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Dietary and isotopic specialization: the isotopic niche of three *Cinclodes* ovenbirds

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Abstract By comparing the isotopic composition of tissues deposited at different times, we can identify individuals that shift diets over time and individuals with constant diets. We define an individual as an isotopic specialist if tissues deposited at different times have similar isotopic composition. If tissues deposited at different times differ in isotopic composition we define an individual as an isotopic generalist. Individuals can be dietary generalists but isotopic specialists if they feed on the same resource mixture at all times. We assessed the degree of isotopic and dietary specialization in three related Chilean bird species that occupy coastal and/or freshwater environments: *Cinclodes oustaleti*, *Cinclodes patagonicus*, and *Cinclodes nigrofumosus*. *C. oustaleti* individuals were both isotopic and dietary generalists. Tissues deposited in winter (liver and muscle) had distinct stable C (δ^{13}C) and stable N isotope ratio (δ^{15}N) values from tissues deposited in the summer (wing feathers) suggesting that birds changed the resources that they used seasonally from freshwater habitats in the summer to coastal habitats in the winter. Although the magnitude of seasonal isotopic change was high, the direction of isotopic change varied little among individuals. *C. patagonicus* included both isotopic specialists and generalists, as well as dietary specialists and generalists. The isotopic composition of the feathers and liver of some *C. patagonicus* individuals was similar, whereas that of others differed. In *C. patagonicus*, there were large inter-individual differences in the magnitude and the direction of seasonal isotopic change. All individuals of *C. nigrofumosus* were both isotopic and dietary specialists. The distribution of δ^{13}C and δ^{15}N values overlapped broadly among tissues and clustered in a small, and distinctly intertidal, region of δ space. Assessing individual specialization and unraveling the factors that influence it, have been key questions in animal ecology for decades. Stable isotope analyses of several tissues in appropriate study systems provide an unparalleled opportunity to answer them.

Keywords *Cinclodes* · Niche width · Stable isotopes · Trophic niche · Stable nitrogen isotope ratio

Introduction

In the mid-20th century G. E. Hutchinson's defined the ecological niche as an *n*-dimensional hypervolume (Hutchinson 1957). The niche received much attention after Hutchinson's definition, but interest in the concept declined in the last two decades of the 20th century (Chase and Liebold 2003). This lull seems to have ended. Ecologists seem to have developed a renewed interest in the niche (Soberón 2007). The renewed interest in the niche is

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perhaps not surprising as niche studies can inform problems as diverse as the evolution of resource use (Bolnick et al. 2003), the success of invading exotics (Holt et al. 2005), and the processes that shape the composition of ecological communities (Wiens and Graham 2005); Bearhop et al. (2004) and Newsome et al. (2007) have proposed that a relatively new technology, stable isotope analyses, can be used to measure many of the dimensions of ecological niches. Using stable isotopes to explore trophic niches is facilitated by the existence of a great variety of isotopic signatures that distinguish the elements within food webs and among food webs (Newsome et al. 2007). Stable isotopes can also be used to assign animals to geographical areas and hence to characterize their bioclimatic niches (Rubenstein and Hobson 2004; West et al. 2006).

Isotopic ecologists often depict the isotopic composition of animal tissues in a multivariate space (called "δ space"; Newsome et al. 2007) in which the axes are the isotopic values for different elements [e.g., stable C (δ^{13}C), N (δ^{15}N), D (δD), and O isotope ratios (δ^{18}O)]. Following Newsome et al. (2007) we call the sub-set of δ space occupied by the isotopic composition of an animal's tissues the "isotopic niche". Layman et al. (2007) and Schmidt et al. (2007) advocated using the distribution of isotopic values in δ space to examine the trophic structure of ecological communities. We used a combination of the quantitative approaches proposed by Layman et al. (2007) and Schmidt et al. (2007) to examine trophic structure within species. Like Newsome et al. (2007) we emphasize that the isotopic niche is not the ecological niche. Newsome et al. (2007) list the limitations of the use of stable isotopes in niche studies and emphasize the differences between the isotopic and the trophic niche. Briefly, the isotopic niche is a construct that in suitable systems can inform questions traditionally considered within the domain of the ecological niche.

For example, several studies have used stable isotope measurements on a single tissue to characterize ecological niches and degrees of dietary specialization (reviewed by Bolnick et al. 2003); Araújo et al. (2007) suggested that the utility of stable isotopes in studies of individual specialization is because "Due to their slow turnover, isotopes will not be subject to the same stochastic sampling effects as gut contents and can be a more reliable way to infer individual temporal consistency in food-resource use." Here we propose an alternative notion, namely that stable isotopes are particularly useful, because by sampling different types of tissues they permit exploration of animal resource use over a variety of temporal scales (Dalerum and Angerbjörn 2005). This application of stable isotopes hinges on the observation that tissues differ in the rate at which they incorporate new materials (reviewed by Martinez del Rio and Wolf 2005). Some tissues, such as liver and plasma proteins have high turnover rates, and their isotopic composition reflects integration of recent dietary inputs. Others, such as bone collagen, exhibit low incorporation rates and their isotopic composition reflects integration of dietary inputs over longer time periods. Furthermore, some tissues, such as feathers and hair, are deposited in a relatively short and discrete interval of time and because they are inert, retain the isotopic composition of resources incorporated while they were manufactured (Bearhop et al. 2003 and references therein). The isotopic variation among tissues within a single individual permits inferring two things: (1) the breadth of an individual's isotopic niche, and (2) the time scale(s) at which animals shift diets/resources.

By comparing among the isotopic composition of tissues with dissimilar incorporation rates we can identify individuals that shift diets over time and individuals with relatively constant diets. "Isotopic specialists" are those individuals in which tissues with different rates of incorporation, or inert tissues deposited at different times, have similar isotopic compositions. In these individuals, the use of resources inferred from isotopic data does not depend on the tissue measured. Note that an isotopic specialist need not feed on a single type of resource, i.e., it need not be a dietary specialist. It may feed for extended periods of time on the same mixture of isotopically distinct sources. Thus, a population of isotopic specialists can include dietary specialists and dietary generalists that feed on the same mixture of resources at all times. In contrast, tissues of an "isotopic generalist" with different incorporation rates would have dissimilar isotopic compositions. This implies that the relative proportion (mixture) of food sources in an isotopic generalist diet changes over time. Using both isotopic and dietary criteria to characterize an animal's degree of specialization emphasizes the complementary nature of these two sources of information.

Our nomenclature refers to individuals. A population can be considered "generalist" in three possible ways: (1) if it comprises a mixture of both isotopic generalists and dietary generalists (Fig. 1a), (2) if it comprises a mixture of isotopic specialists and dietary generalists (Fig. 1b), and (3) if it comprises a mixture of dietary and isotopic specialists that feed on prey with contrasting composition (Fig. 1d). Bearhop et al. (2004) refer to cases 1 and 2 as type A generalist populations, and to case 3 as a type B generalist population. As Bearhop et al. (2004) have emphasized, the notions of specialist and generalist in isotopic studies depend on the tissue analyzed and on the rate of isotopic incorporation of that tissue. Note, for example, that in panel A of Fig. 1, a tissue with slow turnover would tell us that all the individuals are dietary generalists that use a mixture of resources. However, if we

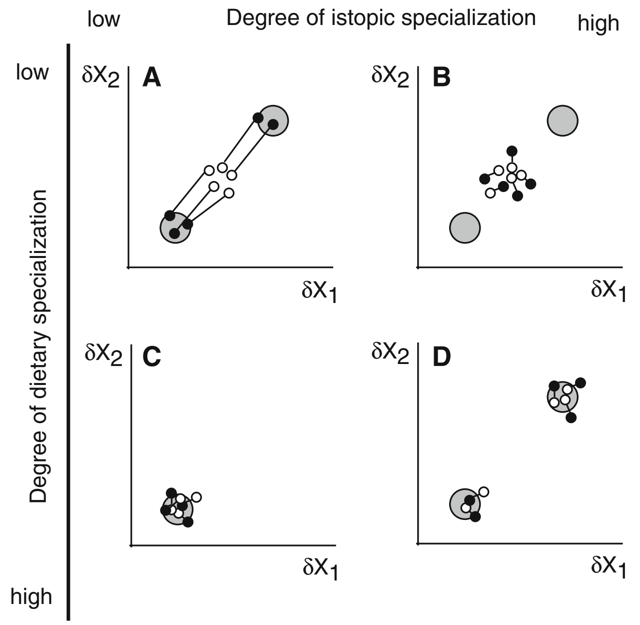

Fig. 1 *Closed points* represents the isotopic composition of a tissue with rapid turnover and *open points* that of one with slower turnover. Point pairs joined by *lines* represent individuals, δX_1 and δX_2 are the isotopic values of two isotopes (e.g., ^{13}C and ^{15}N), and *shaded circles* represent the isotopic composition of two resources. Population A comprises a collection of isotopic generalists, each one of which is also a dietary generalist. Population B comprises isotopic specialists that are dietary generalists and population D is a generalist population comprising individuals that are both isotopic and dietary specialists. Populations A, B, and D should be considered generalist—albeit in different ways; population C represents a specialist population comprising individuals that are both isotopic and dietary specialists, all of which use the same resource

were to measure a tissue with fast turnover we would infer that all individuals "specialize" on one resource or the other. Using stable isotopes to determine degree of individual specialization requires accounting for differences in the time course of isotopic incorporation among tissues.

Here we present an investigation of the isotopic niche of three species of Chilean *Cinclodes* (Furnariidae) ovenbirds: *Cinclodes nigrofumosus* (70 g), *Cinclodes oustaleti* (25 g), and *Cinclodes patagonicus* (39 g; bird masses after Sabat et al. 2006a). The 12 species in this genus represent an adaptive radiation that includes two coastal species, several species that inhabit fresh water streams, and species that shift seasonally between marine and freshwater habitats (Sabat et al. 2006a). All *Cinclodes* species feed on invertebrates. *C. nigrofumosus*, *C. oustaleti*, and *C. patagonicus* are syntopic during the austral winter, but seem to differ in their specialization to a marine diet (Sabat et al. 2006b). *C. nigrofumosus* inhabits only wave-swept rocky shorelines (Sabat et al. 2006a). *C. oustaleti* seems to shift habitats seasonally. During the winter, it shares *C. nigrofumosus*' habitat, but it occupies freshwater and brackish estuaries as well (P. Sabat and C. Martínez del Rio, unpublished observations). During the austral spring and summer, *C. oustaleti* disappears from coastal habitats. It presumably moves inland to freshwater streams and high-elevation bogs. Using qualitative criteria and anecdotal observations, Sabat et al. (2006b) characterized *C. nigrofumosus* and *C. oustaleti* as a habitat specialist and a generalist, respectively. Less is known about the ecological habits of *C. patagonicus*. Jaramillo's (2003) and our own observations indicate that it is possible to find individuals of this species feeding on marine or terrestrial/freshwater environments at all times of the year (Jaramillo 2003; P. Sabat and C. Martínez del Rio, unpublished observations).

Based on the limited information on the natural history of these three species we made the following predictions:

1. *C. nigrofumosus* is both an isotopic and a dietary specialist of marine habitats. Its isotopic niche is the narrowest and does not show seasonal variation.
2. The position of *C. oustaleti*'s isotopic niche varies seasonally, and hence a significant fraction of the magnitude of this species' isotopic niche width is the result of temporal individual shifts in resource use. At temporal scales that include more than one season, *C. oustaleti* is both an isotopic and a dietary generalist.
3. *C. patagonicus* has a broad isotopic niche, but there is less seasonal variation in the position of this niche than in that of *C. oustaleti*. We hypothesized that *C. patagonicus* individuals are dietary generalists, but isotopic specialists.

Materials and methods

We took advantage of specimens collected over a 5-year period for physiological measurements (Sabat et al. 2006a, b). All birds were collected in Chile between 24° and 37°S (Sabat et al. 2006a); 18 individuals each of *C. nigrofumosus* and *C. oustaleti* and nine individuals of *C. patagonicus* were collected during the austral winter (July–September) and stored frozen at −80°C. Because we could not analyze tissues for all *C. nigrofumosus* individuals, the sample sizes for this species range from 17 to 18. We analyzed the isotopic composition of C (δ^{13}C) and N (δ^{15}N) of a tissue with slow incorporation (bone collagen from the tibiotarsus), a tissue with intermediate rates of incorporation (pectoralis muscle), and a tissue with a relatively fast isotopic incorporation rate (liver; Hobson and Clark 1992). Although we have no precise estimates on the rates of incorporation of each of these tissues in *Cinclodes* species, we used data from other bird species to place bounds on their magnitude. The isotopic composition of liver represents that of resources incorporated over a few days to 2 weeks, that of muscle represents the isotopic composition of resources incorporated from 2 weeks to a month, and the isotopic composition of collagen represents incorporation over several months (Table 1). Carleton and Marínez del Rio (2005) demonstrated that the fractional rate of ^{13}C into bird tissues scales allometrically with body mass to the $-1/4$th power. Thus, although there is a roughly threefold difference in mass between *C. oustaleti* and *C. nigrofumosus*, the difference in incorporation rates between their tissues is only $\approx 25\%$. We also analyzed the isotopic composition from a flight feather (primary number 1). We chose primary 1, which in passerines is typically one of the first flight feathers to be molted, to maximize the time of isotopic incorporation between feathers and "winter" tissues. Because *Cinclodes* species molt from late November to January (Bertolero and Zavalaga 2003), the isotopic composition of feathers corresponds to that of resources assimilated for the few weeks during which the feather grew in the austral summer. The isotopic composition of all other tissues, except collagen, corresponds to resources assimilated during the winter. The minimal time separating tissues deposited in "summer" (feathers) from those deposited in "winter" (liver and muscle) is ≈ 120 days (4 months). Because dietary C and N signatures appear to be incorporated into feathers and muscle at roughly similar rates (Bearhop et al. 2003), the isotopic composition of these two tissues corresponds to that of food assimilated during a similar time interval but in different seasons.

Feather samples were washed in a light detergent and a 3:1 chloroform:methanol solution before analysis. To isolate collagen, specimens were cleaned of adhering soft tissue, and lipids were removed with a 3:1 chloroform:methanol solution. Bone samples were demineralized in 1.0 M HCl at room temperature for 72 h. Collagen extracts were rinsed with distilled water and dried. Liver and pectoralis muscle samples were processed as described by Sabat and Martínez del Rio (2002). All samples were dried and ground into a fine powder and had their lipids removed before being loaded (30–70 µg) into tin capsules. Isotope ratios of food were measured on a continuous flow isotope ratio mass spectrometer (Finnigan Delta+XP; University of Wyoming's Light Stable Isotope Facility) with samples combusted in a Costech elemental analyzer. The precision of these analyses was ±0.2‰ for both isotopes. Our standards were vacuum oil [$\delta^{13}\mathrm{C} = -27.5$‰, Vienna Pee Dee belemnite (VPDB)] and ANU sucrose ($\delta^{13}\mathrm{C} = -10.5$‰, VPDB, NIST 8542) for δ^{13}C and peptone ($\delta^{15}\mathrm{N} = 5.60$‰, AIR, USGS40 8542), and glycine ($\delta^{15}\mathrm{N} = 0.73$‰, AIR, IAEAN2) for δ^{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: $\delta X = (R_{\mathrm{sample}}/R_{\mathrm{standard}} - 1) \times 1{,}000$, 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 δ^{13}C and atmospheric N (AIR) for δ^{15}N.

Table 1 Average retention time in days of ^{13}C in the tissues of Japanese quail (*Coturnix japonica*) and house sparrows (*Passer domesticus*)

Species	Liver	Muscle	Bone collagen	References
C. japonica	3.7	17.9	250	Hobson and Clark (1992)
P. domesticus	15.1	32.8	–	Carleton et al. (2008)

Statistical analyses

Our statistical analyses can be divided into three broad sections. First, we compared the isotopic composition among tissues within each species. We used bivariate repeated measures ANOVA to test for differences in (δ^{13}C, δ^{15}N) among tissues. These analyses were followed by univariate repeated measures ANOVA on δ^{13}C and δ^{15}N, respectively. To test whether the isotopic niche changed between seasons, we: (1) estimated the Euclidean distance between individual (δ^{13}C, δ^{15}N) values between feathers (deposited in the summer) and liver (deposited in the winter), and (2) compared the position of the centroid in δ^{13}C and δ^{15}N space of feathers with that of liver (see below). Because we had both feathers and liver samples from each individual, we used a modification of Hotteling's T^2-test that accounts for paired data (Johnson and Wichern 1998). Briefly, we estimated T^2 as $n\delta X'(S^{-1})\,\delta X$, where δX equals the vector of the mean differences between the δ^{13}C of feathers and that of liver, and between the δ^{15}N of feathers and liver (i.e., $\delta X' = \left(\overline{\delta^{13}C_{\mathrm{feather}} - \delta^{13}C_{\mathrm{liver}}}, \overline{\delta^{15}N_{\mathrm{feather}} - \delta^{15}N_{\mathrm{liver}}}\right)$, S^{-1} is the inverse covariance matrix, and n is the number of observations. We compared T^2 with the critical value $(2n-2)(n-2)^{-1}F_{2,\,n-2,\,0.05}$ (Johnson and Wichern 1998). Second, we used the distance of individual points to centroids as estimates of isotopic niche width (Layman et al. 2007). To compare these distances among species and tissues (liver, feathers, and collagen), we used repeated measures ANOVA. Third, and finally, we used circular statistics to compare the magnitude and variation in direction of change in isotopic composition between feathers and liver. Schmidt et al. (2007) advocate the use of circular statistics to assess the direction (measured by the angle of change, α) and magnitude of change (measured by Euclidean distance between points, R) in δ space. We estimated the mean α ($\bar{\alpha}$) and the angular deviation (s) between the (δ^{13}C, δ^{15}N) values of feathers and liver following Zar (1996). We also tested for differences in angular dispersion using a Kruskal–Wallis test on angular distances defined as $|\alpha - \bar{\alpha}|$ (Zar 1996). Our study adapted analyses originally devised to study the trophic structure of communities, including comparisons among communities (Layman et al. 2007) and temporal changes in a community after a disturbance (Schmidt et al. 2007). We adapted these community-wide approaches to compare the isotopic niches of different species and to investigate temporal changes in the isotopic niche of a single species.

The isotopic composition of different tissues can differ within an animal (reviewed by Martínez del Rio et al. 2009). Thus, some studies analyzed isotopic values “corrected” by adding or subtracting the putative tissue-diet discrimination for the tissue in question (e.g., Herrera et al. 2006). Because we have no experimental tissue-diet discrimination values, we analyzed uncorrected isotopic values. Using uncorrected data is unlikely to change our conclusions as most of our analyses involve comparisons of the same tissue across species, or comparisons of different tissues within a single species when the potential food sources (intertidal and freshwater) have isotopic values that are vastly different relative to discrimination factors (see Martínez del Rio et al. 2009).

Results

Differences in δ^{13}C and δ^{15}N among tissues

In all species, there were significant differences in (δ^{13}C, δ^{15}N) values among tissues (Table 2). In *C. oustaleti* there was little or no overlap in the distribution of (δ^{13}C, δ^{15}N) values among tissues deposited in different seasons (Fig. 2a, b). Feathers, deposited in the summer, were significantly depleted in ^{13}C and ^{15}N relative to tissues deposited in the winter (liver and muscle; Table 3). The C and N isotopic composition of collagen was intermediate between that of feathers and liver and muscle (Fig. 2a, b; Table 3). Although, there were significant differences among tissues in *C. patagonicus* (Table 1), these were smaller. In this species there was more overlap in the distribution of (δ^{13}C, δ^{15}N) values among tissues (Fig. 2d, e; Table 3). The difference among tissues in *C. patagonicus* resulted from lower δ^{15}N values in feathers than in all other tissues. *C. nigrofumosus* occupied a much smaller area of the δ^{13}C–δ^{15}N space than its two congeners (Fig. 2f, g). In this species the differences among tissues resulted from slightly, albeit significantly, more positive δ^{13}C and δ^{15}N values in collagen than in other tissues (Table 3).

Table 2 There were significant effects of tissue type on the isotopic composition in all species

	Wilks' λ	*F*	*df*	*P*
Repeated measures MANOVA				
Cinclodes oustaleti	0.17	23.3	6, 98	<0.001
Cinclodes patagonicus	0.43	3.9	6, 46	0.003
Cinclodes nigrofumosus	0.28	13.6	6, 92	<0.001
Repeated measures ANOVA on δ^{13}C				
C. oustaleti		4.7	3, 15	<0.0001
C. patagonicus		2.2	3, 6	0.045
C. nigrofumosus		4.9	3, 14	<0.0001
Repeated measures ANOVA on δ^{15}N				
C. oustaleti		11.2	3, 15	<0.0001
C. patagonicus		1.4	3, 6	0.136
C. nigrofumosus		8	3, 14	<0.001

However, the degree of overlap among tissues in the distribution of isotopic values differed widely among species (Fig. 2). *MANOVA* Multivariate ANOVA, *δ^{13}C* stable C isotope ratio, *δ^{15}N* stable N isotope ratio

The δ^{13}C and δ^{15}N values of liver and feathers in *C. patagonicus* and *C. nigrofumosus* were relatively close to each other. The average Euclidean distance between points was 5.1‰ (±SD = 4.5‰) and 1.9‰ (±SD = 0.8‰) units apart, respectively (Fig. 3), and the centroids of their bivariate distributions did not differ significantly (Hotelling’s $T^2 = 6.3$ and 5.1, $P > 0.05$, respectively). In contrast, the δ^{13}C and δ^{15}N values of liver and feathers in *C. oustaleti* were 13.9‰ (±SD = 4.2‰) units apart, and the centroids of their distributions differed significantly (Hotelling’s $T^2 = 132.3$, $P < 0.001$).

Differences among species in isotopic “niche width”

The distance between (δ^{13}C, δ^{15}N) points and their centroids differed significantly among species ($F_{2,40} = 12.10$, $P < 0.001$), but did not differ among tissues ($F_{2,39} = 0.88$, $P = 0.44$), and there was no significant interaction between species and tissues ($F_{4,78} = 0.57$, $P = 0.69$). Because there was no significant effect of tissue, we compared the distances between (δ^{13}C, δ^{15}N) points and their centroids among species using Tukey–Kramer multiple comparison tests. The average distance between (δ^{13}C, δ^{15}N) points and their centroids was significantly shorter in *C. nigrofumosus* (1.2 ± 1.0‰, $P < 0.01$) than in *C. patagonicus* (3.5 ± 2.3‰) and *C. oustaleti* (3.4 ± 2.5‰). The average distance between (δ^{13}C, δ^{15}N) points and their centroids in these two species did not differ significantly ($P > 0.1$).

Magnitude and direction of change in isotopic niche

There were significant differences in both the magnitude (one-way ANOVA $F_{2,40} = 61.1$, $P < 0.001$) and angular

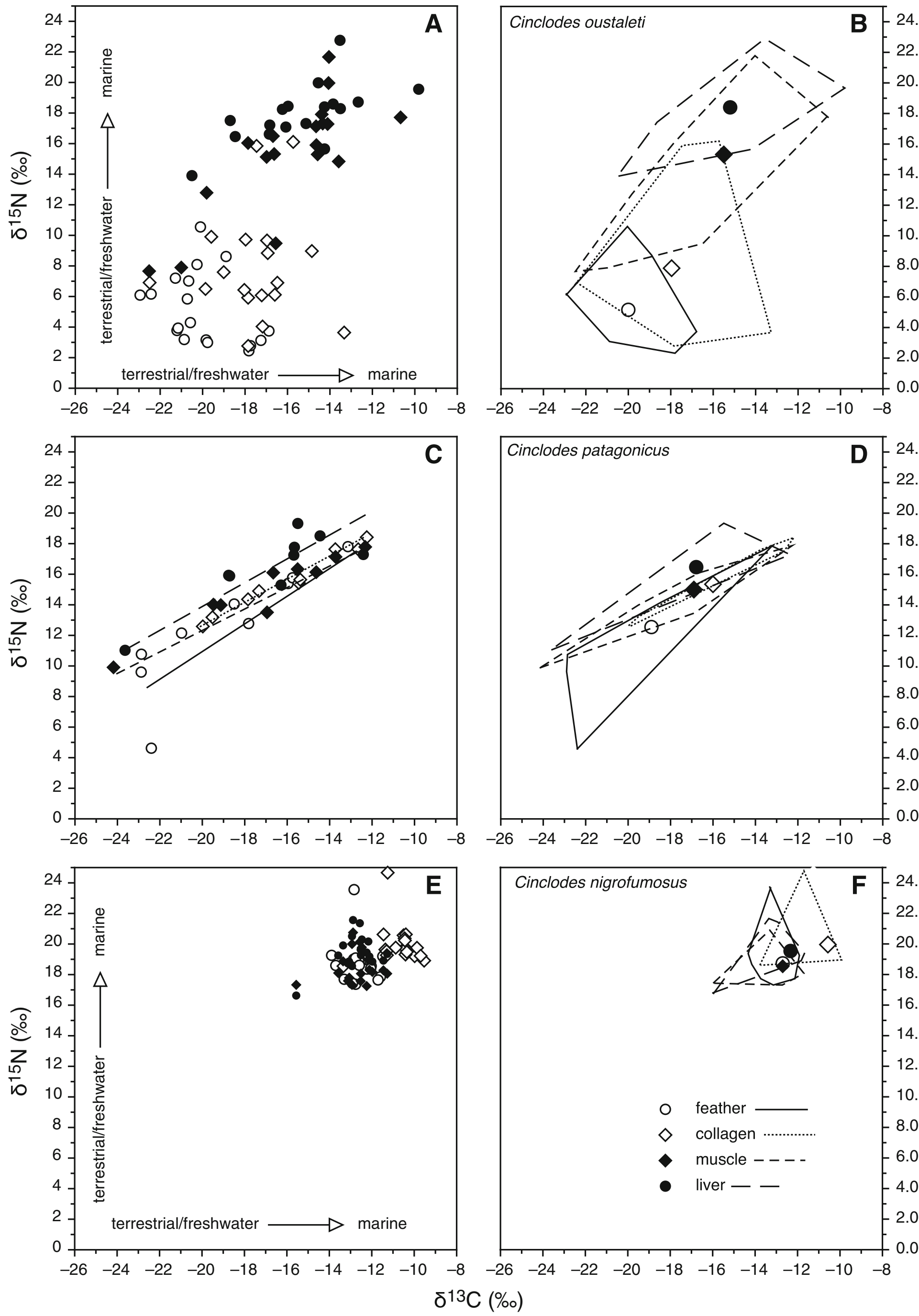

Fig. 2 **a** The tissues of *Cinclodes oustaleti* individuals shifted in stable C ($\delta^{13}C$) and stable N isotope ratio ($\delta^{15}N$) values seasonally. Feathers deposited in summer were relatively depleted in ^{13}C and ^{15}N, whereas muscle and liver, which are deposited in winter, were enriched in ^{13}C and ^{15}N. The isotopic composition of collagen occupied an intermediate position in isotopic space. **b** The convex hulls of feathers did not overlap with those of muscle and liver. *Symbols* represent centroids of each tissues' δ^{13}C and δ^{15}N distribution. **c** The distribution of δ^{13}C and δ^{15}N values of the tissues of *Cinclodes patagonicus* individuals overlapped broadly, and **d** fell along relatively straight lines, suggesting that this species feeds on a mixture of two isotopically distinct sources. **e** The distribution of δ^{13}C and δ^{15}N values of the tissues of *Cinclodes nigrofumosus* individuals overlapped broadly. **f** This species occupied a relatively narrow area within δ space. Convex hulls are shown for descriptive purposes only. *Symbols* for each tissue were chosen arbitrarily

dispersion of seasonal change ($H = 22.2$, $P < 0.0001$; Fig. 4). The magnitude of seasonal change was higher in *C. oustaleti* than in *C. patagonicus* and *C. nigrofumosus* (Tukey's honest significant difference, $P < 0.05$), but this species had lower angular dispersion than its congeners (Nemeyi test, $P < 0.05$; Fig. 4). *C. patagonicus* and *C. nigrofumosus* did not differ significantly in the magnitude and angular dispersion of seasonal changes ($P > 0.05$).

Discussion

The isotopic analysis of four tissues revealed the following patterns:

1. *C. nigrofumosus* had a compact isotopic niche, that did not vary between seasons.
2. The isotopic niche of *C. oustaleti* was relatively broad and varied between seasons. There was no overlap between the summer isotopic niche revealed by the

Table 3 In *C. oustaleti*, tissues deposited in the summer (muscle and liver) were significantly enriched in ^{13}C and ^{15}N compared to feathers deposited in the summer

	Feathers	Collagen	Muscle	Liver
C. oustaleti (n = 18)				
δ^{13}C ± SD‰	−20.0 ± 1.7a	−17.9 ± 2.3b	−15.5 ± 2.3bc	−15.2 ± 2.5c
δ^{15}N ± SD‰	5.2 ± 2.4a	7.9 ± 3.6a	15.3 ± 3.8b	18.4 ± 2.7c
C. patagonicus (*n* = 8)				
δ^{13}C ± SD‰	−18.9 ± 3.6a	−16.0 ± 2.8a	−16.9 ± 3.6a	−16.8 ± 3.2a
δ^{15}N ± SD‰	12.5 ± 3.9a	15.4 ± 2.1ab	15.0 ± 2.4ab	16.5 ± 2.4b
C. nigrofumosus (*n* = 17)				
δ^{13}C ± SD‰	−12.7 ± 0.9a	−10.6 ± 0.9b	−12.7 ± 0.9a	−12.3 ± 0.7a
δ^{15}N ± SD‰	18.7 ± 1.4a	20.0 ± 1.3b	18.5 ± 1.1a	19.4 ± 1.2ab

This temporal shift was also found, albeit with a reduced magnitude, in *C. patagonicus*, but was absent in *C. nigrofumosus*. Mean values in each row labeled with the *same letter* were not different from each other after univariate repeated measures ANOVA and Tukey's honest significant difference multiple comparisons

analysis of feathers, and the winter one revealed by the analysis of liver and muscle.

3. *C. patagonicus* had a broad isotopic niche, but in this species there was no biologically significant difference between the position of the isotopic niche between summer and winter.

Our discussion uses these results for three purposes: first, we elaborate and apply the distinction between isotopic and dietary specialists and generalists to *C. oustaleti*, *C. patagonicus*, and *C. nigrofumosus*; second, we use the isotopic niche as a tool to make biological inferences about the ecological niches of these three species; and third, we comment on the implications of our study for the interpretation of isotopic data that aims to determine degree of individual specialization.

Isotopic specialists and generalists

The relationship between the isotopic composition of tissues with dissimilar time courses of isotopic incorporation can inform the existence of individuals that shift diets over time and of individuals with relatively constant diets. Because the feathers and liver of all *C. oustaleti* individuals had distinctly different isotopic compositions, this species satisfies our criterion of individuals as isotopic generalists. The relatively low angular dispersion observed in *C. oustaleti* indicates that individuals changed the resources that they used seasonally following roughly the same direction (Fig. 4). In contrast, there were wide differences in the magnitude and the direction of seasonal isotopic change observed among *C. patagonicus* individuals (Fig. 4). The isotopic composition of the feathers and liver of some *C. patagonicus* individuals was very similar, whereas that of others differed (Fig. 3). This species appeared to include both isotopic specialists and generalists, as well as isotopic and dietary specialists (Fig. 3). All individuals of *C. nigrofumosus* clustered in a relatively small region of δ space and the distribution of (δ^{13}C, δ^{15}N) values overlapped broadly among tissues (Fig. 2). Therefore we consider the members of this species as both isotopic and dietary specialists. Because the region of δ space occupied by individuals of this species corresponds to a strictly intertidal diet, we consider not only the individuals in it, but the population as specialist.

The ecological and the isotopic niche

Because the tissues of animals differ in isotopic composition as a result of differences in the composition of diets, the isotopic niche is a reflection of the ecological niche. What can we infer about the ecological niche of *Cinclodes* species from the subsets of δ space that they occupy and from the magnitude and direction of seasonal changes in isotopic space? We will consider each species in turn, beginning with *C. nigrofumosus*. With the exception of collagen, the isotopic composition of all tissues in this species clustered in a tight area of δ space. The difference in isotopic composition between collagen and other tissues is likely due to the higher abundance of isotopically enriched amino acids in this tissue (Howland et al. 2003; Fox-Dobbs et al. 2007).

The relatively constant and ^{13}C- and ^{15}N-enriched tissues of *C. nigrofumosus* are consistent with a diet of intertidal invertebrates. The producers at the base of intertidal food webs tend to be, on average, enriched in both ^{13}C and ^{15}N, relative to terrestrial and freshwater foodwebs (Wang and Yeh 2003). Although the value of δ^{15}N among the macroalgae at the base of intertidal food webs varies only slightly, the value of δ^{13}C can vary widely (Raven et al. 2002). In spite of this potential variation, both the δ^{13}C and δ^{15}N values of *C. nigrofumosus*'

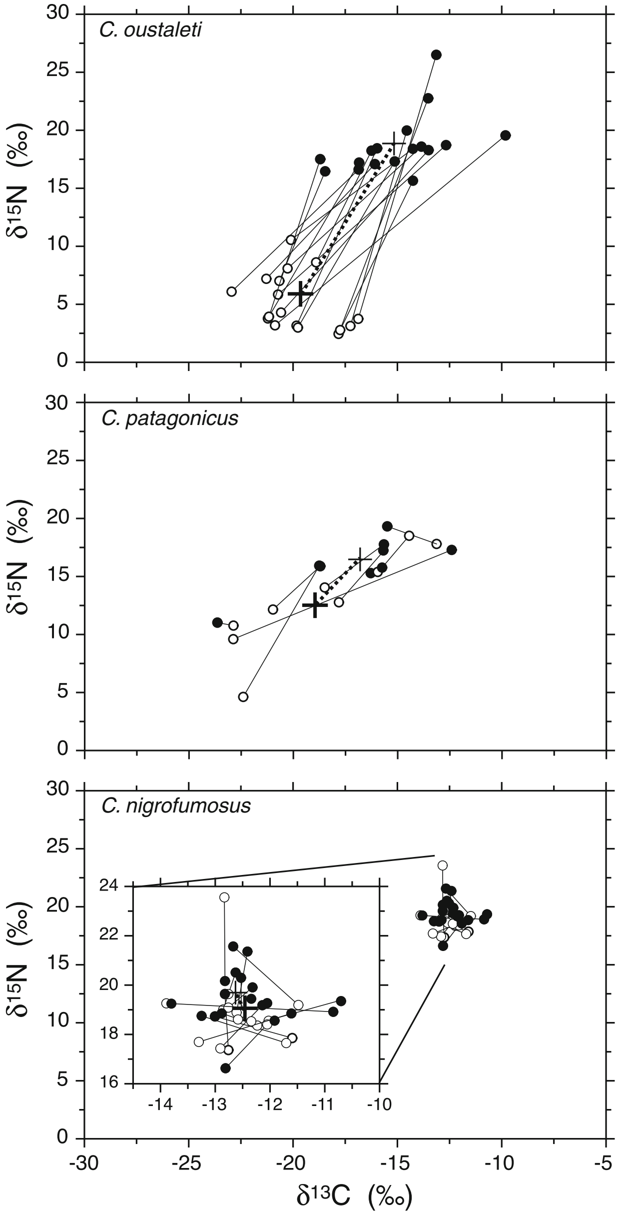

Fig. 3 In *C. oustaleti* the centroids of the isotopic composition of feathers (*open circles*) and liver (*closed points*) differed significantly. Data from each individual are joined by *lines*. In contrast, in *C. patagonicus* and *C. nigrofumosus*, there were no significant differences between the centroids of the δ^{13}C and δ^{15}N of feathers and liver. Note the variation in the magnitude and direction of change in δ^{13}C and δ^{15}N from feathers to liver in *C. patagonicus*. The *inset* in the *lowest panel* represents a rescaling of the distribution of δ^{13}C and δ^{15}N values for *C. nigrofumosus*

tissues fell within a narrow range (Figs. 2, 3). Sabat et al. (2006a) analyzed stomach contents of *C. nigrofumosus* and found that this species feeds primarily on small benthic mollusks and crustaceans. These invertebrates are isotopic specialists that integrate and average the isotopically diverse composition of the coterie of algae that they consume into a relatively narrow range (Vander Zanden and Rasmussen 2001). *C. nigrofumosus*' narrow isotopic niche is a consequence of its position as a secondary consumer in an intertidal foodweb.

δ^{13}C and δ^{15}N were linearly and positively correlated in all the tissues of *C. patagonicus* ($r = 0.75, 0.86, 0.94, 0.96$, for feathers, liver, muscle, and collagen, respectively; Fig. 2d, e). A linear relationship between δ^{13}C and δ^{15}N is evidence of mixing of two isotopically distinct sources that have relatively little variation (Fry 2006). We hypothesize that *C. patagonicus* individuals fed on mixtures of invertebrates that derive their nutrients from terrestrial environments and on invertebrates from intertidal foodwebs. The terrestrial ecosystems within *C. patagonicus*' geographical distribution are dominated by C3 plants with δ^{13}C and δ^{15}N values that fall within a relatively narrow range (δ^{13}C ranges from −28 to −24‰ and δ^{15}N ranges from −3 to 2‰; Ehleringer et al. 1998; Fry 1991). This hypothesis contrasts with the frequent observation of *C. patagonicus* at the edge of streams and lakes (Jaramillo 2003). We hypothesize that this species feeds on terrestrial insects in vegetation at the edges of these bodies of water.

C. oustaleti shifted isotopic niches seasonally (Figs. 3, 4). In the winter, it occupied a broad niche consistent with an intertidal habitat (Fig. 2). Although during the winter both *C. oustaleti* and C. *nigrofumosus* appeared to have a primarily intertidal niche, the winter isotopic niche of *C. oustaleti* was broader than that of *C. nigrofumosus*. Sabat et al. (2006a) reported that although both *C. nigrofumosus*' and *C. oustaleti*'s winter diets include marine invertebrates, *C. oustaleti*'s diet includes a higher proportion of terrestrial insects. This observation might explain why the convex hulls for the liver and muscle of *C. nigrofumosus* were contained within those of *C. oustaleti* (Fig. 2). In the winter, the isotopic niche of *C. oustaleti* seems to include that of *C. nigrofumosus*. The isotopic values of *C. oustaleti*'s feathers spanned a relatively broad range of δ^{13}C values. These values were distinctly more positive than those expected from a terrestrial foodweb, but are consistent with the range of values found in freshwater food webs (Akamatsu et al. 2004). The broad range of δ^{13}C values is also consistent with the wide spatial variation in δ^{13}C that is found both within and among freshwater streams and rivers (Finlay 2001). In contrast with intertidal invertebrates, the δ^{13}C of herbivorous invertebrates of freshwater streams and rivers is tightly correlated with that of the algae that they feed on. Thus, in a stream the δ^{13}C of herbivores found in fast-flowing riffle habitats can be as high as −28‰, whereas that of the same species found in adjacent pool habitats can be as low as −17‰ (Finlay et al. 1999). At a larger spatial scale, there is a positive correlation between watershed area and the δ^{13}C of both algae and the herbivores that feed on them. Large rivers tend to have algae and invertebrates that are relatively enriched in ^{13}C relative to those in smaller streams (Finlay 2001). The resources used

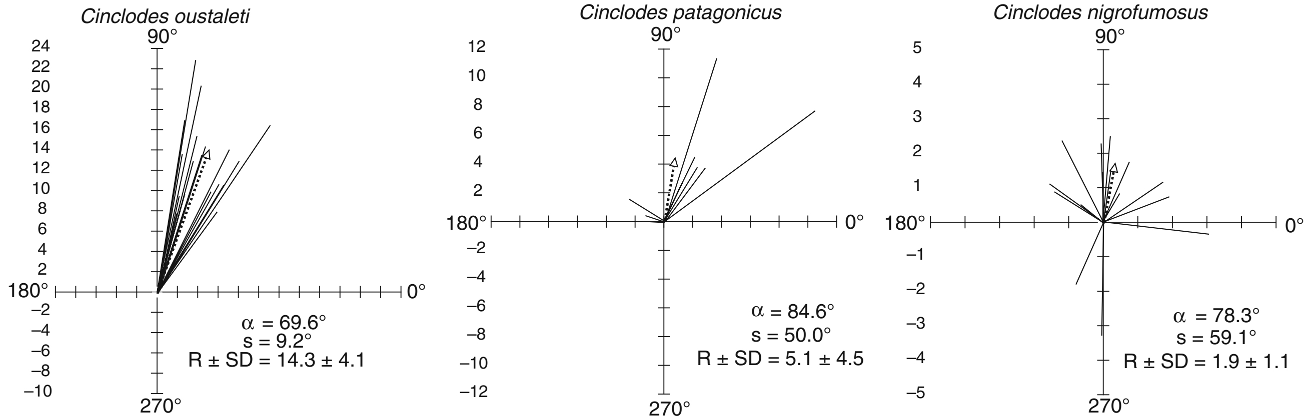

Fig. 4 Both the angular dispersion and the magnitude of change in δ^{13}C and δ^{15}N values between feathers and liver differed among species. The *length* of the *lines* in each one of these plots represents the Euclidean distance between the δ^{13}C and δ^{15}N values of feathers and those for liver for each individual in δ space. The *angle* of each *line* represents the angle of change. Thus, *C. oustaleti* had both significantly lower angular dispersion (all individuals shifted values in roughly the same direction) and higher magnitude of seasonal change in δ^{13}C and δ^{15}N values (i.e., the distance between feather and liver points in δ space was longer for each individual) than *C. patagonicus* and *C. nigrofumosus*. Note the different *scales* in each of the *circular plots*

by an animal can often be diagnosed by specific isotopic values. However, sometimes it is not a value, but a range of isotopic values that allows determining the type of resources used by an animal. We hypothesize that the broad range of δ^{13}C values found in *C. oustaleti* feathers is evidence of the reliance of these birds on aquatic invertebrates from a variety of freshwater systems during the austral summer.

The physiological and morphological traits of *Cinclodes* seem to correlate with their degree of specialization on marine environments. The capacity of the specialist *C. nigrofumosus* to produce concentrated urine, and hence to cope with a salty marine diet, is vastly superior to that of its more generalized congeners (Sabat et al. 2006a). This species has larger kidneys and a higher density of renal medullary cones, which are the structures responsible for urine concentration and salt disposal (Goldstein and Skadhauge 2000), than *C. oustaleti* and *C. patagonicus*. Sabat et al. (2004) investigated the plasticity in osmoregulatory traits in *Cinclodes*. Remarkably, individuals of the generalist *C. oustaleti* exposed to salt water had significantly larger renal medullae and were able to produce more concentrated urine than those exposed to fresh water. There were no significant effects of exposure to salt water in *C. nigrofumosus* or *C. patagonicus*. The broad niche of *C. oustaleti*, which spans from freshwater to marine seasonally, might be facilitated by phenotypic plasticity in the morphology and function of their kidneys.

On the many ways of being a generalist: implications for the interpretation of isotopic data

Stable isotopes are increasingly used to explore the trophic niches of species and the trophic structure of communities (Bearhop et al. 2004; Layman et al. 2007; Schmidt et al. 2007). Our results reveal both the potential of analyzing several tissues with contrasting time courses in a collection of individuals, and the possible interpretative pitfalls of relying on a single tissue and of ignoring its time scale of isotopic incorporation. Using several tissues allowed us to characterize differences in the mode and degree of trophic specialization of three related bird species. Analyses of a single tissue would have led to an incomplete, and potentially misleading, interpretation of the species' trophic biology.

Without further information, the data on collagen would have led us to infer that *C. oustaleti*'s population comprises a mixture of individuals that include both freshwater and marine sources in their diet (Fig. 2). In contrast, a multi-tissue analysis revealed that the individuals of this species shifted seasonally between these two sources. The time averaging of a tissue with low incorporation rates hides the observation that "generalization" in *C. oustaleti* is the result of relative seasonal specialization on different resources. Analyses of liver revealed a high degree of specialization to inter-tidal habitats in winter, whereas analyses of feathers revealed high reliance on freshwater habitats in summer (Figs. 2, 3). In a similar fashion, using only collagen would have hidden the differences in the two alternative ways in which *C. patagonicus* individuals can be dietary generalists: namely by shifting habitat use seasonally (i.e., by being isotopic generalists) or by mixing diets throughout the year (i.e., by being isotopic specialists; Fig. 3). Only in the case of *C. nigrofumosus*, in which all individuals are both dietary and isotopic specialists, would we have reached the correct inference (Figs. 2, 3). Because bone collagen is one of the few proteinaceous tissues with a relatively long preservation window (of the order of 10^5–10^6 years; Koch 2007), it is widely used in paleodietary reconstruction studies (Koch 2007 and references there). Our results suggest that inferring the degree and mode of dietary specialization or generalization from collagen samples can be problematic.

Our analyses were based on the tissues of specimens collected for other purposes. Ecologists can conduct similar studies using tissues with contrasting time courses of isotopic incorporation and that can be sampled non-invasively (e.g., Norris et al. 2004, 2005). By collecting blood cells and plasma, claw, and a feather in small birds, one can infer the isotopic composition of resources at a variety of temporal scales (Bearhop et al. 2003). Ecologists can obtain an almost instantaneous snap-shot of the isotopic composition of the substrate catabolized by an animal by measuring a breath sample (Carleton et al. 2006; Voigt et al., in press). How we assess individual specialization, and what are the factors that influence it have been central questions in animal ecology for decades (Bolnick et al. 2007). Stable isotope analyses of several tissues in a single individual provide an nnparalleled opportunity to answer them (Cherel et al. 2008).

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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

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

Bertolero A, Zavalaga C (2003) Observaciones sobre la biometría y la muda del churrete marisquero en Puerto San Juan, Costa Sur del Perú. Ornitol Neotrop 14:1–7

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

Bolnick DI, Svanbäck R, Araújo MS, Persson L (2007) Comparative support for the niche variation hypothesis that more generalized populations are also more heterogeneous. Proc Natl Acad Sci 104:10075–10079

Carleton SA, Marínez del Rio C (2005) The effect of cold-induced increased metabolic rate on the rate of 13C and 15N 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 Spec 22:3008–3014

Chase JM, Liebold MA (2003) Ecological niches: linking classical and contemporary approaches. University of Chicago Press, Chicago

Cherel Y, Le Corre M, Jaquemet S, Ménard Richard P, Weimerkirch H (2008) Resource partitioning within a tropical seabird community: new information from stable isotopes. Mar Ecol Prog Ser 366:281–291

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

Ehleringer JR, Rundel PW, Palma B, Mooney HA (1998) Carbon isotope ratios of Atacama Desert plants reflect hyperaridity of region. Rev Chil Hist Nat 71:79–86

Finlay JC (2001) Stable carbon isotope ratios of river biota: implications for energy flow in lotic food webs. Ecology 82:1052–1064

Finlay JC, Power ME, Cabana G (1999) Effects of water velocity on algal carbon isotope ratios: implications for river food web studies. Limnol Oceanog 44:1198–1203

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

Fry B (1991) Isotope diagrams of freshwater food webs. Ecology 72:2293–2297

Fry B (2006) Stable isotope ecology. Springer, New York

Goldstein DL, Skadhauge E (2000) Renal and extrarenal regulation of body fluid composition. In: Whittow GC (ed) Sturkey's avian physiology. Academic Press, New York, pp 265–297

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

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

Holt RD, Barfield M, Gomulkiewicz R (2005) Theories of niche conservatism and evolution: could exotic species be potential tests? In: Sax DJ, Stachowicz J, Gaines SD (eds) Species invasions: insights into ecology, evolution, and biogeography. Sinauer, Sunderland, pp 259–290

Howland MR, Corr LT, Young SMM, Jones V, Jim S, Van Der Merwe NJ, Mitchell AD, Evershed RP (2003) Expression of the dietary isotope signal in the compound-specific δ^{13}C values of pig bone lipids and amino acids. Int J Osteoarchaeol 13:54–65

Hutchinson GE (1957) Concluding remarks. Cold Spring Harbor Symp. Quant Biol 22:415–427

Jaramillo A (2003) Birds of Chile. Princeton University Press, Princeton

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

Koch PL (2007) Isotopic study of the biology of modern and fossil vertebrates. In: Michener R, Lajtha K (eds) Stable isotopes in ecology and environmental science, 2nd edn. Blackwell, Boston, pp 99–154

Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide for community-wide measures of trophic structure. Ecology 88:42–48

Martinez 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(1)91-111

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

Norris DR, Marra PP, Kyser TK, Sherry TW, Ratcliffe TM (2004) Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. Proc R Soc Lond B 271:59–64

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

Raven JA, Johnston AM, Kübler JE, Korb B, McInroy SG, Scrimgeour CM, Walker DI, Beardall J, Varderklift M, Fredriksen S, Dunton KH (2002) Mechanistic interpretation of carbon isotope determination by marine macroalgae and seagrasses. Funct Plant Biol 29:355–378

Rubenstein DR, Hobson KA (2004) From birds to butterflies: animal movement patterns and stable isotopes. Trends Ecol Evol 19:256–263

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, Rivera-Hutinel A, Farfan G (2004) Coping with salt without salt-glands: osmoregulatory plasticity in three species of coastal songbirds (ovenbirds) of the genus *Cinclodes* (Passeriformes: Furnariidae). J Comp Physiol B 174:415–420

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

Schmidt SN, Olden JL, Solomon CT, Vander Zanden MJ (2007) Quantitative approaches to the analysis of stable isotope food-web data. Ecology 88:2793–2802

Soberón JM (2007) Grinnelian and Eltonian niches and geographic distribution of species. Ecol Lett 10:1115–1123

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

Voigt CC, Rex K, Michener RH, Speakman J (in press) Nutrient routing in omnivorous animals tracked by stable isotopes in tissue and exhaled breath. Oecologia

Wang W, Yeh H (2003) δ^{13}C values of marine macroalgae from Taiwan. Bot Bull Acad Sin 44:107–112

West JB, Bowen GJ, Cerling TE, Ehleringer JR (2006) Stable isotopes as one of nature's recorders. Trends Ecol Evol 21:408–414

Wiens JJ, Graham CH (2005) Niche conservatism: integrating evolution, ecology, and conservation biology. Annu Rev Ecol Evol Syst 36:519–539

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