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Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird

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Abstract The use of stable isotopes to infer diet requires quantifying the relationship between diet and tissues and, in particular, knowing of how quickly isotopes turnover in different tissues and how isotopic concentrations of different food components change (discriminate) when incorporated into consumer tissues. We used feeding trials with wild-caught yellow-rumped warblers (*Dendroica coronata*) to determine δ^{15}N and δ^{13}C turnover rates for blood, δ^{15}N and δ^{13}C diet-tissue discrimination factors, and diet-tissue relationships for blood and feathers. After 3 weeks on a common diet, 36 warblers were assigned to one of four diets differing in the relative proportion of fruit and insects. Plasma half-life estimates ranged from 0.4 to 0.7 days for δ^{13}C and from 0.5 to 1.7 days for δ^{15}N . Half-life did not differ among diets. Whole blood half-life for δ^{13}C ranged from 3.9 to 6.1 days. Yellow-rumped warbler tissues were enriched relative to diet by 1.7–3.6% for nitrogen isotopes and by −1.2 to 4.3% for carbon isotopes, depending on tissue and diet. Consistent with previous studies, feathers were the most enriched and whole blood and plasma were the least enriched or, in the case of carbon, slightly depleted relative to diet. In general, tissues were more enriched relative to diet for birds on diets with high percentages of insects. For all tissues, carbon and nitrogen isotope discrimination factors increased with carbon and nitrogen concentrations of diets. The isotopic signature of plasma increased linearly with the sum of the isotopic signature of the diet and the discrimination factor. Because the isotopic signature of tissues depends on both elemental concentration and isotopic signature of the diet, attempts to reconstruct diet from stable isotope signatures require use of mixing models that incorporate elemental concentration.

Keywords *Dendroica coronata* · Diet reconstruction · Diet-tissue relationship · Discrimination · Stable isotope turnover

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Introduction

Animal diets are usually determined by foraging observations, gut contents and fecal analysis. Data provided by these methods are often difficult to interpret and restrictive. They are difficult to interpret because retention times and digestibilities of dietary components differ (Levey and Karasov 1994; Afik and Karasov 1995; Klasing 1998) and because a given volume of one component is not nutritionally equivalent to the same volume of another component. The data are restrictive because they only provide information about what an animal has eaten during a brief window of time (i.e., what is in the gut at the time the animal was captured or what the animal was seen eating). There are also observational biases associated with such data because it is often easier to see animals feeding on one type of prey than on another. Finally, different species vary in their ability to assimilate nutrients in food, resulting in an uncertain relationship between what is eaten and what is assimilated (Levey and Martínez del Rio 2001).

Stable isotopes offer an alternative method to reconstruct diets and to evaluate the relative importance of dietary components to consumers. Stable isotopes can be

used to reconstruct diet because the stable isotopic composition of consumer tissues can often be related to the stable isotopic composition of their diet (Peterson and Fry 1987) and can be used to determine the relative contribution of isotopically distinct food sources (e.g., Chisholm et al. 1982; Hobson 1987; Ben-David et al. 1997a, 1997b; Whitledge and Rabeni 1997; Romanek et al. 2000; Wolf and Martínez del Rio 2000). Stable isotopes can also be used to provide quantitative estimates of the flow of nutrients from resources into consumers (Wolf and Martínez del Rio 2000). This approach offers advantages over traditional methods because stable isotopes provide information on assimilated foods (not just ingested foods) and because they provide time-integrated information (Tieszen et al. 1983).

The use of stable isotopes in animal ecology has become popular largely because isotopic data can be gathered and analyzed relatively easily. Yet, many of the assumptions associated with the use of stable isotopes in ecology have still not been validated experimentally (Owens 1987; Hobson et al. 1996; Gannes et al. 1997, 1998). In a call for more laboratory experiments, Gannes et al. (1997) point out that using isotopic signals in an animal's tissues to determine the relative contribution of different food items to diet relies heavily on the assumption that the isotopic composition of the tissues equals the weighted average of the isotopic composition of the dietary constituents. This assumption is invalid because animals assimilate dietary components with varying efficiencies, isotopes fractionate differently among tissues, and animals allocate nutrients in their diet differentially to specific tissues (see reviews in Gannes et al. 1997, 1998). At issue is the extent to which these biases need to be corrected when inferring diet from stable isotopes in tissues. To address this issue requires an understanding of how quickly isotopes turnover in different tissues, how isotopic concentrations of various biochemical components of food change (hereafter, "discriminate") when incorporated into consumer tissues, and how the isotopic signatures of tissues and diet relate.

Mixing models can be used to reconstruct diets from isotopic ratios in animal tissues (Ben-David et al. 1997a, 1997b; Kline et al. 1993; Whitledge and Rabeni 1997). Previous applications of mixing models to reconstruct diet have not accounted for differences in elemental concentrations (Phillips 2001). This simplification may not affect estimates of dietary composition if all food sources are animal matter. If, however, a given diet consists of animal and plant matter, mixing models must incorporate elemental concentrations because these food sources contain fundamentally different ratios of elements (carbon and nitrogen, in particular: Phillips 2001; Phillips and Koch 2002). Phillips (2001) and Phillips and Koch (2002) developed a concentration-weighted linear mixing model, which assumes that for each element, a source's contribution to the isotopic signature of tissue is proportional to the contributed mass times the elemental concentration in that source. An implicit assumption is that both elemental concentration and isotopic signature in the diet influence the discrimination factor and, ultimately, diet-tissue relationships. To our knowledge, the influence of dietary elemental concentration on discrimination factors has not been examined experimentally.

We conducted a captive feeding experiment using a passerine bird, the yellow-rumped warbler (*Dendroica coronata*), that commonly eats fruits and insects. Our goals were to determine: (1) turnover rates of δ^{15}N and δ^{13}C in whole blood and plasma, (2) δ^{15}N and δ^{13}C diet-tissue discrimination factors for plasma and whole blood and apparent discrimination factors for feathers, and (3) the influence of nitrogen (N) and carbon (C) concentration on discrimination factors and on the relationship between the isotopic signature of diet and tissue. Our goal was to quantify isotopic diet-tissue relationships, which can then be used to interpret stable isotope data gathered from omnivorous wild birds. We focused on tissues that can be obtained non-destructively and, consequently, from large samples of birds. In contrast to most previous studies, which have involved large captive-reared species (Kelly 2000), we used a small wild-caught species.

Materials and methods

This study was conducted at the Savannah River National Environmental Research Park in South Carolina, USA (33°20'N, 81°40'W). We captured 32 yellow-rumped warblers shortly after their fall arrival in late-October and early-November. Birds were housed in individual cages at an animal care facility at the University of Georgia's Savannah River Ecology Laboratory, where they were exposed to a natural light cycle and a temperature of approximately 21°C. All were fed ad libitum an acclimation diet consisting of mealworms (*Tenebrio molitor*), bananas, agar, calcium carbonate, and a vitamin and mineral supplement (Table 1) for at least 21 days (mean=28 days, range=21–37 days). The acclimation diet was made in three batches, using bananas purchased at three different times. Each batch was homogenized and stored frozen in small containers that were thawed when needed. All birds were fed from the first batch until it was depleted, then from the second until depleted, and finally the third. Thus, all birds were exposed equally to acclimation diets. We believe,

Table 1 Ingredients of diets fed to captive yellow-rumped warblers (*Dendroica coronata*). All birds were fed the acclimation diet for at least 21 days. The other diets were used during the feeding trial. All values are percentages and are based on dry mass

Ingredient	Acclimation diet	97% Insect	73% Insect	49% Insect	20% Insect
Mealworms	32.4	97.1	72.5	49.4	19.5
Bananas	65.6	0.0	24.4	47.4	76.8
Agar	1.6	2.3	2.5	2.6	2.9
Vitamins	0.2	0.2	0.2	0.3	0.3
Calcium carbonate	0.2	0.3	0.4	0.4	0.4

however, that the isotopic signature of these diets differed because the isotopic signature of ingredients likely varied (see below).

To control for the potential influence of age and sex on isotopic discrimination, we determined the sex and age [hatch year (<1 year of age) or after hatch year (>1 year of age)] of all birds (Pyle 1997). On the same day, approximately equal numbers of each age and sex were randomly assigned to four diet treatments that differed in relative percent of insects (mealworms) and fruit (bananas) (20%, 49%, 73%, and 97% insects; Table 1). These diets were consistent with natural variation in the relative proportions of fruit and insects in yellow-rumped warbler diets (Martin et al. 1951) and thus mimicked the warblers' natural range in elemental concentrations and isotopic signatures. Each experimental diet consisted of one large batch that was homogenized with a blender and stored frozen in small containers that were thawed as needed. Eight birds were held for 21 days on each of the experimental diets.

To determine mass change, birds were weighed every 7–10 days while on the acclimation diet and every 3–4 days after the switch to treatment diets. Blood samples were taken the day prior to the diet switch and on days 1, 3, 6, 9, 12, 16, and 21 (day 0=day of diet switch). Blood samples were approximately 120 µl, taken from the brachial vein. Because we were concerned that taking blood samples too frequently could influence turnover rates and stress the birds, we collected samples from only half the birds in each diet treatment on a collection day, resulting in a minimum interval of 5 days between blood collections for any given bird. Approximately half of the blood sample was centrifuged to separate plasma from cells. Plasma and whole blood were analyzed separately. Blood samples were initially stored at –70°C, then freeze-dried.

To induce adventitious molt, one tail feather was plucked the day prior to switching from the acclimation diet to the experimental diets. This was necessary because fully-grown feathers are metabolically inactive and hence would be unaffected by diet. A growing feather, however, presumably provides a record of diet during its development. At the end of the feeding trial, the entire newly grown feather was removed, washed in ether to remove oils (Hobson and Clark 1992b) and cut into small pieces (ca. 1 mm^3). Approximately 1 mg of each feather and each blood sample (dry mass) were loaded into tins for isotopic analysis. We did not attempt to examine different portions of the feather because it was necessary to use the entire feather in each sample.

The University of California Stable Isotope Facility performed all isotope analyses. Carbon and N stable isotope ratios were determined on CO_2 and N_2 gases, produced by combustion of samples in an elemental analyzer coupled to a continuous flow isotope ratio mass spectrometer (ANCA-CFIRMS; Europa Scientific Crewe, England; ANCA combustion unit and 20–20 mass spectrometer). The ratio of stable isotopes is expressed in delta (δ) notation: $[(R_{sample}/R_{standard})-1]x1{,}000$, where δ is the isotope ratio of the sample relative to a standard. For C, the international standard is the Peedee Belemnite (PDM) limestone formation. For N, the standard is atmospheric N (AIR). R_{sample} and $R_{standard}$ are the fractions of heavy to light isotopes in the sample and standard, respectively. Isotope ratios were derived by comparison of sample with reference gas injections in each analytical cycle. Raw delta values were normalized to international values $\delta^{15}N$ AIR and $\delta^{13}C$ PDB using combusted standard samples (ammonium sulfate + sucrose) inserted at intervals in each batch of samples. Masses of C and N in each sample were derived by integrating the ion beam intensities and comparing the integrals to those of standard samples with known concentrations of C and N. Carbon and N mass were converted to percent by dividing the mass of N and C in the sample by the sample weight and multiplying by 100.

Isotopic concentrations of various biochemical components of food change when incorporated into consumer tissues. The direction and magnitude of this change depends upon the tissue (Tieszen et al. 1983; Mizutani et al. 1991; Hobson and Clark 1992a). We use the term "discrimination factor (Δ_{dt})" following Cerling and Harris (1999) to describe changes in the isotopic signal between consumer tissues and diet: $\Delta_{dt}=\delta_{tissue}-\delta_{diet}$. (Many papers in the ecological literature incorrectly use the term "fractionation" to describe this difference; fractionation is only one process by which a difference in isotopic composition may be generated.) We calculated turnover rates and discrimination factors using the following equation from O'Brien et al. (2000):

$$\delta^{13}C_{tissue}=\delta^{13}C_0+[(\delta^{13}C_{diet}+\Delta_{dt})-(\delta^{13}C_0)\left(1-e^{-r(time)}\right)]$$

This equation, illustrated with C and analogous for N, describes isotopic incorporation kinetics, where $\delta^{13}C_0$ represents the initial $\delta^{13}C$ of tissues, r=the fractional turnover rate, defined as the flow rate into the pool divided by its volume, Δ_{dt}=the discrimination factor, and time=days on a given diet. Half-life was calculated as: [Ln(2)]/r.

Ideally, the parameters of the isotopic incorporation equation (r, Δ_{dt}, and $\delta^{13}C_0$) should be fitted to individual birds. However, the relatively small samples in time for each bird prevented us from obtaining accurate estimates. Therefore, we fitted these parameters for all birds in each treatment. We calculated an "apparent discrimination factor" for feathers by subtracting the delta values for diet from the delta values for feathers ($\Delta_{dt}=\delta_{tissue}-\delta_{diet}$). We refer to this as an "apparent" discrimination factor because it does not describe the isotopic incorporation kinetics of the equation above.

We used linear regression to examine the effects of varying the proportion of insects in the diet on both the elemental concentration and isotopic signature of diet and to examine the effects of varying the elemental concentration in the diet on the discrimination factor. We also used linear regression determine whether the sum of delta value of tissue and that tissue's discrimination factor could accurately predict the delta value of the diet. For this relationship, we assumed that $\delta^{15}N_{tissue}=\delta^{15}N_{diet}+\Delta_{dt}(\%N)$ or that $\delta^{13}C_{tissue}=\delta^{13}C_{diet}+\Delta_{dt}(\%C)$. This equation indicates that N or C isotopic composition in tissues equals the composition in diet plus a discrimination factor. There is a functional dependence of the discrimination factor on %N or C in diet (Phillips 2001). For these analyses we used the isotopic values derived from blood and feather samples taken during the final two days of the experiment.

Results

Body mass

Most birds lost mass immediately after capture and after switching diets, then regained it. During the three-week acclimation period, birds increased in body mass (1.29±0.24 g, Fig. 1). With the exception of the highest percent fruit diet, birds lost mass after switching to the four experimental diets. However, for all diets, average body mass at the end of the experiment was greater than mass at initial capture (Fig. 1), suggesting that the birds

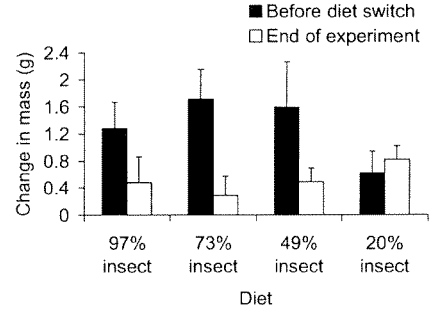

Fig. 1 Mean body mass difference (body mass – body mass at capture ± SE) before switching birds to the four experimental diets and at the end of the experiment. n=8 birds per diet

Table 2 $\delta^{15}N$ and $\delta^{13}C$ (%) and percent nitrogen and carbon (mean±SD) in diets fed to yellow-rumped warblers before the feeding trial (acclimation diet) and the four experimental diets used in the feeding trial. n = number of birds on each diet

Diet	$\delta^{15}N$	N	$\delta^{13}C$	C	n
Acclimation[a]	6.15±0.06	3.0±0.1	−24.21±0.06	43.3±0.4	32
20% Insect	6.17±0.03	2.9±0.1	−24.94±0.03	42.8±0.7	8
49% Insect	6.12±0.02	5.0±0.1	−26.74±0.04	49.4±0.5	8
73% Insect	6.01±0.03	7.0±0.2	−27.25±0.03	52.0±1.1	8
97% Insect	6.07±0.02	8.4±0.2	−27.90±0.10	54.2±0.9	8

[a] The isotopic signature of the acclimation diet may have varied throughout the acclimation diet period (see Materials and methods) however, all birds were exposed to the same diet-isotopic signatures

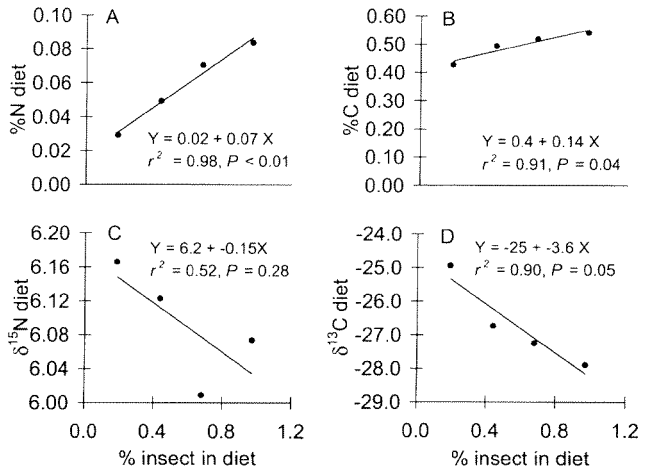

Fig. 2 Concentrations of N and C increased with the percent of insects in the diet (**A, B**). There was no relationship between $\delta^{15}N$ values of the diet and percent insects in the diet and the relationship between $\delta^{13}C$ values of the diet and the percent insects in the diet was linear (**C, D**)

were not nutritionally stressed after acclimation to captive conditions.

Nitrogen and carbon isotopes in diet

The $\delta^{15}N$ and $\delta^{13}C$ values and concentration of C and N in the four experimental diets are presented in Table 2. Concentrations of C and N increased with the percent insects in the diet (carbon: r^2=0.91, $F_{1,3}$=21.2, P=0.04; nitrogen: r^2=0.91, $F_{1,3}$=103.6, P=0.009; Fig. 2). The relationship between $\delta^{13}C$ values of the diet and the percent insects in the diet was linear (r^2=0.89, $F_{1,3}$=17.7, P=0.05; Fig. 2). There was no relationship between $\delta^{15}N$ values of the diet and percent insects (r^2=0.52, $F_{1,3}$=2.1, P=0.28; Fig. 2).

Our goal was to create diets along a linear continuum of both isotopic signatures and concentrations by changing the relative proportion of fruit and insects in the diet. This was the case for concentrations of C and N and for $\delta^{13}C$ (Table 2). However, despite a nearly one trophic level shift in diet (97% insect to 80% fruit), we found only less than 0.2 percentage points difference in the $\delta^{15}N$ values among diets (Table 2). Moreover, and unexpectedly, the diet containing the largest proportion of insects did not have the highest $\delta^{15}N$ value and the diet containing the lowest proportion of insects did not have the lowest $\delta^{15}N$ value. Although we used homogeneous diets, the N isotopic signatures and concentrations of the bananas and mealworms used in each diet must have differed among diets. After completing the experiment, we analyzed nine batches of bananas from different suppliers. $\delta^{15}N$ values ranged from −0.48 to 5.32% (mean=2.86%). This variation in isotopic signature of a key dietary ingredient likely explains the non-linear relationship between percent insects and $\delta^{15}N$ values of the diets we used.

Turnover

Plasma $\delta^{13}C$ and $\delta^{15}N$ values were in equilibrium with dietary values on the day before switching onto the experimental diets and at the end of the experiment, 21 days later. Half-life estimates for $\delta^{13}C$ of plasma ranged from 0.4 to 0.7 days, depending on diet (Table 3). Half-life estimates for $\delta^{15}N$ of plasma ranged from 0.5 to 1.7 days, again depending on diet (Table 3). The 95% confidence intervals surrounding both of these estimates overlapped between diets, suggesting that diet did not influence the turnover rate of C and N in plasma (Table 3). The pattern of C turnover in plasma resembled exponential models and reached asymptotic values during the experiment.

It is less clear whether whole blood $\delta^{13}C$ and $\delta^{15}N$ values were in equilibrium with dietary values on the day before switching onto the experimental diets or at the end of the experiment, presumably because of a slower turnover rate in the cellular fraction of the blood. In general, $\delta^{13}C$ turnover was approximately 4–6 days, except in the 20% insect diet, which was 33 days. Half-life estimate for $\delta^{15}N$ of whole blood were 7.45–27.7 days (Table 3). Variation explained by the nonlinear relationship for $\delta^{15}N$ of blood over time was small and the 95% confidence intervals surrounding these estimates were quite large (Table 3). We only report turnover results for those data for which we have confidence (i.e., data reached an asymptote).

Discrimination

$\delta^{15}N$ values of plasma and whole blood were enriched 1.7 to 3.0% relative to dietary values (Table 3). $\delta^{13}C$ values of plasma and whole blood ranged from depleted to enriched (−1.2 to 2.2%) relative to diet (Table 3). "Apparent" discrimination factors for feathers were enriched for $\delta^{15}N$

Table 3 Parameters estimated by the model describing the isotopic incorporation kinetics. Parameters are presented with Wald confidence intervals (lower, upper). In some diets the non-linear program did not reach an asymptote and hence we do no present estimates

Tissue and isotope	Diet	r^2	Half-life in days (95% C.I.)	Discrimination factor (95% C.I.)
δ^{13}C plasma	20% Insect	0.53	0.5(−0.1,1.1)	−1.5(−1.7,−1.4)
	49% Insect	0.59	0.7(0.04,1.4)	−0.2(−0.4,−0.01)
	73% Insect	0.77	0.4(0.1,0.73)	0.2(0.1,0.3)
	97% Insect	0.88	0.4(0.2,0.6)	0.61(0.5,0.7)
δ^{13}C blood[a]	20% Insect	0.76	33(−72.3,138.3)	-1.2(−5.4,3.1)
	49% Insect	0.80	3.9(1.9,5.9)	1.5(1.4,1.6)
	73% Insect	0.92	5.0(3.1,6.8)	1.8(1.7,2.0)
	97% Insect	0.87	6.1(3.0,9.1)	2.2(2.0,2.5)
δ^{15}N plasma	20% Insect	0.49	1.7(−0.4,3.8)	2.5(2.3,2.8)
	49% Insect	0.31	0.5(−0.4,1.5)	2.6(2.1,3.2)
	73% Insect	0.32		2.9(2.7,3.2)
	97% Insect	0.21	0.6(−1.7,4.1)	3.0(2.7,3.3)
δ^{15}N blood	20% Insect	0.04		1.7(1.6,1.7)
	49% Insect	0.10	7.5(−25.5,40.4)	1.8(1.2,2.3)
	97% Insect	0.11	27.7(−344.8,399.2)	2.7(−8.6,14.0)

[a] Blood = whole blood

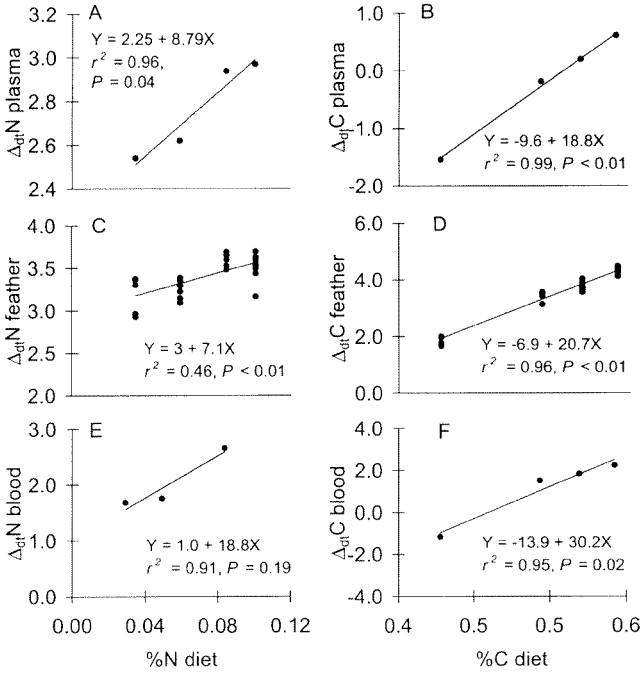

Fig. 3 For plasma and feathers, discrimination factors increased linearly with diet elemental concentration for both N and C (**A–D**). This result indicates that there is a functional dependence of the discrimination factor on elemental concentration. For blood, there is not a linear relationship between discrimination factors and diet elemental concentration for N but there is for C (**E, F**). However, only three points are included in the regression between blood nitrogen discrimination factors and elemental concentration in diet (**E**). This occurred because we were not able to obtain a discrimination factor estimate for the 73% insect diet (see Table 3)

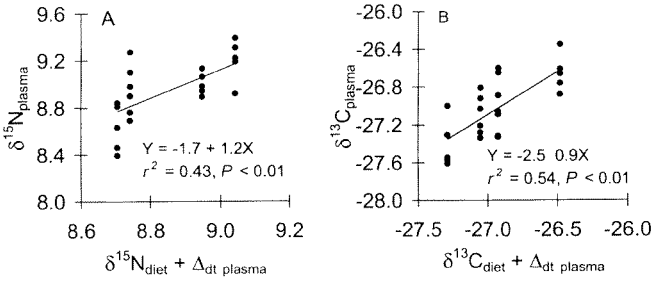

Fig. 4 The isotopic signature of plasma increased linearly with the isotopic signature of the diet plus the discrimination factor for both N and C (**A, B**). Both isotopic signature of diet and discrimination factors influence the ultimate isotopic signature of tissues. This result supports the importance of using concentration dependent mixing models when reconstructing diet

[20% insect=3.2±0.1, 49% insect=3.3±0.04, 73% insect=3.6±0.03, 97% insect=3.5±0.1] and δ^{13}C [20% insect=1.9±0.1, 49% insect=3.5±0.1, 73% insect=3.8±0.1, 97% insect=4.3±0.1]. δ^{13}C values of feathers were more enriched than whole blood and plasma. Whole blood δ^{13}C values were more enriched than plasma (compare the values above for feathers with Table 3). For all tissues and elements, discrimination factors increased in diets with a higher proportion of insects (Table 3).

Effect of elemental concentration on discrimination factors

Discrimination factors increased linearly with elemental concentration in the diet for both N (plasma: r^2=0.92, $F_{1,3}$=25.9 P=0.037; feather r^2=0.46, $F_{1,25}$=21.4, P <0.001; Fig. 3) and C (plasma: r^2=0.99; $F_{1,3}$=530.1; P=0.002; blood: r^2=0.95; $F_{1,3}$=39.3, P=0.025; feather: r^2=0.96, $F_{1,25}$=640.5, P<0.001; Fig. 3). We did not include blood in the analysis for N because of poor confidence surrounding the discrimination factors (Table 3).

Isotopic signatures of diet versus tissues

The isotopic signature of plasma increased linearly with the sum of the diet's isotopic signature and discrimination factor [e.g., $\delta^{15}N_{plasma}=\delta^{15}N_{diet}+\Delta_{dt}(\%N)$] for both N ($r^2$=0.43, $F_{1,21}$=15.9 P<0.001; Fig. 4) and C (r^2=0.54;

$F_{1,24}$=25.1; P<0.001; Fig. 4). We excluded whole blood from this analysis because of the poor confidence surrounding the N discrimination factor estimates (Table 3). Feathers were omitted from this analysis because the "apparent" discrimination factor for feathers was calculated by subtracting the isotopic signature of the feather from that of the diet, which results in a perfectly linear relationship.

Discussion

Accurate discrimination factors are critical in any attempt to reconstruct the relative contribution of two or more types of food to an animal's diet (e.g., Ben-David et al. 1997a, 1997b). Our results indicate that discrimination factors depend on both diet and tissue. Consequently, using discrimination factors in equations to reconstruct diet requires an estimate of elemental concentrations in the diet.

Turnover rates are important for determining how quickly the isotopic signature of an animal's diet is incorporated into its tissues. Our results indicate that plasma turnover occurs in about a day and therefore provides short-term information about diet, while whole blood turnover appears to occur over approximately two weeks and therefore integrates isotopic signatures of dietary components over a longer term.

We also examined the relationship between the isotopic signatures of diet and consumer tissue. For both C and N discrimination factors increase linearly with elemental concentration in the diet. Consequently, the relationship between the isotopic signature of the diet and the sum of a given tissue's isotopic signature and its discrimination factor was also positive and linear. This result indicates the importance of elemental concentration in reconstructing diet and the need to use concentration dependent mixing models when attempting to estimate the relative contribution of different food sources to an animal's diet (Phillips and Koch 2002).

Turnover

Turnover rates of isotopes in a tissue are correlated linearly with the metabolic rate of the tissue (Tieszen et al. 1983). Blood cells have a greater longevity than the constituents of plasma and are thus likely to have a slower turnover rate. As expected, plasma turnover in yellow-rumped warblers was rapid; half-life estimates of δ^{13}C and δ^{15}N ranged from 0.4 to 0.7 days and from 0.5 to 1.7 days, respectively. These estimates did not differ among diets. Our estimates for C half-life in plasma are shorter than those derived for plasma in American crows (*Corvus brachyrhynchos*; 2.9 days; Hobson and Clark 1993), and black bears (*Ursus americanus*; 4.6 days; Hilderbrand et al. 1996). Our estimates of N half-life in plasma are also shorter than those derived for black bears (3.5 days; Hilderbrand et al. 1996). To our knowledge, comparable N isotope half-life estimates are not available for other taxa. Yellow-rumped warbler plasma appears to have faster C and N turnover rates than other vertebrates examined to date, probably because of the warbler's small body size and high mass specific metabolic rate. Thus, C and N isotopes in warbler plasma provide very short-term dietary information.

We believe that our half-life estimates of δ^{13}C for whole blood provided by the isotopic incorporation model provide good estimates (3.9–6.1 days) for all diets other than the 20% insect diet (33 days). In particular, the model yielded narrow confidence intervals surrounding the discrimination factors and explained a high percent of the variability for all diets, except the 20% insect diet. For that diet, the unusually long half-life and the large confidence intervals may have been the result of small δ^{13}C differences between diet and tissues. Our estimate of C half-life (3.9–6.1 days) is shorter than that estimated for whole blood in Japanese quail (*Coturnix japonica*; 11.4 days; Hobson and Clark 1992a) and shorter than the cellular fraction of blood in American crows (29.8 days; Hobson and Clark 1993) and black bears (34.7 days; Hilderbrand et al. 1996).

Half-life estimates of δ^{15}N for whole blood had large 95% confidence intervals and little variation was explained by the non-linear relationship used to estimate them. Turnover rates in wild yellow-rumped warblers may be higher than what we report for captive individuals because of faster metabolic rates associated with natural living conditions.

Discrimination

Although similar experiments have been conducted using mice (*Mus musculus*; DeNiro and Epstein 1978, 1981), we know of no other study that experimentally examined the effects of diet on discrimination using a bird species fed diets differing in N and C isotopic composition. Consistent with the findings of DeNiro and Epstein (1978, 1981), we found that differences in C and N isotope discrimination factors depended on both diet and type of tissue. In birds and mammals, diet-tissue discrimination for N isotopes are enriched 1–5% relative to diet and those of C isotopes are enriched 1–6% (DeNiro and Epstein 1978, 1981; Mizutani et al. 1991, 1992; Hobson and Clark 1992b; Hilderbrand et al. 1996; Hobson et al. 1996). However, diet-tissue discrimination factors for C can be slightly depleted (Hobson and Clark 1992b). Consistent with these results, we found yellow-rumped warbler tissues to be enriched from 1.7 to 3.0% for N isotopes and from −1.5 to 2.2% for C, depending on tissue and diet. Also consistent with the studies cited above, we found feathers to be the most enriched and whole blood and plasma to be the least enriched (except for C in plasma and whole blood, which were slightly depleted in birds on diets dominated by fruit). Tissues from birds on the highest percent fruit diet (lowest N and C concentrations) were the least enriched, while tissues from birds on

high percent insect diets were the most enriched. This pattern was consistent across tissues. These results indicate that isotopic discrimination across trophic levels may depend on dietary N and C concentration. Because C:N ratios decrease across trophic levels (i.e., carnivores ingest more protein than herbivores and omnivores), we expect higher discrimination in higher trophic levels.

There appears to be a functional dependence of N and C discrimination factors on the elemental concentration in the diet. In particular, the correlation between N and C discrimination factors and elemental concentration in the diet was linear for all tissues. The increase in N discrimination factors with an increase in the percent N in the diet is likely the result of increases in excretion of depleted ^{15}N because more amino acids are catabolized for energy as dietary protein increases (Macko et al. 1986). We have no adequate explanation for the enrichment in C with increased protein content in diet.

Diet-tissue relationship

Consistent with previous research, the relationship between the N and C isotopic signatures of diet and those of tissue plus their discrimination factors was linear for plasma, blood and feathers (e.g., DeNiro and Epstein 1981; Hilderbrand et al. 1996). Both the concentration of N and the N-isotopic signature of dietary components influenced the $\delta^{15}N$ value of tissues. Looking at N, most fruits have a lower $\delta^{15}N$ value and a much lower concentration of N than insects (S.F. Pearson, unpublished data). Thus, as a bird shifts from a diet dominated by insects to one dominated by fruit, there will be little change in the overall $\delta^{15}N$ value of diet until the diet is composed primarily of fruit. Previous researchers have used the isotopic values of the diet plus the discrimination factors of the tissue of interest to determine the proportion of the diet derived from two or more isotopically distinct food sources (e.g., Kline et al. 1993; Hobson et al. 1994; Hilderbrand et al. 1996; Ben-David et al. 1997a, 1997b; Whitledge and Rabeni 1997). Our results suggest such a technique may yield misleading results when the food sources vary widely in the concentration of elements. The influence of dietary N concentration on the isotopic signature of a consumer's tissues suggests that when two food sources differ in both isotopic signature and concentration of N such that one food source has a high isotopic signature of N and a high concentration of N (e.g., insects) while the other food source is low in both N concentration and N-isotopic signature (e.g., fruit), the N isotopic signature of the consumer's tissues will reveal only dramatic shifts in diet. However, a concentration dependent mixing model that uses two or more elements in concert should dramatically improve one's ability to reconstruct diet.

Conclusion

Blood, plasma and feathers can be sampled non-destructively and provide time-integrated information about diet. Plasma provides short-term information (approximately 1–4 days), whole blood provides longer-term information (approximately 8–12 days), and feathers reflect the diet at the time they were grown. Because we pulled feathers just prior to switching diet, we were able to induce molt and document that feathers do indeed reflect the isotopic signature of the diets at the time they were grown. Once grown, feathers are no longer connected to the bird's circulatory system and thus become a permanent record of diet during their growth.

Our results indicate that diet-tissue discrimination factors differ among diets and tissues. Thus, using a single discrimination factor in mixing models may not provide an accurate assessment of diet. Another important finding is that when two food sources differ in both isotopic signature and concentration of an element of interest, the isotopic signature of the consumer's tissues will reveal only dramatic shifts in diet when a single element is used to reconstruct diet. Thus, it is critical that concentration of elements be considered when attempting to use isotopic signatures to reconstruct diet (Phillips and Koch 2002).

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