucrose ($\delta^{13}C = -10.5$ %, VPDB, NIST 8542) for $\delta^{13}C$ and peptone ($\delta^{15}N = 5.60$ %, AIR, USGS40 8542), and glycine ($\delta^{15}N = 0.73$ %, AIR, IAEAN2) for $\delta^{15}N$. We included standards in every run to correct raw values obtained from the mass spectrometer. Stable isotope ratios were expressed using standard δ notation in parts per mil (%) as:

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1{,}000,$$

where R_{sample} and R_{standard} are the molar ratios of the heavy/light isotope of the sample and the reference, respectively. Samples were referenced against the international standard, the VPDB for $\delta^{13}C$ and atmospheric N (AIR) for $\delta^{15}N$.

Statistical analyses

Our data set was complex and hence demanded an eclectic analysis. To determine the relationship between variables (typically δX, ΔX, % of insect diet content) we constructed fixed effects linear models that included species as independent variables. The full model included each species as a factor. The reduced model did not include species as factors. We compared the support of models with and without species as a factor using small sample Akaike information theoretic criteria (AICc, Johnson and Wichern 1998). A model was deemed superior if ΔAICc = AICc (with) − AICc (without) >2 (Johnson and Wichern 1998). We estimated $\Delta X_{\text{tissue-diet}}$, and $\Delta X_{\text{liver-muscle}}$ for each individual bird. Because sample sizes for some species were low, we did not attempt to assess whether there were statistically significant differences in discrimination factors among species. Instead, we reported species mean value and estimated sample-size weighted discrimination factors for the whole assemblage as

$$\text{assemblage mean} = \sum\nolimits_i \frac{v_i}{\sum\limits_i v_i}\left(\bar{\Delta}_i\right), \tag{1}$$

where n_i is the number of individuals of species i in the assemblage and $\bar{\Delta}_i$ is its average discrimination factor. We estimated the standard deviation in this sample-size weighted mean as

$$s = \sum_i \left(\frac{n_i}{\sum_i n_i}\right)^2 s_i^2, \tag{2}$$

where s_i^2 is the variance in $\bar{\Delta}_i$ (Zar 1996). We determined the strength of the association between the isotopic values of liver and muscle using Pearson product moment correlation coefficients (r) and in addition to linear models, we estimated the coefficients of linear relationships using reduced major axis (or Deming) regression (RMA). We chose RMA over standard least squares regression for

parameter estimation because in all our bivariate analyses both variables were estimated with error of similar magnitude and in order to obtain symmetric relationships in which neither variable was considered "dependent" or independent" (Smith 2009). The slopes, intercepts, and correlation coefficients of RMA analyses were estimated following Sokal and Rohlf (1981) and Weir (1990). We tested Caut et al.'s (2009) conjecture of a negative relationship between $\Delta X_{\text{tissue-diet}}$ and δX in two ways: first, we tested whether the slope of the RMA regression between δX_{diet} and $\delta X_{\text{tissues}}$ was statistically lower than 1 (see Caut et al. 2010), and second, we estimated the relationship between $\Delta X_{\text{tissue-diet}}$ and δX_{diet}. The latter method is controversial and potentially flawed as $\Delta X_{\text{tissue-diet}}$ and δX_{diet} are not statistically independent (Auerswald et al. 2010). We used it because it depicts the potential dependence of enrichment factors with the isotopic value of diet in a clear and intuitive way. Although we estimated the fraction of the total mass of gut contents represented by insects or plant material in many of the collected specimens, we chose not to use inferential statistics to assess whether this fraction was dependent on whether birds were classified a priori as insectivores, granivores, or omnivores (Table 1). We refrained from using inferential statistics because the samples sizes per species for this measurement were relatively small (they ranged from 1 to 7 individuals per species; Table 1). We simply report all values and averages in "Discussion".

Results

The percentage of nitrogen in gut contents was linearly, and relatively tightly, related with insect content (Fig. 1). Therefore, we used insect content in diet as a variable that summarizes both nitrogen content and protein quality. In general, animal protein is of much higher quality than plant protein (Schaafsma 2000). There was wide variation among species in the carbon and isotopic values of their tissues and in those of their diets (Table 1). The δ^{13}C and δ^{15}N of the diets ingested by the birds in the assemblage varied by $\approx$6 and 14 ‰, respectively (Table 1; Fig. 2). In a similar fashion, the δ^{13}C and δ^{15}N of the birds tissues varied by $\approx$6 and 9.3 ‰, respectively (Table 1; Fig. 2). Several bird species had very broad ranges of δ^{13}C and δ^{15}N tissue values (e.g. *Turdus falklandii* and *Surnella loyca*, Fig. 2). The δ^{13}C of both liver and muscle was significantly and positively related with the δ^{13}C of diet (Fig. 2). Models without species as a factor were better supported than those including species (ΔAICc $>$ 12). The results for δ^{15}N were similar. For both muscle and liver the δ^{15}N of tissues was positively related with that of diet (Fig. 2), and the best-supported models included only the isotopic value of diet as an independent variable (ΔAICc $>$ 3).

Δ^{15}N was not significantly correlated with insect content in diet ($r^2 < 0.007$, $p > 0.5$ for muscle and liver). A model with $\delta^{15}N_{\text{muscle}}$ as a dependent variable and both $\delta^{15}N_{\text{diet}}$ and insect content as independent variables was better supported than a model that included only $\delta^{15}N_{\text{diet}}$ as an independent variable (ΔAICc = 7.4). Insect content had a negative effect on the residuals of the relationship between $\delta^{15}N_{\text{muscle}}$ and $\delta^{15}N_{\text{diet}}$ as predicted by Florin et al. (2011, Fig. 3), but the effect of insect content on δ^{15}N was relatively weak: δ^{15}N decreased by a predicted $\approx$1.5 ‰ from birds feeding only on plant material to those feeding only on insects. This was not the case for $\delta^{15}N_{\text{liver}}$. In this tissue both the full and the reduced models received the same support (ΔAICc = 0.1) and there was no significant decrease in the residuals of the relationship between $\delta^{15}N_{\text{muscle}}$ and $\delta^{15}N_{\text{diet}}$ with increased insect content in diet (Spearman rank $rs = 0.22$, $p = 0.12$).

In support of Caut et al.'s (2009) conjecture, the RMA slope of the relationship between δ^{13}C and δ^{15}N of tissues and the corresponding isotope values of diet was in all cases significantly lower than 1 (Fig. 2). Consequently, $\Delta X_{\text{tissues-diet}}$ was in all cases negatively correlated with δX_{diet} (Fig. 4). For both carbon and nitrogen, the isotopic values of liver and muscle were linearly and tightly correlated (Fig. 5). The RMA slopes of these relationships did not differ significantly from 1 ($t > 1.6$, $p > 0.112$). In the study as a whole, muscle was depleted in both ^{13}C and ^{15}N relative to liver (by a sample-weighted average equal to 0.2 $\pm$ 0.1 and 1.1 $\pm$ 0.3 ‰ for ^{13}C and ^{15}N, respectively; Fig. 5). The assemblage-wide tissue to diet trophic enrichment factors for nitrogen were significantly higher for liver ($\Delta^{15}N_{\text{liver-diet}} = 4.4 \pm 0.5$ ‰) than muscle ($\Delta^{15}N_{\text{muscle-diet}} = 3.5 \pm 0.6$ ‰, paired t test on species mean value = 3.7, $p < 0.023$). The tissue to diet enrichment factors for ^{13}C in liver and muscle had similar values (2.8 $\pm$ 0.2 and 2.8 $\pm$ 0.3 ‰ for liver and muscle, respectively, paired t test on species mean value = 0.21, $p = 0.19$).

Discussion

Our results revealed clear patterns at the same time that they posed intriguing questions. We found clear linear relationships between the isotopic value of tissues and those of diet. However, this relationship did not have a slope equal to one. Rather, the relationship of the isotopic value of tissues with those of diet had shallower slopes that ranged from 0.62 to 0.84. These shallow slopes strongly support Caut et al.'s (2009) perplexing negative relationship between the magnitude of discrimination factors and

Table 1 Dietary category, body mass, liver, muscle and diet isotope signatures of 16 passerine species from central Chile

Species	Symbol	Diet	Body mass	N	$\delta^{13}C_{muscle}$	$\delta^{13}C_{liver}$	$\delta^{15}N_{muscle}$	$\delta^{15}N_{liver}$	$\delta^{13}C_{diet}$	$\delta^{15}N_{diet}$
Pseudasthenes humicola	*	Insectivore	21.42 ± 0.97	8 (3)	−22.21 ± 0.73	−22.18 ± 0.35	4.69 ± 2.89	5.18 ± 0.76	−24.14 ± 0.75	−0.07 ± 1.0
Leptasthenura aegithaloide	+	Insectivore	7.80 ± 0.71	5 (3)	−22.44 ± 0.65	−22.42 ± 0.70	3.82 ± 0.75	5.95 ± 1.49	−24.73 ± 1.28	2.23 ± 3.2
Anairetes palurus	×	Insectivore	5.57 ± 0.57	10 (2)	−22.56 ± 0.76	−22.17 ± 0.72	4.97 ± 2.15	6.60 ± 3.02	−23.82 ± 0.17	−2,42 ± 0.53
Troglodytes aedon	■	Insectivore	8.96 ± 0.91	12 (4)	−23.78 ± 0.86	−23.20 ± 0.79	4.72 ± 1.02	6.47 ± 1.19	−25.64 ± 1.32	2.64 ± 3.86
Xolmis pyrope	□	Insectivore	32.71 ± 2.11	5 (1)	−23.59 ± 0.55	−23.37 ± 0.52	7.19 ± 2.27	9.61 ± 2.32	−25.17	7.87
Elaenia albiceps	●	Omnivore	13.56 ± 1.68	12 (4)	−23.09 ± 0.74	−22.63 ± 0.96	5.40 ± 0.73	6.38 ± 1.17	−24.28 ± 0.87	0.95 ± 1.51
Turdus falklandii	◆	Omnivore	78.08 ± 8.04	11 (4)	−24.07 ± 0.92	−23.09 ± 1.72	6.96 ± 2.12	7.41 ± 1.96	−27.92 ± 1.74	1.13 ± 5.09
Mimus thenca	◇	Omnivore	66.40 ± 7.57	8 (4)	−23.55 ± 0.70	−23.14 ± 0.65	8.51 ± 1.75	9.62 ± 2.07	−24.74 ± 0.76	5.4 ± 1.8
Zonotrichia capensis	○	Omnivore	20.73 ± 1.77	9 (7)	−23.73 ± 0.95	−23.45 ± 1.04	6.16 ± 2.05	6.74 ± 1.75	−26.42 ± 1.41	1.26 ± 2.57
Curaeus curaeus	▲	Omnivore	89.65 ± 10.29	10 (5)	−23.22 ± 0.96	−22.88 ± 0.94	4.69 ± 0.75	5.75 ± 1.36	−25.81 ± 0.95	0.28 ± 2.28
Sturnella loyca	Δ	Omnivore	87.21 ± 11.86	9 (4)	−23.64 ± 1.31	−23.88 ± 1.27	6.34 ± 2.13	7.81 ± 1.79	−26.67 ± 0.28	3.34 ± 3.21
Pteroptochos megapodius	▼	Omnivore	140.6 ± 7.35	2 (2)	−23.07 ± 1.31	−24.70 ± 0.70	7.59 ± 3.37	7.46 ± 0.24	−26.77 ± 1.31	5.16 ± 0.16
Carduelis barbata	▽	Granivore	14.30 ± 2.22	9 (3)	−22.49 ± 0.47	−19.66 ± 7.45	9.13 ± 0.98	10.80 ± 1.41	−24.64 ± 0.03	7.49 ± 1.08
Phrygilus fruticeti fruticeti	◉	Granivore	39.11 ± 1.07	3 (3)	−23.62 ± 1.04	−23.49 ± 0.35	4.97 ± 1.67	4.52 ± 0.45	−28.48 ± 0.04	2.03 ± 1.99
Diuca diuca	✪	Granivore	33.74 ± 2.20	11 (5)	−24,02 ± 0.85	−24.12 ± 0.82	6.84 ± 1.41	7.56 ± 1.28	−27.75 ± 0.59	3.22 ± 1.42
Sicalis luteola	⌑	Granivore	15.47 ± 1.02	10 (2)	−24.16 ± 0.66	−23.99 ± 0.45	6.71 ± 1.08	7.47 ± 0.75	−27.32 ± 0.27	3.43 ± 0.008

Number in parentheses refers to the sample of individual used for dietary isotopic analysis. Symbols refer to those used in all figures

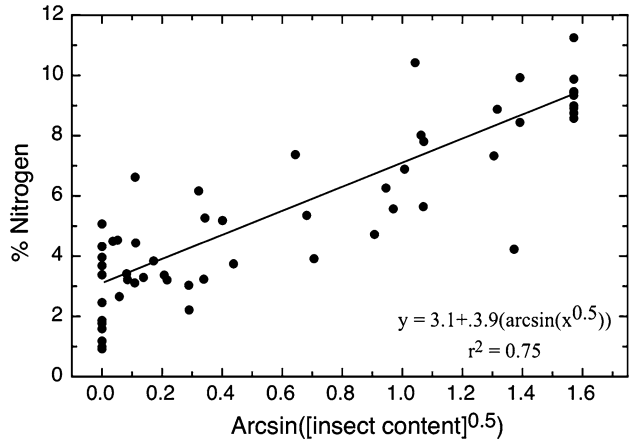

Fig. 1 In an assemblage of Chilean birds (54 individuals of 16 species), the percentage of nitrogen in diet increased linearly with the percentage of insects in gut contents. Regression coefficients were estimates obtained by standard least squares

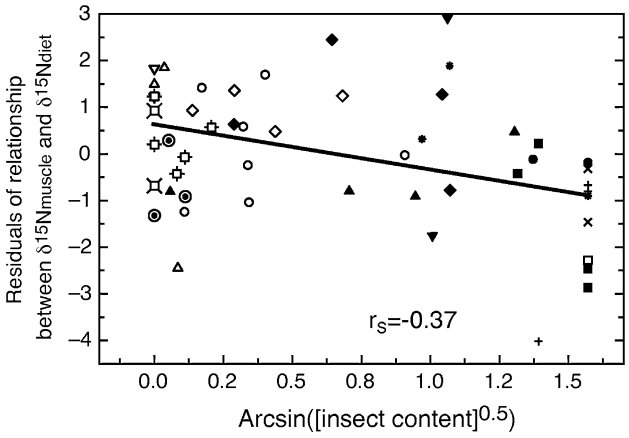

Fig. 3 The residuals of the relationships between the δ^{15}N in muscle and diet decreased with the proportion of insects present in diet. We used Spearman's rank correlation to assess the degree of correlation instead of Pearson product moment correlation because the residuals were not distributed homogeneously around the regression line. Symbols for each species are listed in Table 1

the isotopic value of diet. Furthermore, because these shallow slopes were observed in carbon and nitrogen and in both liver and muscle, our results suggest that Caut's et al. (2009) relationship is robust. Here, we propose an explanation to the pattern. In contrary to our expectations, we found no relationship between trophic level, as measured by the percentage of diet that comprises insects, and nitrogen's isotopic discrimination factor. Here, we consider both the reasons and the significance of this negative result. Finally, our results revealed tight relationships between the isotopic values of liver and muscle with relatively constant, but not insignificant, discrimination factors between these tissues. The final section of our discussion highlights the implications of this result.

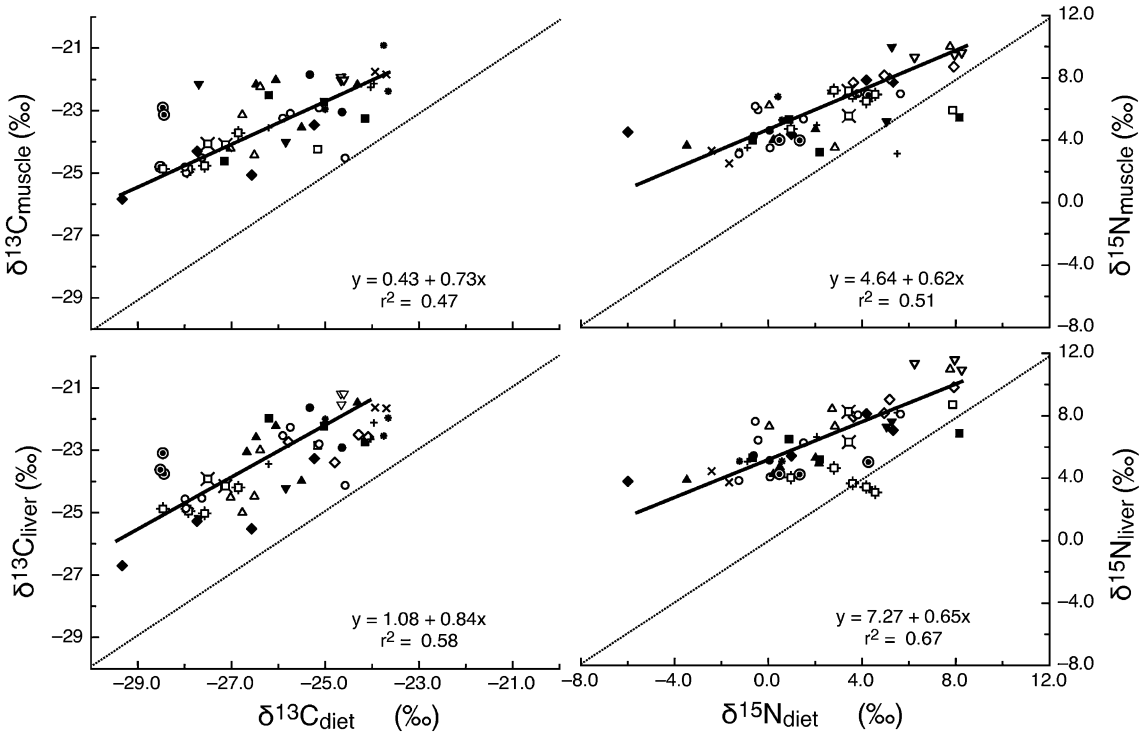

Fig. 2 The isotopic value of tissues increased linearly with the isotopic value of gut contents. However, the reduced major axis slope of these relationships was significantly lower than 1. *Each point* represents one individual. Symbols for each species are listed in Table 1

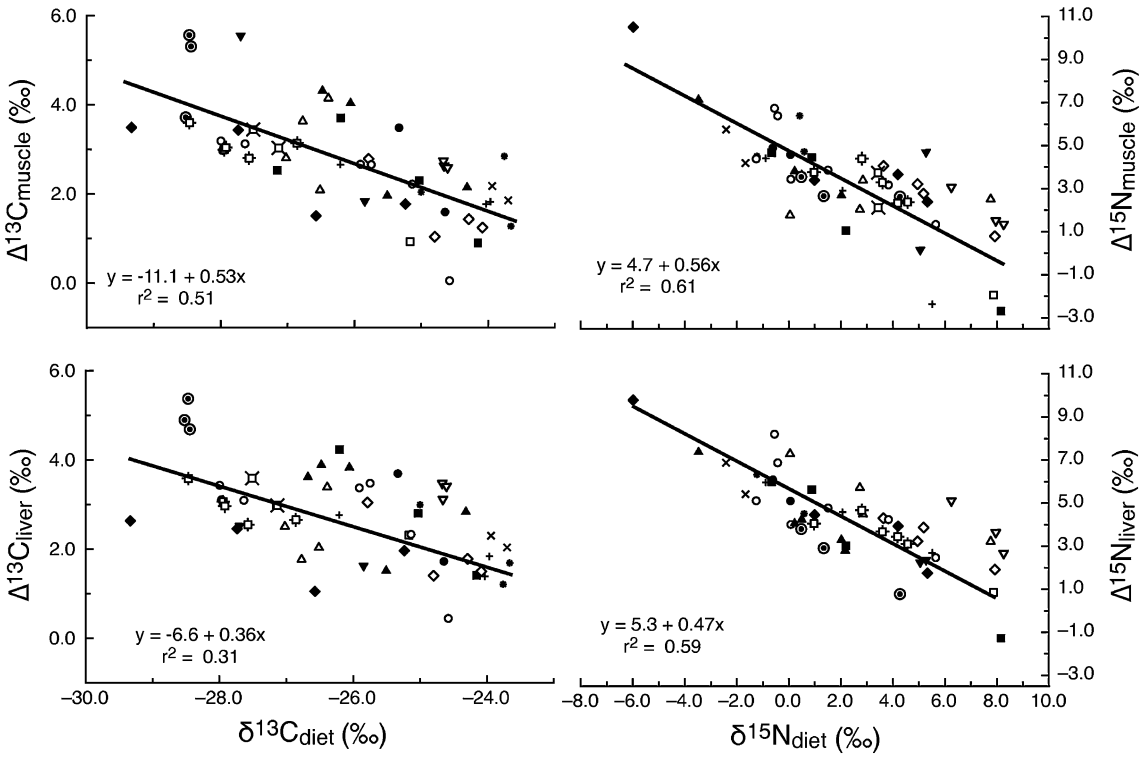

Fig. 4 The discrimination factors for carbon and nitrogen ($\Delta^{13}C$ and $\Delta^{15}N$, respectively) decreased linearly with the isotopic composition of gut contents. Regression coefficients were estimated by reduced major axis. Symbols for each species are listed in Table 1

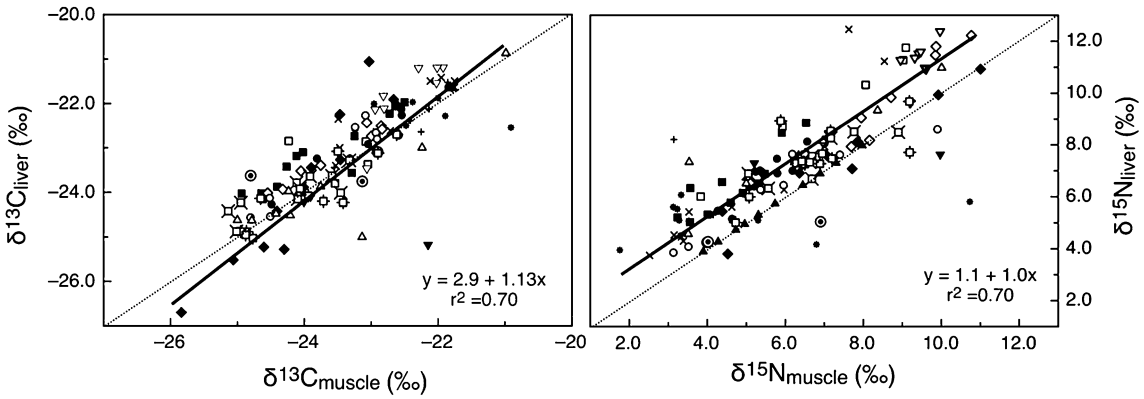

Fig. 5 The isotopic composition of liver and muscle were linearly and tightly correlated. Regression coefficients were estimated by reduced major axis. Symbols for each species are listed in Table 1

Do discrimination factors depend on the isotopic value of diet? A prosaic explanation for a controversial pattern

The report by Caut et al. (2009) of a negative relationship between discrimination factors and the isotopic value of diet was met with skepticism. Perga and Grey (2010) explained the result as a consequence of nutritional heterogeneity in diet and Auerswald et al. (2010) contended that the relationship was a statistical artifact of conducting regression analysis on non-independent variables (see Caut et al. 2010 for a reply to these criticisms). Codron et al. (2012) conducted a set of simulations that suggest that Caut et al. (2009, 2010) observation can arise from mixed diets that are heterogeneous either isotopically or nutritionally, as a result of tissue turnover, or from isotopic routing. Here, we present a more prosaic explanation for this pattern. We suggest that it is the result of perhaps the most ubiquitous and widespread misinterpretation of the results of least squares regression analysis: regression to the mean (RTM, Stigler 1997).

RTM is a very robust statistical result that occurs whenever repeated measures are made on a subject or

observation unit (e.g. a species fed on a certain diet) and when the variable of interest varies randomly within and among subjects. Assume that one conducts a single measurement on a variable with non-systematic (random) error and assume that by chance one obtains an extreme value. If one conducts another measurement on the same variable, its value will be closer to the mean (Davis 1976). Thus, a regression of the first against the second measurement will have a slope that is lower than 1. Statisticians call this effect attenuation of association or regression dilution bias (Barnett et al. 2004). The term "regression" was coined by Francis Galton to refer to this effect (Stigler 1997). In the case of discrimination factors, we are regressing $\delta_{tissues} = \delta_{diet} + \Delta$ as a dependent variable, against δ_{diet}. Thus, if all our values were measured without error, we would expect a line with slope equal to 1 and intercept equal to Δ. However, because measurements on diet isotopic values are made with error and because tissues reflect time averages, we should expect a regression to the mean effect and a slope lower than 1. As implicitly recognized by Codron et al. (2012), the RTM effect is greatly exacerbated in isotopic studies because the isotopic value of tissues reflects a time-integrated average of past diets, whereas diet represents a single measurement in time. The isotopic value of diet is much more likely to represent an extreme isotopic value than the isotopic value of a tissue.

Using incorrect discrimination factors can lead to large errors in dietary reconstruction from isotopic data (references). Thus, Caut et al.'s (2009) observation of diet-dependent discrimination factors is potentially important and useful. Caut et al. (2009) proposed using simple linear equations relating discrimination factors to diet values as a way to estimate discrimination values when these are not measured experimentally (see also Caut et al. 2010). However, if our conjecture is correct and the dependence of the value of discrimination factors on the value of diet is simply a consequence of RTM, then Caut et al.'s (2009) proposal would yield erroneous results.

Our argument for Caut et al.'s (2009) result as a consequence of RTM seems weakened by the observation of tight linear relationships with slopes that did not differ from 1 between the isotopic values of liver and muscle. How come these two tissues do not show regression to the mean effects? The magnitude of the regression to the mean effect increases with the ratio of within subject variance to total variance (Sheppard 2003; Barnett et al. 2004). Indeed, if within-individual variation is nil, then there is no regression to the mean effect. Because the isotopic values of tissues are time-integrated means, their within-individual variance is likely very small relative to the variance among individuals and consequently they should have small RTM effects. We predict that RTM effects on the relationships between the isotopic values of two tissues will be more prevalent as the disparity in time scale of incorporation, and hence as the difference in intra-individual variance in these values, increases between the tissues.

Can we use $\delta^{15}N$ to estimate degree of insectivory in birds?

DeNiro and Epstein (1981) noted that animal tissues were enriched in ^{15}N relative to their diets (i.e. $\Delta^{15}N = \delta^{15}N_{tissues} - \delta^{15}N_{diet} > 0$). This very useful observation led to the conjecture that the content of ^{15}N in animal tissues is biomagnified in an additive fashion along the length of a food chain (Post 2002). The additive biomagnification of ^{15}N allows ecologists to use $\delta^{15}N$ to estimate an animal's trophic level (TL, Cabana and Rassmussen 1994). $\delta^{15}N$ has been used as a trophic level indicator in many freshwater (Vander Zanden and Fetzer 2007) and marine ecosystems (Pauly et al. 1998). In particular, it has been applied to estimate food-chain length from the $\delta^{15}N$ value of apical predators (Takimoto et al. 2008). In terrestrial ecosystems, $\delta^{15}N$ has been used to estimate trophic level (Herrera et al. 2003) and to assess whether bats (Herrera et al. 2001) and birds (Herrera et al. 2006) rely primarily on insects or plants as food. The rationale for the latter application is that consumers such as insects should be enriched in ^{15}N relative to primary producers (DeNiro and Epstein 1981; Peterson and Fry 1987).

Our study included bird species that are considered strict insectivores, strict granivores, and omnivores (Table 1). Our measurements of the insect/plant content in gastrointestinal tracts largely verified this anecdotal classification. Insectivores had mostly insects (mean fraction of insects $\pm$ SD $= 0.94 \pm 0.11$), granivores had mostly plants material (mean fraction of insects $\pm$ SD $= 0.01 \pm 0.01$), and omnivores had both plant and animal material in their gut contents (mean fraction of insects $\pm$ SD $= 0.32 \pm 0.36$). However, we found no correlation between the fraction of gut contents represented by insects and the $\delta^{15}N$ value of either liver of muscle. Indeed, when we accounted for the effect of the diet's isotopic value, we found a weak, but negative, effect of insectivory on the $\delta^{15}N$ value of muscle (Fig. 3).

The lack of a correlation between degree of insectivory and ^{15}N enrichment in bird tissues is probably the result of the wide variation in $\delta^{15}N$ values found among primary producers in the ecosystem that we studied and which likely led to a wide range of $\delta^{15}N$ values in insects (Fig. 6). The $\delta^{15}N$ value of diet in specimens that had exclusively plant material in their guts contents ranged from 0.04 to 8.26 ‰ ($N = 13$). That of birds that had exclusively insect material had an even broader range (from -2.45 to 8.20 ‰, $N = 12$). It is noteworthy that the range of $\delta^{15}N$ values of insect diets was broader because it included more

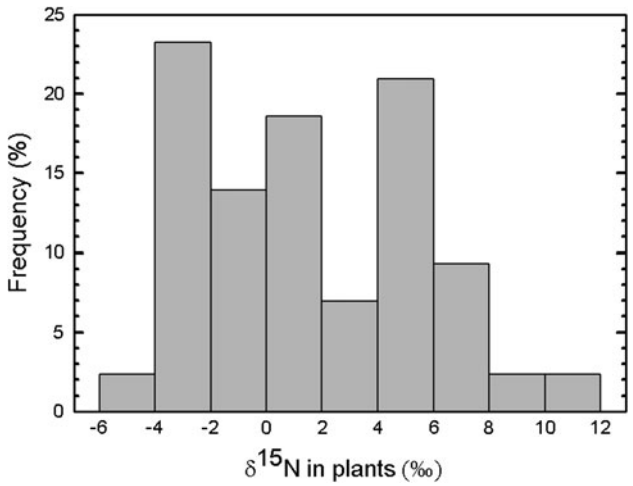

Fig. 6 Frequency of isotopic composition found among 19 primary producers in the Quebrada de la Plata, a Mediterranean ecosystem from central Chile

^{15}N-depleted values. It appears that insects were sampling foods (likely plants) with a broader range of nitrogen isotopic values than that of the plants found in the birds' gastrointestinal tracts. Using the δ^{15}N values of bird tissues to diagnose degree of insectivory (and of trophic level) requires that the insects ingested by birds are enriched in ^{15}N relative to plants, our results on a Mediterranean ecosystem suggest that this assumption might not be satisfied in general. Birds can eat insects that are more ^{15}N-depleted than the plant foods available to birds. Our results are consistent with those of a study by Gagnon and Hobson (2009) who found substantial overlap in δ^{15}N values between insectivorous and omnivorous songbirds in a temperate community. Gagnon and Hobson (2009) suggested that the absence of a large enough δ^{15}N difference between insects and fruits may have prevented detecting dietary differences among birds with these two dietary habits. The use of δ^{15}N as an indicator of insectivory, and presumably of trophic level, in small omnivorous birds is likely not justified in all ecosystems. The δ^{15}N values in plants may vary both within and among ecosystems as a consequence of spatial variation in precipitation, soil nitrogen content, and soil fertilization by anthropogenic sources or by the outputs of consumers (see Robinson 2001 for a review). The variation in δ^{15}N values within ecosystems appears to be higher in temperate than in tropical forest (Martinelli et al. 1999). Thus, the approach must be validated in each ecosystem studied.

Individual "isotopic niche conservatism" in birds?

Analyzing the isotopic signatures of tissues with different turnover rates or the same tissue over time allows assessing the degree of temporal consistency in animal diets (Hobson et al. 1999; Dalerum and Angerbjörn 2005; Newsome et al. 2010; Doucette et al. 2011). Hobson et al. (1999) found a very strong correlation between the carbon and nitrogen isotopic values of liver and that of muscle in invasive Norway rats (*Rattus norvegicus*). These results indicate temporal consistency in isotopic, and hence dietary, choices in these animals. In contrast, although there was a tight relationship between the carbon isotopic composition of liver and the percentage of saguaro fruit in the gut contents of White-winged doves (*Zenaida asiatica*), the relationship between the carbon isotopic value of diet and liver and that of muscle and collagen was poor (Martínez del Rio and Wolf 2005). Similarly, Hobson and Sealy (1991) found that the carbon isotopic values of the muscle of island Northwest saw-whet owls (*Aegolius acadicus*) often reflected a high reliance on marine sources. However, the carbon isotopic composition of bone collagen was that expected from animals feeding primarily on terrestrial sources. Both of these studies are consistent with the notion that some doves and owls relied on a pulse of isotopically distinct sources rather than had a diet that is isotopically consistent temporally.

We found not only a strong correlation between the isotopic composition of diet and that of bird tissues (Fig. 3) but also a strong linear relationships between the isotopic values of liver and muscle. This result suggests that although these two tissues have different rates of incorporation, they give more or less the same information about the isotopic composition of what individual birds eat. In zebra finches (*Taenopygia gutata*) and house sparrows (*Passer domesticus*) the average retention time of carbon in *pectoralis* muscle (21 and 24 days, respectively) was roughly twice as long as that in liver (9 and 12 days, respectively, Carleton et al. 2008; Bauchinger and McWilliams 2009). In spite of these seemingly large differences in isotopic incorporation rates, and with few exceptions (e.g. *Pteroptochos megapodius* for which mean $\Delta^{13}C_{liver\text{-}muscle} \pm SD = -1.6 \pm 1.9$, $N = 2$), the difference in δ^{13}C between liver and muscle was less than 1 ‰ in our species assemblage. Species $\Delta^{13}C_{liver\text{-}muscle}$ mean value spanned from −0.24 to 0.7 ‰ with a weighted average equal to 0.10 ‰ (±SD = 0.51; Fig. 5). When we accounted for the relatively constant 1.1 liver to tissue discrimination value in ^{15}N, we found that these tissues differed in δ^{15}N by less than 1.5 ‰ (Fig. 5). Species $\Delta^{15}N_{liver\text{-}muscle} - 1.1$ mean value spanned from −1.26 to 1.5 ‰ with a weighted average equal to 0.01 ‰ (±SD = 0.64, Fig. 5). The small variation in isotopic composition between tissues within individuals contrasts with the breadth in isotopic composition found between individuals within a species (Fig. 5). In our assemblage, the magnitude of the range in δ^{13}C values varied among species and spanned from 0.7 to 5.5 ‰ and from 1.7 to 4.0 ‰

in liver and muscle, respectively. The magnitude of the range of δ^{15}N values found in each species was broader, it ranged from 0.8 to 8.7 ‰ and from 1.8 to 9.0 ‰.

The positive linear relationship between the isotopic values of liver and muscle, and the narrow range of values found in the difference in isotopic composition between these tissues, implies high consistency in isotopic composition of individual bird diets. We hasten to add that this statement applies only to the time scales integrated by the tissues that we sampled. It does not imply absence of intra-individual variation in the isotopic composition of diet at finer (or broader) temporal scales. This variation can be documented by sampling tissues with faster (i.e. plasma, Hobson and Clark 1993) or slower (collagen, Dalerum and Angerbjörn 2005) rates of isotopic incorporation (see Norris et al. 2005). Finding out the degree of generalization or specialization in animals using stable isotopes depends on the time course of incorporation of the tissues sampled and compared (Martínez del Rio et al. 2009).

In conclusion, our study documented the perplexing and contested pattern documented by Caut et al. (2009) on a community of songbirds. We suggest that this pattern is likely, yet another example of regression to the mean. Economist Milton Friedman lamented that the "regression fallacy (*another name for the RTM effect*) is the most common fallacy in the statistical analysis of economic data" (Friedman 1992). Isotopic ecologists seem to have encountered this fallacy as well. We failed to justify the general use of δ^{15}N as an accurate index of insectivory in songbirds, and found a remarkable degree of intraindividual consistency in the isotopic composition of the resources used by birds at two time scales. Our study highlights the usefulness of a broad comparative field study to understand and interpret isotopic data.

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References

Akamatsu F, Toda H, Okino T (2004) Food source of riparian spiders analyzed by using stable isotopes. Ecol Res 19:655–662

Araújo MS, Bolnick DI, Machado G, Giarreta AA, dos Reis SF (2007) Using δ^{13}C stable isotopes to quantify individual-level diet variation. Oecologia 152:643–654

Auerswald K, Wittmer MHOM, Zazzo A, Schaufele R, Schnyder H (2010) Biases in the analysis of stable isotope discrimination in food webs. J App Ecol 47:936–941

Barnett AG, van der Pols JC, Dobson AJ (2004) Regression to the mean: what it is and how to deal with it. Int J Epidemiol 34:215–220

Bauchinger U, McWilliams S (2009) Carbon turnover in tissues of a passerine bird: allometry, isotopic clocks, and phenotypic flexibility in organ size. Physiol Biochem Zool 82:787–797

Bearhop S, Furness RW, Hilton G, Votier SC, Waldron S (2003) A forensic approach to understanding diet and habitat use from stable isotope analysis of (avian) claw material. Funct Ecol 17:270–275

Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H (2004) Determining trophic niche width: a novel approach using stable isotope analysis. J Anim Ecol 73:1007–1012

Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. Annu Rev Ecol Evol Syst 42:411–440

Bolnick DI, Svanback R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML (2003) The ecology of individuals: incidence and implications of individual specialization. Am Nat 161:1–28

Boyd CE, Goodyear CP (1971) Nutritive quality of food in ecological systems. Archiv für Hydrobiologie 69:256–270

Cabana G, Rassmussen JB (1994) Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature 372:255–257

Carleton SA, Martínez del Rio C (2005) The effect of cold-induced increased metabolic rate on the rate of 13C and 15 N incorporation in house sparrows (*Passer domesticus*). Oecologia 14:226–232

Carleton SA, Bakken BH, Martínez del Rio C (2006) Metabolic substrate use and the turnover of endogenous energy reserves in broad-tailed hummingbirds (*Selasphorus platycercus*). J Exp Biol 209:2622–2627

Carleton SA, Kelly L, Anderson-Sprecher R, Martínez del Rio C (2008) Should we use one, or multi-compartment models to describe ^{13}C incorporation into animal tissues? Rapid Commun Mass Sp22:3008–3014

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. J App Ecol 46:443–453

Caut S, Angulo E, Courchamp F, Figuerola J (2010) Trophic experiments to estimate isotope discrimination factors. J App Ecol 47:948–954

Codron D, Sponheimer M, Codron J, Newton I, Lanham JL, Clauss M (2012) The confounding effect of source isotopic heterogeneity on consumer-diet and tissue–tissue isotope relationships. Oecologia 169:939–953

Dalerum F, Angerbjörn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647–658

Davis CE (1976) The effect of regression to the mean in epidemiological and clinical studies. Am J Epidemiol 104:493–498

DeNiro M, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351

di Castri F, Hajek ER (1976) Bioclimatología de Chile. Editorial Universidad Católica de Chile, Santiago

Doucette JL, Wissel B, Somers CM (2011) Cormorant-fisheries conflicts: stable isotopes reveal a consistent niche for avian piscivores in diverse food webs. Ecol Appl 21:2987–3001

Ehleringer JR, Rundel PW, Nagy KA (1986) Stable Isotopes in physiological ecology and food web research. Trends Ecol Evol 1:42–45

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Ann Rev Plant Physiol 40:503–537

Florin ST, Felicetti LA, Robbins CT (2011) The biological basis for understanding and predicting dietary-induced variation in nitrogen and sulphur isotope ratio discrimination. Funct Ecol 25:519–526

Fox-Dobbs K, Bump JK, Peterson RO, Fox DL, Koch PL (2007) Carnivore-specific stable isotope variables and variation in the foraging ecology of modern and ancient wolf populations: case studies from Isle Royale, Minnesota, and La Brea. Can J Zool 85:458–471

Friedman M (1992) Do old fallacies ever die? J Econ Lit 30:2129–2132

Gagnon C, Hobson KA (2009) Using stable isotopes to track frugivory in migratory passerines. Can J Zool 87:981–992

Gannes LZ, O'Brien DM, Martínez del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory. Ecology 78:1271–1276

Herrera LG, Hobson KA, Manzo A, Estrada D, Sanchez-Cordero V, Mendez G (2001) The role of fruit and insects in the nutrition of frugivorous bats: evaluating the use of stable isotope methods. Biotropica 33:520–528

Herrera LG, Hobson KA, Rodríguez M, Hernandez P (2003) Trophic partitioning in tropical rain forest birds: insights from stable isotope analysis. Oecologia 136:439–444

Herrera LG, Hobson KA, Martínez JC, Méndez CG (2006) Tracing the origin of dietary protein in tropical dry forest birds. Biotropica 38:735–742

Hobson KA (1987) Use of stable-carbon isotope analysis to estimate marine and terrestrial protein content in gull diets. Can J Zool 65:1210–1213

Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. Condor 94:181–188

Hobson KA, Clark RG (1993) Turnover of ^{13}C in cellular and plasma fractions of blood: implications for nondestructive sample in avian dietary studies. Auk 110:638–641

Hobson KA, Sealy SG (1991) Marine protein contributions to the diet of northern saw-whet owls on the Queen Charlotte Islands: a stable-isotope approach. Auk 108:437–440

Hobson KA, Drever MC, Kaiser GW (1999) Norway rats as predators of burrow-nesting seabirds: insights from stable isotope analyses. J Wildl Manage 63:14–25

Johnson RA, Wichern DW (1998) Applied multivariate analysis. Prentice Hall, NY

Karasov WH (2011) Digestive physiology: a view from molecules to ecosystem. Am J Physiol Reg I 301:R276–R284

Klasing KC (1998) Comparative avian nutrition. CAB International, Wallingford

Martinelli LA, Piccolo MC, Townsend AR, Vitousek PM, Cuevas E, McDowell W, Robertson GP, Santos OC, Treseder K (1999) Nitrogen stable isotopic composition of leaves and soil: tropical versus temperate forests. Biogeochemistry 46:45–65

Martínez del Rio C (2007) Comparing modes of inquiry. Nature 450:171

Martínez del Rio C, Wolf BO (2005) Mass-balance models for animal-isotopic ecology. In: Starck M, Wang T (eds) Physiological and ecological adaptations to feeding in vertebrates. Science Publishers, Enfield, pp 141–174

Martínez del Rio C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev 84:91–111

McNab BK (2002) The physiological ecology of vertebrates: a view from energetics. Cornell University Press, Ithaca

Newsome S, Martínez del Rio C, Phillips DL, Bearhop S (2007) A niche for isotopic ecology. Front Ecol Environ 5:429–436

Newsome SD, Bentall GB, Tinker MT, Oftedal O, Ralls K, Fogel ML, Estes JA (2010) Variation in diet-vibrissae $\delta^{13}C$ and $\delta^{15}N$ trophic discrimination factors in a wild population of California sea otters (*Enydra lutris nereis*). Ecol Appl 20:1744–1752

Norris DR, Marra PP, Kyser TK, Ratcliffe TM (2005) Tracking habitat use of a long-distance migratory bird, the American redstart *Setophaga ruticilla*, using stable-carbon isotopes in cellular blood. J Avian Biol 36:164–170

Pauly D, Christensen V, Dalsgaard J, Froese R, Torres F Jr (1998) Fishing down marine food webs. Science 279:860–863

Pearson SF, Levey DJ, Greenberg CH, Martínez del Rio C (2003) Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. Oecologia 135:516–523

Perga ME, Grey J (2010) Laboratory measures of isotope discrimination factors: comments on Caut, Angulo & Courchamp (2008, 2009). J Appl Ecol 47:942–947

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Ann Rev Ecol Syst 18:293–320

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718

Robbins CT, Felicetti LA, Sponheimer M (2005) The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. Oecologia 144:534–540

Robinson D (2001) $\delta^{15}N$ as an integrator of the nitrogen cycle. Trends Ecol Evol 16:153–162

Sabat P, Martínez del Rio C (2002) Inter- and intraspecific variation in the use of marine food resources by three *Cinclodes* (Furnariidae, Aves) species: carbon isotopes and osmoregulatory physiology. Zoology 105:247–256

Sabat P, Maldonado K, Canals M, Martínez del Rio C (2006a) Osmoregulation and adaptive radiation in the ovenbird genus *Cinclodes* (Passeriformes: Furnariidae). Funct Ecol 20:799–805

Sabat P, Maldonado K, Martínez del Rio C (2006b) Osmoregulatory capacity and the ability to use marine food sources in two coastal songbirds (*Cinclodes*: Furnariidae) along a latitudinal gradient. Oecologia 148:250–257

Schaafsma G (2000) The protein digestibility-corrected amino acid score. J Nutr 130:1865S–1867S

Schondube JE, Herrera-M G, Martínez del Rio C (2001) Diet and the evolution of digestion and renal function in phyllostomid bats. Zoology 104:59–73

Sheppard RJ (2003) Regression to the mean: a threat to exercise science. Sports Med 33:575–584

Smith RJ (2009) Use and misuse of the reduced major axis for line-fitting. Am J Phys Anthropol 140:476–486

Sokal RR, Rohlf FJ (1981) Biometry. W. H. Freeman, New York

Sponheimer M, Robinson T, Ayliffe L, Roeder B, Hammer J, West A, Passey B, Cerling T, Dearing D, Ehleringer J (2003) Nitrogen isotopes in mammalian herbivores: Hair d15 N values from a controlled-feeding study. Int J Osteoarchaeol 13:80–87

Stephens DW, Brown JS, Ydenberg RC (2007) Foraging: behaviour and ecology. University of Chicago Press, Chicago

Stigler SM (1997) Regression towards the mean, historically considered. Stat Methods Med Res 6:103–114

Takimoto G, Spiller DA, Post D (2008) Ecosystem size, but not disturbance, determines food-chain length of islands on the Bahamas. Ecology 89:3001–3007

Tieszen LL, Boutton TW, Tesdahland KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: implications for δ13C analysis of diet. Oecologia 57:32–37

Vander Zanden JM, Fetzer WW (2007) Global patterns of aquatic food chain length. Oikos 116:1378–1388

Vander Zanden MJ, Rasmussen JB (2001) Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. Limnol Oceanogr 46:2061–2066

Vander Zanden MJ, Cabana G, Rasmussen JB (1997) Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (δ^{15}N) and literature dietary data. CanJ Fish Aquat Sci 54:1142–1158

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

Weir BS (1990) Genetic data analysis: methods for discrete population analysis. Sinauer, Sunderland

Zar JH (1996) Biostatistical analysis. Prentice Hall, Upper Saddle River