Stable Carbon and Nitrogen Isotope Discrimination and Turnover in Pond Sliders *Trachemys scripta*: Insights for Trophic Study of Freshwater Turtles

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The ecologies of vertebrate species have been increasingly studied via stable isotope analyses of small quantities of body tissues. However, critical assumptions relating to the consistency in stable isotopic values in consumer tissues and their diet as well as the rate of incorporation of diet-derived stable isotopes into consumer tissues remain poorly validated for most taxa despite numerous stable isotope studies targeting natural systems. In this study, we measured stable carbon and nitrogen diet–tissue discrimination and elemental turnover in whole blood, red blood cells, blood plasma, brain, liver, pectoralis major muscle, pubioshiofemoralis internus muscle, stratum corneum (sc) dermis, and stratum germinativum (sg) dermis of Pond Sliders (*Trachemys scripta*) fed *ad libitum* on two isotopically distinct diets. Turtles were fed a soy-based control diet (32% protein, 3% lipids, 4% fiber), and after 146 d, a subset was switched to a fish meal-based experimental diet (44% protein, 24% lipids, 3% fiber). At the diet switch, diet–tissue isotopic equilibrium could not be confirmed by $\delta^{13}C$ values for any tissue, but was established for $\delta^{15}N$ values for all tissues except sc dermis and sg dermis (range = +1.9 to +3.8‰). An exponential-decay regression model and half-life calculation resulted in half-lives for N ranging from 35.6 d (blood plasma) to 52.5 d (liver). These represent the first published stable isotope turnover rates for reptiles and the first diet–tissue isotopic discrimination factors for freshwater turtles. The discrimination factors found here are similar to those established for soft-tissues among other vertebrate taxa; however, elemental nitrogen turnover rates in tissues of *T. scripta* are among the slowest established to date among vertebrate taxa.

S TABLE carbon and nitrogen isotope analysis has become a common method for determining the trophic status and origins of nutrient resources for wildlife populations. Dietary inferences based on stable isotopic profiles in animal tissues are possible because the isotope compositions of a consumer's body tissues are ultimately derived from those in its diet (DeNiro and Epstein, 1978, 1981; Hobson and Clark, 1992a). Within the Reptilia, these examinations have centered primarily on sea turtles (Godley et al., 1998; Hatase et al., 2002; Biasatti, 2004; Wallace et al., 2006), although lizards have been examined to a lesser extent (Magnusson et al., 2001; Struck et al., 2002). However, the large species richness and dietary diversity in reptiles suggest that stable isotope analyses can be applied broadly among reptiles to elucidate their position within a variety of trophic pathways. Further, the small body size of many reptile species limits the collection of stomach and/or fecal samples such that stable isotope analyses of small quantities of skin or other tissues may be the only non-euthanasia technique available for determining their diet strategies.

Stable isotope ratios for carbon ($^{13}C/^{12}C$, expressed as $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, expressed as $\delta^{15}N$) of consumer and prey tissues are usually not identical, and instead exhibit predictable differences due to selectivity for lighter isotopes during a consumer's metabolic processes (DeNiro and Epstein, 1978, 1981). These differences, caused by isotopic discrimination (Farquhar et al., 1982), vary among tissues and taxa, but are often between 0‰ to +1‰ for $\delta^{13}C$, and +3‰ to +5‰ for $\delta^{15}N$ per trophic level for soft tissues (DeNiro and Epstein, 1978, 1981; Miniwaga and Wada, 1984; Peterson and Fry, 1987). Thus $\delta^{13}C$ values more effectively describe different carbon sources (DeNiro and Epstein, 1978; Peterson and Fry, 1987; Rubenstein and Hobson, 2004), whereas $\delta^{15}N$ values are useful for identification of trophic level or trophic structure of the organism or system of interest (Miniwaga and Wada, 1984; Peterson and Fry, 1987).

Incorporation of diet-derived stable isotopic signatures into consumer body tissues occurs at varying rates based primarily on tissue-specific metabolism (Gannes et al., 1997). As a result, different tissues may provide dietary information that is integrated over different time scales. Tissues with higher metabolic activity (liver, whole blood) will reflect more recent diet history, and those with lower metabolism (integument, bone) will represent an integration of diet over a substantially longer period, perhaps approaching the full life of the consumer

(Tieszen et al., 1983; Hobson and Clark, 1992b). Stable isotope analyses of multiple tissues can therefore reveal temporal diet shifts by consumers. By providing information on nutrients assimilated over extended periods, this technique is much less affected by short-term temporal change in diet than conventional dietary analyses (i.e., stomach content analysis, fecal analysis), which only provide dietary 'snapshots' of recently consumed food items.

Knowledge of the patterns of stable isotope incorporation into body tissues of wildlife species is required to interpret field data correctly. Controlled laboratory studies of isotopic discrimination and elemental turnover for reptiles have focused on sea turtles (Seminoff et al., 2006; K. Reich, unpubl. data), and there have been no examinations of these aspects in freshwater turtles. Considering the osmoregulatory differences between marine and freshwater turtles (Bentley, 1976; Lutz, 1997), and the substantial variability in isotopic discrimination and turnover reported within other taxa, determining these aspects for freshwater turtles is required before stable isotopes can be used reliably for dietary reconstructions within this clade.

In this study, we measured stable carbon and nitrogen isotopic diet–tissue discrimination and turnover in body tissues of an emydid turtle, the Pond Slider (*Trachemys scripta*), which is known to eat both plant and animal matter in the wild. We chose this omnivorous species due to its hardiness in captivity, its variable diets, and the extensive information on the biology of this species (Gibbons, 1990). The use of stable isotopes to answer ecological questions about freshwater turtles will increase. Species- and diet-specific discrimination factors and turnover rates will allow accurate interpretations of the data generated from these investigations.

Materials and Methods

Thirty *Trachemys scripta* individuals were captured from ponds at the Savannah River Ecological Laboratory (Aiken, South Carolina) and housed at the animal vivaria at the Department of Zoology, University of Florida (Gainesville) from October 2001 to September 2002. A unique number applied with acrylic paint on the posterior-most central scute identified each turtle. Turtles were maintained in indoor tanks with a water depth of 20 cm and a basking platform. The enclosures were kept at 26 C and lighted for 12 h each day with a 20-W full spectrum natural fluorescent bulb (Vita-Lite) and a 60-W outdoor floodlight for basking. Study animals included only large immature and adult

males (mean initial curved carapace length = 18.8 ± 0.4 cm, range = 15.5–24.0 cm) to minimize potential effects of body size, growth, and reproduction on isotope values.

We carried out a diet-switch experiment in which turtles were fed *ad libitum* on two commercial turtle pellet foods (Melick Aquafeed, Catawissa, PA). Turtles were initially maintained on a control diet (Diet A) having soy meal as the primary protein source (32% protein, 3% lipids, 4% fiber); a subset of turtles was switched to an experimental diet (Diet B) that had fish meal as the primary protein source (44% protein, 24% lipids, 3% fiber). Both diets were from single commercial batches. Analysis of variance (ANOVA) tests demonstrated that lipid-free diet $\delta^{13}C$ and $\delta^{15}N$ values remained constant throughout the course of this study for both Diet A ($F_{1,7} = 0.629$, $P_{Carbon} = 0.63$, $n = 8$; $F_{1,7} = 0.011$, $P_{Nitrogen} = 0.92$, $n = 8$) and Diet B ($F_{1,5} = 0.62$, $P_{Carbon} = 0.47$, $n = 6$; $F_{1,5} = 0.68$, $P_{Nitrogen} = 0.45$, $n = 6$). Stable carbon and nitrogen isotope values of lipid-free control and experimental diets differed by 3.3‰ and 5.7‰, respectively (Table 1). These differences were considered to be of sufficient magnitude to monitor isotopic changes in body tissues of the turtles in response to a dietary shift from Diet A to Diet B.

All turtles underwent a 146-day acclimation period on Diet A to equilibrate the stable isotope composition of soft tissues in all turtles before the switch to Diet B. Turtles were then assigned to a control group ($n = 9$) and an experimental group ($n = 21$). Control turtles were maintained on Diet A, and five of those turtles were randomly selected and humanely euthanized with an injection of sodium pentobarbital (130 mg/kg of turtle) at the start of the treatment (Day 146) and the remaining four were euthanized at the end (Day 338). From the experimental group, three turtles were randomly selected and euthanized after maintenance on Diet B for each of 4, 8, 16, 32, 64, 128, and 192 d. To reduce the effects of body size, one turtle from each of three stratified size groups was randomly selected for each sampling period (mean body mass of turtles remained consistent among all sampling periods: $F_{7,25} = 0.297$, $P = 0.95$). Prior to sacrifice, ca. 0.25 ml of blood was collected from each turtle via the brachial artery with a 21-gauge needle and syringe and transferred to a non-heparinized container (Frische et al., 2001). Approximately half of each blood sample was promptly separated into plasma and cellular components by centrifugation. We then collected 0.25–2.0 g (wet mass) of brain, liver, pectoralis major (pm) muscle, pubioshiofemoralis internus (pi)

TABLE 1.   STABLE ISOTOPE VALUES (δ^{13}C, δ^{15}N) FOR DIETS USED IN THE STUDY OF *Trachemys scripta*. The control diet (Diet A) consisted of a commercial pellet food with soy as the primary protein source (32% protein, 3% lipids, 4% fiber), and the experimental diet (Diet B) consisted of a commercial pellet food with herring (fish) meal as the primary protein source (44% protein, 24% lipids, 3% fiber).

Diet	δ^{13}C	n	δ^{15}N	n
Diet A with lipids	-23.5 ± 0.8	3	$+4.8 \pm 0.5$	3
Diet A without lipids	-22.8 ± 0.3	8	$+4.9 \pm 0.2$	8
Diet B with lipids	-21.9 ± 0.3	3	$+10.3 \pm 0.7$	3
Diet B without lipids	-19.5 ± 0.3	6	$+10.6 \pm 0.4$	6
Mean difference (Diet B-A with lipids)	1.6 ± 0.3		5.5 ± 0.6	
Mean difference (Diet B-A without lipids)	3.3 ± 0.4		5.7 ± 0.5	

muscle, stratum corneum (sc) dermis, and stratum germinativum (sg) dermis.

To track the potentially faster isotopic turnover in blood components as reported in previous stable isotope turnover studies (Tieszen et al., 1983; Pearson et al., 2003), we collected blood from additional turtles in the experimental group after 2, 6, 10, 12, 14, 20, 24, 28, 48, and 96 d on Diet B. Four turtles were sampled during each blood collection period (i.e., on sacrifice days, blood was collected from only one additional non-sacrificed turtle, while on non-sacrifice days, blood was collected from four live turtles). To avoid excessive stress to the turtles, we rotated subjects so that blood sampling occurred a maximum of once every eight days per individual.

Sample preparation and analysis.—Blood samples (whole blood, red blood cells, plasma) were dried at 60 C for 24 h. All other tissues were rinsed with distilled water, dried at 60 C for 48 h, then powdered with a mortar and pestle. Lipids were removed from diet samples and all tissues except blood using a Soxhlet apparatus with a 1:1 solvent mixture of petroleum ether and ethyl ether for at least two 10-hour cycles and then dried at 60 C for 24 h to remove any residual solvent. Approximately 0.50 mg of all food and tissue samples were loaded into precleaned tin capsules and analyzed by a continuous-flow isotope-ratio mass spectrometer in the Stable Isotope Laboratory at the Department of Geosciences, University of Florida. We used a Costech ECS 4010 elemental combustion system interfaced via a ConFlo III device (Finnigan MAT, Bremen, Germany) to a Deltaplus gas isotope-ratio mass spectrometer (Finnigan MAT). Stable isotope ratios are expressed in delta (δ) notation, defined as the parts per thousand (‰) deviation of the ratio of heavy to light isotope in a sample from the sample ratio in a standard material:

$$\delta^{13}C = ([R_{sample}/R_{standard}] - 1)(1000)$$

$$\delta^{15}N = ([R_{sample}/R_{standard}] - 1)(1000),$$

where R_{sample} and $R_{standard}$ are the corresponding ratios of heavy to light isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in the sample and standard, respectively. $R_{standard}$ for ^{13}C was IAEA CH-6 ($\delta^{13}C = -10.4$) normalized monthly against the Vienna Peedee Belemnite (V-PDB) limestone formation international standard (Gonfiantini et al., 1995); $R_{standard}$ for ^{15}N was IAEA N1 Ammonium Sulfate $((NH_4)_2SO_4$; $\delta^{15}N = +0.4$) normalized against atmospheric N_2 and USGS Nitrogen standards. All analytical runs included samples of standard materials inserted at regular intervals to calibrate the system and evaluate any drift over time. Hundreds of replicate assays of standard materials indicated measurement errors of 0.05‰ and 0.095‰ for carbon and nitrogen, respectively.

Statistical analyses.—We concluded that stable isotope compositions of diet and tissues of the control turtles maintained on Diet A were at equilibrium by Day 146 if $\delta^{13}C$ and $\delta^{15}N$ values of tissues were not significantly different (Student's t-test) between Day 146 ($n = 5$) and Day 338 ($n = 4$). Stable carbon and nitrogen isotopic diet–tissue discrimination (Δ_{dt}) in these tissues were calculated as $\Delta_{dt} = \delta_{tissue} - \delta_{diet}$, where δ_{tissue} and δ_{diet} represent the mean stable isotope ratios of each tissue on Day 338 and diet, respectively. We also used this equation to calculate Δ_{dt} for Diet B in tissues with isotopic changes that fit the exponential decay model (see below). For tissues that underwent lipid removal, we determined Δ_{dt} using $\delta_{diet\ w/o\ lipids}$, whereas for tissues that did not have lipids removed (blood fractions), we determined Δ_{dt} using $\delta_{diet\ w/\ lipids}$ (Table 1). We used post hoc Tukey-Kramer honestly significant difference (HSD) tests for pair-wise comparisons

to reveal significant differences in Δ_{dt} among tissues.

To determine the duration for isotope turnover subsequent to the diet shift, we fitted an exponential-decay curve of the form $y = a + be^{ct}$ (Hobson and Clark, 1992b) to the individual $\delta^{13}C$ and $\delta^{15}N$ values for each tissue during each sampling period using non-linear regression. Due to unexpectedly slow elemental turnover, we omitted the 'blood-only' sample periods from Day 2 to Day 28 (Fig. 1). We excluded one turtle from all analyses because its $\delta^{13}C$ and $\delta^{15}N$ signatures were statistical outliers, most likely related to a unique dietary history prior to its inclusion in this study. In the exponential decay equation, y equals $\delta^{13}C$ or $\delta^{15}N$ at time t, a represents the value being approached asymptotically, b equals the overall change in isotopic composition after the diet is switched, and c, which is the component we solved for, is the fractional turnover of the respective isotopes in each tissue (Hobson and Clark, 1992b). Pseudo-replication was likely not a factor since all tissues except blood came from different turtles, and blood was sampled infrequently from any one turtle.

Turnover rates are expressed in terms of half-life ($t_{1/2}$). To estimate the half-life ($t_{1/2}$) from fractional turnover (c) we used the equation $t_{1/2} = \ln(0.5)/c$, where $t_{1/2}$ is the time in days in which half of the stable-carbon and -nitrogen isotopes were exchanged in the corresponding tissue, and 0.5 represents the exchange of 50% of the isotopes. We fit the exponential curves with Sigma Plot (Systat, Pt. Richmond, CA); all other calculations were performed using JMP software (SAS, Belmont, NY). Means are followed by standard error ($\pm$ SE) unless otherwise noted.

RESULTS

Nutritional intake and body mass changes.—We observed feeding by all turtles throughout the study; however, some turtles tended to forage less voraciously for a short period (<7 d) after the diet shift. Body mass did not decrease in any turtle, although the frequency of measurement (once every 2 mo) may have been insufficient to reflect any short-term mass decreases. The mean final body mass of turtles (868 ± 60 g, range = 485–1648 g, $n = 29$) was significantly greater than the mean body mass on Day 0 (842 ± 58 g, range = 442–1551, $n = 29$; paired t-test, $t = -3.89$, $P < 0.01$), indicating that growth may have affected the stable isotope compositions of at least some turtles. No turtle showed a net decrease between their initial and final mass.
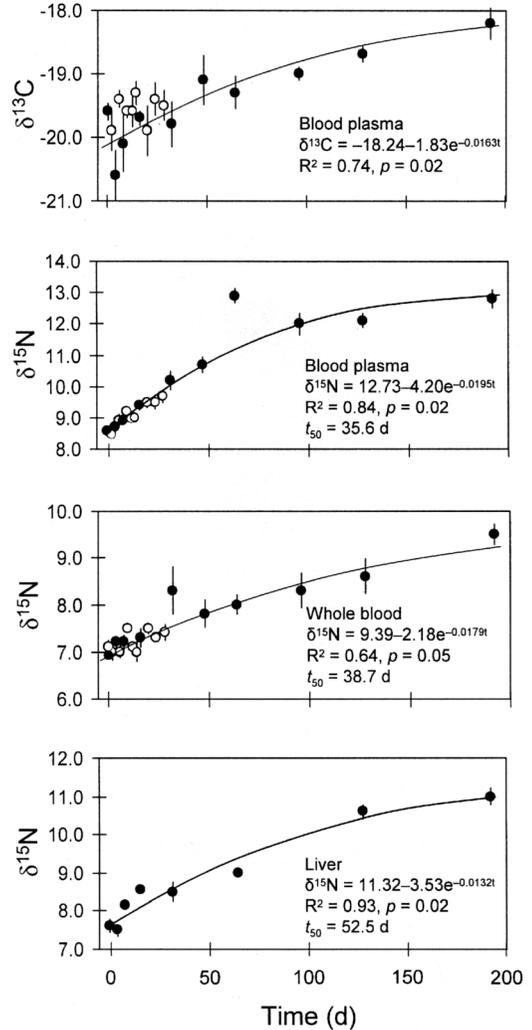
Fig. 1. Stable carbon values of blood plasma, and stable nitrogen values of blood plasma, whole blood, and liver for *Trachemys scripta* as a function of time since their diet was switched from the control to the experimental diet. Data are means (solid circles) $\pm$ SE (vertical lines), and sample sizes are $n = 4$ for each point except Day 0 ($n = 5$), Day 6 ($n = 3$), and Day 16 ($n = 3$). Exponential-decay regression lines of the form $y = a + be^{ct}$ (Hobson and Clark, 1992b) were fitted to individual values for each sample period. Half-life ($t_{1/2}$) is calculated as $t_{1/2} = \ln(0.5)/c$. Open circles represent sample means excluded from model fitting (see text).

Isotope discrimination.—The $\delta^{13}C$ and $\delta^{15}N$ values for soft tissues of *T. scripta* reared on Diet A are presented in Table 2. Among the nine tissues examined, tissue-specific mean $\delta^{13}C$ values ranged from -20.0 ± 0.3‰ to -18.0 ± 1.6‰ on Day 146, and from -21.0 ± 0.4‰ to -18.9 ± 0.3‰ on Day

TABLE 2.   MEAN STABLE ISOTOPIC SIGNATURES FOR *Trachemys scripta* TISSUES FROM THE CONTROL GROUP (MAINTAINED ON DIET A) ON DAY 146 ($n = 5$) AND DAY 338 ($n = 4$). T-statistics and *P*-values compare isotopic values at Day 146 vs. Day 338. We concluded that tissues remaining isotopically unchanged between these two periods were at equilibrium with Diet A at Day 146, except sc dermis and sg dermis (see text).

Tissue	Carbon (δ^{13}C)				Nitrogen (δ^{15}N)			
	Day 146 (‰)	Day 338 (‰)	t-statistic	*P*-value	Day 146 (‰)	Day 338 (‰)	t-statistic	*P*-value
whole blood	-19.2 ± 0.1	-20.1 ± 0.2	4.41	<0.01	$+7.1 \pm 0.1$	$+7.0 \pm 0.2$	0.22	0.42
red blood cells	-18.7 ± 0.2	-19.9 ± 0.3	3.91	<0.01	$+6.7 \pm 0.2$	$+6.7 \pm 0.3$	0.19	0.43
blood plasma	-19.6 ± 0.1	-20.2 ± 0.1	3.22	<0.01	$+8.6 \pm 0.1$	$+8.6 \pm 0.2$	-0.16	0.44
brain	-18.4 ± 0.1	-18.9 ± 0.3	2.68	0.03	$+8.0 \pm 0.3$	$+7.8 \pm 0.2$	0.49	0.32
liver	-20.0 ± 0.3	-21.0 ± 0.4	2.08	0.04	$+7.6 \pm 0.2$	$+7.9 \pm 0.2$	-1.12	0.15
pm muscle	-19.0 ± 0.2	-19.7 ± 0.3	2.18	0.04	$+7.7 \pm 0.3$	$+7.6 \pm 0.2$	0.15	0.44
pi muscle	-18.8 ± 0.3	-19.5 ± 0.4	2.20	0.03	$+8.4 \pm 0.3$	$+8.3 \pm 0.4$	0.18	0.43
sc dermis	-18.9 ± 0.9	-20.1 ± 0.4	0.99	0.18	$+9.8 \pm 0.4$	$+8.4 \pm 0.4$	1.82	0.06
sg dermis	-18.0 ± 1.6	-19.9 ± 0.5	0.86	0.21	$+11.6 \pm 0.7$	$+9.3 \pm 0.6$	2.19	0.03

338. Mean δ^{15}N values ranged from $+6.7 \pm 0.2$‰ to $+11.6 \pm 0.7$‰ on Day 146, and from $+6.7 \pm 0.3$‰ to $+9.3 \pm 0.6$‰ on Day 338. Whereas δ^{13}C comparisons between Day 146 and Day 338 suggest that sc dermis and sg dermis achieved isotopic equilibrium with Diet A prior to the diet switch, comparisons of the δ^{15}N values indicate that all tissues except sg dermis were in isotopic equilibrium with Diet A (Table 3). Concerning sg dermis and sc dermis, although the Student's *t*-tests are indicative of diet–tissue isotopic equilibrium, the large variance in the isotope values and marginal non-significance for these tissues suggest a large potential for Type II error (Table 3). The slower turnover of dermal tissues after the diet switch further suggests that these tissues would be the last of any tissues examined to reach isotopic equilibrium. Based on these considerations, we do not report Diet A Δ_{dt} values for either of the dermal tissues. Thus, the Δ_{dt} factors for turtles reared on Diet A are presented for nitrogen only (Table 3). All seven tissues were significantly ^{15}N-enriched relative to

Diet A, but whole blood and red blood cells were significantly less enriched than the other tissues, and blood plasma was significantly more enriched than all but pi muscle (Tukey HSD, $P < 0.01$; Table 3).

The lack of apparent δ^{13}C-equilibration between *T. scripta* tissues and Diet B precluded the determination Diet B Δ_{dt} factors for carbon. Thus, Diet B Δ_{dt} factors are only calculated for nitrogen in those tissues that showed a significant fit to the exponential decay model (i.e., blood plasma, whole blood, and liver; Table 3). Blood plasma was significantly more enriched in ^{15}N relative to whole blood and liver (Tukey HSD, $P < 0.01$; Table 3). These Diet B Δ_{dt} values for nitrogen should be viewed with caution since asymptotes in the exponential decay curves were not clearly reached prior to the close of the study.

Isotope turnover rates.—Although not all tissues had clearly equilibrated with Diet A by the end of the initial phase of this study, most showed a shift

TABLE 3.   STABLE-NITROGEN ISOTOPE DISCRIMINATION FACTORS AND ELEMENTAL HALF-LIFE ESTIMATES FOR SOFT TISSUES OF *Trachemys scripta*. See Table 1 for description of diets. Results of the Tukey HSD test are indicated by superscripts, where means having at least one superscript in common are not significantly different.

Tissue	Δ_{dt} (‰)		$t_{1/2}$ (days) Diet B
	Diet A	Diet B	
whole blood	$+2.2 \pm 0.2$[a]	-0.8 ± 0.8[a]	38.7
red blood cells	$+1.9 \pm 0.3$[a]	—	—
blood plasma	$+3.8 \pm 0.1$[b]	$+2.5 \pm 0.8$[b]	35.6
liver	$+3.0 \pm 0.3$[c]	$+0.4 \pm 0.5$[a]	52.5
brain	$+2.9 \pm 0.3$[c]	—	—
pm muscle	$+2.7 \pm 0.3$[c]	—	—
pi muscle	$+3.4 \pm 0.4$[b]	—	—

toward the δ^{13}C and δ^{15}N signatures of Diet B after the diet switch. The exponential decay models showed a significant fit for carbon in blood plasma, and nitrogen in blood plasma, whole blood, and liver (non-linear regression, P < 0.05; Fig. 1). For carbon, however, because blood plasma had not reached isotopic equilibrium with Diet A during the acclimation period, we have low confidence in the exponential decay model results and therefore do not report the $t_{1/2}^{13}$C for this tissue. For nitrogen, the half-life durations in blood plasma, whole blood, and liver were 35.6 d, 38.7 d, and 52.5 d, respectively (Fig. 1; Table 3). Because turnover is reached after approximately four half-lives (Hobson, 1993; Martínez del Rio and Wolf, 2005), we estimate that nitrogen turnover times in blood plasma, whole blood, and liver are 142 d, 155 d, and 210 d, respectively. Although we did not estimate $t_{1/2}^{15}$N for the remaining tissues due to their lack of significant curve fits, the fact that stable-nitrogen changes did not show a significant fit to the exponential decay model indicates that the turnover times must be greater than 192 days. Furthermore, the apparent lack of stable carbon isotope diet–tissue equilibrium suggests that carbon turnover time is longer than 146 days, the duration of the acclimation period.

DISCUSSION

Measuring stable isotope discrimination and elemental turnover in emydid turtles is a necessary process to enable the correct interpretation of field data generated for this group. In this study we provide the first stable isotope information for *Trachemys scripta*, one of the most abundant and widespread of all freshwater turtles (Gibbons, 1990). Although reliable stable carbon isotope values proved elusive, we were able to estimate nitrogen isotope discrimination and turnover values for *T. scripta* tissues that reached isotopic equilibrium with the experimental diets.

Of the seven tissues for which we calculated Diet A Δ_{dt}^{15}N (range = +1.9‰ to +3.8‰), all were within the range of values established for other vertebrate taxa. In soft-tissues of birds and mammals, Δ_{dt}^{15}N factors range from +0.2‰ to +5.2‰ (Hobson and Clark, 1992a; Kurle, 2002; Klaassen et al., 2004), whereas those for fish are +2.3‰ to +5.0‰ (Hesslein et al., 1993; Pinnegar and Polunin, 1999). For the Green Sea Turtle, *Chelonia mydas*, Δ_{dt}^{15}N factors range from +0.22‰ to +2.92‰ (Seminoff et al., 2006). In addition to the consistencies in the overall range of discrimination, we found similarities in the relative discrimination levels between tissues of *T. scripta* with homologous tissues of other vertebrates. For

example, the pattern of greater Δ_{dt}^{15}N in *T. scripta* blood plasma (+3.8‰) versus red blood cells (+1.9‰) was also found in a variety of bird (Pearson et al., 2003; Evans Ogden et al., 2004) and mammal species (Roth and Hobson, 2000; Lesage et al., 2002; Klaassen et al., 2004).

The observed Δ_{dt}^{15}N differences among the tissues of *T. scripta* and in tissues of other species likely were caused, at least in part, by variations in the protein and amino acid compositions of different tissues since these components can differ in their nitrogen isotope content (Peterson and Fry, 1987). As suggested previously, the compositional differences may result from selective routing of exogenous nutrients during tissue maintenance and construction, and from differential mobilization of endogenous resources into tissues or tissue components (Macrae and Reeds, 1980; Peterson and Fry, 1987; Gannes et al., 1997). In addition, loss of heavier or lighter isotopes via cellular metabolism may have played a role in the differences among tissues (Gannes et al., 1997; Martínez del Rio and Wolf, 2005). However, we are unable to ascertain the relative contributions of these factors to our results because of the unknown isotopic differences among dietary nutrients and body tissue components. Elucidating the relationships between an animal's diet and the fate of assimilated dietary components, synthesized components, and endogenous nutrients will be important to fully determine the biochemical mechanisms of isotope discrimination.

In addition to stable-nitrogen isotope discrimination, we derived estimates of elemental nitrogen turnover in three *T. scripta* soft tissues that showed a significant fit to the model curve. The half-lives found here (35.6–52.5 d) are among the longest reported to date, and often an order of magnitude greater than those of homologous tissues in other vertebrate species. For example, half-lives among birds and mammals range from 1.7 d to 3.5 d in blood plasma (Hilderbrand et al., 1996; Pearson et al., 2003) and from 0.5 d to 15.7 d in whole blood (Bearhop et al., 2002; Hobson and Bairlein, 2003; Pearson et al., 2003; Evans Ogden et al., 2004). Although the extended longevity of nucleated red blood cells in *T. scripta* (≤11 mo, Frische et al., 2001) likely affected the N half-life in whole blood, we believe the greater N half-lives in *T. scripta* result from the lower basal metabolism and correspondingly slower protein turnover in poikilotherms versus homeotherms (Bennett and Dawson, 1976; Else and Hulbert, 1981; McNab, 2002). In support, Martínez del Rio and Wolf (2005) suggest that fractional isotopic incorporation into body tissues is roughly pro-

portional to protein turnover and that slow metabolism may thereby reduce the uptake of endogenous protein components for tissue maintenance and growth.

With respect to carbon, our derivations of discrimination and half-life were hampered by a lack of apparent isotopic equilibrium between turtle tissues and captive diets and the poor fits of the exponential decay model. The apparent low rates of diet-derived carbon integration into body tissues is intriguing, particularly for tissues of high metabolic activity (blood plasma, liver). Assuming that consumer tissues maintain steady state isotopic equilibrium with their diet, perhaps the feeding trials were of insufficient duration to allow for $\delta^{13}C$ equilibration of *T. scripta* tissues with the captive diets. However, although no tissues equilibrated with the carbon isotope composition of the diet, the trial duration was sufficient for the integration of Diet A $\delta^{15}N$ values into most tissues. This dichotomy suggests that carbon and nitrogen may become decoupled during metabolic processes and raises interesting questions about the metabolic pathways responsible for uptake of exogenous carbon and nitrogen. Interestingly, Hobson and Stirling (1997) report a lack of integration of dietary carbon into body tissues of polar bears (*Ursus maritimus*), and Voigt et al. (2003) report dramatically slow incorporation of dietary carbon into tissues of two nectar-feeding bat species. However, there is little information on the uncoupling of elemental carbon and nitrogen during metabolic processes, nor are there sufficient data on the relative input of endogenous versus exogenous carbon and nitrogen to adequately interpret these results.

The lack of apparent changes in the $\delta^{13}C$ values of *T. scripta* tissues after the diet switch may also relate to composition of the experimental diets. Perhaps the differences in $\delta^{13}C$ values between Diet A and Diet B were not of sufficient magnitude to adequately measure turnover (1.6‰ for Diet B-A with lipids; 3.3‰ for Diet B-A without lipids). This possibility is particularly relevant considering the relatively high variance in $\delta^{13}C$ values among tissues for each sampling period (Fig. 1). However, a more likely scenario relates to the high lipid concentration in Diet B. Because lipids are depleted in ^{13}C relative to protein and carbohydrates (DeNiro and Epstein, 1978; Peterson and Fry, 1987), differential integration of these dietary macromolecules into body tissues may result in isotope discrimination values that are unexpected based on the isotopic compositions of whole diet. In this case, despite the overall lesser ^{13}C-depletion

in Diet B ($\delta^{13}C = -21.9$‰) versus Diet A ($\delta^{13}C = -23.5$‰), the extremely high lipid content of Diet B (24%) may have resulted in tissue carbon reservoirs that were more depleted in ^{13}C, the result of which may be tissue $\delta^{13}C$ values that approach the expected values for turtles maintained on Diet A. Thus, dietary carbon may have been integrated but masked by lipid isotopic compositions. As with previous studies employing stable isotope analysis, our interpretations of carbon and nitrogen turnover (or lack thereof) would benefit greatly from a better understanding of animal nutritional ecology, particularly relating to lipid assimilation (Stott et al., 1997; Schlechtriem et al., 2004).

The results of this study include two important findings that bear on the interpretation of field isotopic data for assessing trophic ecology of freshwater turtles. First, although $\Delta_{dt}^{15}N$ in the species is generally consistent with discrimination factors established previously for homeothermic and poikilothermic vertebrates, the range in values (±1.9‰ for Diet A and ±3.3‰ for Diet B) has substantial consequences on the calculation of trophic structure, nutrient sources, and diet composition. Recall that consumer tissues are generally thought to be enriched in ^{15}N over their diet by 3–5‰ (DeNiro and Epstein, 1981; Miniwaga and Wada, 1984; Peterson and Fry, 1987). With discrimination variability >3‰, our results indicate that calculation of trophic positions could be erroneous by up to a full trophic step if the correct tissue-specific Δ_{dt} value is not used. This underscores the importance of elucidating the isotope discrimination factors for each tissue type prior to its use for making inferences about the trophic status of study organisms. Second, the turnover of blood and liver nitrogen is substantially slower than values reported previously for most taxa. Our results indicate that the temporal diet histories of *T. scripta* reflected by isotopic analyses of tissues will be of long term nature, ranging from ~5 to 7 months. While this supports the value of using stable isotopes to monitor dietary changes over the course of a year or more, it indicates that this technique is not appropriate for addressing intra- and inter-seasonal variability in diet intake. However, isotopic discrimination and turnover in *T. scripta* may vary under different environmental circumstances, particularly for turtles that are nutrient limited (Hobson et al., 1993). Field metabolic rates in wildlife species are often substantially higher than metabolic rates of captive animals (McNab, 2002), and it is thus possible that tissue components in wild turtles turnover more rapidly than those in captivity. Based on these considerations, we encourage additional studies of *T. scripta*

reared in different settings to elucidate the effects of diet type, food availability, growth rate, and metabolic rate. Inquiries into the stable isotope diet–tissue discrimination and turnover in additional reptile species should also be undertaken to provide a better foundation for interpreting results of future isotopic studies in this diverse taxon.

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