Tissue Carbon Incorporation Rates and Diet-to-Tissue Discrimination in Ectotherms: Tortoises Are Really Slow

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ABSTRACT

Understanding carbon incorporation rates and diet-to-tissue discrimination ($\Delta^{13}C_{tissue\text{-}diet}$) in animals is necessary to interpret stable isotope data collected from animals in the field. Our current understanding of the carbon dynamics in terrestrial ectotherms such as snakes, lizards, and turtles is poorly developed. Here we use a diet switch experiment to estimate carbon incorporation rates and diet-to-tissue discrimination factors in growing desert tortoises (*Gopherus agassizii*). Average carbon retention times for red blood cells (RBCs) and plasma were 126.7 ± 40.3 and 32.9 ± 14.5 days, respectively. Tissue carbon incorporation rates were affected by both growth and metabolism, with growth accounting for 50% of the carbon turnover in RBCs and 13% of carbon turnover in plasma. At equilibrium, scute keratin (0.8 ± 0.1) and plasma (1.0 ± 0.2) showed enriched discrimination values ($\Delta^{13}C$) compared to the test diet, but RBC $\Delta^{13}C$ values were indistinguishable from diet (0.2 ± 0.3). We also found that new keratin continued to contribute significant material to previously grown keratin rings on the tortoise's shell. Changes in the $\delta^{13}C$ of previously laid down growth rings indicated that the old rings closest to the region of new growth received about 73% of the carbon from the current diet; these data suggest that the interpretation of dietary history using growth rings must recognize that each ring may represent the weighted average of the diet over several seasons. These results continue to highlight the importance of laboratory experiments in interpreting isotopic data derived from field studies.

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Introduction

Stable isotopes of carbon ($\delta^{13}C$) are routinely used as a tracer for estimating the movement of energy through consumers and their different tissue pools (DeNiro and Epstein 1978; Hobson and Clark 1992; Michener and Schell 1994). The robust interpretation of these data depends on an understanding of the tissue carbon dynamics (i.e., carbon incorporation rates and discrimination factors) in the consumers of interest. The current literature focuses on terrestrial endotherms (birds and mammals) and fishes, with many fewer studies describing these processes in reptiles and amphibians (Dalerum and Angerbjorn 2005; Warne et al. 2010). Differences in the physiology, thermal biology, and habitats used by amphibians and reptiles preclude simply transferring what we know about carbon dynamics of other vertebrates to these groups (Zug 1993; Pough et al. 2004). As a consequence, there are significant gaps in our knowledge of tissue carbon dynamics for many taxa, and these data are needed to provide confidence limits on stable isotope data obtained from field studies (Gannes et al. 1997; Martínez del Rio et al. 2009; Warne et al. 2010). Recent studies of tissue carbon dynamics provide a starting point for understanding how the carbon turnover dynamics of reptiles differ from those of other vertebrates. Warne et al. (2010) have reported on these processes in lizards, Fisk et al. (2009) on juvenile snakes, and Reich et al. (2008) and Seminoff et al. (2006, 2007) on aquatic turtles, but to our knowledge there is no published information available describing these processes in any terrestrial turtle species. Tortoises are expected to differ from other terrestrial ectotherms, such as lizards, because they are long-lived and attain relatively large sizes compared to most lizards. Here, we present tissue carbon retention times and discrimination factors from a diet switch experiment in growing juvenile desert tortoises *Gopherus agassizii*. Desert tortoises are herbivorous reptiles occupying arid regions of the southwestern United States and Mexico characterized by plant communities with carbon isotope values of −32.3‰ to −12.0‰ Vienna Pee Dee belemnite standard (hereafter VPDB; $n = 94$ Sonoran Desert plant species; I. W. Murray and B. O. Wolf, unpublished data). Many past studies have used organisms at or near their adult size, where the only driver of carbon incorporation rates is catabolic tissue turnover. However, we argue that it is important to examine the influence of growth and catabolism because both of these processes are biologically relevant to our understanding of stable isotope tissue kinetics in ecological systems.

Material and Methods

We obtained seven captive-bred hatchling desert tortoises while they were hibernating during their first winter after hatching.

Tortoises were maintained indoors in a stock tank (Rubbermaid model 4243) in the University of New Mexico Biology Department. The vertebral scutes of individual tortoises were marked with unique numbers using a permanent marker. Animals lived on a substrate of gravel, and simulated solar radiation/heat was provided by 100-W heat lamps and ZooMed UVB 10.0 fluorescent bulbs to maintain a diurnal temperature gradient within the enclosure that ranged from 29° to 39°C (mean diurnal body temperature; 32.3° ± 0.6°C). A 14L : 10D photoperiod was maintained, and animals were kept at normal activity temperatures and fed throughout the year. Tortoises were fed and watered daily. Tortoises were weighed (Ohaus model V31XH2; ±0.1 g) and measured (straight carapace length ± 1.0 mm) every 30 d. The project was approved by the University of New Mexico's Institutional Animal Care and Use Committee (UNM-IACUC 10-100471-MCC).

Tortoises were fed diets with unknown isotopic values for up to 2 mo after hatching and before their first hibernation (when we acquired them). We warmed the tortoises in the spring (approximately 6 mo after hatching) and fed them for 307 d on a diet of ZooMed grassland tortoise chow ($\delta^{13}C = -25.0 \pm 0.1$ VPDB). We then switched tortoises to a diet of Mazuri tortoise chow ($\delta^{13}C = -21.9 \pm 0.2$) for 371 d. These two commercial diets differ in their nutritional composition (i.e., 9% vs. 15% protein for ZooMed and Mazuri diets, respectively), which may contribute to different patterns of carbon isotope dynamics.

Blood Tissue Dynamics Experiment

We analyzed the isotopic composition of red blood cells (RBCs), plasma solutes, and scute keratin. We sampled blood from two to seven tortoises on days 0, 2, 6, 10, 20, 40, 70, 99, 181, 282, 293, 321, and 371. Blood was drawn with a 27-ga needle and syringe from the dorsal cervical sinus, transferred to a hematocrit tube, and centrifuged (relative centripetal force = 14,800 *g* for 2–3 min) into plasma and RBC components. Tortoises became noticeably more difficult to extract blood from as the experiment progressed, possibly due to the accumulation of scar tissue. We followed blood preparation and analysis methods detailed by Warne et al. (2010).

Scute Growth Ring Experiment

Turtles generally grow via the addition of successive growth rings visible on keratinized scutes overlain on the bony shell (Cagle 1946; Wilson et al. 2003). Throughout the study, the desert tortoises grew and added two to four rings during the initial 307 d on the baseline diet and between two and five rings during the 371 d on the new test diet. On day 0, the start of the diet switch, and day 371, we sampled scute keratin from all tortoises using nonoverlapping regions of the same scute for both sampling periods. Using a razor saw (Revell 88-6964) and no. 21 scalpel, we cut and lifted a 15-mm-wide strip of keratin bisecting the growth rings on the second left pleural scute. We sampled only the scute and did not cut into the bony carapace. (Tortoises were not harmed during this procedure, and the scute eventually regenerates.) Each strip was scrubbed, washed in a 2 : 1 chloroform/methanol solution to extract any superficial lipids, and dried before being cut to separate individual growth rings with a razor blade under a dissecting scope. Keratin samples from individual growth rings were cut into 0.4–0.7-mg pieces and loaded into tin capsules (Costech 3 × 5 mm, no. 041074) for $\delta^{13}C$ analysis. Some rings were too wide to analyze as a single sample; in these cases, the ring was divided horizontally, and representative parts from each section were loaded into separate tins. Each of these ring parts was then averaged to acquire one $\delta^{13}C$ value per ring.

We measured the $\delta^{13}C$ values of tissue samples using a continuous-flow isotope ratio mass spectrometer (Thermo-Finnigan IRMS Delta Plus) connected to a Costech ECS 4010 Elemental Analyzer in the University of New Mexico Earth and Planetary Sciences mass spectrometry lab. The precision of these measurements was ±0.1‰ SD based on repeated measurements of internal lab standards. All sample runs included regularly spaced lab standards (soy $\delta^{13}C = -27.2$‰ VPDB) throughout the run that were calibrated against international standards used to correct tissue sample raw values. All values are reported using delta notation (δ) in parts per thousand (‰) as $\delta X = (R_{sample}/R_{standard} - 1) \times 1{,}000$. The terms R_{sample} and $R_{standard}$ represent the ratio of heavy to light isotopes ($^{13}C/^{12}C$) for the sample and standard.

Statistical Analyses

Animal tissue isotopic incorporation rates may be best estimated using one-compartment or two-compartment models. One-compartment models assume that stable isotope ratios from ingested foods mix in one compartment or pool and are replaced at constant rates by isotope ratios of newly eaten food items. In two-compartment models, the overall incorporation rates are integrated over multiple compartments in a given tissue with stable isotope ratios turning over, or being replaced, at different rates (Cerling et al. 2007; Kurle 2009). Some data support the existence of multiple carbon compartments operating within an animal (McCue 2007, 2011; Podlesak and McWilliams 2007; McCue et al. 2011), but determining the kinetics of various carbon pools in animals that utilize a microbial gut fauna for nutrient assimilation, such as desert tortoises, may be less straightforward (Nieto and Lobley 1999). The reaction progress variable method (RPV) developed by Cerling et al. (2007) is a valuable tool used to evaluate whether a one- or two-compartment model best fits carbon incorporation rates in a given tissue. In the RPV method, a diet switch experiment is modeled as the fractional approach to equilibrium,

$$\frac{\delta^t - \delta^{eq}}{\delta^{eq} - \delta^{init}} = 1 - F, \quad (1)$$

where δ^t is the isotope value at time t, δ^{eq} is the isotopic equilibrium value, and δ^{init} is the initial isotope value. The RPV

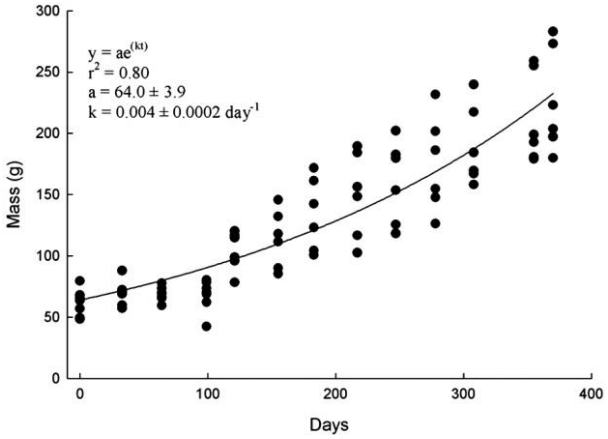

Figure 1. Growth in juvenile desert tortoises *Gopherus agassizii*, after the diet switch over a 371-d experiment. Each point represents the mass of an individual tortoise ($N = 7$ per date for first 100 d; thereafter $N = 6$). Juvenile tortoise growth is well characterized by an exponential function ($y = ae^{(kt)}$; $r^2 = 0.80$; $k = 0.004$ g d^{-1}).

method treats a turnover experiment as the fractional approach to equilibrium, leading to values of $F = 0$ at day 0 and $F = 1$ at equilibrium. Incorporation rates can then be displayed as the fraction of change at time t, between 0 and 1, in essence normalizing them. The RPV results can be log transformed ($\ln(1 - F)$) and graphed versus time, which allows the fractional approach to equilibrium to be represented as a straight line. An intercept less than 0 means that multiple pools are contributing to the isotopic value of the tissue, while an intercept greater than 0 means that there is a delay before the material from the new diet is incorporated into the tissue. A plotted intercept of 0 means that a single pool contributes 100% to isotope exchange. Visual inspection of $\ln(1 - F)$ over time initially determines whether one or multiple compartments are influencing isotope incorporation. We then used the approach of Martínez del Rio and Anderson-Sprecher (2008) to quantitatively select the best-fit model. We estimated isotope incorporation rates with nonlinear regression in SigmaPlot 8.0. If the one-compartment model was deemed most robust, we used the following equation:

$$\delta_t = \delta_{eq} - (\delta_{eq} - \delta_{init})e^{(-T/\tau)}. \quad (2)$$

If the two-compartment model was most robust, we used the following equation:

$$\delta_t = \delta_{eq} - (\delta_{eq} - \delta_{init})[pe^{(-T/\tau_1)} + (1 - p)e^{(-T/\tau_2)}]. \quad (3)$$

In both cases, δ_{eq}, δ_{init}, and δ_t are equilibrium, initial, and time t isotope ratios, respectively; T is time in days; τ is carbon residence time, or the mean length of time that a carbon atom is retained in a particular tissue pool; and p is the fractional contribution of each compartment to the two-compartment model. We follow Martínez del Rio and Anderson-Sprecher (2008) and Warne et al. (2010) and use τ to present isotope incorporation rates. Other investigators report tissue element half-lives ($t_{1/2} = \tau \ln(2)$) derived from fractional rates of incorporation ($\lambda = 1/\tau$; Hobson and Clark 1992; Carleton and Martínez del Rio 2005; Cerling et al. 2007). We used Akaike's Information Criteria corrected for small sample sizes (AICc) to test the goodness of fit of the one- or two-compartment models for each tissue. Here the AICc increases as a function of the number of model parameters. In short, the model exhibiting the lowest AICc is the preferred model. Thus, the one-compartment model is supported if $AICc_1$ is smaller than $AICc_2$, and the two-compartment model is supported if the reverse is true (Burnham and Anderson 2002; Martínez del Rio and Anderson-Sprecher 2008).

There are two processes (growth and catabolism) that potentially contribute to the fractional rate of incorporation of material into the tissues of an animal. The models presented thus far account only for incorporation of materials into tissues due to catabolism (c; Hesslein et al. 1993) and thus fail to account for the second potential source of incorporated material associated with an animal's growth. Our tortoises were hatchlings at the start of the experiment and grew rapidly over the entire course of the experimental treatment, and thus growth (k) must be considered in our models of incorporation. Thus, if we substitute λ for $1/\tau$, equation (2) becomes

$$\delta_t = \delta_{eq} - (\delta_{eq} - \delta_{init})e^{-(k+c)T}. \quad (4)$$

We can then use an exponential model ($y = ae^{(kt)}$) to estimate the fractional growth rate (k, in g d^{-1}) of juvenile desert tortoises in SigmaPlot 8.0. Since $\lambda = k + c$, our estimates of carbon incorporation rates (i.e., $\lambda = 1/\tau$) and fractional growth rates (k) allow us to quantitatively parse out the contributions of catabolism and growth to overall tissue turnover. Thus, if λ and k are indistinguishable, then growth can be assumed to be the only determinant of isotopic incorporation after the diet switch. If λ is higher than k, the difference is the influence of tissue catabolism.

Diet-to-tissue discrimination ($\Delta^{13}C_{tissue\text{-}diet}$) values were reported as the difference between tortoise tissue $\delta^{13}C$ values at equilibrium and $\delta^{13}C$ of the diet. We used t-tests to determine whether there were differences between tissue and diet $\delta^{13}C$ values. We used paired t-tests to examine $\delta^{13}C$ values in growth rings sampled on day 0, with the same rings resampled on day 371. All $\delta^{13}C$ estimates are given as mean ± SE (‰ VPDB).

Results

At the start of the diet switch, desert tortoises had mean plasma and RBC $\delta^{13}C$ values of −23.4 ± 0.4 and −24.2 ± 0.2, respectively, after feeding on an exclusive diet of ZooMed grassland tortoise diet for 10 mo (−25.0 ± 0.1). Five tortoises had three growth rings at the start of the diet switch (day 0), one had two, and one had four. Samples obtained from these rings had mean $\delta^{13}C$ values of −22.3 ± 0.5, −24.0 ± 1.8, −24.3 ± 0.6, −24.6 ± 0.2, and −24.7‰ for rings 0–4, respectively (here we use ring 0 for the neonatal scute and ring 4 for the most distally grown annulus). All of the tortoises avidly fed on the Mazuri tortoise diet (−21.9 ± 0.2) starting at day 0 and running

through day 371. Tortoises grew rapidly, and this growth was well described by an exponential function ($r^2 = 0.80$) with a fractional growth rate (k) of 0.004 ± 0.0002 g d^{-1} (fig. 1).

When the RPV was applied to our results, the linearized output for plasma and RBC supported the use of a two-compartment model, as evidenced by the negative intercepts with confidence intervals that did not overlap the origin (range, −0.70 to −0.14; fig. 2). The negative intercepts evident for plasma and RBC using the RPV method supported selection of the two-compartment model, but the one-compartment model was supported by the AICc comparisons because of the smaller value of $AICc_1$ relative to $AICc_2$ (plasma: $AICc_1$ = 9.9, $AICc_2$ = 15.2; RBC: $AICc_1$ = 13.4, $AICc_2$ = 17.9). Although model goodness-of-fit comparisons were equivocal (plasma: one-compartment adjusted $r^2 = 0.60$, two-compartment adjusted $r^2 = 0.58$; RBC: one-compartment adjusted $r^2 = 0.78$, two-compartment adjusted $r^2 = 0.81$), our selection of the less parameterized one-compartment model for plasma and RBC was informed by the model AICc comparisons (Burnham and Anderson 2002).

Mean (±SE) carbon retention time (τ) in tortoise plasma was 32.9 ± 14.5 and 126.7 ± 40.3 d in RBC (plasma = $-20.9+2.5e^{-T/32.9}$; RBC = $-21.7 + 2.5e^{-T/126.7}$). We do not present carbon retention times for scute keratin because we did not sample this tissue on a continuous schedule.

The estimated values of carbon incorporation rates (λ) for plasma (0.03) and RBC (0.008) are both higher than we would expect if growth ($k = 0.004$ g d^{-1}) alone is the determined rates of tissue carbon incorporation. Thus, catabolic tissue turnover played a key role in carbon incorporation of both plasma and RBCs, and growth contributed significantly to RBC carbon incorporation rates (50% ± 16%) but minimally to that of plasma (13% ± 6%).

Desert tortoise plasma diet-to-tissue discrimination ($\Delta^{13}C_{tissue\text{-}diet}$) was 1.0 ± 0.2, which was significantly enriched over that of diet (−21.9 ± 0.2; one-sample t-test, $t = 5.52$, $P < 0.05$). However, tortoise RBC $\Delta^{13}C$ (0.2 ± 0.3) was not significantly different from 0 (one-sample t-test, $t = 0.85$, $P > 0.05$). Using keratin sampled from the most recently accrued annulus of each tortoise after the diet switch (mean $\delta^{13}C = -21.1 \pm 0.1$), we observed a $\Delta^{13}C$ for scute keratin of 0.8 ± 0.1, which is significantly enriched over diet (one-sample t-test, $t = 6.6$, $P < 0.003$). Desert tortoise plasma (1.6 ± 0.4) and scute keratin (0.6 ± 0.3) $\Delta^{13}C_{tissue\text{-}diet}$ values after 10 mo on

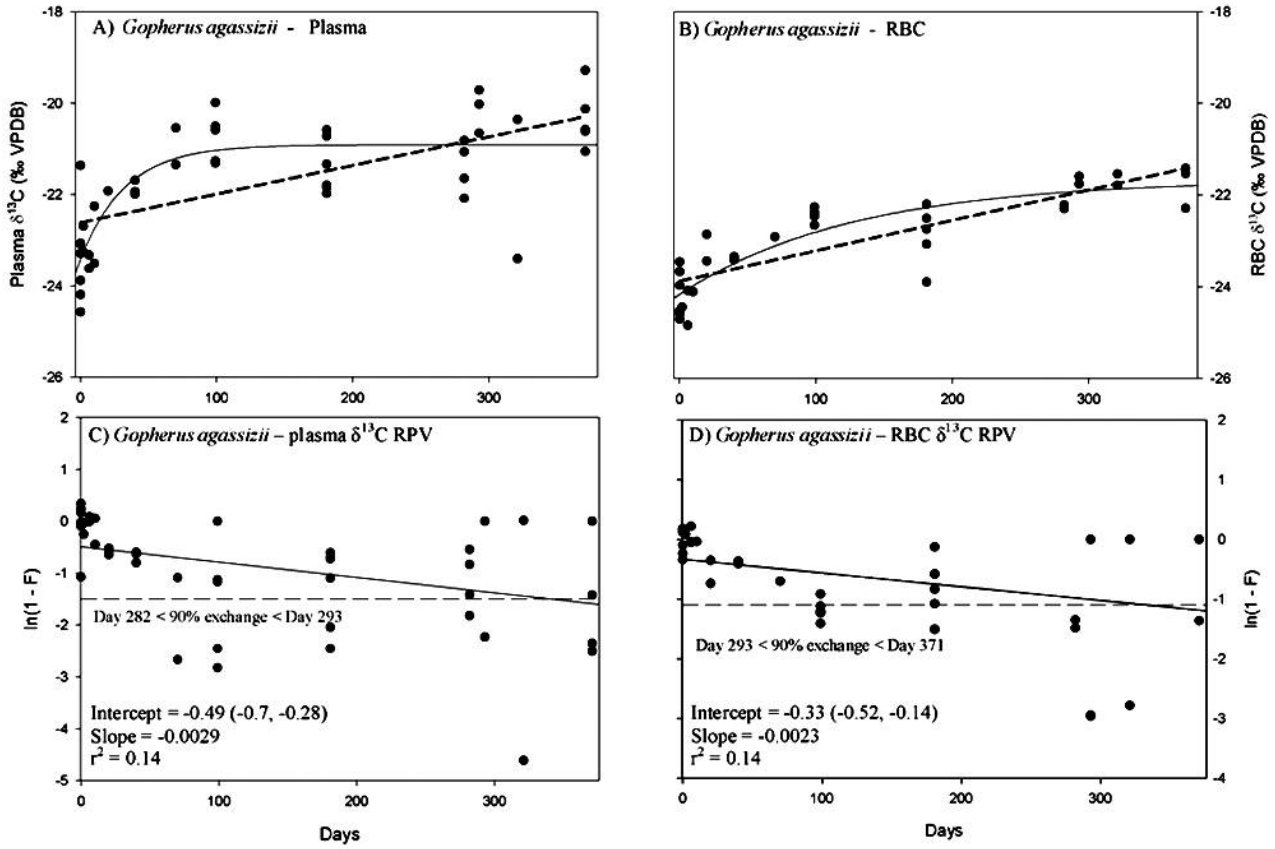

Figure 2. Changes in the $\delta^{13}C$ values of *Gopherus agassizii* blood plasma (*A*) and red blood cells (RBCs; *B*) during a 371-d diet switch experiment. Data best suit a one-compartment model (solid curve). Dashed lines (*A*, *B*) represent the fit if growth is the sole determinant of carbon incorporation rates ($k = 0.004$ g d^{-1}). The negative intercepts on the RPV plots (*C*, *D*) support the use of two-compartment models, but AICc comparisons support the use of one-compartment models for plasma and RBCs.

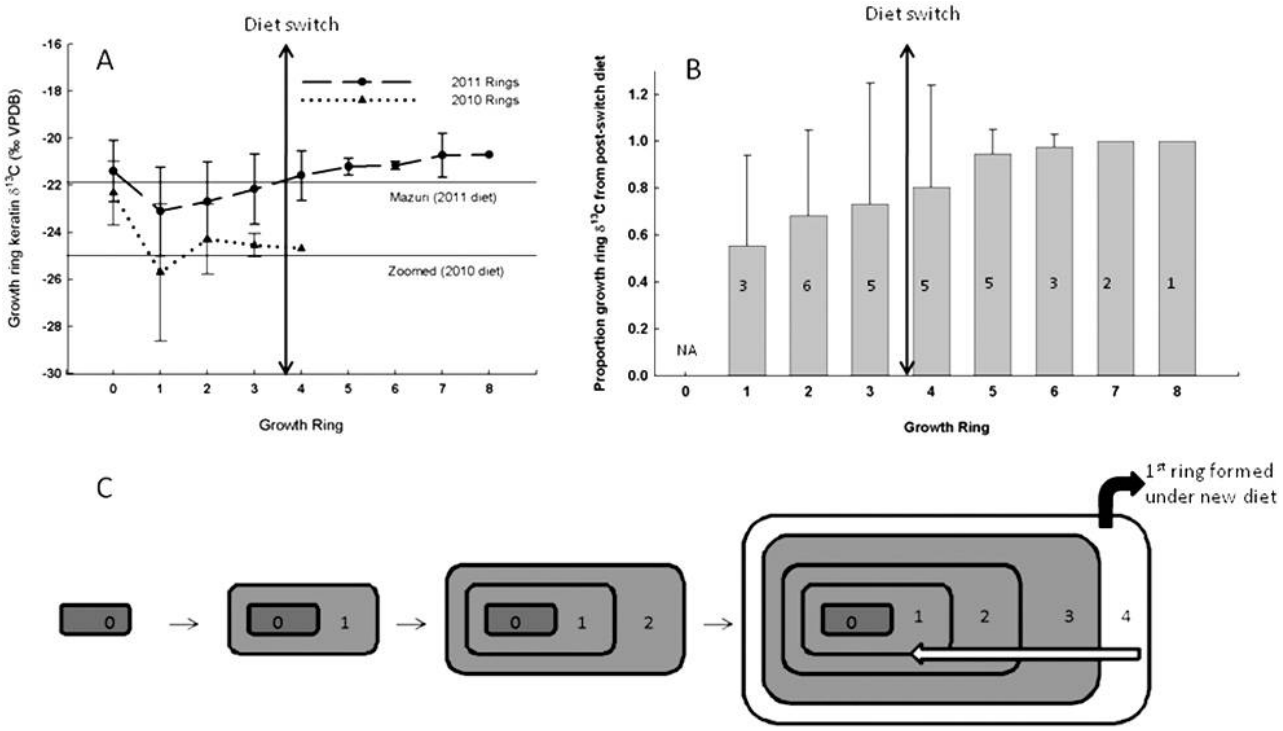

Figure 3. Growth ring keratin $\delta^{13}C$ values for growth rings added while being fed two isotopically distinct diets. *A*, Tortoises accrued two to three growth rings (one animal had four) after 10 mo on the 2010 ZooMed diet. For these rings and the additional rings grown after 371 d on the 2011 Mazuri diet, $\delta^{13}C$ values are plotted for comparison. The distalmost ring present at day 0, before the diet switch (ring 3; only one animal had four rings), is significantly enriched (paired *t*-test, $t = -3.7$, $P < 0.01$) after 371 d on the new diet, while the neonatal scute (ring 0), ring 1, and ring 2 show nonsignificant trends toward carbon enrichment. *B*, Two-source mixing model solving for the relative proportion of the Mazuri diet in growth ring keratin of old rings sampled after 371 d on the new diet. Significant carbon dilution is noted in rings 1–3, which were formed under the ZooMed diet (mean = 73%, 68%, and 55% for rings 3, 2, and 1, respectively). The neonatal scute (ring 0) is not included because its initial $\delta^{13}C$ value reflects unknown maternal inputs. (Mixing model: $\delta^{13}C_{(keratin)} = p(\delta^{13}C_{(2011\ diet)}) + (1 - p)(\delta^{13}C_{(2010\ diet)}) + \Delta$, where Δ = apparent keratin discrimination factor [*Gopherus agassizii* = 0.8‰].) Sample sizes are listed within the bars, and all error bars represent 95% confidence intervals. *C*, Hypothetical schematic illustrating in gray rings 1–3 grown under the ZooMed diet and the first ring (ring 4) in white, formed after the diet switch. A significant proportion of carbon from the new diet is incorporated into previously laid down growth rings; that is, there is carbon creep.

the preswitch ZooMed diet (-25.0 ± 0.1) were indistinguishable from those measured after the diet switch to the Mazuri diet (plasma: one-sample *t*-test, $t = 1.62$, $P > 0.05$; keratin: one-sample *t*-test, $t = -0.5$, $P > 0.05$). However, the tortoise RBC $\Delta^{13}C_{tissue\text{-}diet}$ while eating the more depleted preswitch ZooMed diet (0.8 ± 0.2) was significantly greater than the value measured in animals maintained on the Mazuri diet (one-sample *t*-test, $t = 3.04$, $P < 0.05$).

The $\delta^{13}C$ values for individual growth rings already extant at the start of the diet switch, and sampled before and after diet switch, reflected that of the new diet, in accordance with their proximity to the zone of new ring addition at the distalmost edge of the scute. This regular change in the carbon isotope ratios of preexisting growth rings after a switch to an isotopically distinct diet can be thought of as "carbon creep." Rings 0, 1, and 2 all showed nonsignificant trends toward enrichment (creep toward the new diet) when measured on day 371 relative to day 0 (0.9 ± 0.6, 0.4 ± 1.8, and 1.6 ± 0.8 for rings 0–2, respectively). Ring 3 was significantly enriched by 2.2 ± 0.6 on day 371 compared to day 0 (paired *t*-test, $t = -3.7$, $P < 0.01$). Only one tortoise had four rings at the start of the diet switch, and this fourth ring was enriched 3.4‰ by day 371 (fig. 3).

Discussion

We report on the carbon isotope incorporation and diet-to-tissue discrimination (Δ) in multiple tissues from juvenile tortoises feeding on isotopically distinct diets and explore the influence of growth on incorporation rates in different tissue pools. We also provide a valuable "calibration" of how to interpret the stable isotope chronologies evident in discretely deposited growth ring chronologies on tortoise scutes. Our survey of the literature suggests that these are the first measurements of diet-to-tissue discrimination and carbon incorporation rates reported in terrestrial turtles. Desert tortoise plasma and RBC $\delta^{13}C$ values achieved equilibrium with that of diet but had variable carbon incorporation rates and $\Delta^{13}C_{tissue\text{-}diet}$ values. Use

of the RPV method supported the need for two-compartment models to best characterize incorporation rates in plasma and RBC, but AICc comparisons strongly suggested that a one-compartment model best suited the data. Blood plasma had significantly faster incorporation rates relative to RBCs, and despite the exponential growth shown by all tortoises, catabolism remained an important determinant of incorporation rates, particularly in blood plasma. The carbon isotope ratios in previously deposited growth rings are significantly influenced by current dietary isotope ratios, particularly for those rings closest to actively growing scute edges, an indicator of new tissue addition over multiple seasons after a growth ring is initially deposited. Our study parallels that of Warne et al. (2010), but it is important to note that while lizards and tortoises are both terrestrial ectotherms, differences in diet, body size, growth patterns, and longevity among these groups are factors that importantly influence tissue carbon dynamics. *Gopherus* tortoises are obligate herbivores that grow rapidly for their first 20 yr and can live for 50 yr or more (Germano 1992, 1994), whereas the *Sceloporus* lizards in Warne et al.'s (2010) study are insectivores that mature in about 1 yr and live no longer than 4 yr (Vinegar 1975; Jones and Ballinger 1987). In the following discussion we underscore the similarities and differences between tortoises and other reptiles, put our results into a broader framework based on the literature, and illustrate how this study broadens our understanding of stable isotope dynamics in terrestrial ectotherms in general and chelonians in particular.

Carbon Incorporation Rates

The current literature suggests that carbon incorporation rates increase with decreasing body size, an idea supported by the observation of successively declining isotopic incorporation rates in larger species of birds and lizards (Carleton and Martínez del Rio 2005; Warne et al. 2010) and in line with the metabolic theory of ecology describing the dependence of ecological and physiological processes on metabolic rate and body size (Brown et al. 2004). Carleton and Martínez del Rio (2005) have postulated that rates of carbon incorporation are heavily dependent on tissue-specific protein turnover rates, which tend to be significantly higher as metabolic rate increases. For example, endothermic animals such as mammals and birds, which have total metabolic rates that are seven to 10 times those of ectotherms, have carbon incorporation rates that are seven to 20 times faster than those in similarly sized lizards (Warne et al. 2010). Despite distinct ecological differences and evolutionary trajectories in the tortoise lineage, our data are similar to those reported for other reptiles of comparable size. The half-life ($t_{1/2} = \tau \ln 2$) for carbon in desert tortoise plasma was 23 d, which is similar to the data on plasma half-life in reptiles of similar size (i.e., 14 d for hatchling loggerhead sea turtles; Reich et al. 2008) but four to seven times slower than that in birds (3 d) and mammals (6 d) of the same size (Hobson and Clark 1993; Kurle 2009).

Desert tortoise RBC carbon $t_{1/2}$ (88 d) was greater than those values reported for other similarly sized ectotherms. (Here we assume that reported whole-blood values are similar to RBC values.) For example, Bucheister and Latour (2010) found a half-life of 23 d in flounder blood, while Reich et al. (2008) found values of 53 d in hatchling loggerhead turtle RBC. Reported values for mammalian and avian RBC and whole-blood $t_{1/2}$'s are three to eight times faster than those reported here for tortoises (rat $t_{1/2} = 25$ d [MacAvoy et al. 2006]; rat $t_{1/2} = 30$ d [Kurle 2009]; quail $t_{1/2} = 11.4$ d; crow $t_{1/2} = 30$ d [Hobson and Clark 1992, 1993]). We suggest that tortoise plasma incorporation rates are likely similar to those of other ectotherms because plasma proteins are largely synthesized in the liver, which is a metabolically active tissue, with high rates of turnover and similar function in most organisms (Haschemeyer and Smith 1979; Tieszen et al. 1983). RBCs, however, are in general long-lived and unusually long-lived in turtles, circulating for 11 mo (Krasilnikov 1971), >500 d (Rodnan et al. 1957), or 600–800 d (Altland and Brace 1962). We propose that the relatively slow carbon incorporation rates seen in our study and that by Reich et al. (2008) reflect the exceptionally long life cycle of chelonian RBCs. Because we studied rapidly growing juveniles maintained under constant optimal conditions, our measured rates of carbon incorporation probably represent maximal rates for this species. McCue (2008) found rates of carbon turnover to be six to seven times faster in rapidly growing subadult cockroaches, relative to adults, and Carleton and Martínez del Rio (2010) documented tissue carbon retention times four to six times longer in slow-growing juvenile tilapia (*Oreochromis niloticus*) relative to more rapidly growing juveniles. Mature desert tortoises and/or tortoises experiencing seasonal temperature fluctuations are more likely to have slower incorporation rates than those seen here. If we remove the contribution of growth ($k = 0.004$) from our calculations of carbon retention times ($\lambda = k + c = 1/\tau$) for plasma and RBCs, we can estimate $t_{1/2}$'s ($t_{1/2} = \tau \ln 2$) solely determined by tissue catabolism in the nongrowing animal. This simulation suggests that older tortoises would show plasma $t_{1/2}$'s of approximately 26 d (compared to 23 d in this study) and RBCs would have $t_{1/2}$'s of 173 d (compared to 88 d).

Relative Contribution of Growth and Catabolism to Carbon Incorporation in Tissues

Growth contributed 13% and 50% to the carbon incorporation rates of desert tortoise plasma and RBC, respectively. In rapidly growing animals, whole-body growth rates may obscure the characteristic catabolic turnover rates unique to different tissue pools in the body (Reich et al. 2008). Growth was a minor contributor to carbon incorporation in desert tortoise plasma, and our data were more similar to the ~10% contribution of growth to plasma found in adult lizards and mice (MacAvoy et al. 2005; Warne et al. 2010) relative to the 30% and 48% contribution of growth found in hatchling and juvenile loggerhead sea turtles (Reich et al. 2008). Our result is contrary to the expected result that quickly growing ectotherms (or endotherms) in the early stages of their life cycles will have growth

rates that are major contributors to carbon incorporation rates. Indeed, some studies have shown that in larval fishes growth contributes 90% to carbon incorporation (Herzka and Holt 2000). The minor influence of growth observed in tortoise plasma incorporation rates is probably representative of the relatively rapid turnover rates of plasma solutes; that is, tissue catabolism is the primary determinant of plasma incorporation rate. Carleton and Martínez del Rio (2010) found that growth was a minor contributor to liver turnover in tilapia fish, and because liver and plasma proteins "track" each other, this suggests that this may be a general pattern. The observed contribution of growth to carbon turnover in RBC (50%) was almost identical to the 44% that Reich et al. (2008) reported in juvenile loggerhead turtles and is nearer to values expected for young rapidly growing organisms relative to adult animals at or near their asymptotic masses (MacAvoy et al. 2005; Warne et al. 2010). Our results show that the high growth rates of juveniles did not mask the inherent catabolic turnover rates in these blood compartments even when growth was most rapid, although this result may be tissue specific.

Diet-to-Tissue Discrimination Factors

The carbon isotope ratios of diet and tissue often differ and can be described by a diet-to-tissue discrimination factor (Δ). These discrimination factors represent the sum of the biochemical processes that occur upon the incorporation of ingested nutrients and may be tissue and species specific (Tieszen et al. 1983; Schoeller 1999; reviewed by Caut et al. 2009). Quantifying and understanding how these values vary among different tissues and taxa allow for rigorous interpretation of isotopic data that focuses on individual, population, and community performance in the field. These "offsets" are an important part of any effort to isotopically characterize consumer diets from tissue samples. The factors that influence the isotopic spacing between diet and tissue are not well understood. Growth rate and temperature (for ectotherms) have the potential to impact stable isotope discrimination factors. For example, in sea bass (*Dicentrarchus labrax*) and puffins (*Fratercula cirrhata*), individuals fed ad lib. (and growing more rapidly) had higher $\Delta^{13}C_{\text{tissue-diet}}$ values relative to individuals on restricted diets (Barnes et al. 2007; Williams et al. 2007), but Trueman et al. (2005) found no influence of growth rate on $\Delta^{13}C_{\text{tissue-diet}}$ values in salmon (*Salmo salar*). McCue (2008) found no impact of food restriction or temperature on carbon isotope diet-tissue spacing in cockroaches, whereas Cherel et al. (2005) documented lower carbon isotope tissue-diet spacing in some tissues (plasma) but not others (RBCs) in fasting penguins (*Aptenodytes patagonicus*). However, Hobson et al. (1993) found no effect of limiting food intake on the $\Delta^{13}C_{\text{tissue-diet}}$ values for several tissues in quail (*Coturnix japonica*) and geese (*Chen rossii*). The tortoises in this study experienced no nutritional stress and grew rapidly (as a result of continual access to an optimal thermal gradient), which leads us to postulate that the resulting tissue carbon isotope discrimination factors may be higher than those seen in wild tortoises enduring episodic nutritional constraints and growing more slowly or not at all.

Additionally, the isotopic direction of a diet switch may affect reported isotopic discrimination factors. Some researchers have found that discrimination factors vary depending on the direction of the diet switch (i.e., whether animals are going from an enriched to a depleted diet [elimination] or a depleted to an enriched diet [uptake]), although the biochemical basis behind these observations is not well understood. This is an important observation because it provides a more rigorous framework for the characterization and interpretation of tissue isotope data and consequent linkage of animal resource use with distinct sources available in the environment. These studies have documented a greater offset between tissue and diet in elimination diets (Webb et al. 1998; Bearhop et al. 2002; Olive et al. 2003; Pilgrim 2005). In this study, desert tortoises were fed an uptake diet. Before the switch, tortoises were maintained on a constant diet for 10 mo, which was depleted, or an elimination diet. Samples taken on day 0 of the diet switch reflect 10 mo of an elimination diet. If we assume that plasma, RBC, and scute were in equilibrium with the depleted diet after 10 mo, we can compare $\Delta^{13}C_{\text{tissue-diet}}$ values for tortoises on the uptake and elimination diets. Although tortoise RBCs were more enriched over diet when on an elimination diet, we found no difference between discrimination factors on the two diets for plasma and scute keratin, which is opposite the trend observed in pygmy rattlesnakes (*Sistrurus miliarus*), marine worms (*Nereis virens*), great skuas (*Catharacta skua*), and locusts (*Locusta migratoria*; table 1).

Growth Rings

The growth rings, or annuli, on tortoise scutes are visible indentations on the carapace that mark the boundaries of discrete periods of growth (Cagle 1946; Carr 1952). Captive desert tortoises held under optimal conditions for growth may deposit multiple growth rings in a year (Jackson et al. 1976, 1978; Tracy and Tracy 1995), and our tortoises produced two to four rings during the 371 d after the diet switch. These rings are often thought of as being biologically inert once deposited, but detailed histological and radioimmunological studies by Alibardi (2005, 2006) and Alibardi and Toni (2006) have shown that metabolically active tissue underlies the previously deposited scute keratin and is incorporated into previously accrued growth rings. In short, a thin, living epidermal layer always underlies the nonliving cornified scutes. When a new ring is formed, some of the keratinocytes (beta cells) formed using resources available from the current diet not only form a new keratinized growth ring at the distal edges of the growing scute but also form a thin layer of corneous material that is compressed into the cornified matrix of previously laid down growth rings (Alibardi and Toni 2006). This observation suggests important consequences for using the $\delta^{13}C$ measurements of growth rings to assess tortoise diets. If the $\delta^{13}C$ of the current diet differs from the $\delta^{13}C$ diet of the tortoise when the ring was originally grown, then the $\delta^{13}C$ of the old ring can actually

Table 1: Mean (±SE) $\delta^{13}C$ values at equilibrium and diet-to-tissue discrimination for *Gopherus agassizii* tissues

	Model $\delta^{13}C$ equilibrium	$\Delta^{13}C_{\text{tissue-diet (Mazuri)}}$ (uptake diet)	$\Delta^{13}C_{\text{tissue-diet (ZooMed diet)}}$[a] (elimination diet)
Mazuri diet	−21.9 ± .2	...	...
ZooMed diet	−25.0 ± .1	...	...
Plasma	−20.9 ± .2	1.0 ± .2	1.6 ± .4
RBC	−21.7 ± .3	.2 ± .3	.8 ± .2
Scute keratin	−21.1 ± .1	.8 ± .1	.6 ± .3

Note. Tissue $\delta^{13}C$ equilibrium estimates were derived from fitted models; $\Delta^{13}C_{\text{tissue-diet}}$ values found based on the model are compared with those in tortoise tissues in equilibrium with an isotopically distinct diet fed before the diet switch. Red blood cell (RBC) $\Delta^{13}C_{\text{tissue-diet}}$ values are significantly lower on the enriched uptake diet (Mazuri) relative to the depleted elimination preswitch diet (ZooMed; one-sample t-test, $t = 3.04$, $P < 0.05$). Plasma and scute keratin $\Delta^{13}C_{\text{tissue-diet}}$ values are not significantly different on the depleted and elimination diets.

[a]The listed $\Delta^{13}C_{\text{tissue-diet}}$ values for tortoises eating the preswitch diet (ZooMed diet) are based on the mean tissue values after 10 mo of feeding on this diet.

change as additional rings are added on a new diet and some of this material is deposited under the older rings. As a consequence, some level of dilution, or carbon creep, reflective of the current diet may be expected to influence the $\delta^{13}C$ of older rings more proximally located. We estimated the effects of carbon creep by measuring the $\delta^{13}C$ of all growth rings before the diet switch and then resampled these same rings along with new rings 371 d after the diet switch. Any new material deposited under old rings thus had a $\delta^{13}C$ value that differed from that of the old ring. We observed that there was significant dilution of carbon isotope values in previously grown rings by the new diet. Not unexpectedly, the growth ring adjacent to the growing scute edge showed the most dilution, with the new diet contributing 73% of the material to the older ring (ring 3). This relative contribution of new material decreased with progressively older growth rings (farther from the growing scute edge) and accounted for 68% and 56% of the material in rings 2 and 1, respectively (fig. 3). This finding parallels the findings of Alibardi (2006) and Alibardi and Toni (2006), who found that a majority of radiolabeled histidine and proline appears in cells located at the growing edges of the scutes closest to the currently growing annulus, with a reduction in uptake farther from the actively growing edges. This is an important process to consider when studying the stable isotope ratios in turtle growth rings because the stable isotope signal in a single annulus likely represents a cumulative record of diet over a period of time greater than that required for the deposition of that particular ring. We then conclude that the often widely fluctuating carbon isotope ratios in sequential growth rings in wild tortoises (up to 5‰; I. W. Murray and B. O. Wolf, unpublished data) are in fact very conservative estimates (i.e., we consistently underestimate dietary shifts) of the magnitude of the actual changes in forage intake occurring during the periods of time encapsulated by individual growth rings.

Our data are a step forward in answering the call for more experimental data necessary to better understand stable isotope dynamics in various organisms (Gannes et al. 1997; Martínez del Rio et al. 2009). We add to the paucity of data concerning terrestrial ectotherms and provide the first available data for tortoises. Data such as the reported $\Delta^{13}C_{\text{tissue-diet}}$ values and tissue carbon incorporation rates are vital for the stable isotope ecologist whose quest may be to characterize consumer nutrient fluxes in natural systems. To best interpret data like these, it is critical to know offsets between specific tissues and diet ($\Delta^{13}C_{\text{tissue-diet}}$) as well as the dietary window integrated by those tissues (carbon incorporation rates). In addition, we document and quantify the phenomena of currently growing annuli isotope ratios influencing the carbon values in inert, previously laid down growth rings (carbon creep). These data provide an important framework for interpreting the signal of past diet history locked within the keratin in tortoise growth ring chronologies.

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Literature Cited

Alibardi L. 2005. Proliferation in the epidermis of chelonians and growth of the horny scutes. J Morphol 265:52–69.

———. 2006. Ultrastructural and immunohistochemical observations on the process of horny growth in chelonian shells. Acta Histochem 108:149–162.

Alibardi L. and M. Toni. 2006. Immunolocalization and characterization of beta-keratins in growing epidermis of chelonians. Tissue Cell 38:53–63.

Altland P.D. and K.C. Brace. 1962. Red cell life span in the turtle and toad. Am J Physiol 203:1188–1190.

Barnes C., C.J. Sweeting, S. Jennings, J.T. Barry, and N.V.C. Polunin. 2007. Effect of temperature and ration size on carbon and nitrogen stable isotope trophic fractionation. Funct Ecol 21:356–362.

Bearhop S., S. Waldron, S.C. Votier, and R.W. Furness. 2002. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. Physiol Biochem Zool 75:451–458.

Brown J.H., J.F. Gillooly, A.P. Allen, V.M. Savage, and G.B. West. 2004. Towards a metabolic theory of ecology. Ecology 85: 1771–1789.

Bucheister A. and R.J. Latour. 2010. Turnover and fractionation of carbon and nitrogen stable isotopes in tissues of a migratory coastal predator, summer flounder (*Paralichthys dentatus*). Can J Fish Aquat Sci 67:445–461.

Burnham K. and D. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York.

Cagle F.R. 1946. The growth of the slider turtle, *Pseudemys scripta elegans.* Am Midl Nat 36:685–729.

Carleton S.A. and C. Martínez del Rio. 2005. The effect of cold-induced increased metabolic rate on the rate of $\delta^{13}C$ and $\delta^{15}N$ incorporation in house sparrows. Oecologia 144:226–232.

———. 2010. Growth and catabolism in isotopic incorporation: a new formulation and experimental data. Funct Ecol 24:805–812.

Carr A. 1952. Handbook of turtles. Cornell University Press, Ithaca, NY.

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

Cerling T., L. Ayliffe, M. Dearing, J. Ehleringer, B. Passey, D. Podlesak, A. Torregrossa, and A. West. 2007. Determining biological tissue turnover using stable isotopes: the reaction progress variable. Oecologia 151:175–189.

Cherel Y., K.A. Hobson, F. Bailleul, and R. Groscolas. 2005. Nutrition, physiology, and stable isotopes: new information from fasting and molting penguins. Ecology 86:2881–2888.

Dalerum F. and A. Angerbjorn. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647–658.

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

Fisk A., K. Sash, J. Maerz, W. Palmer, J. Carroll, and M. MacNeil. 2009. Metabolic turnover rates of carbon and nitrogen stable isotopes in captive juvenile snakes. Rapid Commun Mass Spectrom 23:319–326.

Gannes L.Z., D.M. O'Brien, and C. Martínez del Rio. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78:1271–1276.

Germano D.J. 1992. Longevity and age-size relationships of desert tortoises. Copeia 1992:367–374.

———. 1994. Growth and maturity of North American tortoises in response to regional climates. Can J Zool 72:918–931.

Haschemeyer A.E.V. and M.A.K. Smith. 1979. Protein synthesis in liver, muscle, and gill of mullet (*Mugil cephalus* L.) in vivo. Biol Bull 156:93–102.

Herzka S.Z. and G.J. Holt. 2000. Changes in isotopic composition of red drum (*Sciaenops ocellatus*) larvae in response to dietary shifts: potential applications to settlement studies. Can J Fish Aquat Sci 57:137–147.

Hesslein R., K. Hallard, and P. Ramlal. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by ^{34}S, ^{13}C, and ^{15}N. Can J Fish Aquat Sci 50:2071–2076.

Hobson K.A., R.T. Alisauskias, and R.G. Clark. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. Condor 95:388–394.

Hobson K.A. and R.G. Clark. 1992. Assessing avian diets using stable isotopes. I. Turnover of $\delta^{13}C$ in tissues. Condor 94: 181–188.

———. 1993. Turnover of ^{13}C in cellular and plasma fractions of blood: implications for nondestructive sampling in avian dietary studies. Auk 110:638–641.

Jackson C.G., Jr., J.A. Trotter, T.H. Trotter, and M.W. Trotter. 1976. Accelerated growth rate and early maturity in *Gopherus agassizii* (Reptilia, Testudines). Herpetologica 32:139–145.

Jackson C.G., Jr., T.H. Trotter, J.A. Trotter, and M.W. Trotter. 1978. Further observations of growth and sexual maturity in captive desert tortoises (Reptilia: Testudines). Herpetologica 34:225–227.

Jones S.M. and R.E. Ballinger. 1987. Comparative life histories of *Holbrookia maculata* and *Sceloporus undulatus* in western Nebraska. Ecology 68:1828–1838.

Krasilnikov E.N. 1971. Age-conditioned changes of blood cells in turtles and lizards. Arkh Anat Gistol Embriol 61:27–32.

Kurle C.M. 2009. Interpreting temporal variation in omnivore foraging ecology via stable isotope modeling. Funct Ecol 23: 733–744.

MacAvoy S.E., L.S. Arneson, and E. Bassett. 2006. Correlation of metabolism with tissue carbon and nitrogen turnover rate in small mammals. Oecologia 150:190–201.

MacAvoy S.E., S.A. Macko, and L.S. Arneson. 2005. Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis. Can J Zool 83:631–641.

Martínez del Rio C. and R. Anderson-Sprecher. 2008. Beyond the reaction progress variable: the meaning and significance of isotopic incorporation data. Oecologia 156:765–772.

Martínez del Rio C., N. Wolf, S. Carleton, and L.Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev 84:91–111.

McCue M.D. 2007. Stable isotopes track spatio-temporal patterns of nutrient allocation in postprandial pit-vipers. Res Rev BioSci 1:17–26.

———. 2008. Endogenous and environmental factors influence the dietary fractionation of ^{13}C and ^{15}N in hissing cockroaches *Gromphadorhina portentosa*. Physiol Biochem Zool 81:14–24.

———. 2011. Tracking the oxidative and nonoxidative fates of isotopically labeled nutrients in animals. BioScience 61:217–230.

McCue M.D., A. Smith, R. McKinney, B. Rewald, B. Pinshow, and S.R. McWilliams. 2011. A mass balance approach to identify and compare differential routing of ^{13}C-labeled carbohydrates, lipids, and proteins in vivo. Physiol Biochem Zool 84:506–513.

Michener R.H. and D.M. Schell. 1994. Stable isotope ratios as tracers in marine aquatic food webs. Pp. 137–157 in K. Lajtha and R.H. Michener, eds. Stable isotopes in ecology and environmental science. Blackwell Scientific, Oxford.

Nieto R. and G.E. Lobley. 1999. Integration of protein metabolism within the whole body and between organs. Pp. 69–99 in G.E. Lobley, A. White, and J.C. MacRae, eds. Protein metabolism and nutrition. Wageningen Pers, Wageningen.

Olive P.J.W., J.K. Pinnegar, N.V.C. Polunin, G. Richards, and R. Welch. 2003. Isotope trophic-step fractionation: a dynamic equilibrium model. J Anim Ecol 72:608–617.

Pilgrim M.A. 2005. Linking microgeographic variation in pigmy rattlesnake (*Sistrurus miliarius*) life history and demography with diet composition: a stable isotope approach. PhD diss. University of Arkansas at Fayetteville.

Podlesak D.W. and S.R. McWilliams. 2007. Metabolic routing of dietary nutrients in birds: effects of diet quality and macronutrient composition revealed. Physiol Biochem Zool 79: 534–549.

Pough F.H., R.M. Andrews, J.E. Cadle, M.L. Crump, A.H. Savitzky, and K.D. Wells. 2004. Herpetology. 3rd ed. Pearson Prentice Hall, Upper Saddle River, NJ.

Reich K.J., K.A. Bjorndal, and C. Martínez del Rio. 2008. Effects of growth and tissue type on the kinetics of ^{13}C and ^{15}N incorporation in a rapidly growing ectotherm. Oecologia 155: 651–663.

Rodnan G.P., F.G. Ebaugh Jr., and M.R.S. Fox. 1957. The life span of the red blood cell and the red blood cell volume in the chicken, pigeon, and duck as estimated by the use of $Na_2Cr_{51}O_4$ with observations on red cell turnover rate in the mammal, bird and reptile. Blood 12:355–366.

Schoeller D.A. 1999. Isotope fractionation: why aren't we what we eat? J Archaeol Sci 26:667–673.

Seminoff J.A., K.A. Bjorndal, and A.B. Bolten. 2007. Stable carbon and nitrogen isotope discrimination and turnover in pond sliders *Trachemys scripta*: insights for trophic study of freshwater turtles. Copeia 2007:534–542.

Seminoff J.A., T. Jones, T. Eguchi, D. Jones, and P. Dutton. 2006. Stable isotope discrimination ($\delta^{13}C$ and $\delta^{15}N$) between soft tissues of the green sea turtle *Chelonia mydas* and its diet. Mar Ecol Prog Ser 308:271–278.

Tieszen L.L., T.W. Boutton, K.G. Tesdahl, and N.A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37.

Tracy C.R. and C.R. Tracy. 1995. Estimating age of desert tortoises (*Gopherus agassizii*) from scute rings. Copeia 1995: 964–966.

Trueman C.N., R.A.R. McGill, and P.H. Guyard. 2005. The effect of growth rate on tissue-diet isotopic spacing in rapidly growing animals: an experimental study with Atlantic salmon (*Salmo salar*). Rapid Commun Mass Spectrom 19:3239–3247.

Vinegar M.B. 1975. Life history phenomena in two populations of the lizard *Sceloporus undulatus* in southwestern New Mexico. Am Midl Nat 93:388–402.

Warne R.W., C.A. Gilman, and B.O. Wolf. 2010. Tissue carbon incorporation rates in lizards: implications for studies using stable isotopes in terrestrial ectotherms. Physiol Biochem Zool 83:608–617.

Webb S.C., R.E.M. Hedges, and S.J. Simpson. 1998. Diet quality influences the $\delta^{13}C$ and $\delta^{15}N$ of locusts and their biochemical components. J Exp Biol 201:2903–2911.

Williams C.T., C.L. Buck, J. Sears, and A.S. Kitaysky. 2007. Effects of nutritional restriction on nitrogen and carbon stable isotopes in growing seabirds. Oecologia 153:11–18.

Wilson D.S., C.R. Tracy, and C.R. Tracy. 2003. Estimating age of turtles from growth rings: a critical evaluation of the technique. Herpetologica 59:178–194.

Zug G.R. 1993. Herpetology: an introductory biology of amphibians and reptiles. Academic Press, San Diego, CA.