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BLOOD ISOTOPIC ($\delta^{13}C$ AND $\delta^{15}N$) TURNOVER AND DIET–TISSUE FRACTIONATION FACTORS IN CAPTIVE DUNLIN (*CALIDRIS ALPINA PACIFICA*)

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Abstract.—Avian studies are often interpreted using dual (e.g. ^{13}C, ^{15}N) isotope models, assuming turnover of both isotopes occur at similar rates, but only a few studies have quantified turnover rates for more than one of those isotopes simultaneously. To test the generality of previous turnover and fractionation estimates and assumption of synchronous C and N patterns of turnover rates, we captured Dunlin (*Calidris alpina pacifica*) wintering in the Fraser River Delta, British Columbia, and derived isotopic turnover rates and diet–tissue fractionation factors by experimentally manipulating diet. Birds (n = 15) were initially fed a terrestrially derived diet (mean $\delta^{13}C$: –24.7‰, mean $\delta^{15}N$: 3.5‰) for 54 days. A treatment group (n = 11) was then switched to a marine-derived diet (mean $\delta^{13}C$: –18.3‰, mean $\delta^{15}N$: 13.7‰); a control group (n = 4) was maintained on the terrestrial diet for a further 59 days. An exponential model described patterns of isotopic turnover for ^{13}C and ^{15}N, and turnover rates and half-lives of the two isotopes were correlated, confirming the assumption of synchronous patterns of turnover for those isotopes. The half-lives for ^{13}C and ^{15}N in Dunlin whole blood were 11.2 ± 0.8 days and 10.0 ± 0.6 days, respectively, and are among the lowest values obtained to date for wild birds. Variation in turnover rate among individuals was not related to indices of body condition. *Received 5 March 2003, accepted 5 October 2003.*

Résumé.—Les études portant sur la faune avienne sont souvent interprétées en utilisant des modèles isotopiques doubles (e.g. ^{13}C, ^{15}N), assumant que le renouvellement des deux isotopes se fait à des taux similaires. Seulement quelques études ont quantifié les taux de renouvellement pour plus d'un de ces isotopes simultanément. Pour étudier la généralisation de ce renouvellement, le fractionnement des estimés et l'hypothèse de patrons synchrones des taux de renouvellement en C et N, nous avons capturé des *Calidris alpina pacifica* hivernant dans le delta de la rivière Fraser, Colombie Britannique. Nous avons également dérivé les taux de renouvellement isotopiques ainsi que le fractionnement des facteurs diète-tissu en manipulant expérimentalement le régime alimentaire. Les oiseaux (n = 15) ont été tout d'abord nourris avec un régime dérivé de type terrestre (moyenne $\delta^{13}C$: –24.7‰, moyenne $\delta^{15}N$: 3.5‰) pendant 54 jours. Un groupe traitement (n = 11) a été ensuite soumis à un régime dérivé de type marin (moyenne $\delta^{13}C$: –18.3‰, moyenne $\delta^{15}N$: 13.7‰). Un groupe contrôle (n = 4) a été maintenu au régime de type terrestre durant 59 jours supplémentaires. Un modèle exponentiel décrivant les patrons de renouvellement isotopique en ^{13}C et ^{15}N, a été corrélé aux taux de renouvellement et aux demi-vies des deux isotopes, confirmant l'hypothèse de patrons synchrones de renouvellement des ces isotopes. Les demi-vies du ^{13}C et du ^{15}N dans le sang chez *Calidris alpina pacifica* étaient de 11.2 ± 0.8 jours et 10.0 ± 0.6 jours, respectivement, et sont parmi les valeurs les plus basses obtenues jusqu'à aujourd'hui pour des oiseaux sauvages. Les variations inter-individuelles du taux de renouvellement n'étaient pas reliées aux indices de conditions corporelles.

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The use of stable-carbon and nitrogen isotope analysis is expanding as a tool for elucidating the relative proportion of diet gleaned from two isotopically distinct (e.g. marine vs. terrestrial) sources (e.g. Hobson 1986, 1990; Mizutani et al. 1990; Hobson and Sealy 1991; Hobson et al. 2000b). The stable-isotope approach has several advantages over traditional techniques by: (1) providing information for all individuals sampled, including those with empty stomachs; (2) avoiding bias resulting from differential digestion of soft- versus hard-bodied prey items; (3) providing information on foods assimilated into body tissues, not just ingested; and (4) integrating information over a relatively long window of time, rather than just a time "snapshot," as is the case for stomach or fecal analysis (Cree et al. 1999). One important disadvantage of stable-isotope analysis is that different tissues may reflect the isotopic composition of different dietary constituents, and stable isotopes underestimate food used directly for energy, such as carbohydrates (Gannes et al. 1998, Hobson et al. 2000). Also, stable-isotope analysis usually does not provide taxonomic information on prey ingested and thus provides a complementary rather than alternative approach to gut contents analysis (e.g. Hobson et al. 1994).

The period for which tissue isotopic values reflect diet depends upon the isotopic turnover rate in that tissue. Tissues with high turnover rates, such as liver and plasma, reflect recent diet; whereas tissues with slower turnover rates, such as blood cells and muscle, reflect diet over longer terms (Hobson and Clark 1993). Although stable-isotope analysis has been used widely to interpret the diets of free-living bird populations, few controlled studies have been performed to determine isotopic turnover rates and determine how isotopes fractionate or change once they are incorporated into avian tissues (Gannes et al. 1997, Hobson 1999). Avian field studies using stable-isotope analysis have thus far assumed that the results of controlled laboratory studies on captive Japanese Quail (*Coturnix japonica*), Ring-billed Gulls (*Larus delawarensis*), and American Crows (*Corvus brachyrhynchos*) (Hobson and Clark 1992a, b; Hobson and Clark 1993) are representative of a wide taxonomic range of bird species and interpreted their results on the basis of turnover rates and diet–tissue fractionation factors derived from those species. Most recently, Haramis et al. (2001) and Bearhop et al. (2002) provided information on both stable-carbon and nitrogen isotope turnover in whole blood of wintering Canvasback (*Aythya valisineria*) and captive Great Skuas (*Stercorarius skua*), respectively. Those studies were the first to report isotopic turnover rates for both isotopes simultaneously and suggested such patterns were similar. Haramis et al. (2001) reported different turnover rates depending on the nature of the diet switch in captive birds. Although not the focus of those studies, such results imply an interesting physiological complexity that needs to be considered when applying isotopic models to wild birds (Bearhop et al. 2002).

In a recent study (Evans Ogden 2002), we used stable-isotope analysis to infer the dietary contribution from agricultural land versus estuarine intertidal flat to free-living Dunlin (*Calidris alpina pacifica*) wintering in the Fraser River Delta, British Columbia, Canada. Prior to our study, no experimental data for isotopic turnover rates and diet–tissue fractionation factors were available for any shorebird. Dunlin are not closely related to any species that have been investigated experimentally using stable isotopes (Sibley and Ahlquist 1990). Kersten and Piersma (1987) suggested shorebirds have relatively high metabolic rates in comparison with other avian taxa, which would likely increase rates of isotopic turnover in tissues (Hobson and Clark 1992a, Bearhop et al. 2002). We designed a laboratory study to determine the isotopic turnover rates of ^{13}C and ^{15}N in Dunlin whole blood by switching between diets of distinct stable-carbon and stable-nitrogen isotope compositions. We quantified turnover rates for whole blood, the tissue sampled in our field study, and undoubtedly the preferred tissue to sample as a nondestructive assay of diet in wild birds (Hobson and Clark 1993). For comparison with the broader literature, we also determined diet–tissue fractionation factors (i.e. isotopic differences between diet and tissues) for ^{13}C and ^{15}N in whole blood, blood plasma, and cellular fraction of blood, liver, kidney, breast muscle, and newly molted body feathers.

Methods

Dunlin were captured using mist nets at Brunswick Point, (49°03′N, 123°1′W) on the Fraser River Delta, British Columbia, on 24 and 27 November 2000.

Although no studies thus far have suggested sex differences in isotopic turnover rates, to avoid potential sex-related differences in isotopic assimilation or turnover rates, only males were used in the experiment. Birds were sexed by culmen length (Page 1974). Birds were brought into captivity and held in outdoor aviaries at the Animal Care Facility at Simon Fraser University. The experiment was conducted in accordance with Canadian Council for Animal Care Committee protocol. Temporarily using live mealworms (*Tenebrio molitor*) as a lure, birds were trained to feed from containers. Birds were then transitioned onto a terrestrial-based diet consisting of wheat-based poultry grower (Pro-Form 16, Buckerfields Feed, Abbotsford, British Columbia), hard-boiled chicken eggs, blood meal, canola oil, and bird vitamins that were ground and combined with gelatin and water to form a homogeneous mixture. Food and water were provided *ad libitum*. A heat lamp was provided in the enclosure, because the captive facility was at a higher elevation (300 m above sea level) than the estuarine intertidal flat habitat at which birds were captured. Birds were thus subjected to lower temperatures in captivity than those in their natural habitat, particularly at night. Mass of each bird was measured daily for the first 19 days after capture. The experiment was begun on 14 December 2000, by which time all 15 birds had achieved a stable mass, either exceeding or equivalent to initial capture mass.

Initial diet consisted entirely of foods of terrestrial C-3 origin. Food samples were archived weekly throughout the experiment so isotopic variation between weeks could be determined. Blood samples were taken and mass measured from all birds on day 1, and weekly thereafter. After 54 days on the terrestrial diet, birds were randomly assigned to a treatment ($n = 11$) or control ($n = 4$) group. Treatment birds were switched to a diet entirely marine in origin, and control birds remained on the terrestrial-based diet. The marine diet consisted of fish meal (West Coast Reduction Limited, Vancouver) and bird vitamins, ground and combined with gelatin and water to form a homogeneous mixture. Blood sampling of both treatment groups continued, beginning two days after the diet switch (day 56), and weekly thereafter. At the end of the experiment (day 113), the treatment group was released back into the wild at the location of capture; the four control birds were euthanized (via Halothane overdose) to determine diet–tissue fractionation factors. Some ($n = 3$) of the birds had begun molting before the end of the experiment, so samples of newly grown body feathers were also plucked for isotopic analysis.

Stable-isotope composition of feeds differed by more than 6‰ in $\delta^{13}C$ and 10‰ in $\delta^{15}N$ values. Because nutritional stress can cause changes in the stable-isotope values of tissues (Hobson et al. 1993), we ensured diets were isotopically distinct, but nutritionally similar. To reduce the possibility that birds would reject the new diet after the diet switch, texture and appearance of the diets was kept as consistent as possible by means of homogenization of both diets into a gel form and identical presentation of both diets in plastic food dishes. Weekly blood samples were taken from the brachial vein using a 26.5 gauge needle and heparinized microcapillary tubes. The wing that was bled was alternated each week to maximize healing time. For the control group, blood was also centrifuged into cellular and plasma components; when asymptotic values had been reached, muscle, liver, and kidney were extracted to determine diet–tissue fractionation factors. The diet–tissue fractionation factor was calculated as the difference in isotopic abundance between diet and tissue.

Stable-isotope analyses were performed at the University of Saskatchewan, Saskatoon. All but muscle samples were freeze dried and ground to a fine powder in an analytical mill and then directly analyzed isotopically. For muscle tissue, lipids were removed following drying using successive rinses in 2:1 chloroform:methanol solvent. Lipid extraction was not performed on whole-blood samples because of the typically low proportion of lipids in bird blood (see references in Bearhop et al. 2002). Stable-carbon and nitrogen isotope assays for all tissues were performed on 1 mg subsamples of homogenized material by loading into tin cups and combusting at 1,800°C in a Robo-Prep elemental analyzer. Resultant CO_2 and N_2 gases were then analyzed using an interfaced Europa 20:20 continuous-flow isotope ratio mass spectrometer (CFIRMS) with every five samples separated by two laboratory standards. Stable-isotope abundances are expressed in delta (δ) notation as the deviation from standards in parts per thousand (‰) according to $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Standard values were based on the Vienna PeeDee Belemnite (VPDB) for $\delta^{13}C$ measurements and atmospheric N_2 (air) for $\delta^{15}N$ measurements. Hundreds of replicate assays of internal laboratory standards (albumen) indicated measurement errors of ±0.1‰ and ±0.3‰ for stable-carbon and nitrogen isotope measurements, respectively.

Statistical analyses.—As with all previous studies, patterns for the carbon and nitrogen turnover in Dunlin whole blood resembled exponential models (Fig. 1), so we fitted equations to our data of the form $Y(t) = y_a + ae^{-bt}$ using a regression algorithm from SIGMAPLOT (version 5.0). In this equation, $Y(t)$ represents the $\delta^{13}C$ or $\delta^{15}N$ value of blood at time t, y_a is the asympotic condition, a is the absolute difference between initial and asymptotic conditions, b is the turnover rate of carbon or nitrogen in blood, and t is time (days) since the diet switch. Half-lives of isotopes in blood were calculated as $-\ln(0.5)/b$. Regression analysis and t-tests were performed using SAS (2000). To avoid psuedoreplication, we calculated the above

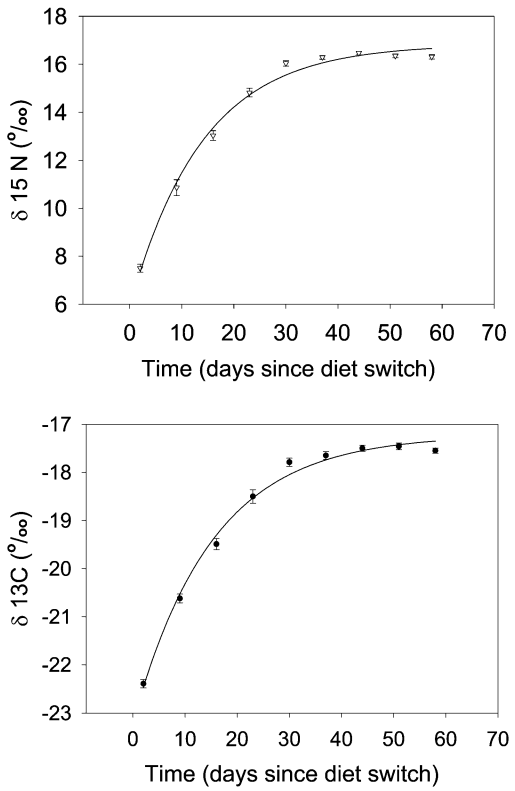

Fig. 1. Stable-nitrogen (open triangles) and carbon (filled circles) isotope turnover patterns for Dunlin whole blood (mean ± SE). Sample size for each experimental period was 11, except for day 2 (n = 10) and day 16 (n = 9). We depict the average response here for illustrative purposes only. Isotopic turnover parameters quoted in the text are based on averages of all individual responses.

parameters for each individual and then averaged those for population-level estimates.

Principal component analysis (PCA) was computed from a correlation matrix using wing, culmen, and tarsus measurements of experimental individuals. The first principal component (PC1) from that analysis was used as an index of structural size. Residual mass was determined by computing the residuals of a linear regression using PC1 as a predictor of body mass. That approach was taken to investigate whether body size or mass corrected for body size explained variance associated with half-lives of each element among individuals.

Results

Terrestrial and marine diets differed in stable-carbon and nitrogen isotopic abundance in the expected directions. The mean terrestrial diet $\delta^{13}C$ value was –24.7 ± 0.07‰ (SE), and the mean $\delta^{15}N$ value was 3.5 ± 0.07‰ (n = 29). The mean marine diet $\delta^{13}C$ value was –18.3 ± 0.1‰, and the mean $\delta^{15}N$ value was 13.7 ± 0.05‰ (n = 16). Both isotopes in whole blood from the treatment group shifted toward more positive values over the course of the experiment, from initial mean $\delta^{13}C$ and $\delta^{15}N$ values of –23.1 ± 0.03‰ and 6.6 ± 0.03‰ , respectively, to asymptotic values approaching –17.2 ± 0.05‰ and 16.9 ± 0.1‰ (n = 11) (Fig. 1). That is consistent with the incorporation into tissues of the isotopically enriched marine diet. In contrast, the isotopic signatures of the control group remained unchanged, with initial values of –23.2 ± 0.1‰ and 6.3 ± 0.1‰ for $\delta^{13}C$ and $\delta^{15}N$ values, respectively, and asymptotic $\delta^{13}C$ and $\delta^{15}N$ values of –23.3 ± 0.1‰ and 6.6 ± 0.05‰ (n = 4).

We found no evidence of nutritional stress when birds changed from the terrestrial to the marine diet, as indicated by no significant change in body mass after the diet switch (paired t-test: mean mass for treatment birds on February 1 (before) = 57.1 g, mean mass on February 15 (after) = 54.0 g, mean difference = –3.2 ± 2.0 g (SE), t-value = 1.7, P = 0.12, n = 11; mean mass for control birds on February 1 (before) = 59.6 g, mean mass on February 15 (after) = 59.7 g, mean difference = 0.1 ± 2.0 g (SE), t-value = –0.06, P = 0.95, n = 4). An exponential model described patterns of isotopic turnover for ^{13}C and ^{15}N, and turnover rates of the two isotopes were correlated (r^2 = 0.98, F = 884.02, df = 1, P < 0.0001), confirming the assumption of synchronous patterns of turnover for those isotopes. On the basis of an analysis of all 11 experimental individuals considered separately, the mean half-life of ^{13}C in male Dunlin whole blood was 11.2 ± 0.8 days and that of ^{15}N was 10.0 ± 0.6 days. Half-lives of each isotope were correlated (r^2 = 0.61, F = 13.76, df = 1 and 10, P = 0.005). In our analysis of body size of experimental birds, PC1 accounted for 61.9% of overall variance and was characterized by the following morphological parameters and factor loadings: wing chord, 0.957; tarsus, 0.027; and culmen, 0.284. Initial mass was correlated with structural size (r^2 = 0.7, F = 8.86, df = 1 and 10, p = 0.016), but half-lives of each element were not correlated with structural size (^{15}N: r^2 = 0.02, F = 0.18, df = 1 and 10, P = 0.89; ^{13}C: r^2 = 0.01, F = 0.01, df = 1 and 10, P = 0.92) or initial mass

corrected for structural size (^{15}N: $r^2 = 0.12$, $F = 1.24$, df = 1 and 10, $P = 0.29$; ^{13}C: $r^2 = 0.02$, $F = 0.15$, df = 1 and 10, $P = 0.70$). Percentage mass change during the experiment was also not associated with turnover rates of each element (^{15}N: $r^2 = -0.10$, $F = 0.10$, df = 1 and 10, $P = 0.76$; ^{13}C: $r^2 = 0.11$, $F = 2.23$, df = 1 and 10, $P = 0.17$).

Diet–tissue fractionation factors for each isotope did not differ between tissues, except for feathers (^{13}C: $F = 3.7$, df = 1 and 6, $P = 0.01$; ^{15}N: $F = 5.8$, df = 1 and 6, $P = 0.001$; Table 1). Feathers grew in asynchronously (between February 15 and March 22) and thus experienced different lengths of exposure to the marine diet. Thus, we considered feathers separately and performed a separate test for differences in diet–tissue fractionation values for tissues with feathers removed from the analysis. For ^{13}C, we determined breast muscle had a higher and plasma a lower diet–tissue fractionation value than all other tissues (Table 1). Cellular fraction of blood, kidney, whole-blood, and liver ^{13}C diet–tissue fractionation factors were not different. For ^{15}N, we determined that kidney and liver diet–tissue discrimination factors were not different from each other but were different from those of all other tissues.

Although sample size was small ($n = 3$), there was higher variability in the feather diet–tissue discrimination factors (means: for $^{13}C = -1.6 \pm 1.3$‰, for $^{15}N = -2.8 \pm 2.1$‰). In one individual, feather diet–tissue discrimination values were positive (for ^{13}C, 2.2‰; for ^{15}N, 3.4‰); whereas in the other two individuals, they were negative (for ^{13}C, –2.5‰ and –3.5‰; for ^{15}N, –4.1‰ and –5.8‰).

Discussion

Isotopic signatures of whole tissues are composites of those of various macro- and micromolecular (e.g. amino acids, fatty acids) components making up those tissues. Turnover rates established for Dunlin whole blood represent integrations of turnover rates of plasma and cellular fractions of blood. Close agreement in turnover rates of both elements suggests close coupling between them, likely reflecting basic bulk-protein turnover versus any other macromolecular components. Although our results are restricted to male Dunlin, we have no reason to suspect that results would differ significantly for females. Nevertheless, potential sex differences in the incorporation of isotopes into tissues has not been adequately studied in birds and merits future investigation, especially with respect to reproductive status of females (Bearhop et al. 2002). On the basis of the $\delta^{13}C$ data, turnover time was slightly shorter than found for Japanese Quail whole blood (11.4 days; Hobson and Clark 1992a), but much shorter than the values derived from Canvasback and Great Skuas. Haramis et al. (2001) reported patterns of isotopic turnover for Canvasback switched from a homogeneous diet to baltic clams (*Macoma balthica*), wild celery tubers (*Vallisneria americana*), and a mixture of baltic clam and corn. With the exception of the tuber diet, half-lives for C and N were similar for each diet switch but differed among diets (clam diet: $^{13}C = 16.0$ days, $^{15}N = 17.8$ days; tuber diet: $^{13}C = 19.8$ days, $^{15}N = 25.4$ days; clam and corn diet: $^{13}C = 26.2$ days, $^{15}N = 26.5$ days).

Table 1. Mean stable isotope values and isotopic diet–tissue fractionation factors for captive Dunlin. All diet–tissue fractionation factors were calculated from control birds after 105 days on the terrestrial diet, except for plasma and cellular fraction of blood (day 88). Letter superscripts indicate results of tissue comparisons (ANOVA with Student Newman Keul's test comparisons). Shared letters indicate no significant differences between mean diet–tissue fractionation factors ($P > 0.05$). Standard error (SE) includes error associated with both diet samples and diet–tissue fractionation factors.

Tissue	Mean $\delta^{13}C$ (‰) ± SE	^{13}C diet–tissue fractionation factor (‰)	Mean $\delta^{15}N$ (‰) ± SE	^{15}N diet–tissue fractionation factor (‰)
Plasma	–24.1 ± 0.21	0.5^{A}	6.8 ± 0.16	3.3^{Y}
Breast muscle	–22.7 ± 0.12	1.9^{B}	6.6 ± 0.12	3.1^{Y}
Cellular fraction of blood	–23.1 ± 0.08	1.5^{C}	6.5 ± 0.12	3.0^{Y}
Whole blood	–23.4 ± 0.07	1.3^{C}	6.4 ± 0.16	2.9^{Y}
Kidney	–23.4 ± 0.12	1.3^{C}	7.5 ± 0.09	4.0^{Z}
Liver	–23.6 ± 0.21	1.1^{C}	7.4 ± 0.16	4.0^{Z}

(However, those results should be interpreted as estimates only because $n = 1$ for each diet.) Bearhop et al. (2002) similarly found half-lives of ^{13}C and ^{15}N in whole blood of captive Great Skuas to be 15.7 and 14.4 days, respectively.

For dietary protein, a macromolecule readily incorporated into body protein of the consumer, we can expect reasonably close coupling between patterns of turnover in carbon and nitrogen. However, for carbohydrates and fats, metabolic pathways for carbon and nitrogen can become decoupled (Hobson and Stirling 1997, Hobson et al. 2000). That may explain the difference in turnover patterns between carbon and nitrogen for Canvasback switched to the high-carbohydrate tuber diet (Haramis et al. 2001). Factors contributing to differences in half-life of C and N in whole blood also undoubtedly involve differences in overall metabolic rate, mediated to some degree by season. Small-bodied migratory shorebirds are expected to have higher elemental turnover rates in blood compared to larger bodied wintering Canvasback or captive skuas (Pearson et al. 2003, Hobson and Bairlein 2003). Within our group of experimental birds, we were unable to determine any structural or body condition factors contributing to the variance in elemental turnover rate among individuals.

The similarity in turnover rates for ^{15}N and ^{13}C in Dunlin is important because in interpreting dual isotope data collected from wild animals, patterns for those elements have been assumed to be similar (e.g. Drever et al. 2000). Asynchronous turnover patterns would make interpretation of diet more complex, because each isotope would reflect different periods. At least for Dunlin consuming high-protein diets, our study confirms that the assumption of synchronous patterns of turnover for ^{13}C and ^{15}N in whole blood is valid, supporting a widely used and important assumption behind techniques used to estimate dietary composition using information from multiple isotopes (Ben-David et al. 1997, Phillips and Gregg 2001).

Although our birds were housed in aviaries allowing for some flight, the extent to which they exercised was less than their wild counterparts. Thus, metabolic rates in our captive birds may have been lower than those for free-living Dunlin (e.g. Nagy 1987). That would make the turnover rates in our captive sample slower than those of their wild counterparts. Nevertheless, our study provides a useful first approximation of stable isotope turnover rates in wild Dunlin, and our results can likely be applied to other migratory shorebird species of similar size. Further studies to determine how variations in metabolic rate might influence isotopic turnover rates and how those are influenced by various life-history traits would be valuable.

Significant differences among different Dunlin tissues underlines the importance of testing diet–tissue fractionation factors experimentally to correctly interpret data collected from free-living birds. We advise caution in making assumptions based on previous studies of unrelated species or of birds confined in captivity without flight and on diets that may differ considerably in macromolecular composition. Between-tissue differences underline the importance of discriminating enrichment of tissues because of physiological effects from those due to diet in interpreting diet from isotopic values. Presence of lipids in samples will greatly influence ^{13}C diet–tissue fractionation factors (see Bearhop et al. 2002), making direct comparisons between studies that have and have not extracted lipids difficult.

Recent papers have used isotopic compositions of feathers to address ecological and biogeographical questions about migratory birds (e.g. Hobson and Wassenaar 2001, Rubenstein et al. 2002). That work assumes a close correspondence between dietary and feather isotopic composition that has rarely been directly assessed. It is not yet clear whether individuals mobilize endogenous resources that have been stored over the long term for feather production (e.g. Austin and Frederickson 1987), or whether individuals use resources directly sequestered from recent diet (e.g. Hobson and Clark 1992a). Indeed, some studies suggest that may be species-specific (e.g. Fox et al. 1999). In our study, the individual that molted later (22 March) had feather $\delta^{13}C$ values of –16.1‰ and $\delta^{15}N$ values of 17.2‰, which are closer to the dietary values of the marine diet; whereas two individuals that began molt earlier (15 February) had $\delta^{13}C$ values of –20.8‰ and –21.8‰, and $\delta^{15}N$ values of 9.6‰ and 7.9‰, which are more intermediate between the two diets. That suggests isotopic incorporation into feathers is closely linked to recent diet, corroborating a study of the feathers of captive American Crows (Hobson and Clark 1992a) in which the isotopic composition

of feathers tracked isotopic changes in diet during feather growth. However, we recognize the limitations of interpreting our small sample size of feathers, and that is an area that requires further investigation with a larger sample size of molting birds. Bearhop et al. (2002) suggest that isotopic composition of feathers may reflect use of endogenous stores if present diet is deficient in particular nutrients such as amino acids.

Our results suggest $\delta^{13}C$ and $\delta^{15}N$ analysis of blood samples taken from wild Dunlin provide a window into the diet over at least a 20 day period (i.e. two half-lives; Hobson and Clark, 1993). The diet–tissue discrimination factors we have established can now be used as correction factors to make tissues directly comparable (e.g. Hobson 1993). Our study broadens the range of species for which isotopic turnover rates and diet–tissue fractionation factors are known, providing the first such data for shorebirds.

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